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PHYTOCHEMICAL SCREENING AND INVESTIGATION OF INVITRO ANTHELMINTIC ACTIVITY OF AERIAL PARTS OF *RUTA GRAVEOLENS* L. (RUTACEAE)

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ABSTRACT

Background: Parasitic roundworms (nematodes) cause substantial morbidity and mortality in livestock animals globally. The control of these nematodes has relied largely on the use of a limited number of anthelmintic. However, resistance to many of these anthelmintic is now widespread and therefore, there is a need to find new drugs to ensure sustained and effective treatment and control into the future. **Methods:** The aerial parts of plant extract of *Ruta graveolens* was prepared by Soxhlet extraction method and phytochemical screening was performed according to the preliminary chemical tests. Then the methanol, ethyl acetate and aqueous extracts were evaluated for its anthelmintic activity against adult Indian earth worms Eudrillus eugeniae (family: Eudrilidiae). Three concentrations (25, 50 and 75mg/ml) of each extract were studied, which involved for the determination of time of paralysis and time of death of the test worms. Albendazole in same concentration as that of extract was used as standard reference and normal saline as control. **Results:** It was found that methanol (49±0.22) and ethyl acetate (65 ±0.24) extracts exhibited significant anthelmintic activity while aqueous extract (89±0.22) show least activity. **Conclusions:** These results suggest that extracts from *Ruta graveolens* have promising anthelmintic activity and that more broadly, plant extracts are a potential rich source of anthelmintic to combat helminthic diseases. Further it would be interesting to isolate the responsible phytoconstituents, which are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory.

KEYWORDS: Anthelmintic activity, Ruta graveolens, albendazole, Eudrillus eugeniae.

INTRODUCTION

Ruta graveolens, commonly called rue, is native to southern Europe. It is a glabrous, glaucous, woody-based, shrubby perennial with aromatic, fern-like, compound leaves. It typically grows in a mound to 2-3' tall. In some parts of the U.S. (particularly the northeast), it has escaped gardens and naturalized along roads, fields and disturbed areas. Notwithstanding its many historical uses, it is primarily grown today for ornamental purposes. Pinnately divided, blue green leaves (to 3-5" long) have oblong/spatulate segments. Foliage has a pungent aroma when bruised and leaves have a bitter taste. Small, 4- to 5-petaled, dull yellow flowers in clusters (flattened corymbs) bloom above the foliage in early summer. Fruit is a brown seed capsule. Ornamental value lies in the delicate blue green foliage. Rue was historically used for a large number of medicinal purposes, but effectiveness and safety concerns now discourage such uses. Leaves are toxic if ingested. Handling plants may cause dermatitis.

Easily grown in moderately fertile, dry to medium moisture, well-drained soils in full sun. Plants tolerate some light shade. Plants also tolerate poor soils as long as they are sharply drained. Drought tolerant once established. Plants perform well in hot and dry sites. Avoid wet soils. Winter mulch is important in the northern parts of this plant's growing range. Prune back plants to old wood in early spring. Propagate by seed or cuttings.[1]

Chemical composition of volatile oil

The essential oil was obtained by hydrodistillation of fresh leaf sample of *R. graveolens* L., and produced a green colour with a yield of 1.29% (v/w). The chemical compositions of essential oil were analyzed by GC and GC–MS and the result was presented in Table 1. In total, seven components were identified, representing 100% of the total amount. 2-Undecanone (47.21%), an aliphatic ketone was found as the main component. 2-Nonanone (39.17%) was the second major aliphatic ketone detected

in rue oil, followed by octyl acetate (7.31%), 2-decanone (2,.03%), diethyl phthalate (1.73%), 2-dodecanone (1.53%), pentadecenoyl acetate (1.02%), and others were found to be the minor components in the essential oil.[2]

Therapeutic use

Ruta graveolens has been administered for numerous types of diseases in Traditional System of Medicine, that it has antispasmodic, anti-inflammatory, analgesic and anthelmintic effects and also exhibit aphrodisiac activity. In addition, this natural medicament has been administered in gastrointestinal, pulmonary and genecology disorders. Later recommended the herb as a vasodilator, ant flatulent, diuretic and a herb to treat amenorrhea and dysmenorrhea. Moreover, leaves of RG have been applied as an appetizer, a medicament for skin disorders, sciatalgia, chill and fever, kidney stones, toxins and poisons, neurological disorders and also a flavour to control the taste and odour of syrup dosage forms. The herb is also widely used in other medical systems. Chinese and Indian traditional physicians applied the herb for syncope, neuralgia, rheumatalgia and ascites. However, the application has been contraindicated in abortions and pregnancies. The abortive effect, menstruation function and management of dyspepsia in veterinary medicines are also other applications of Furthermore, African traditional practitioners have used RG dry leaves for urinary ailments, diabetes, headache, and GI disorders and cardiovascular system. British and Denmark traditional medicines report the antirheumatic, tranquilizing and memory enhancing effects and cardiovascular disorders of the herb. With reference to current knowledge, RG showed antitumor activity against Dalton's lymphoma ascites (DLA), Ehrlich ascites carcinoma (EAC) and L929 cells (IC100 = 16 mg/ml) which may be related to prooxidant and hydroxyl radicals scavenging activities of the extract. The extract has also evaluated in some neurological disorders such as multiple sclerosis which was found effective.[3]

Anthelmintic activity

Anthelmintics or antihelminthics are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may be also called vermifuges (those that stun) or vermicides (those that kill). Anthelmintic are used to treat people who are infected by helminths, a condition called helminthiasis. These drugs are also used to treat infected animals. Helminthiasis or infections with parasitic worms are pathogenic for human beings.[4]

Helminthic infections are now being recognized as cause of many acute as well as chronic ill health among the various human beings. More than half of the population of the world suffers from infection of one or the other and majority of cattle suffers from worm infections. Treatment with an anthelmintic drug kills worms whose genotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Intestinal worm infections in general are more easily treated than those in other locations in the body. Because the worms need not be killed by the drug and the drug need not be absorbed when given by mouth, there is usually a wider margin of safety than with drugs for worm infections in other sites. Indiscriminate use of synthetic anthelmintic can lead to resistance of parasites. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human and could be of value in preventing the development of resistance**.**[5] Additionally, rampant worm resistance to commercially available anthelmintic adds complexity to parasite management plans and researchers have been looking for alternative compounds with effective anthelmintic properties.

This study will attempt to focus on the use of herbs and spices (including plant parts) considered "natural alternative compounds" to reduce gastrointestinal parasitism. Herbs, leaves and spices have been an essential factor in health preservation all over the world and in many cultures. Depending on the use, for external or internal applications, the extraction of the active ingredients varies. Generally, herbs are plants valued for their medicinal, aromatic, coloring and flavor enhancing properties. Hence, herbs are grown and harvested for such unique properties.[6]

The plants are known to provide a rich source of botanical anthelmintic. As we know very well, now a days the medicinal preparation available in the market from which most of them either not effective up to the mark or has to develop resistance resulting in reoccurrence again. Plant derived drug serve as a prototype to develop more effective and less toxic medicines.[7]

MATERIALS AND METHODS

Preparation of *Ruta graveolence* plant extracts.

Collection of plant material

Fresh aerial parts of *Ruta graveolens* were collected in the month of November 2019 from their natural habitat around areacode, mukkam and were authentified by botanist Dr. P.N Krishnan, Emeritus scientist, Malabar botanical garden and institute for plant science, Kozhikode. After botanical identification of the collected plants, voucher specimens, were deposited at the KSCSTE Malabar botanical garden for further verification.

Drying of the plant material.[8,9]

Aerial parts were cleaned and thoroughly washed with water and subjected to drying. The drying operation was carried out under controlled conditions to avoid too many chemical changes occurring and it may be dried under shade for about two weeks. The dried plant materials were homogenized using a grinder to a

moderately coarse (22/60) powder. Moderately coarse powder of size (22/60) is that powder of which all the particles pass through sieve no 22 and not more than 40% pass through a sieve no. 60.

Preparation of ethyl acetate, methanol and aqueous extract.[10]

The crude extracts were prepared by Soxhlet extraction technique. Coarsely powdered plant materials were subjected to extraction methods by using methanol, ethyl acetate and distilled water.

Preparation of ethyl acetate extract

For ethyl acetate extraction, powdered dry plant material (50 gm) was extracted with 200 ml ethyl acetate for 48 hrs by using Soxhlet apparatus. The extracts were filtered and concentrated by re distillation method and recovered the used solvents. The obtained semisolid is then dissolved in normal saline and used for the experiment. The percentage yield of extract was calculated by using initial weight of the herb and final weight of the extract and expressed in %w/w. The concentrate is stored in air tight container and kept for phytochemical analysis.

Preparation of aqueous extract

The dried powder (70g) was extracted with distilled water for 72hr and the same was dried on water bath.

Preparation of methanol extract

The dried powder (70g) extracted in a soxhlet apparatus using methanol (95%) at a temperature range of 45^0C to 60^0C. The filtrate was evaporated to dryness at reduced pressure in vacuum evaporator.

Chemicals and reagents

The following chemicals and drug were used:

- Sodium Chloride – AR grade
- Ethyl acetate – AR grade
- Methanol – AR grade
- Distilled water
- Albendazole suspension
- Reagents for phyto chemical screening

Qualitative phytochemical screening.

Preliminary phytochemical screening[11]

The prepared methanol, ethyl acetate and distilled water extract were subjected to chemical tests to determine the presence of various phytoconstituents like saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids.

Chemical tests for detection of organic chemical constituents:

A. Tests for Carbohydrates

Molish's test (General test): To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H2SO4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Tests for reducing sugars: Fehling's test- To 1 ml of ethanolic extract, 1 ml of the Fehling solution (Fehling A + Fehling B) was added. The mixture was heated on boiling water bath for 5-10 min. Development of yellow precipitates, changing to brick red precipitates indicated the presence of reducing sugars.

B. Tests for proteins

Biuret test (General test): To 3 ml test solution add 4% NaOH and few drops of 1% CuSO4 solution. Violet or pink colour appears.

Million's test (for proteins): Mix 3 ml test solution with 5 ml Million's reagent. White precipitation turns brick red or the precipitate dissolves giving red coloured solution.

C. Tests for Amino acids

Ninhydrin test: Heat 3 ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath 10 min. Purple or bluish colour appears.

D. Tests for fats and oils

Saponification test: Evaporate extract to get 10 ml oil. To oil add 25 ml 10% NaOH. Boil in boiling water bath for 30 min. Cool, add excess Na2SO4 solution. Soap forms and rise to the top. Filter, to filtrate add H2SO4. Evaporate, collect residue, it contains glycerol. Dissolve residue in ethanol. With ethanolic solution perform following tests:

- To ethanolic solution add few crystals of $KHSO_4$. Heat vigorously, pungent odour is produced.
- To ethanolic solution add few drops of $CuSO_4$ and NaOH solutions. Clear blue solution is observed.

E. Tests for steroids

Salkowski reaction: 2ml of chloroform and 2ml conc. H_2SO_4 was added to extract and shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Liebermann - Burchard reaction: Mix 2 ml extract was mixed with chloroform. 1-2ml acetic anhydride and 2 drops conc. H_2SO_4 were added from the side of test tube. First red, then blue and finally green colour appears.

F. Tests for volatile oils

a) Filter paper is not permanently stained with volatile oil
b) Solubility test: Volatile oils are soluble in 90% alcohol.

G. Tests for glycosides

Determine free sugar content of the extract. Hydrolyse the extract with mineral acids (dil. HCl/dil.H_2SO_4). Again, determine the total sugar content of the hydrolysed extract. Increase in sugar content indicates the presence of glycoside in the extract.

- **Tests for cardiac glycosides:**

Keller-Killiani test: To 2 ml extract add glacial acetic acid, one drop 5% $FeCl_2$ and conc. H_2SO_4. Reddish brown colour appears at the junction of two liquid layers and upper layer appears bluish green.

- **Tests for anthraquinone glycosides:**

Modified Borntrager's test: To 5 ml extract add 5 ml 5% $FeCl_2$ and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene, shake well. Separate organic layer. Add equal volume dilute ammonia. Ammoniacal layer shows pinkish red colour.

- **Tests for saponin glycosides**

Foam test: Shake the drug extract vigorously with water. Persistent foam observed.

Haemolytic test: Add drug extract to one drop of blood placed on glass slide. Haemolytic zone appears.

- **Tests for cyanogenetic glycosides:**

To the extract add 3% mercurous nitrate solution. Metallic mercury forms.

- **Tests for coumarin glycosides:**

Alcoholic extract when made alkaline shows blue or green fluorescence.

Take extract in test tube, cover with filter paper soaked in dilute NaOH. Keep in water bath. After sometime expose filter paper to UV light. It shows yellowish green fluorescence.

- **Tests for flavonoids**

Shinoda test: To the extract add 5 ml of 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings. Pink colour observed.

To small quantity of extract add lead acetate solution, yellow coloured precipitate is formed.

Addition of increasing amount of NaOH to the residue shows yellow colouration, which decolourises after addition of acid.

H. Test for alkaloids

The alcoholic extracts added with dilute HCl, shake well and filtered. Filtrate was performed following tests.

Mayer's test: 2-3 ml filtrate added with few drops Mayer's reagent shown precipitation.

Hager's test: 2-3 ml filtrate with Hager's reagent gives yellow precipitation.

Wagner's test- 2-3ml of filtrate added with few drops Wagner's reagent gives reddish brown precipitate.

Dragendorff's test- To 2-3 ml filtrate, few drops Dragendorff's reagent is added and if orange brown colour produced it confirms the presence of alkaloids.

I. Tests for tannins and phenolic compounds

To 2-3 ml of extract add few drops of following reagents:

- 5% $FeCl_2$ solution: deep blue-black colour.
- Lead acetate solution: white precipitation.
- Bromine water: Decolouration of bromine water.
- Acetic acid solution: red colour solution.
- Dilute HNO_3: reddish to yellow colour.

Pharmacological screening of anthelmintic activity by in-vitro method

Worm Collection and Authentication

Adult earthworms Eudrillus eugeniae (family: Eudrilidiae) in a diameter of (4-6 cm in length) were freshly collected from the Botanical garden, Department of Botany; they were washed with distilled water to remove adherent soil particles. The easy availability, anatomical and physiological resemblance of earthworms with the intestinal roundworm, parasites of human beings makes them an ideal model for anthelmintic assay.[12]

Anthelmintic bioassays

Methanol, ethyl acetate and aqueous extracts from the leaves of Ruta graveolens were investigated for anthelmintic activity against Eudrillus eugeniae. Various concentrations (25, 50 and 75 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline water as control. The Anthelmintic assay was carried as per the method followed by Ajaiyeoba et al with minor modifications.[13] The assay was performed on adult Indian earthworms, Eudrillus eugeniae due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings.[14,15,16] Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro.[17,18,19] The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol. The earthworms were divided into thirteen groups containing six earthworms in each group. All the extracts and standard drug solution were freshly prepared in normal saline before starting the experiments. Different extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: Methanol, ethyl acetate, aqueous extract and Albendazole in three different concentrations (25, 50 and 75 mg/ml). Observations were made for the time taken to paralysis and death of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when dipped in warm water (50ºC) followed with fading away of their body colours.[20]

STATISTICAL ANALYSIS

All the data are expressed as mean ± S.E.M. (standard error of the mean). Statistical analysis was performed by oneway analysis of variance (ANOVA) followed by Dunnett's test using graph pad prism ver.7. The threshold for the statistical significance was set at $p<0.05$. At 95% confidence interval $P<0.0001$ was considered significant when compared with standard Albendazole.

RESULTS AND DISCUSSION

Phytochemical screening

Preparation of extract

The aerial parts of *Ruta graveolens* was dried powdered and extracted by hot continuous extraction method using distilled water, ethyl acetate and methanol according to the polarity. Percentage yield was calculated as follows;

Y (%) = (We/ Wv) × 100

Y (%): Yield of extraction in percentage (%).

We: Weight of extract after solvent evaporation.

Wv: Weight of plant part used for extraction.

Table No. 1 Percentage yield of plant extracts.

Sl No.	Solvent	Colour and consistency	Yield(%w/w)
1	Distilled water	Dark brown non sticky	4.1049
2	Ethyl acetate	Brown sticky	1.3405
3	Methanol	Dark green non sticky	4.5436

Chemical test

Qualitative phytochemical examination of extract was carried out and results are shown in Table 2.

Table.2: Preliminary phytochemical tests for extract of Ruta graveolens.

Sl. No	Chemical tests	Observation		
		Ethyl acetate	Methanol	Aqueous
1	Carbohydrate			
a)	Molish's test (General test)	-	-	-
	Tests for reducing sugars: Fehling's test	-	-	-
2	Tests for proteins			
a)	Biuret test (General test)	-	-	-
b)	Million's test (for proteins)	-	-	-
3	Tests for Amino acids			
a)	Ninhydrin test	-	-	-
4	Tests for fats and oils			
a)	Salkowski reaction	+	+	+
b)	Liebermann - Burchard reaction	+	+	+
5	Tests for volatile oils	+	+	+
6	Tests for glycosides			
a)	Tests for cardiac glycosides	-	-	-
b)	Tests for anthraquinone glycosides	-	-	-
c)	Tests for saponin glycosides	+	+	+
d)	Tests for cyanogenetic glycosides	-	-	-
e)	Tests for coumarins glycosides	+	+	+
F)	Tests for flavonoids	+	+	+
7	Test for alkaloids			
a)	Mayer's test	+	+	+
b)	Hager's test	+	+	+
c)	Wagner's test	+	+	+
d)	Dragendorff's test	+	+	+
8	Tests for tannins and phenolic compounds			
a)	5% $FeCl_2$ solution	+	+	+
b)	Lead acetate solution	+	+	+
c)	Bromine water	+	+	+
d)	Acetic acid solution	+	+	+
e)	Dilute HNO_3	+	+	+

In vitro anthelmintic activity of *Ruta graveolens*

Anthelmintic Activity: The in vitro assays showed that crude extracts of aerial parts of plants have promising anthelmintic effects. The extracts produced a dose dependent anthelmintic activity in both distilled water, ethyl acetate and methanol.

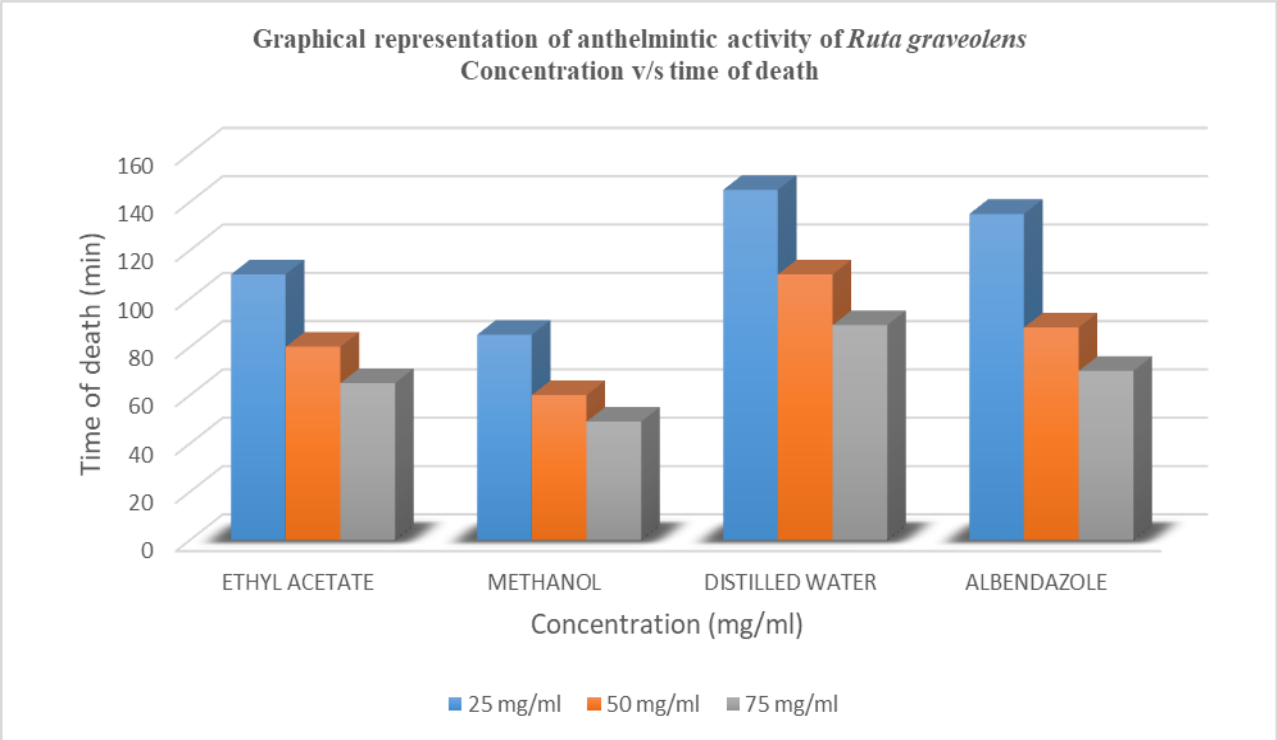
Adult Motility Test: This study indicated that extracts produced a relatively comparable anthelmintic activity with the conventional anthelmintic, albendazole. The activity increased with concentration and time. After 3 hours of exposure of adult earth worms to different concentrations of plant extracts, significant ($p < 0.05$) and dose-dependent reduction in motility was observed for plants (Table 6). At highest concentration (75mg/ml), plants produce mortality of adult earth worms to the level of 95% and 100% after 3 hr exposure to the extracts, respectively (Figure 5). Albendazole, on the other hand killed the parasites in a time-dependent manner and all the adult worms were dead at a concentration of 25 mg/ml within 2 hr after exposure. The adulticidal efficacy profile of the extracts, as measured by the percentage of the adult parasites killed at the end of observation period.

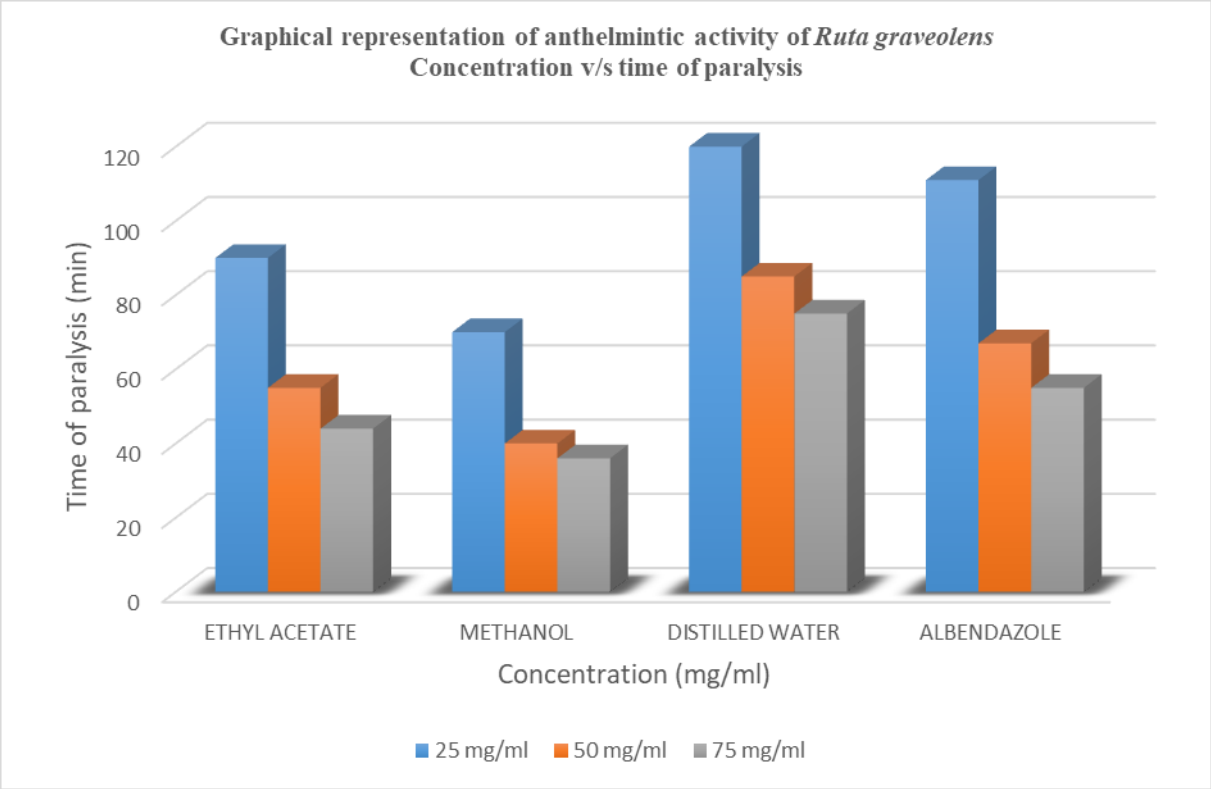
Time of paralysis: It was observed when the worms were not able to move even in normal saline. Death was confirmed when the earthworms lost their motility and fading off their body colours. The average value of each group is considered as the resultant value. Then the plant extracts with different concentrations was compared to the standard drug albendazole.

Table No.3 Invitro Anthelmintic activity of ethyl acetate, methanol and aqeous extract of Ruta graveolens.

Sl.No.	Groups	Conce. (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Normal control	Distilled water	No paralysis	No death
2	Sample I (Ethyl acetate extract)	25	90±0.15	110 ±0.22
		50	55± 0.12	80 ±0.23
		75	44 ±0.16	65 ±0.24
3	Sample II (Methanol extract)	25	70±0.32	85±0.23
		50	40±0.33	60±0.24
		75	36±0.32	49±0.22
4	Sample III (Distilled water extract)	25	120±0.12	145±0.23
		50	85±0.13	110±0.23
		75	75±0.14	89±0.22
5	Albendazole	25	111±0.52	135±0.43
		50	67±0.53	88±0.44
		75	55±0.53	70±0.43

Results expressed as Mean SEM (n=6), p<0.05(statistically significant) as compared to standard.





From the observations made all the extracts of whole plant of *Ruta graveolens* was found to show a potent anthelmintic activity when compared to the standard drug. Methanolic extract of at 75 mg/ml concentration shows paralysis at 36 min and death 49 min, whereas ethyl acetate extract shows paralysis at 44 min and death 65 min respectively while aqueous extracts show paralysis at 75 min and death 89 min by the earth worm. Among the three extracts aqueous extracts show least anthelmintic activity. The reference drug Albendazole exhibited the same at 55 min and 70 min respectively [Table 3]. Albendazole exhibits anthelmintic activity by blocking glucose uptake and depletion of glycogen stores in test parasite. The methanol and ethyl acetate extracts of *Ruta graveolens* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 75 mg/ml in shorter time as compared to that of Albendazole. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins and steroids. Tannins chemically polyphenolic compounds were shown to produce anthelmintic activities. Reported anthelmintic effect of tannins, can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. Further studies are under process to identify the possible phytoconstituents responsible for anthelmintic activity.

DISCUSSION

Results of present study, indicates that all the extracts showed concentration dependent anthelmintic property. The problem of anthelmintic resistance, toxicity, and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant-based drugs. Plant materials evaluated in the current study had been identified from various sources to serve as anthelmintic agents by traditional healers. The in vitro tests using free living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new plant compounds. In vitro techniques are preferred to in vivo methods due to their low cost, simplicity, and rapid turnover. In the current study, a statistically significant association was noted between graded concentrations of the extracts, the exposure test-time interval, and adult parasite mortality.

The whole plant of *Ruta graveolens* is documented to possess medicinal properties in ethnobotanical surveys conducted by ethnobotanists in traditional system of medicine. Moreover, in the present study the methanol extract, ethyl acetate and aqueous extract of aerial parts of *Ruta graveolens* possess anthelmintic activity. The present study showed 100% efficacy of the plant extract against the parasite at the concentration of 75 mg/ml which was the highest efficacy value and was comparable with the standard anthelmintic, albendazole. In the folk medicine, *Ruta graveolens* is an extensively studied medicinal plant throughout the world and has been reported to be used against wide ranges of human and livestock ailments. This is evident from the current study, which showed 95% mortality of adult parasites at a concentration of 75mg/ml in methanolic extracts of *Ruta graveolens*.

As screened in the phytochemical test of *Ruta graveolens* the secondary metabolites, alkaloid and tannin, are

responsible for their anthelmintic activity. In phytochemical screening of *Ruta graveolens*, it is revealed that the plant has secondary metabolites like alkaloids, flavonoids, tannins, and phenols. These classes of plant secondary metabolites are considered the sources of chemical components responsible for wide therapeutic activities of several medicinal plants. Some studies are available for anthelmintic activity of tannins, alkaloids, and flavonoids. The presence of these phytochemicals may be responsible for the observed anthelmintic activity of plant extracts in the present study. Furthermore, tannins have been shown to interfere with coupled oxidative phosphorylation, thus blocking ATP synthesis in these parasites. These drugs may reach the target site in worms either orally, by diffusion or uptake through the cuticle, however the major uptake of the drug is through cuticle. So that the presence of tannins in crude extracts found to produce anthelmintic activity. The presence of alkaloids in extracts may have direct effect on the nervous system of nematodes or can improve tonicity of the gastrointestinal tract and thus expel the worms.

Finally, the in vitro methods provide a means to screen rapidly for potential anthelmintic activities of different plant extracts. Due to drug biotransformation, interaction with food materials, and absorption variations, the results obtained by the in vitro method could not be extrapolated to in vivo activity. Therefore, results should be ascertained by in vivo evaluation

CONCLUSION

The herb is used for treatment of many ailments in traditional medicine. Though most of the remedies have shown positive pharmacological activities, yet a lot of them lack scientific proof and are used superstitiously. Therefore, the aim of the present study was to evaluate the pharmacological action of anthelmintic activity of ethyl acetate, methanol and aqueous extract of *Ruta graveolens*.

The plant material was collected, dried, authentified and herbarium is stored in KSCSTE- Malabar Botanical Garden and Institute for Plant Sciences. The extract of the plant material was prepared by Soxhlet extraction method using ethyl acetate, methanol and distilled water. The extract was concentrated and determine the percentage yield of the extract. Preliminary phytochemical analysis was performed by using standard testing procedure and indicated the presence of alkaloids, saponins, phenols, flavonoids, tannins and coumarin glycosides. The methanol, ethyl acetate and aqueous extracts were evaluated for its anthelmintic activity against adult Indian earth worms Eudrillus eugeniae (family: Eudrilidiae). Three concentrations (25, 50 and 75mg/ml) of each extract were studied, which involved for the determination of time of paralysis and time of death of the test worms. Albendazole in same concentration as that of extract was used as standard reference and normal saline as control. It was found that methanol (49±0.22) and ethyl acetate (65 ±0.24) extracts exhibited significant anthelmintic activity while aqueous extract (89±0.22) show least activity. These findings suggest that extracts from *Ruta graveolens* have promising anthelmintic effects. We propose that future work should focus on attempting to fractionate extract in order to identify and characterise the constituent that are active against helminthiasis and then to explore which biological pathways are affected by these components/fractions.

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