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Research Article

A Comparative Study on Anthelmintic Effects of Citrus Limon and Murraya Koenigii Leaf Extracts

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ABSTRACT

The study explores the anthelmintic properties of Citrus limon (lemon) and Murraya koenigii (curry leaf) leaf extracts as potential natural remedies against helminth infections which are a major global health concern, especially in developing regions. Using Indian earthworms (Lumbricus terrestris) as test organisms, the study assessed the effects of ethanolic extracts at varying concentrations (5, 10 and 20 mg/mL) by measuring the time of paralysis and death. Albendazole (10mg/ml) and 1%v/v Dimethyl sulfoxide (DMSO) were used as positive and negative controls. Results showed that both extracts exhibited dose-dependent anthelmintic activity, while Citrus limon being more effective than Murraya koenigii. At 20 mg/mL, Citrus limon caused paralysis at 78.67 minutes and death at 97.0 minutes, while Murraya koenigii caused paralysis at 83.83 minutes and death at 103.5 minutes. Statistical analysis confirmed significant efficacy compared to the negative control (DMSO), and at higher concentrations, Citrus limon showed effects comparable to the standard drug Albendazole. The findings suggest that Citrus limon and Murraya koenigii could serve as natural alternatives to traditional anthelmintic drugs, addressing concerns over drug resistance and limited treatment access.

INTRODUCTION

Helminthiasis, a condition mainly caused by parasitic worms such as roundworms, hookworms, and whipworms, represents a substantial global health challenge, impacting the lives of over 1.5 billion individuals worldwide and approximately 24% of the global population. This disease predominantly affects populations living in underprivileged and resource poor areas, where access to clean water, adequate sanitation, and basic healthcare services is severely limited. The highest prevalence rates are observed in regions grappling with extreme poverty. In India, the burden of helminthiasis is notably high, with approximately 241 million children aged 1 to 14

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years at risk of soil-transmitted helminth (STH) infections. The most common helminth infections in India include *Ascaris Lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura*, with infection rates suggesting that about one in five people in India are affected. [1]

The term "helminths" encompasses a wide and diverse group of parasitic worms that infect both humans and animals, often leading to significant health problems. They are classified into three main groups: roundworms (nematodes), flatworms (cestodes), and flukes (trematodes). Roundworms, like *Ascaris lumbricoides* spread through contaminated soil and water, leading to malnutrition and anemia. Flatworms, such as tapeworms (Taenia species), infect humans via undercooked meat, sometimes causing severe conditions like cysticercosis. Flukes, like *Fasciolopsis buski*, are transmitted through contaminated water or aquatic plants, affecting digestion and nutrition. [2]

Worm infections range from mild, with no symptoms, to severe, causing abdominal pain, diarrhoea, nutrient loss, and in extreme cases, rectal prolapse. In children, heavy infections can impair growth, cognitive development, and learning. [4] Helminthiasis is diagnosed mainly through clinical symptoms, stool sample analysis and by imaging. Severe infections can lead to anaemia, malnutrition, growth and developmental delays, intestinal obstruction, organ damage, neurological issues, infertility, and even blindness. Preventive measures include proper hygiene, safe food handling, wearing footwear, and access to clean drinking water. Public health initiatives, such as mass deworming and sanitation improvements, help reduce infection rates. Education on transmission and prevention is also thus crucial.[3]

Anthelmintics are antiparasitic drugs used to eliminate or kill parasitic worms such as roundworms, tapeworms, and flukes by disrupting their metabolism, causing paralysis, or impairing reproduction. Different classes of anthelmintics work through various mechanisms: benzimidazoles (e.g., albendazole, mebendazole, thiabendazole) inhibit microtubule formation, leading to parasite death[4]; avermectins like ivermectin enhance GABA release, causing paralysis[5]; praziquantel increases calcium ion permeability, leading to muscle spasms and detachment[6]; pyrantel pamoate acts as a neuromuscular blocker, causing paralysis and expulsion[7]; diethylcarbamazine (DEC) activates calcium channels in filarial parasites, leading to paralysis; and niclosamide inhibits oxidative phosphorylation, depleting parasite energy.[8] Socioeconomic factors further hinder treatment, particularly in low-income regions with poor healthcare access. Addressing these issues requires urgent action to combat resistance, improve drug availability, and enhance treatment strategies.[9]

Herbal remedies have long been used across cultures to treat parasitic infections. Various plants have demonstrated effectiveness against gastrointestinal and external parasites, such as *Azadirachta indica* (Neem) in South Asia, *Acokanthera oppositifolia* (Bushman's poison) in Africa, and *Datura metel* (Thornapple) in China. Other widely used remedies include *Allium cepa* (Onion) and *Allium sativum* (Garlic), *Mentha spicata* (Common mint), *Musa paradisiaca* (Banana), and *Nicotiana tabacum* (Tobacco).[10] This extensive use of herbal remedies underscores their potential as natural alternatives to synthetic anthelmintics, particularly in areas with limited access to conventional treatments.[11,12]

Lemon (*Citrus limon*) is a small evergreen tree of the Rutaceae family, valued for its aromatic leaves, fragrant flowers, and nutrient-rich yellow

fruit. Thriving in warm climates with full sun and well-drained soil, it is rich in essential oils (like limonene and linalool), flavonoids, and vitamin C-making it beneficial for both culinary and medicinal use [13][14]

Curry leaves (Murraya koenigii) are aromatic, nutrient-rich leaves from the Rutaceae family, valued for their distinct nutty-citrus aroma, culinary use, and medicinal properties including antioxidant, anti-inflammatory, and antidiabetic effects due to bioactive compounds like alkaloids and terpenoids; they grow on small trees in tropical climates and are staples in traditional medicine and modern diets. [15][16]

MATERIALS AND METHODS

Apparatus and Equipments

Iodine flasks, beakers, funnel, tripod stand, mechanical grinder, conical flask, petri dish, pipette, measuring cylinder, standard flask, stop watch

Chemicals

Ethanol (90%), Dimethyl Sulfoxide 1%v/v (DMSO), Albendazole, Distilled water

Collection of Raw Materials

Fresh leaves of *Citrus limon* (Rutaceae) and *Murraya koenigii* (Rutaceae) shown in figure no.1 and figure no.2 was collected separately from the local areas of Kottayam and authenticated by Dr. Varghese M.C. Head of Botany Department, Devamatha College, Kuravilangad, Kottayam. A voucher specimen has been kept in the herbarium of Devamatha College, Kottayam with the No. VII [*Citrus limon* (L)] and VIII [*Murraya koenigii* L]. After collection, the leaves were carefully shade-dried, pulverized using a mechanical grinder and stored at room temperature in a sealed container. [17,18]

Fig No.1: *Murraya koenigii* (curry leaves)

Fig No. 2: *Citrus limon* (Lemon)

Extraction of Active Constituents

The maceration method was utilized to extract active constituents from curry leaves and lemon leaves using ethanol (90%) as solvent. For every 25g of fresh, shade-dried and powdered sample 100ml of ethanol (25g/100ml) was used. Ethanol was poured over it until the material is completely submerged. Then the mixture was shaken continuously for an hour and is kept for a period of 7 days to ensure effective extraction. In those days, intermittent agitation was performed. After the maceration period, the mixture was filtered by Whatman filter paper to separate the liquid extract. Then, the ethanolic plant extract was allowed to evaporate at room temperature to get the final extract. This slow evaporation process was chosen to minimize the loss of important volatile compounds, which could be damaged by

evaporation of alcohol on heating. The extract is kept in a sterile container and stored at 4ºC for further experiment. [19,20]

Calculation of Percentage Yield

The percentage yield was calculated for each plant extract and major compounds with reference to the crude material taken using the formula. [19]

Percentage yield with reference to crude plant material =

(Weight in grams of extract obtained /Weight in grams of plant material taken) x 100

Collection of Earth worms

All experiments were conducted on Indian adult earthworms (Lumbricus terrestris), chosen for their anatomical similarity to human intestinal roundworm parasites. [17,21] The earthworms were collected from the moist soil present in the local areas of Pala, Kottayam and thoroughly washed with water to remove any faecal matter. The worm were identified and authenticated by Dr. Priya Joseph, Head of Zoology Department, Devamatha College, Kuravilangad, Kottayam.

In vitro Anthelmintic Activity

The study involved using the ethanolic plant extracts at concentrations of 5 mg/mL, 10 mg/mL, and 20 mg/mL to assess the time until paralysis and death in earthworms. The standard drug, Albendazole, was utilized at a concentration of 10 mg/mL, while a control group was treated with the solvent 1%v/v Dimethyl sulfoxide (DMSO). The 5 to 7cm-long adult Indian earthworms (*Lumbricus terrestris*) were aseptically moved into petri dishes with the proper labels and varying test concentrations (5, 10 and 20mg/ml) of both extracts as shown in figure no. 3.

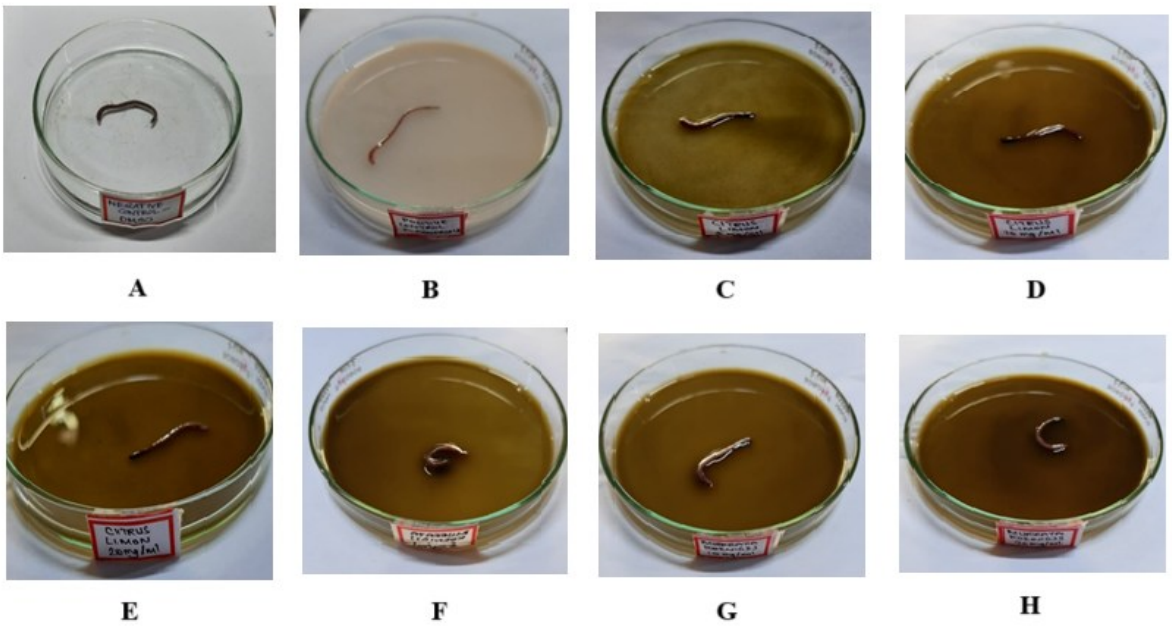

Fig No. 3: (A) Negative control-DMSO, 1%v/v (B) Positive control-Albendazole,10mg/ml *Citrus limon* leaf extract (C) 5mg/ml, (D) 10mg/ml and (E) 20mg/ml *Murraya koenigii* leaf extract (F) 5mg/ml, (G) 10mg/ml and (H) 20mg/ml

The worms were monitored for paralysis and death for a maximum of 8 hours. When no part moved except when the worms were shaken violently, it was considered that paralysis had taken place. The worms were considered dead when they did not move when shaken violently and occasionally when their body colour faded. [22,23] Petri dishes with 10 mg/ml of Albendazole were used as the positive control group and those with same concentration of DMSO (1%v/v) used as negative control group. Their motility and the time taken by the earthworm to become paralyzed and die were noted. The above procedure carried out in groups of six earthworms. [24,25]

Statistical Analysis

Values are expressed as mean ± Standard error mean, where n = 6. The results were analysed by one-way analysis of variance (ANOVA) followed by post Dunnett's post hoc multiple comparison test. The statistical analysis was carried out using Graph Pad Prism version 10.4.1 to analyse the data.[22]

RESULTS

Preparation of extract and calculation of Percentage yield

The powdered leaves of *Citrus limon* and *Murraya koenigii* was extracted by maceration method. For every 25g of fresh, shade-dried and powdered leaf 100ml of 90% ethanol was used (25g/100ml) and the final percentage yield of batches of macerated plant extract was found to be 27.5%w/w for *Citrus limon* and 30.3% w/w for *Murraya koenigii* leaf extracts.

In vitro Anthelmintic Activity Studies

The anthelmintic activity of *Citrus limon* and *Murraya koenigii* leaf extracts was evaluated by taking the ethanolic extracts at three different concentrations like 5, 10 and 20 mg/ml by using earthworms as the test organism. Both the extracts showed a dose-dependent anthelmintic activity against earth worms.

Table 1: Anthelmintic activity of *Citrus limon* leaf extract

Treatment	Concentration (mg/ml)	Time of Paralysis (min) Mean ± SEM	Time of Death (min) Mean ± SEM
Control (DMSO)	-	-	-
Standard (Albendazole)	10	80.00 ± 0.9309	100.00 ±0.9309
Citrus limon leaf extract	5	92.33±0.8819	109.5±0.8851
	10	81.33±1.145	104.7±1.606
	20	78.67 ±0.8819	97.00±1.155

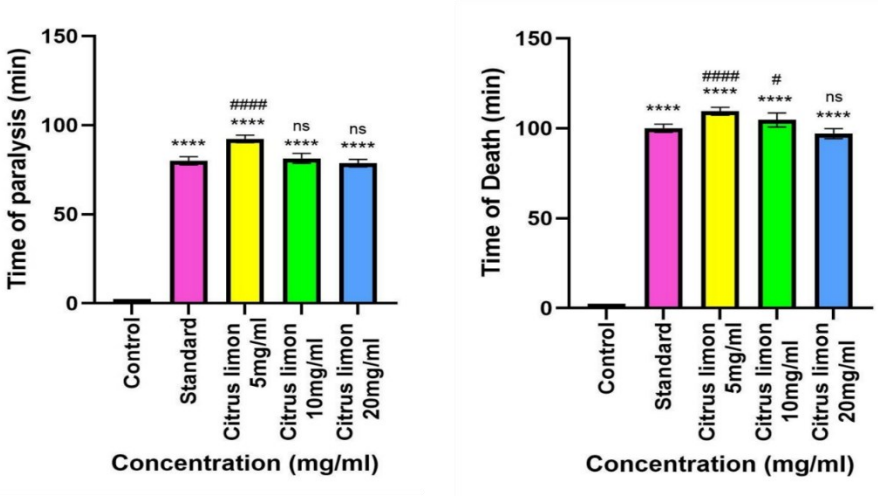

Fig No.4: Anthelmintic activity of *Citrus limon* leaf extract

Albendazole (10 mg/mL) caused paralysis and death of the worms at 80.00 ± 0.9309 and 100.00 ±0.8345min of exposure, respectively. At concentrations 5, 10, and 20mg/mL, *Citrus limon* extracts, significantly (p value < 0.0001****) caused paralysis of worms at 92.33±0.8819, 81.33±1.145, and 78.67±0.8819min respectively, and death at 109.5±0.8851, 104.7±1.606 and 97.00±1.155min respectively, compared to the negative control (DMSO) which showed no paralysis after the maximum time (8h) of exposure. The results of *Citrus limon* extract were shown in table no. 1 and figure no. 4. On statistical comparison with the Standard-Albendazole, *Citrus limon* 20mg/ml group showed no significant difference in ($p>0.05^{ns}$) anthelmintic activity to that of standard drug.

Table 2: Anthelmintic activity of *Murraya koenigii* leaf extract

Treatment	Concentration (mg/ml)	Time of Paralysis (min) Mean ± SEM	Time of Death (min) Mean ± SEM
Control (DMSO)	-	-	-
Standard (Albendazole)	10	80.00 ± 0.9309	100.00 ±0.9309
Murraya koenigii leaf extract	5	117.7 ± 0.7601	134.7±1.116
	10	86.17±1.327	106.5±1.176
	20	83.83±0.7923	103.5±0.9220

Murraya koenigii at concentrations of at 5, 10, and 20mg/mL significantly (p value < 0.0001****) caused paralysis of worms at 117.7 ± 0.7601min, 86.17±1.327 min, and 83.83±0.7923 min of exposure, respectively, and death at 134.7±1.116, 106.5±1.176 and 103.5±0.9220min respectively, which was significant (p value <0.0001****) when compared with the negative control group. The results *Murraya koenigii* leaf extract were shown in table no.2 and figure no. 5. *Murraya koenigii*, at the concentration 10mg/ml and 20mg/ml showed significant difference in anthelmintic activity ($p<0.001^{###}$, $p<0.05^{#}$ respectively), when compared with the standard.

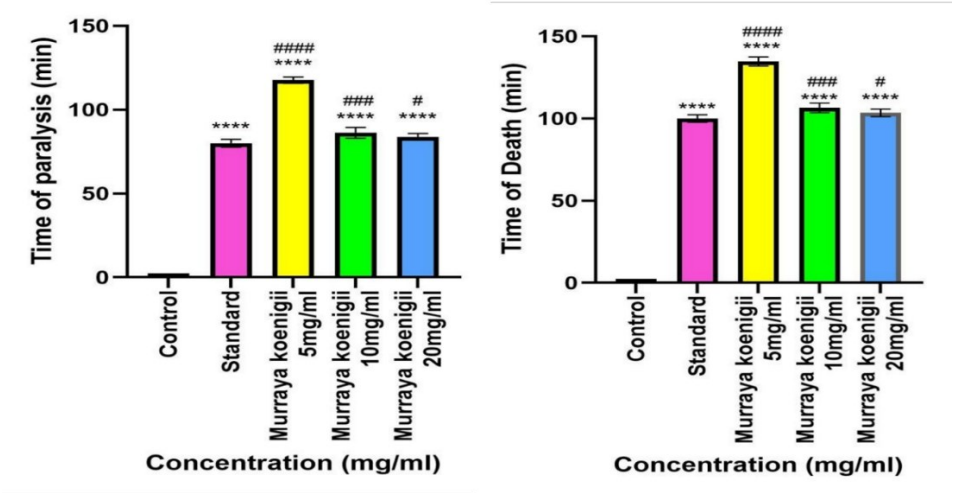

Fig No.5: Anthelmintic activity of *Murraya koenigii* leaf extract

There was significant anthelmintic activity for *Citrus limon* and *Murraya koenigii* leaf extracts in a dose dependent manner and the activity was found to be greater in *Citrus limon* leaf extract when compared to *Murraya koenigii* leaf extract.

DISCUSSION

The study on the anthelmintic activity of *Citrus limon* (lemon) and *Murraya koenigii* (curry leaf) leaf extracts provided significant insights into the potential use of these plants as natural remedies for parasitic infections. The study's methodology involved extraction by maceration, using ethanol (90%) as the solvent that effectively dissolves a wide range of phytochemicals, thus maximizing the yield of bioactive components. The yields of 27.5%w/w for *Citrus limon* and 30.3%w/w for *Murraya koenigii* indicates that both plants contain significant amounts of bioactive constituents. The ethanolic extracts, containing bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids, were tested at concentrations of 5, 10 and 20 mg/mL to assess their effectiveness in inducing paralysis and death. The choice of earthworms (*Lumbricus terrestris*) as test organisms for evaluating anthelmintic activity is grounded in their physiological similarities to parasitic worms that affect humans and animals.

The results demonstrated a marked dose-dependent effect for both extracts. For *Citrus limon,* the time taken for paralysis of earthworms decreased significantly with increasing concentration. At 5 mg/ml, paralysis occurred at an average time of 92.33± 0.8819 min, while at 10 mg/ml and 20 mg/ml, paralysis times were reduced to 81.33 ± 1.145 min and 78.67 ± 0.8819 min, respectively, and death at 109.5±0.8851, 104.7±1.606 and 97.00±1.155 min respectively. The rapid onset of paralysis at higher concentrations suggests that the active compounds in *Citrus limon* may interfere with neuromuscular transmission or disrupt metabolic processes within the worms, effectively incapacitating them. In case of *Murraya koenigii,* concentrations of at 5, 10, and 20mg/mL significantly caused paralysis of worms at, 117.7 ± 0.7601 min, 86.17±1.327 min, and 83.83±0.7923 min respectively and death at 134.7 ± 1.116 min, 106.5 ± 1.176 min, and 103.5±0.9220 min respectively. When compared to *Citrus limon, Murraya koenigii* exhibited less pronounced effects across all concentrations tested.

The Albendazole treated group showed paralysis in earthworms within a time frame of 80.00 ± 0.9309 min and resulted in death at 100.00 ±

0.8345 min. While Albendazole demonstrated effective results, particularly in inducing paralysis, both *Citrus limon* and *Murraya koenigii* showed better comparable effects at higher concentrations, suggesting that these plant-derived extracts could serve as viable alternatives to conventional treatments. The statistical analysis yielded highly significant p-values ($p < 0.0001^{****}$) when comparing both extracts to the negative control group (DMSO), which showed no paralysis or mortality after an extended exposure period of eight hours. This stark contrast highlights the effectiveness of both plant extracts in combating parasitic infections.

The statistical comparison with positive control, Albendazole reveals that *Citrus limon* at both 10mg/ml and 20mg/ml showed no significant difference in anthelmintic activity ($p>0.05^{ns}$) on evaluating the time of death, while at concentration of 10mg/ml extract showed a significant difference ($p<0.05^{\#}$) in time of paralysis when compared to albendazole treated group. The *Murraya koenigii* demonstrated a significant difference in anthelmintic activity at both 10mg/ml ($p<0.001^{\#\#\#}$) and 20mg/ml ($p<0.05^{\#}$) on paralysis and time of death when compared to the Albendazole. Both the extracts at a low concentration of 5mg/ml, exhibited a significant p-values ($p < 0.0001^{\#\#\#\#}$) when comparing to the positive control group (Albendazole).

The study's findings suggest that *Citrus limon* and *Murraya koenigii* extracts have significant anthelmintic potential, paving the way for further research. Future studies should focus on identifying the specific bioactive compounds responsible for their effects using techniques like chromatography and mass spectrometry. Extensive *in-vivo* studies using animals are necessary to assess their safety and effectiveness beyond laboratory conditions. Researches may explore combining these plant extracts with other herbal or conventional anthelmintic drugs to enhance their efficacy.

CONCLUSION

The study investigating the anthelmintic activity of *Citrus limon* (lemon) and *Murraya koenigii* (curry leaf) leaf extracts reveals promising potential for these plants as natural remedies against parasitic infections. There was significant anthelmintic activity for *Citrus limon* and *Murraya koenigii* leaf extracts in a dose dependent manner and the activity was found to be greater in *Citrus limon* leaf extract when compared to *Murraya koenigii* leaf extract on studies using *Lumbricus terrestris* (earthworms) as the test organism. Notably, both extracts showed better comparable efficacy to Albendazole, a standard synthetic anthelmintic drug. In conclusion, these findings underscore the importance of exploring herbal remedies in addressing global health challenges related to parasitic infections, particularly in regions with limited access to synthetic medications. By advancing our knowledge in this area, we can enhance the acceptance and integration of these natural products into modern therapeutic practices, ultimately contributing to more effective and accessible treatment options for communities affected by helminthiasis.

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CONFLICTS OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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