BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
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Articulo Original / Original Article

Evaluation anthelmintic activities of an ethnopharmacological leaves nerium oleander against the earthworms (*Allolobophora caliginosa*)

[Evaluación de las actividades antihelmínticas de un etnofarmacológico de las hojas de *Nerium oleander* contra las lombrices de tierra (*Allolobophora caliginosa*)]

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Received: 1 July 2023
Accepted: 21 July 2023
Accepted corrected: 1 April 2024
Published: 30 November 2024

Citation:
Mares M, Murshed M, Aljawdah HMA, Al-Quraishy S. Evaluation anthelmintic activities of an ethnopharmacological leaves *Nerium oleander* against the earthworms (*Allolobophora caliginosa*)
Bol Latinoam Caribe Plant Med Aromat
23 (6): 839 - 853 (2024)
https://doi.org/10.37360/blacpma.24.23.6.54

Abstract: The emergence of drug-resistant strains reason for the search for natural alternatives. The study aimed to evaluate the anthelmintic activity of *Nerium oleander* leaf extract against *Allolobophora caliginosa in vitro*. The concentrations (25 mg/mL, 50 mg/mL, and 100 mg/mL), were tested, and the results were expressed in paralysis time and the death of worms. Albendazole (40 mg/mL), and distilled water were used as standard drug and control. The FT-IR and GC-MS analysis of the extract showed the presence of 12 and 13 compounds responsible for anthelmintic activity. There was a dose-dependent increase in effectiveness over time during 2.5, 5, 7.5, and 10 minutes. The death reached 97% and 100%, respectively when (100 mg/mL) after 10 minutes. Histological examination and scanning electron microscopy showed noticeable abnormalities and a cuticular layer of the body surface had shrunk in all treated worms. The current experiment results show that oleander has powerful anti-Acaliginosa action.

Keywords: *Allolobophora caliginosa*; *Nerium oleander*; Histopathological; Paralysis; Death.

Resumen: La aparición de cepas resistentes a los medicamentos motiva la búsqueda de alternativas naturales. El estudio tuvo como objetivo evaluar la actividad antihelmíntica del extracto de hoja de *Nerium oleander* contra *Allolobophora caliginosa in vitro*. Se probaron las concentraciones (25 mg/mL, 50 mg/mL y 100 mg/mL), y los resultados se expresaron en tiempo de parálisis y muerte de las lombrices. Se utilizaron albendazol (40 mg/ml) y agua destilada como fármaco estándar y de control. El análisis FT-IR y GC-MS del extracto mostró la presencia de 12 y 13 compuestos responsables de la actividad antihelmíntica. Hubo un aumento de la eficacia dependiente de la dosis a lo largo del tiempo durante 2,5, 5, 7,5 y 10 minutos. La muerte alcanzó el 97% y el 100%, respectivamente, a una concentración de 100 mg/mL después de 10 minutos. El examen histológico y la microscopía electrónica de barrido mostraron anomalías notables y una capa cuticular de la superficie del cuerpo se había reducido en todos los gusanos tratados. Los resultados del experimento muestran que oleander tiene una poderosa acción anti-Acaliginosa.

Palabras clave: *Allolobophora caliginosa*; *Nerium oleander*; Histopatológico; Parálisis; Muerte

INTRODUCTION

The helminths are responsible for many diseases worldwide, causing helminthiases. Several different types of parasites can be generally categorized as roundworms, tapeworms, and flukes. An illness that is considered microparasitic is one in which only a small area of the body is affected by parasitic worms. Gastrointestinal helminths also affect farming systems worldwide (Alzahrani *et al*., 2016). These parasitic diseases cause disruptions in the intake of nutrients by animals and plants, particularly earthworms, which reduces body weight and increases the risk of secondary infections (López-Osorio *et al*., 2020). The use of anti-parasitic medications is the primary focus of the majority of the research initiatives that are being conducted to combat parasites. However, using these treatments without moderation and for extended periods of time has resulted in the development of drug resistance and harmful side effects (Abbas *et al*., 2012).

Traditional medicine is a significant component of a community's cultural legacy, and it has evolved in tandem with the lifestyle and cultural traditions of the civilization in which it has been practiced. The accumulation of first-hand, practical experiences with the therapeutic efficacy of herbal treatments has contributed to the enrichment of several traditional medical systems worldwide. Conventional medical practices from nations such as India, China, and Arabia are highly developed and are also gaining significance in other parts of the world. The traditional medical methods of India have made their way to several other nations, including Malaysia and Latin America. According to a study by the WHO, traditional medicines are the primary form of treatment for almost 80% of the world's population. Compounds derived from plants have been discovered as an alternate method for preventing and treating parasitic diseases (Klimpel *et al*., 2011; Elkhadragy *et al*., 2022). These drugs are effective against the parasites and may also have organ-protective qualities in the target host, which is infested with parasites (Abbas *et al*., 2013; Wunderlich *et al*., 2014). Conventional treatment of nematode infections with traditional anthelmintic agents generated approximately $294 million in veterinary market revenue (Molento *et al*., 2004). The consequences are far greater than the rising costs of animal husbandry. There is no clear evidence that synthetic anthelmintics leave no residue on meat, posing a potential public health hazard (Rodrigues *et al*., 2007).

Nerium oleander, often known as *Nerium oleander* L., is a landscaping and decorative plant. It is an ornamental evergreen shrub or small tree native to the Mediterranean region and Southeast Asia. It is widely cultivated for its showy, fragrant flowers that come in shades of pink, red, white, and yellow. Despite its beauty, all parts of the plant contain toxic compounds, and ingesting the leaves, flowers, or fruit can cause serious health problems. *N. oleander* has been used as a medicinal plant for the treatment of a variety of ailments. Traditional medicine uses oleander to treat epilepsy, cancer, cancerous tumors, and cardiac conditions (Abbas *et al*., 2012).

Most of these behaviors are connected to the presence of substances called cardiac glycosides (Abu Hawsah *et al*., 2023). However, this class of substances also contributes to the toxicity of this herbal medicine. In many parts of the world, most notably in nations like India and China (Sen *et al*., 2017). *Nerium* is employed in the practice of traditional medicine. The flower makes a green dye that is beneficial for treating skin conditions. Moreover, this dye can treat wounds and lessen skin inflammation. The plant treats several reproductive issues in Trinidad and Tobago (Lans, 2007). In Kenya, an extract from the leaves and seeds is used to treat upper respiratory tract and gastrointestinal conditions (Nanyingi *et al*., 2008). The leaves, which may be found in Calabria in southern Italy, have long been utilized in the area's traditional folk medicine to treat malaria. The juice prepared from the stem bark of *Nerium* is used as an earache remedy in the local traditional medical systems in the Kancheepuram region of Tamil Nadu, India (Lans, 2007). Moreover, it is utilized in Morocco to treat diabetes (Bnouham *et al*., 2002).

The plant is used in traditional medicine in Iloilo, Philippines, to treat several illnesses, such as fever, headaches, and skin issues. In the Moroccan province of Errachidia, *Nerium* is used to treat diabetes and hypertension (Tahraoui *et al*., 2007). Also, many secondary metabolites, such as alkaloids, flavonoids, and steroids, have been detected in this plant species. Therefore, several studies have reported that this plant has many pharmacological properties, such as antibacterial, anthelmintic, anti-inflammatory, hepatoprotective, immune-enhancing, antipyretic, antioxidant, antifungal, antitumor, and anti-HIV activity (Toyang & Verpoorte, 2013). According to the findings of the studies, *N. oleander* possesses potent anti-diabetic properties, supporting the claims of numerous ethnopharmacological methods (Pandey, 2007).

The *N. oleander* contains toxic compounds and is also a pharmacological plant, so the current study was designed to consider the possible role that the available chemical constituents of *N. oleander* leaf extracts could play in anthelmintic activity when tested *in vitro*.

MATERIALS AND METHODS

Preparation of plant material

The *Nerium oleander* (Apocynaceae) leaves were collected from Diriyah Botanical Gardens, Riyadh, Saudi Arabia, in the month of December 2022. The plant was identified and authenticated by a botanist at the herbarium of the Botany Department (King Saud University, Riyadh, Saudi Arabia).

Extraction preparation

The collected Nerium oleander leaves were washed thoroughly in water and air dried for a week at 35-40°C; to remove the moisture content, the leaves were kept in a hot air oven at 25°C for 30 minutes. The dried leaves were pulverized using an electric grinder (Senses, MG-503T, Korea). Then the powder was steeped in 70% ethanol (ultrasound-assisted extraction) for several hours (24 hours) to extract various active compounds from the decoction. Maceration extraction was performed on the dried powder of Apocynaceae leaves (200 g) at 4°C, and then total extraction was achieved by percolating the mixture 2–5 times. Based on previous research (Chen *et al*., 2006; Borjigidai *et al*., 2014). the powder underwent a vacuum extraction process at room temperature. After extraction, the extract was separated using two layers of filter paper, gathered, and a concentrated rotating vacuum evaporator (Yamato RE300, Japan), at 50ºC and lowered pressure. Before usage, the extract's concentration was diluted with ethanol to a final concentration of 0.5 mg/mL. After obtaining the crude quote, it was lyophilized and kept at a temperature of -20°C until usage.

Infrared spectroscopy

After completing the processing steps, a minute portion of the material was homogenized with excessive potassium bromide powder (1:99 wt%). After that, the material went through a coarse crushing process before being loaded into a pellet-forming die. The infrared spectrum was analyzed using an optical spectrometer from Thermo Scientific called the NICOLET 6700 Fourier-transform infrared spectroscopy (FT-IR). This allowed for the prediction of the most probable constituent classes. The expression "several waves" (cm^{-1}) denotes the greatest number of absorbed waves absorbed. Spectra were recorded at 25°C, with a resolution of 4 cm, and the spectrum range was from 4000 cm-1 to 400.

The anthelmintic effects of N. oleander

The *in vitro* anthelmintic activity was carried out in adult earthworms (*Allolobophora caliginosa*) collected from moist soil and washed with normal saline to remove all fecal matter. Due to their easy availability and anatomical, and physiological resemblance with the human intestinal roundworm parasite Ascaris lumbricoides, earthworms have been widely used for the initial evaluation of anthelmintic activity (Khan *et al*., 2011). Five different methanolic *N. oleander* leaf extract (NOLE) dosages were used in the anthelmintic investigation against the earthworm, *Allolobophora caliginosa*. The reference medication utilized was albendazole. As a control, worms in pure water were employed. Each group consisted of worms that were roughly the same size. The amount of time needed to reach death and paralysis was given in minutes, according to (Ajaiyeoba *et al*., 2001).

Administration of extract

Methanolic extracts of Nerium oleander leaves at different concentrations (25, 50, 100, 150 mg/mL) were prepared by diluting the stock solution using normal saline (0.9% NaCl), and its final volume was made to 10 mL. Albendazole was prepared in normal saline at 10 mg/mL. The final volume of standard drug solution and different concentrations of extracts were poured into separate Petri dishes. Adult earthworms (*A. caliginosa*) collected from moist soil and washed with normal saline were used for anthelmintic activity; the anthelmintic assay was carried out as per the methods of (Ajaiyeoba *et al*., 2001; Teng *et al*., 2014). The animals were divided into five groups, each containing six earthworms of equal size. Groups first and second received normal saline and the standard drug Albendazole (15 mg/kg), and groups third, fourth, and fifth received different concentrations of a methanolic extract of *Nerium oleander* leaf. Earthworms were placed in 10 ml of the desired concentration of drugs and extracts. Observations were made for the time taken for paralysis (paralysis was said to occur when the worm did not revive in normal saline) and death (time for the end of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor dipped in boil water (100°C), followed with their body colors fading away) for evaluation of the anthelmintic activity of *Nerium oleander* extract (Hadizadeh *et al*., 2009).

Adult motility assay

Worm motility inhibitions were employed as a sign of worm demise or paralysis. After treatment Minutes to anhour approximately, the control follow-up lasted more than four hours, the worms' motility was examined, and the number of motile worms was measured at various intervals. Picked worms without motility were placed in lukewarm PBS for five minutes. If the worms' motility returned, they were considered alive; if not, they were classified as dead.

Examinations of histopathological

The approach was utilized to prepare the treated and control worms for histological examination immediately following the paralysis and death experiment. Specimens were fixed in 10% formalin for 24 hours before being dehydrated in a graded ethanol series and embedded in paraffin. Tissues were then sectioned into thin sections with a microtome, stained with hematoxylin and eosin (H & E), and photographed using an Olympus B61 microscope (Tokyo, Japan) (Roy & Tandon, 1991).

Scanning electron microscopy (SEM)

The treated and control worms were fixed for SEM immediately after paralysis using the usual procedure (1991). Worms were set in 3% buffered glutaraldehyde for 2 hours at 4°C, then dehydrated with successive grades of acetone, air-dried in tetramethyl silane (TMS), mounted on metal stubs, and gold-palladium coated. Specimens were studied and photographed in a Jeol JSM-6060LV with a 15-kV accelerating voltage (Roy & Tandon, 1991).

Statistical analysis

All the results were expressed as Mean ± SEM Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Origin 2018 64 Bit® (Systat Software Inc., Chicago, USA. Differences among groups were considered significant at $p \leq 0.05$. $p < 0.01$.

RESULTS

The FT-IR analysis and qualitative phytochemical investigation of alcoholic extracts of the leaves of *Nerium oleander* showed the presence of 12 compounds of active chemical constituents such as carbohydrates, alkaloids, flavonoids, glycosides, and tannins, which are mainly responsible for anthelmintic activity. The analysis of *Nerium oleander* leaf extracts using FT-IR explained main bands at 3383.10 cm-1, N-H stretching, 2934.35 cm-1, C-H stretching, 2124.09 cm-1, N=C=N stretching, 1633.45 cm-1, C=C stretching, 1515.92 cm-1, N-O stretching, 1423.03 cm-1, O-H bending, 1273.79 cm-1, C-O stretching, 1117.70 cm-1, C-O stretching, 1048.87 cm-1, CO-O-CO stretching, 926.30 cm-1, bending, 828.28 cm-1, C=C bending, and 717.43 cm-1, C=C bending (Figure No. 1 and Table No. 1). In addition to that, C-MS revealed thirteen chemically active components in the Nerium oleander extract (Figure No. 2). Table No. 2 lists each chemical and its pharmacological usefulness against parasitic worms.

It is demonstrated here that a crude methanolic extract of *Nerium oleander* possesses anthelmintic activity, as measured by the mortality and motility of *A. caliginosa*, which showed a relatively competitive anthelmintic activity in comparison with the conventional anthelmintic agent (Albendazole) against live adult *A. caliginosa* worms (Figure No. 3) in the study. The findings demonstrated that both the plant extract from *N. oleander* and the pharmaceutical product was effective against the parasites in a dose-dependent manner. We note that the rate of death increased with increasing the duration of exposure of the worm to the oleander extract, which is directly proportional to the increase in the concentration of the section. The ethanolic extract of *N. oleander* has been found to possess the highest anthelmintic efficacy at its highest dosage of 100 mg/mL of NOE, causing paralysis and death after 10 minutes at 97% and 100%, respectively. Of the three concentrations of NOE (25, 50, and 100 mg/mL) exposed to the parasite, the highest dose causes paralysis and leads to death in 7.5 and 10 minutes, respectively. The untreated control showed physical activity up to 4 ± 1 h, where the highest percentage of paralysis of earthworms in the tenth minute of exposure was 58%, 69%, 100%, and 79% at concentrations of 25, 50, 100, and 10 mg/mL of albendazole, respectively (Figure No. 4).

Figure No. 1
FT-IR chromatogram of *Nerium oleander* leaf extracts in methanolic medium showing the functional characteristic of the active chemical compounds

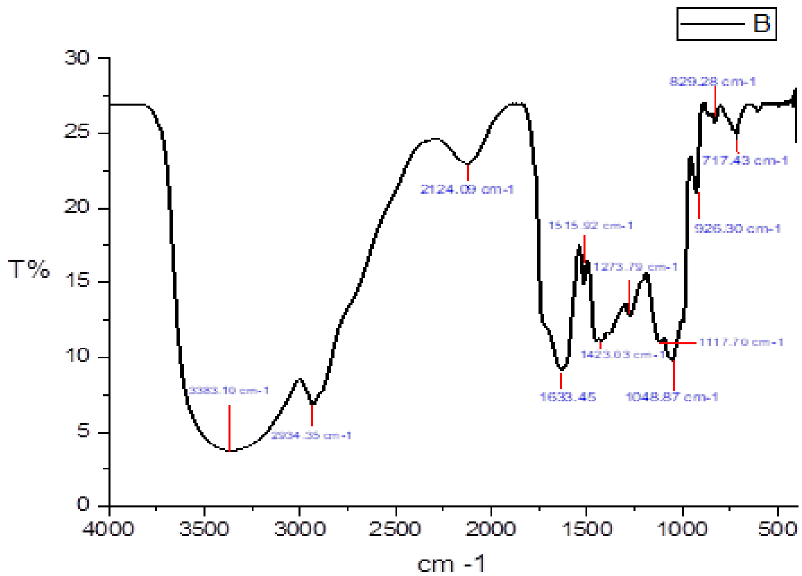


Table No. 1
Analyze NOLE to identify potential active chemical compounds using FT-IR.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound class
3383.10	Medium	4	N-H stretching	aliphatic primary amine
2934.35	medium	7	C-H stretching	Alkane
2124.09	Strong	23	N=C=N stretching	Carbodiimide
1633.45	medium	9	C=C stretching	Alkene
1515.92	strong	15	N-O stretching	nitro compound
1423.03	medium	11	O-H bending	Alcohol
1273.79	strong	12	C-O stretching	alkyl aryl ether
1117.70	strong	10	C-O stretching	secondary alcohol
1048.87	strong, broad	9	CO-O-CO stretching	Anhydride
926.30	strong	21	C=C bending	Alkene
829.28	medium	26	C=C bending	Trisubstituted
717.43	strong	25	C=C bending	disubstituted (cis)

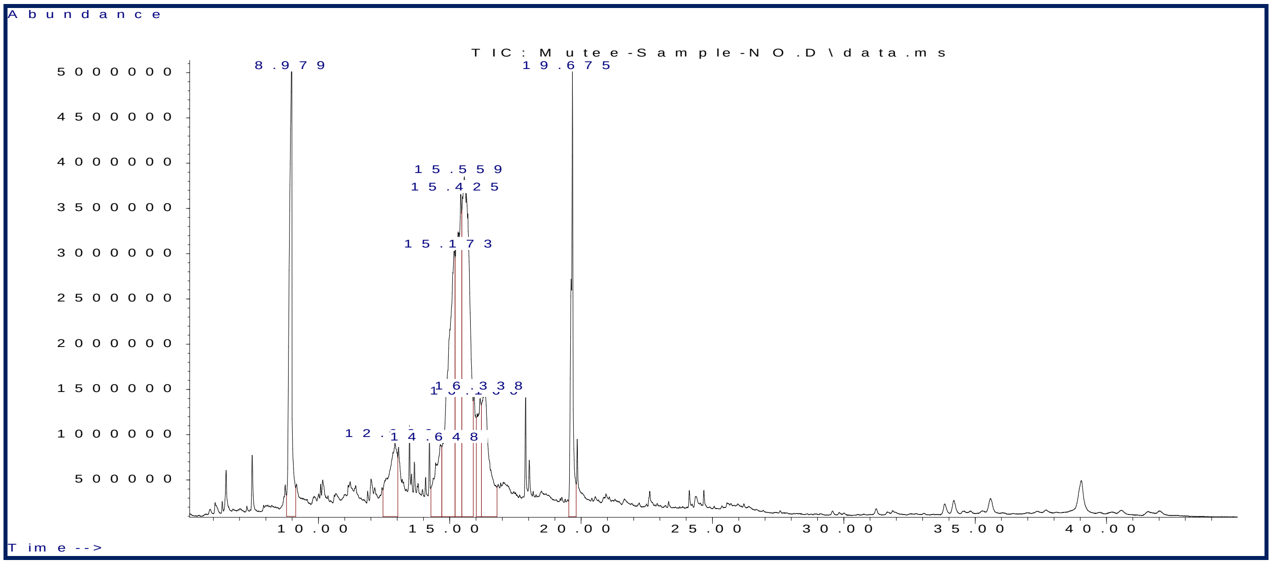
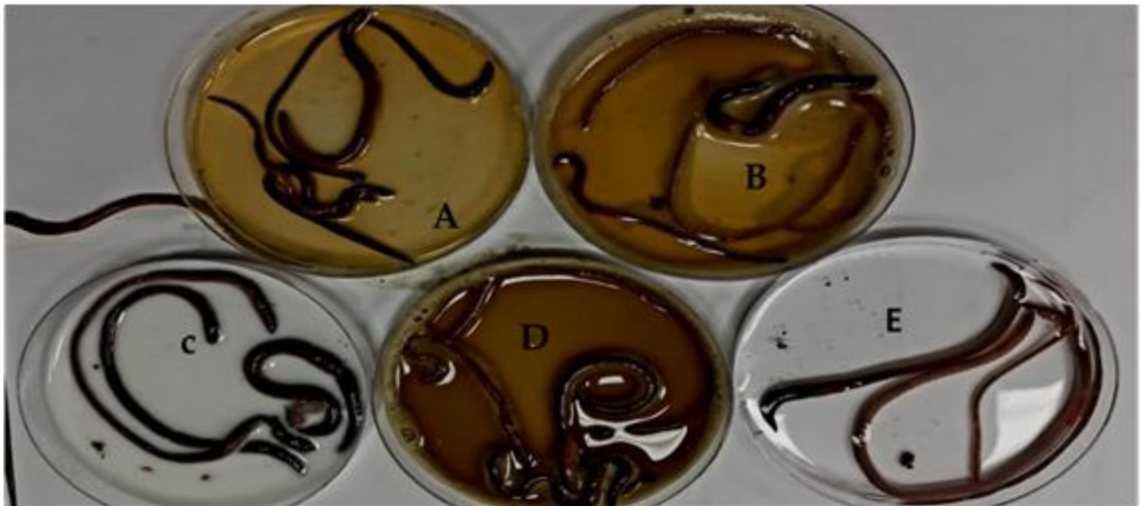


Figure No. 2
The functional characteristics of the active chemical components in GC-MS chromatogram of *Nerium oleander* leaf extracts in a methanol medium

Table No. 2
Identification of phytochemical compounds by GC-Mass in *Nerium oleander* extracts

tR (min)	Proposed compound	MW	Formula	Peak área%
6.49	3-Methoxycarbonylpyrazole	126	C5H6N2O2	0.95%
7.48	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	C6H8O4	0.86%
8.98	5-Hydroxymethylfurfural	126	C6H6O3	15.64%
13.47	2,5-Dimethoxy-4-ethylamphetamine	223	C13H21NO2	0.81%
14.08	Megastigmatrienone	190	C13H18O	0.20%
14.23	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl-	190	C13H18O	0.65%
15.56	3-O-Methyl-d-glucose	194	C7H14O6	69.78%
17.88	n-Hexadecanoic acid	256	C16H32O2	1.14%
18.03	Mandelic acid, 3,4-dimethoxy-, methyl ester	226	C11H14O5	0.43%
19.62	Linoleic acid	280	C18H32O2	2.97%
19.67	Oleic Acid	282	C18H34O2	5.85%
19.85	Octadecanoic acid	284	C18H36O2	0.54%
24.68	Pregn-5-en-20-one, 3-hydroxy-	316	C21H32O2	0.18%

Figure No. 3
Evaluating *Allolobophora caliginosa* mortality and motility using a methanolic extract of *Nerium oleander* leaf extracts



(A) 25 mg/mL, (B) 50 mg/mL, (c)10 mg/mL Albendazol, (D) 100 mg/mL, (E)Distilled water

Whereas demonstrating that the mortality rate increased as the worm's exposure to the oleander extract with the highest concentration lasted longer in the section. The maximum percentage of killed worms was 58%, 69%, 100%, and 79% at concentrations of 25, 50, 100, and 10 mg/mL of albendazole, respectively, in the tenth minute of exposure (Figure No. 5)

After 10 minutes of testing, the concentration of 50 mg/ml of NOLE showed slight changes in activity but was not lower than 80% of the concentration of 100 mg/mL. At 2.5 minutes, there were no deaths or damage to the body segments in the worms at a concentration of 25 mg/mL. Two concentrations of 100 mg/ml and the reference drug 10 mg/ml of albendazole had good effects in the tests at 25 and 50 mg/mL, with significant decreases in activity at 7.5 and 10 min, respectively, and loss of motility and death in a short period (Figure No. 6 & Figure No. 7).

In vitro main effects of *N. oleander* leaf extracts on Paralysis%, death%, of *A. caliginosa* at different concentrations (25, 50, 100, and 10 mg/mL) of ONLE and albendazole. The most efficient dose, 100 mg/mL of *N. oleander* extracts, showed the time to paralysis and death after 10 minutes were 97 and 100%, respectively, compared to the reference drug albendazole (40 mg/mL), with less effect (75 and 88%) for paralysis and death time, respectively, compared to the 75 mg/kg NOE.

In Figure No. 8 & Figure No. 9, the main effects were shown for different periods (2.5, 5, 7.5, and 10 min) of exposure of the worm to concentrations (25, 50, and 100 mg/mL) and for 10 mg/mL albendazole, which indicated that a long exposure period leads to paralysis and killing of the worm in a short period (Figure No. 8 & Figure No. 9).

Microscopical investigations of LM and SEM of control worms revealed homogeneous normal body architecture without any changes to the worms' surface. While all worms treated with NOLE exhibited alterations in the general appearance of the worm and external wall shape, including shrinkage of the homogeneous body wall and an increase in cuticular thickness, as well as a reduction in size along the length. On the other hand, the reference drug albendazole caused identical types of disruption in all worms treated with it (Figure No. 10 & Figure No. 11).

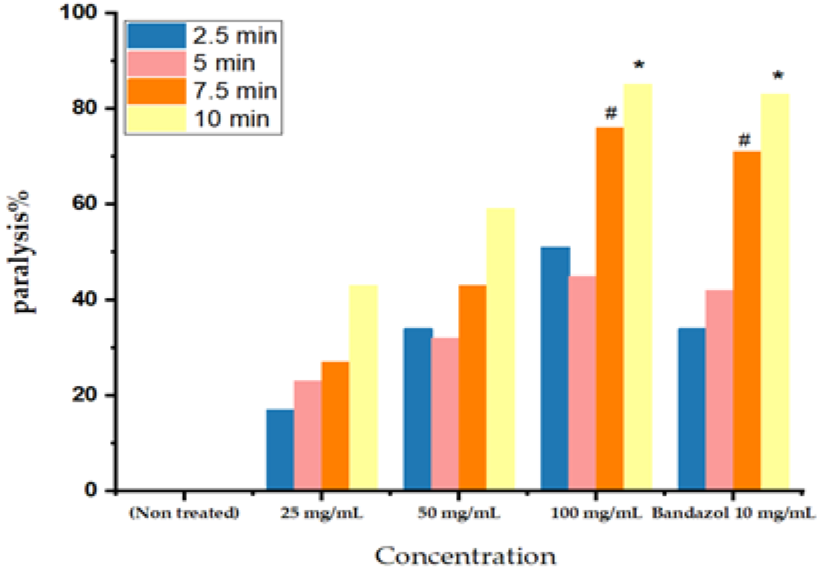


Figure No. 4
Effect of different concentrations of *Nerium oleander* leaf extracts on the paralysis of *Allolobophora caliginosa.* *Significance in comparison to the infected group (*p*<0.01). # Significance in comparison to the infected group (*p*<0.05)

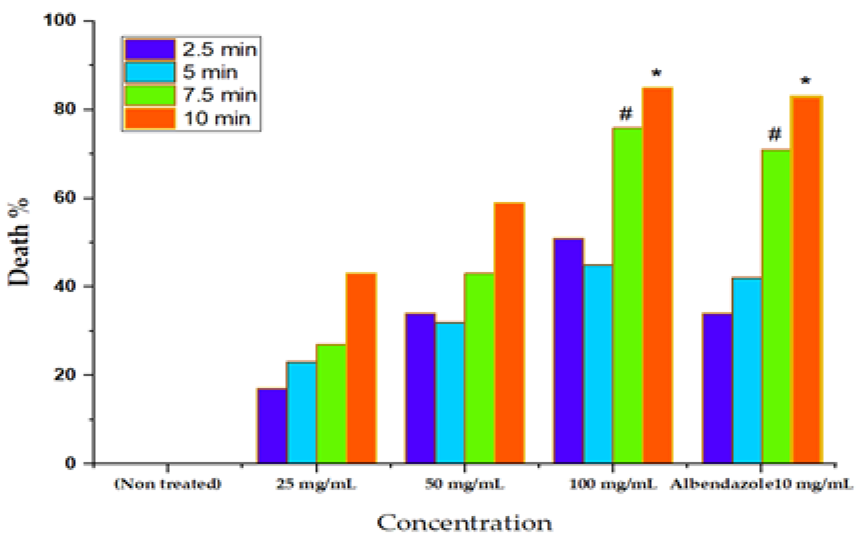


Figure No. 5
Effect of different concentrations of *Nerium oleander* leaf extracts on the mortality of *Allolobophora caliginosa.* *Significance in comparison to the infected group (*p*<0.01). # Significance in comparison to the infected group (*p*<0.05)

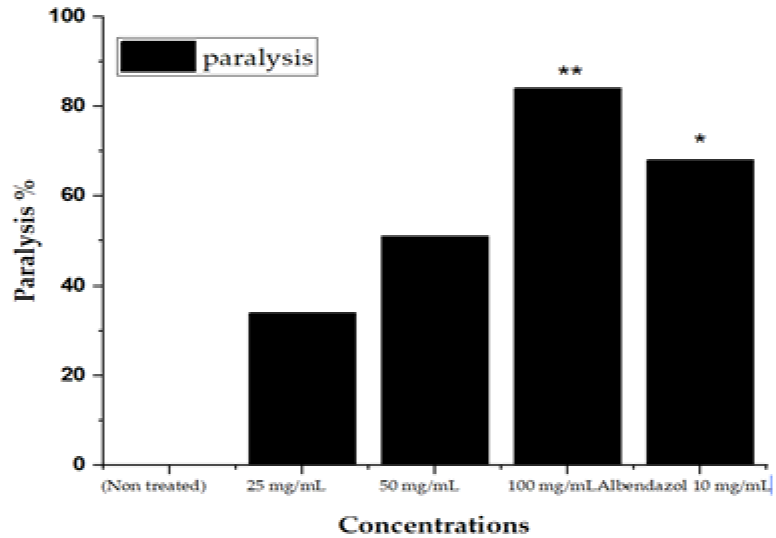


Figure No. 6
Cumulative effect of different concentrations of *Nerium oleander* leaf extracts on the paralysis of *Allolobophora caliginosa.* *Significance in comparison to the infected group (*p*<0.01). # Significance in comparison to the infected group (*p*<0.05)

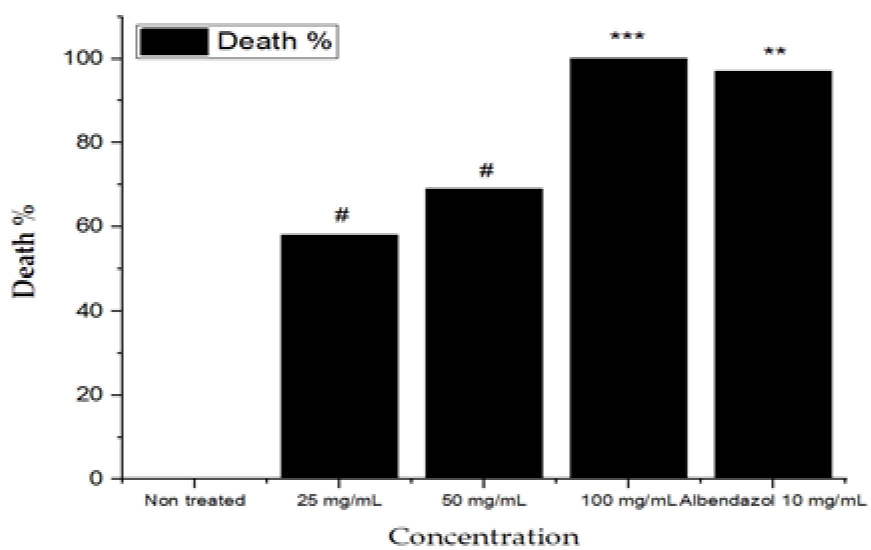


Figure No. 7
Cumulative effect of different concentrations of *Nerium oleander* leaf extracts on the mortality of *Allolobophora caliginosa.* *Significance in comparison to the infected group (*p*<0.01). # Significance in comparison to the infected group (*p*<0.05)

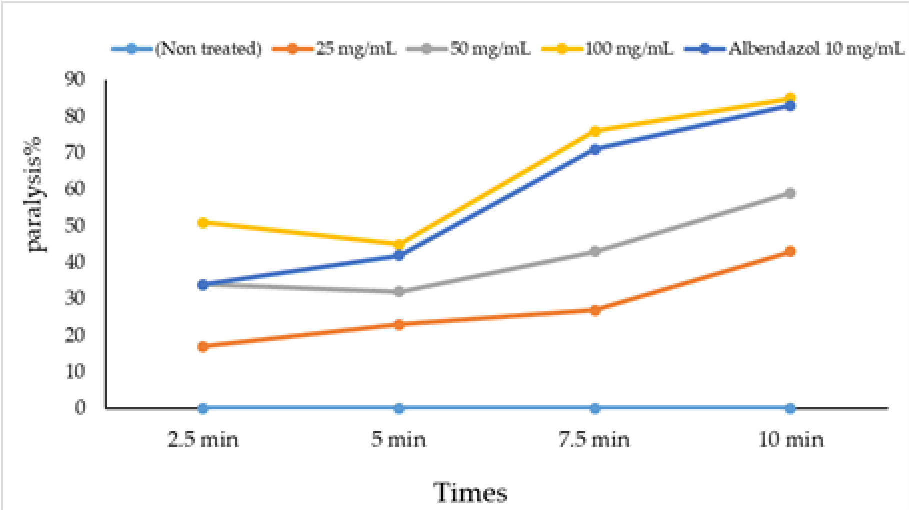


Figure No. 8
***In vitro*, main contact time effects of *Nerium oleander* leaf extracts on paralysis%, of *Allolobophora caliginosa* at different concentrations of ONLE and albendazole compared to control**

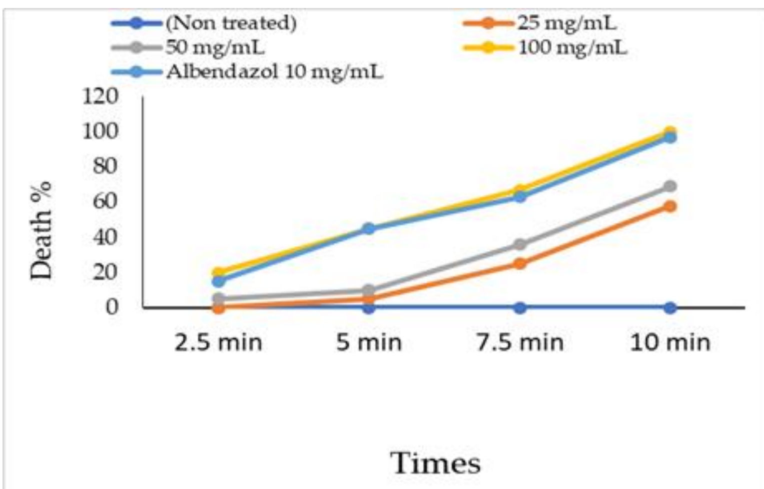


Figure No. 9
***In vitro* main contact time effects of *Nerium oleander* leaf extracts on death% of *Allolobophora caliginosa* at different concentrations of ONLE and albendazole compared to control**

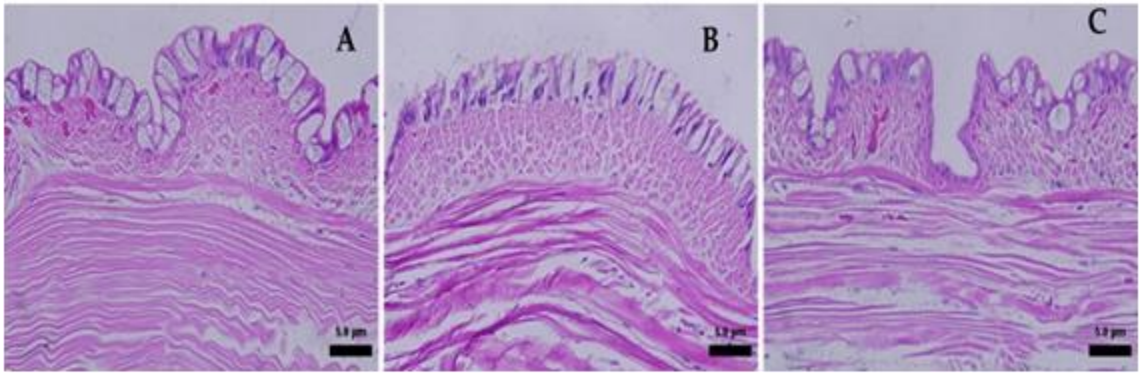


Figure No. 10

Figure 3. Histological changes in the cortex thickness of *A. caliginous* were affected by the various therapeutic extracts (A) worms in distilled water (Non-treated). (B) worms in 100 mg/*mL N. oleander* leaf extracts. (C) worms in the reference drug (Abebendazole). Scale bar 5 µm

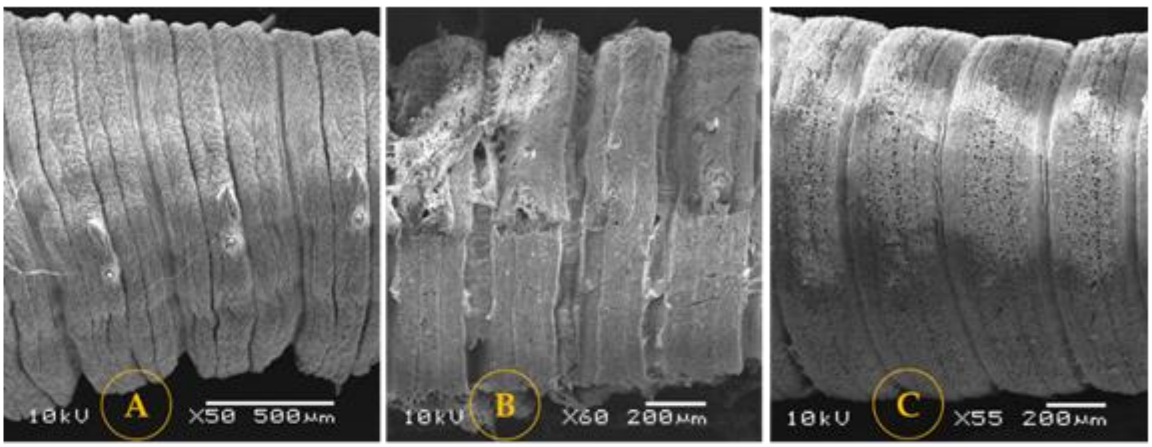


Figure No. 11

SEM of *Allolobophora caliginosa* with various treatments. (A) worms in distilled water (Non-treated), Scale bar 500 µm. (B) worms treated with 100 mg/mL *Nerium oleander* leaf extracts, Scale bar 200 µm. (C) worms treated with the reference drug Albendazole, Scale bar 200 µm. (SEM): A scanning electron microscope.

DISCUSSION

Parasitic worms are a common cause of concern and pose many problems affecting human and animal health, especially those in the digestive system. Searching for alternatives to anti-helminthic pharmaceuticals to combat and treat helminths is a crucial area of research. Various antiparasitic treatments, including plant extracts that received much attention for their therapeutic uses as an alternative anthelmintic, have been tested in multiple trials *in vitro* and *in vivo*, with promising results and few adverse effects (Ranasinghe *et al*., 2023). Many medicinal plants claim to possess anthelmintic activity and can be effectively used against these disorders. In this study, the possible function of NOLE as anthelmintic effects was investigated, and earthworms were used as a model for evaluating the effectiveness of *Nerium oleander* extract as an anthelmintic where he noticed that the concentration of 200 of *Nerium oleander* extract is highly effective in expelling worms compared to the commercially used treatment Albendazole *in vitro*.

When treated with various doses of *Lysimachia ramosa* extract, test helminths, including *F. buski*, likewise showed a dose-dependent anthelmintic property similar to the current findings, where concentrations of 5 mg and 50 mg/mL of PBS, respectively, paralyzed the parasite at 3.64 h, 0.14 h, and 1.20 h, respectively (Challam *et al*., 2010). According to a recent study by Ahmed *et al*. (2013), the ethanolic crude extracts of *Ananas comosus*, *Aloe ferox*, *Allium sativum*, *Lespedeza cuneata*, and *Warburgia salutaris* exhibit the best efficacies against the sheep worm *Haemonchus contortus* (Ahmed *et al*., 2013). Like our investigation, Dutta *et al*. (2012) evaluated the anthelmintic activity of several solvent fractions of the foliar portions of *Glinus oppositifolius* (Linn.) DC. They found that the ethyl acetate fraction had the maximum effectiveness against the assay parasite (Dutta *et al*., 2012).

These results align with (Morsi *et al*., 2022) found in the effect of *Nerium oleander* leaf extract on the schistosomiasis parasite. Also, these results are consistent with (Begum *et al*., 1999; Siddiqui *et al*., 2012) confirmed: that the extract of oleander leaves contains many effective compounds, including flavonoid glycosides, oleanderocinoic acid, a cardenolide, oleandigoside, neridiginoside, and odoroside-H. These compounds have CNS depressant effects and growth inhibitory and cytotoxic activities, leading to paralysis and killing worms (Rashan *et al*., 2011) reported that mono glycosidic cardenolides from *N. oleander* possessing the 3β,14β-dihydroxy-5β-cardenolide-20 (22)-enolide structure with or without an acetoxy group at C-16 exhibited significant anticancer activity. The results indicated that the inhibition of plasma membrane-bound Na^+/K^+-ATPase induces cytotoxic effects; this affects the permeability of the cell membrane of the parasite and causes vacuolization, the disintegration of teguments, and eventual death. Also, the worms work to swallow the extract, which in turn works to cause worm poisoning, which results in the end of the worms. This is consistent with the mention in (Bandara *et al*., 2010) that *Nerium oleander* is a plant that can be fatal if ingested. All parts of the plant are toxic and contain various cardiac glycosides. Ingestion of oleander causes nausea, vomiting, abdominal pain, diarrhea, cardiac arrhythmias, and hyperkalemia in animals. In a study that proved the presence of a group of effective compounds in the Nerium oleander extract, such as alkaloids, phenols, flavonoids, tannins, and actions (Huang *et al*., 2009), as for flavonoids, they act to reduce sugars, causing an imbalance in carbohydrate metabolism and reducing the number of energy units (ATP) supplied in the parasite's vital activity (Hamad, 2021). Also, *N. oleander* leaf extract is important in eliminating many insects. The aqueous leaf extract of *N. oleander* has ovicidal and larvicidal activity against Culex tritaeniorhynchus and Culex glides (Kumar *et al*., 2013). showed that *N. oleander* has toxic effects on the development of larval and pupal stages for *Bemisia tabaci* species (Rathi *et al*., 2011).

In the current study, the changes obtained from histopathology and SEM on the worm's skin surface were confirmed due to the effect of the *Nerium oleander* leaf extracts on the worms. The cuticle is an important structure in annelids because it provides coverage and protection for the worm's body and supports internal organs (Abu Hawsah *et al*., 2023). In this study, we observed the occurrence of many changes on the skin of worms treated with Nerium oleander leaf extracts, among which were the occurrence of ruptures in the skin's surface, the occurrence of cracks on the skin, and the presence of necrosis on the surface of the worms. This study agrees with what (Sambodo *et al*., 2018) stated: any changes on the surface of the parasite due to antihelminthic treatments or medicinal plant extracts lead to paralysis and then the death of the parasite. The reason may be that phenols affect the enzyme acetylcholine esterase, which controls the elasticity and permeability of the cell membrane. The phenols made the membrane lose this permeable property, leading to the entry of various toxic substances without regulation and then the parasite's death. Phenol can distort proteins and stop the action of enzymes involved in many basic metabolic reactions, thus causing an inhibitory process and causing microorganisms to lose viability (Campêlo *et al*., 2019). Also, the worms work to swallow the extract, which in turn works to cause worm poisoning, which results in the death of the worms.

Limitations

Some limitations of this study include the following: a) The results are not representative of the entire helminth, and the sample consisted of earthworms. Future studies will need to cover more diverse worms. b) causality: incomplete information was found in some cases to evaluate causality assessment and herbal products containing different metabolites. Therefore, the causality is attributed to a mixture of several components. However, causality assessment scales do not consider a mixture of components. We need to separate the chemical compounds to find out which substance kills the worms. c) There is a significant possibility of undeclared medicinal herbs.

CONCLUSIONS

The helminths are responsible for disruptions in the intake of nutrients by animals, plants, and humans, which leads to an increased risk of secondary infections. Furthermore, it causes significant economic losses around the world. The majority of parasite-fighting research endeavors are focused on the use of anti-parasitic drugs. However, long-term use of these treatments without moderation has resulted in the development of drug resistance and serious side effects, prompting the investigation of new therapies. *Nerium oleander* leaf extract is an interesting potential candidate for an alternative treatment approach. Our study examined the pharmacological potential of NOLE, and the possible role that extracts of N. oleander leaves might play in anthelmintic activity when tested *in vitro*.

Our study indicates *in vitro* that *Nerium oleander* leaf extract bioactive compounds have strong dose-dependent anthelmintic effects against *Allolobophora caliginosa*. However, more research is required to understand this compound's toxicological characteristics fully. A study must be conducted in vivo to evaluate *N. oleander* extract in the eradication of worms in animals, and to determine the new pharmacodynamic effects of bioactive components, a more extensive pharmacological study should be done. Furthermore, the primary mechanisms underlying bioactivity should be thoroughly studied. Also, further studies are needed to isolate the pharmacologically active compounds responsible for these activities.

ACKNOWLEDGEMENT

Thank you to Researchers Supporting Project (RSP-2023R3), King Saud University, Riyadh, Saudi Arabia, for the financial support.

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