

The antiparasitic and antioxidant bioactive chemical components of *Nerium oleander* leaf extracts cultivated in Saudi Arabia

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[Componentes químicos bioativos antiparasitários e antioxidantes dos extratos de folhas de *Nerium oleander* cultivadas na Arábia Saudita]

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ABSTRACT

Natural products play a significant role in medicine, where they serve as alternative treatments for parasitic diseases. The investigation aims to evaluate the effectiveness of the bioactive compounds in *Nerium oleander* leaf extracts, and its effect on killing *Aspiculuris tetraptera* worms that infect laboratory mice. Total phenols, tannins, and flavonoids were determined. Antioxidant activity was estimated (DPPH and ABTS). The experiment was divided into 5 transactions, containing three concentrations (50, 100, and 200mg/mL) of *Nerium oleander* leaf extract. In addition, Groups 3 and 4 have positive and negative control. The death time for the worms was recorded at 15, 30, 60, and 120 minutes after treatment. The study showed that the extract is rich in flavonoids, tannin, and phenolics in different proportions. The results showed a significant degree of antioxidant activity, and as the plant extract content increased, so did the DPPH radical and hydrogen peroxide scavenging. The extract showed effectiveness with increasing dose and time during 15, 30, 60, and 120 minutes, which reached 89% and 96% after 120 minutes at the highest concentrations (100 and 200mg/mL). The findings imply that oleander leaves contain active phytochemicals that might be used to create new medication candidates.

Keywords: bioactive, antioxidant, radical scavenging, anthelmintic, *Aspiculuris tetraptera*

RESUMO

Os produtos naturais desempenham um papel importante na medicina, onde servem como tratamentos alternativos para doenças parasitárias. A pesquisa tem como objetivo avaliar a eficácia dos compostos bioativos nos extratos de folhas de *Nerium oleander* e seu efeito na eliminação de vermes *Aspiculuris tetraptera* que infectam camundongos de laboratório. Foram determinados os fenóis totais, taninos e flavonoides. A atividade antioxidante foi estimada (DPPH e ABTS). Dividiu-se o experimento em 5 transações, contendo três concentrações (50, 100 e 200mg/mL) de extrato de folha de *Nerium oleander*. Além disso, os Grupos 3 e 4 têm controle positivo e negativo. Registrou-se o tempo de morte dos vermes aos 15, 30, 60 e 120 minutos após o tratamento. O estudo mostrou que o extrato é rico em flavonoides, taninos e fenólicos em diferentes proporções. Os resultados mostraram um grau significativo de atividade antioxidante e, à medida que o conteúdo do extrato da planta aumentou, o mesmo ocorreu com a eliminação do radical DPPH e do peróxido de hidrogênio. O extrato mostrou eficácia com o aumento da dose e do tempo durante 15, 30, 60 e 120 minutos, que alcançou 89% e 96% após 120 minutos nas concentrações mais altas (100 e 200 mg/mL). Os resultados indicam que a folha de oleandro contém fitoquímicos ativos que podem ser usados para criar novos candidatos a medicamentos.

Palavras-chave: bioativo, antioxidante, eliminação de radicais, anti-helmíntico, *Aspiculuris tetraptera*

INTRODUCTION

The natural product extracts are rich sources of bioactive chemicals and are eco-friendly. Consequently, the identification of novel bioactive metabolites through natural product screening has been a crucial element of numerous drug development endeavors (Atanasov *et al.*, 2021). It is estimated that more than 70 % of approved pharmaceuticals were either derived from or structurally related to chemicals that may be found in nature (Cragg and Newman, 2013). This suggests the need for a fast method for identifying novel plant extract chemicals (Cieřla and Moaddel, 2016). The primary biologically active components of these naturally occurring products are typically present, and they may take the shape of tiny molecules, macromolecular polysaccharides, or other similar compounds (Shi 2016).

Bacteria's fermentation byproducts or plants' secondary metabolites can yield these naturally occurring substances (Granato *et al.*, 2022). Extraction, separation, and purification methods are necessary for successfully isolating bioactive chemicals (Lemes *et al.*, 2022). This information serves as a reference for the potential future development of medicines, including healthcare compounds (Granato *et al.*, 2022; Lemes *et al.*, 2022). The discovery of bioactive chemicals ushered in an exciting new era in the field of biotechnology, which has important implications for the health of people (Demain, 2014). In vitro and/or in vivo biological activities could prove the therapeutic potential of natural health products, as their major goal is to prevent or alleviate a variety of chronic and degenerative diseases (Forman and Zhang, 2021). Natural extracts, including byproducts, have the potential to serve as valuable substrates to produce new goods (Johnson *et al.*, 2023). The in vitro and in vivo correlations for the actions (antioxidant, anti-inflammatory, or antibacterial) that these compounds exhibit are quite low due to their bioavailability, which is one of their key limiting variables (Caponio *et al.*, 2022). They ameliorate chronic disorders, and the administration of these medicines could have powerful benefits (for conditions such as type 2 diabetes, cardiovascular dysfunctions, or neurodegenerative diseases) (Alam *et al.*, 2021). The ability to manufacture these secondary metabolites is associated with their antimicrobial

properties. The process involves creating and identifying phytochemicals, as well as determining the physiological pathways involved in the health advantages they provide (Alamgir and Alamgir, 2018).

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 (Potapenko *et al.*, 2020). People have used N. oleander as a medicinal plant to treat a variety of ailments. Traditional medicine uses oleander to treat epilepsy, cancer, cancerous tumors, and cardiac conditions (Ayouaz *et al.*, 2023). The plant treats several reproductive issues in Trinidad and Tobago (Lans, 2007). Kenyans use an extract from the leaves and seeds to treat upper respiratory tract and gastrointestinal conditions (Ayouaz *et al.*, 2023). Traditional folk medicine in Calabria, southern Italy, has long used the leaves to treat malaria (Murshed *et al.*, 2022). Researchers have detected numerous secondary metabolites, including alkaloids, flavonoids, and steroids, in this plant species. Several studies have found that this plant has many medical benefits, including the ability to fight bacteria, parasites, and inflammation, protect the liver, boost the immune system, lower fever, fight infections, fight tumors, and fight HIV (Toyang and Verpoorte, 2013).

This study showed contemporary methodologies for phytochemical analysis and the identification of physiologically active components. This helps to further illustrate that isolated chemicals may have potential bioactivities anthelmintic.

MATERIALS AND METHODS

Fresh leaves from the herbal plant of plant *N. oleander*, were collected from the Riyadh Gardens, Saudi Arabia. Voucher specimens were deposited in the herbarium of the Botany and Microbiology Department, King Saud University to verify their botanical identity.

The leaves were ground after washing and dried at room temperature for further investigation. Then, 50 g of the powder was extracted in 400 ml of 75% methanol and kept in a shaker at room temperature for 24 h. The extract was filtered using Whatman paper. Then, the resulting extract

was concentrated using a rotary evaporator under vacuum (Yamato RE300, Tokyo, Japan) at -10 °C and low pressure for further use (Manikandan *et al.*, 2017).

The Ainsworth method was utilized to estimate the total phenolic content (TPC) of the (*N. oleander*) leaf extract (Luczaj *et al.*, 2014). The mixed-up volume of 100µL the oleander extract with the Folin–Ciocalteu reagent and 300µL of a solution of sodium carbonate (20%). After that, the sample was kept at room temperature and incubated in the dark for 30 minutes, and the recorded wavelength was 765nm, utilizing a UV-visible spectrophotometer (SHIMADZU, UV-1800). The total phenolic content (mg/g DW) of the samples was calculated by using the linear equation ($y = 0.0021x + 0.0021$ with $R^2 = 0.9995$).

The method has been applied by Luczaj *et al.* (2014) to determine the total tannin content (TFC) of the material. 0.1mL of the sample was added to 1.5mL of Milli-Q water and 0.1mL of the Folin-Ciocalteu phenol reagent for 8 min. After that, 0.3mL of a sodium carbonate solution that was 35% was added to the mixture so that it could be neutralized. Next, the ingredients were thoroughly combined and placed in a dark, room-temperature area for 20 minutes. The absorbance was measured at 700nm. The following equation was utilized: ($Y = 0.0013x + 0.0052$ with $R^2 = 9937$). Determined the total tannin concentration and represented it in mg TAE/g DW for TTC.

The method applied by Ordonez, *et al.* (2006) was used to determine the total flavonoid content (TFC) in extract. Methanol extract 0.5mL was mixed with a water solution containing 2% AlCl₃ having the same volume. The wavelength was measured at 420 nm at 25 °C after 2 hrs. The TFC was calculated using a calibration curve that was constructed using different concentrations (50–0400g/mL) of quercetin standard and the following equation ($y = 0.0172x + 0.0507$ with $R^2 = 0.995$). The calculated TFC has been represented as quercetin (mg/g DW).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to test the antioxidant potential of the leaf methanolic extract of *N. oleander* (Liyana-Pathirana and Shahidi, 2006). In total, 1 milliliter of leaf extract ranging from 31.25 to 500 micrograms per milliliter was combined with the

same volume of DPPH dissolved in methanol at a concentration of 0.135 millimoles per liter. After a vigorous round of vortexing, the ingredients were allowed to sit undisturbed at room temperature for half an hour. At a wavelength of 517nm, the optical density of both the extract and the control mixture (1mL DPPH and 1mL methanol) was measured. Using the following formula, we were able to estimate the proportion of DPPH scavenging activity that the extract or the standard possessed:

DPPH scavenging activity (%) = [(absorbance of control absorbance of the sample)/ absorbance of control] *100,

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

The total antioxidant activity, abbreviated as TAC, was determined by applying the methodology outlined. In a nutshell, 0.15 mL of leaf extract was combined with 1.5mL of reagent solution that had 4mM of ammonium molybdate, 28mM of sodium phosphate, and 0.6 M of sulfuric acid. After that, the reaction mixture was kept warm for 90 minutes at 95°C. 695nm was found to be the optical density of the samples when they were measured. The total antioxidant capacity was given the unit of measurement of milligrams of ascorbic acid equivalence (AAE) per gram of dry weight (DW).

$$\% \text{Inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Mice exhibiting signs of illness were isolated in the experimental animal house and necropsied to determine the cause of the weakness. *Aspicularis tetraptera* pinworms were the source of the infection. Mice were dissected and their intestines were washed with saline (0.9% NaCl). Then, adult worms of the cecum and colon. Worms were cultured in approximately 10 ml of sterile physiological saline at 23°C. The worms were identified under a stereomicroscope (BX51TF, OLYMPUS, Tokyo, Japan). Healthy worms were selected with a standard microscopic structure and good motility. Immediately upon collecting the worms, they were tested with extract.

The larval or adult motility test is the preferred method for investigating the medication effects of different worm species. In vitro sensibility analyses employed the subculture method. *A. tetraptera* worms were tested in vitro using five different treatments, each containing three

concentrations (50, 100, and 200mg/mL) of *Nerium oleander* leaf extract. We then placed ten actively moving adult worms at room temperature in each petri dish. We prepared a saline solution and 10mg/mL of Albendazole as negative and positive controls. The number of test worms in each petri dish is 10 (5 males and 5 females). We repeated the test three times for all concentrations. The death time for the worms was recorded at 15, 30, 60, and 120 minutes after treatment. After touching their body parts with a surgical needle and shaking the petri dish, we consider worms dead if they do not move for 30 seconds. The % inhibition and motility were calculated in the treated worms for the motility assay per concentration, to determine their vitality. The worm motility was examined under a microscope after test times of exposure to all doses and rated the findings. Loss of motility is identified as the inability of worms to regain motility at pre-treatment level after incubation in fresh medium at 37°C for 10-20 min and is reported as a (%) of control. The following formula was used to calculate the parasite death rate for each activity in the extract:

Mortality of worm (%) = control-tested sample with extract/control * 100%.

The standard deviations (SD) of the given data in the tables and figures are the average across three replicates. The means of the separation and significant level determination at ($p < 0.05$) were determined using Duncan's test, a one-way analysis of variance (ANOVA), and SPSS software.

RESULTS

Natural plants are still one of the rich sources of biologically active compounds, so academic and pharmaceutical researchers have devoted their efforts to constantly searching for biologically active components in plants (Pagare *et al.*, 2015). The present study was designed to evaluate the biological activity of oleander leaf extract and its antioxidant and anthelmintic properties. Zaid *et al.* (2022) report that traditional medical approaches utilize *Nerium oleander* leaves extensively. Chemical structures primarily classify phenolic compounds into phenolic acids, flavonoids, tannins, phenolic lignans, and phenolic stilbenes (Alu'datt *et al.*, 2017). Tannins are a group of phenolic compounds that are widely present in plant herbs and many fruits and

vegetables. They possess various biological activities, including antimicrobial, antiparasitic, antiviral, antioxidant, anti-inflammatory, immunomodulatory, and so on (Smeriglio *et al.*, 2017).

In this study, the total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC), in the *Nerium oleander* leaf extracts were estimated (Figure 1). The obtained results showed that the methanolic extract exhibited the highest amount of TPC ($160.217 \pm 0.652 \text{ mg/g DW}$), followed by TTC ($65.872 \pm 0.263 \text{ mg/g DW}$), while TFC ($34.0463 \pm 0.545 \text{ mg/g DW}$).

ABTS radical scavenging activity was positively correlated to the concentration of the methanolic extracts. In this analysis, the concentrations highest samples showed higher radical scavenging activity of ABTS than the lower concentrations (Fig. 2, 3). According to Balkan *et al.* (2018), *Nerium oleander* extract has a high level of antioxidant activity, which offers significant protection against a significant number of diseases. In this study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method determines the antioxidant activity of the methanolic extract of leaves. Researchers have observed that the antioxidant effect of plant products primarily stems from the radical scavenging properties of phenolic components like polyphenols, flavonoids, tannins, and phenolic terpenes (Rahman *et al.*, 2007). Referring to the redox characteristics of phenolic compounds, which are regarded as free radical scavengers, it was demonstrated that these compounds have an antioxidant character (Mustafa *et al.*, 2010).

The effect of oleander extract on *A. tetraptera* was tested in vitro at therapeutic doses of 50, 100, and 200mg/mL and compared with negative (10mg/mL albendazole) and positive (distilled water) control groups. After 15, 30, 60, and 120 minutes, it was determined whether they were dead or alive (Table 1). The worms exposed to a low concentration of extracts (50 and 100 mg/mL) had lower mortality rates after 15, and 30, min (Figure 4), while the worms subjected to a strong concentration of extracts (100 and 200mg/mL) had a high death rate during the periods of 60 and 120 minutes, respectively. Despite not observing drug exposure, the death rate for the positive control was practically zero.

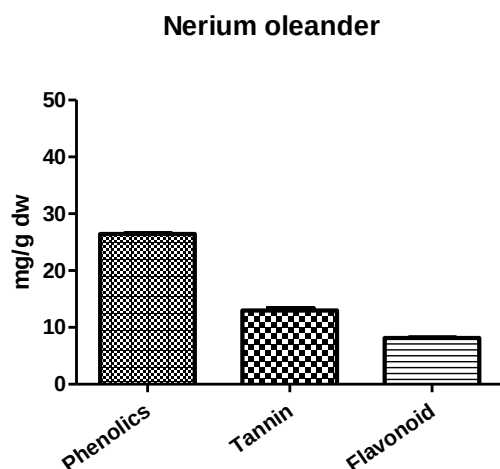


Figure 1. Flavonoids, tannin, and total polyphenols in the leaves methanolic extract of the *Nerium oleander* leaf extracts plant.

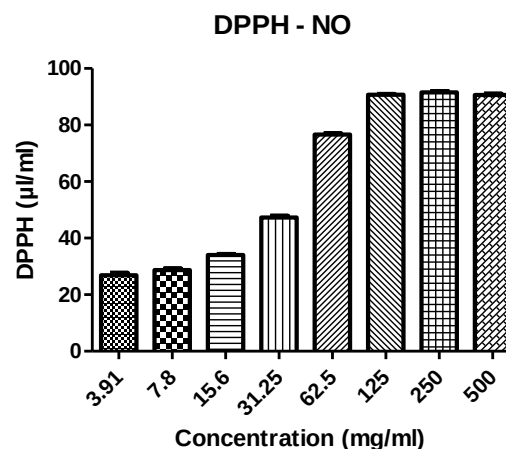


Figure 2. DPPH estimation: the data that were reported are the mean values based on three replicates $\pm$ SD.

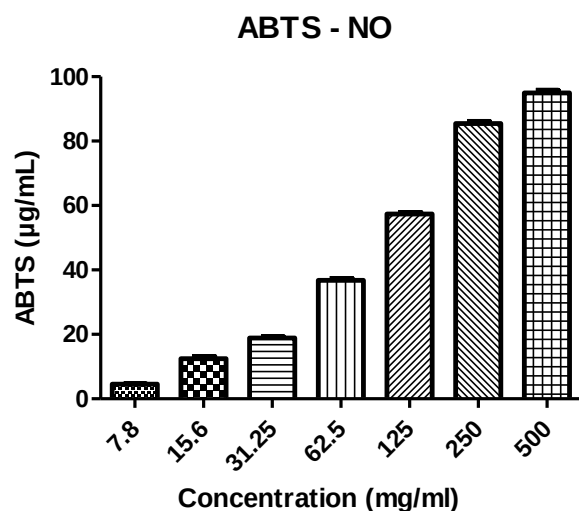


Figure 3. ABTS estimation: the data that were reported are the mean values based on three replicates $\pm$ SD.

Observed mortality rates of 82% and 100% for *A. tetraptera* worms with doses of 100 and 200 mg/mL of *N. oleander* extract and 90% at 10 mL/mL albendazole following treatment periods of 120 minutes. After 60 min of exposure, the death rate stayed below 2%. Higher mortality rates were shown by the 200 and 100mg/mL concentrations as well as the reference medication (10mg/mL) than in the control

group—distilled water. limited mortality in the periods following 15 and 30 minutes at the other values (50 and 100mg/mL) suggested limited motility during all times. Simultaneously, these mortality rates were common in other concentrations. 97% of the *A. tetraptera* worms that were treated with 200mg/mL of *Nerium oleander* leaf extract died after 120 minutes. The albendazole dose, on the other hand, had an 80%

death rate, and the control group had no deaths at all. Conversely, an increase in treatment times led to an increase in the death proportion. The *A. tetraptera* worms died a lot more after being

treated for up to 120 minutes ($p \leq 0.01$) and 60 minutes ($p \leq 0.05$); there were statistically different levels of viability between 30, 60, and 120 minutes (Figs. 4, 5).

Table 1. Means of mortality % ($\pm$ SE) of leaf oleander extracts at various exposure periods (12, 24, 36, and 48hrs) against *Tribolium castaneum*.

Concentration (%)	Mortality (%)			
	15 min	30 min	60 min	120 min
Control	0.0 $\pm$ 0.0 ^c	0 $\pm$ 0.0 ^c	0 $\pm$ 0.0 ^c	0 $\pm$ 0.0 ^c
50 mg/mL	0.0 $\pm$ 0.0 ^c	0 $\pm$ 0.0 ^c	0 $\pm$ 0.0 ^c	3.3 $\pm$ 2.7 ^{bc}
100 mg/mL	3.1 $\pm$ 2.5 ^{bc}	3.4 $\pm$ 2.5 ^{bc}	13.3 $\pm$ 2.7 ^{abc}	23.3 $\pm$ 5.4 ^{abc}
200 mg/mL	3.3 $\pm$ 2.4 ^{bc}	3.3 $\pm$ 2.4 ^{bc}	23.7 $\pm$ 11.9 ^{abc}	28.7 $\pm$ 7.2 ^{abc}
10 mg/mL Albendazole	3.3 $\pm$ 2.4 ^{bc}	3.3 $\pm$ 2.4 ^{bc}	23.3 $\pm$ 11.9 ^{abc}	40 $\pm$ 4.7 ^a

The means with different lowercase letters in columns or rows are significantly different ($p < 0.05$). Those with the same lowercase letters are not significantly different ($p > 0.05$) based on Duncan's Multiple Range Test following ANOVA. Each value is expressed as mean $\pm$ standard error.

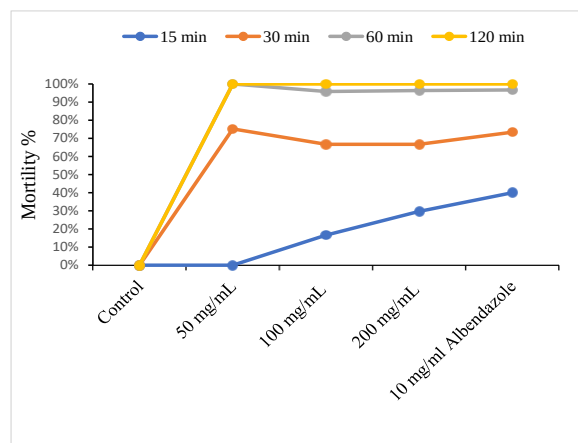


Figure 4. Mortality % of *A. tetraptera* assayed with *Nerium oleander* leaf extracts at various exposure periods (15, 30, 60, and 120 hrs).

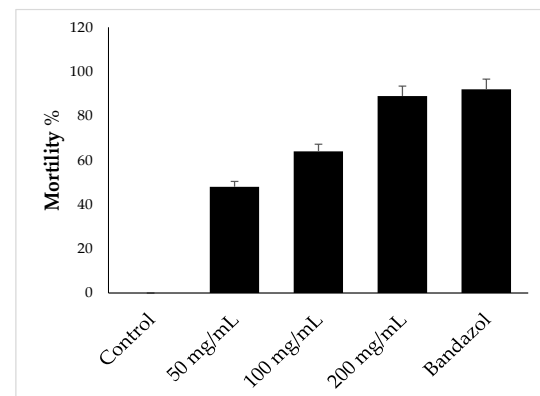


Figure 5. The general influences of various dosages of leaf extracts (50, 100, 200mg/mL) of *Nerium oleander* and reference drug (10mg/mL Albendazole) on the mortality rate of *A. tetraptera* of 30 to 120 minutes.

This study is consistent with many other studies that verified the effectiveness of *Nerium oleander* leaves against various parasites (Mares et al., 2024). In vivo, anthelmintic potential was found in *N. oleander* extract by Sebai et al. (2022). After 7 days of treatment, the eggs of *Heligmosomoides polygyrus* were no longer laid, which led to an 80% drop in the total number of worms. Infected animals' worm burden decreased (Benson et al., 2015). The *Nerium oleander* plant was rich in linalool, which plays a vital role in

eliminating worms in the intestines of infected mice (Sebai et al., 2022). Studies have reported potent antioxidant, antimicrobial, and antibacterial activity in *N. oleander* (Kivçak and Mert, 2002). People have traditionally used *Nerium oleander* leaves to treat gastrointestinal symptoms like eructation, epigastric bloating, impaired digestion, and flatulence (Jemâa et al., 2012).

CONCLUSIONS

Extract from Nerium oleander leaves revealed different degrees of antioxidant activity. It has a lethal ability against worms. These results will help future studies to pinpoint, separate, and define the particular molecule causing the increased antioxidant activity.

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