Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 3, 2025, 166
https://doi.org/10.33263/LIANBS143.166

In vitro Antimicrobial Potential of *Nerium oleander* Linn Flower Extract Obtained from Bahrain Against Selected Foodborne Pathogens Associated with Gastroenteritis

Adnan A. Bekhit [1,2,*], Huda J. Habib [1], Abd El-Aziz Ashraf Alansari [1], Abrar S. Husaini [1], Sherry N. Nasralla [1], Salma A. Bekhit [3]

[1] Pharmacy Program, Allied Health Department, College of Health and Sport Sciences, University of Bahrain, Zallaq, Kingdom of Bahrain
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt; adnbekhit@pharmacy.alexu.edu.eg;
[3] High Institute of Public Health, Alexandria University, Alexandria 21568, Egypt; salma2010@alexu.edu.eg;
* Correspondence: adnbekhit@pharmacy.alexu.edu.eg;
Received: 20.05.2024; Accepted: 12.07.2025; Published: 7.09.2025

Abstract: *Nerium oleander* is an evergreen shrub known for its multiple therapeutic properties. The study aimed to evaluate the antimicrobial potential of the hydroalcoholic extract of *Nerium oleander* flowers (NOFE) against selected foodborne pathogens, besides assessing the potential synergistic interaction between NOFE and ciprofloxacin against MRSA. The antibacterial activity of NOFE (30 µg/ml) and ciprofloxacin (10 µg/ml) was evaluated against selected Gram-negative, Gram-positive, and drug-resistant bacteria using agar well diffusion assay. The MIC values were assessed using the broth microdilution method. Additionally, NOFE and ciprofloxacin's synergistic antibacterial activity was examined against MRSA. NOFE inhibited the growth of tested bacteria, producing an inhibition zone (IZ) diameter ranging from 24±0.4 mm to 48±0.2 mm. NOFE produced the largest IZ diameter against *L. monocytogenes* and the smallest IZ diameter against MRSA. Moreover, NOFE had more potent activity against MRSA and MDR K. *pneumonia* (24±0.4 and 28±0.2 mm) than ciprofloxacin (12±0.5 and 14±0.4 mm). NOFE at 12.5 µg/ml was the lowest MIC value against *E. coli*, *B. subtilis*, and *L. monocytogenes*, while it had the highest MIC value of 200 µg/ml against MRSA. The antibacterial potential of *N. oleander* against a broad range of foodborne pathogens is evident, supporting its use in folk medicine.

Keywords: *Nerium oleander*; flower; antimicrobial resistance; drug-resistant bacteria; MRSA.

1. Introduction

Foodborne gastroenteric diseases are a major public health dilemma, depleting healthcare systems, especially in developing countries. Gastroenteritis is the inflammation of the stomach and intestinal walls caused by consuming contaminated food or water. Symptoms of gastroenteritis vary from mild to severe diarrhea, nausea, vomiting, and stomach cramps; however, more severe life-threatening symptoms may occur, especially in children under 5 years of age [1]. Bacterial gastroenteritis is widely reported in developing countries [2]. The most commonly reported causative bacteria are *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium botulinum*, *Vibrio cholerae*, *Escherichia coli*, *Listeria* *monocytogenes*, *Salmonella*, and *Shigella* [3–5]; however, unusual gastroenteritis outbreaks have been associated with *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA) [6–10]. Since bacteria mainly cause gastroenteritis, antibiotics are often prescribed to treat resulting infections [1]. However, the resistance of bacteria against the commonly prescribed antibiotics has become a matter of concern.

Recently, great interest has been devoted to medicinal plants, and thus, the term Green Medicine has emerged widely. Plants are believed to be a safe, reliable, and cheaper alternative to synthetic drugs, which are usually associated with more side effects [11]. Historically, plant extracts have been globally used to prevent and treat a wide range of diseases since they can produce a diverse range of bioactive molecules and medicinal compounds [12]. These compounds are of great medical significance since their bioactivity generally does not produce resistance [13].

Nerium oleander (*N. oleander*) is a little shrub belonging to the family *Apocynaceae* that is widely distributed worldwide and remains green throughout the year [14,15]. *N. oleander* is widely used in folk medicine for the treatment of cancer, leprosy, and skin diseases (e.g., foot fungal infections). The oleander plant's fresh parts, including the wood, bark, leaves, and flowers, contain lethal glycosides that are highly toxic to humans, animals, and insects [16–18]. Despite its documented toxicity in both fresh and dry forms, *N. oleander* has been reported to exhibit several therapeutic effects derived from different parts of the plant, such as antidiabetic [19,20], antioxidant [21], antitumor [22,23], antiviral [24], and hepatoprotective activities [25,26]. Moreover, *N. oleander* plant extracts have been demonstrated to inhibit the growth of a broad range of bacteria, such as *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, MRSA, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Bacillus cereus* [27–32]. *N. oleander* has been thought to demonstrate antibacterial activity due to the presence of certain constituents such as conessine, digitoxigenin, amorphane, eucalyptol, α-pinene, calarene, limonene, terpinene-4-ol, and sabinene [26,33–35].

The objective of this study was to evaluate the antibacterial activity of the *N. oleander* flowers extract (NOFE) obtained from the Kingdom of Bahrain against a diverse range of Gram-negative and Gram-positive foodborne pathogens. Furthermore, the study aimed to investigate the potential synergistic antibacterial effects of NOFE when combined with ciprofloxacin against MRSA.

2. Materials and Methods

2.1. Bacterial strains.

Six foodborne bacterial isolates were used throughout the current study. Multidrug-resistant clinical isolate *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* were provided by the Surveillance Microbiology Department strain bank of Al-Shatby Pediatric Hospital, Alexandria, Egypt. Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 2785) and Gram-positive bacteria (*Bacillus subtilis* ATCC 19659 and *Listeria monocytogenes* ATCC 679) were provided by the Faculty of Sciences, Alexandria University, Egypt. All strains were maintained at -80°C in nutrient broth (Oxoid, England) with 20% (v/v) glycerol and were propagated three times in nutrient broth for activation before experimental use.

2.2. Flower samples.

One kilogram (1000 g) of the matured pink flowers of the *Nerium oleander* Linn plant was collected from the garden of the College of Health and Sports Sciences, University of Bahrain, in March 2020 (Figure 1). The flowers' taxonomy was identified at the Faculty of Sciences, Alexandria University, Egypt.

Figure 1. Fresh pink *Nerium oleander* Linn flowers.

2.3. Preparation of plant extract.

Flowers of *Nerium oleander* Linn (1000 g) were thoroughly washed with running water to remove unwanted materials, air-dried in the shade for a week at ambient temperature, and ground. Half of the powdered flowers (500 g) were stored at 4°C for further use, while the other half (500 g) was then extracted using a hydro-ethanol (20:80) mixture by maceration for three nights under agitation. The mixture was then filtered through a 0.22 µm sterile filter (Millipore) and concentrated under vacuum at 45°C to obtain a yellowish-pink paste. The paste obtained was then left to air dry. The dried extract was conserved at -20°C till further use.

2.4. Preparation of the bacterial inoculum.

Gram-positive (*Bacillus subtilis* ATCC 19659, *Listeria monocytogenes* ATCC 679, and MRSA) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2785, and MDR *Klebsiella pneumoniae*) bacteria were pre-cultured in nutrient broth overnight at 35±2°C. Later, the turbidity of each strain was adjusted to a concentration of 1.5×10^{8} CFU/mL using a 0.5 McFarland standard.

2.5. Preparation of the flower extract stock solutions.

The *N. oleander* flower extract stock solutions were prepared by dissolving crude dried extracts in pure dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA). Several concentrations of NOFE were prepared: 10, 20, or 30 µg/ml.

2.6. Screening of the antibacterial activity.

The antibacterial activity of NOFE and ciprofloxacin (Sigma Aldrich, USA) against different indicator organisms was evaluated using the agar well diffusion assay in a semi-solid Mueller-Hinton Agar (MHA) plate (Oxoid, England). After cooling to 40°C and mixing well, 25 ml of the semi-solid MHA seeded with the bacterial indicator pathogens was poured into Petri dishes and left to solidify under UV light in a laminar flow hood. After solidification, a sterile cork borer (6 mm diameter) was used to make wells into agar plates containing inoculums. Then, 100 µL of DMSO solution containing 30 µg/mL of NOFE or 10 µg/mL of ciprofloxacin was added to the wells. Plates were placed in the refrigerator for 30 minutes to let the flower extract diffuse into the agar. Finally, plates were placed in the incubator at 37°C for 18 hours. Antibacterial activity was detected by measuring the inhibition zone (IZ) that appeared following incubation. This was repeated three times for each indicator strain to calculate the inhibition zone's mean diameter. DMSO at a concentration of 10% was used as a negative control [36].

2.7. Determination of minimum inhibitory concentration (MIC).

The minimum inhibitory concentration (MIC) values of NOFE and ciprofloxacin against the indicator strains were determined according to the Clinical and Laboratory Standards Institute (CLSI) with minor modifications (Clinical and Laboratory Standards Institute, 2019). An overnight culture at 37°C in Muller-Hinton broth medium of each tested indicator strain was adjusted to the logarithmic-phase growth, yielding approximately 10^6 CFU/ml. Using the indicator strains, the broth dilution method was carried out in 96-well microtiter plates. A stock solution of NOFE at a concentration of 30 µg/mL was prepared aseptically in DMSO and then transferred to sterile 96-well microtiter plates containing the Mueller-Hinton broth. Two-fold serial dilutions of the tested flower extract were prepared with a final concentration of 800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL per well. The contents of wells (containing either NOFE or ciprofloxacin in DMSO and bacterial cultures in Mueller-Hinton broth) were mixed thoroughly and incubated overnight at 37°C. The MIC was recorded as the lowest concentration of NOFE or ciprofloxacin, which completely inhibited visible bacterial growth in wells. Wells not containing the extracted flower solutions were used as a negative control.

2.8. Evaluation of the interaction between ciprofloxacin and NOFE.

The broth microdilution checkerboard method was used to evaluate the interaction between NOFE extract and ciprofloxacin against MRSA [37]. The MIC values of NOFE and ciprofloxacin against MRSA at a concentration of $1.5\text{x}10^6$ CFU/ml were measured individually using the broth microdilution (BMD) method. Then, the synergism between different combinations was evaluated according to the fractional inhibitory concentration index (FICI), which was calculated as the sum of the FICs of stock solutions of both NOFE dissolved in nutrient broth containing 10% DMSO and ciprofloxacin. The FIC of each substance was calculated as follows:

$$FIC = \frac{\text{MIC of the substance in combination}}{\text{MIC of the substance alone}} \qquad (1)$$

The FICIs were interpreted as follows: synergy, FICI ≤0.5; additivity, FICI >0.5 to ≤1; indifference, FICI >1 to ≤4, and antagonism, FICI >4.

3. Results and Discussion

3.1. Antibacterial activity of NOFE by agar well diffusion assay.

NOFE showed promising antibacterial activity against all the tested foodborne pathogenic strains (Table 1). Three concentrations of NOFE (10, 20, and 30 µg/ml) were tested in pilot experiments. Based on the pilot study results, the flower extract concentration (30 µg/ml) was selected. The activity of NOFE (30 µg/ml) was determined and compared to the reference standard of ciprofloxacin (10 µg/ml).

Table 1. Antibacterial activity of *N. oleander* flower extract (NOFE) against the reference standard ciprofloxacin.

Indicator strains	**Zone of inhibition (mm)** [1]	
	NOFE (30 µg/ml)	**Ciprofloxacin (10 µg/ml)**
***E. coli* ATCC25922**	38±0.6	26±0.2
***P. aeruginosa* ATCC 2785**	42±0.4	30±0.6
MDR *K. Pneumonia*	28±0.2	14±0.4
***B. subtilis* ATCC 19659**	45±0.8	28±0.5
***L. monocytogenes* ATCC 679**	48±0.2	36±0.8
MRSA	24±0.4	12±0.5

[1] IZ values recorded are means of triplicate determination (n=3)± standard deviations.

The highest antibacterial effect of NOFE was observed against *L. monocytogenes* with an IZ of 48±0.2 mm, followed by *B. subtilis* and *P. aeruginosa* with an IZ of 45±0.8 mm and 42±0.4 mm, respectively. However, the lowest antibacterial activity of NOFE was shown against MDR *K. pneumoniae* and MRSA with an IZ of 28±0.2 mm and 24±0.4 mm, respectively. This contrasts with a study that evaluated the antibacterial effect of the essential oils extracted using hexane from the *N. oleander* flowers cultivated in Morocco against *E. coli*, *P. aeruginosa*, and *S. aureus* using the disc diffusion method. Their results demonstrated a stronger antibacterial activity against the Gram-negative pathogens (*E. coli* and *P. aeruginosa* with IZ diameters = 28.89 mm and 18.22 mm, respectively) compared to the Gram-positive pathogen (*S. aureus* with IZ diameter = 6.32 mm) [38].

Another study assessed the antibacterial activity of n-hexane, dichloromethane, and methanol extracts of the Iranian *N. oleander* flowers against different pathogens (*E. coli* (PTCC 1047) and *S. aureus* (PTCC 1112)) using the disc diffusion method. Results indicated that dichloromethane and methanol extracts displayed a more potent antibacterial activity against *E. coli* (14 mm and 15 mm, respectively) and *S. aureus* (13 mm and 16 mm, respectively). However, the n-hexane extract showed no effect against the *E. coli* and showed only 9 mm IZ for *S. aureus* [29].

Variations in the antibacterial activity of NOFE observed across different studies may be attributed to geographical factors, such as environmental conditions and soil type differences between regions. These factors may influence the concentration and potency of active constituents in plants grown in different countries. Furthermore, using different solvents may result in variations in the types and the nature of chemical compounds dissolving in each solvent [39]. For example, some studies used alcoholic extracts and n-hexane that may confer an additive antibacterial effect during antimicrobial testing. In the current study, we used hydro-ethanol for the extraction process, followed by filtration and drying under a vacuum to remove any residual alcohol that could interfere with the results. DMSO was chosen as the solvent for the dried flower extract as it does not exhibit antibacterial activity, ensuring that any observed antibacterial effects against the target pathogens are solely attributed to the *N. oleander* flower extract, not the solvent [40].

The NOFE (30 µg/ml) demonstrated better antibacterial effects against all the tested foodborne pathogens than that produced by ciprofloxacin (10 µg/ml). Nitave and Patil [41] reported a similar observation, in which the antimicrobial effects of the ethanolic extract of the Indian *N. oleander* flowers at different concentrations (30, 60, and 100 mg/ml) were compared to those of ciprofloxacin (10 µg/ml) against *E. coli*, *P. aeruginosa*, and *S. aureus*.

3.2. MIC of Nerium oleander flower extract.

The NOFE MIC values ranged from 12.5 µg/ml to 200 µg/ml. The data revealed variability in NOFE MIC values against the different tested strains (Table 2). The lowest NOFE MIC values were demonstrated against *E. coli* ATCC 25922, *B. subtilis* ATCC 19859, and *L. monocytogenes* ATCC 679 at 12.5 µg/ml. However, ciprofloxacin showed the lowest MIC values against these organisms, with MIC values that ranged from 0.125 to 0.5 µg/ml.

Table 2. Minimum inhibitory concentration (MIC) of the *Nerium oleander* flowers extract and ciprofloxacin against all tested bacterial strains.

Indicator Strains	**MIC (µg/mL)**	
	NOFE	**Ciprofloxacin**
***E. coli* ATCC 25922**	12.5	0.125
***P. aeruginosa* ATCC 2785**	25	0.5
MDR *K. Pneumonia*	100	64
***B. subtilis* ATCC 19659**	12.5	0.5
***L. monocytogenes* ATCC 679**	12.5	0.5
MRSA	200	128

On the other hand, the current study demonstrated that the resistant isolates (MDR *K. pneumoniae* and MRSA) recorded the highest MIC values of 100 µg/ml and 200 µg/ml, respectively, by the flower extract of *N. oleander*. Likewise, ciprofloxacin showed the highest MIC values (64 µg/mL and 128 µg/mL) against the MDR *K. pneumoniae* and MRSA, respectively. Differences in the MIC values of plant extracts can be attributed to variations in the susceptibility of the tested organisms [42].

3.3. Synergistic effect.

The accelerated emergence of drug-resistant pathogenic microbes necessitates evaluating the joint activity of conventional commercial antibiotics and plant-based natural antibacterial extracts. The checkerboard method was used to determine the FIC index value of NOFE and ciprofloxacin against MRSA. NOFE and ciprofloxacin were combined, and the synergy of their combination was recorded. Results of this study showed that the FICI value was less than 0.5 (Table 3). Evidence has already been reported for augmenting conventional antibiotics by acting synergistically with plant-derived compounds [43].

Table 3. FIC index value of both NOFE and ciprofloxacin against MRSA.

Antibacterial agent	**MIC (µg/mL)**		**FIC**	**FICI**	**Interpretation**
	Single drug	**Combined drug**			
NOFE	200	40	0.2	0.325	**Synergy**
Ciprofloxacin	128	16	0.125		

4. Conclusions

In conclusion, *Nerium oleander* is currently used traditionally for the treatment of a variety of diseases in the Kingdom of Bahrain. The positive findings from this study and many other studies in the literature provide a scientific basis for the traditional use of this plant for its antibacterial activity. *N. oleander* flower extract demonstrated potent antibacterial activity against all the studied foodborne microorganisms (*Bacillus subtilis* ATCC 19659, *Listeria monocytogenes* ATCC 679, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2785, MDR *Klebsiella pneumoniae*, and MRSA). Flower extract showed the highest antibacterial activity against *L. monocytogenes* ATCC 679, and the lowest activity was recorded against MRSA. Surprisingly, the antibacterial activity of NOFE was superior to that of ciprofloxacin against all the tested foodborne strains at the studied concentrations. Synergistic antibacterial activity between NOFE and ciprofloxacin against MRSA was reported. Further studies are needed to isolate, purify, and determine the mechanism of action of the active phytocompound(s) present in the *Nerium oleander* flower extract. Extending the antimicrobial testing against different bacterial strains and determining their synergistic effects with other antibiotics should also be studied.

Author Contributions

Conceptualization, A.B.; Antimicrobial testing, S.B.; methodolog, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, All authors (A.B., H.H., A.A., A.H., S.N. and S.B.) have contributed equally to these parts of the work. All authors have read and agreed to the published version of the manuscript. All authors confirm their agreement with the contribution statement before submission.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

The authors would like to acknowledge the faculty of the Pharmacy Program, College of Health and Sport Sciences, University of Bahrain, for their support during the preparation of this article.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bruzzese, E.; Giannattasio, A.; Guarino, A. Antibiotic treatment of acute gastroenteritis in children. *F1000Research* **2018**, *7*, 193, https://doi.org/10.12688/f1000research.12328.1.
2. Giddings, S.L.; Stevens, A.M.; Leung, D.T. Traveler's Diarrhea. *Med. Clin. North Am.* **2016**, *100*, 317–330, https://doi.org/10.1016/j.mcna.2015.08.017.
3. Makun, H.A. Significance, Prevention and Control of Food Related Diseases. InTechOpen: : Rijeka, **2016**; http://dx.doi.org/10.5772/60612.
4. Stanyevic, B.; Sepich, M.; Biondi, S.; Baroncelli, G.I.; Peroni, D.; Di Cicco, M. The evolving epidemiology of acute gastroenteritis in hospitalized children in Italy. *Eur. J. Pediatr.* **2022**, *181*, 349-358, https://doi.org/10.1007/s00431-021-04210-z.
5. Collins, J.P.; King, L.M.; Collier, S.A.; Person, J.; Gerdes, M.E.; Crim, S.M.; Bartoces, M.; Fleming-Dutra, K.E.; Friedman, C.R.; Francois Watkins, L.K. Antibiotic prescribing for acute gastroenteritis during ambulatory care visits—United States, 2006–2015. *Infect. Control Hosp. Epidemiol.* **2022**, *43*, 1880-1889, https://doi.org/10.1017/ice.2021.522.
6. Rennie, R.P.; Anderson, C.M.; Wensley, B.G.; Albritton, W.L.; Mahony, D.E. Klebsiella pneumoniae gastroenteritis masked by Clostridium perfringens. *J. Clin. Microbiol.* **1990**, *28*, 216–219, https://doi.org/10.1128/jcm.28.2.216-219.1990.
7. Calbo, E.; Freixas, N.; Xercavins, M.; Riera, M.; Nicolás, C.; Monistrol, O.; Solé, M.d.m.; Sala, M.R.; Vila, J.; Garau, J. Foodborne Nosocomial Outbreak of SHV1 and CTX-M-15–producing *Klebsiella pneumoniae*: Epidemiology and Control. *Clin. Infect. Dis.* **2011**, *52*, 743-749, https://doi.org/10.1093/cid/ciq238.
8. Chuang, C.-H.; Janapatla, R.P.; Wang, Y.-H.; Chang, H.-J.; Huang, Y.-C.; Lin, T.-Y.; Chiu, C.-H. *Pseudomonas aeruginosa*-associated Diarrheal Diseases in Children. *Pediatr. Infect. Dis.* **2017**, *36*, 1119–1123, https://doi.org/10.1097/INF.0000000000001567.
9. da Silva, A.C.; Rodrigues, M.X.; Silva, N.C.C. Methicillin-resistant *Staphylococcus aureus* in food and the prevalence in Brazil: a review. *Braz. J. Microbiol.* **2020**, *51*, 347-356, https://doi.org/10.1007/s42770-019-00168-1.
10. Mishra, S.; Mann, B.; Besmanos, C.; Raza, N.; Heidari, A. A A Unique Case of Pseudomonas aeruginosa-Associated Diarrhea in a Long-Term Hospitalized Adult Patient. *Cureus* **2023**, *15*, e39978, https://doi.org/10.7759/cureus.39978.
11. Gebre, S.H. Bio-inspired Synthesis of Metal and Metal Oxide Nanoparticles: The Key Role of Phytochemicals. *J. Clust. Sci.* **2023**, *34*, 665–704, https://doi.org/10.1007/s10876-022-02276-9.
12. Sanna, G.; Silvia, M.; Alessandra, S.; David, C.; Thomas, E.; Lukmanul, H.F.; and Rashan, L. Anti-poliovirus activity of *Nerium oleander* aqueous extract. *Nat. Prod. Res.* **2021**, *35*, 633-636, https://doi.org/10.1080/14786419.2019.1582046.
13. Adewale Akintelu, S.; Kolawole Oyebamiji, A.; Charles Olugbeko, S.; Felix Latona, D. Green chemistry approach towards the synthesis of copper nanoparticles and its potential applications as therapeutic agents and environmental control. *Curr. Res. Green Sustain. Chem.* **2021**, *4*, 100176, https://doi.org/10.1016/j.crgsc.2021.100176.
14. Battal, A.; Dogan, A.; Uyar, A.; Demir, A.; Keleş, Ö.F.; Celik, I.; Baloglu, M.C.; Aslan, A. Exploring of the ameliorative effects of *Nerium* (*Nerium oleander* L.) ethanolic flower extract in streptozotocin induced diabetic rats via biochemical, histological and molecular aspects. *Mol. Biol. Rep.* **2023**, *50*, 4193-4205, https://doi.org/10.1007/s11033-023-08332-5.
15. Giordano, M.; Petropoulos, S.A.; Cirillo, C.; Rouphael, Y. Biochemical, Physiological, and Molecular Aspects of Ornamental Plants Adaptation to Deficit Irrigation. *Horticulturae* **2021**, *7*, 107, https://doi.org/10.3390/horticulturae7050107.
16. Rónavári, A.; Igaz, N.; Adamecz, D.I.; Szerencsés, B.; Molnar, C.; Kónya, Z.; Pfeiffer, I.; Kiricsi, M. Green Silver and Gold Nanoparticles: Biological Synthesis Approaches and Potentials for Biomedical Applications. *Molecules* **2021**, *26*, 844, https://doi.org/10.3390/molecules26040844.

17. Ceci, L.; Girolami, F.; Capucchio, M.T.; Colombino, E.; Nebbia, C.; Gosetti, F.; Marengo, E.; Iarussi, F.; Carelli, G. Outbreak of Oleander (*Nerium oleander*) Poisoning in Dairy Cattle: Clinical and Food Safety Implications. *Toxins* **2020**, *12*, 471, https://doi.org/10.3390/toxins12080471.
18. Azzalini, E.; Bernini, M.; Vezzoli, S.; Antonietti, A.; Verzeletti, A. A fatal case of self-poisoning through the ingestion of oleander leaves. *J. Forensic Leg. Med.* **2019**, *65*, 133–136, https://doi.org/10.1016/j.jflm.2019.05.016.
19. Karimi, A.; Kohpeyma, F.; Asadi, E.; ziyaee, M.; Karimi, S. Protective efficacy of *Nerium oleander* extract on spermatogenesis in streptozotocin-induced diabetic rats. *Zygote* **2024**, *32*, 139-148, https://doi.org/10.1017/S0967199423000643.
20. Ayouaz, S.; Arab, R.; Mouhoubi, K.; Madani, K. *Nerium oleander* Lin: A Review of Chemical, Pharmacological and Traditional uses. *J. Biomed. Res. Environ. Sci.* **2023**, *4*, 641–650, https://doi.org/10.37871/jbres1720.
21. Singhal, K.G.; Gupta, G.D. Hepatoprotective and antioxidant activity of methanolic extract of flowers of *Nerium oleander* against CCl_4–induced liver injury in rats. *Asian Pac. J. Trop.* **2012**, *5*, 677–685, https://doi.org/10.1016/S1995-7645(12)60106-0.
22. Singh, Y.; Nimoriya, R.; Rawat, P.; Mishra, D.K.; Kanojiya, S. Structural Analysis of Diastereomeric Cardiac Glycosides and Their Genins Using Ultraperformance Liquid Chromatography-Tandem Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2021**, *32*, 1205–1214, https://doi.org/10.1021/jasms.1c00017.
23. Ahmed, O.H.; Ezghayer, M.A.; Jabir, M.S.; Tawfeeq, M.F. Cytotoxic activity of methanolic extract of Nerium Oleander Naturally Grown in Iraq. *J. Surv. Fish. Sci.* **2023**, *10*, 2517-2524.
24. Salama, M.; Rashed, S.A.; Fayez, A.; Hassanein, S.S.; Sharaby, M.R.; Tawfik, N.M.; Mansour, H.; Adel, M. Medicinal plant-derived compounds as potential phytotherapy for COVID-19: Future perspectives. *J. Pharmacognosy Phytother.* **2021**, *13*, 68-81, https://doi.org/10.5897/JPP2021.0603.
25. Akhtar, T.; Sheikh, N.; Abbasi, M.H. Alternative therapeutic approaches: Hepatoprotective effect of *Nerium oleander* extract in thioacetamide-induced hepatotoxicity. *Int. J. Clin. Exp. Med.* **2016**, *9*, 16118–16124. Available online: https://e-century.us/files/ijcem/9/8/ijcem0018989.pdf
26. Pandey, A.; Usmani, S.; Ahmad, M.; Khatoon, S.; Wahab, S.; Prakash, O. Phytochemical and pharmacological attributes of *Nerium oleander*: A review. *Curr. Nutr. Food Sci.* **2024**, *20*, 570-585, https://doi.org/10.2174/1573401319666230522160742.
27. Bakir Çilesizoğlu, N.; Yalçin, E.; Çavuşoğlu, K.; Sipahi Kuloğlu, S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. *Sci. Rep.* **2022**, *12*, 21421, https://doi.org/10.1038/s41598-022-26087-0.
28. Shafiq, Y.; Naqvi, S.B.S.; Rizwani, G.H.; Asghar, M.A.; Bushra, R.; Ghayas, S.; Rehman, A.A.; Asghar, M.A. A mechanistic study on the inhibition of bacterial growth and inflammation by *Nerium oleander* extract with comprehensive in vivo safety profile. *BMC Complement. Altern. Med.* **2021**, *21*, 135, https://doi.org/10.1186/s12906-021-03308-z.
29. Namian, P.; Talebi, T.; Germi, K.G.; Shabani, F. Screening of Biological Activities (Antioxidant, Antibacterial and Antitumor) of *Nerium oleander* Leaf and Flower Extracts. *Am. J. Phytomed. Clin. Ther.* **2013**, *10*, 378-384.
30. Fartyal, M.; Kumar, P.; Bioactivity of crude extracts of *Nerium oleander* Linn. extracted in polar and non polar solvents. *J. Sci. Innov. Res.* **2014**, *3*, 426–432, https://doi.org/10.31254/jsir.2014.3406.
31. Hussain, M.A.; Gorsi, M.S. Antimicrobial Activity of *Nerium oleander* Linn. *Asian J. Plant Sci.* **2004**, *3*, 177–180, https://doi.org/10.3923/ajps.2004.177.180.
32. Dardona, A.W.Y.; Shahbuddin, D. Evaluation Of Antimicrobial Activity OfMethanolic And Ethanolic Extracts Of ThreeVarieties Of Nerium Oleander. *J. Pharm. Negat. Results* **2022**, *13*, 1821-1829.
33. Sharma, R.; Singh, S.; Tewari, N.; Dey, P. A toxic shrub turned therapeutic: The dichotomy of *Nerium oleander* bioactivities. *Toxicon* **2023**, *224*, 107047, https://doi.org/10.1016/j.toxicon.2023.107047.
34. Daware, P. A PHARMACOLOGICAL SCREENING OF *NERIUM OLEANDER* LINN: A REVIEW. *Int. J. Pharm. Sci. Rev. Res.* **2012**, *15*, 125–126.
35. Zaid, R.; Canela-Garayoa, R.; Ortega-Chacón, N.M.; Mouhouche, F. Phytochemical analyses and toxicity of *Nerium oleander* (Apocynaceae) leaf extracts against *Chaitophorus leucomelas* Koch, 1854 (*Homoptera: Aphididae*). *J. Saudi Soc. Agric. Sci.* **2022**, *21*, 310-317, https://doi.org/10.1016/j.jssas.2021.10.011.

36. Tanhaeian, A.; Sekhavati, M.H.; Moghaddam, M. Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. *Chem. Biol. Technol. Agric.* **2020**, *7*, 13, https://doi.org/10.1186/s40538-020-00181-9.
37. Siqueira, I.B.; Andrea, T.B.A.; Sona, J.; Pereira, M.F.R.; Soares, T.S.A.R.; Henrique, F.B.F.; Marta, S.D.; Fitzgerald, B.A.; Guimarães, S.A.; Aparecida, d.C.N.D.; and Dolabella, S.S. *In vitro* Antibacterial Activity of Essential Oils of *Croton tetradenius* Baill. From the Brazilian Caatinga Biome and Its Synergistic Effect With Ciprofloxacin and Meropenem. *J. Essent. Oil-Bear. Plants* **2021**, *24*, 12-21, https://doi.org/10.1080/0972060X.2020.1871079.
38. Derwich, E.; Benziane, Z.; Boukir, A. ANTIBACTERIAL ACTIVITY AND CHEMICAL COMPOSITION OFTHE ESSENTIAL OIL FROM FLOWERS OF NERIUM OLEANDER. *Electron. J. Environ. Agric. Food Chem.* **2010**, *9*, 1074-1084.
39. Gonelimali, F.D.; Lin, J.; Miao, W.; Xuan, J.; Charles, F.; Chen, M.; Hatab, S.R. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Front. Microbiol.* **2018**, *9*, 1639, https://doi.org/10.3389/fmicb.2018.01639.
40. Ginovyan, M.; Petrosyan, M.; Trchounian, A. Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC Complement. Altern. Med.* **2017**, *17*, 50, https://doi.org/10.1186/s12906-017-1573-y.
41. Nitave, S.A.; Patil, V.A. Study of antibacterial and antifungal activity of *Nerium oleander* flower extract and its phytochemical screening. *World J. Pharm. Res.* **2016**, *5*, 640-647.
42. Mostafa, A.A.; Al-Askar, A.A.; Almaary, K.S.; Dawoud, T.M.; Sholkamy, E.N.; Bakri, M.M. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J. Biol. Sci.* **2018**, *25*, 361-366, https://doi.org/10.1016/j.sjbs.2017.02.004.
43. Basavegowda, N.; Baek, K.-H. Combination Strategies of Different Antimicrobials: An Efficient and Alternative Tool for Pathogen Inactivation. *Biomedicines* **2022**, *10*, 2219, https://doi.org/10.3390/biomedicines10092219.

Publisher's Note & Disclaimer