University of Thi-Qar Journal of agricultural research
Thi-Qar Journal of agricultural research
ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

Fungitoxicity and Inhibitory Activity of *Nerium oleander* Leaf Extracts Against *Fusarium solani*

Nour Al-Huda Khudair Samir[1], Dr. Majid Hanoon Sharhan[2]

[1] **Department of Biology, College of Science, Wasit University, Wasit, Iraq**

[2] **Department of Biology, College of Science, Wasit University, Wasit, Iraq**

mhanon@uowasit.edu.iq

std2024204.nooralhudahussein@uowasit.edu.iq

I. Abstract

The results of this study demonstrated the effective role of biochemical compounds extracted from the leaves of *Nerium oleander* L. as a natural alternative for inhibiting the growth of the pathogenic fungus *Fusarium solani* (T18TAL-Rashaed), through a comparison between aqueous and alcoholic (95% ethanol) extracts. In this study, a pure fungal isolate was used, obtained from the Scientific Research Authority, Agricultural Research Center in Iraq, and registered in the international GenBank database affiliated with the National Center for Biotechnology Information (*NCBI*).

The laboratory experiment conducted in the Plant Laboratory, College of Science, University of Wasit, revealed variation in the results depending on the type of extract and the concentration used. Four concentrations were prepared for each extract (5, 10, 15, and 20%). The alcoholic extract showed qualitative superiority over the aqueous extract, achieving complete inhibition (100%) at concentrations of 10%, 15%, and 20%, whereas the highest inhibition percentage recorded for the aqueous extract did not exceed 33.80% at a concentration of 20%, compared with the negative control treatment (6.42%).

The results further indicated that the Minimum Inhibitory Concentration (MIC) of the alcoholic extract was 10%, which resulted in complete inhibition of fungal colony growth. This variation in the results confirms the high efficiency of the alcoholic solvent in extracting phenolic compounds, alkaloids, resins, glycosides, and tannins, which were qualitatively identified as possessing antifungal activity from the plant leaves, compared with the aqueous solvent. Therefore, the alcoholic extract can be considered an effective biological option for fungal control.

II. Introduction

The rapid development of agricultural technologies and the excessive reliance on chemical pesticides have led to ecological imbalance and depletion of biological resources. This has created an urgent need to search for natural alternatives derived from toxic medicinal plants capable of limiting the growth and reproduction of phytopathogenic fungi. The present study focused on *Nerium oleander* L., the only species within the genus *Nerium*, which belongs to the family *Apocynaceae* (Ayouaz et al., 2023). This plant requires careful investigation of its chemical properties and biological effects, as it is an evergreen perennial shrub characterized by its ability to tolerate harsh climatic conditions, in addition to its abundance and richness in bioactive compounds.

Recent studies have revealed that this plant possesses compounds with significant antifungal and antibacterial activities. However, the high toxicity of its plant parts necessitates extreme caution and precise medical considerations in determining safe dosages (Bandara et al., 2010). The toxicity of this plant is attributed to toxic cardiac glycosides of various types, including Oleandrin, Neriine, Cardenolides, and Strychnine (Barbosa et al., 2008). Furthermore, the plant contains several important phytochemicals such as alkaloids, flavonoids, and steroids (Farkhondeh et al., 2020). A previous study confirmed that extracts of *N. oleander* leaves contain antifungal substances (Siddiqui et al., 2016).

In another study evaluating the biological activity of this plant, qualitative phytochemical screening was conducted for aqueous and alcoholic (ethanolic) extracts of *N. oleander* leaves to identify the compounds present and their effect on inhibiting the growth of the pathogenic fungus *Fusarium solani*, the causal agent of root rot disease. The study revealed the presence of alkaloids, flavonoids, and cardiac glycosides (Upadhyay, 2024). This pathogen causes severe economic and financial losses to farmers, which highlights the importance of utilizing these plant extracts as natural antifungal agents. Extracts of *N. oleander* exhibit inhibitory activity against several pathogenic fungal species, including *F. solani*, which is considered a facultative parasitic and saprophytic fungus capable of producing three types of spores: macroconidia (fusiform), microconidia (cylindrical), and chlamydospores (Hussein, 2020).

The current study investigated the inhibitory effects of aqueous and alcoholic extracts of *N. oleander* in order to determine the efficiency of each extract in suppressing the growth of *F. solani*, as well as to evaluate the differences among treatments according to extract type and concentration used in the experiment. The objectives of the study were as follows:

1. To evaluate the biological activity and toxicity of aqueous extracts prepared with distilled water and alcoholic extracts prepared with 95% ethanol from *N. oleander* leaves.
2. To compare the efficiency of the two solvents (water and ethanol) in extracting antifungal bioactive compounds.
3. To determine the Minimum Inhibitory Concentration (MIC) of the alcoholic extract.

1. Materials and Methods

4. **Fungal Isolate**
5. The pathogenic isolate of *Fusarium solani* (T18TAL-Rashaed) was obtained from the Scientific Research Authority, Agricultural Research Center in Iraq. Genetic analysis revealed amplification of the Internal Transcribed Spacer (ITS) region containing the 5.8S rRNA gene, with a length of 207 base pairs. The isolate was registered in the GenBank database affiliated with the National Center for Biotechnology Information (*NCBI*). The isolate was maintained and cultured on Potato Dextrose Agar (PDA) medium at a temperature of 25 ± 2°C.
6. **Collection of Plant Leaves**
7. Leaves of *Nerium oleander* were collected from the gardens of the College of Science, University of Wasit. The leaves were carefully cleaned and washed thoroughly with water to remove dust and impurities, then air-dried at room temperature after being cut into small pieces using a grinder. The dried leaves were subsequently ground using an electric mill and stored in sterile glass containers wrapped with aluminum foil to protect them from light, as some chemical compounds are light-sensitive. The samples were preserved until use.
8. **Preparation of PDA Culture Medium**
9. The culture medium was prepared according to the manufacturer's instructions by dissolving 41 g of PDA powder in 1000 mL of distilled sterile water. The medium was sterilized in an autoclave at 121°C under a

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

pressure of 15 psi for 50 minutes. After cooling to 45–50°C, 0.05 g of chloramphenicol was added to prevent bacterial growth.

10. **Preparation of the Aqueous Extract**
11. The plant extract was prepared by soaking 20 g of powdered *N. oleander* leaves in 400 mL of sterile distilled water. The mixture was then placed in a water bath at 40°C for 4 hours and left at room temperature for 24 hours. Subsequently, the extract was filtered through layers of medical gauze and centrifuged at 2000 rpm for 15 minutes. The supernatant was collected and stored in sterile dark containers at 4°C for the preparation of the required concentrations according to the method of Sasidharan et al. (2011), with some modifications.
12. **Preparation of the Alcoholic Extract**
13. The alcoholic extract was prepared following the same procedure used for the aqueous extract, except that 95% ethanol was used instead of distilled water, according to the method of Sasidharan et al. (2011).
14. **Fungal Cultivation and Evaluation of Extract Activity**
15. After preparing the PDA medium, the extracts were incorporated into the medium using the Poisoned Food Technique at different concentrations (5, 10, 15, and 20 mL per 100 mL PDA), with three replicates for each concentration according to the method described by Khanzada et al. (2006).
16. Plates containing only PDA medium served as the negative control for comparison with all treatments. Additional plates containing ethanol at the same concentrations used in the experiment were prepared as solvent controls for alcoholic extract comparison. Plates containing the fungicide Hyxat 30% at a concentration of 1% were used as a positive control.
17. The plates were inoculated with a 6 mm disc taken from an actively growing culture of *F. solani* using a cork borer, and the disc was placed at the center of each plate. The cultures were incubated at 25 ± 2°C for 7 days. Thereafter, two perpendicular colony diameters were measured, and the average fungal growth was calculated. The inhibition percentage was then determined according to the equation described by Wanchaitanawong et al. (2005):
18. **Evaluation of Qualitative Inhibitory Efficiency and Its Relationship with the Chemical Composition of the Extracts**
19. The inhibitory activity of the plant extracts was evaluated and the scientific explanation for the variation in their effectiveness was determined by calculating the percentage of inhibition and correlating it with the nature of the chemical compounds extracted by each solvent.
20. **Preliminary Screening of Bioactive Compounds**
21. **1. Detection of Resins**
22. Five milliliters of the filtrate from the alcoholic extract of *Nerium oleander* leaves were heated in a boiling water bath for two minutes. Subsequently, 5 mL of diluted hydrochloric acid were added. The appearance of turbidity or a visible precipitate indicated the presence of resins (Shihata, 1951).
23. **2. Detection of Tannins**
24. An aqueous solution was prepared by mixing 2 g of plant powder with 10 mL of distilled water in a glass flask. The mixture was boiled, filtered, and completely cooled. Thereafter, 1% ferric chloride solution ($FeCl_3$) was added. The appearance of a bluish-green coloration indicated the presence of tannins (Evans, 2002).
25. **3. Detection of Flavonols and Flavonoids**
26. One milliliter of concentrated sulfuric acid (H_2SO_4) was carefully mixed with an equal volume (1 mL) of the aqueous extract in a sterile test tube. After thorough mixing, the development of a dark yellow color was considered a positive indication for the presence of flavonols and flavonoids (Al-Khazraji, 1991).
27. **4. Detection of Alkaloids**
28. An acidic aqueous extract was prepared by mixing 5 g of plant powder with 25 mL of distilled water containing 4% hydrochloric acid (HCl). The mixture was heated, filtered, and cooled. Wagner's reagent was then used as a qualitative detection reagent. The reagent was prepared by dissolving 2 g of potassium

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

iodide in 100 mL of distilled water, followed by the addition of 1.3 g iodine with continuous stirring until complete dissolution. The appearance of a brown precipitate indicated the presence of alkaloids (Harborne, 1984).

29. **5. Detection of Cardiac Glycosides**
30. One to two drops of 2% ferric chloride solution ($FeCl_3$) were added to 1 mL of the alcoholic plant extract. The mixture was then transferred into another test tube containing 2 mL of concentrated sulfuric acid (H_2SO_4). The formation of a brown ring at the interface indicated the presence of cardiac glycosides (Yadav & Agarwala, 2011).
31. **6. Measurement of pH**
32. The pH values of the aqueous and alcoholic extracts of *N. oleander* leaves were determined using a digital pH meter. Measurements were carried out by directly immersing the electrode into the solutions at laboratory temperature, ensuring proper calibration of the instrument using buffer solutions (Adeloye et al., 2007; Shihata, 1951), as shown in Table (1).

Table (1): pH values of aqueous and alcoholic extracts of *Nerium oleander*

Parameter	Aqueous extract	Alcoholic extract
PH	6.8	6.3

Statistical Analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS). A Completely Randomized Design (CRD) was adopted for conducting the experiment. Analysis of Variance (ANOVA) was performed to determine significant differences among the study treatments. To compare the means of the different concentrations of plant extracts (aqueous and alcoholic), in addition to the control treatments, the Least Significant Difference (LSD) test was used to determine statistical significance at the probability level of $P > 0.05$ (Daniel & Cross, 2018).

1. Results and Discussion

The study employed two types of *Nerium oleander* extracts prepared using two solvents, distilled water and 95% ethanol, to ensure efficient extraction of the plant's bioactive compounds.

1. Effect of Alcoholic Extracts of

Nerium oleander

on

Fusarium solani

Nerium oleander L. is considered one of the important local medicinal plants that has demonstrated significant effectiveness in inhibiting pathogenic fungi. The experimental results showed that the alcoholic extract exhibited clear inhibitory activity even at the lowest concentration (5%), where the mean fungal growth diameter was 22.7 mm with an inhibition percentage of 64.18%. Although this low concentration did not achieve complete inhibition, it significantly reduced fungal growth.

In contrast, concentrations of 10%, 15%, and 20% showed a pronounced effect on the growth of *F. solani*, resulting in complete inhibition of fungal growth in all three replicates. The mean colony diameter was 0 mm, corresponding to an inhibition percentage of 100% compared with the control treatment, which exhibited normal fungal growth with a mean colony diameter of 64.2 mm. These findings indicate the high efficiency of the alcoholic extract in suppressing fungal growth.

When compared with the positive control treatment (fungicide), the alcoholic extract exhibited inhibitory activity equivalent to the antifungal agent Hyxat 30% at concentrations of 10%, 15%, and 20%. These results confirm that the alcoholic extract possesses antifungal activity that increases with increasing concentration (Al-Hasnawi & Al-Khaikani, 2017).

The inhibitory effect on fungal growth may be attributed to the bioactive compounds present in the alcoholic extract, particularly cardiac glycosides such as oleandrin, nerine, cardenolides, and gentiobiosyl, which are highly toxic even at low concentrations (Ayouaz et al., 2023). In addition, tannins, resins, flavonoids, and alkaloids contribute to antifungal activity by disrupting the fungal cell membrane, inhibiting vital enzymes, and coagulating cellular proteins (Al-Snafi, 2020). Furthermore, Upadhyay (2024) reported that *N. oleander* extracts possess significant inhibitory activity against certain pathogenic fungal species, supporting the findings of the present study and confirming the antifungal effectiveness of *N. oleander* against *F. solani* (Table 2).

Table (2): Growth rate (mm) and inhibition (%) of *F. solani* by *N. Oleander* alcoholic extract

Treatment (v/v)	Growth Rate (mm)	Inhibition %
Control 0%	64.2	0.00
5%	22.7	64.18
10%	0.0	100
15%	0.0	100
20%	0.0	100
Hyxat 1%	0.0	100
L.S.D	3.54	

Mean of 3 replicates

.2Effect of Aqueous Extracts of

Nerium oleander on

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

Fusarium solani

The aqueous extract exhibited a noticeable inhibitory effect on the growth of the studied fungus. A reduction in fungal growth was observed even at the lowest concentration (5%), where the mean colony diameter reached 56.6 mm with an inhibition percentage of 11.73%; however, this concentration did not result in complete inhibition.

At concentrations of 10%, 15%, and 20%, the mean colony diameters of the fungal colonies were 55.8, 53.3, and 42.5 mm, respectively, with corresponding inhibition percentages of 13.03%, 16.93%, and 33.80%, respectively. These inhibition percentages were calculated in comparison with the control treatment, which exhibited normal fungal growth with a mean colony diameter of 64.2 mm. Nevertheless, even the highest concentration (20%) did not achieve complete inhibition of fungal growth when compared with the fungicide Hyxat 30%. These findings confirm that the aqueous extract was less efficient than the alcoholic extract, which may be attributed to the polarity of the solvent used in extract preparation, as solvent polarity plays a crucial role in determining the inhibitory efficiency of the extract (Table 3).

The statistical analysis (ANOVA) revealed significant differences among the extracts at the probability level of 0.05, with the alcoholic extract showing superior inhibitory efficiency against fungal growth compared with the aqueous extract.

The variation in inhibitory activity between the alcoholic and aqueous extracts may be explained by the differences in the solvents and their dielectric constants. The dielectric constant of water is 79.99, whereas that of ethanol is 25.02. Solvents with lower dielectric constants, such as ethanol, are generally more effective in penetrating plant tissues and extracting less polar compounds. This explains the superiority of the alcoholic extract compared with water, which possesses high polarity and a higher dielectric constant. These findings are consistent with those reported by Mohsen-Nia et al. (2010).Based on the results, which demonstrated significant differences in extraction efficiency and inhibitory activity, the Minimum Inhibitory Concentration (MIC) of the alcoholic extract of *N. oleander* against *F. solani* was determined to be 10%, as this concentration completely inhibited fungal growth in all replicates. In contrast, the aqueous extract did not achieve a comparable MIC value within the concentrations tested in the present study. These findings further confirm the efficiency of organic solvents, such as ethanol, in extracting secondary metabolites responsible for strong inhibitory activity against phytopathogenic fungi.

Table (3): Growth rate (mm) and inhibition (%) of F. solani by N. Oleander Aqueous extract

Treatment (v/v)	Growth Rate (mm)	Inhibition %
Control 0%	64.2	0.00
5%	56.7	11.73
10%	55.8	13.03
15%	53.3	16.93
20%	42.5	33.80
Hyxat 1%	0.0	100
L.S.D	6.69	

Mean 3 replicates

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

It is observed from the results that there is a clear positive relationship between the different concentrations of *Nerium oleander* extracts and the percentage inhibition of *Fusarium solani*. As the concentration increased, the inhibitory effect also increased. This is attributed to the higher availability of bioactive compounds such as alkaloids and cardiac glycosides in the extract, which are responsible for growth inhibition (Figure 1).

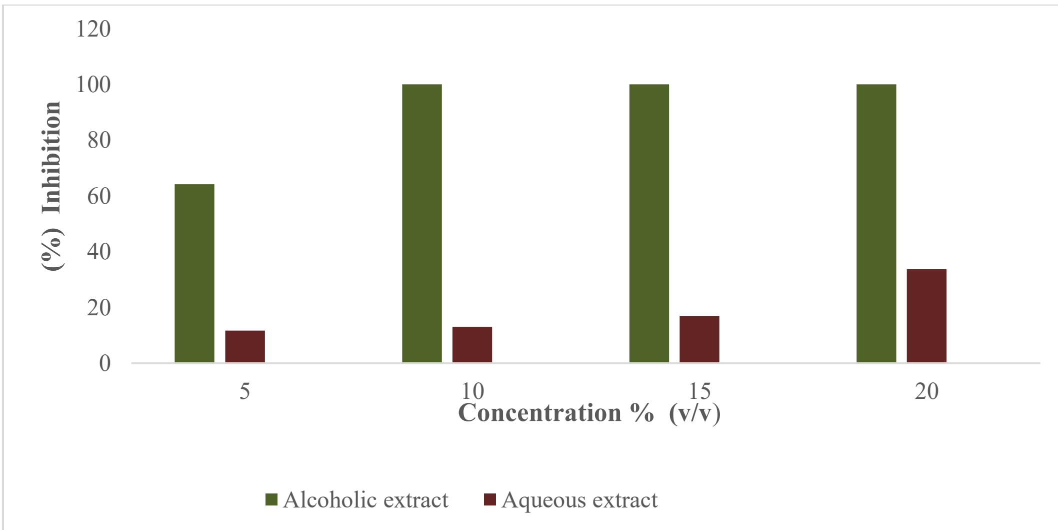

Fig. 1: Antifungal activity of *Nerium oleander* extracts against *Fusarium solani*

Results of Phytochemical Screening

In order to understand the chemical nature of *Nerium oleander* extracts, a series of qualitative phytochemical tests were conducted to identify the active compounds. The results presented in Table (4) revealed that the plant is rich in bioactive constituents, most notably glycosides, alkaloids, and tannins. These compounds are considered to play a major role in the observed inhibitory activity against the plant pathogenic fungus *Fusarium solani*.

Table (4): Phytochemical screening of *Nerium oleander* extracts

Compounds	Resins	Tannins	Flavonoids	Alkaloids	Glycosides
Result	+	+ +	+	+ +	+ + +

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

Conclusions

1. The study demonstrated that *Nerium oleander* extracts exhibit a clear inhibitory effect against *Fusarium solani*, the causal agent of root rot disease.
2. A positive correlation was observed between extract concentration and inhibition percentage; as concentration increased, the percentage of fungal growth inhibition also increased.
3. The alcoholic extract of *Nerium oleander* showed superior antifungal activity compared with the aqueous extract, which is attributed to the higher efficiency of ethanol in extracting bioactive compounds.
4. Both alcoholic and aqueous extracts of *Nerium oleander* represent a safe and environmentally friendly alternative when compared with synthetic fungicides.

Recommendations

1. Testing *Nerium oleander* extracts (aqueous and alcoholic) against other pathogenic fungal species.
2. Conducting further studies using different extraction methods to determine the most efficient technique for obtaining bioactive compounds from *Nerium oleander*.
3. Performing field experiments to evaluate the effectiveness of the extracts under natural environmental conditions outside the laboratory.
4. Investigating other plant parts (stems and roots) and comparing them with leaf extracts to determine the most effective plant part in inhibiting fungal growth.
5. Studying the potential phytotoxic effects of different extract concentrations on plant growth to determine whether they cause any harmful effects.

References

Adeloye, O. A., Akinpelu, A. D., Ogundaini, O. A., & Obafemi, A. C. (2007). Studies on antimicrobial, antioxidant and phytochemical analysis of Urena lobata leave extract. *J Phys Nat Sci*, *1*(2), 1-9 .

Al-Hasnawi, I. M., & Al-Khaikani, D. H. (2017). Effect of using some plant extracts in the control of the pathogenic fungus Fusarium oxysporum f. sp. cucumerinum, the cause of cucumber root rot disease (In Arabic). *Al-Qadisiyah Journal of Pure Science*, *22* .(1)

Al-Khazraji, S. (1991). Biopharmacological study of Artemisia herba alba. *M. Sc* .

Al-Snafi, A. E. (2020). Bioactive ingredients and pharmacological effects of Nerium oleander. *IOSR Journal of Pharmacy*, *10*(9), 19-32 .

Ayouaz, S., Arab, R., Mouhoubi, K., & Madani, K. (2023). NERIUM OLEANDER LIN: a review of chemical, pharmacological and traditional uses. *J. Biomed. Res. Environ. Sci*, *2766*, 2276 .

Bandara, V., Weinstein, S. A., White, J., & Eddleston, M. (2010). A review of the natural history, toxinology, diagnosis and clinical management of Nerium oleander (common oleander) and Thevetia peruviana (yellow oleander) poisoning. *Toxicon*, *56*, 273-281 .

Barbosa, R., Fontenele-Neto, J., & Soto-Blanco, B. (2008). Toxicity in goats caused by oleander (Nerium oleander). *Research in veterinary science*, *85*(2), 279-2 .81

Daniel, W. W., & Cross, C. L. (2018). *Biostatistics: a foundation for analysis in the health sciences*. John Wiley & Sons .

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 150.

Evans, W. (2002). Trease and Evans Pharmacognosy. 15th (ed.) Saunders Publishers, an imprint of Elsevier Science Ltd. In: India.

Farkhondeh, T., Kianmehr, M., Kazemi, T., Samarghandian, S., & Khazdair, M. R. (2020). Toxicity effects of Nerium oleander, basic and clinical evidence: A comprehensive review. *Human & experimental toxicology*, *39*(6), 773-784 .

Harborne, J. B. (1984). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (2nd ed.). Chapman & Hall .

Hussein, S. N. (2020). Diagnosis and control of the cucumber root rot causes using some chemical and biological agents

Khanzada, S. A., Iqbal, S. M., & Akram, A. (2006). In vitro efficacy of plant leaf extracts against Sclerotium rolfsii Sacc. *Mycopathologia*, *4*(1), 5 .53-1

Mohsen-Nia, M., Amiri, H., & Jazi, B. (2010). Dielectric constants of water, methanol, ethanol, butanol and acetone: measurement and computational study. *Journal of Solution Chemistry*, *39*(5), 701-708 .

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African journal of traditional, complementary and alternative medicines*, *8* .(1)

Shihata, I. (1951). A pharmacological study of Anagallis arvensis. *MD Vet Thesis. Cairo University of Egyp* .

Siddiqui, I., Bokhari, N., & Perveen, K. (2016). Antifungal ability of Nerium oleander against Fusarium oxysporum, Sclerotium rolfsii and Macrophomina phaseolina. *JAPS: Journal of Animal & Plant Sciences*, *26* .(1)

Upadhyay, R. K. (2024). Phytochemistry, therapeutic and
pharmacological potential of *Nerium oleander L. International Journal of Green Pharmacy* .

Wanchaitanawong, P., Chaungwanit, P., Poovarodom, N., & Nitisinprasert, S. (2005). In vitro antifungal activity of Thai herb and spice extracts against food spoilage fungi. *Agriculture and Natural Resources*, *39*(3), 400-405 .

Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, *3* .(12)