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PHYTOCHEMICAL CHARACTERIZATION, THIN LAYER CHROMATOGRAPHY PROFILING, AND ANTIMICROBIAL ACTIVITY EVALUATION OF NERIUM OLEANDER FLOWER

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Abstract: Nerium oleander is a rich source of secondary metabolites with potential biological activities. In the present study, the methanolic extract of Nerium oleander flowers was evaluated for its phytochemical constituents, chromatographic profile, and antimicrobial activity. Preliminary phytochemical screening was conducted using standard qualitative chemical tests to detect major classes of bioactive compounds, including alkaloids, flavonoids, phenolics, tannins, and cardiac glycosides, which are known to possess antimicrobial and antioxidant properties. Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plates using suitable solvent systems to separate the chemical constituents of the extract. Distinct spots with characteristic Rf values were observed under visible and ultraviolet light, confirming the presence of multiple phytochemical components and demonstrating the complexity of the flower extract. The TLC fingerprint obtained can serve as a reference for the identification and quality control of Nerium oleander flower extract. The antimicrobial activity of the extract was evaluated against selected gram-positive and gram-negative bacterial strains, as well as fungal species, using the agar well diffusion method. The methanolic flower extract exhibited appreciable antimicrobial activity, with zones of inhibition increasing in a concentration-dependent manner. The observed activity may be attributed to the synergistic effects of phenolic compounds, flavonoids, tannins, and glycosides present in the extract. Overall, the findings suggest that Nerium oleander flower extract contains diverse bioactive compounds with significant antimicrobial potential. The combination of phytochemical screening, TLC profiling, and antimicrobial evaluation supports the possible use of this plant as a natural source of antimicrobial agents and provides a scientific basis for further isolation and characterization of its active constituents.

Index Terms - Nerium oleander, phytochemical screening, thin-layer chromatography, antimicrobial activity, methanolic extract, medicinal plants

INTRODUCTION

Nerium oleander is an evergreen shrub in the family Apocynaceae[1]. It is the only species currently classified in the genus Nerium. It is commonly known as oleander because of its superficial resemblance to the unrelated olive. It is widely cultivated and is thought to have originated from southwest Asia[5]. Oleander grows well in warm subtropical regions, where it is extensively used as an ornamental plant in landscapes, parks, and along roadsides[4]. Nerium oleander contains a high variety of bioactive compounds and shows antioxidant, anticancer, antimicrobial, and antidiabetic activities[1]. Nerium oleander exhibits many biological and pharmacological activities owing to its rich phytochemical content[3]. Nerium oleander leaves are rich in

carbohydrates, flavonoids, alkaloids, steroids, cardiac glycosides, and tannins[4]. It should be handled carefully during laboratory studies because of the presence of toxic cardiac glycosides[8]. Despite the danger, oleander is of great medicinal importance and is used for heart conditions, asthma, epilepsy, cancer, painful menstrual disease, leprosy, malaria, ringworm, indigestion, and venereal disease, and to induce abortions; drugs derived from this plant are also used in the treatment of cancer, and research is ongoing for its future implementation. Extraction is an essential step in the isolation and characterization of bioactive compounds from medicinal plants. Among the various techniques, Soxhlet extraction is one of the most widely used conventional methods for obtaining phytochemical constituents from plant materials. The Soxhlet apparatus enables continuous extraction by repeatedly washing the plant material with fresh solvent under reflux conditions, resulting in efficient extraction of both polar and semi-polar compounds[3]. In the Soxhlet process, dried and powdered Nerium oleander flowers are placed in a thimble holder, while a suitable solvent, such as methanol or ethanol, is heated in a round-bottom flask. The solvent evaporates, condenses, and repeatedly percolates through the plant material, dissolving the phytochemicals. The extract-rich solvent siphons back into the flask, and the cycle continues until exhaustive extraction is achieved[4].

Phytochemicals are naturally occurring bioactive compounds present in plants that contribute to their therapeutic and pharmacological properties. Preliminary phytochemical screening is an important step in the evaluation of medicinal plants, helping to identify the presence of various classes of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds; it is also useful in qualitative analytical procedures that involve specific chemical reactions producing characteristic color changes or precipitates in the presence of particular classes of compounds4. These tests provide valuable information regarding the chemical composition of plant extracts and serve as a basis for further chromatographic and pharmacological investigations5.

Thin-layer chromatography is a simple, rapid, and cost-effective chromatographic technique widely employed for the qualitative analysis, identification, and monitoring of phytoconstituents in plant extracts. This method is based on the differential migration of compounds over a stationary phase, usually silica gel-coated plates, under the influence of a suitable mobile phase. Separation occurs because of the differences in the adsorption and partition coefficients of compounds, resulting in distinct spots characterized by their retention factor (Rf) values.

The increasing prevalence of antimicrobial resistance among pathogenic microorganisms has created an urgent need for the discovery of new antimicrobial agents from natural sources. Medicinal plants have long been recognized as valuable reservoirs of bioactive compounds with therapeutic potential, including antimicrobial properties. Plant-derived secondary metabolites, such as flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, and glycosides, have been reported to exhibit significant inhibitory effects against a wide range of bacterial and fungal pathogens1,2. The assessment of antimicrobial activity in column chromatography fractions of Nerium oleander flower extract facilitates the identification of biologically active constituents and contributes to the development of novel plant-based antimicrobial agents3,6.

METHODOLOGY

Soxhlet Extraction

Fresh Nerium oleander flowers were collected, thoroughly washed with water to remove dust and impurities, and shade-dried at room temperature. The dried flowers were then ground into a coarse powder. Nerium oleander flower powder (25 g, accurately weighed) was placed into a cellulose extraction thimble and inserted into the Soxhlet extractor. The extractor flask was filled with 250 ml of ethanol or methanol, and the Soxhlet apparatus was assembled and heated at 54°C. The solvent boiled, evaporated, condensed in the condenser, and repeatedly percolated through the plant material. This cycle continued for 6–8 hours, obtaining 15 cycles, or until the siphon tube became nearly colorless, indicating exhaustive extraction. After completion of extraction, the extract was filtered through Whatman No. 1 filter paper. The solvent was removed under reduced pressure using a rotary evaporator to obtain a concentrated crude extract.

Figure 1 Nerium oleander flowers

Figure 2 Fine powder of flower

figure 3: Soxhlet extraction

Phytochemical Screening

Preliminary phytochemical screening of Nerium oleander flower extract was carried out using standard qualitative methods to identify the presence of alkaloids, flavonoids, phenolics, saponins, terpenoids, cardiac glycosides, and so on.

Preparation of test solution: 100 mg of dried methanolic Nerium oleander flower extract was accurately weighed and dissolved in 10 ml of methanol or ethanol.

[1] Test for alkaloids (Dragendorff's test): 1 ml of extract solution was taken in a test tube and a few drops of Dragendorff's reagent were added. Formation of an orange or red precipitate indicates the presence of alkaloids.

[2] Test for alkaloids (Wagner's test): 1 ml of Nerium oleander flower extract was taken in a test tube and a few drops of Wagner's reagent solution were added. Formation of a reddish-brown or brown precipitate indicates the presence of alkaloids.

[3] Test for phenolics (Ferric chloride test): 1 ml of flower extract was taken in a test tube and 2–3 drops of 5% ferric chloride solution were added. Formation of a blue, black, or green colour indicates the presence of phenolics.

[4] Test for phenolics (Gelatin test): 1 ml of flower extract was mixed with 1 ml of 1% gelatin solution containing 10% sodium chloride solution. Observation of a white precipitate indicates the presence of phenolics.

[5] Test for flavonoids (Alkaline test): 1 ml of flower extract solution was taken in a test tube and a few drops of 10% sodium hydroxide (NaOH) solution were added. Formation of a yellow colour that disappears upon addition of dilute HCl indicates the presence of flavonoids.

[6] Test for flavonoids (Lead acetate test): 1 ml of flower extract solution was mixed with a few drops of 10% lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.

[7] Test for saponins (Foam test): 1 ml of flower extract was mixed with 5 ml of distilled water and shaken vigorously for 5 minutes. Formation of a persistent froth (foam) layer of about 1 cm or more indicates the presence of saponins.

[8] Test for cardiac glycosides (Keller–Kiliani test): To 1 ml of flower extract solution, 1 ml of glacial acetic acid containing one drop of ferric chloride solution was added. 1 ml of concentrated sulfuric acid was then carefully added along the side of the tube. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

[9] Test for cardiac glycosides (Legal's test): 1 ml of flower extract solution was mixed with 1 ml of pyridine, followed by 1 ml of freshly prepared sodium nitroprusside solution and a few drops of 10% sodium hydroxide (NaOH) solution. Development of a pink to blood-red colour indicates the presence of cardiac glycosides containing an unsaturated lactone ring.

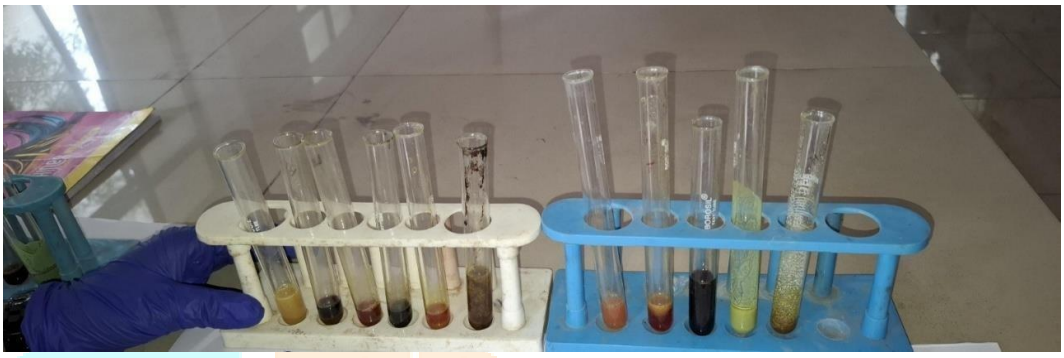


figure 4: observed phytochemical constituents in Nerium oleander flower extract

Thin Layer Chromatography

The phytochemical components of the Nerium oleander flower extract were separated and identified using thin layer chromatography (TLC). The stationary phase consisted of pre-coated silica gel 60 F254 plates. After Soxhlet extraction, the dried flower extract was dissolved in a small amount of methanol. 5–10 µl of the extract solution was carefully spotted 1 cm above the lower edge of the TLC plate using a capillary tube. The plate was placed in a developing chamber saturated with a mobile phase of toluene: ethyl acetate: formic acid (5:4:1, v/v/v), commonly used for separating plant secondary metabolites. The solvent ascended by capillary action until it reached about 8 cm from the origin. The plate was then removed, air-dried, and observed under UV light at 254 nm and 366 nm to visualize the separated compounds. Visible spots were marked, and retention factor (Rf) values were calculated using the formula:

Rf = Distance travelled by compound / Distance travelled by solvent

The number of spots, their color, and corresponding Rf values were recorded and compared to characterize the phytochemical profile of Nerium oleander flower extract. Thin layer chromatography serves as a rapid and reliable method for the preliminary identification and monitoring of plant constituents.

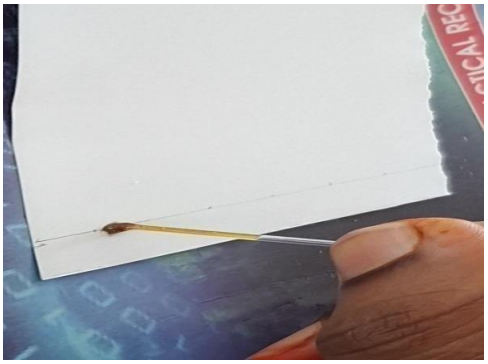


Figure 5: spotting the extract

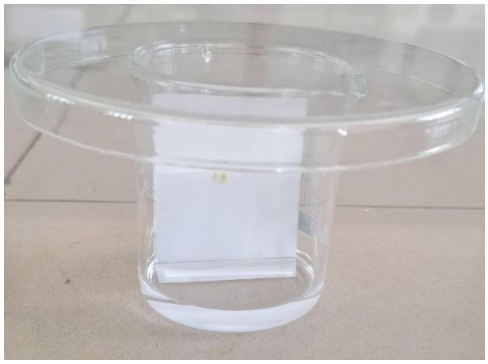
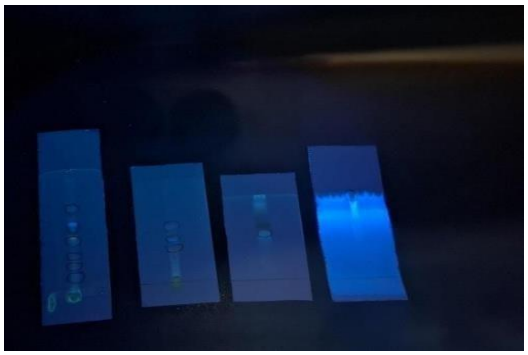


Figure 6: placing the TLC plate in mobile phase



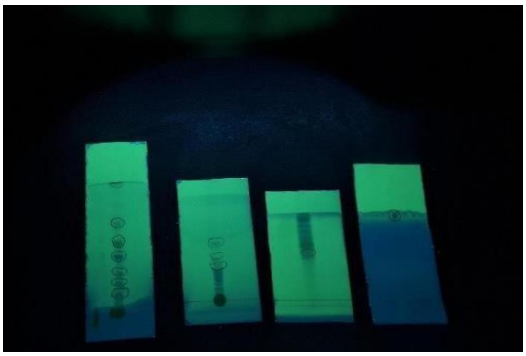


Figure 7 & 8: development of spots in TLC plate under the UV chamber

Antimicrobial Activity

The antimicrobial activity of the Nerium oleander flower extract was evaluated using the agar well diffusion method. Fresh flowers were collected, shade-dried, powdered, and extracted using ethanol in a Soxhlet apparatus. Before use, the concentrated extract was kept at 4°C. Test microorganisms included Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus.

Using a sterile cotton swab, the agar plates were prepared and evenly inoculated with the bacterial suspension. A sterile cork borer was used to create 6 mm diameter wells in the agar. The corresponding wells were filled with varying concentrations of the flower extract, namely 25, 50, and 100 mg/ml. The positive control was a common antibiotic, ciprofloxacin (5 µg/disc), while Tween-20 or the extraction solvent was used as the negative control.

To help the extract diffuse into the agar, the plates were left at room temperature for half an hour. The inoculated plates were then incubated at 37°C for a full day. Using a ruler or vernier caliper, the diameter of the zone of inhibition surrounding each well was measured to ascertain the antimicrobial activity following incubation. The experiment was conducted in triplicate, and the results were expressed as mean ± standard deviation. Greater antimicrobial activity of the flower extract against the tested microorganism was indicated by a larger zone of inhibition.

Figure 9: placing the TLC plate in mobile phase

Figure 10: sampling through micropipette

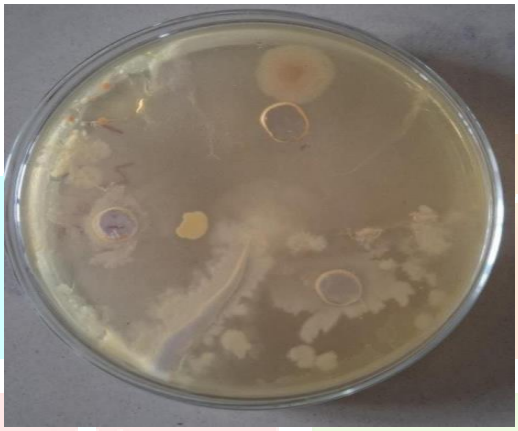


Figure 11: 25 mg/ml staphylococcus aureus

Figure 13: 100 mg/ml staphylococcus aureus

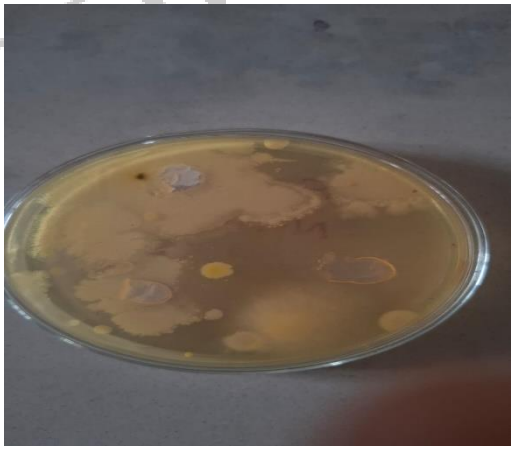


Figure 14: 100 mg/ml Escherichia coli

RESULTS

The observed results are tabulated below.

TABLE 1: PHYTOCHEMICAL SCREENING OF NERIUM OLEANDER FLOWER

Chemical tests	Observation	Result
Alkaloids – Dragendorff's test	Orange or red precipitate	Positive
Alkaloids – Wagner's test	Reddish-brown precipitate	Positive
Phenolics – Ferric chloride test	Green colour	Positive
Phenolics – Gelatin test	White precipitate	Positive
Flavonoids – Alkaline test	Yellow colour disappears	Positive
Flavonoids – Lead acetate test	Yellow precipitate	Positive
Saponins – Foam test	Persistent foam layer	Positive
Glycosides – Keller–Kiliani test	Brown ring	Positive
Glycosides – Legal's test	Pink to blood-red colour	Positive

TABLE 2: THIN-LAYER CHROMATOGRAPHY ANALYSIS OF NERIUM OLEANDER FLOWERS

Mobile phase	Stationary phase	Ratio	Detecting agent	Rf value
n-Butanol : Water : Acetic acid	Silica gel	4:5:1	Vanillin sulphuric acid	0.59 (Tannins)
Formic acid : Toluene : Ethyl acetate	Silica gel	1:4:5	Vanillin sulphuric acid	0.46 (Phenolics)
Glacial acetic acid : Formic acid : Ethyl acetate : Water	Silica gel	11:11:100:27	Vanillin sulphuric acid	0.73 (Flavonoids)

TABLE 3: ANTIMICROBIAL ACTIVITY OF NERIUM OLEANDER FLOWER (ZONE OF INHIBITION)

Concentration	Staphylococcus aureus	Escherichia coli	Plant extract	Ciprofloxacin (standard)
25 mg/ml	1 mm	0 mm	1 mm	2 mm
50 mg/ml	3 mm	1 mm	2 mm	4 mm
100 mg/ml	5 mm	4 mm	3 mm	5 mm

DISCUSSION

Alkaloids, phenolic compounds, flavonoids, saponins, and cardiac glycosides were among the significant secondary metabolites found in the initial phytochemical screening. These substances may contribute to the plant's therapeutic qualities, which are well known for their biological activities. The rich phytochemical composition of the flower extract was confirmed by the positive results obtained in the Dragendorff's, Wagner's, ferric chloride, gelatin, alkaline, lead acetate, foam, Keller–Kiliani, and Legal's tests.

The presence of several phytochemical constituents was further confirmed by thin layer chromatography (TLC) analysis. Distinct spots with Rf values of 0.38, 0.47, and 0.95 were produced by different solvent systems, indicating the separation of different compounds contained in the extract. For Nerium oleander flower extract identification, standardization, and quality control, the TLC fingerprint can serve as a helpful reference.

The flower extract showed inhibitory effects against Escherichia coli and Staphylococcus aureus, according to the antimicrobial activity study. A concentration-dependent antimicrobial effect was demonstrated, with the zone of inhibition growing as extract concentration increased. The extract exhibited the strongest antimicrobial activity at 100 mg/ml, indicating that the bioactive phytochemicals in the extract may work in concert to combat microbial pathogens. The results show that Nerium oleander flowers have promising antimicrobial qualities, even though the activity was less than or equal to that of ciprofloxacin.

CONCLUSION

The phytochemical characterization, TLC profiling, and antimicrobial activity of the methanolic extract of Nerium oleander flowers were successfully demonstrated in this study. The significant bioactive components, including alkaloids, flavonoids, phenolic compounds, saponins, and cardiac glycosides, were confirmed by preliminary phytochemical screening, suggesting the plant's rich medicinal potential. TLC analysis produced unique chromatographic spots with varying Rf values, confirming the existence of several phytochemical components and offering a distinct fingerprint profile for the extract's quality evaluation. The flower extract showed inhibitory activity against the tested bacterial strains according to the antimicrobial evaluation, and the zone of inhibition grew with increasing extract concentration. A concentration-dependent effect is suggested by the observation of the highest antimicrobial activity at 100 mg/ml. The results suggest that Nerium oleander flower extract is a promising source of natural antimicrobial agents. To investigate their therapeutic uses and determine the safety and effectiveness for pharmaceutical use, further research involving the isolation and structural characterization of the active compounds is advised.

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REFERENCES

[1] IJCRT2410059.pdf, available at: https://www.ijcrt.org/papers/IJCRT2410059IJCRT2410059.pdf
[2] Available at: https://www.uoanbar.edu.iq/catalog/file/lects/WomenEducationcollege/drOmarhamd/56.pdf
[3] Research Journal of Pharmacy and Technology, available at: https://share.google/s7VS3jE1LglO5dG4O
[4] Nerium oleander: its application in basic and applied science: a review, available at: https://share.google/BdeOTrS3FCxVXMpGh
[5] Available at: https://www.pnrjournal.com/index.php/home/article/view/4824
[6] Instrumental Thin-Layer Chromatography: Analysis of Plant Material. Elsevier, 2015.
[7] Kowalska T, Sajewicz M. Thin Layer Chromatography (TLC) screening of botanicals – its versatile potential and selected applications. Molecules. 2022;27(19):6607.
[8] Trease GE, Evans WC. Pharmacognosy. 16th ed. Saunders Elsevier; 2009.
[9] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 54th ed. Nirali Prakashan; 2014.
[10] Bhuvaneshwari L, Arthy E, Anitha C, Dhanabalan K, Meena M. Phytochemical analysis and antibacterial activity of Nerium oleander. Ancient Science of Life. 2007;26(4):24–28.
[11] El-Sawi NM, Geweely NS, Qusti S, Mohamed M, Kamel A. Cytotoxicity and antimicrobial activity of Nerium oleander extracts. Journal of Animal Research. 2010;37(1):25–31.
[12] Makia RSA. Antibacterial evaluation of Nerium oleander extract enhanced by titanium oxide nanoparticle. Al-Nahrain Journal of Science. 2018;20(2):25–30.