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Comprehensive Analysis of Anti-microbial Activity in *Nerium oleander* L. Latex Extracts

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***Abstract*:** This study explored the antibacterial and antifungal potential of *Nerium oleander* latex extracts. In antibacterial tests, *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited superior inhibition zones in acetone extracts, while *Bacillus subtilis* and *Proteus vulgaris* showed inhibition in diethyl ether extracts. The diethyl ether extract surpassed the standard drug tetracycline against *Proteus vulgaris*. All extracts demonstrated maximum inhibition at a 15% concentration. In antifungal evaluations against *Aspergillus niger, Fusarium oxysporium*, and *Penicillium sp,* acetone, chloroform, and diethyl ether extracts displayed significant inhibition zones, with concentrations correlating positively with inhibition size. This concentration-dependent relationship indicates *Nerium oleander* latex extracts' potential as a valuable resource for diverse phytopharmacological activities, suggesting promising applications in future disease treatments.

***Keywords*:** *Nerium oleander,* Latex, Antifungal, Antibacterial, Zone of inhibition

Since time immemorial, plants have been the source of medicine throughout the world and still continue to occupy an important place in traditional as well as modern systems of medicine. Even today, rural and tribal communities depend heavily on biodiversity for human and veterinary healthcare, using traditional knowledge is inherited orally from one generation to the other through trial and error methods (Sinha 1996). Plants are also an important source of the world's pharmaceuticals medicine, which are widely used in the treatment of various skin diseases. Ethno-botany is the study of the direct relationship between humans and plants (Adhikari et al., 2021). India, rich in medicinal plant diversity, offers numerous plant-based remedies that are integral to socio-cultural and spiritual practices (Mary, 2017). These plants are also a key source of pharmaceuticals, yielding compounds with antimicrobial, antibacterial, and antifungal properties (Tapsell 2006, Ghosh et al., 2007).

Nerium oleander L.**,** an evergreen shrub or small tree belongs to Apocynaceae family, is a vital medicinal plant in Indian folk medicine. Its flowers, leaves, bark, and latex contains secondary metabolites like flavonoids, alkaloids, steroids, and cardiac glycosides (e.g., oleandrin and neriine) with significant pharmacological applications. Traditionally, it has been used to treat heart conditions, asthma, epilepsy, cancer, malaria, menstrual pain, leprosy, indigestion, ringworm, and venereal diseases, as well as for inducing abortions. The roots possess healing properties for hemorrhoids and ulcers, while its flowers are used in tinctures. *Nerium oleander* exhibits antidiabetic, anti-inflammatory, hepatoprotective, cardiotonic, antioxidant, antibacterial, and antiviral properties (Yogeshwari et al., 2022). This study focuses on the antimicrobial properties of *Nerium oleander* L. against antibiotic-resistant microorganisms, contributing to advancements in natural product-based drug development.

MATERIAL AND METHODS

Collection and preparation of *Nerium oleander* latex extracts: The latex was obtained from *Nerium oleander* plant in the early morning using capillary action and carefully stored in two separate amber glass screw-cap bottles, each holding around 5 ml. The collected latex was subsequently preserved in a refrigerator at -40°C until required. Working solutions, consisting of acetone, chloroform, diethyl ether, and the plant latex, was prepared based on their respective solubility. Following this, a sequence of dilutions was carried out on the stock solutions, resulting in different concentrations (5%, 10%, and 15%).

Preliminary phytochemical analysis: The plant extracts were subjected to phytochemical analysis to detect the presence of alkaloids, flavonoids, tannins, resins, saponins, cardiac glycosides, and steroids (Okeke et al., 2005).

Collection of bacterial cultures: All the bacteria, i.e., *Staphylococcus aureus (*MTCC 7443), *Bacillus subtilis* (MTCC 121), *Proteus vulgaris*, and *Pseudomonas aeruginosa* (NCIM 2200) used for the detection of antibacterial activity were collected from the Department of Microbiology, Yuvaraja's College, Mysuru, and sub cultured on nutrient agar slants. The nutrient agar high medium was prepared as per the requirement according to the number of

plates, and the pH was adjusted to 7. The media were sterilized by autoclave at 121°C for 15 min. After sterilization, the nutrient agar medium was poured into sterile Petri plates under aseptic conditions and allowed for solidification.

Preparation of bacterial inoculums: Two ml volume of double-distilled water was placed in a sterile test tube, and a loop full of bacterial culture was suspended in it. Subsequently, one ml of this sample was inoculated into the agar medium and left for culturing.

Antibacterial activity: The bacterial subculture was evenly spread across the medium using an L-shaped glass rod. Various concentrations of different extracts (acetone, chloroform, and diethyl ether) were applied onto sterile discs with a diameter of 0.1 mm and positioned on the plates. Additionally, a preparation of latex extract, along with the standard drug tetracycline (50 mg/ml), was impregnated onto separate discs to serve as the standard for assessing antibacterial activity. Subsequently, the plates were incubated overnight at 37°C (Harborne 1973).

Antifungal assay: Fungal cultures, including *Aspergillus niger, Penicillium* sp, and *Fusarium oxysporum,* were acquired from the Department of Microbiology at Yuvaraja's College, Mysuru. These cultures were sub cultured in potato dextrose broth solution for subsequent applications. A potato dextrose agar high medium was formulated as per specifications and autoclaved at 121°C for 15 minutes. Following sterilization, the potato dextrose agar medium was carefully poured into sterile Petri plates under aseptic conditions and allowed to solidify.

Preparation of fungal inoculum: One ml aliquot of the fungal inoculum from the sub cultured broth solution is transferred to a sterile test tube, which is then adjusted to a total volume of two ml with sterile distilled water. The mixture is thoroughly shaken. Subsequently, one ml of this inoculum is applied to the plates for further inoculation.

Antifungal activity: The fungal subculture was evenly distributed across the medium using an L-shaped glass rod. The stock solutions, resulting in different concentrations (10%, 20% and 30%) were employed for the antifungal activity. Sterile discs with a diameter of 0.1 mm were impregnated with various concentrations of different extracts (acetone, chloroform, and diethyl ether) and positioned on the plates. Additionally, a preparation of latex extract, along with the standard drug Fluconazole (50 mg/ml), was impregnated onto separate discs to serve as the control for assessing antifungal activity. The treated plates were then incubated at 30°C for 24-48 hours. Each experiment was conducted in triplicate for accuracy and reliability.

RESULTS AND DISCUSSION

In the present study to assess antibacterial activity*of N. oleander* latex extracts were tested against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris,* and *Pseudomonas aeruginosa* at 5%, 10%, and 15 % of acetone, chloroform, and diethyl ether solvent extracts. All extracts exhibited maximum inhibition zones at a concentration of 15% (Table 1, Fig. 1). It was reported that diethyl ether extract of latex of *E. heterophylla* exhibited significant antibacterial activity against *S. aureus* and *P. aeruginosa* (Pruthvi et al., 2020). In present study acetone latex extract demonstrated the highest potential zone against *Staphylococcus aureus* compared to chloroform and diethyl ether extracts. Similarly, diethyl ether latex extract showed the highest potential against *Bacillus subtilis* compared to chloroform and acetone

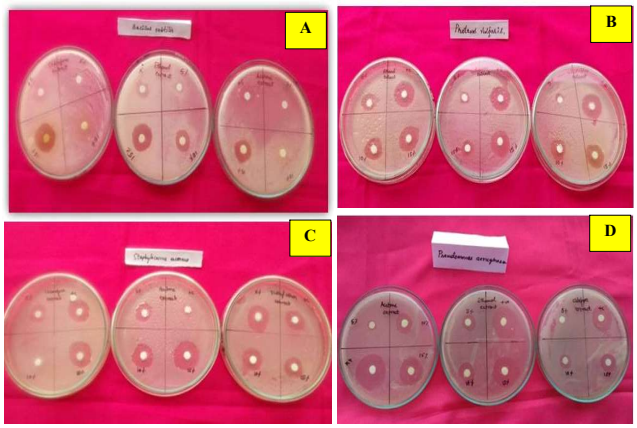

Fig. 1. Antibacterial activity of *N. oleander* latex extracts against (A) *Bacillus subtilis,* (B) *Proteus vulgaris,* (C) *Staphylococcus aureus and* (D) *Pseudomonas aeruginosa*

Table 1. Antibacterial activity of *Nerium oleander* latex extracts against four Bacterial strains

Bacterial strains	Acetone			Chloroform			Diethyl ether latex			+ve control (Tetracycline) 5%		
	5%	10%	15%	5%	10%	15%	5%	10%	15%			
Bacillus subtilis	0.0	0.0	6.0	0.0	0.0	8.0	0.0	6.0	9.0	6.0	10.0	4.0
Proteus vulgaris	2.0	4.0	10.0	3.0	6.0	8.0	6.0	7.0	12.0	7.0	8.0	8.0
Staphylococcus aureus	7.0	8.0	13.0	0.0	4.0	9.0	5.0	8.0	11.0	12.0	11.0	12.0
Pseudomonas aeruginosa	4.0	7.0	10.0	0.0	3.0	6.0	3.0	6.0	10.0	14.0	9.0	12.0

Table 2. Antifungal activity of *Nerium oleander* latex extracts against three fungal strains

Bacterial strains	Acetone latex extract			Chloroform latex extract			Diethyl ether latex extract			+ve control (Fluonozole) 5%		
	5%	10%	15%	5%	10%	15%	5%	10%	15%			
Aspergillus niger	2.0	4.0	7.0	3.0	5.0	7.0	3.0	4.0	8.0	10.0	12.0	12.0
Fusarium oxysporum	4.0	6.0	8.0	3.0	5.0	6.0	2.0	4.0	7.0	10.0	9.0	6.0
Penicillium sp.,	3.0	4.0	6.0	0.0	3.0	5.0	0.0	4.0	6.0	9.0	6.0	7.0

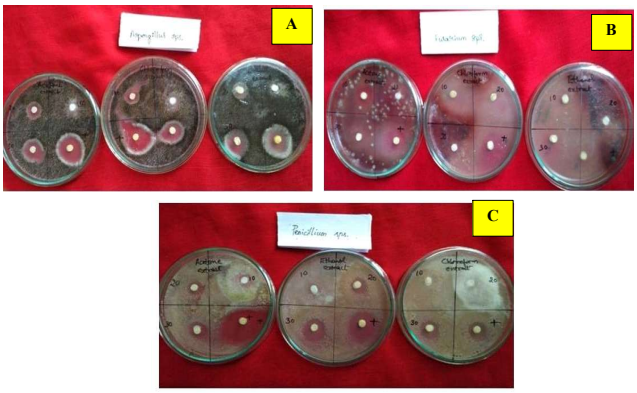

Fig. 2. Antifungal activity of *N. oleander* latex extracts against (A) *Aspergillus niger,* (B) *Fusarium oxysporum* and (C) *Penicillium* sp.

extracts. The acetone, chloroform, and diethyl ether latex extracts displayed consistent inhibition zones, diethyl ether latex extract exhibited the highest inhibition zone against *Proteus vulgaris*. The diethyl ether extract displayed a remarkable inhibition zone compared to the standard drug tetracycline used as a control. Similar potential zones were observed in diethyl ether and acetone latex extracts against *Pseudomonas aeruginosa* compared to chloroform latex extract. At concentrations of 900 mg/ml and 500 mg/ml, *Nerium oleander* exhibited inhibition zones of 22 mm and 13 mm, respectively, against *Staphylococcus aureus* (Wong et al., 2013). Notably, a remarkable zone of inhibition was observed in the diethyl ether extract when compared to the standard drug tetracycline, used as a control, particularly in tests against *Proteus vulgaris.*

The antifungal efficacy of *N. oleander* latex was assessed using the disc diffusion method against various fungal strains. The plant extracts exhibited substantial zones of inhibition against *Aspergillus niger, Fusarium oxysporium*, and *Penicillium sp*. (Table 2, Fig. 2). The acetone, chloroform, and diethyl ether *N. oleander* latex extracts demonstrated significant zones of inhibition against *Aspergillus niger*. The zone of inhibition increased proportionally with the concentration of the latex extract, indicating a concentration-dependent effect. Acetone latex extract exhibited a particularly high potential zone of inhibition. When tested against *Fusarium oxysporium*, an increase in the zone of inhibition was observed with a higher concentration of *N.oleander* latex extract in all three solvent, acetone extract displayed a higher potential zone of inhibition compared to chloroform and diethyl ether extracts. The broad spectrum of antibacterial and antifungal activities demonstrated by *N. oleander* latex suggests the potential discovery of new chemical classes of antibiotic substances. These findings could contribute to the development of alternatives or second-line treatments for infectious diseases and their control.

CONCLUSION

The antimicrobial activity assessment demonstrates the ability of *Nerium oleander* latex to inhibit the growth of bacteria and fungi. These findings collectively provide valuable insights into the potential therapeutic applications of *N. oleander* latex, suggesting its possible use in the development of antimicrobial agents for treating various diseases. Further research and clinical studies would be essential to explore and validate its efficacy and safety in practical therapeutic applications.

AUTHORS' CONTRIBUTIONS

Dr. Sahaya Mary and Kavitha RS authored the research and contributed to the preparation of the manuscript. Dr. Mahesh provided guidance on the manuscript structure and contributed to the development of the methodology

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