

## STUDY OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *NERIUM OLEANDER FLOWER* EXTRACT AND ITS PHYTOCHEMICAL SCREENING

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### ABSTRACT

*Nerium oleander* belongs to family Apocyanaceae. The objective of the present work was to identify the phytochemical constituents and to study of antibacterial and antifungal activity of extract of *Nerium oleander* flower. Recently, natural products have been evaluated as sources of antimicrobial and antifungal agents with efficacies against a variety of microorganisms. Present study was designed to evaluate the antibacterial and antifungal activity of *Nerium oleander* flower against human pathogens. The extract was prepared using ethanol. Antimicrobial activity was tested against one gram positive bacteria and three gram negative bacteria while antifungal activity was tested against two fungi. The various concentrations (30, 60, 100mg/ml) of the ethanolic extract were tested. Evaluations were based on the zone of inhibition using Agar well diffusion assay. The inhibitory activity

was found to be dose dependent. This study represents that ethanol extracts of flower of *Nerium oleander* may be utilize as a potential source of antimicrobial and antifungal agents. Ciprofloxacin (10µg/ml) used as standard for antibacterial activity while fluconazole (10µg/ml) used as standard for antifungal activity. The phytochemical investigation showed the presence of active chemical constituents such as alkaloids, tannins, flavanoids, steroids, cardiac glycosides and terpenoids, carbohydrates, amino acids, proteins, triterpenoids.

**KEYWORDS:** Nerium oleander, Antibacterial, Antifungal, Phytochemicals, Extraction.

## INTRODUCTION

Since ancient times herbal drugs are used for the treatment of infectious diseases in human without any side effects. To eradicate the side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient peoples used.<sup>[1]</sup> Herbal medicine also known as botanical medicine or phytomedicine refers to using plants seeds, flowers, roots for medicinal purpose. Herbals have a long tradition of use of outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease.<sup>[2]</sup> In this way to create the scientific evidence for the natural *Nerium oleander* selected for the antibacterial and antifungal activity and taken for Phytochemical screening. *Nerium oleander* belonging to family *Apocyanaceae* is an evergreen, shrub or small tree with white and red flowers both possess similar properties. The leaves, bark and flowers are majorly used for the treatment of various diseases and functional disorders. The leaves are used for cardiovascular diseases, as well as for skin diseases. The leaves and roots have a number of active constituents including glycosides, terpenoids, steroids and other compounds. It is an important herbal drug used as Anti inflammatory, Antioxidant, Antibacterial, Anti fungal<sup>[3]</sup>, Anticonvulsant, Antidiabetic, Anticancer<sup>[4]</sup>, Anxiolytic<sup>[5]</sup>.

The phytochemical constituents were studied by qualitative analysis for performing various chemical tests. Although many studies have reported the antibacterial activity of *Nerium oleander* but it did not revealed enough studies about its effect on bacterial resistance and did not determined the most effective part of the plant in dealing with bacteria, whether flowers, leaves, stem of the *Nerium oleander*. The objective of present study were to evaluate the antibacterial and antifungal activity of ethanolic extract of *Nerium oleander* flower on selected bacterial gram positive and gram negative and fungal cultures.

## MATERIAL AND METHOD

### Collection of Plant Material

The *Nerium oleander* (*Apocynaceae*) flowers was collected from the city Nandani, Maharashtra, India 416102. The sample is identified and authenticated by Dr. (Miss.) K. R. Datar, Head Dept. of Botany, Deccan Education Society, Pune Willingdon College, Sangli. Collected material is subjected to drying at room temperature for about a week in open air.

This air dried material was grind into powdered and stored under refrigeration until their further utilization.

### Preparation of Extracts

The extract of *Nerium oleander* flower was prepared in 6% concentration (6gm of weighed *Nerium oleander* flower powder and 100ml of solvent). While soxhlet extraction was carried out using solvent ethanol. The filtrates were evaporated to get concentrated extract. The extract was stored at 4°C.<sup>[6,7,8]</sup>

### Assessment of Antimicrobial and Antifungal Activity

The screening of ethanolic extraction of flower of *Nerium oleander* was carried out using agar well diffusion method. The bacterial and fungal strains for the study were obtained from Govt. Medical college, (Microbiology and bacteriology department) Miraj. The Gram negative bacterial strains used for study are *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and Gram positive *Staphylococcus aureus*. Fungal strains used in the study are *Candida albicans* and *Aspergillus flavus*.

From bacterial and fungal cultured slants, several colonies were transferred to 5ml of sterile distilled water. It is mixed for some seconds to ensure homogeneity and further diluted to match the turbidity with 0.5 McFarland standard solution which is roughly equivalent to 150 million cells per mL. (Corresponding to  $1.5 \times 10^8$ CFU/ml).<sup>[9,10]</sup>

*Nerium oleander* flower ethanolic extract was prepared at 30mg/ml, 60mg/ml & 100mg/ml concentrations in sterile water. Ciprofloxacin 10µg/ml and fluconazol 10µg/ml were taken as standard for antibacterial and antifungal activity respectively.

Nutrient agar was prepared as bacterial media and Sabouraud Dextrose agar is prepared as fungal media and sterilized.<sup>[11]</sup> All glassware's, borer, petri plates, extract dilutions were sterilized in autoclave. In aseptic technique, using sterile swab a bacterial lawn is made on sterile petri plates from microbial inoculums suspension. Swab is made in one direction by rotating plate at 90°.

An agar-well diffusion method was employed for determination of antibacterial and antifungal activities.<sup>[12]</sup> Wells (4.6mm in diameter) were cut from the agar with a sterile borer and 60µL extract solutions were delivered into them. Sterile water is kept as control. The inoculated plates were incubated at 37°C for 24 h. Antibacterial and antifungal activity was

evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. The inhibitory DIZ was expressed in millimetres. All tests were performed in triplicates. After 24 hours the plates was examined for zone of inhibition.

### PHYTOCHEMICAL SCREENING

Phytochemical analysis of the extract was carried out using various procedures describe by various authors.<sup>[13,14]</sup> Phytochemical screening was performed to detect the presence of several phytochemicals like Alkaloids, Flavonoids, Steroids, Saponins, Cardiac glycoside, Tannins, Terpenoids and free Amino acid etc. “Table 1”

**Table 1. Phytochemical Analysis of *Nerium oleander* flower extract.**

Sr. No.	Test For	Chemical Tests	Peel Ethanolic extract
01	Test for alkaloids	A. Hager's test	Positive
		B. Wagner's test	Positive
		C. Dragandroffs test	Positive
02	Test for flavanoids	A. Lead acetate test	Positive
03	Test for saponins	A. Foam test	Negative
04	Test for steroids	A. Salkowski test	Positive
05	Test for cardiac glycosides	A. Keller-killiani test	Positive
		B. Legal's test	Positive
06	Test for tannins	A. 5% w/v FeCl <sub>3</sub> solution	Positive
		B. Acetic acid solution.	Positive
		C. Dil. KmnO <sub>4</sub> solution.	Positive
07	Test for amino acids	A. Ninhydrine test	Positive
08	Test for carbohydrates	A. Molisch's test	Positive
		B. Fehling's test	Positive
		C. Benedict's test	Positive
09	Test for anthraquinone glycosides	A. Borntragers test	Positive
10	Test for proteins	A. Tri chloro acetic acid test	Positive
11	Test for amino acids	A. Ninhydrine test	Positive

### RESULT AND DISCUSSION

The qualitative phytochemical investigation of aqueous and ethanolic extracts of flower of *Nerium oleander* showed the presence of active chemical constituents such as Carbohydrates, Alkaloids, Flavonoids, Steroids, Cardiac Glycosides, Anthraquinone Glycosides, Proteins, Amino Acids, Triterpenoids and Tannins. Absence of phytochemical such as Saponins (Table 1). Ethanolic extract shows significant antibacterial and antifungal activity towards clinically significant microbes “Table 2 and 3”.

Nearly 80% of the world populations depend on the traditional medicine for primary health care, mainly including the use of natural products. Researchers have extensively studied the

biological properties of *Nerium oleander* and their results showed that this plant is ethno medically valuable.

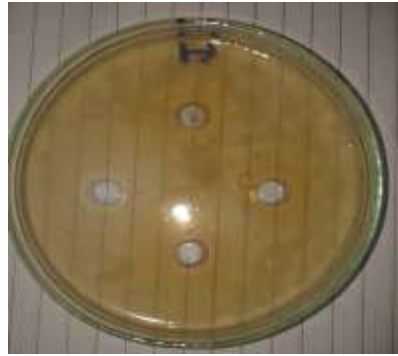
The study shows the pharmacological importance of flower of *Nerium oleander* showing antimicrobial and antifungal activity and thus substantiates traditional medicinal use. The separation and further activity mediated approach was emphasize to conduct in future to demonstrate active phytochemicals to be utilize as lead compounds for antimicrobials. Thus, the study provides a strong direction for proper investigation of various plants to explore molecules having antimicrobial and antifungal properties against human pathogens.

**Table 2: Dose Dependent Antimicrobial Activity of Ehanolic Extract of *Nerium oleander* Flower.**

Sr. No.	Name of Pathogens	Extract Conc. In mg/ml	Zone of Inhibition in mm of diameter	Standard Ciprofloxacin 10µg/ml Zone of Inhibition in mm of diameter
01	Escherichia coli	30	25	25
		60	30	
		100	33	
02	Proteus vulgaris	30	20	19
		60	24	
		100	28	
03	Pseudomonas aeruginosa	30	12	18
		60	14	
		100	18	
04	Staphylococcus aureus	30	19	17
		60	23	
		100	27	

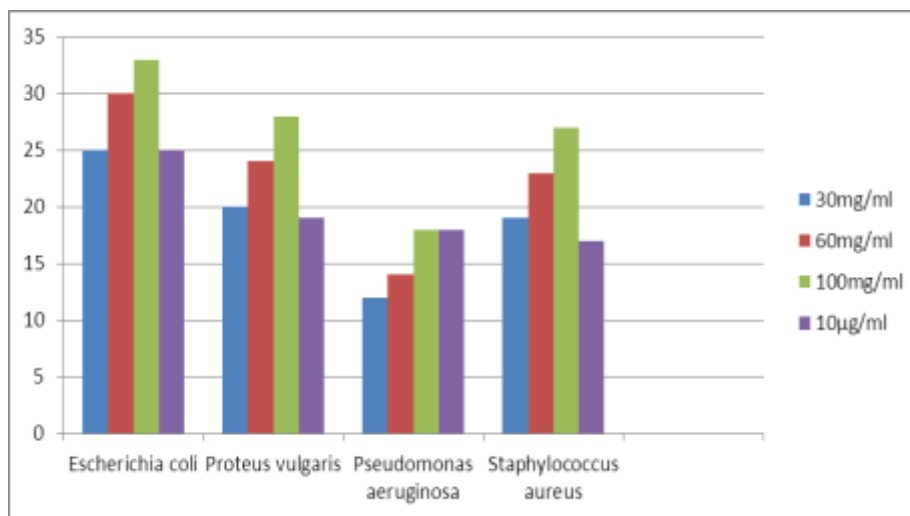
**Table 3: Dose Dependent Antifungal Activity of Ethanolic Extract of *Nerium oleander* Flower.**

Sr. No.	Name of Pathogens	Extract Conc. In mg/ml	Zone of Inhibition in mm of diameter	Standard Fluconazol 10µg/ml Zone of Inhibition in mm of diameter
01	Candida albicans	30	12	09
		60	14	
		100	17	
02	Aspergillus flavus	30	12	10
		60	16	
		100	19	



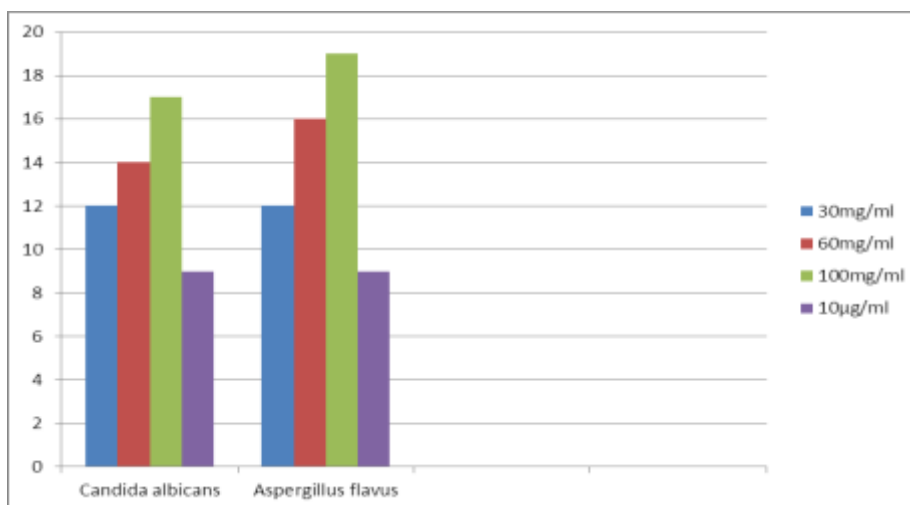
**E. COLI.**

**Antimicrobial Activity of Ehanolic Extract of *Nerium Oleander* Flower and Standard Drug.**



**CONCENTRATION IN MG/ML.**

**Antifungal Activity of Ehanolic Extract of *Nerium Oleander* Flower and Standard Drug.**



**CONCENTRATION IN MG/ML.**

## CONCLUSION

In the present study an attempt has been made to make out the antimicrobial and antifungal activity of ethanolic extract of flower of *Nerium oleander*. The extract has antibacterial activity against bacterial strains (*E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. aureus*) and shows more potency than that of standard ciprofloxacin. The extract also has antifungal activity against fungal strains (*Candida albicans* and *Aspergillus flavus*) and shows more potency as that of standard fluconazol. After further purification and characterization of the active metabolites present in flower of *Nerium oleander* followed by a detailed study of toxicity and pharmacological effects of the compound, the flower extracts of *Nerium oleander* may be used as remedy against various diseases without any side effects and the plant species can be a good pharmacophore source in future.

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