



## STUDIES ON NERIU OLEANDER LEAF EXTRACT FOR ANTIMICROBIAL ACTIVITY FROM SOIL MICROFAUNA IN FAIZPUR AREA OF NORTH MAHARASHTRA REGION (M.S.) INDIA

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### ABSTRACT

The study was carried out on antibacterial activity of essential oils obtain from Nerium oleander leaf with the help of extract like ethanol. The aqueous solution of leaf extract confirms the presence of phytochemicals like standard ciprofloxacin. Evaluation for antibacterial activity were determine by disc diffusion method. The leaf extract was tested in specific concentration i.e. 25, 50 and 100mg/ml against bacterial strains. In the present investigation, we observed zone of inhibition which shows, extract has antibacterial activity against tested bacterial strains i.e. E. coli, P. aeruginosa, S. aureus and shows more effectiveness than that of standard ciprofloxacin. The inhibitory activity was found to be dose dependent. This study may contribute to the development of standardization parameters of the plant, which helps in the botanical identification of Nerium oleander that extracts may be develop as a possible source of antimicrobial agents.

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

### INTRODUCTION

Nerium oleander is evergreen shrub of Apocynaceae family normally known as kaner that grows up to 5 m. in height, the leaves are long, simple, whorled, linear-lance late, 9-14 cm in length with horizontal nerves, leathery, narrowly elliptic to linear entire. Since ancient times herbal treatments are used for the treatment of infectious diseases in human without any side effects. To eliminate the side effects of the present allopathic drugs now scientists are moving towards the herbal drugs what our ancient peoples used, Kokate, et. al., 2015. Herbal medicine refers to using plants seeds, leaves, roots for remedial persistence. The flowers grow in clusters at the end of each branch. Nerium fruit is long 15-20 cms narrow parallel lines, cylindrical, deep, longitudinal, ridges and paired growing with the stem. Herbals have a long ritual of use of outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along through developments in clinical research show the value of herbal remedy in the treating and preventing disease. The leaves are used for cardiovascular diseases, as well as for skin problems. The leaves and roots have several active ingredients with glycosides, steroids and other compounds. It is an important herbal drug used as Anti-inflammatory, Antioxidant, Antibacterial.

The objective of present study was to evaluate the antibacterial activity of extract of Nerium oleander leaf on bacterial gram positive and gram negative cultures under taken to investigate to phytochemical analysis of the plant. There are numbers of secondary metabolic compounds found in plants which may protect humans from various diseases. Phytochemicals are plant chemicals that have non-nutritive but have disease protective or preventive properties. We observed that in all parts of the plants, but are most concentrated in the Sap. Many of oleander's relatives have similar leaves and contains toxic compounds. Oleander contain many other unknown or un-researched compounds that may have dangerous effects to the entire plant including the milky white sap which is toxic and any part can cause an adverse reaction. Oleander is also known to hold its toxicity even after drying, Goktas et.al.,2003. Many researchers have studied oleander species on different activities such as, Murphy, 1990; Nair and Burke,1990; Digrak et.al. 1999; Sen et.al. 2002; Khare, 2004; Garima Zibbu and Amla Batra, 2010; Suganya et.al., 2012; Shinde et.al., 2012; Anmar Saadi Aboud, 2015.

### MATERIAL AND METHOD

**Collection of Plant Material:** The Nerium oleander (Apocyanaceae) leaves was collected from the village Faizpur, Maharashtra, India.

The collected material was dried 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.

**Screening procedure for Preparation of Extracts:** Ethanol leaf extract of Nerium oleander were screened for the presence of phytochemical compound. The extract of Nerium oleander leaf was prepared in 6% concentration (6gm of weighed Nerium oleander leaf powder and 100ml of solvent). While extraction was carried out using solvent ethanol. The filtrates were evaporated to get concentrated extract. Stalk was prepared by captivating 2mg of the extract was taken and diluted to 20ml with appropriate solvent and used as standard. The extract was stored at 40C. Rafi Khan P et al., 2011.

**Antibacterial effect of pant extract:** The antibacterial activity of Nerium oleander plant extract was evaluated by disc diffusion method Chung et.al. 1990. Nutrient agar medium was prepared and poured into the petri-plates and allow to solidify. Then it was inoculated with the swab of culture and spread throughout the medium uniformly with sterile cotton swab. A sterile filter paper disc was prepared and dipped with plant leaf extract (Ethanol) at different concentrations of 25, 50 and 100mg/ml and then placed on the surface of agar plates. All these plates were incubated at 370C for 24hrs. The antagonistic test was performed in triplicates and their efficiency was determined by measuring the diameter of zone of inhibition around well as per Plate-I and II.

**Assessment of Antimicrobial and Activity:** The transmission of ethanolic extraction of leaf of Nerium oleander was carried out with agar well-diffusion method. The bacterial strains for the study were obtained from Dhanaji Nana Mahavidyala, (Microbiology and biotechnology department) Faizpur. The Gram negative bacterial strains used for study are Escherichia coli, Pseudomonas aeruginosa and Gram Positive Staphylococcus aureus. From bacterial cultured slants, several colonies were transferred to 5ml of sterile distilled water. Nerium oleander leaf ethanolic extract was prepared at 25mg/ml, 50mg/ml & 100mg/ml concentrations in sterile water. Ciprofloxacin 10 g/ml was taken as standard for antibacterial activity respectively. Nutrient agar was prepared as bacterial media sterilized. (Tannu et. al., 2011) All glass wares, 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 activities. Sterile water

is kept as control. The inoculated plates were incubated at 37°C for 24 Hrs. Antibacterial action was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. The inhibitory DIZ was expressed in millimeters. All tests were performed in triplicates. After 24 hours the plates were examined for zone of inhibition.

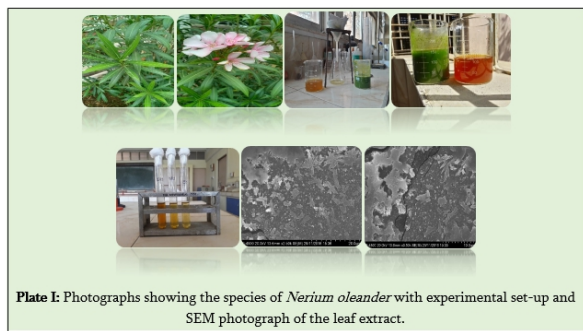


Plate I: Photographs showing the species of *Nerium oleander* with experimental set-up and SEM photograph of the leaf extract.

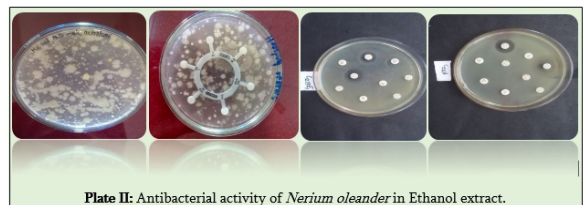
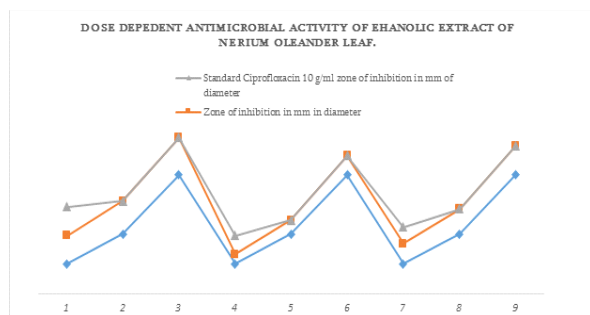


Plate II: Antibacterial activity of *Nerium oleander* in Ethanol extract.

Table 1: Dose dependent Antimicrobial Activity of Ethanolic Extract of *Nerium oleander* leaf.

Sr. No.	Name of Pathogens	Extract conc. In mg/ml	Zone of inhibition in mm in diameter	Standard Ciprofloxacin 10g/ml zone of inhibition in mm of diameter
01	Escherichia Coli	25	24	24
		50	28	
		100	31	
02	Pseudomonas aeruginosa	25	08	16
		50	12	
		100	16	
03	Staphylococcus aureus	25	17	14
		50	21	
		100	24	

Graph 1: Dose dependent Antimicrobial Activity of Ethanolic Extract of *Nerium oleander* leaf.



## RESULT AND DISCUSSION

Oleander contains tremendous immune boosting action with inhibits angiogenesis, inhibits NF- $\kappa$ B factor in cancer cells, causes apoptosis in cancerous cells (natural cell death) and causes and increased rates of autophagic cancer cell death when tested on pancreatic cancer. Plant is used as a rat poison and an insecticide Kirtikar and Bassu, 1999. Pounded leaves and bark are used as an insecticide. Green dye is obtained from the flowers and the plant is commonly used for informal heading in the Mediterranean. Leaves

contain small amount of latex that can be used to make rubber, though the amount is too small for commercial utilization. Plant has an extensive root system and are often used to stabilize soil in warmer areas.

Recently it is noted that the possible health benefits of heavily diluted oleander i.e. helping with muscle cramps, natural support for asthma, epilepsy and paralysis, help for skin conditions such as eczema, powerful all natural organic insecticide, usefulness in aiding heart conditions, natural support for healthy blood sugar levels, possible support for the HIV virus, usefulness in increasing healing times for wounds, when crushed and applied topically, powerful immune-system stimulator, promoting normal menstrual cycles, aiding the body in the fight against arthritis, psoriasis & hepatitis C. Ethanolic soluble extractive values were found to be greater than ciprofloxacin as presented in graph 1. The investigation of ethanolic extracts of leaf *Nerium oleander* ethanolic extract shows significant antibacterial activity towards clinically significant microbes as shown in Table 1. Cases have been noted of poisoning and death due to intake of oleander leaf, tea and its extracts even eating honey made from the nectar of the plant can produce dangerous effects, Jacob L. et. al., 2009. One raw leaf has enough poison in it to kill a small child, as well as any pet, Pratt and Chase, 1949; Muller, 1991; Shankar et.al. 2004; Lokesh et.al. 2010; Makuvebthan and Murugesan, 2010; Shanti et.al. 2011; Tannu et. al. 2011 and Anmar Saadi About, 2015. The study shows with the help of SEM photographs and Table no. 1 that the pharmacological importance of leaf of *Nerium oleander* antimicrobial activity and thus substantiates traditional medicinal use. The separation and further activity mediated approach was emphasizing to conduct in future to demonstrate active phytochemicals to be apply as lead compounds for antimicrobials. Thus, the research offers a strong direction for proper investigation of various plants to explore molecules having antimicrobial properties against human pathogens.

## CONCLUSION

People are using herbal medicines from centuries for safety, efficacy, culture acceptability and lesser side effects. An attempt has been made to make out the antimicrobial activity of ethanolic extract of leaf of *Nerium oleander*. The extract has antibacterial activity against bacterial strains i.e. *E. coli*, *P. aeruginosa*, *S. aureus* and shows more effectiveness than that of standard ciprofloxacin. After further purification and characterization of the active metabolites existing in leaf of *Nerium oleander* followed by a complete study of toxicity and pharmacological effects of the compound, we conclude that the leaf extracts of *Nerium oleander* may be used as remedy against many diseases deprived of any side effects and the plant species can be a good pharmacophore source in future.

## ACKNOWLEDGMENT

We are thankful to Rajiv Gandhi National Fellowship (RGNF) for the funding of this research, basically this research paper is based on initial research work done in Faizpur area of Khandesh region. I am also thankful to the Director and head of the school for providing necessary facilities to carry out this research work.

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