

ANTIBACTERIAL ACTIVITY OF *NERIUM INDICUM* AGAINST SOME GRAM POSITIVE BACTERIAL SPECIES

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ABSTRACT

Phytochemicals presents in plants probably explain the various uses of plants for traditional medicine. In this study *Nerium indicum* leaves was selected for assessing antimicrobial activity against different bacterial species. Leaves were collected and methanolic, chloroform and hexane extracts were prepared by using soxhlet apparatus. Then the antibacterial activity of these plant extracts was studied against *Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium perfringens* and *Streptococcus mutans*. According to results methanolic extract was most effective then chloroform or hexane extract.

Keywords: Microbiostatic, Extract, Antibacterial activity, Phytochemicals.

INTRODUCTION

Plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.⁴ Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Many of the herbs and spices used by humans to season food yield useful medicinal compounds.⁷ Phytochemicals are protective and diseases-preventing metabolites produced by plants. The presence of these secondary metabolites in plants probably explains the various medicinal & antioxidant activities of these plants.⁵ *Nerium indicum* is a plant that was used for this study. This plant belongs to Apocynaceae Antibiotics are sometimes associated with adverse effects on hosts which include hypersensitivity, depletion of beneficial gut, mucosal microorganisms, immuno suppression and allergic reactions.¹ Bacteria have the genetic ability to transmit and acquire resistance to drugs.⁶ Essential oils and extracts of

certain plants have been shown to have antimicrobial effects, as well as imparting flavor to foods.³ The *Nerium indicum* extracts decrease the microbial growth, this suggests that it is, having microbiostatic effects. This study mainly concerned with the study of antibacterial activity of *Nerium indicum* against some Gram positive bacteria.

MATERIALS AND METHODS

Collection of the Sample

Nerium indicum leaves were collected from college botanical garden.

Preparation of the Sample

The leaves selected for the sample after collection were washed under tap water. After washing they were left to dry under shade for one week. After the drying process leaves were crushed in pestle mortar to fine powder.

Preparation of Extraction

Extraction is a process whereby the desired constituents of a plant are removed using a

solvent. The primary ways for extraction of organic molecules of interest to biologists and medical investigators involve breaking open the cells. We used soxhlet extractor for the extraction in different solvents (methanol, chloroform, n-hexane).

Soxhlet Extraction

It is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. It is required where the desired compound has limited solubility in a solvent and the impurity is insoluble in that solvent. We took dried leaves powder of neem in small pouches of glass wool and loaded into main chamber of soxhlet extractor. The soxhlet extractor was placed onto a flask containing the extraction solvent. The soxhlet was then equipped with a condenser. The temperature of distillation port was set to boiling point of the solvent used. Repeated cycles were allowed till the colored extraction mixture changes to colorless. Then the liquid extract was evaporated using water bath to get dried extract, the extract was weighed and dissolved in that solvent. Then the herbal extracts were used to check their antimicrobial activity.

Microbial Culture

Cultures of five microbial strains each of *Bacillus subtilis* (MTCC 61), *Staphylococcus aureus* (MTCC62), *Clostridium perfringens* (MTCC 69), *Streptococcus mutans* (MTCC 70) and *Candida albicans* (MTCC 79) microbial cultures were maintained in Nutrient broth. Purification of cultures was done by streaking method. Colonies were picked from petriplates and inoculated into the sterilized broth present in flask. Then the cultures were incubated at 37°C for 24 hours.

Antimicrobial Agents Susceptibility Test

Susceptibility to antimicrobial agents was determined by well diffusion method of Kirby Bauer on Muller Hinton agar as described by Clinical and Laboratory Standard Institute (CLSI, 2009). Plates of sterilized Muller Hinton agar were prepared and kept in incubator for 24 hours to check any contamination. After that cultures

were inoculated by spreading inoculums over the entire surface of Muller Hinton agar plates in the laminar air flow. The surface was allowed to dry for 3-5 minutes before the wells were created on the plates of Muller Hinton agar with a sterile Cork borer. Extracts used for susceptibility tests were of different concentrations. Then plates (in duplicates) were incubated at 37°C for 24 hours and zone of inhibition was measured. Each and every herbal extract was assayed for antimicrobial activity.

RESULTS AND DISCUSSION

In the present study, antibacterial activity of plant extracts were studied by agar well diffusion method and diameters zone inhibition was measured and Results are presented in Table 1.

The present study shows that methanolic extract was most effective then other two extracts. Methanolic extract shows a maximum of 19mm zone against staphylococcus aureus while hexane extract was least effective against these bacterial species Bhuvaneshvari *et al.*, (2007) studied the Phytochemical analysis & Antibacterial activity of *Nerium oleander*, *Staphylococcus aureus*, showed zone of inhibition which is 10mm in our study it was 19 mm.

CONCLUSION

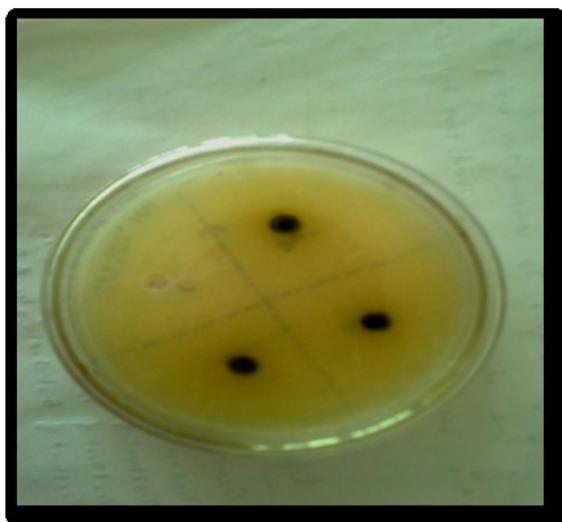
All the extracts displayed broad spectrum of activity against gram +ve bacteria and fungus. The *Nerium indicum* extracts decrease the microbial growth, this suggests that it is, having microbiostatic effects. The results obtained are encouraging as the methanolic, chloroform, hexane extracts have shown considerable antimicrobial activity. The activity of the plant is appreciable considering the importance of microorganisms, further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies.

ACKNOWLEDGEMENT

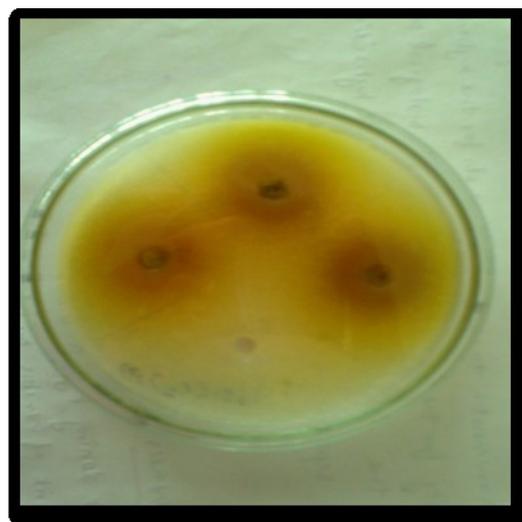
We are extremely thankful to Dr. Monika Dept. of Biotechnology Mata Gujri College for providing us such a good Research Lab and equipments for completing this work.

Table 1: Showing antibacterial activity of plant extracts

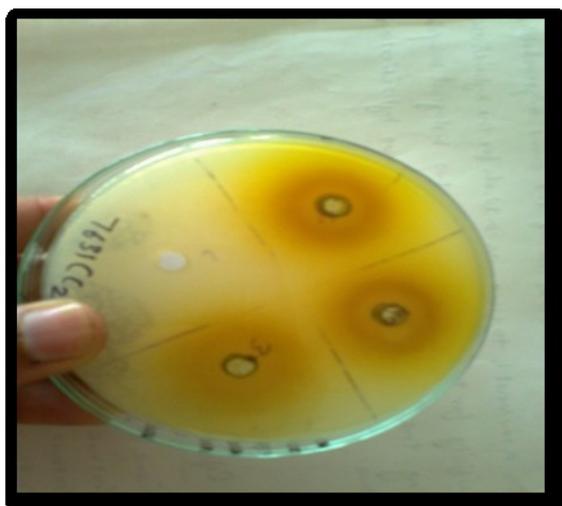
Extracts 20mg/ml	Bacterial species				
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Clostridium perfringens</i>	<i>Streptococcus mutans</i>	Control
Methanol Extract	12mm	19mm	16mm	15mm	4mm
Chloroform Extract	17mm	15mm	15mm	16mm	2mm
Hexane Extract	11mm	15mm	12mm	14mm	-



Antibacterial activity against Clostridium perfringens



Antibacterial activity against Streptococcus mutans



Antibacterial activity against Bacillus subtilis



Antibacterial activity against Staphylococcus aureus

Pictures: Showing antibacterial activity of *Nerium indicum*

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Cite This Article: Shashi, Chauhan; Manjeet, Singh; Amit, Thakur and Manjeet Singh, Dogra (2013), "Antibacterial Activity of *Nerium Indicum* against Some Gram Positive Bacterial Species", *Int. J. Drug Res. Tech.*, Vol. 3 (1), 8-11.