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ANTIMICROBIAL ACTIVITY OF *NERIUM OLEANDER* L. AND *NICOTIANA TABACUM* L.: A COMPARATIVE STUDY

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Abstract

The antimicrobial activity of aqueous ethanolic extract of two plant species i.e. *Nerium oleander* L. and *Nicotiana tabacum* L. from Multan district were checked, against three pathogenic bacteria viz: *Staphylococcus aureus* (gram positive), *Escherichia coli* and *Pseudomonas aeruginosa;* (gram negative). Disc diffusion technique was used to check the antimicrobial activity. The bacterial strains under question were found susceptible to plant extracts. *Nerium oleander* and *Nicotiana tabacum* exhibited strong antibacterial action against tested bacteria. The ethanolic extract of *Nerium oleander* leaves showed highest antibacterial action against *Pseudomonas aeruginosa* at 900mg/ml concentration, whereas, *Nicotiana tabacum* showed maximum zones of inhibition against *Staphylococcus aureus* at 900mg/ml concentration. The results showed that the leaves extracts of these plants have great power as anti-microbial agents against these common bacterial isolates. However, for future pharmacological implication of these plants more research is needed for the isolation of active ingredient of these plants. The results obtained could be attributed to the differential environmental conditions on the ecology of these plants.

Key words: Antimicrobial effect, *Nerium oleander*, *Nicotiana tabacum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.*

Introduction

Environmental circumstances play a vital role in defining the function and floristic composition of plants. From the beginning of human civilization plant and plant products are usually used to treat different diseases (Joshi *et al.*, 2009). As plants have substances of medicinal values, therefore, they are used to treat number of diseases since long time. Use of plants had minimal or less side effect on human beings (Doughari, 2006). With the passage of time the usage of plants is increasing in pharmaceutical industries, suggested that if a plant is used as remedy of disease then it would have some important ingredients (Nostro *et al.*, 2000). Researchers have great interest in those substances which are derived from plants because they are versatile in their applications. Various phytochemicals can be obtained from plants which are very beneficial for mankind and medicinal plants have become the richest biological resource of such chemicals which are used in manufacturing of traditional drugs as well as in modern nutraceuticals, food supplements, medicines, folk medicines, raw material and pharmaceutical intermediates for synthetic drugs **(**Tumwine, 2011).

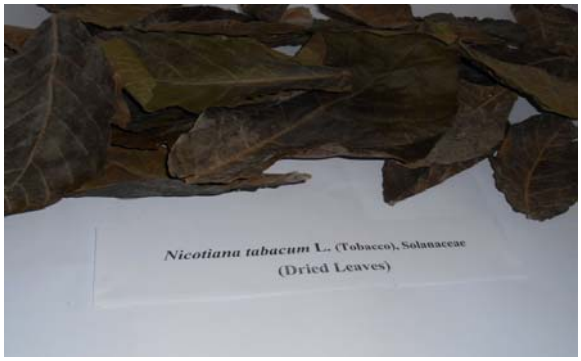
Antimicrobial compounds are a group of chemical compounds which are synthetically or biosynthetically produced which either destroy or usefully suppress the growth and metabolism of variety of microorganisms (Lavanya & Brahmaprakash, 2011). Pharmacologically important plants present a large source of antimicrobial agents and serve as a drug in many countries (Mahesh & Satish, 2008). This antimicrobial activity of plant is due to their constituents of oils and extracts which are used in pharmaceutical and others **(**Hammer *et al.*, 1999). Globally, researchers are using extracts of plants for their antiviral, antibacterial, and antifungal activities. The characteristics of the plants that retard the growth of micro-organisms have been investigated in different laboratories around the world since 1926 (Bakht *et al.*, 2012).

The plants are selected for study due to their following contrasting characteristics (Table 1).

Materials and Methods

The study was carried out during October 2012-2013. In this study leaves of two different plants *Nerium oleander* L. (Apocynaceae), *Nicotiana tabacum* L. (Solanaceae) were used. The disc diffusion technique (Anon., 2002) was used to evaluate the antibacterial activity. For antibacterial sensitivity extracts of these leaves were taken and processed. Experimental work was categorized as following: 1) Collection of plant material, 2) Preparation of aqueous ethanolic leaves extracts and 3) Determination of Zone of Inhibition (Fig. 1). The leaves of both plants (*Nicotiana tabacum* L. and *Nerium oleander* L.) were collected from local field of Multan, Pakistan. The leaves were thoroughly washed and air dried under shade for about 15-20 days. The dried leaves were then grinded in a herbal grinder into a coarse form. Before extraction the powder was stored at room temperature in air tight polythene bags. The extract was prepared and crude extract was stored in dried and air tight Petri dishes (Pyrex), labeled and stored in refrigerator for further study (Lavanya & Brahmaprakash, 2011), then incubated for overnight at 37^{o}C.The bioassay used was the standard disk diffusion assay adapted from (Parekh & Sumitra, 2007)**.** By dipping and saturating commercially sterilized discs in plant extract test discs were prepared. Same sized sterilized disks absorbed the same volume of the extract. Aqueous ethanol paper discs were used for control, which prepared by dipping the disks into aqueous ethanol solution (Sermakkani & Thangapandian, 2010).

Collection



Drying

Grinding

Soaking

Filtering

Fig. 1. Pictures showing methodology of experiment.

Table 1. Characteristics of selected plants species (*Nerium oleander &Nicotiana tabacum*) for antimicrobial assay.

Sr. No.	Plants	Family	Part used	Chemical Constituents	Medicine	Uses	References
1.	*Nerium oleander*	Apocynaceae	Leaves extract	Cardiac glycosides, Neriin & Oleandrin	Folk medicine, An oral anticancer drug known as Anvirzel	Used in suicide equipment' insecticidal properties	Haynes *et al.*, 1985; Edward *et al.*, 1993; Nostro *et al.*, 2000; Doughari, 2006; Joshi *et al.*, 2009 and Lavanya & Brahmaprakash, 2011.
2.	*Nicotiana tabacum*	Solanaceae	Leaves extract	Nicotine	Insecticide, pesticide	Raw material for cigarette industry, Smoking	Denduangboripant *et al.*, 2005 and Jude, 2013

Observation of results: On the basis of absence or presence of zone of inhibition results were measured. Zone reader was used to measure zone of inhibition in mm (Ahmad & Arina, 2011).

Antimicrobial assay of plant extract: Three species of bacteria *(Escherichia coli, Staphylococcus aureus & Pseudomonas aeruginosa)* were used for antimicrobial assay. For further tests nutrient agar broth, nutrient agar suspension and Hi-sensitivity test agar were prepared by following manufacture's instruction. The commercially sterilized discs (HIMEDIA, Mumbai) were used by incorporating 20µl of plant extract using micropipette and then discs were kept into sterile petri plates (Pyrex) and incubated for overnight for further use after checking sterility. A swab dipped in standard inoculum was used for the transformation of bacteria of petri dish with nutrient agar. These were

Results

Antibacterial effect of *Nerium oleander*: The plant extracts of both the species are applied on three tested bacteria, different zone of inhibitions are exposed in (Fig. 2). *Escherichia coli* had highest diameter for zone of inhibition (24mm) at 900mg/ml concentration of *Nerium oleander* extract while at 500mg/ml concentration showed the minimum diameter (15mm) of zone of inhibition (Fig. 3(A). A decline in diameter of zone of inhibition 22mm was observed at 800mg/ml, then zone of inhibition showed gradual decline 20mm and 17mm with decreasing dilution of the extract from 700-600mg/ml concentrations. The mean values of antibacterial activity (Zones of inhibition in mm) of *Staphylococcus aureus* under different concentration (900mg/ml, 800mg/ml, 700mg/ml, 600mg/ml and 500mg/ml) of *Nerium oleander* leaf extract is represented in Fig. 3(B). The data indicated that *Staphylococcus aureus* had highest diameter for zone of inhibition (22mm) at highest 900mg/ml concentration while at lowest level of extract 500mg/ml concentration the minimum diameter (13mm) for zone of inhibition was observed. A slight difference in antimicrobial activity 19mm and 18mm was observed at 800-700mg/ml, then zone of inhibition showed gradual decline (15mm) with decreasing dilution of the extract 600mg/ml concentration. Fig. 3(C); indicated that *Pseudomonas aeruginosa* had highest diameter for zone of inhibition (28mm) at 900mg/ml concentration while at lowest level of extract 500mg/ml concentration the minimum diameter (18mm) for zone of inhibition was observed. A slight difference in antimicrobial activity 24mm, 23mm and 22mm was observed at 800-600mg/ml dilutions respectively.

Antibacterial effect of *Nicotiana tabacum*: Fig. 3(D); indicated that *Staphylococcus aureus* had highest diameter for zone of inhibition (26mm) at highest plant extract (900mg/ml) concentration while at lowest extract concentration (500mg/ml) the minimum diameter (15mm) for zone of inhibition was observed. A gradual decline in diameter of zone of inhibition i.e. 23, 20 and 17mm was observed at 800, 700 and 600mg/ml dilutions respectively. Fig. 3(E); data indicated that *Escherichia coli* had highest diameter for zone of inhibition (20mm) at highest 900mg/ml concentration while at lowest level of extract 500mg/ml concentration the minimum diameter 13mm for zone of inhibition was observed. A slight difference in antimicrobial activity 19mm was observed at 800mg/ml. A gradual decline in diameter of zone of inhibition 17mm and 14mm was observed at 700-600mg/ml dilutions respectively. Fig. 3(F); data indicated that *Pseudomonas aeruginosa* had highest diameter for zone of inhibition (21mm) at highest 900mg/ml concentration. While at lowest level of extract 500mg/ml concentration the minimum diameter (11mm) for zone of inhibition was observed. A gradual decline in diameter of zone of inhibition 18mm, 16mm and 13mm was observed at 800-600mg/ml dilutions respectively.

Plants contain many biologically active compounds which have potential as medicinal agents (El-Mahmood *et al.*, 2010). The main theme of the present research work was to analyze anti-bacterial actions of *Nerium oleander* and *Nicotiana tabacum*. The bacterial species had shown different susceptibilities for different concentration i.e. (900, 800, 700, 600 and 500mg/ml) of ethanolic extracts of leaves of these two species as also observed by Garima *et al*. (2011). The results showed that both plants are affected against all tested organisms/bacteria. The effects against gram-negative bacteria were more dominant than those gram-positive but *N. tabacum* showed maximum results against *Staphylococcus aureus at* maximum concentration (900mg/ml) and zone of inhibition was 26mm.

In most of the cases the leaves of the plants have reported to contain more phytoconstituents than any other part of the plant. The occurrence of metabolites in these plants is more concentrated that's why this indicated that they contain more active metabolites. So this was the strong reason to choose the leaves of these species (Kawalekar, 2012).

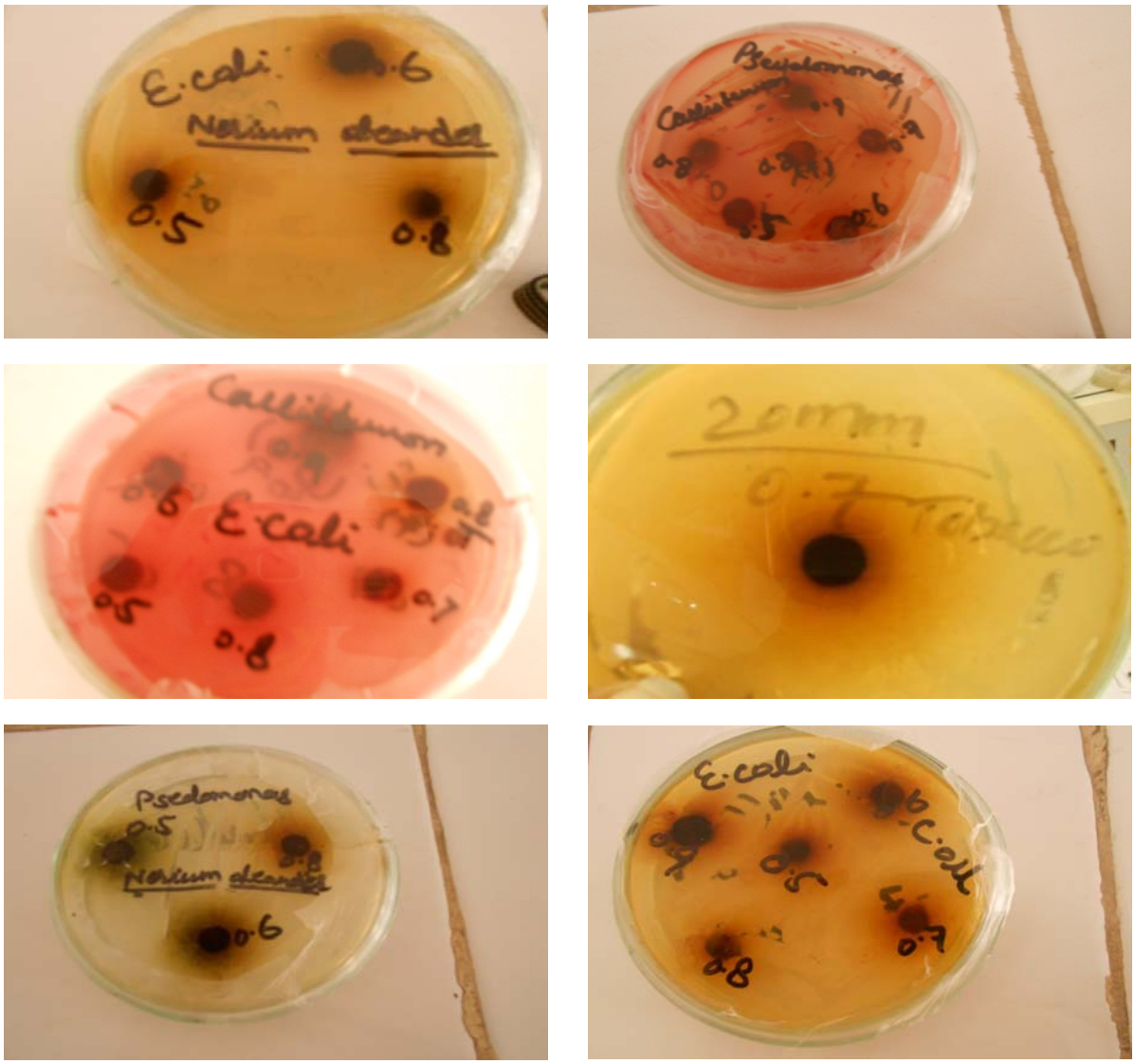


Fig. 2. *Nerium oleander and Nicotiana tabacum* plates showing zones of inhibition againt various bactreial strains.

Discussion

The disc-diffusion method was mainly used for the determination of antibacterial action. Among the different methods, disk diffusion has been used more frequently to examine the antimicrobial activity of natural antimicrobials (Kim & Kim, 2007; Mayachiew *et al*., 2010). The antibacterial activities monitored by different concentration of extracts to investigate that which concentration showed maximum antimicrobial activity. Simultaneously increasing the concentrations of extracts and notifies the trend in activity. The antibacterial activities of both the plants were checked on all three bacterial strains. The two plant species showed the highest values for zone of inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Among these, the least activity was shown by *N. tabacum* against *Escherichia coli*. The results showed that *Nerium oleander* had biggest diameter for zone of inhibition (28mm) at highest (900mg/ml) concentration, while at lowest concentration (500mg/ml) of extract showed the minimum diameter (18mm) for zone of inhibition was observed against *Pseudomonas aeruginosa*. While at the concentration of (900, 500mg/ml) *N. oleander* showed different zones of inhibition which were 22mm and 13mm respectively against *Staphylococcus aureus* as also observed by Wong *et al*. (2013).

In case of *N. tabacum*, the highest diameter for zone of inhibition was found at (26mm) at highest 900mg/ml concentration, while at lowest level of extract 500mg/ml concentration the minimum diameter (15mm) for zone of inhibition was observed against *Staphylococcus aureus*. At the concentration of 900mg/ml and 500mg/ml *N. tabacum* showed different zones of inhibition which were 21mm and 11mm respectively against *Pseudomonas aeruginosa*. Ethanolic extract of *Nerium oleander* showed the maximum diameters for zone of inhibition against three bacterial species. The highest activity of this plant might be due the presence of terpenoids, saponins and amino acids.

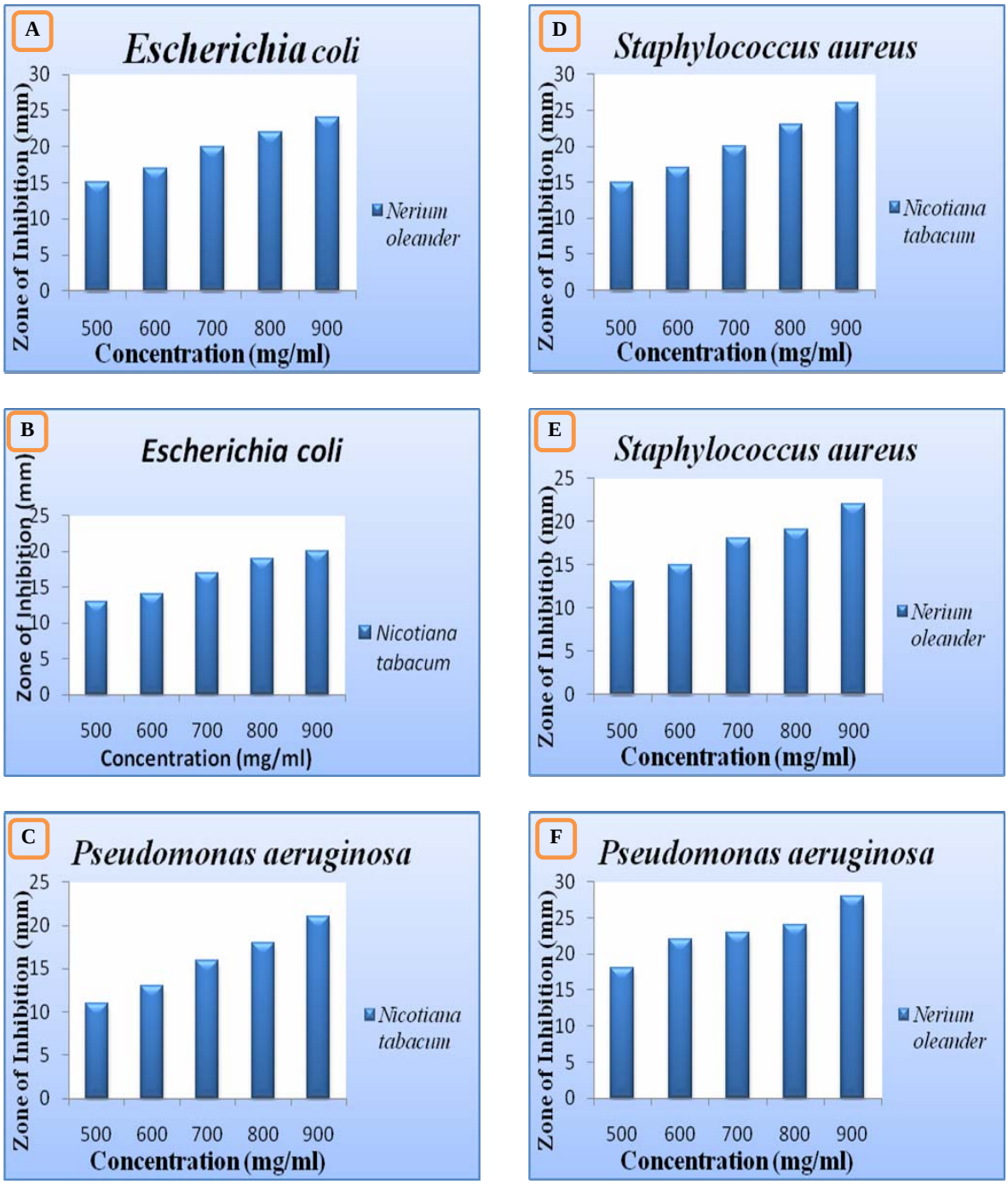


Fig. 3. A, B, C, D, E & F showing different zones of inhibition against *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* for both plants (*Nicotiana tabacum* L. and *Nerium oleander* L.)

Ethanolic extract of *N. tabacum* showed the maximum diameters for zone of inhibition (26mm) against *Staphylococcus aureus* while there was a less difference against *Escherichia coli* and *Pseudomonas aeruginosa* which were 20mm and 21mm respectively. The phytoconstituents of this specie were mainly saponins, flavoids and anthraquinones. While amino acids were absent in *N. tabacum*.

According to the Jude (2013) *Nerium oleander, Lippia nodiflora, Wattakaka volubilis* and *Weinmannia tinctoria* were possessed the highest inhibitiory zone against *E. coli, K. pneumoniae, S. typhi, P. vulgaris* and *P. mirabilis*. Methanol extract of *Nerium oleander* exhibited the highest activity (28 mm) against *S. typhi* than standard antibiotics around 22 mm. According to the report of Jeyachandran *et al.* (2010) the methanolic extract of *Nerium oleander* had maximum zone of

inhibition (28mm) against *S. typhi*. Results in the present investigation showed the similar zone of inhibition of ethanolic extract *N. oleander* against *Pseudomonas aeruginosa*.

Conclusion

1. These plant species have antimicrobial potential against common pathogenic bacterial strains.
2. The maximum concentration 900mg/ml is found to be most efficient against all bacterial strains.
3. So, we can use this level of plant extracts as a source of natural medicine against these bacteria.

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