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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL PROFILE OF FLOWER EXTRACT OF PINK OLEANDER

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ABSTRACT

In the present study, antibacterial activity and phytochemical analysis of flowers of pink oleander (*Nerium oleander* L.) were carried out. Methanolic extract of dried flowers was prepared and its 25, 50 and 75 µL were used in disc diffusion method to check antibacterial activity against *Listeria monocytogenes*, *Staphylococcus caprae*, *Erwinia carotovora* and *Escherichia coli*. *L. monocytogenes* and *E. carotovora* were inhibited more than the others. The highest quantity of the extract (75 µL) caused 12–18 mm inhibition zones in case of different bacterial species. GC-MS analysis was carried out for phytochemical profile of the extract. There were 11 constituents in the pink flower extract of *N. oleander*. The major compound was benzene, 1-ethenyl-4-methoxy- (51.11%) followed by propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]- (22.44%). Moderatley abundant compounds included nonanoic acid, methyl ester (5.12%), *cis*,*cis*,*cis*-7,10,13-hexadecatrienal (4.96%), benzofuran, 2,3-dihydro- (3.88%) and propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester (3.06%).

Key words: Antibacterial activity, *Nerium oleander*, Pink flowers, Phytochemicals.

INTRODUCTION

Plants are being used to cure a wide range of disorders as they have a number of biologically active molecules (Ferdosi *et al.,* 2021; Khan and Javaid, 2022; Javaid *et al.,* 2022). There is a years of record for safe use of plant-derived natural products against different ailments (Rizwana *et al.,* 2016), and about 80% population of the world still has confidence in conventional medicines as reported by WHO (El Mihyaoui *et al.,* 2023). The natural compounds of plants are known to possess antibacterial (Javed *et al.,* 2011; Ferdosi *et al.,* 2020), antifungal (Banaras *et al.,* 2020; Jabeen *et al.,* 2022), insecticidal (Ahmad *et al.,* 2022), herbicidal (Javaid and Khan, 2020), antioxidant, anticancer (Khan and Javaid, 2020), anti-inflammatory (Thitinarongwate *et al.,* 2022) and many other activities (Naqvi *et al.,* 2020). Mostly bacteria are responsible to spoil food products and also cause diseases like diarrhea (Kirk *et al.,* 2017). *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes* are the main bacterial pathogens, which are responsible for food-borne illnesses (Abebe *et al.,* 2020). Consumers have great concern about the risks associated with the use of synthetic additives for human health (Kalem *et al.,* 2017). Extracts of plants can be utilized as safe natural alternative preservatives (Clarke *et al.,* 2017). Plants possess many phytochemicals like alkaloids, flavonoids, terpenoids and tannins, which have antimicrobial potential (Talib and Mahasneh, 2010).

Nerium oleander L. is a toxic plant of Apocynaceae that has pharmacological properties. Its different parts contain lethal alkaloids and glycosides (Barbosa *et al.,* 2008). It is generally used to treat cancer and hyperglycemia around the globe and particularly in Turkey, Morocco, India and China (Sharma *et al.,* 2023). Moreover, phytochemicals of this plant also possess anti-viral (Plante *et al.,* 2021), antibacterial (Almanaa *et al.,* 2021), insecticidal (Zaid *et al.,* 2022), and anti-diabetic (Dey *et al.,* 2015) properties. It naturally grows in the region Mediterranean basin along rivers up and also grows in other parts of the world having a subtropical climate like Mediterranean region (Bañon *et al.,* 2006). In Pakistan, it is cultivated in gardens and along road-sides as an ornamental plant. Its flowering season is from spring to late summer. There are different varieties of this plant species with white, pink and reddish flowers. In a recent study, Saeed *et al.* (2023) showed antibacterial activity of white flowers of this plant species and identified the

possible antibacterial compound. However, similar studies on pink flowers of *N. oleander* from Pakistan are missing. Thus, this study was undertaken to evaluate the antibacterial activity and phytochemical profile of methanolic extract of pink flowers of *N. oleander* collected from Lahore, Pakistan.

MATERIALS AND METHODS

Sample collection

Flowers of the *N. oleander* var. pink were collected manually at early morning time during May 2022. Since the plant contains high amounts of allergins in the form of white exudes so the hand gloves were used to avoid skin allergy while plucking the flowers. The flowers were placed in paper bags and then carefully handled during shifting to laboratory for experimentation. The flowers were placed on paper sheets and allow to dry out for a week. In continuation of the drying process, the remaing moisture content of the sample was palced in an oven for 8 hours at 40 ºC.

Preparation of methanolic extract of flowers

After the drying process, 100 g flowers were crushed into very fine powder, placed in a flask and 500 mL of methnol were added. The flask was kept for 14 days for extraction of possible phytochemicals. The solvent was then filtered in a beaker by using double layered filter papers. The obtained solvent was transferred to flask attached with a rotary evaporator for the evaporation process of methanol. The material obtained as a result of evaporation process was kept in a 100-mL volume beaker. To completely evaporate the solvent, beaker was placed in an oven at 40 ºC for 10 h.

Antibacterial bioassays

The bacterial species used for the current study were obtained from Jinnah Hospital Lahore. The bacteriral species which were used for study were *Listeria monocytogenes, Staphylococcus caprae, Erwinia carotovora* and *Escherichia coli.* Extract doses applied to check their effect on bacterial growth were 25, 50 and 75 µL. Agar well technique was used to study the inhibition size zones formed due to antibacterial activity of *N. oleander* flowers extract (Saeed *et al.,* 2023).

GC-MS analysis

The sample was subjected to GC-MS (Gas Chromatography and Mas spectroscopy) analysis to identify phytochemical compounds. The gas chromatography machine 7890B, with a column DB5 (30 m × 0.25 µm × 0.25 µm) was used. Injection volume was 1 µL while helium was used as carrier gas. The intial run temperature was 80 ºC that was raised up to 300 ºC at the rate of 10 ºC per minute, and the inlet temp. was 280 ºC. The mass spectroscopy machine model 5977A made by Agilent technologies USA was used. The spectra were compared with NIST 2020 library to identify the chemical compounds (Ferdosi *et al.,* 2022).

Statistical analysis

Data regarding the effect of methanolic extract of pink *N. oleander* flowers on bacterial growth in terms of inhibition zones was subjected to two-way analysis of variance followed by application of LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity of extract

ANOVA showed a highly significant ($P \leq 0.001$) effect for the bacterial species, extract concentration and their interactions (Table 1). *E. carotovora* was the most susceptible bacterial species where 13.7 to 18 mm zones of inhibition were recorded due to 25–75 µL extract of pink flowers of *N. oleander.* Similarly, *L. monocytogenes* growth was also very sensitive to the applied extract. In this case, different doses of the flower extract caused 9.2–18 mm inhibition zones. The other two bacterial species were somewhat resistant to the flower extract where 10.7–12.7 mm and 10–14.3 mm zones of inhibition were found for *S. caprae* and *E. coli*, respectively. In general, the inhibitory effect of the flower extract was dose dependant and was generally increased by increasing the extract quantity, and the extract had variable inhibitory activity against the different target bacterial species (Fig. 1). These findings are in agreement with some previous studies that reported the antibacterial activity of *N. oleander.* However, generally, previous studies were

carried out on leaves and studies regarding flower extracts are very limited. Methnolic extract of white flowers of *N. oleander* showed antibacterial activity against *Salmonella* sp., *Shigella dysenteriaea*, *Listeria monocytogenes* and *Staphylococcus carpae* (Saeed *et al.,* 2023). Hussain and Gorsi (2004) demonstrated that methanol leaf and root extracts of *N. oleander* were very effective to control the growth of *E. coli, Bacillus pumilus, B. subtilis* and *S. aureus*.

Table 1. Two-way ANOVA for the effect of different concentrations of methanolic extract of *Nerium oleander* var. Pink on the size of inhibition zone.

Sources of variation	df	SS	MS	F values
Bacterial species (B)	3	79.55	26.51	27.98*
Concentration (C)	2	30.38	15.19	16.03*
B × C	6	176.19	29.36	30.99*
Error	24	22.74	0.95	
Total	35	308.86		

*, Significant at P≤0.001.

Table 2. Compounds in methanolic pink flower extract of *Nerium oleander* var. Pink.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	2-Cyclopenten-1-one, 3-methyl-	C_6H_8O	96.13	4.634	1.58
2	Phenol	C_6H_6O	94.11	4.743	1.61
3	Benzene, 1-ethenyl-4-methoxy-	$C_9H_{10}O$	134.17	7.206	51.11
4	Benzofuran, 2,3-dihydro-	C_8H_8O	120.14	8.019	3.88
5	Propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]-	$C_{14}H_{14}N_2O$	226.27	10.173	22.44
6	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	$C_{16}H_{30}O_4$	286.40	12.856	3.06
7	Pentadecanal	$C_{15}H_{30}O$	226.40	13.144	1.59
8	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	172.26	16.505	5.12
9	9-Octadecyne	$C_{18}H_{34}$	250.5	18.147	2.53
10	*cis,cis,cis*-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234.38	18.206	4.96
11	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.47	18.437	2.06

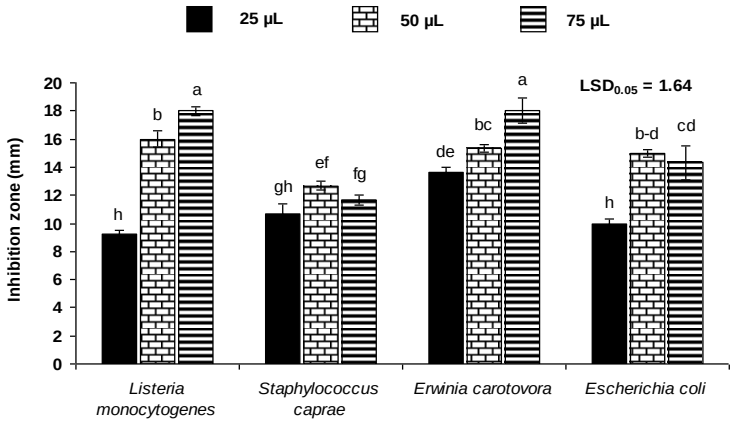

Fig. 1. Antibacterial activity of methanolic pink flower extract of *Nerium oleander*. Values with different letters show significant difference (P≤0.05) as determined by LSD test.

GC-MS analysis

In total, 11 compounds were identified in this study as shown in Fig. 2 and Table 2. Chemical composition, especially the major compounds identified in pink flowers of *N. oleander* in the present study are different from those reported in the extract of white flowers of this plant. The predominant compound in this study was benzene, 1-ethenyl-4-methoxy- (51.11%) in contrast to the major compound 2-O-methyl-D-mannopyranosa reported in methanolic extract of white flowers (Saeed *et al.,* 2023). It is also known by other common names such as *p*-vinylanisole or *p*-methoxystyrene, and is an aroma compound occurring naturally. It was also found as a major volatile compound (24.4%) in a liverwort *Cyathodium foetidissimum* (Sakurai *et al.,* 2018). It was found one of the major compounds in essential oil of Bingol propolis that had antibacterial activity against *Bacillus cereus*, *S. aureus* and *E. coli* (Arserim-Ucar *et al.,* 2020). This compound was found among the three major compounds found in volatiles of *Streptomyces albulus*, having remarkable activity against two fungal species namely *Fusarium oxysporum* and *Sclerotinia* sclerotiorum (Kumar *et al.,* 2021). Extracts of *N. oleander* contain terpenoids, tannins, alkaloids and saponins, which could be responsible for antibacterial activity (Bhuvaneshwari *et al.,* 2007).

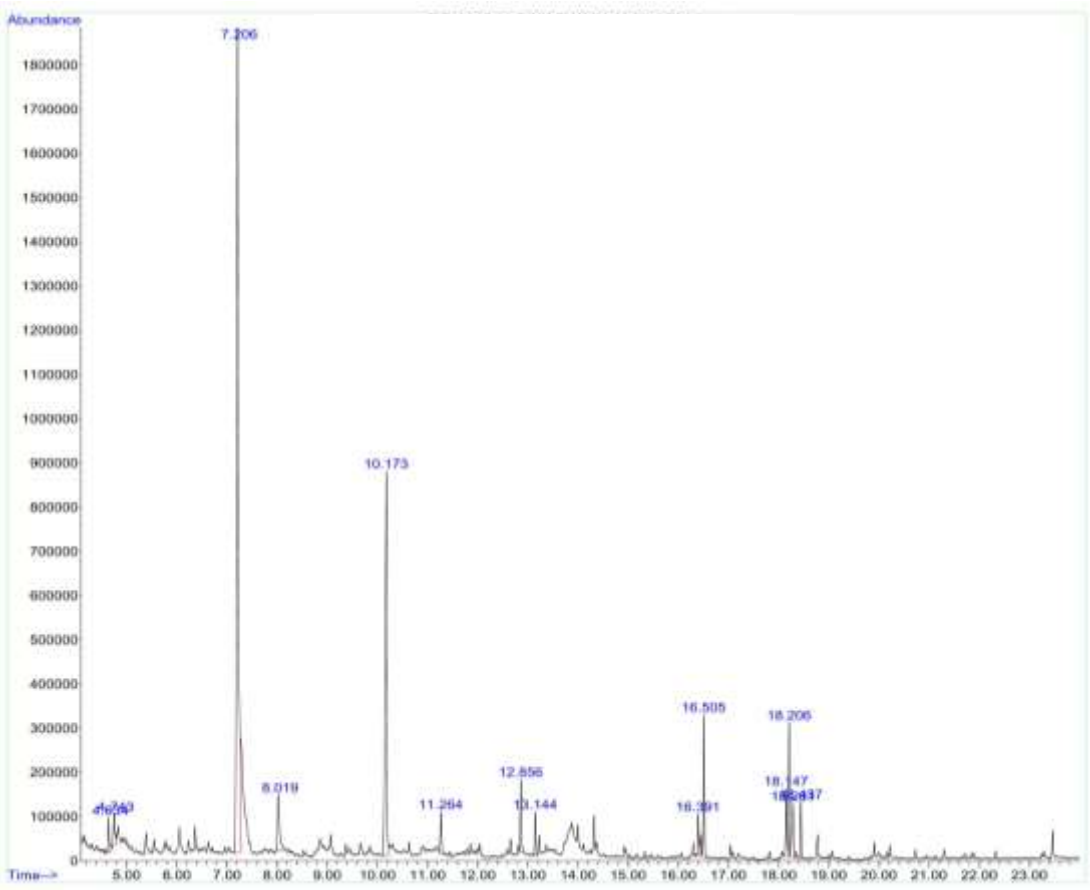

Fig. 2. GC-MS chromatogram of flower pink extract of *Nerium oleander*.

The second most occurring compound was propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]- (22.44%). Four compounds namely nonanoic acid, methyl ester (5.12%), *cis,cis,cis*-7,10,13-hexadecatrienal (4.96%), benzofuran, 2,3-dihydro- (3.88%), and propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester (3.06%) were found moderately occurring ones (Table 2). The *cis,cis,cis*-7,10,13-hexadecatrienal has been reported in *Skimmia anquetilia* and *Litsea cubeba* with antibacterial activity (Li *et al.,* 2014; Nabi *et al.,* 2022). Similarly, benzofuran compounds are very common in nature and many compounds of this group possess various biological activities including antibacterial (Miao *et al.,* 2019).

Five compounds including 9-octadecyne (2.53%), and hexadecanoic acid, 15-methyl-, methyl ester (2.06%), phenol (1.61%), pentadecanal (1.59%) and 2-cyclopenten-1-one, 3-methyl- (1.58%) were less abundant as presented in Table 2. Among these, pentadecanal showed antibacterial activity against *Listeria monocytogenes* (Venuti *et al.,* 2022).

Conclusion

Methanolic extract of pink flowers of *N. oleander* is highly inhibitory to growth of *E. carotovora* and *L. monocytogenes* causing up to 18 mm inhibition zones due to 75 µL of the extract. Compounds namely benzene, 1-ethenyl-4-methoxy-; pentadecanal; benzofuran, 2,3-dihydro- and *cis,cis,cis*-7,10,13-hexadecatrienal might be responsible for antibacterial activity.

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