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ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF WHITE FLOWERS EXTRACT OF *NERIUM OLEANDER* L.

Hafiz Muhammad Saeed[1], [*]Malik F. H. Ferdosi[1], Iqra Haider Khan[2], Arshad Javaid[2] and Malik Waqar Sultan[3]

[1]*Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan*
[2]*Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan*
[3]*Department of Zoology, Government College University Lahore*

[*]Corresponding author's email: fiaz.iags@pu.edu.pk, malikferdosi@yahoo.com

ABSTRACT

Nerium oleander L., a poisonous plant of Apocynaceae family, is cultivated in Pakistan for its beautiful white, pink and red flowers. In this study, antibacterial activity and GC-MS profiling of its white flowers was carried out. Four bacterial species namely *Listeria monocytogenes, Staphylococcus carpae, Shigella dysenteriaea* and *Salmonella* sp. were used in the experiment. Two doses of the extract *viz.* 50 and 75 µL were used using agar well diffusion technique. The extract showed a moderate antibacterial activity with the formation of 5.66 to 6.33 mm inhibition zones in different treatments. GC-MS analysis demonstrated 15 constituents in the extract. The major compound was 2-O-methyl-D-mannopyranosa with 17.27% peak area followed by 1,2,3-propanetriol, 1-acetate (16.77%), maltol (12.26%), glyceraldehyde (8.33%), *n*-hexadecanoic acid (8.12%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.53%), oleic acid (6.09%), N-formyl-β-alanine (5.13%), octadecanoic acid (3.91%), 1-propanamine, N-methyl-N-nitroso- (2.87%), *cis*-vaccenic acid (2.85%), benzene, 1-ethenyl-4-methoxy- (2.66%), 9,12-octadecadienoic acid (Z,Z)- (2.20), eicosane (2.08%) and propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]- (1.93%).

Keywords: Antibacterial, Apocynaceae, Methanolic extract, *Nerium oleander*, White flowers.

INTRODUCTION

Antimicrobial resistance to available drugs is a serious dilemma that adversely affects the treatments of different diseases caused by bacterial and fungal pathogens (El-Shiekh *et al.*, 2020). *Staphylococcus aureus*, a Gram-positive bacterium, has developed resistance against methicillin (O'Donnell *et al.*, 2010). Similarly, some Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* have evolved resistance against various antibiotics (Mulyaningsih *et al.*, 2011). A viable alternate to overcome this issue is the identification of natural products from plants with biological activities (Naqvi *et al.*, 2020; Javaid *et al.*, 2021), especially those compounds having antimicrobial characteristics (Khan and Javaid, 2019, 2020; Ferdosi *et al.*, 2020). Naz *et al.* (2014) verified antibacterial activity of *Trachyspermum ammi* essential oil against *Acidovorax* sp. Recent studies have shown the antibacterial activities of extracts of *Chenopodium album*, *Mimosa diplotricha*, *Enydra fluctuans*, *Aristolochia tagala* against a variety of bacterial species including species of *Staphylococcus*, *Enterobacter*, *Proteus*, *Pseudmonas* and *Escherichia* (Acharjee *et al.*, 2023; Mariyammal *et al.*, 2023). Zhao *et al.* (2023) identified two new antibacterial terpenoids namely salviaterpenoid A and ligurobustoside T from *Salvia cavaleriei*. The natural bioactive metabolites have numerous advantages over synthetic medicines such as lesser toxicity and cost, greater biodegradability, and novel mechanisms of action (El-Shiekh *et al.*, 2020).

Nerium oleander L. is a toxic plant of family Appocynaceae that naturally grows along rivers in the Mediterranean basin countries and is also grows in numerous other regions having similar subtropical environment (Bañon *et al.*, 2006). Its toxicity is because of some glycosides and alkaloids in its different parts ((Barbosa *et al.*, 2008). The cardiotonic active constituents are heterosides as reported by Bruneton (2001). The plant is known to have insecticides, antibacterial and cytotoxic effects (Mijatovic *et al.*, 2007; Derwich *et al.*, 2010; Zaid *et al.*, 2022). Recently, Nawaz *et al.* (2023) reported a new bioactive steroid 3*β*-acetoxy-5, 25 (26) diene, 24*β*-hydroxy lanostane from this plant. In Pakistan, it is cultivated as an ornamental plant for its white, pink and red colored flowers. Numerous earlier phytochemical studies have been carried out on leaves of *N. oleander* while little is known about chemical profile of its flowers especially from Pakistan. Therefore, this study was undertaken to investigate antibacterial activity and phytochemical analysis of white flowers of *N. oleander* collected from Lahore, Pakistan.

MATERIALS AND METHODS

Collection of white flowers of *Nerium oleander*

During early in the morning, the flowers of white colored *N. oleander* were hand plucked. The hand gloves were used to avoid any kind of allergy on hands as the plant exudes white fluid. The flowers were kept in the paper bags and very carefully shifted into the lab for further procedure. The flowers then dried out for almost 7 days and the remaining moisture was finally evaporated by keeping them into an oven at 40 ºC for about 6 hours.

Extraction procedure

The dried flowers were weighed 100 g and then crushed into powder form. The dry powdered form was placed in a graduated flask and a 200 mL of analytical grade methanol was added into it. The flask was kept for 10 days to extract possible secondary metabolites into the solvent and then filtered by using double layered filter papers into a beaker and that solvent was placed into rotary evaporator. The rotary apparatus was used to evaporate the organic solvent. The obtained concentrate was freezed before shifting into the lab for GC-MS analysis. The sample was put into an air tight glass vial and shifted into the lab for analysis (Javaid *et al.,* 2021b).

Antibacterial activity

Four bacterial species namely *Listeria monocytogenes*, *Staphylococcus carpae, Shigella dysenteriaea* and *Salmonella* sp. were tested in the present study for their response to two doses (50 and 75 µL) of methanolic extract of white flowers of *N. oleander.* Agar well diffusion technique was used to check the size of inhibition zone formed due to antibacterial effect of the flower extract (Ferdosi *et al.,* 2021).

GC-MS analysis

The Gas Chromatography and Mas spectroscopy was used for the identification of possible antimicrobial phytochemical constituents. The Gas chromatography machine 7890B, Agilent; USA, column; DB5, dimensions (30 m × 0.25 µm × 0.25 µm), Injection Vol. 1 micro litre, carrier gas helium. Intitial temperature 80 ºC which was raised up to 300 ºC @10 ºC per minute with inlet temp. 280 ºC. Mass spectroscopy model 5977A by Agilent USA; scan 50–500 m/z, the source temp. 230 ºC with quadrupole temperature was 150 ºC. The chemical constituents were identified by comparing the spectra of these with NIST 2020 library. The peak areas were used to report relative abundance of these chemicals (Ferdosi *et al.* 2022). The structures of the important compounds were drawn by using ChemDraw software.

Statistical analysis

Data regarding antibacterial activity of the extract of white flowers of *N. oleander* was analyzed by one-way ANOVA following by separation of treatments by LSD test at $P \leq 0.05$ using software Statistix 8.1.

RESULTS AND DISCUSSION

Antibacterial activity

Extract of white flowers of *N. oleander* showed moderate antibacterial activity against all the four tested bacterial species namely *L. monocytogenes, S. carpae, S. dysenteriaea* and *Salmonella* sp. Both the doses of the extract showed almost similar antibacterial behavior. Size of inhibition zone was in the range of 5.66 to 6.33 mm in case of different tested bacterial species (Fig. 1). Earlier, Saranya *et al.* (2017) reported that ethanolic flower extract of *N. oleander* found very effective against *P. aeruginosa, E. coli, S. aureus* and *Salmonella* sp. causing 28, 20, 21 and 25 mm inhibition zones, respectively. Previous antibacterial studies were mostly carried out using leaf extract of *N. oleander.* Malik *et al.* (2015) evaluated antibacterial activity of ethanolic extract of *N. oleander* leaves (collected from Multan) using disc diffusion method that strongly suppressed the growth of *S. aureus*, *P. aeruginosa* and *Escherichia coli*. Similarly, Bhuvaneshwari *et al.* (2007) reported that alcoholic and chloroform leaf extracts of *N. oleander* were highly inhibitory against *P. aeruginosa, S. aureus* and *Salmonella typhimurium* with 9, 10 and 7 mm zones of inhibition, respectively. However, Mouhcine *et al.* (2019) reported moderate antibacterial activity of ethanolic and aqueous leaf extract of this plant. They found that the activity was limited to the Gram-positive bacteria. Flowers of *N. oleander* contain a number of bioactive compounds such as alkaloids, saponins, flavonoides, phenols and tannins (Saranya *et al.,* 2017), that might be responsible for antibacterial activity (Kanwal *et al.,* 2009; Wei *et al.,* 2021; Du *et al.,* 2022).

Table 1. List of compounds in methanolic flower extract of *Nerium oleander* var. White identified by GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	1-Propanamine, N-methyl-N-nitroso-	$C_4H_{10}N_2O$	102.13	6.216	2.87
2	Benzene, 1-ethenyl-4-methoxy-	$C_9H_{10}O$	134.17	6.282	2.66
3	Maltol	$C_6H_6O_3$	126.11	10.315	12.26
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	11.718	5.53
5	N-Formyl-β-alanine	C_4H_7NO	117.10	11.818	5.13
6	Propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]-	$C1_4H_{14}N_2O$	226.27	11.854	1.93
7	Glyceraldehyde	$C_3H_6O_3$	90.08	11.990	8.33
8	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13	13.809	16.77
9	2-O-Methyl-D-mannopyranosa	$C_7H_{14}O_6$	194.18	21.469	17.27
10	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	25.871	8.12
11	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	28.199	2.20
12	Oleic acid	$C_{18}H_{34}O_2$	282.86	28.275	6.09
13	*cis*-Vaccenic acid	$C_{18}H_{34}O_2$	282.86	28.344	2.85
14	Octadecanoic acid	$C_{18}H_{36}O_2$	284.47	28.584	3.91
15.	Eicosane	$C_{20}H_{42}$	282.54	32.665	2.08

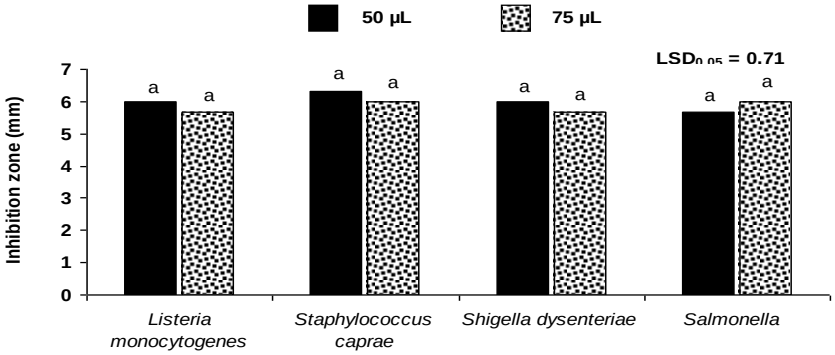

Fig. 1. Antibacterial activity of methanolic flower extract of *Nerium oleander* var. White. Values with different letters show significant difference (P≤0.05) as determined by LSD test.

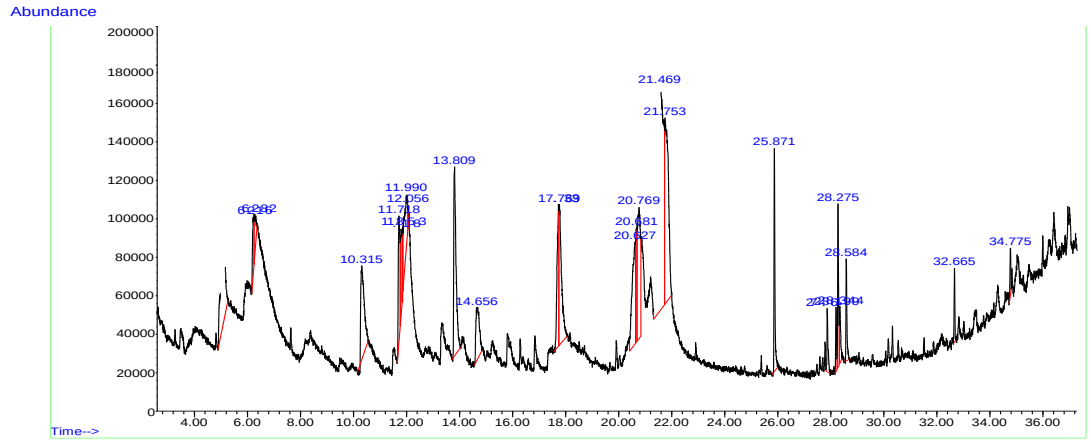

Fig. 2. GC-MS chromatogram of methanolic flower extract of *Nerium oleander* var. White.

GC-MS analysis

GC-MS chromatogram of the white flowers extract of *N. oleander* is shown in Fig. 2. According to this chromatogram, there were 15 compounds. Details of the identified compounds are mentioned in Table 1. The major compounds in the extract were 2-O-methyl-D-mannopyranosa (17.27%), 1,2,3-propanetriol, 1-acetate (16.77%), maltol (12.26%). Glyceraldehyde (8.33%), *n*-hexadecanoic acid (8.12%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (5.53%), oleic acid (6.09%), N-formyl-β-alanine (5.13%), and octadecanoic acid (3.91%) were categorized as moderately abundant compounds. The remaining six compounds namely 1-propanamine, N-methyl-N-nitroso- (2.87%), *cis*-vaccenic acid (2.85%), benzene, 1-ethenyl-4-methoxy- (2.66%), 9,12-octadecadienoic acid (Z,Z)- (2.20), eicosane (2.08%) and propanedinitrile, [3-(4-methoxyphenyl)-1-methylpropylidene]- (1.93%) were identified as less abundant ones.

Some of the compounds of this study have previously been reported to possess various biological activities including antibacterial activity. The major compound 2-O-methyl-D-mannopyranosa was previously identified in stem-bark extract of *Brachystegia eurycoma* with 3.61% peak area. The extract exhibited activity against *S. aureus*, *E. coli* and *Salmonella typhi* (Igwe and Okwa, 2013). Likewise, 1,2,3-propanetriol, 1-acetate was a major compound (13.73%) in acetone extract of rhizome of *Homalomena cochinchinensis* that showed antibacterial activity against *Salmonella enteritidis, Bacillus cereus, E. coli* and *S. aureus* (Van *et al.,* 2021). Maltol, one of the major compounds in the present study, has been found earlier in many plant species like *Cercidiphyllum japonicum* (Tiefel and Berger, 1993), *Passiflora incarnata* (Hayata, 1974), and *Citharexylum spinosum* (Mar and Pripdeevech, 2014). It is a safe favoring agent and biomedical compound that also possesses antimicrobial characteristics (Ziklo *et al.,* 2021). Octadecanoic acid has been found in a number of plant species such as *Jatropha curcas* (Rahman *et al.,* 2014) and quinoa (Khan and Javaid, 2022). It is known to have antibacterial activity in addition to anti-inflammatory, antioxidant, anti-amoebic and anticancer activities (Kumar *et al.,* 2010; Manivannan *et al.,* 2017). *cis*-Vaccenic acid was the most abundant compound (94.84%) in *Ricinus communis* with antimicrobial activity (Maliehe *et al.,* 2021).

Conclusion

Methanolic extract of white flowers of *N. oleander* possess moderate antibacterial activity. GC-MS analysis of this extract revealed the presence of 15 compounds including maltol, octadecanoic acid, and *cis*-vaccenic acid, which might be responsible for its activity against the tested bacteria.

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