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# A NEW BIOACTIVE STEROID ISOLATED FROM *NERIUM OLEANDER* L.

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## ABSTRACT

This research project was designed to explore the biologically active constituents from *Nerium oleander*. A new steroid was isolated and purified from chloroform soluble fraction of *N. oleander*. It was characterized using state of the art spectroscopic techniques including 1D and 2D NMR and mass spectrometry. The isolated compound **1** was identified as 3*β*-acetoxy-5, 25 (26) diene, 24 *β* hydroxy lanostane. It inhibited lipoxygenase enzyme significantly when compared to Bacailene.

**Keywords:** Steroid, *Nerium oleander*, lipoxygenase, 1D and 2D NMR

## INTRODUCTION

Because of microbial resistance development and adverse effects of synthetic drugs, people turned to ethnopharmacognosy. Thousands of phytochemicals have been found as effective and safe alternatives to synthetic drugs (Sasidharan *et al.*, 2010). Many important biological activities including wound healing, antioxidant, anticancer, antidiarrheal, antimicrobial, and analgesic have been reported in literature associated with natural products (Khan and Javaid, 2019, 2020a). Natural compounds such as from plants namely *Ageratum conyzoides*, *Sonchus oleraceous* and *Monotheca buxifolia* (Banaras *et al.*, 2020, 2021; Javed *et al.*, 2021), and their derivatives (Uroos *et al.*, 2022) have been reported very effective against *Macrophomina phaseolina*, a devastating plant and an opportunistic human pathogen. These bioactive compounds have been reported from all parts of plants *viz.* leaves (Khan and Javaid, 2020b), stems (Naqvi *et al.*, 2020), roots (Javaid *et al.*, 2021), flowers (Ferdosi *et al.*, 2020, 2021) and fruits (Khan and Javaid, 2013).

*Nerium oleander* L. is an imperative member of *Apocynaceae* family (Zhao and Bai, 2007). The genus name *Nerium* is derived from Greek word 'neros' meaning water as the plant is habitant of river sides. It is called oleander due to its resemblance to olive plant (Chaudhay and Prasad, 2014). *N. oleander* is a poisonous flowering shrub which is thought to be originated from Southwest Asia but as it is extensively cultivated so no specific origin has been identified. The natural spread of this plant is Mediterranean region and subtropical Asia, as well as India–Pakistan subcontinent. It is dispersed in Himalaya ranges from Nepal westwards to Kashmir, spreading to Baluchistan, Afghanistan, and India (Patel, 2010). *N. oleander* has been used for treatment of various diseases. Literature survey revealed the use of various plant parts of oleander like flowers, bark, and latex, leaves, and their juice for the cure of microbial diseases. In ancient time, all plant parts were used as therapeutic agents against various ailments. The roots of this plant were used externally for treating haemorrhoids, ulcers (Hseini and Kahouadji, 2007), skin diseases, herpes, and ringworm infections after boiling with water while roots powder was used to cure venereal diseases (Shaw and Pearn, 1979). The bark of oleander was used as heart tonic, diaphoretic, emetic, diuretic and as expectorant (Patel, 2010). In Homoeopathy, tincture of leaves used for treatment of various diseases like hemiplegia, paralysis, and nervous system diseases (Khare, 2004). The objective of this research project was isolation, purification, characterization, structure elucidation and pharmacological activity of phytochemicals present in *Nerium oleander*.

## MATERIALS AND METHODS

### Plant Material

The plant was collected from Cholistan Desert and the voucher specimen was kept in Biochemistry and Biotechnology Department of Islamia University Bahawalpur.

**Extraction and Isolation**

The air-dried plant (15 kg) was grounded and extracted thrice with methanol for fifteen days at room temperature. The combined methanolic extract was evaporated on a rotary evaporator under reduced pressure to yield the greenish gummy solid (0.85 kg).

The methanolic extract was suspended in water and extracted with *n*-hexane. The process was repeated with hexane till no color in hexane appeared and the combined *n*-hexane solvent was evaporated under reduced pressure on a rotary evaporator to obtain a thick oily material (0.14 kg) of hexane soluble extract. The methanolic extract was again fractionated with chloroform, and on solvent evaporation of this fraction, 0.30 kg chloroform extract was obtained (Khan *et al.,* 2021).

The chloroform soluble fraction was subjected to vacuum liquid chromatographic column eluting with *n*-hexane, *n*-hexane-chloroform, chloroform, chloroform-methanol in increasing order of polarity to obtain 10 fractions labelled as Fraction-1→10.

Fraction 10 was collected and again chromatographed over silica gel column chromatography using the solvent system *n*-hexane: ethyl-acetate in increasing order of polarity to obtain three fractions 10A-10C. Repeated column chromatography on 10A using the same solvent system (*n*-hexane: ethyl acetate) yielded several semi-pure compounds in variable amounts. These compounds were purified using argentic silver gel coated PTLC using the solvent system *n*-hexane:ethylacetate (2.0:8.0) yielded compound **1** (9 mg).

**Characterization of Compound 1:**

Colorless amorphous solid **MP:** 210 °C; **IR ($CHCl_3$):** $\upsilon_{max}$ $cm^{-1}$ 3479 (OH), 2950 (CH- stretching), 1730 (acetate), 1622 (C=C), 1650, 895 (terminal double bond), 1089 (C-C), 812 (C-H bending); **$^1$H-NMR** ($CDCl_3$, 500 MHz), and **$^{13}$C-NMR** ($CDCl_3$, 125 MHz) See Table 1; **HREIMS:** $[M]^+$ at *m/z* 484.3916 (Calcd. for $C_{32}H_{52}O_3$, 484.7535); **EIMS** *m/z* (rel int %): $[M]^+$ 484, 443 (17), 425 (23),357 (47), 345 (21), 316 (23), 168 (55), 139 (45), 127 (77), 59 (33), 41 (22). Details are shown in Table 1.

**Biological activity:**

The isolated and purified compound **1** was tested for its lipoxygenase activity using Tappel method (Nawaz *et al.,* 2018).

**RESULTS AND DISCUSSION**

**Characterization of isolated compound**

The compound **1** (Fig. 1) was isolated and purified as colorless amorphous solid from chloroform soluble fraction. It gave positive Salkowski as well as Lieberman Burchard tests for steroids (Harborne, 1973). HREIMS displayed molecular ion peak at *m/z* 484.3916 corresponding to molecular formula $C_{32}H_{52}O_3$ with unsaturation index of six. FTIR spectrum disclosed the presence of hydroxyl (3479 $cm^{-1}$), acetoxy (1730 $cm^{-1}$) and terminal olefinic (1650 $cm^{-1}$, 895 $cm^{-1}$) moieties in the molecule. In EIMS spectrum, two characteristics peak appeared at *m/z* 316 and 168 due to ring D fission which is specific for steroidal molecules. These peaks depicted the presence of an extra hydroxyl moiety in the side chain of molecule.

The $^1$H-NMR spectra of compound **1** was a typical of steroidal nucleus which showed two quaternary methyl singlets at $\delta$ 1.12 and 0.88 due to Me-19 and Me-18 respectively. Furthermore, appearance of sharp singlets at $\delta$ 2.01 (Me-2′), 1.72 (Me-27), 1.05 (Me-28), 1.04 (Me-29) and 0.91 (Me-30) revealed the existence of five tertiary methyl groups in the structure. While doublet at $\delta$ 0.92 ($J$ = 7.0 Hz) was consistent with the presence of a secondary methyl at C-21. A trisubstituted olefinic proton resonated as triplet at $\delta$ 5.52 ($J$ = 2.0 Hz) due to $\Delta^5$ moiety. Spectrum further suggested the presence of terminal olefinic moiety and hydroxymethine proton at $\delta$ 4.90, 4.80 (1H each, s, H-26) (Dai *et al.,* 2001) and $\delta$ 3.91 (1H, m, H-24) in the molecule, respectively.

BB and DEPT $^{13}$CNMR spectral data confirmed the presence of thirty-two carbons including eight methyl, ten methylene, seven methine and seven quaternary carbon atoms respectively. Among these resonances, signals at $\delta$ 171.0 (C-1′), 145.1 (C-5), 121.9 (C-6), 147.2 (C-25) and 110.6 (C-26) were assigned to acetyl functionality and olefinic carbons of steroid, respectively.

Further confirmation of structure of the compound was done by 2D NMR spectroscopy. In HMBC experiments (Fig. 2) the proton at C-3 (δ 4.66 $J$ = 8.8, 5.3) showed $J^2$ correlation with C-4 (δ 38.4) and C-2 (δ 23.2). It also exhibited $J^3$ correlations with C-1 (δ 38.2), C-5 (δ 145.1) and C-1′ (δ 171.0) and C-28 (δ 1.05). The methyl protons at C-28 (δ 1.05) and C-29 (δ 1.04) gave $J^2$ correlations with C-4 (δ 38.4) and $J^3$ correlations with C-3 (δ 79.2) and C-5 (δ 145.1). All these correlations helped in placing the acetate moiety at C-3 position and higher $J$ value indicated the $\beta$ conformation of hydroxyl moiety which is analogous to biogenesis of steroidal molecules. An important task

was to give the appropriate position to hydroxyl moiety present in the side chain either it is present at C-23 or C-24. The most important correlation which helped to complete this task is the correlations of hydrogen present at C-24 (δ 3.91) position. It showed $J^2$ correlations with C-23 (δ 32.5) and C-25 (δ 147.2) and $J^3$ correlations with C-22 (δ 32.0), C-26 (δ 110.6) and C-27 (δ 17.1). Terminal olefinic protons (δ 4.90, 4.80) gave $J^2$ correlations with C-25 (δ 147.2) and $J^3$ correlations with C-24 (δ 75.2) and C-27 (δ 17.1). These correlations showed that the olefinic moiety is attached at C-25 position while hydroxyl group is present at C-22 (Mendes *et al.,* 2009). The hydroxyl moiety at C-24 assigned *β* configuration because in NOESY correlation the hydrogen at this carbon was showing signal with C-21 which is in *α* configuration in steroidal structure (Fig. 3). This cumulative data leads us to assign the structure as 3*β*-acetoxy-5, 25 (26) diene, 24 *β* hydroxy lanostane.

**Table 1.** $^{1}$H-NMR (500 MHz, $CDCl_3$) and $^{13}$C-NMR (125 MHz, $CDCl_3$) of compound **1** with *J* values (Hz) in parenthesis.

| **Position No.** | **$^{\delta}$H** | **$^{\delta}$C** |
|---|---|---|
| 1 | 2.01 m<br>1.21 m | 38.2 |
| 2 | 2.07 m<br>1.90 m | 23.2 |
| 3 | 4.60 dd (8.8, 5.3) | 79.2 |
| 4 | - | 38.4 |
| 5 | - | 145.1 |
| 6 | 5.52 t (2.0) | 121.9 |
| 7 | 1.85 m<br>1.84 m | 28.7 |
| 8 | 1.71 m | 38.3 |
| 9 | 1.23 m | 49.4 |
| 10 | - | 35.1 |
| 11 | 2.82 m<br>1.72 m | 21.4 |
| 12 | 1.34 m<br>0.86 m | 21.3 |
| 13 | - | 45.0 |
| 14 | - | 43.1 |
| 15 | 1.84 m<br>1.74 m | 33.3 |
| 16 | 1.66 m<br>1.62 m | 32.0 |
| 17 | 1.47 m | 51.5 |
| 18 | 0.88 s | 16.0 |
| 19 | 1.12 s | 19.9 |
| 20 | 1.49 m | 36.1 |
| 21 | 0.92 d (7.0) | 18.5 |
| 22 | 1.41 m<br>1.31 m | 32.0 |
| 23 | 1.70 m<br>1.80 m | 32.5 |
| 24 | 3.91 m | 75.2 |
| 25 | - | 147.2 |
| 26 | 4.90 s<br>4.80 s | 110.6 |
| 27 | 1.72 s | 17.1 |
| 28 | 1.05 s | 26.2 |
| 29 | 1.04 s | 24.5 |
| 30 | 0.91 s | 14.1 |
| 1′ | - | 171.0 |
| 2′ | 2.01 s | 20.5 |

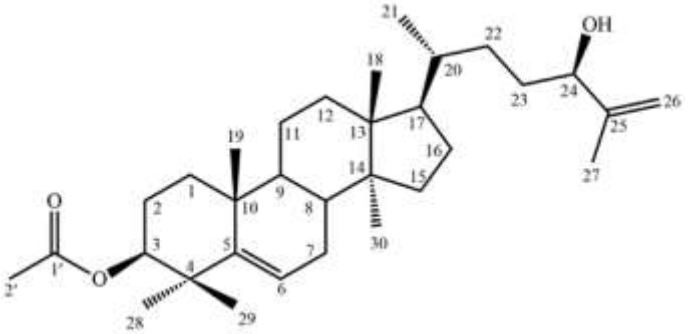

**Fig. 1.** Structure of isolated compound.

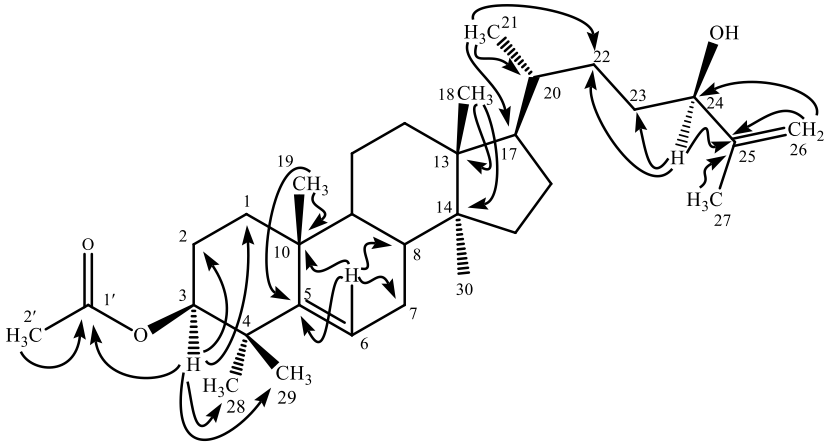

**Fig. 2.** Structure of compound **1** with important HMBC correlations.

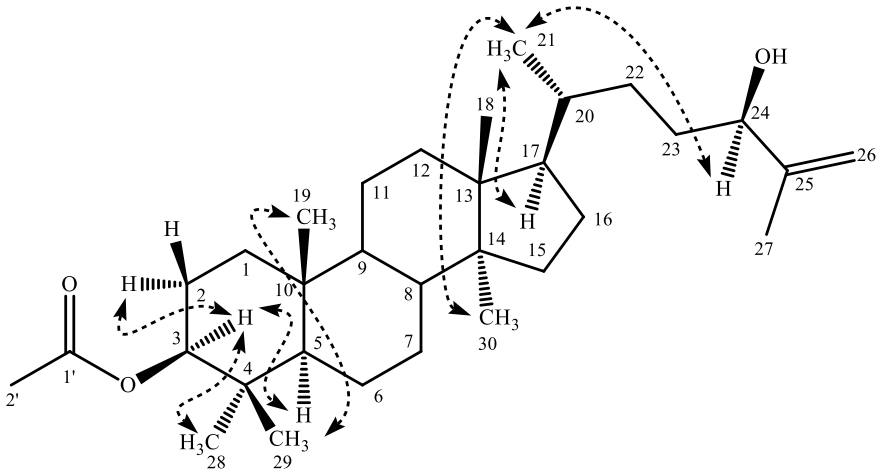

**Fig. 3.** Structure of compound **1** with important NOESY correlations.

**Biological activity**

The purified compound was tested for inhibition of lipoxygenase enzyme. Lipoxygenases (LOXs) are important iron containing groups of enzymes which are responsible for biosynthesis of different bioregulatory compounds in animals including leukotrienes, lipoxins and hepoxylines (Werz and Steinhilber, 2006). This study focused on inhibition of 5-LOX pathway as it leads to the formation of 5,6-epoxy leukotrienes, which are responsible for inflammatory disorders in human body. This compound significantly inhibited lipoxygenase enzyme with $IC_{50}$ value of 42.5 ± 0.10. Baicalein was used as positive control with $IC_{50}$ of 22.0 ± 0.04. This value of baicalein is in

accordance with that reported in literature (Choudhary *et al.,* 2009). In literature various steroids have been reported with moderate to significant lipoxygenase inhibition (Arshed *et al.,* 2017, Sajid *et al*., 2018)**.**

## CONCLUSIONS

This study was conducted to explore the pharmacological potential of phytochemicals present in *Nerium oleander*. A new steroid from lanostane class was isolated and after characterization and structure elucidation, it was identified as 3*β*-acetoxy-5, 25 (26) diene, 24 *β* hydroxy lanostane. This lanostane exhibited moderate inhibition of lipoxygenase (5-LOX) enzyme and may be a part of medicine for inflammation after future studies.

## CONFLICT OF INTEREST

Authors have no conflict of interest

## REFERENCES


Arshed, F., E. Ahmed, A. Sharif, A. Sajid, M. Arshad, H.N. Khalid and A. Razaq (2017). Two new lipoxygenase inhibiting constituents from *Pervoskia abrotanoides*. *Journal of Chemical Society of Pakistan,* 39(6): 1084-1088.

Banaras, S., A. Javaid and I.H. Khan (2020). Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina*. *International Journal of Agriculture and Biology,* 24(5): 1376-1382.

Banaras, S., A. Javaid and I.H. Khan (2021). Bioassays guided fractionation of *Ageratum conyzoides* extract for the identification of natural antifungal compounds against *Macrophomina phaseolina*. *International Journal of Agriculture and Biology,* 25(4): 761-767.

Chaudhay, K. and D. N. Prasad (2014). A Review on *Nerium oleander*. *International Journal of Pharmacognosy and Phytochemical Research,* 6: 593-597.

Choudhay, I., Azizuddin, S, Jalil, S.A. Nawaz, K.M. Khan, R.B. Tareen and A. Rahman (2009). Antiinflammatory and Lipoxygenase inhibitory compounds from *Vitex agnus-castusand. Phytotherapy Research*, 23: 1336-1339.

Dai, J., C. Zhao, Q. Zhang, Z. Liu, R. Zheng and L. Yang (2001). Taraxastane type triterpenoids from *Saussurea petrovii*. *Phytochemistry,* 58: 1107-1111.

Ferdosi, M.F.H., I.H. Khan, A. Javaid, T. Sattar and A. Munir (2020). Identification of antimicrobial constituents in essential oil of *Paulownia fortunei* flowers. *Mycopath,* 18(2): 53-57.

Ferdosi, M.F.H., A. Javaid, I.H. Khan, S. Ahmad and N. Shad (2021). Analysis of *n*-butanol flower extract of *Cassia fistula* through GC-MS and identification of antimicrobial compounds. *Pakistan Journal of Phytopathology,* 33(1): 103-107.

Harborne, J.B. (1973). *Phytochemical method*. 1st Ed. Tokyo, Japan; Toppao Company Ltd.

Hseini, S. and A. Kahouadji (2007). Ethnobotanical study of medicinal flora in the region of Rabat (Morocco Western). *Lazaroa,* 28: 79-92.

Javaid, A, I.H. Khan and M.F.H. Ferdosi (2021). Bioactive constituents of wild *Cannabis sativa* roots from Pakistan. *Pakistan Journal of Weed Science Research,* 27(3): 359-368.

Javed, S., Z. Mahmood, K.M. Khan, S.D. Sarker, A. Javaid, I.H. Khan and A. Shoaib (2021). Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. *Scientific Reports,* 11: 8417.

Khan, I.H. and A. Javaid (2013). Antifungal activity of *Melia azedarach* L. fruit extract against *Sclerotium rolfsii*, the cause of collar rot disease of chickpea. *Mycopath,* 11(1): 9-13.

Khan, I.H. and A. Javaid (2019). Antifungal, antibacterial and antioxidant components of ethyl acetate extract of quinoa stem. *Plant Protection,* 3(3): 125-130.

Khan, I.H. and A. Javaid (2020a). Anticancer, antimicrobial and antioxidant compounds of quinoa inflorescence. *Advancements in Life Sciences,* 8(1): 68-72.

Khan, I.H. and A. Javaid (2020b). Antifungal activity of leaf extract of *Cannabis sativa* against *Aspergillus flavipes*. *Pakistan Journal of Weed Science Research,* 26(4): 447-453.

Khan, I.K., A. Javaid and S.F. Naqvi (2021). Molecular characterization of *Penicillium expansum* isolated from grapes and its management by leaf extract of *Chenopodium murale*. *International Journal of Phytopathology,* 10(1): 29-35.

Khare, C.P. (2004). *Encyclopedia of Indian Medicinal Plants.* Springer-Verlag Heidelberg, pp. 328-330.

Mendes, C.C., L.Q. Sandes, F.G. Cruz and D.F. Roque (2009). New (9bH) -Lanostanes and lanostanes from *Mikania* aff. *jeffreyi*, (Asteraceae). *Chemistry & Biodiversity,* 6: 1463-1470.

Naqvi, S.F., I.H. Khan and A. Javaid (2020). Hexane soluble bioactive components of *Chenopodium murale* stem. *Pakistan Journal of Weed Science Research,* 26(4): 425-432.

Nawaz, Z., E. Ahmed, A. Sharif, M. Arshad, A. Sajid, F. Arshed and A. Razaq (2018). Two triterpenyl fatty acid esters from *Fagonia cretica* as lipoxygenase inhibitors. *Journal of Chemical Society of Pakistan,* 40: 406-409.

Patel, G. (2010). Physiological evaluation and qualitative chemical examination of methanolic extract of *Nerium Indicum*. *International Journal of Biomedical Research*, 1: 209-213.

Sajid, A., E. Ahmed, A. Sharif, F. Arshed, M. Arshad, M. Sher, A. Sajid and S. Amanat (2018). Bioassay directed isolation studies on *Hypericum oblongifolium*. *Journal of Chemical Society of Pakistan,* 40(1): 249-254.

Sasidharan, S., Y. Chen, D. Saravanan, K.M. Sundram and L.Y. Latha (2010). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary, and Alternative Medicines,* 8(1): 1-10.

Shaw, D. and J. Pearn (1979). Oleander poisoning. *The Medical Journal of Australia,* 2: 267-269.

Uroos, M., A. Javaid, A. Bashir, J. Tariq, I.H. Khan, S. Naz, S. Fatima and M. Sultan (2022). Green synthesis of coumarin derivatives using bronsted acidic pyridinium based ionic liquid [MBSPy][HSO4] to control an opportunistic human and a devastating plant pathogenic fungus *Macrophemina phaseolina*. *RSC Advances,* 12: 23963-23972.

Werz, O and D. Steinhilber (2006). Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacology and Therapeutics,* 112: 701-718.

Zhao, M. and L. Bai (2007). Bioactive cardenolides from the stems and twigs of *Nerium oleander*. *Journal of Natural Product,* 70: 1098-1103.

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