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Alkaloid constitution of *Nerium oleander* using gas chromatography-mass spectroscopy (GC-MS)

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Oleander is an evergreen shrub or small tree from 5 to 25-ft tall containing gummy sticky sap in the dogbane family Apocynaceae. In this study, the alkaloid compounds of *Nerium oleander* (NO) have been evaluated. The chemical compositions of the leaf ethanol extract of *N. oleander* were investigated using gas chromatography-mass specroscopy (GC-MS). GC-MS analysis of *N. oleander* alkaloid leaf ethanol extract revealed the existence of the 5-hydrooxy methylfurfural, β-d-allopyranoside, methyle 6-dioxy-2-o methyl, cycloheptasiloxane, tetradecamethyle, cyclooctasiloxane, hexadecamethyl, cyclononasiloxane, octadecamethyl, cyclodecasiloxane, eicosamethyl, 2-cyclopenten-1-one, 2-hydroxy-3-methyl, 9.12.15-octadecatrienoic acid, 2,3bis[trimethylsilyl]oxy] propyl ester, octadecane, 3-ethyl-5-(2-ethylbutyl), 1-monolinoleoylglycerol trimethylsilyl ether, 1.1.3.3.5.5.7.7.9.9-decamethyl-9-(2-methyl propoxy)pentasiloxane, 2-cyclohexen-1-one, 4-(hydroxybutyle)-3.5.5-trimethyl, octasiloxane, 1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15-hexadecamethyl and 3-eicosene. Eight chemical alkaloids constituents have been identified from methanolic extract of the *Nerium oleander*.

Key words: Alkaloids, methanol, gas chromatography-mass spectroscopy (GC-MS) analysis, *Nerium oleander*.

INTRODUCTION

Nerium oleander (NO), common as wild plant in stony torrent beds or dry rocky valleys in foothills of Kurdistan up to altitude 800 m and as ornament plant throughout the country (Figure 1). The leaves contain neriin and oleandrin. Oleandrin is 3-glucoside-16-acyle derivative of gitoxigenin. They are poison to man, sheep, goat, cattle and horses. Root, bark and seeds contain the toxic principles neriodorin and karabin. The effects of poison are nausea, vomiting, colic dizziness, decreased pulse rate, irregular heart action, bloody diarrhea, and respiratory paralysis and death. Oleander is originally a Mediterranean and Asian plant and is widely distributed in the world, especially in tropical and subtropical regions. The plant is grown throughout Iran and is more common in eastern and southern provinces (Aslani, 2004). Oleander has long been known to be poisonous to animals and human beings. All parts of the plant either fresh or dried are toxic and contain cardiac glycosides,

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where the roots and seeds having the highest concentrations. Themost prominent of those glycosides are oleandrin and neriin (Aslani et al., 2007).

The main cardiac glycoside of *N. oleander* is oleandrin with a molecular formula of $C_{32}H_{48}O_9$ and a molecular mass of 576.3. The common oleander is one of most poisonous plants that have been shown to contain digitalis cardiac glycosides. Oleander is an idiom for plants of the *Nerium oleander* L, *Nerium indicum*, and *Nerium odorum* species. Common names include soland, lorier bol, rosebay, and rose laurel and kaner (Sazada et al., 2009). All parts of the oleander plant contain cardiac glycosides, including the roots and the smoke produced from burning, as heat does not inactivate the glycosides. The toxic components are the two potent cardiac glycosides; oleanderin and neriin, which can be isolated from all parts of the plant, both are very similar to the toxin of Foxglove (Shumaik et al., 1988; Cheeke, 1998; Singh et al., 1998). Oleandrin and neriin are the most potent of those glycosides (Longforad and Boor, 1996; Aslani et al., 2004). Oleander is one of the most toxic and lethal plants in birds. Accidental and/or experimental cases of oleander poisoning have been described in several species, including cattle (Aslani and Rezakhani, 2000; Soto-Blanco et al., 2006), sheep (Adam et al., 2002), goats (Aslani et al., 2007; Barbosa et al., 2008), camels (Kozikowski et al., 2009), horses (Hughes et al., 2002), donkeys (Smith et al., 2003), cats (Giuliano and Nebbia, 2004), dogs (Szabuniewicz et al., 1972), monkeys (Schwartz et al., 1974), turkeys, geese (Alfonso et al., 1994), canaries (Arai et al., 1992), ducks and budgerigars (Shropshire et al., 2003). The objective of this study was to determine the alkaloid compounds of *N. oleander*.

MATERIALS AND METHODS

Collection and preparation of plant

N. oleander leaves were collected from Hilla city Iraq. After collection of the required quantity of the plant material, it was then carefully segregated, cleaned and dried in shade to constant weight. The completely dried plant material free of moisture was powdered and sieved through a BSS Mesh No. 85 sieve and then stored in an airtight plastic container. Fresh areal parts of plant were collected for preparation of decoction.

Extraction and identification of alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniac layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

Wagner's test: To the filtrate in tube III, 1 ml of Wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test: To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Mayer's test: To the filtrate in test tube I, 1 ml of Mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis of the methanol extract of *N. oleander* was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo Mass Gold Perkin-Elmer Turbo Mass 5.1 spectrometer with an elite – 1 (100% Dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system, and was operated in electron impact mode with ionization energy of 70 eV. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature rose up to 280°C, at the rate of an increase of 5°C min^{-1}, and maintained for 9 min. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 ml was employed (split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 100°C min^{-1} to 200°C, then 5°C min^{-1} to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45 to 450 (m/z). The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.2 (Imad et al., 2014b; Imad et al., 2015b; Mohammed et al., 2015) .

RESULTS AND DISCUSSION

GC-MS analysis of alkaloid compound clearly showed the presence of fifteen compounds. The alkaloid compound, formula, molecular weight and exact mass are as shown in Table 1. The GC-MS chromatogram of the 15 peaks of the compounds detected are as shown in Figure 2. Chromatogram GC-MS analysis of the methanol extract of *N. oleander* showed the presence of fifteen major peaks and the components corresponding to the peaks were determined as follows. The first set up peaks were determined to be 5-hydrooxy methylfurfural, β-d-allopyranoside as shown in Figure 3. The second peaks indicated to be methyle 6-dioxy-2-o methyl (Figure 4). The next peaks was considered to be cycloheptasiloxane, tetradecamethyle, cyclooctasiloxane, hexadecamethyl, cyclononasiloxane, octadecamethyl, cyclodecasiloxane, eicosamethyl, 2-cyclopenten-1-one, 2-hydroxy-3-methyl, 9.12.15-octadecatrienoic acid 2,3bis

Table 1. Compounds present in the leaves extract of *N. oleander* using GC-MS analysis.

Alkaloid compound	RT (min)	Formula	Molecular Weight	Exact mass	Structure
2-Cyclopenten-1-one, 2-hydroxy-3-methyl	4.952	$C_6H_8O_2$	112	112.0524297	Figure 3
5-Hydrooxy methylfurfural	6.686	$C_6H_6O_3$	126	126.031694	Figure 4
β-d-allopyranoside, methyle 6-dioxy-2-o methyl	9.043	$C_8H_{16}O_5$	192	192.099773	Figure 5
2-Cyclohexen-1-one, 4-(hydroxybutyle)-3.5.5-trimethyl	11.904	$C_{13}H_{22}O_2$	210	210.16198	Figure 6
3-Eicosene	13.449	$C_{20}H_{40}$	280	280.313002	Figure 7
1-Monolinoleoylglycerol trimethylsilyl ether	15.938	$C_{27}H_{54}O_4Si_2$	498	498.356064	Figure 8
9.12.15-Octadecatrienoic acid,2,3bis[trimethylsilyl]oxy] propyl ester	17.329	$C_{27}H_{52}O_4Si_2$	496	496.340414	Figure 9
Octadecane, 3-ethyl-5-(2-ethylbutyl)	19.818	$C_{26}H_{54}$	366	366.422552	Figure 10

Figure 1. Plant of *Nerium oleander*.

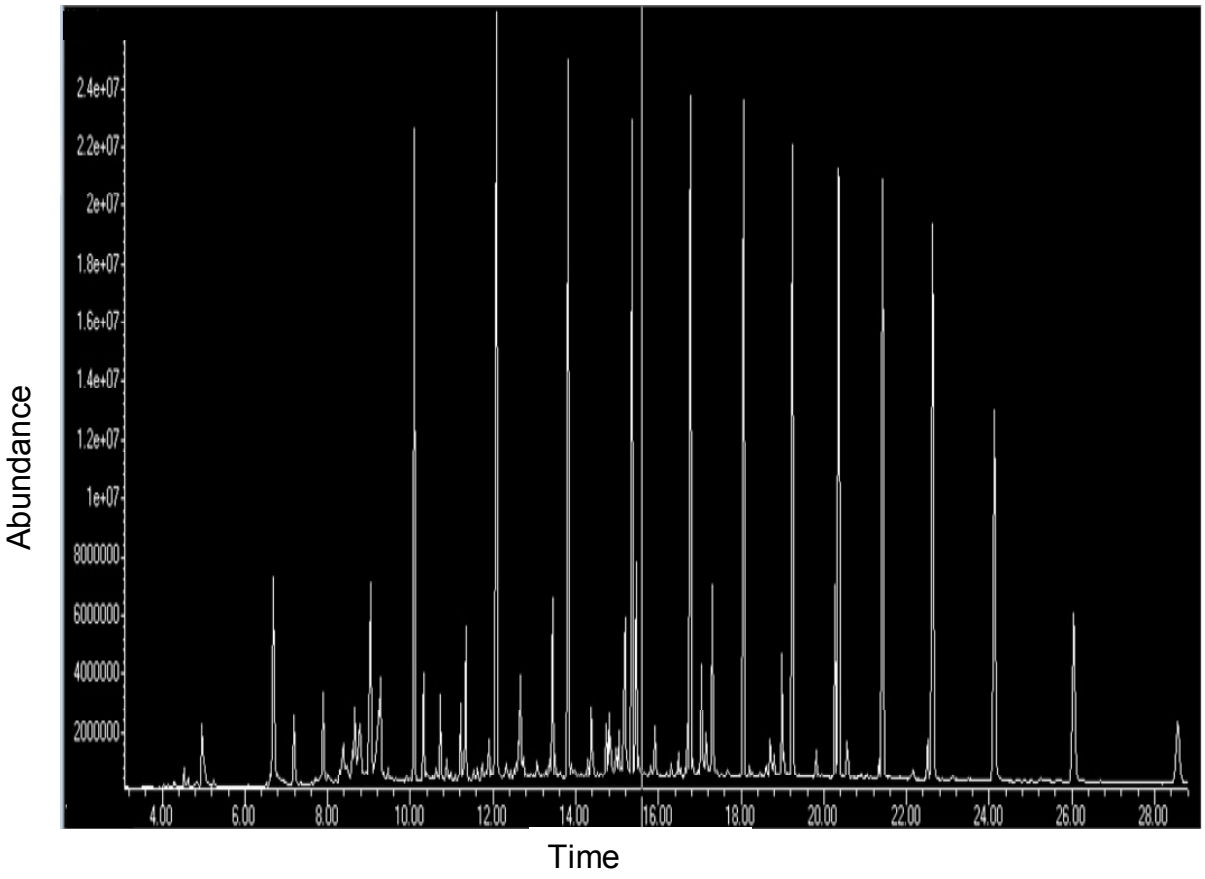

Figure 2. GC-MS Profile of leaves extract of *Nerium oleander*.

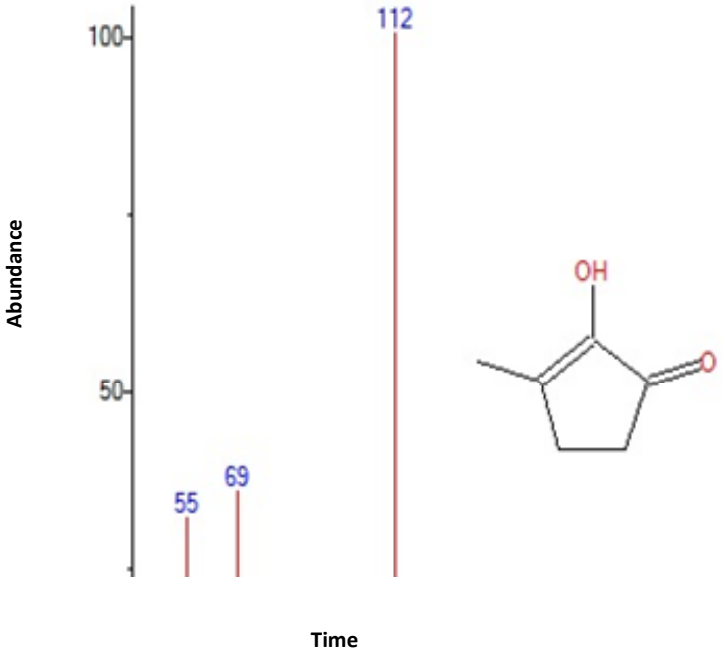

Figure 3. Structure of 2-Cyclopenten-1-one, 2-hydroxy-3-methyl present in the leaves extract of *N. oleander* using GC-MS analysis.

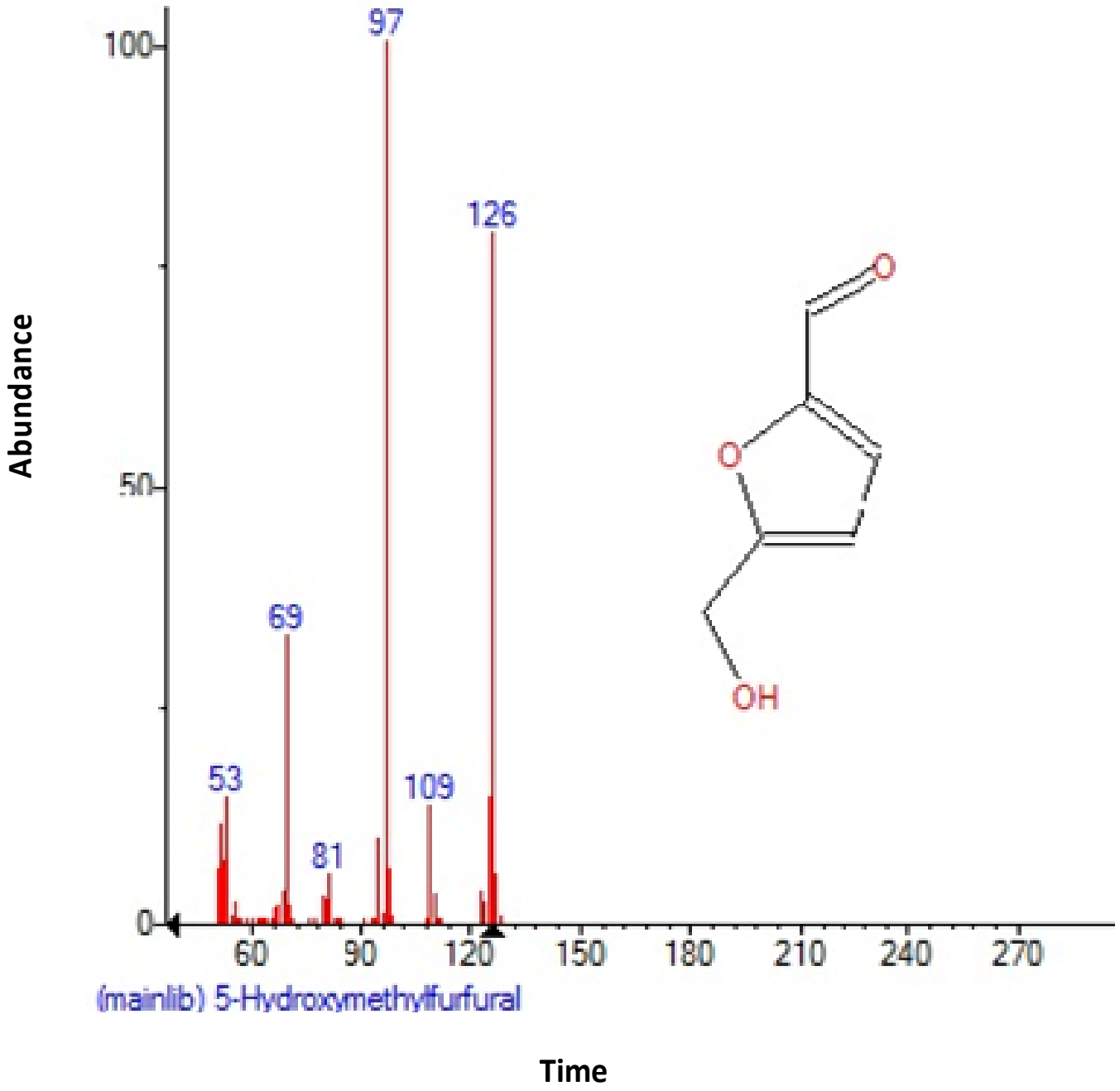

Figure 4. Structure of 5-Hydrooxy methylfurfural present in the leaves extract of *N. oleander* using GC-MS analysis.

[trimethylsilyl]oxy] propyl ester, octadecane, 3-ethyl-5-(2-ethylbutyl), 1-monolinoleoylglycerol trimethylsilyl ether, 1.1.3.3.5.5.7.7.9.9-decamethyl-9-(2-methyl propoxy)pentasiloxane, 2-cyclohexen-1-one, 4-(hydroxybutyle)-3.5.5-trimethyl, octasiloxane, 1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15-hexadecamethyl and 3-eicosene (Figures 5 to 10). Among the identified phytocompounds have the property of anti oxidant and antimicrobial activities (Kumar et al., 2001; Avci and Dik, 2014; John and Senthilkumar, 2005; Venkatesan et al., 2005; Santh, 2006). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects. Continued further exploration of plant derived antimicrobials is needed today.

Conclusion

Fifteen chemical alkaloids constituents have been

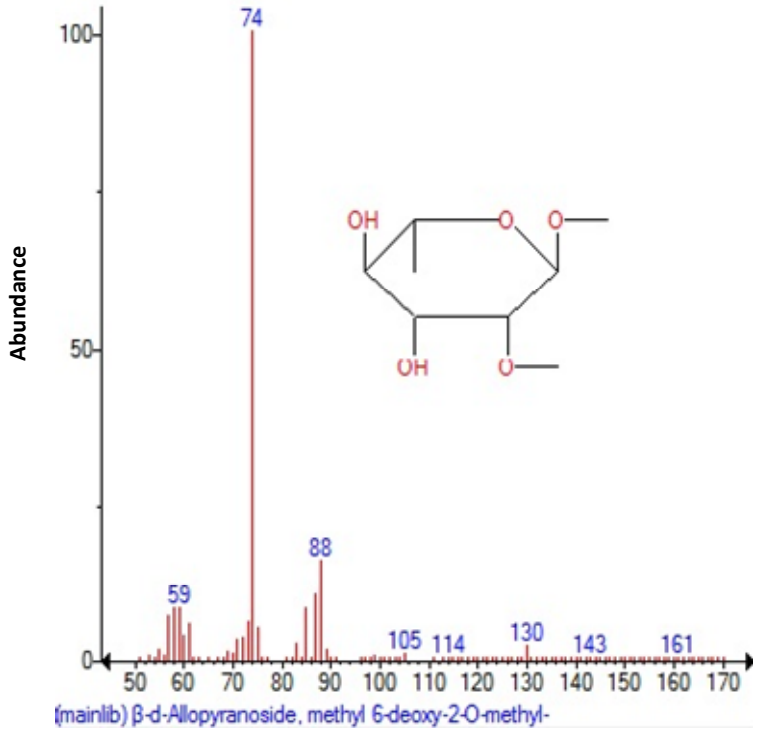

Figure 5. Structure of β-d-allopyranoside, methyle 6-dioxy-2-o methyl present in the leaves extract of *N. oleander* using GC-MS analysis.

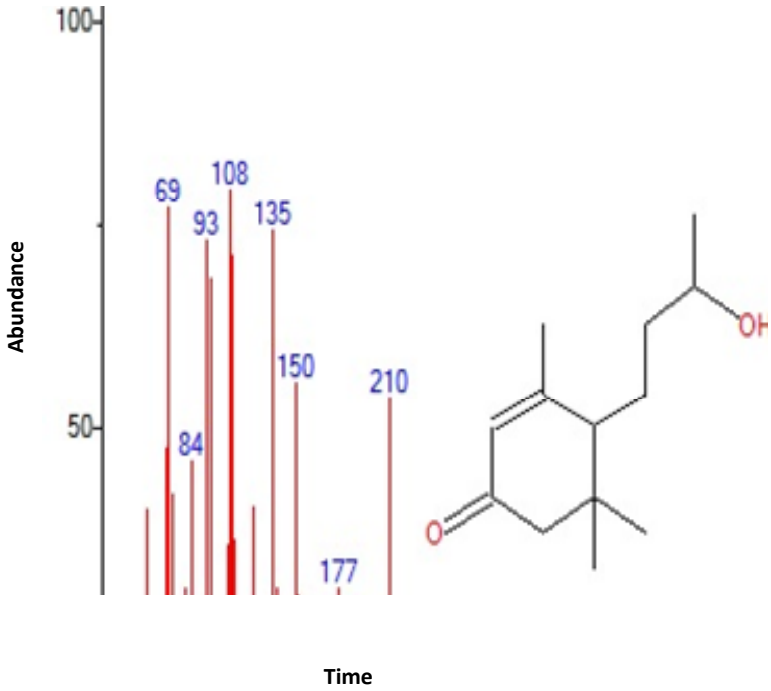

Figure 6. Structure of 2-Cyclohexen-1-one, 4-(hydroxybutyle)-3.5.5-trimethyl present in the leaves extract of *N. oleander* using GC-MS analysis.

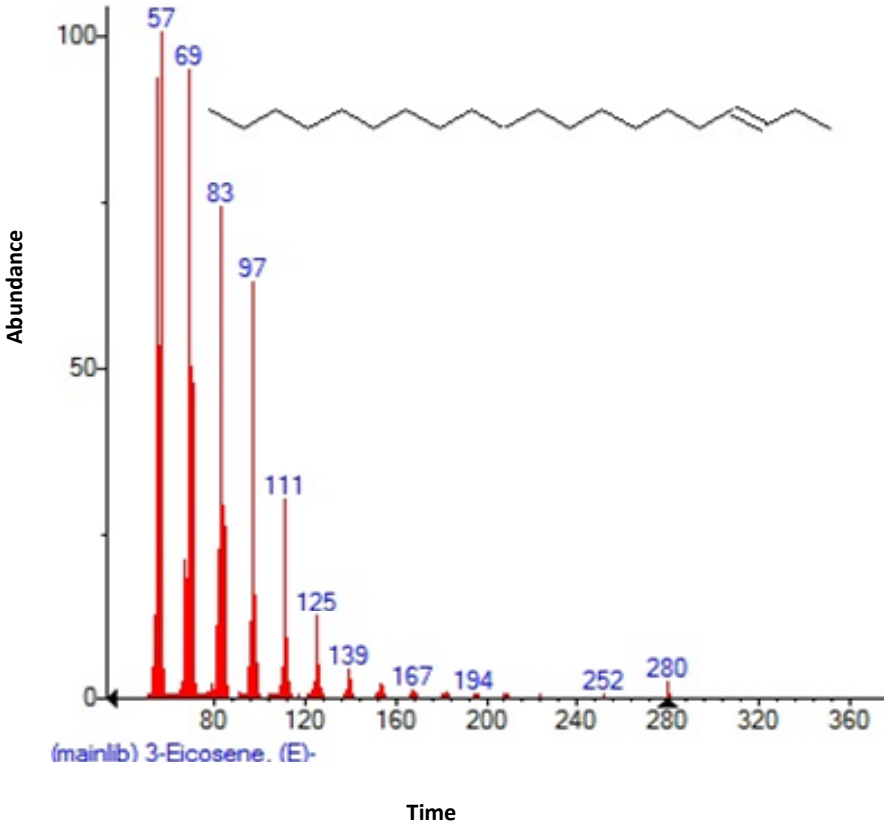

Figure 7. Structure of 3-Eicosene present in the leaves extract of *N. oleander* using GC-MS analysis.

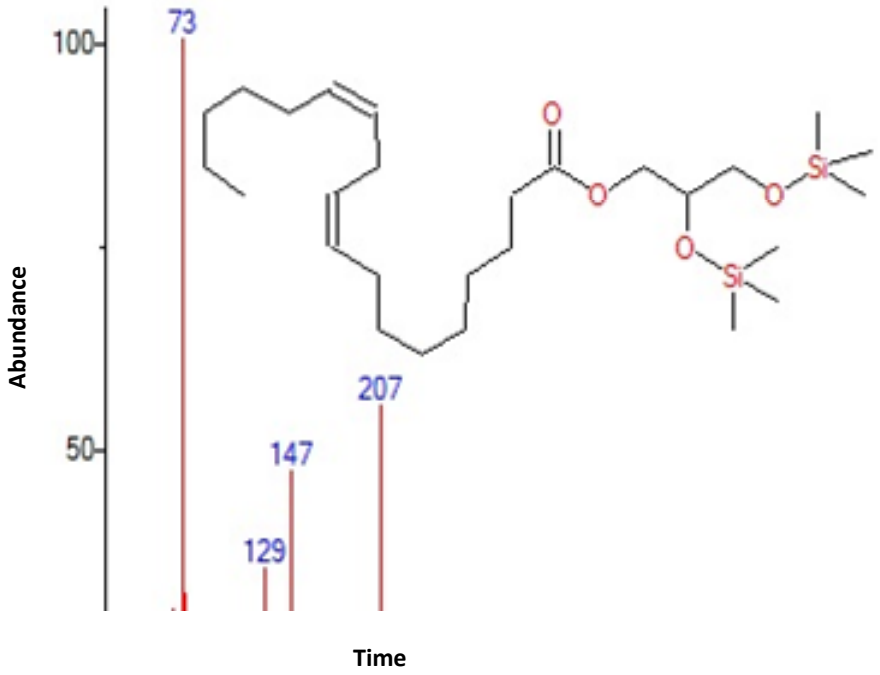

Figure 8. Structure of 1-Monolinoleoylglycerol trimethylsilyl ether present in the leaves extract of *N. oleander* using GC-MS analysis.

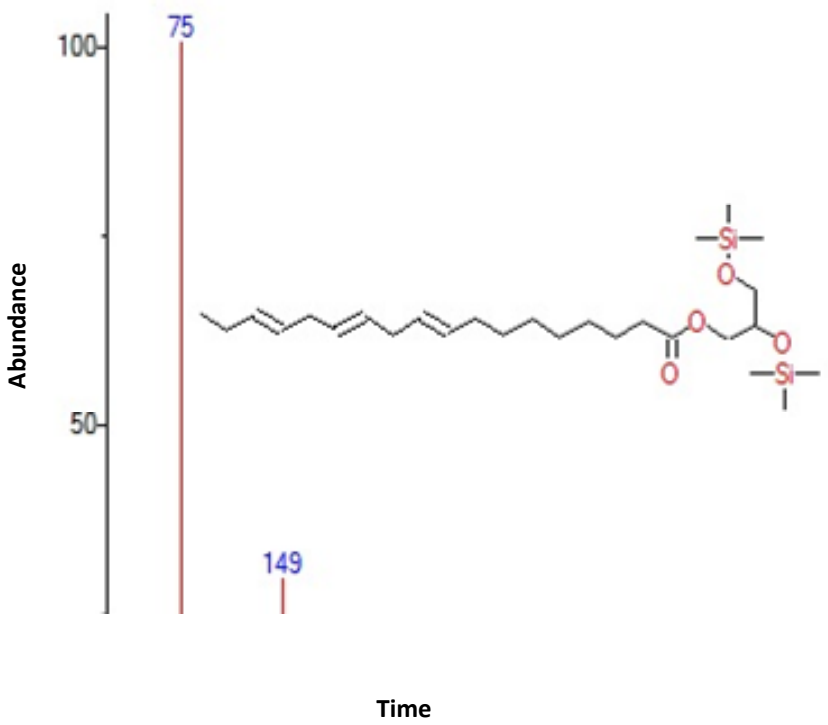

Figure 9. Structure of 9.12.15-Octadecatrienoic acid, 2,3 bis[trimethylsilyl]oxy] propyl ester present in the leaves extract of *N. oleander* using GC-MS analysis.

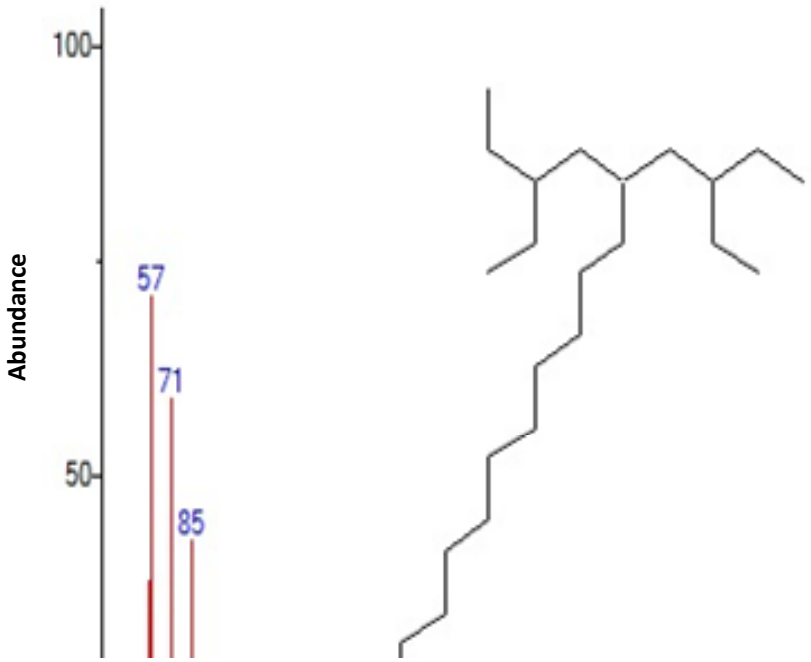

Figure 10. Structure of Octadecane, 3-ethyl-5-(2-ethylbutyl) present in the leaves extract of *N. oleander* using GC-MS analysis.

from methanolic extract of the Nerium oleander by Gas Chromatogram Mass spectrometry (GCMS).

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Conflict of interests

The author(s) have not declared any conflict of interests.

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