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GC-MS Profile and toxicity study of essential oil, hydrosol and crude extracts of *Nerium oleander* L. Leaves

Idowu, Mary O.* and Aboaba, Sherifat A.

Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria

*Corresponding author: Idowumaryolamilekan@gmail.com

Abstract: *Nerium oleander* L. also known as oleander, belongs to the Apocynaceae family. It is an evergreen ornamental shrub that grows in tropical, subtropical, and Mediterranean climates. Essential oil and hydrosol were obtained from the plant material by hydro-distillation, methanol extract by solvent extraction which was fractionated with n-hexane and ethyl acetate. Gas chromatograph**y-m**ass spectrophotometr**y** (GC-MS) was employed for analyses of the extracts, and Brine Shrimp Lethality Assay for the toxicity study. The GC-MS analyses of the essential oil, hydrosol, n-hexane, ethyl acetate and methanol extracts afforded 38, 32, 27, 31, and 37 compounds, respectively, representing 99.99, 99.98, 99.99, 100, and 100 % of the total constituent**s** respectively. The profile of the constituents can be described as terpene, fatty acid, amide, phenolic and aromatic compounds, respectively. Major components were (+)-intermedeol (18.63 %), 1-chloroheptacosane (23.76 %), squalene (15.02 %), 2-O-methyl-d-xylose (14.04 %), 2-O-methyl-d-xylose (48.77 %) respectively. The **b**rine **s**hrimps **l**ethality **a**ssay yielded LC_{50} values of 317332.39, 0.00, 0.00, 1532.00 and 185587578.80 ppm**,** respectively. The LC_{50} value**s** reveal that essential oil, ethyl acetate and methanol extracts were non-toxic, indicating their safety for biological applications in pharmaceuticals, cosmetics, and food products, while hydrosol and n-hexane extract demonstrated significant toxicity, suggesting promising bioactive potential. These findings highlight the variability in the oil composition and toxicity profiles of *N. oleander* leaf extracts obtained using different solvents and identify the potential of the extracts for exploration in the treatment of diseases.

Key words: *Nerium oleander,* GC-MS, *Artemia salina*, Toxicity.

Introduction

Nerium oleander L. also known as oleander is an evergreen ornamental shrub. It grows widely in tropical, subtropical, and Mediterranean climates and belongs to the Apocynaceae family. It has linear and leathery leaves with various colors from dark green to grey-green with separate light yellowish veins. The flowers of *N. oleander* are funnel-shaped and fragrant, with white to pink to deep red colors. *N. oleander* is 2-5 m in height and the leaves are 5-20 cm long. The fruit has a narrow sheath containing many silky-haired seeds.[1,2] *N. oleander* has pink, red, white and yellow varieties. The phytochemical screening as previously reported showed that *N. oleander* contained alkaloids, flavonoids, carbohydrates, tannins, phenolics, saponins, cardenolides, cardiac glycosides, pregnanes, triterpenoids, triterpenes and steroids.[1,2] Prior pharmacological studies revealed that *N. oleander* possessed antioxidant, anticancer, antimicrobial, antiparasitic, anti-inflammatory, dermatological, analgesic hypolipidemic, antidiabetic, cardiovascular and central nervous effects as well as antibiofilm activities.[1,2] *Nerium oleander* seed oil exhibited good physiochemical properties for biodiesel production.[3] Recent studies have also highlighted that oleander possesses favorable anti-tumor and anti-viral properties.[4]

This paper reports the constituents and toxicity profile of the essential oil, hydrosol, methanol crude extract and fractions of *N. oleander* leaves grown in Ibadan, South Western Nigeria. It also compares the constituents of the essential oil with the previous work. This study is the first to investigate the GC-MS profile of both hydrosol and ethyl acetate extracts of *N. oleander* leaves.

Materials and methods

Sample collection and preparation

Fresh leaves of *N. oleander* were collected at the University of Ibadan botanical garden, Ibadan, Oyo state, Nigeria during the rainy season and were identified and authenticated by Mr. Donatus at Botany Department (Herbarium section) of the University of Ibadan where the voucher number UIH-23460 was deposited. The fresh leaves were air-dried and pulverised using an electronic pulverizing machine to increase the surface area and achieve effective extraction of the

constituents of the essential oil and crude extracts from the plant sample.

Extraction of essential oil and hydrosol

Essential oil was extracted from 300 g air-dried pulverised *N. oleander* leaves by hydro-distillation for 3 hours using an all-glass Clevenger-type apparatus. The essential oil was trapped in 2.0 mL of n-hexane and then collected in a pre-weighed sample vial. It was stored in a refrigerator at 4 ^{o}C before to analysis.

Hydrosol was extracted by adding 1 mL of chloroform to 50 mL of *N. oleander* essential oil distillate in a separating funnel and was shaken at intervals for 10 minutes. The organic layer was collected in a pre-weighed sample vial, weighed and kept in a refrigerator before spectroscopic analysis and cyto-toxicity bioassay.

Extraction and preparation of crude extract

Crude extract was obtained from 850 g of air-dried pulverized plant sample using methanol for 72 hours (3 days) after which it was concentrated. Methanol extract was fractionated with n-hexane and the residue was solubilized with water for ethyl acetate fraction. The n-hexane, ethyl acetate and methanol extracts were prepared before the toxicity test by dissolving 20 mg of each extract in 2 mL of their corresponding solvent.

GC-MS analysis of *Nerium oleander* extracts

The GC-MS analysis was performed using Agilent technologies 7890 coupled with MS Agilent technologies 5975 and separation was carried out with the column model Agilent technologies HP5MS (length 30 m, internal diameter 0.320 mm and thickness 0.25 µL). The carrier gas was Helium. The column temperature was programmed as follows: initial temperature was 80 ^{o}C held for 2 minutes at 12^{o}C per minute to the final temperature of 240 ^{o}C held for 6 minutes. Volume injected was 1 µL. The scan ranges between 50 to 500. The mode of analysis was splitless. The interface temperature between GC and MS was 250 ^{o}C.

The constituents were identified using the NIST library by comparing their retention times with those of authentic samples and by comparison of their mass spectra with published spectra as well as reference compounds from the NIST library.

Brine shrimp lethality test

The preliminary toxicity test of the extracts from *N. oleander* leaves was conducted following the **b**rine **s**hrimp **l**ethality test method.[5,6]

Results

The essential oil and hydrosol of *N. oleander* yielded (w/w); 0.47 % and 1.21 % respectively. The compounds discerned from the GC-MS analysis of the essential oil are presented in table 1. Thirty-eight (38) comp-ounds representing 99.99 % of the essential oil constituents were characterised. Monoterpenes (9.72%), oxygenated monoterpenes (20.57%), sesquiterpenes (20.59%), oxygenated sesqui-terpenes (34.25%), diterpenes (0.90%), oxygen-ated diterpenes (1.28%), and non-terpenes (12.68%) were the classes of compounds distributed in the leaf oil. Dominant constituents were the terpenes; (+)-intermedeol (18.63%) and fenchone (14.33%). Moderate constituents were caryophyllene (8.07%), ι-cardinol (7.61%), ɣ-terpinene (3.51%), copaene (2.64%), (Z,E)-α-farnesene (2.43%), ι-muurolol (2.02%) while others are minor. Almanaa *et al*.[7] have reported that the essential oil of *N. oleander leaves* had abundance mono- and sesquiterpenes which is in correlation with this study. The constituents listed were 2-thujene, terpineol, pinene, terpenyl acetate, borneol, spathulenol and octanone. This present work has pinene, borneol and terpeneol, in common with the previous work. However, the present study contained β-caryophyllene, (+)-intermedeol, copaene, (Z,E)-α-farnesene and dehydroabietol which were not reported by Almanaa *et al*. This variation may be as a result of extraction time, purification of the plant material, season and location of collection. The most abundant constituent, (+)-intermedeol, is a sesquiterpene alcohol with diverse biological activities including anti-inflammatory, antimicrobial, antitumor, anti-oxidant, neuroprotective, insecticidal, repellent, and immunomodulatory activity.[1] These observed activities are possibly responsible for the wide applications of *N. oleander* in pharma-ceutical development, agricultural industries and cosmetic formulations.[1]

The characterisation of the hydrosol extract resulted in the identification of 29 compounds representing 99.98% as recorded in table 1. The classes of compounds found in the hydrosol are; monoterpenes (0.85%), oxygenated mono-terpenes (16.86%), sesquiterpenes (1.23%), oxygenated sesquiterpenes (2.89%), fatty acids (10.91%) and non-terpenes (67.24%). The dominant constituents were the non-terpenes; with 1-chloroheptacosane (23.76%) as the highest. It also comprises hexacosane (23.29%) and bis (2-ethylhexyl) phthalate (7.33%). Some of the terpenes present were thymol (14.47%), caryophyllene oxide (1.54%), and caryophyllene (1.23%). The analysis also showed that oleic

acid and palmitic acid, which are fatty acids were present in 4.04% and 3.02%, respectively while others were present in low amounts. The compound, 1-chloroheptacosane is a chlorinated long-chain alkane. Comparing the constituents of hydrosol to that of the essential oil revealed that hydrosol is of more of non-terpene class of compounds than the essential oil due to the physicochemical nature of terpenes (lipophilic, volatile and are heath sensitive in nature). Although hydrosol biological activities have not been as extensively studied as more common bioactive compounds, some reported activities include antimicrobial activity and insecticidal activity.[8]

The analysis of n-hexane extract afforded 27 compounds representing 99.98 % of the total constituents as listed in table 1. Sesquiterpenes (7.00%), diterpenes (1.94%), oxygenated diterpenes (19.10%), triterpenes (15.02%), fatty acids (27.84%) and non-terpenes (29.09%) were classes of compounds distributed in the crude extract. The dominant constituents were the non-terpenes with 29.09% and the dominant compound is squalene (15.02%). Squalene, a natural triterpene hydrocarbon known for diverse biological activities that makes it valuable in pharmaceuticals, cosmetics, and nutraceuticals. Some of the key biological activities of squalene include anti-inflammatory activity, anticancer properties, cardioprotective effects, antioxidant activity, antimicrobial activity, skin benefits, anti-aging effects, immune system modulation.[9,10]

Thirty-one (31) compounds were identified in the ethyl acetate extract (Table 1) representing 100% of the total constituents. The characterised compounds can be classified as; oxygenated monoterpenes (1.41%), sesquiterpenes (2.98%), oxygenated sesquiterpenes (1.69 %), oxygenated diterpenes (0.87%), fatty acids (13.16%) and non-terpenes (79.89%). The dominant constituents were the non-terpenes with; 2-O-methyl-d-xylose (14.04%) been the highest. Others include 2,3-dihydro benzofuran (12.04%), N-methoxy methanamine (11.75%), bis (2-ethylhexyl) phthalate (9.09%) and 2-methoxy-4-vinylphenol (7.35%). The dominant terpene found in the leaf ethyl acetate extract is caryophyllene (2.98%). Other terpenes are fenchone (1.41 %), dehydroabietol (0.87 %), and 1-(1-cyclohexen-1-yl) ethanone (0.77 %) in low amount. It also has palmitic acid (3.83 %) as the dominant fatty acid. 2-O-methyl-d-xylose has its application in nutraceuticals, pharmaceuticals and biotechnology (as a building block in glycan or glycoconjugate research) with antioxidant, anti-inflammatory, glycosylation modulation, anti-tumour and antimicrobial activities.[11]

Table 1 also reveals that methanol extract has 37 compounds representing 100% of the analysed extract. Oxygenated monoterpenes (1.45%), sesquiterpenes (7.00%), oxygenated sesquiterpenes (2.24%), diterpenes (1.29%), oxygenated diterpenes (1.26%), fatty acids (7.11%) and non-terpenes (79.66%) were the classes of compounds profiled in the leaf crude extract. The dominant constituents were the non-terpene derivatives whereby 2-O-methyl-d-xylose (48.77%) dominated as it does for ethyl acetate extract. Others include; 2,3-dihydro benzofuran (8.34%), 2-methoxy-4-ninyphenol (4.98%), chloromethyl methyl sulfide (3.99%), and N-acetyl-di-alanine methyl amide (1.60%). The dominant terpene found in the leaf methanol extract is caryophyllene (4.86%). It also has palmitic acid (2.47 %) as the dominant fatty acid. Zaid *et al.*[12] reported the dominant constituents of methanol extract to be terpenes while in this study the dominant constituents were the non-terpene. This variation may be due to extraction method, season and location of the collection of the plant material.

Generally, all the extracts, essential oil, hydrosol, n-hexane, ethyl acetate and methanol possess caryophyllene as a constituent, but the essential oil has the highest composition of caryophyllene (8.07%). Also, the extracts, except hydrosol and n-hexane, possess fenchone with essential oil having the higher percentage (14.33%). These properties reveal the antioxidant, antimicrobial, antifungal and cardioprotective activities of the extracts.

The n-hexane, ethyl acetate and methanol extracts have stearic acid (2.65%, 0.78% and 0.65%, respectively), 2-methoxy-4-vinylphenol (1.01%, 7.35% and 4.98%, respectively) and 2,3-dihydrobenzofuran (2.66%, 12.04% and 8.34%, respectively) in common. *Nerium oleander* has been reported to exhibit antimicrobial, antioxidant, antidiabetic, anti-inflammatory, and anticancer activities.[13,14] 2-methoxy-4-vinylphenol, also known as 4-vinyl guaiacol, has been reported to possess allelopathic property that influence the growth of neighbouring plants or microorganisms.[15] The minority of some of the constituents may act as contributory factors to the biological activities of the extracts directly or through synergistic interactions with the major compound. The GC-MS chromatograms of the essential oil, hydrosol and crude extract are presented as supplementary data.

Table 1: Chemical constituents of the essential oil, hydrosol, methanol, ethyl acetate and n-hexane of *Nerium oleander*

S/N	CONSTITUENT	Rt (min)	COMPOSITION (%)				
			EO	**Hydrosol**	**Methanol**	**Ethyl acetate**	**n-Hexane**
1	Toluene	3.534	4.81	-	-	-	-
2	p-Xylene	5.565	1.00	-	-	-	-
3	α-Pinene	6.824/6.806	1.53	0.85	-	-	-
4	Cis-sabinene	7.579	0.83	-	-	-	-
5	β-Pinene	7.665	0.86	-	-	-	-
6	1-octen-3-ol	7.762	1.18	-	-	-	-
7	O-Cymene	8.523	1.91	-	-	-	-
8	D-Limonene	8.603	1.91	-	-	-	-
9	Eucalyptol	8.660	1.24	-	-	-	-
10	ɣ-Terpinene	9.107	3.51	-	-	-	-
11	Fenchone	9.645/9.662	**14.33**	-	0.96	1.41	-
12	Fenchol	10.160	0.86	-	-	-	-
13	(+)-2-Bornanone	10.600	1.08	-	-	-	-
14	Endo-Borneol	11.007	0.76	0.49	-	-	-
15	Terpinene-4-ol	11.121	1.77	-	-	-	-
16	Thymol	12.725	1.61	14.47	-	-	-
17	1,5,5-Trimethylene-cyclohexene	13.295	1.21	-	-	-	0.95
18	**β-Caryophyllene**	14.503	**8.07**	**1.23**	**4.86**	**2.98**	**4.87**
19	Trans-α-Bergamotene	14.634	1.02	-	0.80	-	0.84
20	Humulene	14.966	0.75	-	-	-	-
21	β-Selinene	15.395	1.29	-	-	-	0.83
22	(+)-ɣ-Gurjunene	15.470	1.63	-	-	-	-
23	(-)-Spathulenol	16.477	1.17	-	-	-	-
24	Caryophyllene oxide	16.545	1.29	1.54	-	-	-

25	Humulene-6,7-epoxide	16.877	2.51	-	-	-	-
26	Cardina-1(2),4-diene	17.060	0.92	-	-	-	-
27	α-Bulnesene	17.118	1.25	-	-	-	-
28	ι-Cardinol	17.226	7.61	-	-	-	-
29	Copaene	17.272	2.64	-	-	-	-
30	ι-Muurolol	17.392	2.02	-	-	-	-
31	(+)-Intermedeol	17.524	**18.63**	-	-	-	-
32	(Z,E)-α-Farnesene	17.730	2.43	-	-	-	-
33	Isocaryophyllene	17.976	0.76	-	-	-	-
34	α-Cyperone	18.388	0.85	-	-	-	-
35	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	19.990	0.99	2.04	-	-	-
36	Palustradiene	21.129	0.90	-	1.29	-	1.94
37	Dehydroabietinol	23.881	1.28	-	0.64	0.87	1.22
38	Bis(2-ethylhexyl) phthalate	25.506	1.58	7.33	-	9.09	-
39	N-Hydroxymethylacetate	3.590	-	0.51	-	-	-
40	3-Methylbenzyl alcoho TBDMS derivative	5.473	-	0.51	-	-	-
41	(S)-3-Ethyl-4-methylpentanol	8.529	-	0.52	-	-	-
42	Methyl ester benzoic acid	9.748	-	1.47	0.55	1.12	-
43	Undecane	9.793	-	0.70	-	-	-
44	Terpen-4-ol	11.127	-	1.90	-	-	-
45	Trans-Z-α-Bisabolene epoxide	17.392	-	0.60	-	-	-
46	1,6-dimethyl-4-(1-methylethyl)-Naphthalene	17.564	-	0.88	-	-	-
47	2-Bromotetradecane	18.256	-	0.52	-	-	-
48	Hemimellitene	18.960	-	0.64	-	-	-
49	Hexahydrofarnesyl acetone	19.321	-	0.75	-	-	-
50	Methyl palmitate	20.167	-	1.33	1.03	-	1.98
51	Palmitic acid	20.539/20.556	-	3.02	2.47	3.83	14.62

52	(E)-methyl ester-9-octadecenoic acid	21.850	-	1.50	-	-	-
53	Methyl stearate	22.090	-	1.02	-	-	-
54	Oleic acid	22.216	-	4.04	-	-	-
55	Eicosyl vinyl ester carbonic acid	22.422	-	1.52	-	-	-
56	Eicosane	23.629	-	0.68	-	-	-
57	Octacosanol	25.214	-	0.68	-	-	-
58	Heneicosane	25.271	-	2.19	-	-	-
59	Hexacosane	26.885	-	**23.29**	-	-	-
60	1-chloro heptacosane	29.071	-	**23.76**	-	-	-
61	Dimethyl silanediol	3.453	-	-	-	-	4.65
62	2,4,6-trimethyl octane	9.793	-	-	-	-	1.46
63	2,3-dihydrobenzofuran	11.756/11.802	-	-	8.34	12.04	2.66
64	2-methoxyl-4-vinylphenol	13.003/13.009	-	-	4.98	7.35	1.01
65	Bicyclogermacrene	15.464	-	-	-	-	1.27
66	2,4-di-tert-butylphenol	15.567	-	-	-	-	0.79
67	4-O-methylmannose	17.329	-	-	-	-	9.67
68	Pentaldecanal	17.970	-	-	-	-	1.24
69	1,4-Eicosadiene	19.515	-	-	-	-	1.30
70	3,7,11,15-tetramethyl-2-hexadece-1-ol	19.704	-	-	-	-	9.22
71	Abitatriene	21.523	-	-	-	-	1.10
72	2-methylene cis bicyclo[4,3,0]nonane	21.844	-	-	-	-	2.07
73	Phytol	21.941	-	-	0.62	-	8.66
74	Linoleic acid	22.204	-	-	1.48	-	1.57
75	α-linoleic acid	22.216	-	-	-	-	7.02
76	Stearic acid	22.422/22.427	-	-	0.65	0.78	2.65
77	2-(pentyloxycarbonyl) benzoic acid	25.512	-	-	-	-	1.38
78	Squalene	27.989	-	-	-	-	**15.02**
79	Hydroxy-methyl ester acetic acid	3.373/3.293	-	-	1.06	1.68	-

80	N-methoxy methanamine	3.625	-	-	-	11.75	-
81	3,5-dimethylpyrazone	5.033	-	-	-	0.98	-
82	5-methyl-2(3H)-Furanone	6.807	-	-	-	0.73	-
83	Ethyl thiokyanat (Ethyl ester thiocyanic acid)	8.964	-	-	-	1.23	-
84	β-oxo-ɣ-butyrolactone	9.095	-	-	-	0.84	-
85	2,2,3,3-tetramethyl butyramide	9.404	-	-	-	1.47	-
86	1-(1-cyclohexen-1-yl) Ethanone	9.605	-	-	-	0.77	-
87	Methyl-4-(methylthio) butyrate	12.443	-	-	-	0.88	-
88	Trans-Isoeugenol	14.835	-	-	-	1.56	-
89	2-methoxybenzylamine	14.920	-	-	-	1.42	-
90	Nonyl cyclopropane	15.138	-	-	-	4.05	-
91	4-methyl-2,5-dimethoxybenzaldehyde	16.208	-	-	-	1.08	-
92	2,3,5,5,8,8-hexamethyl cycloocta-1,3,6-triene	16.443	-	-	-	0.99	-
93	3,4-dihydro-4,5,7-trimethyl Coumarin	16.986	-	-	-	1.00	-
94	2-O-methyl-d-xylose	17.558/17.427	-	-	**48.77**	**14.04**	-
95	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	18.273	-	-	0.52	4.12	-
96	Fluoroatropine	18.834	-	-	-	1.19	-
97	6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-3-ol	20.104	-	-	1.16	1.69	-
98	Trans-Sinapyl alcohol	20.837	-	-	0.89	1.39	-
99	(Z)-methyl ester-9-octadecenoic acid	21.844	-	-	-	0.73	-
100	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	22.205	-	-	-	2.27	-
101	26-Nor-5-cholesten-3-β-ol-25-one	25.180	-	-	-	4.67	-
102	1,1-bis(methylthio)pentane	3.373	-	-	0.72	-	-
103	Chloromethylmethyl sulfide	3.682	-	-	3.99	-	-
104	3-furaldehyde	5.033	-	-	0.54	-	-
105	Methoxyphenyl oxime	6.853	-	-	0.63	-	-
106	Phenol	7.962	-	-	0.57	-	-

107	1-methyl cyclohexanol	9.101	-	-	0.51	-	-
108	N-acetyl-di-alanine methylamide	9.410	-	-	1.60	-	-
109	Methyl-6-deoxy-2-methyldiacetate mannofuranoside	12.443	-	-	0.65	-	-
110	1-ethenyl-1-methyl cyclohexane	13.293	-	-	0.77	-	-
111	Lactose	14.840	-	-	0.54	-	-
112	(1S,2E,6E,10R)-3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene	15.464	-	-	1.34	-	-
113	1,1-diphenyl ethylene	16.213	-	-	0.71	-	-
114	3-hydroxy-β-damascone	16.826	-	-	0.77	-	-
115	Megastigmatrienone	16.968	-	-	1.17	-	-
116	9-hydroxyl megastigma-4,7-dien-3-one	17.192	-	-	1.07	-	-
117	2-Mercaptobenzothiazole	18.491	-	-	0.53	-	-
118	Loliolide	18.651	-	-	0.49	-	-
119	Neopentyl-4-methoxyphenyl ester carbonic acid	18.828	-	-	0.85	-	-
120	Methyl ester oleic acid	21.844	-	-	1.48	-	-
	TOTAL		**99.99**	**99.98**	**100**	**100**	**99.99**
	Monoterpenes		**9.72**	**0.85**	**-**	**-**	**-**
	Sesquiterpenes		**20.59**	**1.23**	**7.00**	**2.98**	**7.00**
	Diterpenes		**0.90**	**-**	**1.29**	**-**	**1.94**
	Triterpenes		**-**	**-**	**-**	**-**	**15.02**
	Monoterpenoids		**20.57**	**16.86**	**1.45**	**1.41**	**-**
	Sesquiterpenoids		**34.25**	**2.89**	**-**	**1.69**	**-**
	Diterpenoids		**1.28**	**-**	**1.26**	**0.87**	**19.10**
	Sesterterpenoids		**-**	**-**	**2.24**	**-**	**-**
	Fatty acids		**-**	**10.91**	**7.11**	**13.16**	**27.84**
	Others		**12.68**	**67.24**	**79.65**	**79.89**	**29.09**

***EO = Essential Oil**

Table 2 indicates LC_{50} of the brine shrimp lethality test of essential oil, hydrosol, n-hexane, ethyl acetate and methanol extracts. The extract(s) with $LC_{50} \leq 1000$ ppm are considered to be toxic, while extracts with $LC_{50} \geq 1000$ ppm are non-toxic.[16] Therefore, the essential oil, ethyl acetate and methanol extracts of *N. oleander* leaf are non-toxic, while the hydrosol and n-hexane extracts are toxic based on their LC_{50}. The toxicity of extracts has been linked to possession of pesticidal and insecticidal properties.[17]

Table 2: The LC_{50} of the extracts

S/N	Extract	LC_{50} (ppm)
1	Essential oil	317332.39
2	Hydrosol	0.00
3	N-hexane	0.00
4	Ethyl acetate	1532.00
5	Methanol	185587578.80

Conclusion

The GC-MS analysis of the extracts of *N. oleander* revealed that their unique components compounds included terpenes for essential oil, fatty acids for hydrosol, aromatic compounds for methanol, lipophilic substance for n-hexane and amides and phenolic for ethyl acetate extracts. They ~~can~~ have varied constituents base on extraction method and time, purification of the plant material, period and location of plant collection. The LC_{50} of essential oil, methanol and ethyl acetate extracts from the brine shrimps lethality assays showed non-toxic properties, indicating their safety for biological applications in pharmaceuticals, cosmetics, and food products while hydrosol and n-hexane extract demonstrated significant cytotoxicity, suggesting promising pesticidal potential. These findings highlight the variability in the toxicity levels of *N. oleander* leaves extracts and identify the potential of the toxic extracts for further exploration in therapeutic uses. This study enhances the understanding of the toxicity spectrum of plant-derived compounds from *N. oleander* leaves extracts, suggesting their targeted application and usefulness in various applications. To the best of our knowledge, this represents the first report on the essential oil, hydrosol, hexane, ethyl acetate and methanol of *N. oleander* leaves from South West of Nigeria. This is also the first time ~~that~~ the full GC-MS profiling of hydrosol and ethyl acetate extracts of *N. oleander* leaves will be presented.

Conflict of interest

The authors declared no conflict of interest.

Supplementary data

Supplementary data to this article can be found online at Supplementary data section.

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