The Pharmaceutical and Chemical Journal, 2022, 9(1):31-37

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

GC-MS Analysis and Antimicrobial Activity of Sudanese *Nerium oleander* (Apocynaceae) Oil

Abdel Karim, M.*, Sima, A., Mai Mekki

Sudan University of Science and Technology, Faculty of Science, Sudan

Abstract The chemical constituents and antimicrobial activity of *Nerium oleander* oil have been investigated. *Nerium oleander* is an evergreen small tree in the family Apocynaceae. For many years *Nerium oleander* has been mentioned in ancient texts and folklore medicine. All parts of the plant have been used in ethnomedicne. The leave juice is used against eye diseases and snake bite. Bark is expectorant, diuretic, emetic and heart tonic. GC- MS analysis of *Nerium oleander* oil revealed the presence of three major constituents: 9,12-octadecenoic acid methyl ester (34.95%) ; hexadecanoic acid methyl ester (24.85%); methyl stearate (9.66%); 9-octadecenoic acid (Z)-, methyl ester (7.69%) and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-(6.36%). The oil has been evaluated for antimicrobial activity using the cup-plate agar diffusion method. *Nerium oleander* oil showed moderate activity against all test organisms with the exception of the fungal species *Candida albicans*.

Keywords *Nerium oleander*, Oil, Constituents, Antimicrobial Activity

Introduction

Nerium oleander is an evergreen small tree in the family Apocynaceae. The centre of origin of this species is the Mediterranean region and the Indo-Pakistan subcontinent [1]. This draught- tolerant plant is widely grown in tropics, subtropics and temperate regions as an ornamental plant [2].

For many years *Nerium oleander* has been mentioned in ancient texts and folklore medicine. All parts of the plant have been used in ethnomedicne. The leave juice, in small doses, is used against eye diseases and snake bite. Bark is expectorant, diuretic, emetic and heart tonic [1]. Root is used for leprosy, ulcer, hemorrhoids and cancer [3,4]. Roots showed digoxin-like cardiac activity beside antimicrobial effect [5]. Leaves are applied externally for scabies. The flowers are diuretic, cardiotonic, expectorant and emetic [6]. Leaves are also used for baldness and diabtes [7]. The stem extracts showed antimicrobial potency [8]. The antiviral [9], anti-inflammatory [9-11], anticancer [9,12], antimicrobial [13], larvicidal [13-17], immunomodulating [11], antidiabetic [18-20] and diuretic [11] activities of *Nerium oleander* have been reported.

Materials and Methods

Plant Material

Seeds of *Nerium oleander* were collected from Kordofan-western Sudan. The plant was authenticated by direct comparison with reference herbarium sample.

Instruments

GC-MS analysis was carried out on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness).

Test organisms

Nerium oleander oil was assessed for antimicrobial activity using the standard microorganisms shown in Table(1).

Table 1: Test organisms

S. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungi

Methods

Extraction of oil from *Nerium oleander*

Powdered seeds of *Nerium oleander* (350g) were macerated with n-hexane. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

Nerium oleander oil was studied by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium was used as carrier gas. Chromatographic conditions are as follows: **column oven temperature**: 150.0^{o}C ; **injection temperature** : 300.0^{o}C ; **injection mod**e : split : **flow control mode** : linear velocity ; **pressure** : 139.3KPa ; **total flow** : 50.0ml/ min ; **column flow** : 1.54ml/sec. ; **linear velocity** : 47.2cm/sec. ; **purge flow** : 3.0ml/min. ; **spilt ratio** : -1.

Antimicrobial Assay

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for 3 days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for Antimicrobial Activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates , which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test samples.

The agar discs were removed, alternate cup were filled with 0.1 ml sample using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for antifungal assay, but Sabouraud dextrose agar was used for fungal growth instead of nutrient agar. After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

Results and Discussion

Nerium oleander oil was studied by GC-MS which revealed 23 components (Table 2). Fig. shows the total ions chromatograms.

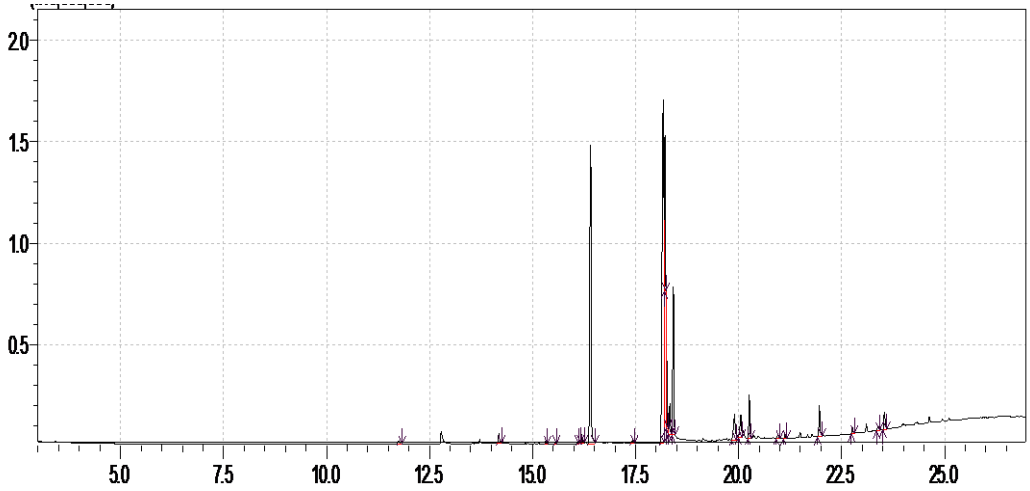

Figure 1: Total ions chromatograms

Table 2: Constituents of the oil

ID#	Name	Ret. Time	Area%
1.	Dodecanoic acid, methyl ester	11.715	0.30
2.	Methyl tetradecanoate	14.135	0.77
3.	Pentadecanoic acid, methyl ester	15.285	0.17
4.	2-Pentadecanone, 6,10,14-trimethyl-	15.525	0.05
5.	7,10-Hexadecadienoic acid, methyl ester	16.065	0.03
6.	7-Hexadecenoic acid, methyl ester, (Z)-	16.130	0.05
7.	9-Hexadecenoic acid, methyl ester, (Z)-	16.175	0.53
8.	Hexadecanoic acid, methyl ester	16.345	24.85
9.	Heptadecanoic acid, methyl ester	17.380	0.20
10.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.100	34.95
11.	9-Octadecenoic acid (Z)-, methyl ester	18.195	7.69
12.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.235	6.36
13.	Phytol	18.305	1.93
14.	Methyl stearate	18.380	9.66
15.	8,11,14-Docosatrienoic acid, methyl ester	19.830	2.71
16.	cis-11-Eicosenoic acid, methyl ester	19.995	1.97
17.	Eicosanoic acid, methyl ester	20.220	2.82
18.	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	20.915	0.33
19.	Heneicosanoic acid, methyl ester	21.085	0.31
20.	Docosanoic acid, methyl ester	21.920	2.18

21. Tricosanoic acid, methyl ester	22.735	0.44
22. 15-Tetracosenoic acid, methyl ester, (Z)-	23.345	0.36
23. Tetracosanoic acid, methyl ester	23.500	1.34

Dominant constituents of the oil are:

i- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (34.05%).

ii- Hexadecanoic acid, methyl ester (24.85%)

iii- Methyl stearate (9.66%)

iv- 9-Octadecenoic acid (Z)-, methyl ester (7.69%)

v- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(6.36%).

Fig. 2 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 18.100) corresponds $M^+ [C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3. The peak at m/z 270 (RT. 16.345) is due to $M^+ [C_{17}H_{32}O_2]^+$. Fig. 4 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T. 18.380) corresponds $M+[C_{19}H_{38}O_2]+$, while the peak at m/z 267 accounts for loss of a methoxyl. The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 5. The signal at m/z296 (RT.18.195) corresponds $M^+ [C_{19}H_{36}O_2]^+$. Fig. 6 illustrates the mass spectrum of 12,15-octadecatrienoic acid, methyl ester. The molecular ion $[C_{19}H_{32}O_2]^+$ appeared at m/z 292 (RT. 18.235).

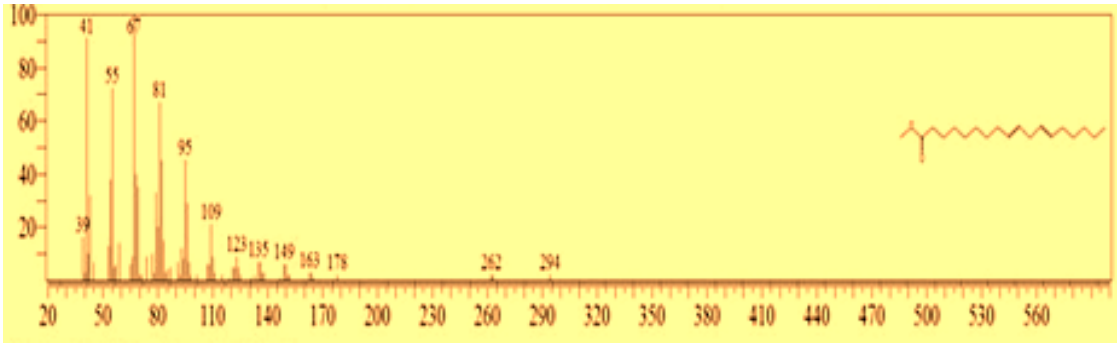

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

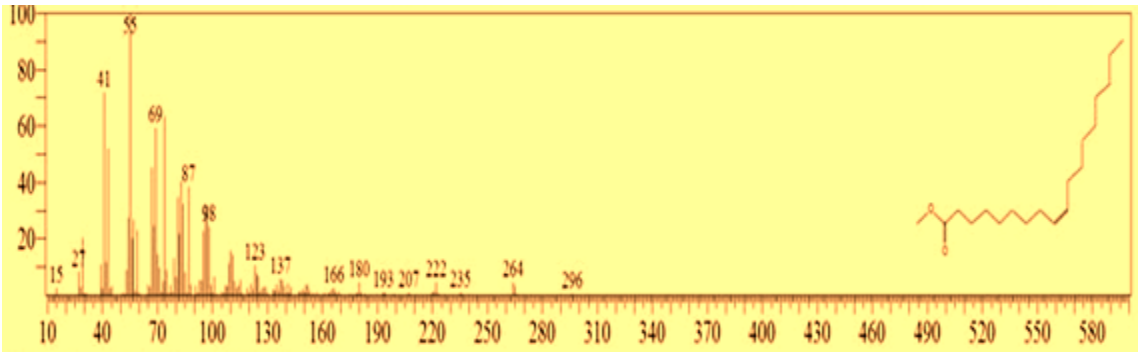

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

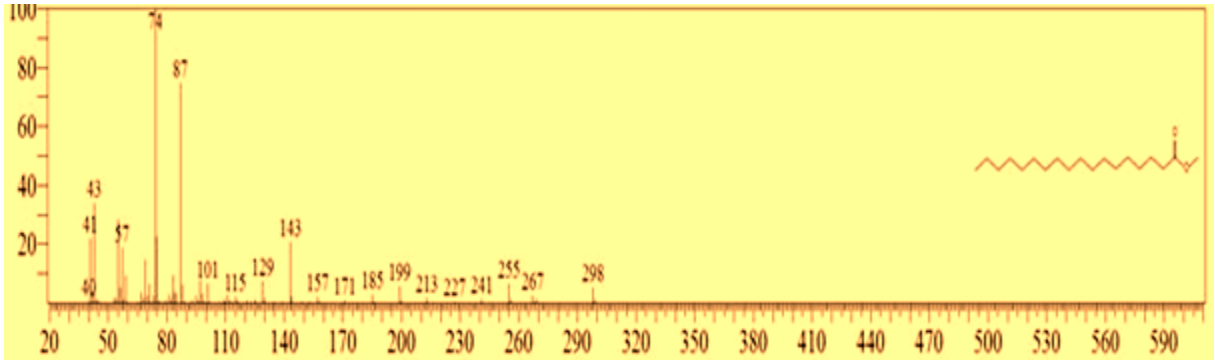

Figure 4: Mass spectrum of methyl stearate

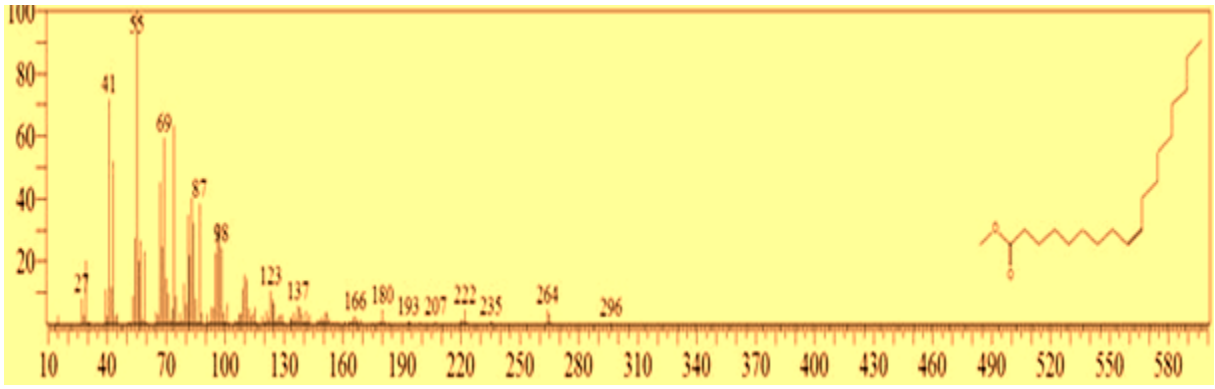

Figure 5: Mass spectrum of 9-octadecenoic acid methyl ester

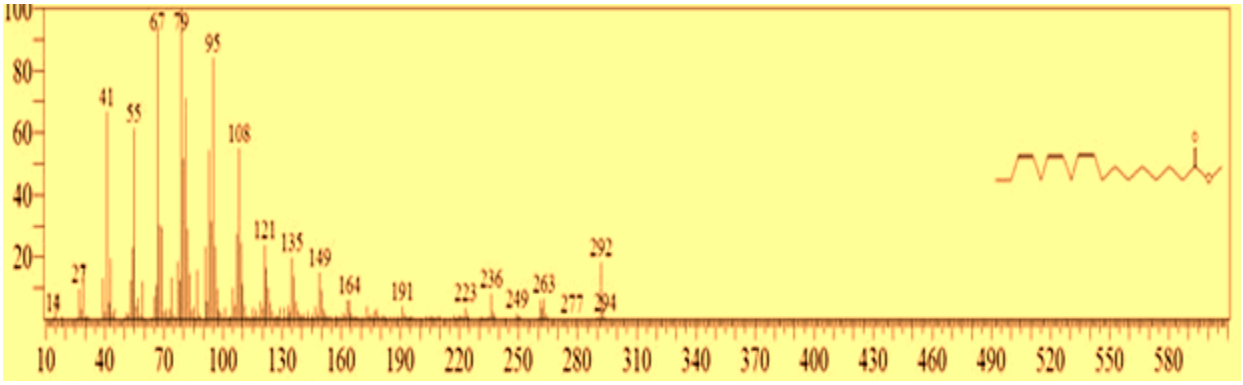

Figure 6: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

Antimicrobial activity

Nerium oleander oil was assessed for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are presented in Table 3. Results were interpreted in the following terms: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed moderate activity against all test organisms with the exception of the fungal species *Candida albicans*.

Table 3: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	14	13	14	15	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*
Bs.: *Bacillus subtilis*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*

References

[1]. Patel G., Physiological evaluation and qualitative chemical examination of Methanolic extract of *Nerium indicum. International Journal of Biomedical Research*, 2020, 1(5): 209–213.
[2]. Kingsbury JM, Poisonous plants of the United States and Canada. *Soil Science*, 1964, 98(5): 349.
[3]. Ahmed US, Ali MS, Begum F & Alimuzzaman M, Analgesic Activity of Methanolic Extract of *Nerium indicum* Mill. *Dhaka University Journal of Pharmaceutical Sciences*, 2006,(1–2): 85–87
[4]. Chauhan S, Singh M, Thakur M & Dogra MS, Antibacterial activity of *Nerium indicum* against some Gram positive bacterial species. *International Journal of Drug Research and Technology*, 2013, 3(1): 8–11.
[5]. Huq MM, Jabbar A, Rashid MA & Hasan CM, A novel antibacterial and cardiac steroid from the roots of *Nerium oleander*. *Fitoterapia*, 1999, 70(1): 5–9.
[6]. Hseini S & Kahouadji A, Ethnobotanical study of medicinal flora in the region of Rabat (Morocco Western). *Lazaroa*, 2007, 28: 79–92.
[7]. Lahsissene H, Kahouadji A & Hseini S, Catalog of medicinal plants used in the region of Zaer (Morocco Occidental). *Lejeunia*, 2009, 186: 0457–4184.
[8]. Jude CA, Extraction, Characterization and industrial applications of Tobacco Seed Oil (*Nicotiana tabacum*). *Chemistry and Materials Research*, 2013, 3(2): 19–21.
[9]. Dey, P. Chaudhuri, T.K., Pharmacological aspects of Nerium indicum Mill: A comprehensive: review. Pharmacognosy Reviews, 2014, 8(16): 156-162.
[10]. Farooqui, S. Tyagi, T., Nerium oleander: it's application in basic and applied science: a review. International Journal of Pharmacy and Pharmaceutical Sciences, 2018, 10(3): 1-4.
[11]. Zibbu, G. Batra, A.: A review on chemistry and pharmacological activity of Nerium oleander L. Journal of Chemical and Pharmaceutical Research, 2010, 2(6): 351-358.
[12]. Ali HFM, El-Ella FMA & Nasr NF, Screening of chemical analysis, antioxidant antimicrobial and antitumor activities of essential oil of oleander (*Nerium oleander*) flower. *International journal of biological Chemistry*, 2010, 4(4): 190–202.
[13]. Sinha, S.N. Biswas, K., A concise review on Nerium oleander L. - An important medicinal plant. Tropical Plant Research, 2016, 3(2): 408-412.
[14]. Gupta PD & Thorsteinson AJ, Food plant relationships of the diamondback moth (*Plutella maculipennis* (Curt)). I. Gustation and olfaction in relation to botanical specificity of the larva. Entomologia Experimentalis et Applicata, 1960, 3: 241–250.
[15]. Jacobson M (1975) Insecticides from plants a review of the literature, 1954–1971. Agriculture Handbook 461, USDA, Washington, DC, P. 138.
[16]. Grainge M (1985) *Plant species reportedly possessing pest-control properties: an EWC/UH database*. Resource Systems Institute, East-West Center (EWC).
[17]. Kumar G, Karthik L, Rao KVB, Kirthi AV & Rahuman AA., Phytochemical composition and mosquito controlling property of *Nerium oleander* leaves (Apocynaceae) against *Culex tritaeniorhynchus* and *Culex gelidus* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, 2012, 2: 1–6.
[18]. Bas, A.L. Demirci, S. Yazihan, N. Uney, K. Kaya, E.E., Nerium oleander distillate improves fat and glucose metabolism in high-fat diet-fed streptozotocin-induced diabetic rats. International Journal of Endocrinology. 2012: Article ID 947187.
[19]. Sikarwar, M.S. Patil, M.B. Kokate, C.K. Sharma, S. Bhat, V. ; Antidiabetic activity of Nerium indicum leaf extract in alloxan-induced diabetic rats. Journal of Young Pharmacists, 2009, 1(4): 333-335.

[20]. Yanardag, S.B. Akkoca, A. Çiçek, F. Ayaz, M.; Effect of distillated Nerium oleander etract on diabetic neuropathy: Animal model study. Journal of Advanced Neuroscience Research., 2015, 2: 16-21.