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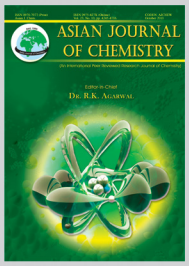

Chemical Constituents and Biological Activity of Lipids, Proteins and Flavonoids of *Nerium oleander* L. Growing in Egypt

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The lipid contents of *Nerium oleander* L. grown in Egypt were analyzed by GLC. A series of hydrocarbons ranging from C_{19}-C_{31} in addition to cholesterol, campasterol, stigmasterol and the triterpenoid α-amyrin were identified. The GLC of the fatty alcohol fraction revealed the presence of 8 fatty alcohols represented about 88 % of the total alcohol. Also, the analysis of the fatty acid fraction revealed the presence of 11 fatty acids in which palmitic acid (27.88 %) represented the main acid. The study of the flavonoidal fraction revealed the isolation and identification of four compounds which proved to be luteolin 4'-methyl ether, luteolin-7-O-glucouronid, apigenin-7-O-galactoside and isorhamnetin-3-O-galactoside. Identification of the isolated compounds was carried out by spectroscopic analysis *viz.*, UV, MS, NMR, TLC, PC and acid hydrolysis of the glycoside compounds. The crude protein of *Nerium oleander* L. was found to be 12.33 %. The analysis of the amino acids isolated from the plant using the amino acid analyzer, revealed the presence of 14 amino acids, in which alanine (15.49 %) represented the main component. The mucilage hydrolyzate of the aerial parts of the plant was found to contain rhamnose, arabinose and galactose by using PC. The radical scavenging effects of the tested extracts and isolated compounds on DPPH free radical were studied, in which fatty alcohol and fatty acid fractions showed low antioxidant activity, also *n*-butanol and the unsap. fractions showed moderate activity. Whereas, the four isolated flavonoidal compounds showed a strong antioxidant activity compared to Trolox (standard antioxidant compound). The different extracts and isolated compounds of the plant exhibited no cytotoxic activity against Ehrlich-ascitis carcinoma cell line at the tested concentrations.

Key Words: *Nerium oleander* L., Lipids, Flavonoids, Protein, Antitumer, Spectroscopic analysis, Radical scavenging.

INTRODUCTION

A great variety of Egyptian medicinal plants is indigenous and yield a wide range of natural products of medicinal values. *Nerium oleander* is an ornamental, ever green shrub with leathery dark green leaves. In Egypt the plant is cultivated in gardens and sometimes grows naturally as escaped from cultivation and belongs to the family Apocynaceae[1,2]. The plant is classified as medicinal plant[3]. It is used in traditional medicine against snake bits and claimed to have cardiotonic, diuretic, antibacterial and insecticidal properties and as cure for boils and guineaworm[4-6] and is mostly used in veterinary practice for toxicity in which all plant parts are toxic if ingested and they can cause death by heart paralysis[7]. From literature, it was found that several taraxasterane-type triterpenes were isolated from the plant[8]. In addition, several steroid glycoside were isolated[9]. The methanol extract of the plant showed antischistosomal and antimicrobial activities[10].

A new triterpenoid, kanerocin, has been isolated from the plant and its structure established through chemical and spectroscopic methods[11]. Also, two new coumarayloxy triterpenoids, neriu coumaric and isoneriucoumacid acid has been isolated from the fresh leaves of the plant[12].

The phytochemical data of *Nerium oleander* L. growing in Egypt revealed the presence of terpenes, flavonoids, coumarins, cardiac glycosides and sterols.

Recently, much attention has been focused on natural antioxidants. Although many natural antioxidants have been found in numerous plant materials[13], tocopherols are only now widely used as the safe natural antioxidant. However, they have the limitation that they are not effective as synthetic antioxidants when used alone[14], beside their high manufacturing costs. With these objectives we tried to isolate a new type of anti-oxidant from medicinal plants, in particular from *Nerium oleander*.

The present work deals with the study of the lipid and flavonoid constituents as well as the protein contents, amino acids and mucilage composition of *Nerium oleander* growing in Egypt. It deals also with evaluation of the antitumer and the antioxidant activity of both the total extracts and the isolated compounds.

EXPERIMENTAL

Nerium oleander L. was collected from the Northern Mediterranean cost (Borg El-arab) near Alex during May 2009. The plant was identified by Dr. M. El-Gebaly and Dr. S. El-Kawashty, Taxonomists, National Research Centre (NRC), Cairo, Egypt. The aerial parts of the plant were air dried and ground into fine powder. A voucher specimen was kept in the herbarium of NRC.

Antitumer activity: The antitumer activity of the extracts and the isolated compounds were tested against *Ehrlich ascites* carcinoma *in vitro* in the National Institute of Cancer, Cairo.

Antioxidant activity: 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Aldrich Chemical Co.), 1,1-Diphenyl-2-picrylhydrazyl (DPPH, Sigma Chemical Co.) and Methanol HPLC.

Shimadzu UV. Pc. 2401 spectrophotometer. Mass spectrophotometer GC-MS finnigan mat SSQ 7000 mass spectroscopy 70 ev. Gas liquid chromatography Hewlett HP 6890 series. Amino acid composition: Lc 3000 Amino acid analyzer (Shimadzu) was used for determination of amino acid composition, the analysis were performed in Pharmaco Central Lab., National Research Centre. Preparative, centrifugally, accelerated, radial, thin layer chromatography by using Chromatotron apparatus. ^{1}H NMR spectra were recorded in (DMSO-d_6) on a Jeol-EX-270 MHz spectrometer.

The chemical composition of mucilage was qualitatively studied by analyzing their hydrolyzate by using PC [Whatmann No. 1, ethyl acetate-pyridine-water (12:5:4) and *n*-butanol-benzene-pyridine-water (5:1:3:1)]. GLC analysis were carried out according to the following conditions:

For the unsaponifiable matter and fatty alcohol: Column: HP-1 (methyl siloxane) 30 m length/0.53 μm × 2.65 μm, Temp. program: Ini. temp. 60 ºC, Ini. time 2 min., program rate 10 ºC/min, F. temp. 280 ºC, Final time 0.5 h, injection temp. 260 ºC, detector (FID), T = 300 ºC, flow rate of carrier gas N_2: 30 mL/min, H_2: 35 mL/min, air: 30 mL/min.

For the fatty acids: Methyl esters of fatty acids were analysed on HP-6890 GC. Column: HP-5 (phenyl methyl siloxane) 30 m length/0.32 μm × 2.25 μm, temp. program: Ini. temp. 60 ºC, Ini. time 2 min, program rate 8 ºC/min, F. temp. 270 ºC, final time 10 min, injection temp. 270 ºC, detector (FID), T = 300 ºC, flow rate of carrier gas N_2: 30 mL/min, H_2: 35 mL/min, air: 30 mL/min.

Isolation of lipids[15]: About 800 g of the air dried powdered plant material of *Nerium oleander* was extracted with pet. ether (40-60 ºC). The purified extract (fullers earth) was evaporated and the residue (17.3 g) was dissolved in boiling acetone (250 mL), cooled and the amorphous precipitate formed was separated out (acetone precipitate, 1.32 g). The acetone soluble fraction was saponified (0.5N alc. KOH) and the unsaponifiable matter (1.12 g) was separated. The liberated fatty acid mixture, was extracted, methylated (methanol, 4.5 % HCl). Samples of the isolated fatty alcohols, isolated from the unsaponifiable fraction and the methyl esters of fatty acids were subjected to GLC analysis.

Isolation of flavonoides[16]: About 750 g of the air dried powdered plant material of *Nerium oleander* was macerated with 80 % methanol (3 × 2 L). The combined aqueous alcoholic extracts were evaporated *in vacuo* at 40 ºC. The residue (23.5 g) was dissolved in hot distilled water and left over night. The aqueous filtrate was extracted with successive portions of chloroform (3 × 150 mL), followed by ethyl acetate (3 × 150 mL) and finally with *n*-butanol (3 × 150 mL). The ethyl acetate fraction (0.92 g) was subjected to preparative paper chromatography PPC (3 MM, 20 % acetic acid). The main flavonoidal bands (R_f 0.46 and 0.29) were cut and eluted separately by 90 % methanol. The *n*-butanol fraction (1.05 g) was also subjected to preparative paper chromatography PPC (3 MM, 15 % acetic acid). The main flavonoidal bands (R_f 0.61 and 0.67) were cut and eluted separately by 90 % methanol. The eluted fractions were further purified using Sephadex LH-20 column using 85 % methanol or subjected to centrifugally accelerated rotatory TLC using (silica gel 60 PF_{254}) discs and elution was carried out with 95 % chloroform in methanol.

Preparation of protein[17]: About 50 g of the defatted powdered plant material was stirred in 10 % sodium chloride solution for 1 h then filtered, an equal volume of 10 % trichloroacetic acid solution was added to the filterate. The protein was precipitated as white flocculent amorphous precipitate, collected by centrifugation, washed with 5 % trichloroacetic acid solution, ether and absolute ethanol, then dried in a vacuum desiccator. The crude protein was dialyzed by parchment membrane. The non-dialyzable fraction was collected and lyophilized by freeze drying to afford the protein.

Amino acid analysis: The protein (26.6 mg) of the plant was hydrolyzed with 6N HCl at 105 ºC for 24 h in a sealed tube[18]. After cooling and filtering, the residue was washed with distilled water and the combined filtrates were completed to 25 mL in a volumetric flask. Portion of the filtrate (5 mL) was evaporated to dryness at room temp. in a desiccator under vacuum. The residue was dissolved in 5 mL buffer (0.2N sodium citrate, pH 2.2) and the solution was filtered through 0.22 μm membrane. Twenty μL of the final filtrate were injected in the LC 3000 amino acid analyzer for quantitative determination of the amino acids in the plant. Retention time and amino acids percentages of the plant were calculated as shown in Table-5. The total protein content of *Nerium oleander* was determined by using the Micro-kjeldal method[19].

Study of the mucilage and free sugars of the plant[20]: Twenty grams of the powdered plant material was mixed with 1 L of distilled water slightly acidified with HCl, stirred for 3 h at 25 ºC and left overnight. The solution was filtered and the process was repeated twice again. The aqueous extract was concentrated under vacuum to about 250 mL then mucilage was precipitated from the aqueous extract by adding slowly while stirring 4 volumes of absolute ethanol. The precipitate was separated by centrifugation then washed several times with absolute ethanol, followed by acetone with stirring and filtered and kept in vacuum desiccator over anhydrous calcium chloride. The isolated mucilage from the plant was an odourless substance with mucilaginous taste, soluble in water, insoluble in ethanol, ether and chloroform.

Hydrolysis of the mucilage: 100 mg of the obtained mucilage was heated in 2 mL of 0.5 M sulphuric acid in a sealed tube for 20 h on a boiling water bath. At the end of hydrolysis a brown flocculent precipitate was noticed, which was filtered off and the filtrate was freed of SO_4^{2-} by precipitation

with barium carbonate, filtered and the filtrate was evaporated under vacuum and the residue dissolved in 10 % isopropyl alcohol and chromatogramed on PC using two different systems.

Determination of scavenging effect on DPPH radicals[21]: The decrease of the absorbance at 516 nm of the DPPH solution after addition of the sample (plant materials) was measured in a glass cuvette. An aliquot of 0.1 mL M. methanol solution of DPPH was mixed with the methanolic solution of the sample, so that the relative concentration of plant materials *versus* the stable radical in the cuvette was 0.13, then the solution with tested sample was shaken vigorously. The absorbance was mentioned at the start and at 20 min after being kept in the dark against a blank of methanol without DPPH. All tests were run in duplicate and averaged. The antioxidative of these samples were compared with Trolox.

Where

$$\text{RSA (\%)} = 100\ \% \times \frac{\text{Abs of blank}_{516\ nm} - \text{Abs of sample}_{516\ nm}}{\text{Abs of blank}_{516\ nm}}$$

The results are expressed as radical scavenging activity (RSA %) as shown in Table-6.

RESULTS AND DISCUSSION

Unsaponifiable fraction: GLC analysis for the unsaponifiable fraction of *Nerium oleander* proved to be a mixture of of hydrocarbons ranging from C_{19}-C_{31}, sterols and triterpenes. Identification of the compounds was carried out by comparison of their retention time with the available reference compounds, Table-1.

TABLE-1
GLC ANALYSIS OF UNSAPONIFIABLE FRACTION OF *Nerium oleander*

Peak No.	RT	Relative (%)	Constituents
1	14.71	0.94	C_{19} *n*-nonadecane
2	17.31	2.86	C_{21} *n*-uncosane
3	18.36	2.81	C_{22} *n*-dodacosane
4	19.52	3.06	C_{23} *n*-tricosane
5	21.66	3.52	C_{25} *n*-pentacosane
6	22.04	7.11	C_{26} *n*-hexacosane
7	22.62	1.04	Un-identified
8	24.10	3.67	C_{27} *n*-heptacosane
9	24.63	0.45	Un-identified
10	25.45	9.65	C_{28} *n*-octacosane
11	26.96	12.23	C_{29} *n*- nonacosane
12	27.40	3.57	C_{30} *n*-tricentane
13	28.32	5.74	C_{31} *n*-unitricontane
14	30.81	11.72	Cholesterol
15	32.84	2.9	Un-identified
16	33.26	5.6	campasterol
17	34.88	4.12	Stigmasterol
18	36.47	3.22	Un-identified
19	38.75	6.24	α-amyrin

Fatty alcohol fraction: GLC analysis of the acetone precipitate fraction of *Nerium oleander* proved to be a mixture of 7 fatty alcohols in which heptatriacontanol ($C_{37}H_{76}O$) represented the main component (29.99 %) as illustrated in Table-2.

Fatty acid fraction: GLC analysis of the fatty acid methyl esters resulted in the identification of 11 fatty acids in which palmitic acid (27.88 %) and linolinic acid (13.36 %) were the main components as illustrated in Table-3.

TABLE-2
GLC ANALYSIS OF THE FATTY ALCOHOL FRACTION OF *Nerium oleander*

Peak No.	RRT*	Relative (%)	Constituents
1	0.346	17.84	Hexacontanol ($C_{26}H_{54}O$)
2	0.605	2.84	Hentriacontanol ($C_{31}H_{64}O$)
3	0.707	3.96	Tritriacontanol ($C_{33}H_{78}O$)
4	0.763	7.78	Tetratriacontanol ($C_{34}H_{70}O$)
5	1.00	29.99	Heptatriacontanol ($C_{37}H_{76}O$)
6	1.04	4.69	Un-identified
7	1.07	7.62	Un-identified
8	1.124	14.43	Octatriacontanol ($C_{38}H_{78}O$)
9	1.271	10.64	Nonatriacontanol

RRT* = Relative to retention time of heptatriacontanol ($C_{37}H_{76}O$) 33.6 min.

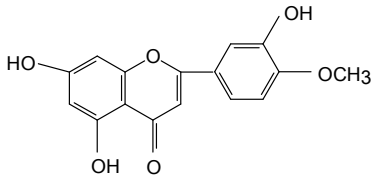
TABLE-3
GLC ANALYSIS OF THE FATTY ACID METHYL ESTERS OF *Nerium oleander*

Peak No.	RRT*	Relative (%)	Constituents
1	0.430	6.452	$C_{10\ (0)}$ decanoic acid
2	0.490	2.53	Un-identified
3	0.730	4.97	C_{12} lauric acid
4	0.802	1.17	Un-identified
5	0.865	7.45	C_{14} myristic acid
6	0.928	4.33	C_{15} pentadecanoic
7	1.000	27.88	$C_{16\ (0)}$ palmitic acid
8	1.176	3.65	$C_{18\ (0)}$ stearic acid
9	1.258	7.91	$C_{18\ (1)}$ oleic acid
10	1.308	6.81	$C_{18\ (2)}$ linoliec acid
11	1.426	13.36	$C_{18\ (3)}$ linolenic acid
12	1.448	2.48	Un-identified
13	1.488	1.84	Un-identified
14	1.557	5.31	$C_{20\ (0)}$ arachidonic acid
15	1.630	1.98	C_{22} docosanoic acid

RRT* = Relative to retention time of palmitic acid C_{16} (0) (17.49 min).

Flavonoids

Compound I: Luteolin 4′-methyl ether: The flavonoidal band (R_f 0.13) isolated from the ethyl acetate fraction by preparative paper chromatography (3 MM, 20 % acetic acid) and eluted by 90 % methanol, gave after purification on Sephadex LH_{20} column (90 % methanol), a single flavonoidal compound corresponding to that of luteolin 4'-methyl ether (13 mg) which identified by TLC, PC, UV[22], Table-4 and MS [M^+ 300 and fragment ions at m/e 258 (M^+-CH_3), 272, 152, 148] which are characteristic for luteolin 4'-methyl ether[23].



Luteolin 4`-methyl ether

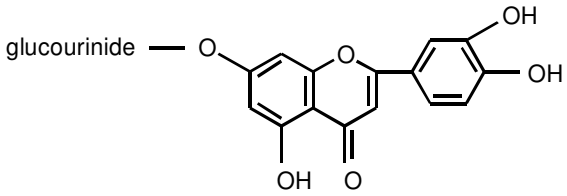
Compound II: Luteolin 7-O-glucouronide: The flavonoidal band (R_f 0.29) isolated from the ethyl acetate fraction by preparative PC. (3 MM, 20 % acetic acid) and eluted by 90 % methanol, gave upon purification by on Sephadex LH_{20} column (90 % methanol), a single flavonoidal compound corresponding to that of Luteolin 7-O-glucouronide (16 mg) which identified by TLC, PC, UV[22]. Acid hydrolysis (2N HCl) gave luteolin which identified by TLC, PC, UV[22] and MS.

TABLE-4
UV SPECTRAL DATA (nm) OF THE ISOLATED FLAVONOIDS

Flavonoids	UV. abs in MeOH	NaOMe	$AlCl_3$	$AlCl_3$ + HCl	NaOAc	NaOAc + H_3BO_3
Compound **I**	348, 290 sh, 269, 252	387,301sh 271	394, 356 292,270	381,352 294,278	372,324 274	345, 263, 253sh
Compound **II**	349, 266, 252	397,302sh, 266	436, 333, 292 sh, 277	389, 361, 276	409, 371 sh, 271 sh, 262	375, 261
Compound **III**	331, 294 sh, 271 sh, 268	397, 272, 267	376 sh, 331 271, 264	331, 289, 265	381, 272, 267	354, 273, 266
Compound **IV**	351, 30 6sh 266	413, 273	406, 352, 293, 272	399, 345, 294 sh, 274	382, 272	357, 307sh 273

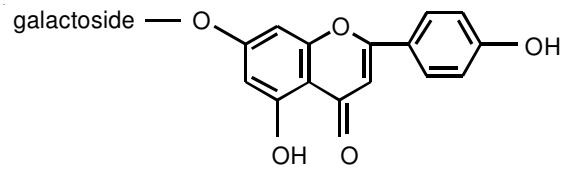
Sh = Shoulder, Compound **I** = Luteolin 4'- methyl ether, Compound **II** = Luteolin 7-O-glucouronide, Compound **III** = Apigenin 7-O-galactoside, Compound **IV** = isorhamnetin 3-O-galactoside.

The sugar moiety was identified as glucuronic acid by using [PC. Whatman No. 1, *n*-butanol-benzene-pyridine-water (5:1:3:3) and ethyl acetate- pyridine-water (12:5:4)].



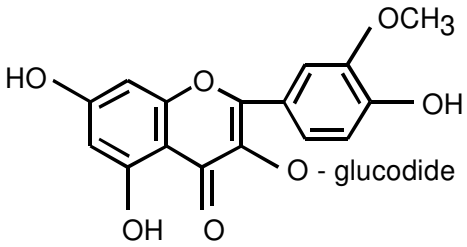
Luteoline 7- O - glucouronide

Compound III: Apigenin 7-O-galactoside: The flavonoidal band (R_f 0.44) isolated from the *n*-butanol fraction by preparative PC. (3 MM, 20 % acetic acid) and eluted by 90 % methanol, gave after purification by using centrifugally accelerated rotatory TLC using (silica gel 60 PF_{254}) discs and elution with chloroform/methanol (85:15), a single flavonoidal compound corresponding to that of apigenin 7-O-galactoside (25 mg) which identified by TLC, PC, UV[22]. Acid hydrolysis (2N HCl) gave apigenin which identified by TLC, PC, UV[22] and MS [M^+ 272, in addition to fragment ions at m/e 243, 152, 154,118 which are characteristic to apigenin[23]. The sugar moiety was identified as galactose by [PC. Whatman No. 1, ethyl acetate-pyridine-water (12:5:4)].



Apigenin 7 - O - galactoside

Compound IV: Isorhamnetin 3-O-galactoside: The flavonoidal band (R_f 0.67) isolated from the *n*-butanol fraction by preparative PC. (3 MM, 20 % acetic acid) and eluted by 85 % methanol, gave after purification on Sephadex LH-20 column (85 % methanol), a single flavonoidal compound corresponding to that of isorhamnetin 3-O-galactoside (19 mg) which identified by TLC, PC, UV[22]. Acid hydrolysis (2N HCl) gave isorhamnetin which identified by TLC, PC, UV[22] and MS [M^+ 316 and fragment ions at m/e 301, 285, 164, 152 which are characteristic for that of isorhamnetin[23]. The sugar moiety was identified as glucose by [PC. Whatman No. 1, ethyl acetate-pyridine-water (12:5:4)].



Isorhamnetin 3 - O - galactoside

The study of the flavonoidal fraction revealed the isolation and identification of four compounds which were proved to be luteolin 4'-methyl ether, luteolin 7-O-glucouronide, apigenin 7-O-galactoside and isorhamnetin 3-O-galactoside. Their identification was proved by TLC, PC, UV and MS analysis, in addition to acid hydrolysis of the flavonoidal glycosides. The preparative centrifugally accelerated TLC using (silica gel 60 PF_{254}) discs has been recommended as one of the best techniques for separation and purification for flavonoids.

Fractionation of the constituents of the pet. ether extract of *Nerium oleander* was carried out and the components of various fractions were identified by GLC. The unsaponifiable fraction was found to contain a series of hydrocarbons ranged from C_{19}-C_{31} in which C_{29} was the main component (12.23 %), cholesterol, campasterol and stigmasterol were represented by 11.72, 5.6 and 4.12 %, respectively and the triterpenoid α-amyrin was also represented by 6.24 %. GLC analysis of the fatty alcohol fraction was proved to be a mixture of 7 fatty alcohols in which heptatriacontanol ($C_{37}H_{76}O$) represented the main alcohol (29.99 %). The study of the fatty acids methyl esters was carried out by GLC analysis which revealed the presence of 11 fatty acids in which palmitic acid (27.88 %) represented the main acid.

The crude protein of *Nerium oleander* was found to be 12.33 %. The analysis of the amino acids isolated from the plant using the amino acid analyzer, revealed the presence of 14 amino acids, in which alanine (15.49 %), threonine (9.84 %) and tyrosine (8.04 %) representing the major components (Table-5). The chemical composition of the mucilage of the aerial parts of the plant (1.53 %) was qualitatively studied by analyzing their hydrolysate using PC (Whatman No. 1), in which arabinose, rhamnose and galactose were characterized from the plant.

The values of the radical scavenging activity (RSA) of the tested extracts and isolated flavonoidal compounds were represented in Table-6. From the RSA % values represented

TABLE-5
AMINO ACIDS ANALYSIS OF THE PROTEINS OF *Nerium oleander*

Peak No.	Amino acids	RT (min)	Amino acids (%)
1	Aspartic acid	10.94	5.16
2	Threonine	14.06	9.84
3	Serine	15.31	5.11
4	Glutamic acid	16.89	6.72
5	Glycine	23.66	4.47
6	Un-identified	24.14	3.11
7	Alanine	25.15	15.49
8	Cystine	26.13	0.12
9	Un-identified	28.61	7.49
10	Valine	29.52	1.64
11	Isoleucine	35.02	2.89
12	Leucine	35.92	5.84
13	Tyrosine	38.16	8.04
14	Phenylalanine	39.85	4.22
15	Histidine	51.06	5.91
16	Un-identified	56.2	6.5
17	Arginine	60.44	7.63

TABLE-6
RADICAL SCAVENGING EFFECT OF SAMPLES ON DPPH FREE RADICAL

Tested compounds	*Absorbance 516 nm/reaction period (min) 10 mins 20 min		RSA (%)
	10 min	20 min	
Trolox	0.017	0.028	93.18
Fatty alcohol	0.157	0.159	52.12
Fatty acid	0.118	0.125	62.73
Unsap.	0.077	0.074	77.12
Compound **I**	0.066	0.067	80.0
Compound **II**	0.051	0.053	84.24
Compound **III**	0.041	0.041	78.43
Compound **IV**	0.049	0.052	84.69
Ethyl acetate extract	0.071	0.078	69.78
Butanol extract	0.085	0.092	73.18

*Absorbance reading at each reaction period are means of two measurements. Compound **I** = Luteolin 4'-methyl ether. Compound **II** = Luteolin 7-O-glucouronide. Compound **III** = Apigenin 7-O-galactoside. Compound **IV** = Isorhamnetin 3-O-galactoside.

TABLE-7
RESULTS OF SCREENING OF ANTITUMOR ACTIVITY OF *Nerium oleander*

Tested compounds	Concentration (µg/mL)	Inhibition of cell viability (%)
Ethanolic extract	25	5
	50	5
	100	10
Ethyl acetate extract	25	10
	50	14
	100	20
Butanol extract	25	10
	50	10
	100	25
Compound **I**	25	15
	50	20
	100	28
Compound **II**	25	5
	50	25
	100	43
Compound **III**	25	7
	50	16
	100	35
Compound **IV**	25	11
	50	14
	100	48

in the table, it is noticed that the unsaponifiable fraction, butanol and ethyl acetate extracts possess a moderate antioxidant activity followed by fatty acid and fatty alcohol fractions. The results also revealed that, all the isolated flavonoidal compounds showed high antioxidant activity compared to Trolox (standard antioxidant compound).

The results of screening for the antitumor activity of different extracts and isolated compounds from *Nerium oleander* L. are summarized in Table-7. It could be concluded that the different extracts and isolated compounds showed low antitumor activity against Ehrlich-ascitis carcinoma cell line at the tested concentrations (25, 50 and 100 µg/mL).

REFERENCES

1. E.A. Omino and L.G. Maesen, The Plant Family Apocynaceae in East Africa. The Biodiversity of African Plants, Proceedings of the 14th AETFAT Congress, Netherlands, pp. 504-506 (1996).
2. F.W. Andrews, Flowering Plants of the Anglo-Egyption Sudan, T. Runcle and Company Ltd., Arbroath Scotland, pp. 1-21 (1985).
3. M. Mulas, B. Perinu, A. Francesconi, C. Johnson and C. Franz, *J. Herbs Species Med. Plants*, **9**, 121 (2002).
4. S. Sushma, D. Singh and S. Singh, *Fitoterapia*, **68**, 545 (1997).
5 R.N. Irvine, Glossary of Indian Medicinal Plant (CSIR), New Delhi, p. 24 (1994).
6. A.M. Sofowoya, Medicinal Plants and Traditional Medicine in Africa, John Wiley & Sons Ltd., England, pp. 196-197 (1982).
7. A. Taklitajan, Woody Plants of Ghana and Africa, Oxford University Press, London, p. 37 (1998).
8. N. Kuniahi, Kokai Tokkyo Koho JP (Japan), **8**, 879 (2007).
9. M. Zhao, L.W. Fu, N. Li, W.X. Tang, M.J. Li, T. Hasegawa, H. Ogura, T. Kataoka, K. Hirose and M. Ando,*Recent Prog. Med. Plants*, **16**, 83 (2007).
10. E.S. Abdel-Hameed, H.A. El-Nahas and S.A. Abo-Sedera, *Pharm. Biol*., **46**, 626 (2008).
11. S. Siddiqui, B.S. Siddiqui, S. Begum and F. Hafeez, *Planta Med.*, **55**, 292 (1989).
12. S. Siddiqui, B.S. Siddiqui, F. Hafeez and S. Begum, *Planta Med.*, **53**, 424 (1987).
13. L.R. Dugan, In eds.: M.G. Simic and M. Karol, Antioxidation in Food and Biological Systems, Plenums: New York, Ch. 17 (1979).
14. N.M. Emanuel and Y.N. Lyskovskaya, In The Inhibition of Fat Oxidation Processes, Pergamon: London; Ch. IV (1967).
15. H.M. Radwan, M.M. El-Missiry and M.M. Seif El-Nasr, *Bull. Fac. Pharma. Cairo Univ.*, **35**, (1997).
16. H.M. Radwan, N.M. Nazif and A.A. Hamdy, *Egypt. J. Appl. Sci*., **17** (2002).
17. J.B. Harbone, Phytochemical Methods, Chapman & Hall, London, New York, edn. 2 (1984).
18. J.L. Bailey, Techniques in Protein Chemistry, Elsevier Publishing Co., London (1967).
19. R.H.A. Plimmer, Practical Organic and Biochem, Longmans Green and Co., New York, pp. 25 amd 1371 (1962).
20. D.J. Bell, Modern Methods of Plant Analysis, Ed., Peach and M.V. Tracey, Springer Verlage, Berlin, Vol. 11, p. 11 (1955).
21. J.H. Chen and C.T. Ho, *J. Agric. Food Chem*., **45**, 2374 (1997).
22. T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identification of Flavonoids Spectrum, Springer Verlage, Berlin (1970).
23. T.J. Mabry and K.R. Markham, The Flavonoids, Chapman & Hall, London (1975).