International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 4 (4): 190-202, 2010
ISSN 1819-155X

Screening of Chemical Analysis, Antioxidant Antimicrobial and Antitumor Activities of Essential Oil of Oleander (*Nerium oleander*) Flower

[1]H.F.M. Ali, [2]F.M.A. El-Ella and [3]N.F. Nasr
[1]Department of Chemistry, Faculty of Science,
Taif University, Kingdom Saudi Arabi
[2]Department of Biochemistry,
[3]Department of Microbiology, Faculty of Agriculture, Cairo University, Egypt

Abstract: In this study, the GC/MS analysis and *in vitro* screening of antioxidant antimicrobial and antitumor activities of essential oil extracted from Oleander flower (*Nerium oleander*) were investigated. The GS/MS analysis recorded 64 components account 94.69% of the total essential oil were composition. Amount of total phenolics was 136.54±3.32 mg as gallic acid/g essential oil. The antioxidant activity was studied by three methods (DPPH assay; β-Carotene/linoleic acid a bleaching assay and ferric reducing power assay). Oleander essential oil had significantly antioxidant activity compared to synthetic antioxidants (trolox and BHT). Antitumor activity was tested as ability of inhibition the growth of ehrlich ascites carcinoma cells line and obtained result indicated gradually increase of antitumor activity with increasing of oil concentration. Antimicrobial activity was observed by agar disc diffusion technique against different strains of Gram-positive; Gram-negative; yeast and mold. The essential oil displayed a variable degree of antimicrobial activity against the different strains tested compared with that of the standard antibiotics tested and the Minimum Inhibition Concentration (MIC) values ranged from 125 to 500 and 250 to 2000 μg mL^{-1} for bacteria and fungi respectively. The toxicity of oleander essential oil was studied in animal model system by different parameters including LD_{50}, diarrhea; (GPT) activity; lactate dehydrogenase (LDH) activity and createnine levels. Toxicity results indicated that no adverse effect recorded with all concentrations of oleander oil range used in present study.

Key words: Oleander, essential oil, antimicrobial, antioxidant, antitumor, chemical analysis, screening

INTRODUCTION

Nerium oleander Linn. (Syn. *N. odorum* Soland; *N. indicum* Mill), distributed in the Mediterranian region and sub-tropical Asia, is indigenous to the Indo-Pakistan subcontinent. The plant is commonly known as kaner and widely cultivated as ornamental plants, but all the parts of the plant are poisonous to man, animals and certain insects and investigations on deferent parts of the plant have revealed the presence of several glycosides, tri-terpenes and straight chain compounds (Langford and Boor, 1996; Begum *et al*., 1999; Soto-Blanco *et al*., 2006; Barbosa *et al*., 2007).

Corresponding Author: Hanaa F.M. Ali, Department of Biochemistry, Faculty of Agriculture, Cairo University ,Giza, Egypt

The chemical composition and possible biological effects of the flower essential oil of *Nerium oleander* has not been studied to date.

A great number of plant essential oils from different origin have been chemically analyzed and reported to possess stronger antioxidant and antimicrobial activities. The biological activity of different essential oil based on their content of phenolic compounds (Wang *et al*., 1998) and terpens compounds according several studies (Burt, 2004; Sacchetti *et al*., 2005; Wu *et al*., 2006; Li *et al*., 2009; Jaroslav *et al*., 2009; Oke *et al*., 2009; Mehmet *et al*., 2009).

The aim of the present study are GC/MS analysis and *in vitro* screening of antioxidant; antimicrobial and antitumor activities of essential oil extracted from Oleander flower.

MATERIALS AND METHODS

Plant Material

Fresh flower of *Nerium oleander* was collected from the different sites of Taif desert, Saudi Arabia during May to June 2009. The plant was identified and authenticated by a botanist at the Plant Department, Faculty of Science Taif university Saudi Arabia (Fig. 1).

Chemicals

Diphenylpicrylhydrazyl (DPPH), methanol, n-hexane, 2-deoxy-2-ribose, β-carotene and linoleic acid, were procured from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Folin-Ciocalteu's phenol reagent and tween 40 and dimethyl sulphoxide (DMSO) were from Merck (Darmstadt,Germany). All chemical reagent used were of analytical grade.

Extraction Method

Batches of 500 g of plant material were submitted to hydrodistillation for 5 h using a Clevenger-type apparatus according to the method recommended in the Council of Europe (2004), using n-hexane (10 mL) as collector solvent. After evaporation of the solvent under N_2 flow, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at -20°C before analyses by Gas Chromatography-Mass Spectroscopy (GC-MS).

GC-MS Chemical Analysis

The GC-MS analysis of the volatile oil was performed on a Varian gas chromatograph interfaced to a Finnigan SSQ 7000 Mass Selective Detector (MSD) with ICIS V2.0 data system

Fig. 1: The fresh flower of *Nerium oleander*

for MS identification of the GC components. The column used was a DB-5 (J and W Scientific, Folsom, CA) cross-linked fused silica capillary column (30 m, 0.25 mm i.d.), coated with polydimethylsiloxane (0.5 mm film thickness). The oven temperature was programmed from 40°C for 3 min, isothermal, then heating by 4°C min^{-1} to 250°C and isothermally for 15 min at 250°C. Injector temperature was 210°C and the volume was 0.5 μL which auto-injected. Transition-line and ion source temperatures were 250 and 150°C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from m/z 40 to m/z 550. Ionization energy was set at 70 eV. The identification of volatile components was based on computer matching with the WILEY275, NIST05 and ADAMS libraries, as well as authentic compounds for major identified compounds (Adams, 2007; NIST, 2005). The quantitative determination was carried out by peak area integrated by the analysis program.

Determination of Total Phenolic Content

Total phenolic contents of the volatile oil were determined as described by Tsai *et al.* (2008), with Folin-Ciocalteu reagent and gallic acid used as a standard. An aliquot (0.2 mL) of the volatile oil was added to a volumetric flask. Then, 46 mL distilled water and 1 mL Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, a 3 mL solution of Na_2CO_3 (7.5%) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The results were expressed as milligrams of Gallic Acid Equivalents (GAEs) per gram of extract.

Antioxidative Assays

DPPH Assay

The antioxidant activity of oleander oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Sahin *et al*., 2004; Sharma and Bhat, 2009). Fifty microliter of oleander oil were added to 2 mL of a 60 μM buffered methanol solution of DPPH (pH = 5.5). Absorbance measurements were read at 517 nm, after 20 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of buffered methanol and DPPH solution acted as the negative control, BHT and trolox (10 mg mL^{-1}) were used as positive control. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\text{Inhibition (\%)} = \left(\frac{A_B - A_A}{A_B}\right) \times 100$$

where, A_B is the absorption of the blank sample (t = 0 min) and A_A is the absorption of the tested oil or standards substance solution (t = 20 min). The EC_{50} value, defined as the concentration of antioxidant in the reactive system necessary to decrease the initial DPPH concentration by 50% and was calculated from the results.

β-Carotene/Linoleic Acid Bleaching Assay

In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated dienehydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi (2008). A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 mL chloroform, 25 μL of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 mL of aerated

distilled water was then added to the residue. The oleander oil was dissolved in DMSO (g L^{-1}) and 350 μL of solution was added to 2.5 mL of th above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 2 h, together with three tubes, two contained the antioxidant BHT and trolox as a positive control and the other contained the same volume of DMSO instead of the extracts. The test tubes with BHT and trolox maintained its yellow colour during the incubation period. The absorbencies were measured at 470 nm on an ultraviolet spectrometer (Cintra 6, GBC, Australia). Antioxidant Activities, (AA) (inhibition percentage, I%) of the samples. The AA of sample was evaluated in terms of bleaching of β-carotene using the following equation:

$$\text{Inhibition (\%)} = \left(\frac{A_{\beta\text{-carotene after 2h assay}}}{A_{\text{initial }\beta\text{-carotene}}} \right) \times 100$$

where, $A_{\beta\text{-carotene after 2h assay}}$ is the absorbance of-carotene after 2 h assay remaining in the samples and $A_{\text{initial }\beta\text{-carotene}}$ is the absorbance of and-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as Mean±SD of triplicates.

Ferric Reducing Antioxidant Power Assay

The reductive potential of the oils and the standards positive controls (BHT and trolox) was determined according to the method of Oyaizu (1986). The oleander oil or standards were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$; 2.5 mL, 1%). The mixture was then incubated at 50°C for 20 min. Afterwards, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Results expressed as EC_1 value, which means the concentration of antioxidant in the reactive system having ferric reducing ability equivalent to that of 1% ferric cyanide.

Antimicrobial Activity Assay

The antimicrobial activity of oleander oil was evaluated by the standard disc diffusion technique as described by Gillies and Dodds (1984) and Lngolfsdottir *et al.* (1997). Species; strains and cultivation conditions of used microorganism are shown in Table 1.

Table 1: The species, strains and cultivation conditions of used microorganism

Species of microorganisms	Strains	Cultivation conditions
Gram-positive bacteria		
G^+ bacilli, spore-forming	*Bacillus subtilis*[a]	TSA+YE, 30°C for 24 h
G^+ cocci	*Staphylococcus aureus* (ATCC 29213)	TSA+YE, 37°C for 24 h
Gram-negative bacteria		
G^- short rods	*Escherichia coli* (ATCC 25922)	TSA+YE, 37°C for 24 h
G^- short rods	*Salmonella typhimurium* (ATCC 19430)	TSA+YE, 37°C for 24 h
Yeast	*Saccharomyces cerevisia*[a]	TSA+YE, 30°C for 48 h
Mold	*Fusarium oxysporum*[b]	PDA, 25°C for 72 h
Mold	*Rhizoctonia solani*[b]	PDA, 25°C for 72 h
Mold	*Macrophoma mangiferae*[b]	PDA, 25°C for 72 h

[a]Obtained from Department of Microbiology, Agriculture Faculty, Cairo University, [b]Obtained from Plant Pathology Institute, Agricultural Research Center, Egypt, G^+: Gram-positive bacteria, G^-: Gram-negative bacteria, TSA+YE: Trypticase Soy Agar + 0.6% Yeast Extract, PDA: Potato Dextrose Agar

Procedure

Target organisms were inoculated in melted (at 50°C) trypticase soy agar + 0.6% yeast extract medium (APHA, 1978) and mold strains were inoculated in melted (at 50°C) potato dextrose agar medium (APHA, 1978) with heavy inoculum. Then the inoculated medium were poured over a solid layer of uninoculated agar medium in sterilized Petri-dishes and left to solidify at 4°C (surface layer should be constant in volume and horizontally homogenous). Discs of Whatman No. 1 filter paper (6.0 mm in diameter) were sterilized by autoclaving at 121°C for 15 min. An accurate volume (10 μL) of undiluted essential oil was aseptically added to each disc and left to dry. Each disc was aseptically placed on the middle of agar surface (in triplicate) and left at 4°C for 1 h then plates were incubated. Gentamicin for bacterial strains and nystatin for fungal strains (10 μL $disc^{-1}$) were used as positive reference standards.

The antimicrobial activity of essential oil was evaluated by measuring the average of inhibition zone diameter against the test microorganisms and the values were expressed as Mean±SE.

Determination of the Minimum Inhibitory Concentration (MIC)

The MICs of the oleander oil against the test bacterial strains and yeast were determined by tube dilution method (Sokmen *et al*., 2004) using Trypticase soy broth + 0.6% yeast extract (APHA, 1978; Evans, 1996). Inocula suspensions of the microorganisms were prepared from 12 h cultures. Oleander essential oil was dissolved in 10% dimethylsulfoxide (DMSO) and serial two fold dilutions of the oil were prepared in sterilized tubes, ranging from 7.8 to 4000 μg mL^{-1}. The MIC was defined as the lowest concentration of the essential oil which the microorganism did not demonstrate visible growth (visible turbidity). The MIC value for each of fungus was determined by agar dilution method (Sokmen *et al*., 2004) using Potato Dextrose Agar (PDA) (APHA, 1978). The oil was added aseptically to sterile melted PDA medium containing 0.5% DMSO at the appropriate volume to produce concentrations ranging from 7.8 to 4000 μg mL^{-1}. The resulting PDA agar solutions were immediately poured into Petri dishes. The plates were spot inoculated with fungus strains. The MIC value was determined as the lowest concentration of the essential oil at which an absence of fungus growth. The MICs of the standards (Gentamicin and Nystatin) were also determined in parallel experiments as control for the microorganism's sensitivity.

Anti-Tumor Assay

Ehrlich Ascites Carcinoma(EAC) cells was used to study the anti-tumor activity of oleander oil. The tumor line is maintained in the National Cancer Institue (NCI) Cairo, Egypt in female Swiss albino mice by weekly intrapertoneal (i.p.) transplantation of 2.5×10^6 cells.

Similar line was proceeded in Biochemistry department, Cairo university. The *in vitro* study as described by Kandasamy *et al.* (2005) with slight modification. Cells were taken from tumor transplanted animals after ≈7 days of transplantation then the number of cells mL^{-1} was calculated by using microscope counting technique (≈2×10^7 cells mL^{-1}). The cells were centrifuged at 1000 rpm for 5 min, washed with saline then the needed numberof cells was prepared by suspending the cells in the appropriate volume of saline.

The cells culture medium was prepared using RPMI 1640, 10% fetal bovine serum, 10% L-glutamine and 0.01% dimethyl sulphoxide (DMSO). Trypan blue (0.4%) was prepared by dissolving of 0.4 g of the dye in 100 mL distilled water then kept in brown closed glass bottle.

Viability of Ehrlish Ascites Carcinoma Cells (EACC)

The viability percentages of tumor cells were measured after incubation with each oils extract as well as saline and DMSO as control. Two milliliter of media containing EACC (2×10^6 cells) were transferred into a set of tubes each, then different concentrations (0.0, 0.2, 0.4, 0.8, 1.0, 1.4, 2,4, 8, 10 μL) oleander oil were added into the appropriate tube as well as saline. The tubes were incubated at 37°C for 2 h then centrifuged at 1000 rpm for 5 min and the separated cells were suspended in 2 mL saline.

Viable/Non-Viable Tumor Cell Percentage Count

For each examined materials (and control), a new clean, dry small test tube was used and 10 μL of cell suspension, 80 μL saline and 10 μL trypan blue were added and mixed, then the number of non-viable cells (stained) and viable cells (non-stained) counted under microscope by using a homocytometer slide in the 50 small squares.

Acute Toxicity of Oleander Oil

Toxicity of oleander essential oil was monitored in animal model system by different biochemical profiles including LD_{50}, diarrhea; GPT; LDH and createnine. Male Albino mice of 6 animals per group and weighing between 20 and 25 g were administered with graded doses of (100-400) mg kg^{-1} b.wt. intra peritoneal of the oleander oil suspended in DEMSO. The toxicological effects were observed after 48 h of treatment in terms of mortality and expressed as LD_{50}. The number of animals dying during the period was noted (Ghosh, 1984). Others biochemical parameters determined after 10 days of administration according to methods of Reitman and Frankel (1957) for GPT activity; Bergmeyer (1974) for LDH activity and Husdan and Rapoport (1968) for createnine.

Statistical Analysis

Each of the measurements described was carried out in three replicate experiments and the results are recorded as mean ± standard deviation. The significantly different calculated at level of $p\leq 0.05$.

RESULTS AND DISCUSSION

Chemical Composition and Total Yield of Oleander Essential Oil

Total yield of essential oil was 0.1%. Most chemical constituents of the oleander essential oil were determined by GS/MS analysis and WILEY275, NIST05 and ADAMS libraries validation. Sixty four components, representing 94.69% of the total essential oil, were identified and listed in order of elution from capillary column in GC-MS and their retention times and area percentages (concentrations) in Table 2. Among which 34.2% were oxygenated compounds, 60.54% were terpenes compounds and alkane compounds (2.02%). The major components were, Camphore (12.76%), Eugenol (10.45%), α-, α-Campholenal (5.05%), thymol (8.43%) and which accounted for 38.12% of the essential oil.

Amount of Total Phenolics

Based on the absorbance values of the oleander essential oil solution, reacting with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents The amount of total phenolics was 136.54±3.32 mg gallic acid g^{-1} essential oil.

Table 2: Chemical composition of the essential oil of oleander

No.	Compound[a]	Rt	Area (%)	No.	Compound[a]	Rt	Area (%)
1	2-Heptanol	4.2	0.38	42	Trans-calamenene	35.7	0.82
2	α-Thujene	4.9	0.43	43	β-Sesquiphellandrene	35.9	1.98
3	α-Pinene	5.7	0.94	44	δ-Cadinene	36.3	1.27
4	Camphene	7.8	2.75	45	Myristicin	37.2	1.04
5	Sabinene	8.1	0.61	46	γ-Cuprenene	37.6	0.92
6	α-Pinene	8.6	0.77	47	Spathulenol	38.3	0.06
7	2-Heptanol-5-methyl	9.5	0.54	48	Caryophyllene	39.1	3.43
8	Myrcene	10.3	1.31	49	Carotol	39.7	0.97
9	α-Phellandrene	10.8	1.43	50	Guaiol	40.2	1.88
10	Octanal	11.0	0.70	51	α-Humulene	40.6	2.43
11	Thymol	11.9	8.43	52	Unknown	40.7	1.17
12	β-Ocimene	12.3	1.05	53	(E)- and-Farnesene	43.1	0.22
13	γ-Terpinene	13.2	0.98	54	α-Acoradiene	45.6	0.08
14	unknown	14.1	1.51	55	Unknown	50.2	0.02
15	Camphore	14.3	12.76	56	β-Acoradiene	53.1	0.05
16	Terpinolene	16.3	0.86	57	β-Chamigrene	56.8	1.08
17	Perillene	16.7	0.48	58	unknown	60.2	0.26
18	Nonanal	17.0	0.32	59	Germacrene D	60.4	2.76
19	Octyl acetate	17.4	0.21	60	β-Selinene	61.3	1.98
20	α-Campholenal	17.8	5.05	61	γ-Curcumene	61.9	1.09
21	Ocimene	18.3	1.79	62	unknown	64.1	0.88
22	Nonen-1-al	20.1	0.72	63	Cuparene	67.3	1.76
23	Nonanol	20.5	0.26	64	α-Zingiberene	70.1	0.76
24	α-Cubebene	20.9	3.43	65	β-Bisabolene	70.5	1.01
25	Eugenol	21.5	10.45	66	unknown	73.2	0.09
26	unknown	22.1	0.43	67	α-Farnesene	75.7	0.04
27	α-Copaene	22.4	1.50	68	γ-Cadinene	75.9	0.02
28	Daucene	22.7	0.97	69	γ-Bisabolene	77.1	0.76
29	β-Bourbonene	23.5	0.32	70	Trans-calamenene	77.3	1.03
30	β-Cubebene	24.1	1.87	71	Tetracosane	78.5	0.76
31	β-Elemene	24.8	1.08	72	Pentacosane	79.0	0.95
32	Italicene	25.1	0.1	73	Heptacosane	79.6	0.34
33	β-Funebrene	25.3	2.77		Total unknown		5.31
34	α-Cedrene	27.8	0.31		Total identified compounds		94.69
35	Aristolene	29.3	0.98				
36	β-Barbatene	30.3	0.21		Oxygenated compounds		34.20
37	unknown	32.7	0.11		terpenes		60.54
40	unknown	33.9	0.86		Alkane compounds		2.05
41	γ-Bisabolene	35.2	0.78				

[a]Compounds quantified on the DB-5 capillary column and listed in order of elution time from the same column and identified by comparison with MS libraries and cited literature except majors compounds were identified by comparison with MS libraries and with authentic compounds

Table 3: Antioxidants activities of the essential oil of oleander

Sample	EC_{50} (μg mL^{-1})	EC_1(μg mL)	AA (%)
Control	1.51±0.09	1.51±0.15	75.26±2.31
Oleander oil	2.11±0.12	2.61±0.45	65.73±3.11
BHT	21.51±1.61	4.61±0.35	90.20±1.81
Trolox	6.75±0.22	8.35±0.22	54.31±2.51

[a,b,c,d]Values with different letter(s) in the same raw were significantly different ($p<0.05$). Each value in presented as Mean±SD (n = 3)

Antioxidant Activity

The potential antioxidant activity of the oleander essential oil was determined on the basis of three methods, the scavenging activity of the stable free radical DPPH (EC_{50} value); inhibition of the coupled oxidation of linoleic acid and beta-carotene (AA% value) and Ferric reducing antioxidant power (EC_1 value). Since, the reaction followed a concentration-dependent pattern, only values of EC_{50}; AA% and EC_1 of oleander essential oil; BHT and Trolox are shown in Table 3. In general the lower the EC_{50} value the higher the free radical scavenging activity of a sample.

Oleander essential oil had significantly lower EC_{50} value (2.11±0.12) compared to trolox (6.75±0.22 μg mL^{-1}) and BHT (21.51±1.61 μg mL^{-1}). Regarding the EC_1 values, the lower EC_1 value the higher the ferric reducing activity of the sample. In present study, the oleander essential oil had significantly higher activity and lower EC_1 (2.61±0.12 μg mL^{-1}) than trolox (8.35±0.22 μg mL) and BHT (4.61±0.35 μg mL^{-1}).

In the β-carotene linoleic acid system assay, oleander essential oil also possessed better antioxidant activity (65.73±3.11%) than trolox (54.31±2.51%) and BHT (90.20±1.81%).

The efficiency of an antioxidant component to reduce DPPH essentially depends on its hydrogen donating ability, which is directly related to the number of phenolic hydroxyl moieties. In the β-carotene linoleic acid system assay, phenolics block the chain reaction of lipid peroxidation mainly by scavenging the intermediate lipid peroxyl radicals which are generated (Haslam, 1996). This also depends on the hydrogen-donating ability of antioxidants. Studies on oxidation potentials and redox reactions between polyphenols and transition metal ions have shown that the o-dihydroxyl feature is a crucial factor for the reducing efficiency (Makris and Kefalas, 2005). Regarding the three assay systems in present study, the aromatic hydroxyl groups, especially the o-dihydroxyl configuration, are important, either for hydrogen donation or interaction with Fe^{3+}. The potential activity of essential oils as natural antioxidant has been studied by several authors. Wang *et al.* (1998) studied the antioxidant activity of phenolic compounds from sage essential oil. Ruberto and Baratta (2000) have tested about 100 pure components of essential oils from different groups of chemicals largely phenolics and terpens compounds for their antioxidant effectiveness.

Also, Ozkan *et al.* (2010) and Albayrak *et al.* (2010) recorded the same correlation in essential oil between antioxidant activities and phenolics compounds. From the major components of oleander essential oil are phenolic; Camphore (12.76%), eugenol (10.45%) and thymol (8.43%), which accounted for 31.54%. This result suggests that there may a close relationship between these three phenolics compounds and antioxidant activity specially reducing power of oleander essential oil, due to hydroxyl substitutions in aromatic ring, which possess potent hydrogen donating abilities as described by Haslam (1996).

Other compounds of oleander essential oil also seem to play an important role and have widely-varying capabilities towards all the radicals, for example α-Campholenal; Caryophyllene; Camphene and α-Humulene which represent 5.05%, 3.43, 2.75 and 2.43% of oleander essential oil.

Anti-Tumor Activity

Different concentration of oleander oil were tested for their ability to inhibit the growth of ehrlich ascites carcinoma cells line. The anti-tumor activity expressed as non-viable (dead) tumor cell percentage count is shown in Fig. 2. Anti-tumor activity gradually increase with concentration of oleander essential oil and reach to 100% with 8 μL mL^{-1} of oleander essential oil. The obtained result in anti-tumor experiment in present study indicated that essential oil of oleander is very active on tested tumor cell line. This may related to some compounds present in oleander essential oil, for instance terpenic compounds and carvacrol which represent 60.54% and phenolic compounds which accounted for 31.54% from oleander essential oil. This compound recorded to has *in vitro* activity on tumor cell resistant to chemotherapy as well as significant antitumor effect on mice (Ulubelen *et al.*, 2000; Mbarek *et al.*, 2007). The effect of essential oil on tumor cell may due to toxic effect of terpenes and their ability to bonding with critical causing the death of tumor cell Omidbeygi *et al.* (2007).

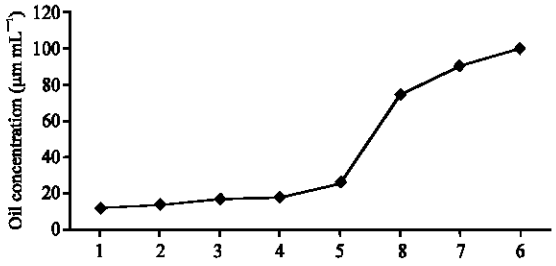

Fig. 2: Effect of oleander essential oil concentration (µL mL^{-1}) on ehrlich tumor cell death

Table 4: The zone of inhibition (mm) and Minimum Inhibitory Concentration (MIC) values (µg mL^{-1}) of the essential oil of oleander; gentamicin and nystatin

Species of organisms	Zone of inhibition (mm)			MIC (µg mL^{-1})		
	Oleander oil	Gentamicin	Nystatin	Oleander oil	Gentamicin	Nystatin
Bacillus subtilis	18	25	0	125	15.60	
Staphylococcus aureus	16	22	0	250	31.25	
Escherichia coli	15	20	0	250	62.50	
Salmonella typhimurium	13	21	0	500	62.50	
Saccharomyces cerevisia	18	0	23	125		31.25
Fusarium oxysporum	14	0	17	250		125.00
Rhizoctonia solani	10	0	13	2000		500.00
Macrophoma mangiferae	13	0	15	500		250.00

Antimicrobial Activity

Antimicrobial activity of oleander oil was observed by agar disc diffusion technique against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhimurium*) and yeast (*Saccharomyces cerevisia*) and three strains of mold (*Fusarium oxysporum*; *Rhizoctonia solani* and *Macrophoma mangiferae*). Results from the agar disc diffusion tests for antimicrobial activity of oleander essential oil are shown in Table 4. The essential oil displayed a variable degree of antimicrobial activity against the different strains tested It was found to be active against all the microbes used for the activity compared with that of the standard antibiotics tested. According to inhibition disc zone diameter; oleander oil antimicrobial activity was in order of against *B. subtilis*>*S. aureus*>*E. coli*>*S. cerevisia*>*S. typhimurium*> *M. mangiferae* and moderately active against *R. solani*. Regarding the MIC values of oleander essential oil ranged ranged from 125 to 500 µg mL^{-1} and 250 to 2000 µg mL^{-1} for bacteria and fungi, respectively. The MIC values for standard ranged from 15.5 to 62.5 µg mL^{-1} and 31.25 to 500 µg mL^{-1} for Gentamicin and Nystatin, respectively. In general, the essential oil showed better antibacterial activity than antifungal activity (Table 3). The Gram-positive bacterium (*Bacillus subtilis* and *Staphylococcus aureus*) is more susceptible to the antimicrobial properties of essential oils than Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) and it is considered to be due to its outer membrane which more permeable for essential oil compounds (Cox *et al*., 2001; Benli *et al*., 2007).

Several studies have been conducted to understand the mechanism of action of essential oils as antimicrobial agent. Cox *et al*. (2001) attributed this effect to the ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action. Veldhuizen *et al*. (2006) attributed this function to the phenolic compounds, which has their interactions with biomembrane of microorganism and thus the antimicrobial activity. On the

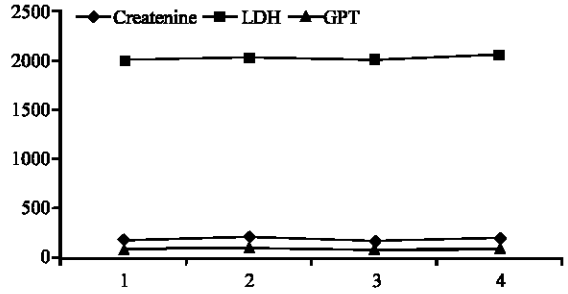

Fig. 3: Effect of different doses of oleander essential oil on GOT, LDH and createnine

other hand, Cristani *et al.* (2007) and Omidbeygi *et al.* (2007) reported that the antimicrobial activity of essential oils is related to ability of terpenes to penetrating into the interior of the microorganism cells and interacting with critical interacellular sites causing the death of cells.

This studies in agreement with results of oleander oil, which has 31.54 and 60.54% of phenolic and terpenes compounds, respectively. The Gram-positive bacterium (*Bacillus subtilis* and *Staphylococcus aureus*) is more susceptible to the antimicrobial properties of essential oils than Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) and it is considered to be due to its outer membrane which more permeable for essential oil compounds (Cox *et al.*, 2001; Hanamanthagouda *et al.*, 2010).

The variety of antimicrobial activity of the essential oil with its concentration and kind of bacteria, may due to the differences in the susceptibility of the test organisms to essential oil, this could be attributed to a variation in the rate of the essential oil constituent's penetration through the cell wall and cell membrane structures (Cox *et al.*, 2001).

This difference between concentrations of the essential oil and the standard antibiotic can be explained in terms of the fact that the active components in the essential oil comprise only a fraction of the oil used. Therefore, the concentration of the active components could be much lower than the standard antibiotics used Hanamanthagouda *et al.* (2010).

Toxicity of Oleander Essential Oil

Toxicity of oleander essential oil was monitored in animal model system by different biochemical profiles including LD_{50}, diarrhea; GPT and LDH and createnine. Obtained results are shown in (Fig. 3). Toxicity results indicated that no mortality or diarrhea observed with administration with all concentrations range of oleander oil. The GPT, an enzyme which allows determining the liver function as indicator on liver cells damage. Results indicated that no-significant effect after administration with oleander oil with different doses (in comparison with positive control). The LDH enzyme is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis (Butt *et al.*, 2002).

According to recorded results, no-significant effect after treatment with oleander oil with different doses (in comparison with positive control). Measuring serum creatinine is a simple test most commonly used indicator of renal function, increasing of createnine levels indicator of post stage of kidney failure (Delanghe *et al.*, 1989). Results indicated that no-influence on the levels of creatinine in all treated groups with vanadyl sulphate at different doses, this indicated that the oleander oil has no side effect on kidneys tissue in animal model system under conditions of present experiments. Generally, the toxicity data indicated that the oleander essential oil has no side effect on animal model system under concentrations used in present study.

CONCLUSION

Essential oil of oleander flower had significant antioxidant activities; antimicrobial activities and antitumor activity, these biological properties without adverse effect. Therefore, it is suggested that further work be performed on the isolation and identification of the biologically active. These results indicated that the essential oil of oleander flower could be considered as a natural food preservatives and enhance the human health as natural antioxidant.

ACKNOWLEDGMENT

This study was financial supported by a grant from the Taif University, Kingdom of Saudi Arabia (grand No.1/430/458). Authors thank National Cancer Research Institute, Egypt For providing tumor cell line and experimental animals.

REFERENCES

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Allured Publishing Corporation, IL, USA., ISBN-10: 1932633219.

Albayrak, S., A. Aksoy, O. Sagdic and E. Hamzaoglu, 2010. Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. Food Chem., 119: 114-122.

APHA, 1978. Standard Methods for the Examination of Dairy Products. 17th Edn., Public Health Association, Washington DC., ISBN: 087553175X.

Barbosa, R.R., J.D. Fontenele-Neto and B. Soto-Blanco, 2007. Toxicity in goats caused by oleander (*Nerium oleander*). Res. Vet. Sci., 85: 279-281.

Begum, S., B.S. Siddiqui, R. Sultana, A. Zia and A. Suria, 1999. Bio-active cardenolides from the leaves of *Nerium oleander*. Phytochemistry, 50: 435-438.

Benli, M., K. Güney, l. Bing, F. Geven and N. Yigit, 2007. Antimicrobial activity of some endemic plant species from Turkey. Afr. J. Biotech., 6: 1774-1778.

Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis. 2nd Edn., Academic Press, New York, ISBN: 0895732424, pp: 534.

Burt, S.A., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol., 94: 223-253.

Butt, A.A., S. Michaels, D. Greer, R. Clark, P. Kissinger and D.H. Martin, 2002. Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS Read, 12: 317-321.

Cox, S.D., C.M. Mann, J.L. Markham, J.E. Gustafson, J.R. Warmington and S.G. Wyllie, 2001. Determination the antimicrobial action of tea tree oil. Molecules, 6: 87-91.

Cristani, M., M. Arrigo, G. Mandalari, F. Castelli, M.G. Sarpietro and D. Micieli, 2007. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. J. Agric. Food Chem., 55: 6300-6308.

Council of Europe, 2004. European Pharmacopoeia. 5th Edn., Directorate for the Quality of Medicines, Council of Europe, Strasbourg, France.

Delanghe, J., J.P. De Slypere, M. De Buyzere, J. Robbrecht, R. Wieme and A. Vermeulen, 1989. Normal Reference values for creatine, creatinine and carnitine are lower in vegetarians. Clin. Chem., 35: 1802-1803.

Evans, W.C., 1996. Trease and Evans Pharmacognosy. 14th Edn., WB. Saunders Co. Ltd., London, ISBN: 0-7020-1899-6, pp: 248.

Ghosh, M.N., 1984. Fundamentals of Experimental Pharmacology. 2nd Edn., Scientific Book Agency, Kolkatta, ISBN: 81-902965-0-7, pp: 175-176.

Gillies, R.R. and Dodds, 1984. Bacteriology Illustrated. 5th Edn., Churchill Livingstone, Edinburgh London Melbourne and New York, ISBN-10: 0443028095, pp: 160-165.

Hanamanthagouda, M.S., S.B. Kakkalameli, P.M. Naik, P. Nagella, H.R. Seetharamareddy and H.N. Murthy, 2010. Essential oils of *Lavandula bipinnata* and their antimicrobial activities. Food Chem., 118: 836-839.

Haslam, E., 1996. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J. Nat. Prod., 59: 205-215.

Husdan, H. and Rapoport, 1968. Estimation of creatinine by jaffe reaction a comparison of three methods. Clin.Chem., 14: 222-238.

Jaroslav, H., B. Milos, K. Pavel, K. Ladislav, V. Irena, V. Sona and Z. Vaclav, 2009. Norsesquiterpene hydrocarbon, chemical composition and antimicrobial activity of *Rhaponticum carthamoides* root essential oil. Phytochemistry, 70: 414-418.

Kandasamy, M., V. Yeligar, B. Maiti and T. Maity, 2005. Antitumor and antioxidant of potential of *Heliotropium zeylanicum* against ehrlich ascites carcinoma in swiss albino mice. Eur. Bull. Drug Res., 13: 99-107.

Langford, S.D. and P.J. Boor, 1996. Oleander toxicity: An examination of human and animal toxic exposures. Toxicology, 109: 1-13.

Li, C., Y. Jian, L. Yan, S. Hong, J. Wen and Z. Li *et al*., 2009. Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. Food Chem., 115: 801-805.

Lngolfsdottir, K., M.A. Hajalmarsdottir, A. Sigurdsson, G.A. Gud-jonsdottir, A. Bryonjolfsdottir and O. Steingreimsson, 1997. Agents and chemotherapy. Antimicrobe, 4: 215-215.

Makris, D. P. and P. Kefalas, 2005. Association between *in vitro* antiradical activityand ferric reducing power in aged red wines: A mechanistic approach. Food Sci. Technol. Int., 11: 11-18.

Mbarek, A.L., A.H. Mouse, A. Ja andfari, R. Aboufatima and A. Benharref *et al*., 2007. Cytotoxic effect of essential oil of thyme (*Thymus broussonettii*) on the IGR-OV1 tumor cells resistant to chemotherapy. Brazilian J. Med. Biol. Res., 40: 1537-1544.

Mehmet, M., T. Olga and C. Maria, 2009. Essential oil composition of the turpentine tree (*Pistacia terebinthus* L.) fruits growing wild in Turkey. Food Chem., 114: 282-285.

Miraliakbari, H. and F. Shahidi, 2008. Antioxidant activity of minor components of tree nut oils. Food Chem., 111: 421-427.

NIST, 2005. NIST/EPA/NIH Mass Spectral Library Software. Version 2.0, National Institute of Standards and Technology, USA.

Oke, F., B. Aslim, S. Ozturk and S. Altundag, 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem., 112: 874-879.

Omidbeygi, M., M. Barzegar, Z. Hamidi and H. Nafhdibadi, 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food Control, 18: 1518-1523.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jap. J. Nutr., 44: 307-315.

Ozkan, G., O. Sagdic, S. Gokturk, O. Unal and S. Albayrak, 2010. Study on Chemical composition and biological activities of essential oil and extract from *Salvia pisidica*. LWT-Food Sci. Technol., 43: 186-190.

Reitman, S. and S. Frankel, 1957. Determination of GOT and GPT. Am. J. Clin. Pathol., 28: 56-56.

Ruberto, G. and M.T. Baratta, 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 69: 167-174.

Sacchetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in food. Food Chem., 91: 621-632.

Sahin, F., M. Güllüce, D. Daferera, A. Skmen, M. Skmen and M. Polissiou, 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* sp. vulgare in the Eastern Anatolia region of Turkey. Food Contry, 15: 549-557.

Sharma, O.P. and T.K. Bhat, 2009. DPPH antioxidant assay revisited. Food Chem., 113: 1202-1205.

Sokmen, A., M. Gulluce, H.A. Akpulat, D. Daferera, B. Tepe and M. Polissiou, 2004. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Contry, 15: 627-634.

Soto-Blanco, B., J.D. Fontenele-Neto, D.M. Silva, P.F.C.C. Reis and J.E. No and´brega, 2006. Acute cattle intoxication from *Nerium oleander* pods. Trop. Anim. Health Prod., 38: 451-454.

Tsai, T.H., T.H. Tsai, Y.C. Chien, C.W. Lee and P.J. Tsai, 2008. *In vitro* antimicrobial activities against *Cariogenic streptococci* and their antioxidant capacities: A comparative study of green tea versus different herbs. Food Chem., 110: 859-864.

Ulubelen, A., G. Topcu and U. Sonmez, 2000. Chemical and biological evaluation of genus teucrium. Stud. Nat. Prod. Chem., 23: 591-648.

Veldhuizen, E.J., J.L. Tjeerdsma van Bokhoven, C. Zweijtzer, S.A. Burt and H.P. Haagsman, 2006. Structural requirements for the antimicrobial activity of carvacrol. J. Agric. Food Chem., 54: 1874-1879.

Wang, M., J. Li, M. Rangarajan, Y. Shao, E.J. La Voie, T.C. Huang and C.T. Ho, 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). J. Agric. Food Chem., 46: 4869-4873.

Wu, C.P., G.X. Wu, M.Y. Chen, Y.N. Lin and L.Q. Huang, 2006. Studies on chemical component and antibacterial activity of essential oil from *Mosla scabra*. J. Plant Resour. Environ., 15: 26-30.