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Full Length Research Paper

Cytotoxicity and Bioactive Compounds from Diethyl Ether Extract of *lotus halophilus* Boiss Et Spruner Growing in Egypt

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In the present study we carried out the GC-MS of lipid diethyl ether extract of the *Lotus halophilus* boiss et spruner aerial parts to identify its chemical compositions. Fatty acids of the saponifiable fraction revealed the presence of twelve saturated fatty acids and eleven unsaturated fatty acids, in addition a series of (C_4-C_{69}) hydrocarbons and three sterols were identified in the unsaponifiable fraction. Cytotoxic and antioxidant activities were carried out for the ether extract of the plant. The ether extract showed cytotoxic activity against HEPG2 and MCF7, while it was unable to inhibit the growth of HCT; also it showed significant antioxidant activity.

Keywords: *Lotus halophilus*, GC-MS, fatty acids, hydrocarbons, sterols, cytotoxic andanti-oxidant activities.

INTRODUCTION

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in therapy (Aggarwal *et al.,* 2003). Cancer is a disease in which there is uncontrolled multiplication and spread with abnormal forms in the body's own cell and it is the second leading cause of more than six million deaths each year in the world (Rang *et al.,* 2007). Natural compounds have provided many effective anticancer agents in current use. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources (Newman and Gragg, 2007). Family Fabaceae (Leguminosae) commonly known as legume, pea, or bean family is the third-largest plant family with 630 genera and over 18,860 species and rich in medicinal plants. Fabaceae is the most common family found in tropical rain forests and in dry forests in the Americas and Africa (Judd *et al.,* 2002, Stevens, 2002, Burnham and Johnson 2004). Leaf extract of *Parkiabiglobosa (Jacq) benth* as a species of leguminosae has anti-ulcer activity (Chinasa *et al.,* 2014), while the aqueous stem bark of *Pentaclethra macrophylla Benth* has hypoglycaemic activity (Igbe and Osigwe 2012). The methanolic extract of *S. grandiflora* has antiviral and cytotoxic activities (Arthanari *et al.,* 2012). GLC analysis of the n-hexane extracts of *Saraca indica* L. *and Enterolobium cyclocarpum Jacq* showed that the lipid content especially the saturated fatty acid (palmitoleic acid) have a moderate to good antimicrobial activity against Gram (+ve) and Gram(-ve) bacteria (Hawas *et al.,* 2012). Saturated and unsaturated fatty acids aspalmitic, oleic, linoleic and linolenic acids were

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determined in *Lotus corniculatus* with different concentrations (Bakoglu *et al.,* 2012).

To the best of our knowledge, no reports could be traced concerning the constituents of *Lotus halophilus* boiss et spruner. Therefore, the aim of this study is to explore some phytochemical constituents, cytotoxic and antioxidant activity of *Lotus halophilus* boiss et spruner.

MATERIALS AND METHODS

Collection of plant material

Aerial parts of *Lotus halophilus* boiss et spruner; Were collected during spring, 2014 from Al Omaied reserve, Matrouh governorate, northwest coast, Egypt. The plant was kindly identified by Prof. Dr. Azza El Hadidy, Professor of Plant Taxonomy and Flora, Botany Department, Faculty of Science Cairo University, Egypt. The plant material was air dried in shade and grinded to fine powder.

Materials for cytotoxic activity

Human tumor cell lines: [(HEPG2 liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT (colon carcinoma cell line)].

Methods

Preparation of extract

The plant was extracted exhaustively with Di ethyl ether. The extraction was left 24 hours, with the solvent in a Soxhlet apparatus (Rosenthaler, 1930). The extract was collected after filtering through filter paper and concentrated on a Rota-vapor.

Chromatographic Analysis of saponifiable and unsaponifiable matters

The dried powdered plant material was extracted with petroleum ether (b.p 40-60 ˚C) in a soxhlet apparatus. The combined petroleum ether extract was filtered, dried over anhydrous sodium sulphate and evaporated in vacuo at 50 ˚C till dryness. The residue was dissolved in hot acetone and left in the refrigerator overnight, then filtered; the saponifiable and unsaponifiable fractions were separated according to (Ackman, 1998 and Gomez *et al.,* 2015).

Investigation of the saponifiable matter (SM)

The saponifiable fraction was subjected to GC/MS using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5 MS fused silica capillary column (30m, 251mm, 0.1mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 ev was used, Helium gas was used as the carrier gas at constant flow rate of 1ml/min. The injector and MS transfer line temperature was set as 280 ˚C. The oven temperature was programmed at an initial temperature 150 ˚C (hold 4 minutes) to 280˚C as a final temperature at an increasing rate of 5˚C/min (hold 4 minutes).

Investigation of the unsaponifiable matter (USM)

The unsaponifiable fraction was subjected to GC/MS using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS , TG-5 MS fused silica capillary column (30m, 251mm, 0.1mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 ev was used, Helium gas was used as the carrier gas at constant flow rate of 1ml/min. The injector and MS transfer line temperature was set as 280 ˚C. The oven temperature was programmed at an initial temperature 50 ˚C (hold 2 min) to 150 ˚C at an increasing rate of 7 ˚C/min, then to 270 at an increasing rate of 5 ˚C/min (hold 2 min) then to 310 as a final temperature at an increasing rate of 3.5 ˚C/min (hold 10 minutes).

The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Methods of antitumor activity

Potential cytotoxicity of the successive extracts of the aerial parts of Lotus *halophilus* boiss et spruner was tested using the method of (Skehan *et al.,* 1990). The human cancer cell lines used in the test were HEPG2 (Liver carcinoma cell line), MCF7 (Breast carcinoma cell line) and HCT (Colon carcinoma cell line). The cell lines were obtained from National Cancer Institute, Kasr El-Einy, Cairo, Egypt.

Procedures of Cytotoxic activity

- Cells were plated in 96-multiwell plate (104 cells /well) for 24 hours before treatment with the extracts to allow attachment of cell to the wall of the plate.
- Different concentrations of the extract under test (0, 5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
- Monolayer cells were incubated with the extracts for 48 hours at 37 ˚C and atmosphere of 5 % CO_2.
- After 48 hours. cells were fixed, washed and stained with sulforhodamine B stain (SRB).
- Excess stain was washed with acetic acid and attached stain was recovered Tris-EDTA buffer.

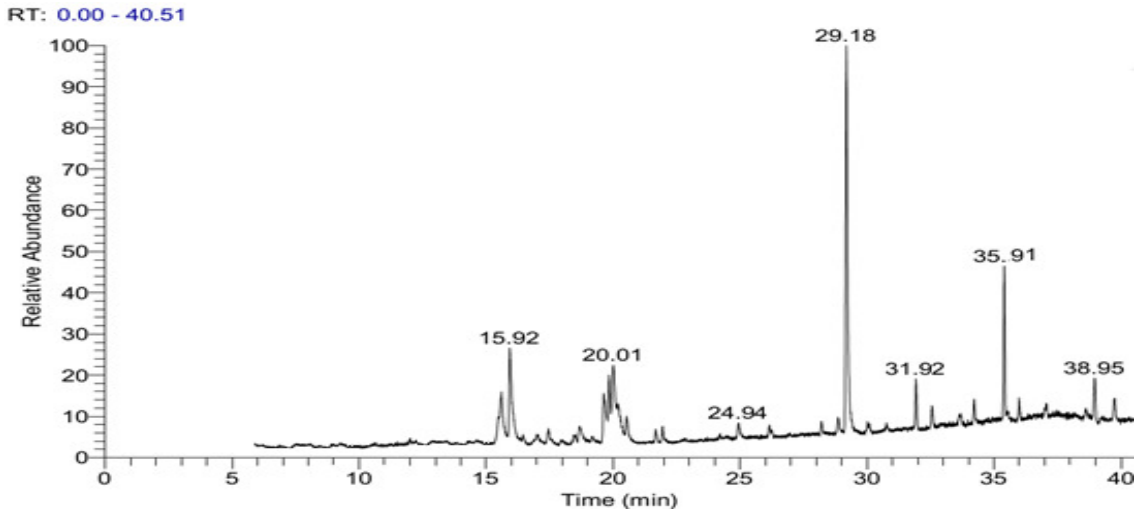

Figure 1. GC-MS chromatogram of saponifiable fraction of *Lotus halophilus*aerial parts

- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line after the specified extract under investigation was added.

Antioxidant activity (Determination of free radical scavenging activity)

Free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl radical ((DPPH) Sigma-Aldrich) was carried out according to Yildirim *et al.,* (2001). This method is based on the reduction of alcoholic DPPH solution, in the presence of a hydrogen donor (the antioxidant). Different concentrations (50, 125, 250, 500 and 1000 µg/ml) of the tested extract and of ascorbic acid as a reference antioxidant (reference control) were prepared in 80% (v/v) ethanol. A volume of 3ml from each extract and ascorbic acid concentrations were mixed with 1ml of 1mM of DPPH radical. A control tube was prepared by mixing 3ml of 80 % ethyl alcohol with 1ml of alcoholic solution of DPPH radical. The tubes were kept at room temperature in the dark for 30 minutes. The degree of disappearance of purple color was measured against blank (80% ethyl alcohol) at 517nm. The percentage of DPPH Scavenged can be calculated as follows:

$$(\%)\ \text{Scavenged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

EC_{50} (Effective concentration) and MI (Maximal Inhibition) were calculated by means of Graph Pad Prism software (Ver.4).

RESULTS AND DISCUSSION

Chromatographic Analysis of saponifiable and unsaponifiable fractions

GC-MS analysis of fatty acids esters content of the aerial part of *Lotus halophilus* boiss et spruner revealed the presence of twenty three compound of fatty acids under the experimental conditions. The unsaturated fatty acids represented (59.06 %), predominated by 1, 2-benzene dicarboxylic acid, dioctyl ester (dioctyl phthalate) (30.35%) and methyl 9,12,15-octadecatrienoate (4.83%). The major identified saturated fatty acids of (40.94 %) were palmitic acid, methyl ester (7.3%) and methyl hexacosanoate (methyl cerotate) (4.80 %) as shown in table (1) and figure (1). Chromatographic investigation of the unsaponifiable fraction of the lipoidal matters of *Lotus halophilus* achieved by GC-MS, showed the presence of a series of (C_4-C_{69}) hydrocarbons amounting to (99.92%) and predominated by 3,5,5-trimethyl-2-cyclohexenone (91.47) with significant percentage. Moreover, three sterols that have been identified were acetic acid,17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta[a]phenanthren-10-yl methyl ester, 7,8-epoxylanostan-11-ol, 3-acetoxy and 5à-cholestane, 3á,5-dichloro-6á-nitro representing (0.08%) of the total unsaponifiable matter as shown in table (2) and figure (2).

Table 1. GC-MS analysis of fatty acids of of *Lotus halophilus* aerial parts

No	Rt	Area %	MoleculaFormula	No. of carbon atoms	Compound Name
1	7.17	0.34	$C_{19}H_{30}O_2$	C19:2	Methyl 12,15-octadecadiynoate
2	8.37	0.36	$C_{28}H_{40}O_4$	C28:6	9,12,15-Octadecatrienoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester
3	15.48	2.20	$C_{16}H_{31}N$	C16:0	Palmitonitrile (palmitic acid nitrile)
4	15.60	4.83	$C_{14}H_{18}N_2O_4$	C14:3	2-Acetylamino-3-phenylpropionic acid, 1-carbamoyl ethyl ester
5	15.93	7.39	$C_{17}H_{34}O_2$	C17:0	Palmitic acid, methyl ester
6	17.00	2.02	$C_{32}H_{48}O_8$	C32:2	Phorobl 12,13-dihexanoate
7	17.46	3.80	$C_{19}H_{36}O_2$	C19:1	Oleic acid, methyl ester
8	18.49	4.03	$C_{36}H_{72}O_3$	C36:0	1-Hexadecyl-2-stearoyl ethanediol
9	18.68	1.54	$C_{17}H_{24}O_4$	C17:3	1,2-Benzenedicarboxylic acid, octyl methyl ester
10	19.63	4.09	$C_{18}H_{33}N$	C18:1	Oleanitrile (oleic acid nitrile)
11	19.84	3.73	$C_{19}H_{34}O_2$	C19:2	6,9-Octadecadienoic acid , methyl ester
12	20.02	4.52	$C_{19}H_{32}O_2$	C19:3	Methyl 9,12,15-octadecatrienoate
13	20.20	3.03	$C_{19}H_{37}N$	C19:0	Nondecanoic acid nitrile
14	20.54	5.66	$C_{19}H_{38}O_2$	C19:0	Methyl stearate (stearic acid methyl ester)
15	21.68	2.56	$C_{25}H_{48}O_5$	C25:0	2-(Acetyloxy)-1-[(octadecyloxy)methyl]ethyl acetate
16	24.94	3.52	$C_{21}H_{42}O_2$	C21:0	Arachidic acid methyl ester
17	28.21	2.41	$C_{39}H_{78}O_3$	C39:0	Stearic acid, 3-(octadecyloxy)propyl ester
18	28.86	2.49	$C_{23}H_{46}O_2$	C23:0	Methyl behenate (Behenic acid, methyl ester)
19	29.19	30.35	$C_{24}H_{38}O_4$	C24:3	1,2-Benzenedicarboxylic acid, dioctyl ester (Dioctyl phthalate)
20	30.77	2.69	$C_{34}H_{68}O_5Si$	C34:0	Glycerine -1,3-dimyristate , 2-O-trimethyl silyl
21	35.99	6.63	$C_{27}H_{54}O_2$	C27:0	Methyl hexacosanoate (methyl cerotate)
22	37.06	2.26	$C_{39}H_{78}O_3$	C39:0	Stearic acid, 3-(octadecyloxy)propyl ester
23	39.74	3.30	$C_{24}H_{22}O_8$	C24:4	Dimethyl(1RS,5RS,6SR)-6-Benzoyl-3,5-dimethyl-1phenyl-4,7,8-trioxabicyclo[3.2.1]oct-2-ene-2,6-dicarrbboxyloate

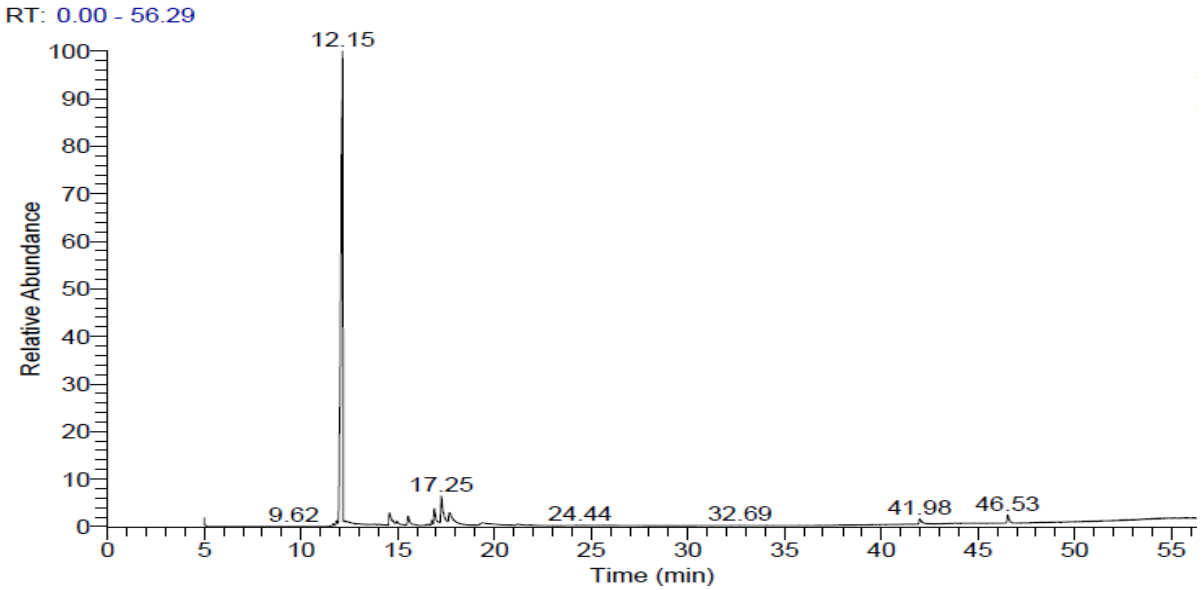

Figure 2. GC-MS chromatogram of unsaponifiable fraction of *Lotus halophilus* aerial parts

Table (2):GC-MS analysis of unsaponifiable fraction of *Lotus halophilus* aerial parts

No	Rt	Area %	Molecular Formula	Compound name
1	11.66	0.37	$C_8H_{12}O$	4,5-Dimethylcyclohexen-2-one
2	11.83	0.21	$C_9H_{14}O$	S-Diisopropylidene acetone
3	11.99	0.01	$C_{66}H_4O$	Benzo[b]furano[2',3':1,2][60]fullerene
4	12.15	91.47	$C_9H_{14}O$	3,5,5-Trimethyl-2-cyclohexenone
5	12.28	0.05	$C_{12}H_{10}ClNO$	3,4-Dihydro-2-(4'-chlorophenyl)-2H-pyran-6-carbonitrile
6	12.38	0.09	$C_{11}H_{16}O_2$	(3S,4S)-3-Isopropenyl-3-(formylmethyl)-4-methylcyclopentanone
7	12.44	0.06	$C_6H_{12}O$	Cis-2-Hexen-1-ol
8	12.51	0.03	$C_{12}H_9ClN_2O$	1H-Benzoimidazole, 5-chloro-1-(furan-2-yl)methyl
9	12.55	0.02	$C_{10}H_{16}O$	p-Menth-3-en-9-al
10	12.64	0.01	$C_{22}H_{42}O_2Si_2$	3,6-Bis[(t-Butyl)dimethylsilyloxy]-3,6-dimethylocta-1,7-diyne
11	12.80	0.04	$C_{11}H_{14}O_2$	1,1-Dimethylethyl benzoate
12	12.86	0.03	$C_9H_{14}O_2$	(1S,5R)-5-Methyl-2-oxabicyclo[4.3.0]nonan-3-one
13	13.00	0.02	$C_{11}H_{14}N_2$	2-Methylspiro[2.5]octane-1,1-dicarbonitrile
14	13.29	0.02	$C_6H_8N_2O$	6-Methylpyridazin-3-methanol
15	13.38	0.02	$C_4H_2N_2$	Fumaronitrile
16	13.49	0.05	$C_9H_{14}O$	Phorone
17	13.64	0.02	$C_9H_{18}O$	Cis-3-Nonen-1-ol
18	13.75	0.02	$C_8H_{13}NO$	N,N,5-Trimethylfurfurylamine
19	13.85	0.03	$C_9H_{15}NO_2$	2,3-Heptadienylaminium hydrogen oxalate
20	14.22	0.02	$C_{11}H_{17}N$	2,7-Imino-3,6-methanonaphthalene,decahydro
21	14.27	0.02	$C_{69}H_{78}N_4O$	10,15,20-Tris[3",5"-di(t-butyl)phenyl]-5-(4'-formylphenyl)porphyrine
22	14.57	1.64	$C_8H_{12}O_2$	Allyl tiglate
23	15.54	0.27	$C_{12}H_{18}O$	Dimethyl - (methylpropenyl) – cyclohexenone
24	16.43	0.03	$C_{15}H_{26}$	Cedrane
25	16.51	0.03	$C_9H_{15}N_3O$	5-Methyl-1,3-diazaadamantan-9-one Oxime
26	16.77	0.24	$C_{12}H_{18}O$	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-1,8-dimethyl
27	16.90	0.59	$C_{24}H_{38}O_3$	4,7-Methanobenzofuran,2,2'-oxybis[octahydro-7,8,8-trimethyl-
28	17.25	1.79	$C_{12}H_{18}O$	2-Propanone, 1-(3,5,5-trimethyl-2-cyclohexen-1-ylidene)
29	17.69	0.85	$C_{10}H_{10}O_3$	3'-Hydroxy-4'-methoxy-(trans)-cinnamaldehyde
30	19.35	0.24	$C_{16}H_{15}N_3OS_2$	5-(1-methyl-1H-pyrazol-4-ylmethylene)-3-(1-phenyl-ethyl)-2-thioxo-
31	19.48	0.07	$C_9H_{10}N_2O_2$	Pyridine, 2,6-epidioxy-5-ethyl-3-iminomethyl-4-methyl
32	41.98	0.59	$C_{24}H_{38O4}$	Bis(2-ethylhexyl) phthalate
33	46.54	0.90	$C_{30}H_{50}$	Squalene
34	54.83	0.01	$C_{32}H_{66}O_5Si_4$	Trimethylsilyl (5E,13E)-9,11,15-tris[(trimethylsilyl)oxy]prosta-5,13-dien-
35	55.49	0.02	$C_{25}H_{39}NO_5$	Acetic acid,17-acetoxy-3-hydroxyimino-4,4,13-trimethyl -hexadecahydrocyclopenta[a]phenanthren-10-yl methyl ester
36	55.70	0.03	$C_{32}H_{54}O_4$	7,8-Epoxylanostan-11-ol, 3-acetoxy
37	55.98	0.03	$C_{27}H_{45}Cl_2NO_2$	5à-Cholestane, 3á,5-dichloro-6á-nitro
38	56.06	0.02	$C_{17}H_{21}BrO_5$	(Z)-6-bromo-4,5,7-trimethoxy-2,2-dimethyl-3-(3-oxobutylidene)-2,3-
39	56.18	0.04	$C_{36}H_{43}NO_5$	(1R,3R,5P) and(1R,3R,5M)-(2'-Hydroxymethyl-5'-isopropoxy-4'-methoxy-1'-(N-benzyl-6-hydtoxy-8-isopropoxy-1,3-dimethyl-1,2,3,4-

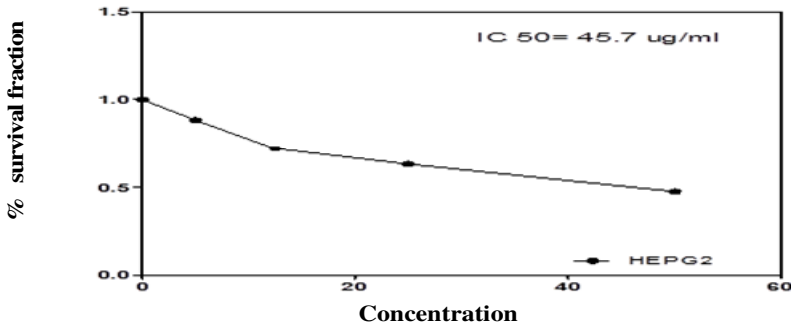

Figure 3. Percentage of survival fraction against concentration (µg/ml) of the ether extract of *Lotus halophilus* aerial parts of liver carcinoma cell line.

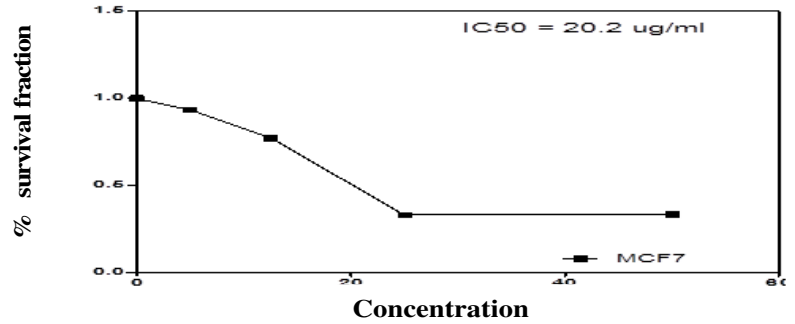

Figure 4. Percentage of survival fraction against concentration (µg/ml) of the ether extract of *Lotus halophilus* aerial parts of breast carcinoma cell line.

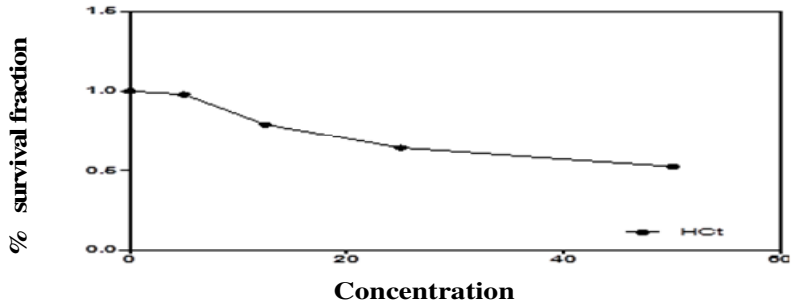

Figure 5. Percentage of survival fraction against concentration (µg/ml) of the ether extract of *Lotus halophilus* aerial parts of colon carcinoma cell line.

Table 3. IC_{50} of the ether extract of *Lotus halophilus* aerial parts on MCF7 and HEPG2.

Sample	IC_{50} (µg/ml) for HEPG2	IC_{50} (µg/ml) for MCF7
Diethyl ether extract	45.7 µg/ml	20.2 µg/ml

Cytotoxic activity

Potential cytotoxicity of the ether extract of the plant aerial parts with different concentrations was tested against HEPG2 (Liver carcinoma cell line), MCF7 (Breast carcinoma cell line) and HCT (Colon carcinoma cell line). Results indicated that the extract showed different cytotoxic activity against HEPG2 and MCF7 as showed in table (3), but it was unable to inhibit the growth of HCT (Colon carcinoma cell line). A relation between concentration and surviving fractions of the extract on the three cell lines was showed as follow in figures (3, 4 and 5).

It was found from the above data that ether extract have inhibition effect against MCF7 and moderate inhibition with HEPG2 but no inhibition with HCT.

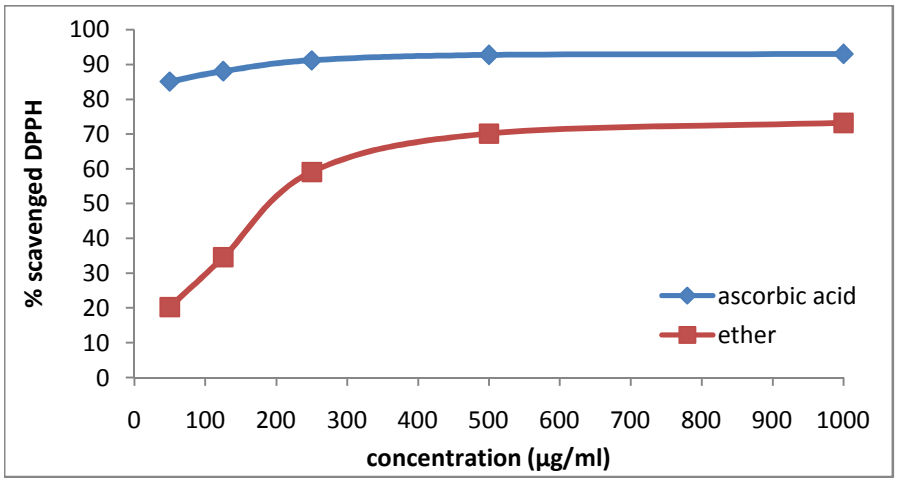

Figure 6. Scavenging activity of di ethyl ether extract of *Lotus halophilus* aerial parts compared with ascorbic acid.

Table 4. Antioxidant activity of the ether extract of *Lotus halophilus* aerial parts against log concentration.

Log concentration	% of DPPH Scavenged
3.000	73.20
2.690	70.12
2.397	59.12
2.096	34.56
1.600	20.23

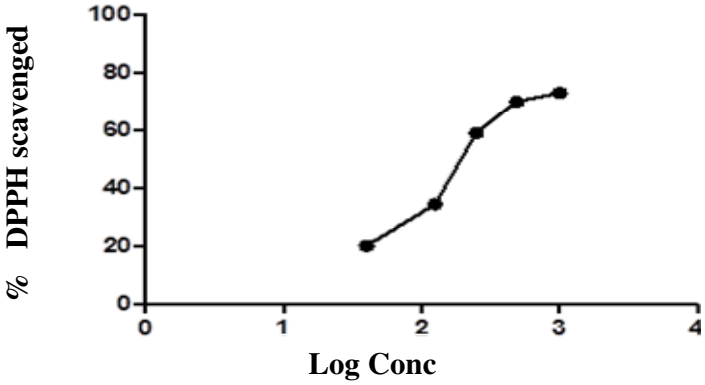

Figure 7. % DPPH scavenged curve of the ether extract of *Lotus halophilus* aerial parts against log concentration.

Antioxidant activity (Determination of free radical scavenging activity using DPPH)

Antioxidant activity using DPPH free radical scavenging activity was carried out on the ether extract of the plant to evaluate its antioxidant properties with reference to ascorbic acid "natural antioxidant". Ascorbic acid showed the highest radical scavenging activity attaining aplateau pattern of activity (91.92 %) of concentration (1000 µg/ml).

Results of antioxidant activity revealed that the scavenging activity of diethyl ether extract of *Lotus halophilus* aerial parts was a dose dependent as shown in figures (6 and 7) and table (4). The percent of DPPH scavenging activity was (59.12 %) for diethyl ether extract.

Table 5. The median effective concentration (EC_{50}) and percentage of maximal inhibition(MI) for ether extract of *Lotus halophilus* aerial parts in DPPH radical scavenging assay.

Tested extract	Scavenging activity of DPPH radicals (EC_{50}) value µg/ml	(% MI)
Diethyl ether	200 µg/ml	73.20 (1000 µg/ml)

Table (5): summarizes the antioxidant properties of the diethyl ether extract of *Lotus halophilus* boiss et spruner. The median effective concentration (EC_{50}) that produced scavenging of DPPH radical for diethyl ether (200 µg/ml). But the percentages of maximal scavenging of DPPH radical (% MI) for ether extract (73.20 % at 1000 µg/ml).

CONCLUSION

It was founded that fatty acid constituents of the saponifiable fraction revealed the presence of twelve saturated fatty acids and eleven unsaturated fatty acids, in addition a series of (C_4-C_{69}) hydrocarbons and three sterols were identified in the unsaponifiable fraction. Cytotoxic and antioxidant activities were carried out for the ether extract of *Lotus halophilus* aerial parts. The ether extract showed cytotoxic activity against HEPG2 and MCF7, while it was unable to inhibit the growth of HCT; also it showed significant antioxidant activity. Attributing to; the presence of free radical scavenging molecules in the ether extract of the plant, such as (fatty acids, hydrocarbons and sterols) which have antioxidant activity.

REFERENCES

Ackman RG (1998). Remarks on official methods employing boron trifluoride in the preparation of methyl esters of the fatty acids of fish oil. *J. the Am. Oil Chem. Soc.* 75(4): 541-545.

Aggarwal BB, Kumar A, Bharti AC (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Antican. Res.* 23:363–398.

Arthanari SK, Vanitha J, Ganesh M, Venkateshwaran K, Clercq D (2012). Evaluation of antiviral and cytotoxic activities of methanolic extract of *S. grandiflora* (Fabaceae) flowers. *Asian Pacific J. Trop. Biomed.* S8: 55-58.

Bakoglu A, Bagci E, Ciftci H (2009). Fatty acids, protein contents and metal composition of some feed crops from Turkey. *J. Food, Agric. Environ.* 7(2): 343-346.

Burnham RJ, Johnson KR (2004). South American palaeobotany and the origins of neotropical rain forests. *Phil. Trans. Roy. Soc. London* B, 359: 1595–1610.

Chinasa EC, Chukwuemeka ES, Chika AC, Chima OC, Chinedu AF, Nwamaka OL (2014). Investigation of the anti-ulcer potentials of the menthanol leaf extract of *Parkia biglobosa* (Jacq) benth (Fam: Fabaceae). *Asian J. Biochem. Pharm. Res.* 4(3): 154-160.

Gomez-Coca RB, Perez-Cameno MC, Moreda W (2015). Neutral Lipid Unsaponifiable. Handbook of Food Analysis, 3rded, volume (2), by Nollet ML,Fidel Toldra, Pp 460.

Hawas UW, Eltomy SA, Abou-Zid S, El-Hossary GA, Nassif RM (2012). Lipid content and antimicrobial activity of some Egyptian Fabaceae (Leguminosae) plants. *J. Med. Plants Res.* 6(44): 5604-5608.

Igbe I, Osigwe C (2012). Hypoglycaemic activity of aqueous extract of *Pentaclethra macrophylla* (Fabaceae) stem bark in streptozotoc-ininduced diabetic rats. *J. Pharm. Bioresources*. 9(1): 39-44.

Judd WS, Campbell CS, Kellogg EA,Stevens PF, Donoghue MJ (2002). Plant systematics: a phylogenetic approach, Sinauer Axxoc, 287-292,ISBN 0-87893-403-0.

Newman DJ, Gragg GM (2007). Natural Products as Source of New Drugs over the Last 25 Years. *J. Nat. Prod*, 70: 461-477.

Rang HP, Dale MM, Ritter JM, Moore PK (2007). *Pharmacology*. Churchill Livingstone, Edinburgh, Pp.718.

Rosenthaler L (1930). The chemical investigation of plants 3rded. G. Bell and Sons, Ltd., London, 63.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990). New coloremtric cytotoxicity assay for anti-cancer drug screening. *J. Natl. Cancer Inst*. 82: 1107 – 1112.

Stevens PF (2006). "Fabaceae". Angiosperm Phylogeny Website. Version Retrieved 28 April 2008.

Yildirim A, Mavi A, Kara AA (2001). Determination of antioxidant and antimicrobial activities of *Rumex Crispus* L extracts. *J. Agric. food chem.* 49: 4083-4089.