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Metabolites profiling of *Limonium tubiflorum* (Delile) Kuntze var *tubiflorum via* UPLC-qTOF-MS technique in relation to its cytotoxic activity

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Abstract

Ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-qTOF-MS) technique led to the detection of 55 metabolites in *Limonium tubiflorum* (Delile) Kuntze var *tubiflorum* flowers extract for the first time including 38 flavonoids, 7 phenolic acids and 10 anthocyanins. The total phenolic content was (231.225 mg GAE g DW^{-1}) and the total flavonoid content was (136.66 mg rutin g DW^{-1}) in *L. tubiflorum* (Delile) Kuntze var *tubiflorum*. The flower plant powder has been prepared by successive fractionation. The successive extracts have been tested for *in vitro* cytotoxic activity against MCF7, HEPG2, and HCT116 and exhibited moderate to weak cytotoxic activities compared to Doxorubicin (positive control) which showed in vitro cytotoxic effect with IC_{50} values of 2.97 ± 0.9, 4.57 ± 0.5 and 3.73 ± 0.6 µg/mL, respectively. The results also showed that the 95% ethanolic successive extract exhibited the strongest cytotoxic activity among all the tested extracts against HEPG2 and HCT116 cell lines with IC_{50} values of 21 ± 0.5 and 21 ± 0.8 µg/mL, respectively, while 70% ethanolic successive extract showed the highest cytotoxic activity against MCF7 cell line with IC_{50} value (26.6 ± 0.7 µg/mL). The hydro-ethanolic plant flower extracts are enriched in phenolic compounds (flavonoids, phenolic acids and anthocyanins) which are likely to mediate for cytotoxic activity.

Keywords: *Limonium tubiflorum* (Delile) Kuntze var *tubiflorum*, UPLC-qTOF-MS, flavonoids, anthocyanins, cytotoxicity

1. Introduction

Plumbaginaceae is a family of flowering plants cosmopolitan in distribution. It is sometimes referred to as the leadwort family or the plumbago. It consists of about 30 genera and 725 species (Christenhusz and Byng, 2016). *Limonium* genus, also known as (Statice or Sea-lavender), is one of the largest genus of this family comprising about 180 species of halophytic plants as perennial shrubs, subshrubs spread in Africa, Europe, America and Asia (Ksouri *et al*., 2011).

The salt-tolerant halophyte *Limonium* genus has many potentially useful plants. It has traditionally been used to treat a wide range of diseases and ageing symptoms, and continues to be used for such medical purposes in rural areas (Ksouri *et al*. 2011). For examples, *Limonium brasiliense* Kuntze exhibits anti-inflammatory and antibacterial properties (Murray *et al*. 2004), *L. wrightii* (Hance) Kuntze is used to treat arthritis and fever (Aniya *et al*. 2002), *L. sinense* (Girard) Kuntze has been reported to possess antiviral properties (Yuh-Chi *et al*. 2002), and *L. axillare* (Forssk.) Kuntze and *L. californicum* (Boiss.) A. Heller have been shown cytotoxic and antibacterial effects (Kandil *et al*. 2000). *Limonium* is reported to have a wide range of chemical diversity (Medini *et al*. 2014).

L. tubiflorum (Delile) Kuntze var *tubiflorum* is a perennial subshrub 10–35 cm, woody at the base, densely covered with scale-like tubercles; stems 1–5 cm, simple to richly branched, leafy; leaves restricted to basal part of the short stems, 0.5–2 x 0.2–0.5 cm, flowering stems branched, brittle; flowers 1 cm diam, the margin membranous; outer bracts 1.5–3.5 x 2 mm, unequal, broadly ovate,acute, the inner 7–9 x 2.5 mm; calyx-teeth ending in a reddish–brown awn 2–3 mm; petals . 1.5 cm vivid rose, fruit not seen. The plant has been recorded to grow in Egypt (South of Mersa Matruh region) in calcareous ridges (Boulos, 2000).

To the best of our knowledge, there are no previous studies regarding the chemical constituents of *L. tubiflorum* (Delile) Kuntze var *tubiflorum*. Therefore, this study targets the chemical profile of the flower extract of the plant and investigates *in vitro* cytotoxic activity of the successive extracts of the flower plant powder against MCF7, HEPG2 and HCT116 in order to evaluate its potential medicinal uses.

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2. Materials and Methods

2.1. Plant collection and Preparation

The aerial parts of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* were collected in April 2017 from Wadi Habis at Mersa matrouh governorate, Northwestern coast, Egypt. The plant sample was identified by Prof. Dr. Azza El Hadidy, Professor of plant taxonomy and flora, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen of the plant (18/4/2017-H) was deposited at the Herbarium of Faculty of Science, Cairo University, Egypt. The flowers were separated then stored in plastic bags under dark, chilled conditions during transportation to the laboratory, then washed under tap water and air dried at Laboratory temperature till constant weight. The flowers were ground to fine powder to be used for chemical analysis.

2.2. Human tumor cell lines for the cytotoxic activity

Human tumor cell lines [HEPG2 (liver carcinoma cell line), MCF7 (breast carcinoma cell line) and HCT116 (colon carcinoma cell line)] were obtained in frozen state under liquid nitrogen (-180 ºC) from the American Type Culture Collection. The cancer cell lines were maintained by serial sub-culturing in the National Cancer Institute, Cairo, Egypt. The cells were suspended in RPMI 1640 medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA) in presence of 1% antibiotic antimycotic mixture (10.000 U/mL K-penicillin, 10.000 U/mL streptomycin sulphate and 25 µg/mL amphotericin B) and 1% L-glutamine (all purchased from Lonza, Belgium).

2.3. Extraction procedure for L. tubiflorum (Delile) Kuntze var tubiflorum flowers

For chemical profile of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* via high resolution UPLC-qTOF-MS analysis, 20 mg of the flowers plant powder was homogenized with 4 mL 70% MeOH using an ultrasonic bath for 30 min. Extract was then vortexed and centrifuged at 10,000g for 10 min to remove plant debris and filtered through 22 µm millipore filter. For cytotoxic assay, other quantity (25 g) of the air-dried powder of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers was subjected to successive extraction with gradient organic solvents in polarity (petroleum ether, chloroform, ethyl acetate, 95% ethanol and 70% ethanol) using soxhlet apparatus. The obtained residue from each solvent was dried and weighed.

2.4. Determination of Total Phenolic Content

The total phenol content was analyzed using a colorimetric measurement at 630 nm (Attard, 2013). A standard series of gallic acid (GA) was used for quantification. Each sample was measured as technical triplicate. Results were given as GA equivalents (GAE)/g dry weight. Gallic acid stock solution of 1mg/mL in methanol was prepared, and seven serial dilutions were prepared in the concentrations of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 µg/mL. Solution of flower sample was prepared in concentration of 5mg/mL in methanol. Then the results were recorded using microplate reader FluoStar Omega. Each of the 7 standards and the sample were pipetted in the plate wells in 6 replicates. Measurement was performed at 630 nm.

2.5. Determination of Total Flavonoid Content

The amount of total Flavonoid content was determined by aluminum chloride assay through colorimetric method at 510 nm (Herald *et al*. 2012). Distilled water (100 µL) was added to each of the 96 wells, followed by 10 µL of 50 g/L $NaNO_2$ and 25 µL of standard or flower sample solution. After 5 min, 15 µL of 100 g/L $AlCl_3$ was added to the mixture; 6 min later, 50 µL of 1 mol/L NaOH and 50 µL of distilled water were added. The plate was shaken for 30 seconds in the plate reader prior to absorbance measurement at 510 nm. Rutin was used as a standard, its stock solution of 1mg/mL in methanol was prepared, and 6 standards were prepared in the concentrations of 1000, 500, 250, 150, 100, and 50 µg/mL generate a calibration curve. Then, the results were recorded using microplate reader FluoStar Omega. Each of the 6 standards and the sample were pipetted in the plate wells in 6 replicates. Measurement was performed at 510 nm.

2.6. Metabolites analysis via high-resolution UPLC-qTOF-MS analysis

Chromatographic separations were performed on Sciex ExionLC chromatographic separation coupled with TripleTOF 5600+ equipped with a Xbridge C18 column (3.5 µm, 2.1x50 mm; Waters) applying the following elution binary gradient at a flow rate of 0.3 mL min^{-1}: 0–1 min, isocratic 90% A (5 mM ammonium formate buffer pH=8 containing 1% methanol [v/v]), 10% B (100 % acetonitrile); 1–21 min, linear from 10–90% B; 21–25 min, isocratic 90% B; 25–28 min, isocratic 10% B. The injection volume was 10µL. Eluted compounds from UPLC were detected from *m/z* 100 to 1000 using a MicroTOFQ hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics) equipped with an Apollo-II electrospray ion source in negative ion modes using the following instrument settings: nebulizer gas, nitrogen,1.6 bar; dry gas, nitrogen, 190 ºC; capillary,5500 V (+4000 V); end plate offset, 500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp. MicroTOF-Q: Precursor ions were selected in Q1 with an isolation width of ±2 D and fragmented in the collision cell applying collision energies in the range of 10–30 eV. Argon was used as collision gas. Product ions were detected using the following parameter settings: collision RF 150/400 Vpp (timing 50/50); transfer time, 70 ls; pre pulse storage, 5 ls; pulser frequency, 10 kHz; spectra rate, 1.5 Hz. The MSn spectra were recorded by using the following conditions: MS/MS analysis with starting collision-induced dissociation energy of 30 eV and an isolation width of ±2 D in a data dependent, negative ionization mode. UPLC–MS files were converted to netCDF file format using the File Converter tool in Bruker Daltoniks software and further processed using AMDIS software to assist in adjacent peak deconvolution and background subtraction (Halket *et al.* 1999). Metabolites were characterized by their retention times, mass spectra and phytochemical dictionary of natural products database.

2.7. Cytotoxicity assay

Cytotoxicity assay of the successive extracts of the flowers of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* was tested using the method of Skehan *et al.* 1990. Cells were plated in 96-multiwell plate (10^4 cells /well) for 24 hours before treatment with the extracts. Different concentrations of each 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 ºC 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 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. The IC_{50} value was defined as the concentration of the tested extract (µg/mL) that decreased the number of viable cells by 50%. Results are expressed as the mean value of triplicate data points ± SD.

3. Results

3.1. Determination of Total Phenolic Content

Total phenolic content of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers was calculated and represented as gallic acid equivalent (mg GAE g DW^{-1}), where the total phenolic content was 231.225± 14.899 mg GAE g DW^{-1}.

3.2. Determination of Total Flavonoid Content

The total flavonoid content of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* was expressed as rutin equivalents in [mg g DW^{-1}] and its value equal 136.66± 11.168 mg Rutin g DW^{-1}.

3.3. Metabolites profiling via UPLC-qTOF-MS

Chemical composition of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* was performed via UPLC/qTOF-MS in negative ionization mode to provide a comprehensive coverage of metabolites composition. The metabolites profile of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flower extract led to the identification of 55 metabolites belonging to phenolic compounds including flavonoids, phenolic acids, and anthocyanins (Table 1). These compounds include 38 flavonoids, namely Luteolin **(1)**, Luteolin-7-*O*-glucoside **(2)**, Kaempferol-7-*O*-neohesperidoside **(3)**, Hesperidin **(4)**, Quercetin-4'-*O*-glucoside **(5)**, Quercetin **(6)**, Luteolin-3', 7-di-*O*-glucoside **(7)**, 3, 5, 7-trihydroxy-4'-methoxyflavone **(8)**, Isorhamnetin-3-*O*-glucoside **(9)**, Isorhamnetin-3-*O*-rutinoside **(10)**, Kaempferol-3-*O*-*α*-L-rhamnoside **(11)**, Apigenin 8-*C*-glucoside **(12)**, Syringetin-3-*O*-glucoside **(13)**, Apigenin **(14)**, 3'-methoxy-4',5,7-trihydroxyflavonol **(15)**, Kaempferol-3-*O*-glucuronide **(16)**, Naringenin **(17)**, Syringetin-3-*O*-galactoside **(18)**, Rhoifolin **(19)**, Formononetin **(20)**, Acacetin-7-*O*-rutinoside **(21)**, Quercetin-3-*O*-arabinoglucoside **(22)**, Quercetin-3-D-xyloside **(23)**, Catechin **(24)**, Hesperetin **(25)**, Myricitrin **(26)**, Myricetin **(27)**, Quercetin-3-*O*-glucuronide **(28)**, Naringenin-7-*O*-glucoside **(29)**, Apigenin-7-*O*-glucoside **(30)**, Quercetin-3,*4*'-*O*-di-glucopyranoside **(31)**, Quercetin-3-arabinoside **(32)**, Acacetin **(33)**, Kaempferol-3-*O*-*α*-L-arabinoside **(34)**, Kaempferol-3-*O*-robinoside-7-*O*-rhamnoside **(35)** Luteolin-6-*C*-glucoside **(36)**, Isoquercitrin **(37)**, Baicalein-7-*O*-glucuronide **(38)** and 7 phenolic acids namely D-(-)-Quinic acid **(39)**, Chlorogenic acid **(40)**, 3,4-dihydroxybenzoic acid **(41)**, Homogenentisic acid **(42)**, Caffeic acid **(43)**, Rosmarinic acid **(44)**, *p*- hydroxybenzoic acid **(45)**, and 10 anthocyanins namely Delphinidin-3-O-*β*-glucopyranoside **(46)**, Pelargonidin-3-*O*-glucoside **(47)**, Cyanidin-3,5-di-*O*-glucoside **(48)**, Peonidin **(49)**, Cyaniding-3-*O*-glucoside **(50)**, Cyanidin-3-*O*-(2"-O-*β*-xylopyranosyl-*β*-glucopyranoside) **(51)**, Petunidin-3-*O*-*β*-glucopyranoside **(52)**, Malvidin-3,5-di-*O*-glucoside chloride **(53)**, Peonidin-3-*O*-glucoside chloride **(54)**, Malvidin-3-*O*-galactoside **(55)**. The total ion chromatogram is shown in Fig. 1.

Figure 1. Total ion chromatogram of metabolities of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers

Table 1. Metabolities identified in 70% methanol flowers extract of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* via UPLC-qTOF-MS in negative ionization mode.

Peak	R_t(min)	$[M-H]^-$ *m/z*	Formula	MS/MS	Identification
1	6.9611	477.1048	$C_{22}H_{22}O_{12}$	317,316,286	Petunidin-3-*O*-*β*-glucopyranoside
2	8.8432	285.0431	$C_{15}H_9O_6$	285,217,175 , 151	Luteolin
3	6.7060	447.0946	$C_{21}H_{19}O_{11}$	285,199,175	Luteolin-7-*O*-glucoside
4	6.4047	593.1537	$C_{27}H_{29}O_{15}$	447,285	Kaempferol-7-*O*-neohesperidoside
5	5.7482	609.1475	$C_{28}H_{33}O_{15}$	578,463,301	Hesperidin
6	5.8947	463.0890	$C_{21}H_{19}O_{12}$	301	Quercetin-4'-*O*-glucoside
7	9.0820	301.0370	$C_{15}H_9O_7$	301,271,151	Quercetin
8	8.2692	609.1293	$C_{27}H_{29}O_{16}$	447,285	Luteolin-3', 7-di-*O*-glucoside
9	1.9536	353.0896	$C_{16}H_{17}O_9$	191,179	Chlorogenic acid
10	1.1835	191.0567	$C_7H_{11}O_6$	173,127,109,85	D-(-)-Quinic acid
11	9.7857	299.0577	$C_{16}H_{11}O_6$	284,268	3', 5, 7-trihydroxy-4'-methoxyflavone
12	1.2210	153.0185	$C_7H_5O_4$	109	3,4-dihydroxybenzoic acid
13	7.7153	477.1033	$C_{22}H_{21}O_{12}$	315,299,285	Isorhamnetin-3-*O*-glucoside
14	6.6572	623.1620	$C_{28}H_{31}O_{16}$	477,315	Isorhamnetin-3-*O*-rutinoside
15	5.4726	431.1931	$C_{21}H_{19}O_{10}$	285	Kaempferol-3-*O*- *α*-L -rhamnoside
16	6.1194	431.0995	$C_{21}H_{20}O_{10}$	341,311, 269	Apigenin 8-*C*-glucoside
17	7.4670	507.1133	$C_{23}H_{23}O_{13}$	477,345,314	Syringetin-3-*O*-glucoside
18	10.1651	269.0464	$C_{15}H_9O_5$	269,151	Apigenin
19	10.7898	315.0530	$C_{16}H_{11}O_7$	300,199,285	3'-methoxy-4',5,7-trihydroxyflavonol
20	6.2827	461.0753	$C_{21}H_{17}O_{12}$	285	Kaempferol-3-*O*-glucuronide
21	6.5392	167.0346	$C_8H_7O_4$	167	Homogenentisic acid
22	10.0298	271.0725	$C_{15}H_{11}O_5$	271, 175	Naringenin
23	10.0298	507.0399	$C_{23}H_{23}O_{13}$	345	Syringetin-3-*O*-galactoside
24	5.2471	179.0337	$C_9H_7O_4$	179,135,107	Caffeic acid
25	4.9041	577.1582	$C_{27}H_{29}O_{14}$	431,269	Rhoifolin
26	11.8527	267.0665	$C_{16}H_{11}O_4$	253,237	Formononetin
27	6.5637	591.0120	$C_{28}H_{31}O_{14}$	445,283,268	Acacetin-7-*O*-rutinoside
28	8.4151	595.0636	$C_{26}H_{27}O_{16}$	463,301	Quercetin-3-*O*-arabinoglucoside
29	8.8432	433.1046	$C_{20}H_{17}O_{11}$	301	Quercetin-3-D-xyloside
30	7.7851	497.1087	$C_{22}H_{22}ClO_{11}$	463,335,301	Peonidin-3-*O*-glucoside chloride
31	5.1964	447.0913	$C_{21}H_{20}O_{11}$	285	Cyanidin-3-*O*-glucoside
32	4.0883	359.0535	$C_{18}H_{15}O_8$	161	Rosmarinic acid
33	1.3099	431.0312	$C_{21}H_{20}O_{10}$	271, 270	Pelargonidin-3-*O*-glucoside
34	7.7974	491.1168	$C_{23}H_{24}O_{12}$	330,329,314, 299	Malvidin-3-*O*-galactoside
35	7.1186	289.1456	$C_{15}H_{13}O_6$	271,247,125	Catechin
36	2.8862	283.0232	$C_{16}H_{11}O_5$	283,253	Acacetin
37	6.9123	739.2557	$C_{33}H_{39}O_{19}$	593,431,285	Kaempferol-3-*O*-robinoside-7-*O*-rhamnoside
38	6.7305	447.1602	$C_{21}H_{19}O_{11}$	285	Luteolin-6-*C*-glucoside
39	1.3973	298.9926	$C_{16}H_{12}O_6$	283,270,269	Peonidin
40	1.4101	137.0243	$C_7H_5O_3$	137,93	*p* - hydroxybenzoic acid
41	4.4711	301.0936	$C_{16}H_{13}O_6$	301,258,143	Hesperetin
42	7.4033	653.1713	$C_{29}H_{34}ClO_{17}$	654,653,491, 329	Malvidin-3,5-di-*O*-glucoside chloride
43	12.2830	463.1032	$C_{21}H_{19}O_{12}$	317	Myricitrin
44	6.4410	317.0325	$C_{15}H_9O_8$	287,178,151	Myricetin
45	2.9110	477.0414	$C_{21}H_{17}O_{13}$	301	Quercetin-3-*O*-glucuronide
46	6.2322	579.1379	$C_{26}H_{28}O_{15}$	448,447,286	Cyanidin-3-*O*-(2"-*O*-*β*- xylopyranosyl-*β*-glucopyranoside)
47	9.7118	433.1039	$C_{21}H_{21}O_{10}$	271	Naringenin-7-*O*-glucoside

48	1.3099	463.0483	$C_{21}H_{20}O_{12}$	302	Delphinidin-3-*O*-*β*-glucopyranoside
49	1.2210	431.1375	$C_{21}H_{19}O_{10}$	269	Apigenin 7-*O*-glucoside
50	9.6628	445.1456	$C_{21}H_{17}O_{11}$	269	Baicalein-7-*O*-glucuronide
51	7.0216	624.1066	$C_{27}H_{29}O_{17}$	462,301	Quercetin-3,4'-*O*-diglucopyranoside
52	7.3153	417.0930	$C_{20}H_{17}O_{10}$	285	Kaempferol-3-*O*-*α*-L-arabinoside
53	5.5012	433.0931	$C_{20}H_{17}O_{11}$	301	Quercetin-3-arabinoside
54	7.7533	463.1581	$C_{21}H_{19}O_{12}$	301	Isoquercitrin
55	1.3099	609.0966	$C_{27}H_{30}O_{16}$	448,447,286	Cyanidin-3,5-di-*O*-glucoside

3.4. Successive Extraction

Successive extraction showed that the percentages of residue of petroleum ether, chloroform, ethyl acetate, 95% ethanol and 70% ethanol extracts were 1.2, 1.4, 1.66, 9.06 and 4.52% respectively. The obtained percentage of total residues was 17.84%. The 95% ethanol extract has the highest percentage among the extracts, while the lowest one was that of petroleum ether extract.

3.5. Cytotoxic Activity

Cytotoxicity of the successive extracts of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers were tested against HEPG2, MCF7, and HCT cell lines. Results showed that successive extracts of the plant flowers exhibited moderate to weak cytotoxic activity against HEPG2, MCF7 and HCT116 compared to Doxorubicin (positive control) which showed *in vitro* cytotoxic effect with IC_{50} values of 4.57 ± 0.5, 2.97 ± 0.9 and 3.73 ± 0.6 µg/mL, respectively (Table 2). The 95% ethanolic extract of the flower plant powder exhibited the strongest cytotoxic activity among all the tested successive fractions against HEPG2 and HCT116 cell lines with the same IC_{50} value of 21 ± 0.5 and 21 ± 0.8 µg/mL, respectively, whereas 70% ethanolic successive extract showed the highest cytotoxic activity against MCF7 cell line among the tested extracts with IC_{50} value of 26.6 ± 0.7 µg/mL.

Table 2. IC_{50} (Inhibition concentration) of the successive extracts of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers on MCF7, HEPG2 and HCT116

Successive extracts & Doxorubicin (+ve control)	IC_{50} (µg/mL)		
	HEPG2	MCF7	HCT116
Petroleum.ether	37 ± 0.6	33 ± 1.0	38.5 ± 1.1
Chloroform	36 ± 0.3	34.5 ± 1.2	39 ± 1.2
Ethylacetate	27 ± 0.2	28.9 ± 0.8	27.5 ± 1.2
Ethanol 95%	21 ± 0.5	28.7 ± 0.7	21 ± 0.8
Ethanol 70%	31.5 ± 0.3	26.6 ± 0.7	34 ± 1.2
Doxorubicin (positive control)	4.57 ± 0.5	2.97 ± 0.9	3.73 ± 0.6

* The activity was shown as IC_{50} value, which was the concentration of the tested extract (µg/mL) that decreased the number of viable cells by 50%. Results are expressed as IC_{50} ± SD (n = 3).

4. Discussion

From the above results, it was found that the number flavonoids were the major chemical constituents present in *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers extract. Flavonoids (flavone, flavonols, and flavanones) and/or their conjugates, *O*-glycosides resulted from UPLC-qTOF-MS, were previously reported in *Limonium* species (Lin and Chou, 2000). In MS analysis, the type of the sugars were observed by the loss of 132 amu (pentose), 146 amu (monodeoxyhexose), 162 amu (hexose), and 176 amu (hexouronic acid) residues. Tandem MS spectra of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* identified quercetin as its aglycone (*m/z* 301.037, $C_{15}H_9O_7$) in peaks (6-7, 28-29, 45, 51 and 53) and luteolin (*m/z* 285.0431, $C_{15}H_9O_6$)- in peaks (2-3, 8 and 38), (Table 1). For example, hesperidin with *m/z* 609.1475 and molecular formula ($C_{28}H_{33}O_{15}$) were detected in peak 5. Fragmentation pattern of peak 5 showed losses of 31 amu (-methoxy group), 146 amu (-monodeoxyhexose) and 162 amu (-hexose unit), identified as hesperidin.

Another class of compounds that was detected includes phenolic acids. For instance, chlorogenic acid was recognized from its $[M-H]^-$, *m/z* 353.0896 detected at R_t 1.9536 min (peak 9) and showed also fragment ions at 191 amu and 179 amu corresponding to quinic acid and caffeic acid found in the tandem MS spectra of peak 10 and 24, respectively (Table 1).

Anthocyanins and/or their conjugates, *O*-glycosides were also characterized in the negative ion MS spectra including 10 compounds. For example, Petunidin-3-*O*-*β*-glucopyranoside (peak 1) with (*m/z* 477.1048, $C_{22}H_{22}O_{12}$) which has fragmentation pattern showed losses of 162 amu (-hexose unit) and 31 amu (-methoxy group). Anthocyanins have been previously recorded in the species *Limonium* (Asen *et al*., 1973).

UPLC–MS profiling revealed the enrichment of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flower extract with number of flavonoids especially flavone and flavonol types and their *O*-glycosides in the plant, which is in agreement with Iwashina, 2013. The successive fractions of *L. tubiflroum* (Delile) Kuntze var *tubiflorum* flowers especially the hydro-ethanolic extracts (95% Ethanol and 70% Ethanol) showed a promising cytotoxic activity as a result of their enrichment in phenolic compounds. Our findings were consistent with the results obtained from the previous cytotoxic studies on *L. globuliferum* Kuntze (Eren, 2016).

Data of this study showed that *L. tubiflroum* (Delile) Kuntze var *tubiflorum* collected in Egypt contains appreciable amounts of total phenolic and total flavonoid contents. Also, the current study describes the screening of the metabolic profile of *L. tubiflroum* (Delile) Kuntze var *tubiflorum via* UPLC/qTOF/MS for the first time resulting 55 metabolities dominated by flavonoids of about 38 compounds matches with the high total flavonoid content. Flavonoids are synthesized by plants with *in vitro* cytotoxic activity against various types of cancer cell lines (Oueslati *et al.* 2012, Boulaaba *et al.* 2013 and Boulaaba *et al.* 2019). Flavonoids such as kaempferol, quercetin and their glycosides have been found to possess a protective

effect against cancer by their effect on signal transduction in cell proliferation and angiogenesis (Fatemeh and Khosro, 2013). Recently, flavonoids enhanced a great interest in the beneficial human health effects such as antitumor, anti-inflammatory, antiviral, anti-diabetic and anti-ageing properties (Cook and Samman, 1996, Ren *et al*. 2003, Zhou *et al*. 2009 and Boulaaba *et al.* 2019). It was declared that these compounds contribute to all the former biological effects via their strong antioxidant and free radical scavenger capabilities (Sharififar *et al*. 2009 and Abdel-Mawgoud *et al*. 2019). Phenolic acids determined in UPLC/qTOF/MS analysis could contribute as well enhance the cytotoxic activity and have the ability to scavenge the free radical that caused cell damage and apoptosis. Various phenolic acids have this activity such as caffeic acid and chlorogenic acid with antiproliferative effect (Ye *et al*. 2010, Yagasaki *et al*. 2000). This effect is attributed to the presence of two OH groups on phenyl group in their structure, giving them the same polyphenols properties.

Furthermore, UPLC/qTOF/MS analysis was able to identify 10 anthocyanin compounds; anthocyanins are a type of flavonoids found naturally in a number of foods and plants possessing antioxidant effects. Anthocyanins were previously characterized for genus *Limonium* (Iwashina, 2013). Numerous studies have confirmed that anthocyanins have anti-proliferative properties (Thomasset *et al.* 2009, Jeong *et al.* 2010 and Szymanowska *et al.* 2018).

5. Conclusion

In summary, this is the first study on the metabolite profile of *L. tubiflorum* (Delile) Kuntze var *tubiflorum* flowers via UPLC-qTOF-MS resulting in tentatively identification of 55 metabolites of phenolic compounds including 38 flavonoids, 7 phenolic acids and 10 anthocyanins. This approach permitted identifying several phenolic compounds in the plant flowers. Also, the results suggest that *L. tubiflorum* (Delile) Kuntze var *tubiflorum* could represent a promising source of natural products. Additionally, the total flavonoid and phenolic content of the plant flowers were determined. *In vitro* cytotoxic activity of the successive plant extracts were investigated against MCF7, HEPG2 and HCT116. The results showed that the 95% ethanolic successive extract among all the tested successive extracts exhibited the strongest cytotoxic activity against HEPG2 and HCT116 cell lines with the same IC_{50} value of 21 ± 0.5 and 21 ± 0.8 µg/mL, respectively. The hydro-ethanolic plant flower extracts are enriched in phenolic compounds which are likely to mediate for cytotoxic activity. This research provides useful knowledge for further studies on isolation and characterization of the active compounds as well as the biological screening of these isolated metabolites providing the potential uses of *L. tubiflorum* (Delile) Kuntze var *tubiflorum*.

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