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# Comparative Volatile Profiling of *Ludwigia stolonifera* Aerial Parts and Roots Using VSE-GC-MS/MS and Screening of Antioxidant and Metal Chelation Activities

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**ABSTRACT:** Ludwigia stolonifera (Guill. & Perr.) P.H.Raven belonging to the family Onagraceae is an important aquatic herbal plant of economic importance in water bioremediation. We explored the compositional heterogeneity in the aroma profile of *L. stolonifera* aerial parts and roots. Volatile profiling was employed for the first time using volatile solvent extraction (VSE-GC-MS/MS) of both aerial parts and roots. A total of 85 volatiles were identified belonging to eight classes, viz., aliphatic, aromatic, and oxygenated hydrocarbons, monoterpenes, diterpenes, alcohols, acids/esters, and sterols. Aliphatic and aromatic hydrocarbons were found to be the most abundant metabolite groups in both aerial parts and roots. Furthermore, antioxidant and metal chelation activities of aerial parts and roots were investigated, revealing a potent activity as an antioxidant and high metal chelation capacity for heavy metals.

# ■ INTRODUCTION

Onagraceae, also known as evening primrose or willow herb family, is a family of flowering plants which comprises about 650 species of herbs, shrubs, and trees distributed in 17 genera.<sup>1,2</sup> Several phytochemicals which are widely abundant in different species such as phenolic compounds, flavonoids, essential oils, triterpenenoids, and saponins were reported. Essential oils are a strong-smelling secondary metabolite group of complex chemical composition and myriad pharmacological and medicinal value such as antimicrobial, anti-inflammatory, and antioxidant properties.<sup>3</sup> Ludwigia is a pantropic genus and comprises about 82 species of aquatic plants, well represented in South and North America.<sup>4</sup> Ludwigia species were reported for their and traditional importance and medicinal uses such as antidiabetic,<sup>5</sup> cytotoxic activity,<sup>6</sup> and anti-inflammatory activity.<sup>7</sup> In Egyptian flora, genus Ludwigia is represented by two species, Ludwigia stolonifera (Guill. & Perr.) P.H.Raven (the dominant aquatic macrophytes) and Ludwigia erecta (L.) Hara, in canals and drains crossing the cultivated lands in the Nile Delta.<sup>8</sup> L. stolonifera is an important aquatic herbal plant used for eliminating various toxic pollutants associated with hazardous effects on ecosystems. Also, L. stolonifera is used for water bioremediation helping to improve the quality of drinking water.9 Roots and leaves of L. stolonifera were used as heavy metal biofilters for cadmium (Cd) and nickel (Ni) where leaf biomass showed the best metal binding capacity.<sup>10</sup> Metabolite profiling of different plants of nutritional and economical value



has lately started to implement modern analytical approaches, for example, metabolomics, where samples are examined in a rather untargeted, comprehensive manner.<sup>11</sup> Gas chromatography–mass spectrometry (GC–MS) analysis has been extensively adopted for metabolomic profiling in different plant parts, viz., fruits, leaves, seeds, or even the whole plant, to characterize their metabolite components, for example, volatiles and nonvolatiles.<sup>12</sup> Essential oil identification in plant materials can be achieved by several methods such as steam distillation, headspace solid-phase microextraction, supercritical fluid extraction, microwave-assisted extraction, and solvent extractions. Unlike common thermal methods for volatile extraction, for example, distillation, volatile solvent extraction (VSE) is a simple analytical technique for the enhancement of volatile recovery from the plant sample.<sup>13</sup>

To the best of our knowledge, this is the first report of volatile profiling of *L. stolonifera* aerial parts and roots using GC-MS. Also, *in vitro* antioxidant and metal chelation potentials of the two parts were investigated using DPPH radical scavenging capacity assay and iron metal chelation assay, respectively.

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# Table 1. Relative Percentile of Volatile Metabolites Detected in *Ludwigia stolonifera* Aerial Part and Root Hexane Extracts Analyzed Using GC–MS

peak	$R_{ m t}$	RI	compound	aerial part	roots		
total alcoh	ol			1.56	1.76		
1	14.63	1548.9	2-undecen-1-ol	0.35			
2	12.8	1447	1-octanol, 2-butyl		1.07		
3	14.64	1585.7	2-dodecen-1-ol		0.33		
4	15.09	1622	cvclododecanemethanol	0.41	0.36		
5	11.43	1351.3	11-methyldodecanol	0.8			
total aliph	5 11.45 1551.5 11-illeulyidodecalioi 0.8						
6	8 06	11/1 8	tritatracontana	0.23	10.7		
7	0.00	1201.2	decape	3.47	4.61		
8	9.12	1201.2	4 methyl decane	1.50	7.01		
0	7.02 10.22	1240.7	a methyl decane	1.57	0.81		
9 10	10.33	1200	Josefier	1.5	0.81		
10	10.82	1311.0		1.58			
11	10.93	1318.8	2,5-dimethyl nonane	0.49	1.42		
12	11.14	1332.8	2-methyl decane	1.42	1.43		
13	11.76	13/2./	1-etnyl-1-metnyl cyclonexane	0.75	0.51		
14	12.31	1410	undecane	9.38	7.41		
15	12.59	1430.9	2-methyl trans-decalin	1.01	<i></i>		
16	12.81	1447.6	dodecane	9.44	6.47		
17	14.02	1538.7	5-methyl-undecane	1.19			
18	14.14	1547.8	4-methyl-undecane	0.74	0.64		
19	14.27	1557.6	2-methyl-undecane	1.89	1.56		
20	14.47	1572.9	3-methyl-undecane	1.01	0.84		
21	15.79	1681.4	2,5-dimethyl-undecane	0.37			
22	42.22	4830.2	heneicosane	2	2.65		
23	45.44	5238.2	hexacosane	0.74			
24	46.96	5430.9	tetracontane	1.23	3.76		
25	6.64	1062.1	1,2,3-trimethoxycyclohexane		0.26		
26	6.95	1079.2	propyl-cyclohexane		0.37		
27	7.06	1085.4	3-methyl-nonane		1.96		
28	7.22	1094.7	1-ethyl-2,4-dimethyl-cyclohexane		1.5		
29	7.46	1108	1-methyl-3-propyl-cyclooctane		1.95		
30	7.7	1121.7	(E)-3-tetradecene		0.56		
31	8	1138.1	2-methyl-nonane		0.46		
32	9.99	1258	3,7-dimethyl-decane		0.33		
33	10.33	1280.1	3,7-dimethyl-nonane		1.03		
.34	11.5	1356	1-isopropyl-1-methylcyclohexane		0.56		
35	11.76	1373	1-ethyl-1-methyl-cyclohexane		0.79		
36	12.14	1235	1-methyl-2-propyl cyclohexane		0.73		
37	13.98	1536	2 5-dimethyl-decane		1.04		
38	15.8	1681.9	3.6-dimethyl-undecane		0.29		
39	49.32	5729.8	2-methyloctacosane		0.09		
40	0.25	1200.0	1.2 dimothowardonantana		0.53		
41	9.25	1265.4	butyleyclobayana	1 1 2	1.21		
+1	10.11	1203.4	butyicyclonexane	1.15	27.71		
		2616	6 nhanri dagang	40.28	37.71		
42	24.74	2010	4 should should be	1.19	0.90		
43	24.95	2043.2	4-phenyl elcosane	1.14	0.91		
44	25.41	2700	3-phenyl decane	1.41	1.16		
45	26.31	2814.5	2-phenyl-decane	2.08	1.79		
46	27.14	2919.5	5-phenyl undecane	4.48	3.57		
47	27.37	2949.6	4-phenyl undecane	2.77	2.21		
48	27.86	3011.3	3-phenyl undecane	3.43	2.76		
49	28.74	3122.2	2-phenyl undecane	4.93	3.94		
50	29.31	3195.2	6-phenyl dodecane	2.17	1.76		
51	29.42	32.9.1	5-phenyl dodecane	2.36	1.93		
52	29.69	3243.7	4-phenyl dodecane	2.09	1.69		
53	30.18	3304.8	3-phenyl dodecane	2.67	2.18		
54	31.03	3413.1	2-phenyl dodecane	3.93	3.17		
55	31.49	3470.8	6-phenyl tridecane	2.61	2.11		
56	31.63	3489.5	5-phenyl tridecane	2.01	1.59		
57	31.9	3522.8	4-phenyl tridecane	1.73	1.45		

# Table 1. continued

peak	$R_{ m t}$	RI	compound	aerial part	roots
58	32.4	3586.3	3-phenyl tridecane	2.1	1.77
59	33.23	3691.2	2-phenyl tridecane	3.18	2.63
60	26.06	3759.7	3-phenyl tridecane		0.13
total diter	penes			1.36	2
61	13.12	1471.5	phytol	1.36	1.52
62	9.58	1231.3	tetraprenol		0.48
Total fatty	v acid/ester			4.33	3.71
63	14.95	1609.9	oxalic acid, cyclohexylmethyl isohexyl ester	0.91	0.8
64	33.52	3728.3	palmitic acid methyl ester	0.71	0.62
65	44.8	5158.1	bis(2-ethylhexyl)phthalate	1.61	1.53
66	11.63	1364.2	oxalic acid, di(cyclohexylmethyl) ester	0.38	
67	36.88	4154.3	methyl linoleate	0.33	0.43
68	37.02	4171.8	methyl linolenate	0.91	0.8
total monoterpenes				5.34	7.34
69	12.6	1431.7	2-methyl-trans-decalin		0.69
70	6.81	1071.5	1,1,4,4-tetramethyl-cyclohexane		0.35
71	7.55	1113.1	1-butyl-2-propyl-cyclopentane		0.15
72	10.54	1293.5	1-ethyl-2-propyl-cyclohexane		0.33
73	10.63	1299.3	tetrahydrocarvone		0.2
74	10.83	1312.1	decalin		2.15
75	11.87	1380.3	1-methyl-2-pentyl-cyclohexane		1.49
76	13.36	1489.3	pentyl cyclohexane	0.98	0.92
77	13.5	1499.6	hexylcyclopentane	0.58	0.58
78	7.87	1130.9	1-ethyl-2,4-dimethyl-cyclohexane	0.46	
79	8.24	313	p-menthane	0.83	0.48
80	8.56	1151.7	1-methyl-2-propyl-cyclohexane	0.86	
81	8.94	1191	1,2-diethyl-, <i>cis</i> -cyclohexane	0.2	
82	11.94	1384.6	1-ethyl-2-propyl-cyclohexane	0.76	
83	13.77	1520	1-methyl-2-pentyl-cyclohexane	0.67	
total oxyg	enated hydrocarbons	0.1			
84	9.25	1209.8	1,1-bis(dodecyloxy)-hexadecane	0.1	
total stero	ls			0.22	0.92
85	56.37	6623.6	γ-sitosterol	0.22	0.92



Figure 1. (a) GC–MS spectrum of the *L. stolonifera* aerial part *n*-hexane extract. (b) GC–MS spectrum of the *L. stolonifera* root *n*-hexane extract, 7: decane, 14: undecane, 16: dodecane, 47: 4-phenyl undecane, 52: 4-phenyl dodecane, 57: 4-phenyl tridecane, 24: tetratetracontane, and 85: γ-sitosterol.

# RESULTS AND DISCUSSION

**GC–MS Volatile Profiling in** *L. stolonifera* **Aerial Parts and Roots.** The goal of this study was to explore the volatile metabolite composition in *L. stolonifera* aerial part and root hexane extract. GC–MS analysis revealed the identification of 85 volatile components (Table 1, Figure 1) belonging to eight classes, viz., aliphatic, aromatic, and oxygenated hydrocarbons, monoterpenes, diterpenes, alcohols, acids/esters, and sterols. Each group includes various numbers of different compounds in which their percentage differs in both hexane extracts of the plant, as shown in Figure 2. ACS Omega



Figure 2. Chemical composition of *L. stolonifera* aerial part and root hexane extracts.

**Aliphatic Hydrocarbons.** Aliphatic hydrocarbons were found to be the major constituents detected in aerial part and root hexane extracts which accounted for 41.16 and 46.70%, respectively, of the volatile composition. Undecane (peak 14), dodecane (peak 16), and decane (peak 7) were prevalent by 9.38, 9.44, and 3.47% in aerial parts, respectively, while in roots were 7.41, 6.47, and 4.61%, respectively, whereas tritetracontane (peak 6), decalin (peak 10), and 2,5-dimethylnonane (peak 11) were represented by 0.23, 1.58, and 0.49%, respectively, and detected only in the aerial part extract. Undecane is a naturally occurring alkane hydrocarbon and has been reported to have anti-inflammatory, antiallergic, and immunosuppressant effects.<sup>14</sup>

**Aromatic Hydrocarbons.** Aromatic hydrocarbons constituted the second most abundant volatile class in aerial part and root hexane extracts which represented 46.28 and 37.71% of the total identified compounds, respectively. The highest compounds detected were 5-phenylundecane (peak 46) which constituted 4.48 and 3.57%, 2-phenylundecane (peak 49) which constituted 4.93 and 3.94%, and 2-phenyldodecane which constituted 3.93 and 3.17%, respectively, in both aerial parts and roots. Oxygenated hydrocarbons were detected in trace levels only in the aerial part and represented by 1,1-bis(dodecyloxy) hexadecane (peak 84) and was detected by 0.13%. Aromatic hydrocarbons and their phenylundecane derivatives have been reported for their good antifungal and antibacterial activity.<sup>15</sup>

Acids/Esters. The relative amounts of fatty acids/esters were detected in the volatile blend of L. stolonifera aerial parts and roots by 4.33 and 3.71%, respectively. Both saturated and unsaturated fatty acid esters were detected in both aerial parts and roots. Palmitic acid methyl ester (peak 64) 0.71 and 0.62%, methyl linoleate 0.33 and 0.43%, and methyl linolenate 0.91 and 0.8%, respectively, were detected as the major components. Oxalic acid derivatives were detected in both aerial parts and roots and represented by oxalic acid, cyclohexylmethyl isohexyl ester (peak 63, 0.91, and 0.8%, respectively), while oxalic acid, di(cyclohexylmethyl)ester (peak 66) was detected only in the aerial parts. In addition, the abundance of phthalic acid derivative (bis(2-ethylhexyl)phthalate, peak 65) was observed in both aerial parts and roots.<sup>16</sup> Organic acids such as oxalic acid and phthalic acid were reported to be effective in metal chelation and produced by plants as typical extracellular metal chelators in phytoremediation.<sup>17,18</sup> Interestingly, the presence of oxalic acid and phthalic acid derivatives may interfere with the metal chelation capacity of L. stolonifera.

**Terpenes/Sterols.** Monoterpene compounds were detected by 5.34 and 7.34% in aerial parts and roots, respectively. Dekalin

(peak 74), 1-methyl-2-pentyl cyclohexane (peak 75), and 1methyl-2-pentyl-cyclohexane (peak 75) were detected only in the root extract. Other monoterpenes were detected in both aerial part and root hexane extracts by different percentages such as pentyl cyclohexane (peak 76) 0.98 and 0.92% and *p*menthane (peak 79) 0.83 and 0.48%, respectively. Phytol, a diterpene member of the long-chain unsaturated acyclic alcohols, was detected in the aerial part and root extracts and accounted 1.36 and 1.52%, respectively. Phytol is a valuable essential oil of pleasant fragrance and has been focused for its myriad biological activities such as anxiolytic, metabolismmodulating, cytotoxic, antioxidant, apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects.<sup>19</sup>

Sterols were detected in trace levels in both aerial part and root extracts represented by  $\gamma$ -sitosterol which accounted for 0.22 and 0.92%, respectively. Plant sterols are found in the highest amounts in vegetable oils, nuts, and seeds and considered as potent hypolipidemic agents.<sup>20</sup>

**Alcohols.** Alcohols were detected by comparable levels in both aerial part and root hexane extracts of 1.56 and 1.76%, respectively. Cyclododecanemethanol (peak 4) was detected in aerial and root extracts at levels ca. 0.41 and 0.36%, respectively, whereas 11-methyldodecanol (peak 5) and *trans*-2-undecen-1-ol (peak 3) were predominant only in the aerial part extract by 0.8 and 0.35%, respectively. On the other hand, 1-octanol, 2-butyl (peak 2) and *trans*-2-dodecen-1-ol (peak 3) were detected only in the root extract by 1.07 and 0.33%, respectively.

#### BIOLOGICAL ACTIVITY

**Antioxidant Assay.** Free radicals could be produced as a result of different metabolic reactions or initiated by other environmental factors. Therefore, there are great interests in antioxidants, especially those from the natural origin as being safer and desirable than synthetic drugs, which can reduce these free radicals in the human body.<sup>21</sup> Medicinal plants are rich in phenolic compounds that possess antioxidant activity. 2,2-Diphenyl-1-picrylhydrazyl assay (DPPH) is the most common assay for antioxidant activity evaluation via testing radical scavenging capacity of the plant extract.

DPPH inhibition results of the different concentrations used compared with ascorbic acid as reference antioxidants are summarized in Table 2 and Figure 3. Calculated  $SC_{50}$  for ascorbic acid and different fractions of *L. stolonifera* are shown in Figure 4.

The samples showed variable antioxidant scavenging activity against the DPPH radical concluded from the  $SC_{50}$  value of the aerial part total methanolic extract, ethyl acetate, *n*-butanol fractions, and root total methanolic extract recorded as 19.2, 7.6,

Table 2. Calculated SC<sub>50</sub> for Ascorbic Acid and Different Fractions of *L. stolonifera* and Metal Chelation Activity Represented by % of Inhibition and  $\mu$ M EDTA eq/mg Extract

radical scavengin	g capacity	metal chelation assay		
SC <sub>50</sub> (µg/1	mL)	% of inhibition	μM EDTA eq/mg extract	
aerial total ex.	19.2 ± 1.6	52.8	36.36 ± 3.58	
ethyl acetate Fr.	$7.6\pm0.4$			
n-butanol Fr.	$16.0 \pm 1.2$			
roots total ex.	$61.6 \pm 3.4$	43.6	$29.67 \pm 1.52$	
ascorbic acid R. S.	$10.6 \pm 0.8$			

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**Figure 3.** (a) Antioxidant activity of *Ludwigia stolonifera* different fractions represented as % inhibition. (b) Calculated SC<sub>50</sub> *L. stolonifera* fractions compared to ascorbic acid expressed as average  $\pm$  standard deviation (scanning electron microscopy), and *P* < 0.05 was used.



**Figure 4.** (a) Linear dose–inhibition curve of EDTA. (b) *L. stolonifera* aerial part and root metal chelation activity represented by % inhibition and  $\mu$ M EDTA eq/mg extract expressed as average ± standard deviation (SD).

16, and 61.6  $\mu$ g/mL, respectively, compared to ascorbic acid SC<sub>50</sub> 10.6  $\mu$ g/mL (Figure 3). The aerial part ethyl acetate fraction showed to be the most effective sample (SC<sub>50</sub>: 7.6  $\mu$ g/mL), and the root total methanolic extract showed the modest

effect with SC<sub>50</sub>: 61.6  $\mu$ g/mL. The results are confirmed by the reported biological review for the family Onagraceae which has common potent antioxidant activity as in the *Circaea lutetiana L*. aqueous methanolic extract and aerial part which possess strong scavenging activity toward DPPH (SC<sub>50</sub> 33.1  $\mu$ g/mL).<sup>22</sup>

**Metal Chelation Assay.** Reactive oxygen species are formed *in vivo* by the presence of the iron metal (Fe<sup>3+</sup>) such as superoxide radicals (O2<sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals (<sup>-</sup>OH), which are very strong oxidant agents, so they need strong antioxidants to overcome the oxidant agents.<sup>23</sup> Antioxidants may be classified according to the mechanism of action as chelation of transition metals, electron transfer, or hydrogen atom transfer.<sup>24</sup> In this study, we assessed the ability of samples to chelate transition metals such as Fe<sup>2+</sup> which may induce degenerative diseases such as cardiovascular, Parkinson's, and Alzheimer's diseases.

The results of the tested samples (aerial and root total methanolic extracts) are presented as  $\mu$ M EDTA equivalent/mg sample using the linear regression equation extracted from the calibration curve (Figure 4), and compounds of samples bind a certain amount of Fe<sup>2+</sup>, but the remaining Fe<sup>2+</sup> reacts with ferrozine, forming a blue-colored complex that can be monitored spectrophotometrically, and the data were labeled in Table 2 and Figure 4.

Samples were prepared in a concentration of 1 mg/mL in methanol using the linear regression equation. The results revealed that 1 mg of the aerial part total extract is equal to 36.36  $\mu$ M EDTA, and from the calibration curve, the percentage of inhibition was 52.8%. On the other hand, 1 mg of the root total extract equal to 29.67  $\mu$ M EDTA revealed 43.6% of inhibition. Data showed that the same concentration for two samples was equivalent to different concentrations of EDTA and so was recorded different % of inhibition. The extract of the aerial parts was more effective than the root extract of *L. stolonifera* as the metal chelator.

# EXPERIMENTAL SECTION

**Plant Material.** *L. stolonifera* (Guill. & Perr.) P.H.Raven was collected from the Nile River at Al-Qanatir Al-Khayriyah, September 2019, and kindly identified by Dr. Rim Hamdy, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen (01Lst/2019) was deposited at the Herbarium of Pharmacognosy department, Faculty of Pharmacy, Helwan University. The plant materials (roots and aerial parts), 500 g each, were dried in shade and then coarsely grinded separately. Part of the coarsely divided powdered roots and aerial parts were extracted with *n*-hexane for GC–MS analysis. Another part of both aerial parts and roots was macerated and extracted with the methanol solvent and then further fractionated with ethylacetate and *n*-butanol for biological study.

**GC**–**MS Analysis.** GC–MS analysis was performed at Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Mass spectra were recorded using Shimadzu GCMS-QP2010 (Koyoto, Japan) equipped with an Rtx-SMS fused bonded column (30 m × 0.25 mm i.d. X 0.25  $\mu$ m film thickness) (Restek, USA) equipped with a split–splitless injector. The initial column temperature was kept at 50 °C for 3 min (isothermal) and programmed to 300 °C at a rate of 5 °C/ min and kept constant at 300 °C for 10 min (isothermal). The injector temperature was 280 °C. The helium carrier gas flow rate was 1.37 mL/min. All the mass spectra were recorded by applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; and ion source, 220 °C. Diluted samples (1% v/v) were injected with the split mode (split ratio 1: 15).

Identification of essential oil composition was performed by comparing their retention indices in relation to *n*-alkanes (C6–C20), mass matching to NIST, Wiley library database, and standards if available. Peaks were first deconvoluted using AMDIS software (www.amdis.net (accessed on 28 November 2019)) before mass spectral matching.<sup>12,25</sup>

## BIOLOGICAL INVESTIGATION

**DPPH Radical Scavenging Activity.** Radical scavenging activity of *L. stolonifera* aerial part and root total methanolic extracts, *n*-butanol, and ethyl acetate fractions against the stable free radical DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. The 3 mL DPPH (0.2 mM) solution was mixed with 77  $\mu$ L extract solution in 1 cm path length cuvettes, and the samples were kept in the dark for 30 min at room temperature, and then, the decrease in absorption was measured at 517 nm. Ascorbic acid was used as a positive control, and the results were expressed as % inhibition for DPPH.<sup>26</sup> All measurements were performed in three replicates, and results are calculated as mean and standard deviation. The percentage inhibition of the DPPH radical was calculated according to the formula

% inhibition =  $[(AB - AA)/AB] \times 100$ 

where AB = absorbance of the control at t = 0 min and AA = absorbance of the sample at t = 16 min.

**Metal Chelation Activity.** Iron metal chelation assay was used to determine the chelating activity of *L. stolonifera* aerial part and root methanol extracts.<sup>27</sup> Trolox stock solution of 0.1 mM in methanol was prepared, and 10 serial dilutions were prepared in different concentrations and preparation of samples at a concentration of 1 mg/mL in methanol. The freshly prepared ferrous sulfate ( $20 \mu$ L, 0.3 mM) was mixed with  $50 \mu$ L of the sample/compound in 96-well plates (n = 6); then,  $30 \mu$ L of ferrozine (0.8 mM) was added to each well. The reaction mixture was incubated at room temperature for 10 min. At the end of the incubation time, the decrease in the produced color intensity was measured at 562 nm and EDTA was used as the standard chelating agent. Finally, the data are represented as means  $\pm$  SD according to the following equation, and the results were recorded using microplate reader FluoStar Omega.

 $[(Abs blank - Abs sample)/Abs blank] \times 100$ 

# CONCLUSIONS

*L. stolonifera* (Guill. & Perr.) P.H.Raven is an important aquatic macrophyte in the Nile Delta. This plant is common with its economic importance and high potential in eliminating various toxic pollutants from the aquatic environment. In this study, we investigated the difference between aerial part and root essential oil contents by GC–MS analysis. A total of 85 metabolites were identified in both aerial parts and roots with the abundance of aliphatic and aromatic hydrocarbons. Biological investigation on the plant parts revealed its potential activities as antioxidants and metal chelation capacity. Further studies on the nonvolatile metabolites and more deep biological evaluation are recommended.

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