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Chemical composition variations, allelopathic, and antioxidant activities of *Symphyotrichum squamatum* (Spreng.) Nesom essential oils growing in heterogeneous habitats

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Abstract The present study aimed to analyze the chemical composition of *Symphyotrichum squamatum* EOs growing in two different habitats to explore the ecological implication on the EOs production and evaluate their antioxidant and allelopathic potentialities. The EOs from the aerial parts collected from coastal Mediterranean belt and inland abandoned habitats in the Nile Delta of Egypt, were extracted and analyzed using gas chromatography-mass spectrometry. Sixty compounds were characterized as overall constituents of EOs from both samples. Sesquiterpenes were the main component and represented by 69.77% and 88.68% from coastal and inland sample, respectively. The coastal sample attained a relatively high content of monoterpenes compared to the inland sample. Major compounds from the EOs of the coastal habitat sample, were humulene epoxide, (-)-spathulenol, (-)-caryophyllene oxide, germacrene D, and α-humulene representing

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59.72%. However, β-pinene, germacrene D, α-humulene, α-muurolene, humulene epoxide, (-)-caryophyllene oxide, and β-cadinene were the major compounds of EOs of the inland habitat sample, representing 63.70%. The correlation analysis revealed more correlation between the Egyptian inland *S. squamatum* and the Japanese ecospecies. However, the Egyptian coastal *S. squamatum* and Turkish ecospecies were more correlated to each other. The present data suggested that chemotypes of *S. squamatum* maintain their typical pattern despite ecological or climatic differences. The EOs of *S. squamatum* showed moderate antioxidant activity, wherein coastal and inland EOs have an IC_{50} value of 382.53 and 559.63 μL L^{-1}, respectively. Also, the EOs from both habitats showed moderate allelopathic activity against the noxious weed *Bidens pilosa*. However, the activity of the coastal sample was more than inland one and could be attributed to the content of the major compounds, especially the oxygenated terpenes.

1. Introduction

Essential oils (EOs) derived from different plant parts exhibit aromatic odors and are valuable resources of various secondary metabolites. In most of the cases, the major components of EOs are terpenes (Kim et al., 2014a). Essential oils have been commonly used in several applications in aromatherapy (Sharifi-Rad et al., 2018a, 2018b). Since ancient times, EOs have been used in folklore medicine due to their potent biological activities (Lang and Buchbauer, 2012). The pleasant fragrance or flavor of EOs have encouraged their use in significant amounts in cosmetic, perfume, pharmaceutical and food industries (Raut and Karuppayil, 2014; Salehi et al., 2018; Sharifi-Rad et al., 2018c).

Family Asteraceae represents about 10% of the overall of the flora around the world, with approximately 1535 genera and 23,000 plant species (Nakajima and Semir, 2001). Also, Asteraceae is one of the biggest family of the Egyptian flora (Barakat et al., 2014). The EOs from Asteraceae species include various components that might have several commercial applications in perfume and liquor industries. Also, several pharmacological potentials such as larvicide, nematicide, antispasmodic, genotoxic, antimicrobial, antifungal, and allelopathic properties were stated for the EOs of these plants (Dias et al., 2009).

The species of genus *Aster* (syn. *Symphyotrichum*) are widely distributed around the world, including Africa, Asia, North America, and Europe (Mabberley, 2008). The *S. squamatum* introduced to Egypt from Latin America, and the first record in Egypt back to early of the 1970s. Now it is a wide and common distributed plant in Egypt, and it is found with high biomass in different habitats such as wastelands, abandoned fields, fields of orchards, fields of crops, railways, high ways, edges of canals, drains, and lakes (Boulos and El-Hadidi, 1994).

It has been reported that several candidates of this genus are used in traditional medicines for the treatment of chronic bronchitis, pertussis, and pneumonia. Several biological activities associated with these plants and their metabolites were tested for antifungal, (Lanzotti, 2005), antitumor (Kim et al., 2014b), antioxidant and cytotoxicity properties (Bibi et al., 2011). Several bioactive metabolites were characterized from *Aster* species including diterpenes, monoterpene, sesquiterpenoids, flavones, coumarins, polyacetylenes, triterpene glycosides, phenolic compounds, saponins, peptides, benzofurans, sugars and esters (Choi, 2012; Dias et al., 2009; Kim et al., 2014b; Miyazawa and Kameoka, 1977; Nemanja et al., 2015; Shao et al., 1995a, 1995b).

Up to our knowledge, there are only two studies investigating the chemical composition of the EOs of *Symphyotrichum squamatum* (Spreng.) Nesom (syns: *Aster subulatus* Michx. & *Aster squamatus* (Spreng.) Hieron.) collected from Turkey (Ayaz et al., 2017) and Japan (Miyazawa and Kameoka, 1977). The results of these studies indicated that sesquiterpene, elemol, and the hydrocarbon, hexadecanoic acid, represented the main components of the Turkish ecospecies, while β-pinene and *p*-cymene were the main compounds of the Japanese one (Ayaz et al., 2017; Miyazawa and Kameoka, 1977). Various studies confirmed that the chemical composition of EOs is dependent on the plant organ, season, prevailing temperature, water availability, altitude, soil fertility, and genetic pool (Abd El-Gawad et al., 2019; Abd El-Gawad, 2016; Khazaie et al., 2008). Further, no study has examined the antioxidant or allelopathic activities of the EOs from *S. squamatum*.

Therefore, our study is aimed to (i) determine the chemical composition of the EOs of *S. squamatum* collected from two different habitats in Egypt, (ii) study the correlation between the chemical composition of the EOs and the soil variables of these habitats, (iii) correlate the EOs of the Egyptian ecospecies of *S. squamatum* from different habitats with other reported ecospecies, (iv) asses the antioxidant activity of the EOs, and (v) evaluate the allelopathic effect of the EOs against the noxious weed *Bidens pilosa* as potential green eco-friendly bioherbicide.

2. Material and methods

2.1. Plant materials

The aerial parts of *S. squamatum* were collected during March (flowering stage) from two different habitats in Egypt. The first sample was collected from the Mediterranean coastal belt at Gamsa City, Al-Dakahlia Governorate, Egypt (31°26′35.4″N 31°33′35.5″E), and was called coastal sample. The second sample was collected at the same time from an abandoned area near Mansoura University, Mansoura, Egypt (31°02′22.2″N 31°21′09.7″E). This location is in the middle of the Nile Delta and was called inland sample. The collected samples were cleaned from dust and dried at room temperature (25 °C

± 2). The dried plant materials were ground into a fine powder using a grinder (IKA® MF 10 Basic Microfine Grinder Drive, Breisgau, Germany) and stored in paper bags until further analyses. Voucher specimen with code: Mans.0010272703 was added in the herbarium of Botany Department, Faculty of Science, Mansoura University, Egypt.

2.2. Extraction and analysis of the EOs

The EOs were separately extracted from 300 g of aerial parts of *S. squamatum* from each sample/habitat (coastal and inland) immediately after preparation by hydrodistillation using a Clevenger-type apparatus for three h. The oil layer of the oil was separated using diethyl ether and dried with anhydrous sodium sulfate (0.5 g). This extraction was repeated twice afforded two samples of EOs for each location. The extracted EOs were stored in sealed air-tight glass vials at 4 °C until further analysis.

The EOs components of the extracted samples were analyzed separately and identified following GC-MS analysis. The GC-MS analysis was performed via chromatography-mass spectrometry instrument at the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt. The GC-MS systems consist of TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), accompanied by thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer; Model ISQ spectrometer). The GC-MS system contained a TR-5 MS column with a dimension of 30 m × 0.32 mm ID × 0.25 µm. Helium gas was used as a carrier gas with a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10. The temperature cycle was as follows: 60 °C for one min and then rising at 4.0 °C per min until 240 °C and held for one min. The detector and injector were held at 210 °C. An aliquot of one µL of the diluted samples (1:10 hexane, v/v) was always injected. Mass spectra were derived by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450.

2.3. Identification of EOs constituents

The chemical compounds were identified according to their retention indices (relative to *n*-alkanes C_8-C_{22}), comparison with the authentic constituents available in our laboratories, and comparison of their mass spectra with that of Wiley spectral libraries databases (NIST AMDIS). The compound percentage was calculated based on the peak area derived from gas chromatography.

2.4. Antioxidant activity

The antioxidant scavenging activity of the extracted EOs from coastal and inland samples was evaluated according to their ability to scavenge the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical. In accordance with Sharma and Bhat (2009), a reaction mixture of two mL of DPPH (0.15 mM) and equal amount of various concentrations (50, 100, 200, 300, 400, 500, and 600 µL L^{-1}) of either EOs, in methanol, or ascorbic acid (standard antioxidant) was prepared in test tubes. The test tubes were shaken vigorously and incubated in dark condition at 25 °C for 30 min. The absorbance was measured by a spectrophotometer (Spectronic® 21D model) at 517 nm. The antioxidant scavenging activity was expressed in percentage as follows:

$$\text{Radical scavenging activity}\% = [1 - (\text{Absorbance}_{\text{sample}}/\text{Absorbance}_{\text{control}})] \times 100$$

The IC_{50} (the concentration of the EOs required to scavenge 50% of the initial concentration of DPPH) was also calculated graphically.

2.5. Allelopathic bioassay

The allelopathic activity of the extracted EOs was evaluated against *B. pilosa* as one of the dangerous noxious weeds. Ripe seeds of *B. pilosa* weed were collected in June from the garden of Mansoura University, Mansoura, Egypt (30°38′33.5″N 31°59′50.4″E). The collected seeds were surface sterilized by 0.3% NaOCl for three min. To examine the allelopathic effect of the EOs, various concentrations (100, 200, 400, 600, 800, and 1000 µL L^{-1}) were prepared in Tween® 80 (Sigma–Aldrich, Germany) as an emulsifier. Twenty *B. pilosa* seeds were placed in Petri dishes (9 cm) lined with a filter paper Whatman No. 1. and five mL of each EOs concentration or tween (as control treatment) was added. The Petri dishes were sealed with Parafilm® tape and incubated in a growth chamber at 27 °C light regime conditions of 16 h/8h light/dark for five days (Abd El-Gawad, 2016). The shoot and root lengths of all seedlings per each plate were measured, and the allelopathic inhibition of root and shoot growth was calculated, with respect to control, as follows:

$$\text{Inhibition}\ (\%) = 100 \times \frac{(\text{No/Length of control} - \text{No/Length of treatment})}{\text{No/Length of control}}$$

2.6. Statistical analysis

The data of the allelopathic bioassay of the EOs were expressed as the percentage of inhibition with respect to control and were subjected to Generalized Linear Models (GLM) analysis, using the STATISTICA (version 7) software system (StatSoft, Inc. Tulsa, Oklahoma, USA, www.statsoft.com). The data of antioxidant, in triplicates, were subjected to one-way ANOVA followed by Duncan's test at probability level 0.05 using CoStat (version 6.311, CoHort Software, USA, www.cohort.com). The data of the EOs composition derived from GC-MS analysis of the coastal and inland samples from the Egyptian ecospecies as well as the data of the EOs reported from the Japanese and Turkish ecospecies were subjected to Pearson similarity correlation. The dataset consists of the concentrations (%) of 99 chemical compounds that were identified in the three investigated ecospecies. Also, the dataset was submitted to principal component analysis (PCA) to determine whether a significant difference exists between different ecospecies, based on the EOs composition. The software XLSTAT (version 2018, Addinsoft, NY, USA, www.xlstat.com) was used in the analysis of Pearson correlation and PCA.

3. Results and discussion

3.1. Chemical constituents of *S. squamatum* EOs

The hydro-distillation extraction of the aerial plant parts of coastal and inland *S. squamatum* yielded 0.017% and 0.014% (v/w) of the EOs, respectively. The Japanese ecospecies of *S. squamatum* yielded more EOs (0.03%) than that of the Egyptian ecospecies (Miyazawa and Kameoka, 1977). The yield was more pronounced in the coastal sample, where the habitat is affected by salinity from the Mediterranean Sea. Bourgou et al. (2010) reported an increase of the EOs yield in *Nigella sativa* by increasing the salinity.

An overall of sixty compounds of EOs from both coastal and inland samples, representing 100% of the total mass, were characterized based on GC-MS analysis. Forty-six compounds were identified from the coastal sample and forty-nine from the inland sample of *S. squamatum* (Table 1). Among the identified compounds, sesquiterpenes represented the main component comprising 69.77% and 88.68% of the total EOs from the coastal and inland sample, respectively (Fig. 1). Sesquiterpene hydrocarbons were characterized as major components of EOs of the inland sample (47.39%), while the EOs of the coastal sample has 27.13% as sesquiterpene hydrocarbons of the total mass. On the other hand, the oxygenated sesquiterpenes were the major components in the coastal sample (61.55%) while they were represented by 22.38% in the inland sample (Fig. 1). These findings were in consonance with that of the Turkish ecospecies which attained high content of sesquiterpenes in its EOs (Ayaz et al., 2017). It is pertinent to mention here that sesquiterpenes have been reported as dominant group of the EOs of other related species of *Aster* such as *A. albanicus* (Nemanja et al., 2015), *A. handelii* (Xiao-ping and Xiaoping, 2006), *A. lanceolatus* (Dias et al., 2009), and *A. tataricus* (Choi, 2012).

Contrarily, the EOs of the Japanese ecospecies was characterized by the dominance of monoterpenes (Miyazawa and Kameoka, 1977) which represented 84.88% of the total mass. Also, our results showed that the Egyptian ecospecies have a relatively high content of monoterpenes, but the coastal habitat sample attained higher content than that of the inland sample. Moreover, other species of *Aster* have been reported to contain monoterpenes as the major class of their EOs such as *A. ageratoides* (Miyazawa et al., 2008), *A. poliothamnus* (Tu et al., 2006), *A. spathulifolius* (Kim et al., 2014a), and *A. scaber* (Lee et al., 2012). In the present study, diterpenoids and hydrocarbons were determined as minor classes of the EOs in both inland and coastal samples.

In the EOs of the coastal sample, humulene epoxide, (-)-spathulenol, (-)-caryophyllene oxide, germacrene D, and α-humulene were identified as major compounds representing 59.72% of the EOs total mass. However, β-pinene, germacrene D, α-humulene, α-muurolene, humulene epoxide, (-)-caryophyllene oxide, and β-cadinene were the major compounds of the inland sample of the Egyptian *S. squamatum* ecospecies, where they represented 63.70% of its EOs (Table 1). Other minor compounds are listed in detail in Table 1.

In a general overview of the chemical composition of EOs of the two samples, we observed that the two samples include most of the components with different percentages. All the major components of the two samples especially germacrene D, β-pinene, spathulenol, and caryophyllene oxide were already reported as major compounds in the EOs of other species of *Aster* such as *A. subulatus*, *A. albanicus*, *A. spathulifolius* and *A. lanceolatus* (Ayaz et al., 2017; Dias et al., 2009; Kim et al., 2014a; Nemanja et al., 2015).

3.2. Correlation between S. squamatum ecospecies based on EOs composition

The analysis of PCA showed that the two samples of the Egyptian *S. squamatum*, as well as the Turkish and Japanese plants, were more comparable in the composition of the EOs (Fig. 2). Specifically, more correlation was found between the Egyptian inland *S. squamatum* of the present study and the Japanese ecospecies. These two samples were also correlated to the β-pinene, D-limonene, β-cadinene, and *trans*-caryophyllene. Also, β-pinene represented 40.9% and 24.2% of the total EOs from the Japanese and Egyptian inland *S. squamatum*, respectively.

On the other hand, the other two samples (Egyptian coastal and Turkish *S. squamatum*) were more correlated to each other, and showed a close relation to elemol, (-)-caryophyllene oxide, (-)-spathulenol, humulene epoxide, α-humulene, α-muurolene, and germacrene D. This correlation could be ascribed to the similarity in the climate of both geographical regions of the Mediterranean climate (Ayaz et al., 2017). The formation of EOs in plants is profoundly affected by climatic conditions, particularly temperature, solar radiation, day length, and water supply (Baser and Buchbauer, 2015). Moreover, the variation in the EOs can be attributed to the habitat structure, population genetics, soil type, and stress condition (Abd El-Gawad, 2016; Khazaie et al., 2008).

Pearson correlation coefficient analysis revealed a significant positive correlation between the inland sample and the Japanese ecospecies (0.786) as well as the coastal sample (0.376) (Table S1). However, the Japanese ecospecies showed a negative correlation with the Turkish ecospecies (−0.023).

Generally, the rest of major compounds didn't show a specific correlation to any samples (Fig. 2). The present data revealed that the chemotypes of *S. squamatum* maintain their typical pattern despite ecological or climatic differences. Environmental factors induce the production of specific chemicals pattern by activation of the gene expression. This means that the potential to produce bioactive compounds is genetically coded (Franz, 1993).

On the other hand, our results of soil analysis revealed a significant variation in the organic carbon and salinity between the two locations (Table S2). Salinity stress is one of the most important factors that can affect the composition of EOs in plants. Changes in the composition of the EOs in response to salinity have been reported in various plants such as *Salvia officinalis* (Taarit et al., 2010), *Nigella sativa* (Bourgou et al., 2010), and *Ocimum basilicum* (Tarchoune et al., 2013). Germacrene D and caryophyllene oxide (major compounds in the present study) were induced in *Ocimum basilicum* due to salinity (Hassanpouraghdam et al., 2011).

3.3. Antioxidant activity of S. squamatum EOs

The EOs from the aerial parts of *S. squamatum* showed moderate radical scavenging activity of the DPPH in a concentration-dependent manner (Table 2). The EOs from

Table 1 The composition of the EOs from the aerial parts of the coastal and inland samples of *S. squamatum*.

No	Rt[a]	KI_{Lit}[b]	KI_{Exp}[c]	Compound	Area %		Identification[e]
					Inland	Coastal	
Monoterpene hydrocarbons							
1	4.44	933	939	(-)-α-Pinene	1.07[d] ± 0.02	–	MS; KI
2	5.43	1005	1001	α-Phellandrene	0.28 ± 0.01	–	MS; KI
3	5.58	974	975	β-Pinene	24.16 ± 0.04	1.99 ± 0.02	MS; KI
4	5.92	991	993	α-Myrcene	0.25 ± 0.01	–	MS; KI
5	7.12	1024	1028	D-Limonene	0.63 ± 0.01	–	MS; KI
Oxygenated monoterpenes							
6	11.33	1183	1180	Pinocarveol	0.52 ± 0.01	1.15 ± 0.01	MS; KI
7	12.25	1162	1161	Pinocarvone	1.09 ± 0.02	1.53 ± 0.02	MS; KI
8	13.68	1193	1190	(1*R*)-(-)-Myrtenal	0.96 ± 0.01	2.28 ± 0.02	MS; KI
9	17.12	1285	1287	Bornyl acetate	0.21 ± 0.01	0.70 ± 0.01	MS; KI
Sesquiterpene hydrocarbons							
10	19.29	1432	1429	β-Gurjunene	0.48 ± 0.01	–	MS; KI
11	20.67	1376	1379	α-Copaene	1.38 ± 0.01	0.50 ± 0.01	MS; KI
12	21.00	1505	1509	(-)-α-Bourbonene	0.90 ± 0.01	0.43 ± 0.01	MS; KI
13	21.30	1386	1383	α-Elemene	2.83 ± 0.02	2.55 ± 0.03	MS; KI
14	21.50	1402	1398	Longifolene	0.31 ± 0.01	–	MS; KI
15	22.48	1337	1331	*trans*-Caryophyllene	4.71 ± 0.03	1.66 ± 0.02	MS; KI
16	22.95	1351	1356	α-Cubebene	2.13 ± 0.02	0.52 ± 0.01	MS; KI
17	23.07	1436	1439	*trans*-α-Bergamotene	–	0.22 ± 0.01	MS; KI
18	24.15	1439	1434	Aromadendrene	–	0.44 ± 0.01	MS; KI
19	24.00	1454	1450	α-Humulene	7.43 ± 0.04	5.46 ± 0.03	MS; KI
20	24.84	1477	1472	γ-Muurolene	3.43 ± 0.02	0.84 ± 0.02	MS; KI
21	25.06	1480	1483	Germacrene D	8.59 ± 0.05	5.87 ± 0.03	MS; KI
22	25.42	1494	1490	α-Selinene	1.14 ± 0.01	1.31 ± 0.01	MS; KI
23	25.65	1494	1492	Bicyclogermacrene	0.32 ± 0.01	2.30 ± 0.02	MS; KI
24	25.82	1499	1502	α-Muurolene	6.84 ± 0.04	1.60 ± 0.01	MS; KI
25	26.13	1503	1503	Germacrene A	1.77 ± 0.01	1.31 ± 0.01	MS; KI
26	26.41	1506	1508	α-Amorphene	–	0.22 ± 0.01	MS; KI
27	26.58	1473	1470	β-Cadinene	4.78 ± 0.03	1.90 ± 0.02	MS; KI
28	27.65	1542	1541	α-Calacorene	0.35 ± 0.01	–	MS; KI
Oxygenated sesquiterpenes							
29	24.94	1659	1662	Patchouli alcohol	1.18 ± 0.02	0.31 ± 0.01	MS; KI
30	26.78	1646	1644	Isolongifolan-8-ol	–	0.31 ± 0.01	MS; KI
31	28.60	1482	1481	1,5-epoxysalvial-4(14)-ene	–	0.52 ± 0.01	MS; KI
32	28.90	1580	1583	Cubedol	0.22 ± 0.01	–	MS; KI
33	28.99	1578	1580	(-)-Spathulenol	0.43 ± 0.01	15.49 ± 0.04	MS; KI
34	29.13	1580	1577	(-)-Caryophyllene oxide	6.00 ± 0.02	14.42 ± 0.04	MS; KI
35	29.65	1625	1631	Aromadendrene oxide-1	0.47 ± 0.01	0.39 ± 0.01	MS; KI
36	30.25	1605	1601	Humulene epoxide	6.28 ± 0.03	18.48 ± 0.05	MS; KI
37	30.65	1794	1799	Verrucarol	0.81 ± 0.01	0.67 ± 0.01	MS; KI
38	30.94	1642	1637	Cubenol	0.51 ± 0.01	0.67 ± 0.01	MS; KI
39	31.17	1625	1628	Isospathulenol	–	0.94 ± 0.01	MS; KI
40	31.64	1653	1656	α-Cadinol	1.58 ± 0.03	1.03 ± 0.02	MS; KI
41	31.74	1645	1649	Torreyol	1.11 ± 0.02	0.59 ± 0.01	MS; KI
42	31.87	1633	1630	Alloaromadendrene oxide-2	–	0.29 ± 0.01	MS; KI
43	32.08	1636	1631	.tau.Muurolol	0.84 ± 0.01	1.44 ± 0.02	MS; KI
44	32.22	1620	1626	Calarene epoxide	0.53 ± 0.02	1.20 ± 0.01	MS; KI
45	32.63	1591	1596	(+) spathulenol	0.61 ± 0.01	1.47 ± 0.03	MS; KI
46	33.02	1613	1618	Geranyl isovalerate	0.59 ± 0.01	0.67 ± 0.01	MS; KI
47	33.34	1678	1673	*Z*-Nerolidol-epoxyacetate	–	0.22 ± 0.01	MS; KI
48	33.23	1584	1589	Isoaromadendrene epoxide	0.35 ± 0.01	–	MS; KI
49	33.44	1634	1636	Ledene oxide II	0.25 ± 0.01	–	MS; KI
50	34.07	1631	1637	8-Cedren-13-ol	0.28 ± 0.01	0.59 ± 0.01	MS; KI
51	34.39	1666	1669	Alloaromadendrene oxide-1	–	0.36 ± 0.01	MS; KI
52	34.71	1634	1632	*trans*-Longipinocarveol	0.34 ± 0.01	0.75 ± 0.01	MS; KI
53	35.55	1763	1758	Aristolene epoxide	–	0.29 ± 0.01	MS; KI
54	38.73	1845	1848	Hexahydrofarnesyl acetone	–	0.45 ± 0.01	MS; KI

(*continued on next page*)

Table 1 (*continued*)

No	Rt[a]	KI_{Lit}[b]	KI_{Exp}[c]	Compound	Area %		Identification[e]
					Inland	Coastal	
Diterpenoids							
55	38.26	1811	1808	Phytane	–	0.25 ± 0.01	MS; KI
56	38.80	1845	1839	Phytone	0.25 ± 0.01	–	MS; KI
57	40.25	2218	2215	Phytol, acetate	–	0.34 ± 0.01	MS; KI
Oxygenated hydrocarbons							
58	28.19	1623	1629	2-Hydroxy-2,4,4-trimethyl-3-(3-methylbuta-1, 3-dienyl)cyclohexanone	–	0.56 ± 0.01	MS; KI
59	31.06	1655	1650	Acetic Acid 1-[2-(2,2,6-trimethylbicyclo [4.1.0]hept-1-yl)-ethyl]-vinyl ester	0.33 ± 0.01	0.88 ± 0.01	MS; KI
60	36.17	2139	2144	Ethyl linoleate	0.48 ± 0.01	1.64 ± 0.02	MS; KI

[a] Rt: Retention time.
[b] KI_{Lit}: Kovats retention index on DB-5 column in reference to *n*-alkanes.
[c] KI_{Exp}: Experimental Kovats retention index.
[d] Values are the mean ± standard deviation.
[e] The identification of EO components was established depending upon (ii) mass spectral data of compounds (MS), and Kovats indices (RI) with those of Wiley spectral library collection and NIST library databases.

Table 2 Antioxidant activity of the essential oil from aerial parts of *S. squamatum* and ascorbic acid as a reference, estimated by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. Values are mean ± standard error (n = 3).

Conc. (μL L^{-1})	Scavenging activity %			$LSD_{0.05}$
	Coastal sample	Inland sample	Ascorbic acid	
50	17.77 ± 0.74	5.00 ± 0.39	44.02 ± 1.21	2.00
100	24.88 ± 0.51	13.32 ± 0.82	50.94 ± 1.33	3.89
200	39.30 ± 0.86	23.44 ± 0.80	58.95 ± 1.29	4.15
300	48.83 ± 0.78	28.32 ± 0.82	64.22 ± 0.62	3.62
400	51.05 ± 1.13	39.14 ± 0.78	82.77 ± 0.66	3.96
500	59.77 ± 1.33	45.00 ± 1.17	88.87 ± 0.82	5.86
600	64.57 ± 1.76	51.37 ± 1.29	98.13 ± 1.48	7.38
IC_{50} (μL L^{-1})	382.53	559.63	107.81	

the coastal sample of *S. squamatum* revealed more antioxidant activity than that of the inland sample, where they attained an IC_{50} value of 382.53 μL L^{-1} and 559.63 μL L^{-1}, respectively. However, the IC_{50} value for ascorbic acid (standard antioxidant) was 107.81 μL L^{-1} (Table 2). The significant variation in the antioxidant activity between the two samples could be ascribed to the variation in the composition of the EOs.

The EOs are considered as an important class of the allelochemicals that are enhanced in the plant under stress conditions such as salinity stress. Thus, as we expect, the extracted EOs sample from the coastal area of the Mediterranean Sea to show more antioxidant activity. Under salt stress condition, the reactive oxygen species (ROS) are generated in plant cells, and in consequence, the antioxidant defense systems (enzymatic and non-enzymatic) are triggered (El-Shora and Abd El-Gawad, 2015a, 2015b). It is worth mentioning here that plants produce a vast array of chemical compounds specific to their habitats. These compounds play a significant role in stress amelioration, enhancement of the defense system, as well as communication with other organisms including herbivores, insects, pathogens, and other plants (Jones and Dangl, 2006).

However, the general profile of the EOs of the two samples of *S. squamatum* was comparable, though the composition is so different. The sample of the coastal location has a high content of oxygenated compounds, particularly sesquiterpenes, whereas it was triple fold for the inland sample (Fig. 1). This explains the more antioxidant activity of the coastal sample compared to the inland one. Therefore, the antioxidant activity of the coastal sample of *S. squamatum* might be attributed to the synergistic or singular effect of the major compound(s) identified in this sample such as humulene epoxide, (-)-spathulenol, (-)-caryophyllene oxide, germacrene D, and α-humulene. The synergistic activity of the terpenoid compounds was reported for other terpenoid compounds in other plants (Chaubey, 2012; Mitić et al., 2018).

On the other hand, the antioxidant activity of the inland sample could be attributed to *β*-pinene, germacrene D, α-humulene, α-muurolene, humulene epoxide, (-)-caryophyllene oxide, and *β*-cadinene. Nevertheless, the other minor compounds could play a significant role in antioxidant activity, even at low concentration (Carrillo and Tena, 2006). It is possible that the minor constituents may be implicated in synergism with the other active compounds (Hou et al., 2007; Lattaoui and Tantaoui-Elaraki, 1994).

Caryophyllene oxide was reported as an antioxidant compound in the EOs of *Cullen plicata* and *Allophylus africanus* (Abd El-Gawad, 2016; Balogun et al., 2014). Usually, the EOs rich in caryophyllene and its isomers α-humulene and

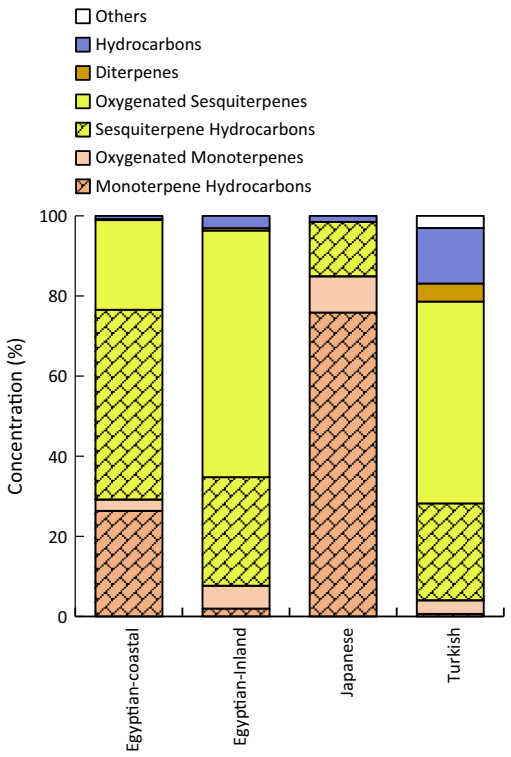

Fig. 1 Percentage of various classes of the chemical compounds of EOs from coastal and inland samples of Egyptian *S. squamatum* as well as Japanese (Miyazawa and Kameoka, 1977) and Turkish ecospecies (Ayaz et al., 2017).

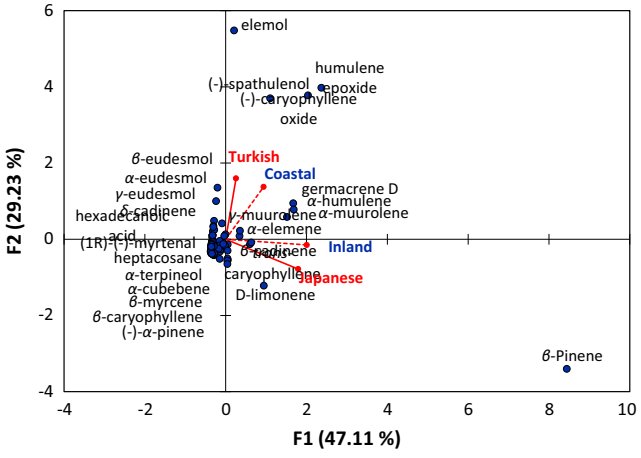

Fig. 2 Principal component analysis (PCA) based on the chemical composition of the EO derived from Egyptian ecospecies of *S. squamatum* collected from Mediterranean coastal desert (coastal) and inland abandoned habitat (inland) as well as Turkish and Japanese ecospecies.

β-caryophyllene possess biological activity (Sabulal et al., 2006). The α-humulene and β-pinene have been reported as antimicrobial agents (Jirovetz et al., 2006), while α-humulene, caryophyllene oxide were showed cytotoxic activity (Hou et al., 2007). Therefore, these major compounds could be responsible for the observed antioxidant activity of the EOs of *S. squamatum*.

3.4. Allelopathic activity of S. squamatum EOs

The EOs of *S. squamatum* aerial parts showed significant allelopathic activity against the root and shoot growth of the noxious weed *Bidens pilosa* (Fig. 3 & Table S3). The inhibition was dose-dependent. The root system of *B. pilosa* was inhibited more than shoot system under the effect of the EOs. This inhibition could be ascribed to the direct contact of the radicle with the EOs or even due to the higher permeability of the root cells (Abd El-Gawad et al., 2018). The present results showed significant variation in the allelopathic activity between the coastal and inland samples of *S. squamatum* (Fig. 3). This variation could be attributed to variation in the content of the EOs which is affected indirectly either by the soil properties (Table S2) or the climatic conditions. Soil analysis of the coastal desert sample revealed significantly high salinity compared to the inland sample due to the effect of the Mediterranean Sea. Also, salinity has been reported as a stress factor responsible for the induction of the EOs in plants (Hassanpouraghdam et al., 2011).

The variation in the allelopathic activity is consonant with the antioxidant activity, and it might be correlated to the variation in composition of the EOs between the two samples. The

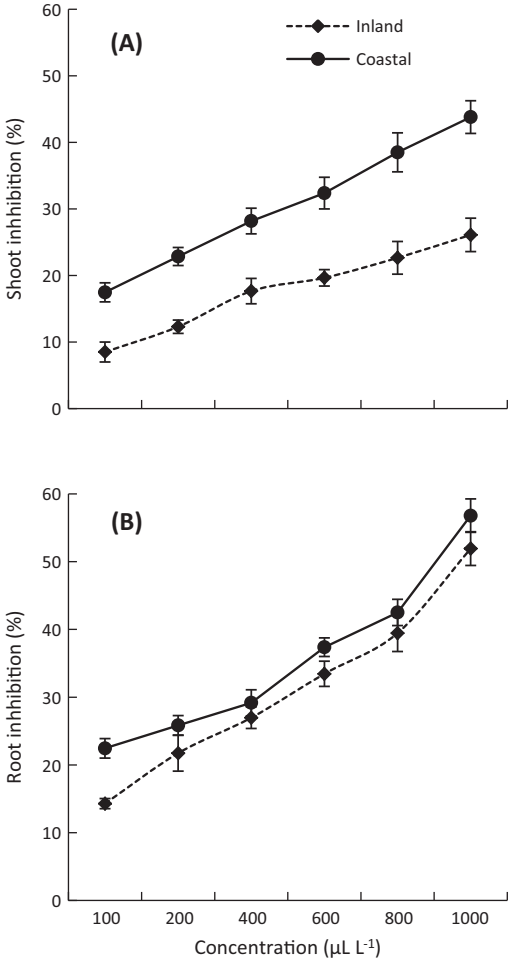

Fig. 3 Inhibitory effect of the EOs from the aerial parts of *S. squamatum* collected from inland and costal deserts on the shoot (A), and root (B) growth of *B. pilosa*.

variations in soil factors, light, temperature, nutrients, water content, daylength, genetic pool, and harvesting time have been reported to influence the EOs composition and hence affect biological activities such as allelopathy (Abd El-Gawad et al., 2019).

The allelopathic activity of the EOs against *B. pilosa* was studied for other donor species such as *Xanthium strumarium* (Abd El-Gawad et al., 2019), *Cullen plicata* (Abd El-Gawad, 2016), *Artemisia scoparia* (Kaur and Batish, 2010), *Tagetes minuta* (Arora et al., 2017), *Eucalyptus citriodora* (Setia et al., 2007). The activity of the EOs from these plants against *B. pilosa* could be ranked in the following order: *E. citriodora* > *C. plicata* > *X. strumarium* > *T. minuta* > *S. squamatum* (present study) > *A. scoparia*. This variation in the allelopathic activity might be ascribed to the variation in the EOs composition.

The allelopathic activity of the EOs from *S. squamatum* could be ascribed to the activity of the major compounds which act either singular or synergistic. Most of the major compounds of the EOs were reported as allelochemicals such as germacrene D (Dali and Xinru, 1996), spathulenol (Nishimura and Mizutani, 1995), caryophyllene oxide (Abd El-Gawad, 2016), humulene epoxide (Tellez et al., 2000), and pinene (Wang and Zhu, 1996).

Although *S. squamatum* produce a little amount of the EOs, it showed promising biological activities (Antioxidant and allelopathic). Also, this plant has a wide range of distribution in Egypt, so significant biomass can be obtained easily and could be integrated in EOs production. As a weed, this may be a potential way to control this plant as well as to become a good resource for bioactive compounds.

4. Conclusion

The EOs of the Egyptian *S. squamatum* is richer than the Turkish or Japanese ecotypes in the chemical compounds where it contained 60 compounds with the predominance of sesquiterpenes. A substantial variation was observed in the compound diversity in the EOs between the coastal and inland samples of Egyptian *S. squamatum*, while the variation in the concentration was little different. This might be attributed to the soil factors particularly the salinity and organic matter content. Humulene epoxide, (-)-spathulenol, (-)-caryophyllene oxide, germacrene D, and α-humulene were identified as major compounds in the coastal sample. However, β-pinene, germacrene D, α-humulene, α-muurolene, humulene epoxide, (-)-caryophyllene oxide, and β-cadinene were the major compounds of the inland sample of the Egyptian *S. squamatum* ecospecies. The PCA showed more correlation between the Egyptian inland *S. squamatum* and the Japanese ecospecies, while the Egyptian coastal *S. squamatum* and Turkish were more correlated to each other, reflecting the effect of the climatic factor. The present data revealed that the chemotypes of *S. squamatum* keep their typical pattern in spite of the ecological or climatic difference. The EOs of *S. squamatum* showed moderate antioxidant and allelopathic activities. However, the coastal sample had more allelopathic potential than that of the inland one and may be attributed to the oxygenated terpenes content. Further study is needed for the separation, characterization, and evaluation of the modes of action of the identified major compounds either singular or in combination.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2019.07.005.

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