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Sesquiterpenes-Rich Essential Oil from Above Ground Parts of *Pulicaria somalensis* Exhibited Antioxidant Activity and Allelopathic Effect on Weeds

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Abstract: *Pulicaria* genus (fleabane) is characterized by its fragrant odor due to the presence of essential oil (EO). According to the literature reviews, the EO of *Pulicaria somalensis* O.Hoffm. (Shie) is still unexplored. For the first time, 71 compounds were characterized in EO derived from above-ground parts of *P. somalensis* collected from Saudi Arabia. Sesquiterpenes represented the main components (91.8%), along with minor amounts of mono-, diterpenes, and hydrocarbons. Juniper camphor (24.7%), α-sinensal (7.7%), 6-epi-shyobunol (6.6%), α-zingiberene (5.8%), α-bisabolol (5.3%), and T-muurolol (4.7%) were characterized as main constituents. The correlation analysis between different *Pulicaria* species showed that *P. somalensis* has a specific chemical pattern of the EO, thereby no correlation was observed with other reported *Pulicaria* species. The EO showed significant allelopathic activity against the weeds of *Dactyloctenium aegyptium* (L.) Willd. (crowfoot grass) and *Bidens pilosa* L. (hairy beggarticks). The IC_{50} value on the germination of *D. aegyptium* was double that of *B. pilosa*. The IC_{50} values on the root growth of *B. pilosa* and *D. aegyptium* were 0.6 mg mL^{-1} each, while the shoot growths were 1.0 and 0.7 mg mL^{-1}, respectively. This variation in the activity could be attributed to the genetic characteristics of the weeds. Moreover, the EO exhibited significant antioxidant effects compared to ascorbic acid. Further studies are necessary to verify if these biological activities of the EO could be attributable to its major compounds.

Keywords: *Pulicaria somalensis*; essential oil; sesquiterpenes; phytotoxicity; antioxidant activity; juniper camphor

1. Introduction

Since their early presence on Earth, humans have depended largely on plants for food, energy, and medicine [1]. Nowadays, even with highly scientific and technological developments, aromatic and medicinal plants are still the main source of food and medicinal products. Most of the scientists focused on finding and developing new products derived from plants, plant extracts, and constituent choices for the treatment of different diseases and illnesses [2].

Asteraceae or Compositae is a widely distributed family throughout the world and contains around 1600 genera with more than 23,000 plant species [3]. *Pulicaria* (fleabane) genus (family Asteraceae) comprises around 75 species widely distributed in Africa, Europe, and Asia [4]. *Pulicaria* species are used in the treatment of several diseases such as cancers, fever, hypoglycemia, microbial, inflammation, and spasmodic diseases [5–7].

The chemical characterization of *Pulicaria* plants revealed the presence of various secondary metabolites, such as mono-, sesqui-, and diterpenoids [8–12], flavonoids, and phenolics [5–7]. Several reports described the chemical characterization of essential oils (EOs) from *Pulicaria* species such as *Pulicaria dysenterica* (L.) Bernh. (common fleabane) [13], *Pulicaria gnaphalodes* (Vent.) Boiss. (false fleabane) [14], *Pulicaria mauritanica* Batt. (fleabane) [15], *Pulicaria jaubertii* E.Gamal-Eldin. (Araar) [16], and *Pulicaria undulata* (L.) C.A. Mey (Gethgath). [17]. All these studies deduced that members of this genus are rich with terpenoids, especially mono- and sesquiterpenoids. The EOs extracted from *Pulicaria* species exhibited numerous biological potentialities, such as antibacterial, antioxidant, and antifungal activities [15,18]. A previous chemical study of *P. somalensis* O.Hoffm. (Shie) described that this plant is rich with diterpenoids as well as flavonoids [19]. The methanolic extract of this plant has remarkable antioxidant, antifungal, and antibacterial activities [20].

To the best of our knowledge, there are no reports concerning *P. somalensis* EO. Thereby, in the present study, we aimed to (1) determine the chemical composition of the EO from the above-ground parts of *P. omalensis*, (2) assess the allelopathic activity of the EO against two weeds, *Dactyloctenium aegyptium* (crowfoot grass, Poaceae) and *Bidens pilosa* (hairy beggarticks, Asteraceae), and (3) evaluate the antioxidant properties of the EO.

2. Materials and methods

2.1. Plant Materials

We collected the above-ground parts of *P. somalensis* from three populations at Alwashla, Riyadh region, Saudi Arabia (24°25′36.1″ N 46°39′07.3″ E). Within each population, we collected samples from five individuals and mixed them as one composite sample. At the laboratory, we cleaned the samples from dust, dried them in a shaded place at room temperature, and ground them into powder using a grinder (IKA®MF 10 Basic Microfine Grinder Drive, Breisgau, Germany). We identified the plant according to Chaudhary [21] and deposited a voucher specimen (RIY-15647) in the National Herbarium and Genebank, Riyadh, Saudi Arabia.

2.2. Extraction of EO

Hydro-distillation of the prepared plant materials from above-ground parts of *P. somalensis* (400 g) was achieved using a Clevenger-type apparatus for three hours. The dark yellow oil (0.5% w/w) was separated and then dried with anhydrous $NaSO_4$. EOs from all the three samples of *P. somalensis* populations were extracted in the same way and stored at 4 °C until further gas chromatography-mass spectrometry (GC-MS) analysis was performed.

2.3. GC-MS Analysis and Identification of Components of EO

The chemical composition of the extracted EO samples was analyzed separately by GC-MS according to our published protocol [22].

2.4. Allelopathic Activity of the EO

To assess the allelopathic activity of the extracted EO from the above-ground parts of *P. somalensis*, we targeted two weeds from different families: *D. aegyptium* (Poaceae) and *B. pilosa* (Asteraceae). The seeds of *D. aegyptium* were collected from newly reclaimed fields near New Mansoura City, northern Nile delta, Egypt (31°29′57.3″ N 31°21′59.3″ E), while the seeds of *B. pilosa* were collected from the gardens of Mansoura University campus, Mansoura, Egypt (31°02′38.1″ N 31°21′01.7″ E). We selected

seeds of both weeds with homogenous size and color. We surface sterilized the seeds with sodium hypochlorite (0.3%), rinsed them with water (distilled and sterilized), and then dried them over a sterilized Whatman® cellulose filter paper (Sigma-Aldrich, Taufkirchen, Germany) [23].

We prepared different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg mL^{-1}) of the extracted EO using 1% Tween® 80 (Sigma–Aldrich, Darmstadt, Germany). For bioassay, we placed 20 seeds of each weed in sterilized Petri plates (Ø: 9 cm) lined with sterilized Whatman No. 1 filter paper and immediately added 4 mL of each concentration. The plates were sealed with Parafilm® and incubated at 27 ± 2 °C in a growth chamber with a light cycle of 8 h dark and 16 h light. Tween (1%) was used as a negative control. After seven days for *B. pilosa* and ten days for *D. aegyptium*, we counted the number of germinated seeds and measured the length of the seedling root and shoot for both weeds. The inhibition of seed germination, root growth, and shoot growth was calculated according to the following equation:

$$\text{Inhibition } (\%) = 100 \times \frac{(\text{Length/Number of control} - \text{Length/Number of treatment})}{\text{Length/Number of control}} \quad (1)$$

The bioassay experiment was repeated three times with three replications (three plates), and the IC_{50} (the concentration of EO required for 50% inhibition)was calculated graphically as the amount of the EO necessary for 50% inhibition.

2.5. Antioxidant Activity of the EO

The antioxidant activity of the extracted EO from *P. somalensis* was performed based on 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity.

2.5.1. DPPH Radical Scavenging Activity

The EO capability to react with the free DPPH radical (Sigma-Aldrich, Darmstadt, Germany) and reduce its color was determined according to the method of Miguel [24]. In brief, we prepared different concentrations (10, 20, 40, 60, 80, 100 μg mL^{-1}) of the EO in methanol (70%). A reaction mixture of 2 mL of each concentration and 2 mL of DPPH (0.3 mM) was prepared in screwcap test tubes, shaken well, and incubated in dark conditions at 25 °C for 15 min. Negative control was performed using 2 mL of 1% Tween instead of the EO. We measured by a spectrophotometer (Milton Roy Spectronic 21D UV-Visible Spectrophotometer, California, USA) at 512 nm. In addition, positive control with ascorbic acid (as a standard antioxidant) was prepared in a range of 1–25 mg mL^{-1} and treated as previously mentioned for the EO treatments. We calculated the scavenging activity according to the following equation:

$$\text{Scavenging activity } (\%) = 100 \times \frac{\left(Absorbance_{control} - Absorbance_{sample}\right)}{Absorbance_{control}} \quad (2)$$

Also, the IC_{50} was calculated as the concentration of the EO required for 50% scavenging of the DPPH.

2.5.2. ABTS-Free Radical Scavenging Activity

To confirm the antioxidant activity of the extracted EO, we determined the scavenging of the ABTS radical (Sigma-Aldrich, Darmstadt, Germany) according to the method of Re et al. [25]. The free radical was prepared using 7 mM of ABTS and 2.45 mM of $K_2S_2O_8$. The mixture (1/1, v/v) was kept at room temperature (25 ± 2 °C) in dark conditions. We then diluted the radical by MeOH until it reached the absorbance of 0.700 ± 0.02 at 734 nm. A reaction mixture of 2 mL of each concentration of the EO and 2 mL of the freshly prepared ABTS was prepared, mixed well, and incubated at room temperature (25 °C) for 6 min. We then measured the absorbance at 734 nm using a spectrophotometer (Milton

Roy Spectronic 21D UV-Visible Spectrophotometer, California, USA). Ascorbic acid was also used as a positive control. We calculated the scavenging activity and the IC_{50} as mentioned in DPPH method.

2.6. Treatment of Data

We repeated the experiments of allelopathic and antioxidant activity three times with three replications for each. We subjected the data of antioxidant experiments, as a percentage of scavenging activity in triplicates, to a one-way analysis of variance (ANOVA) test followed by Duncan's test, where the significant differences among the various tested concentrations were assessed at $p \leq 0.05$ using CoStat software program, version 6.311 (CoHort Software, Monterey, CA, USA). However, the data of allelopathic activity, as a percentage of inhibition in triplates, were subjected to two-way ANOVA at $p \leq 0.05$ using the CoStat program, version 6.311 (CoHort Software, Monterey, CA, USA), which afforded the concentration of the EO and the types of weed as two factors.

Based on the EO composition, the correlation between the present studied plant (*P. somalensis*) and other reported *Pulicaria* species, including *P. dysenteric* [13,26], *P. glutinosa* (Boiss.) Jaub. & Spach [27], *P. gnaphalodes* [14,28], *P. incisa* (Lam.) DC. (wild tea) [29,30], *P. jaubertii* [16], *P. mauritanica* (Ifenzi oudaden) [31,32], *P. odora* (L.) Rchb. (Mediterranean fleabane) [18], *P. sicula* (L.) Moris (fleabane) [33], *P. stephanocarpa* Balf.f. (derbeb) [17], *P. undulata* [34–37], and *P. vulgaris* Gaertn. (false fleabane) [38–40], was assessed by agglomerative hierarchical cluster (AHC) as well as principal component analysis (PCA). We constructed a data matrix from the percentage of various classes of the EO (mono-, di-, sesquiterpenes, and others) in different *Pulicaria* species (12 species representing 21 samples) and then subjected them to PCA. However, we performed the AHC based on a data matrix of a total of 44 major identified chemical compounds, with concentration >5%, from the EO of 12 *Pulicaria* species. We performed the AHC based on the similarity using Pearson's coefficient of correlation and with the agglomeration method of unweighted pair-group average. The AHC and the PCA analyses were performed using XLSTAT statistical computer software package, version 14 (Addinsoft, New York, USA).

3. Results and Discussion

3.1. Chemical Composition of the EO

The EO with a dark yellow color from the above-ground parts of *P. somalensis*, collected from Saudi Arabia, was extracted by the hydrodistillation method and yielded 0.5% (v/w) oil. The EO was analyzed via GC-MS. The chromatogram, including the main components, is indexed in Figure 1. All the identified constituents of EO comprising 71 compounds are listed in Table 1, representing 100% of the total mass. Mono-, di-, and sesquiterpenes as well as hydrocarbons and aromatic phenolic compounds were characterized as components of the EO.

Sesquiterpenes represented the main characterized class (91.8%) of compounds, including both oxygenated (72.4%) and non-oxygenated (19.3%) sesquiterpenes. Oxygenated monoterpenes were one of the identified compounds with a concentration of 3.7% from overall identified monoterpenes (4.8%) in addition to minor monoterpenes hydrocarbons (1.0%). Diterpenoids were the usual minor compounds in EOs derived from aromatic and medicinal plants [22]. Herein, diterpenes are characterized as minor constituents with a concentration of 2.5%, including the concentration of 1.8% of oxygenated and 0.7% of non-oxygenated diterpenes. A concentration of 2.0% from overall mass represented the other compounds, including oxygenated and non-oxygenated hydrocarbons (0.8% and 0.2%) as well as 0.94% of volatile aromatic compounds.

In our findings, sesquiterpenes were the backbone of the characterized compounds in the EO. From 36 identified oxygenated sesquiterpenes, juniper camphor (24.7%), α-sinensal (7.6%), 6-epi-shyobunol (6.6%), α-bisabolol (5.3%), and T-muurolol (4.7%) represented the main components, while isoaromadendrene epoxide was a minor one with a concentration of 0.1%. By comparing our results with the literature survey of EOs of other *Pulicaria* species, it was clear that the chemical

composition EO of *P. somalensis* is comparable to some other *Pulicaria* species, with a preponderance of sesquiterpenes such as *P. dysenterica* [13] and *P. gnaphalodes* [14].

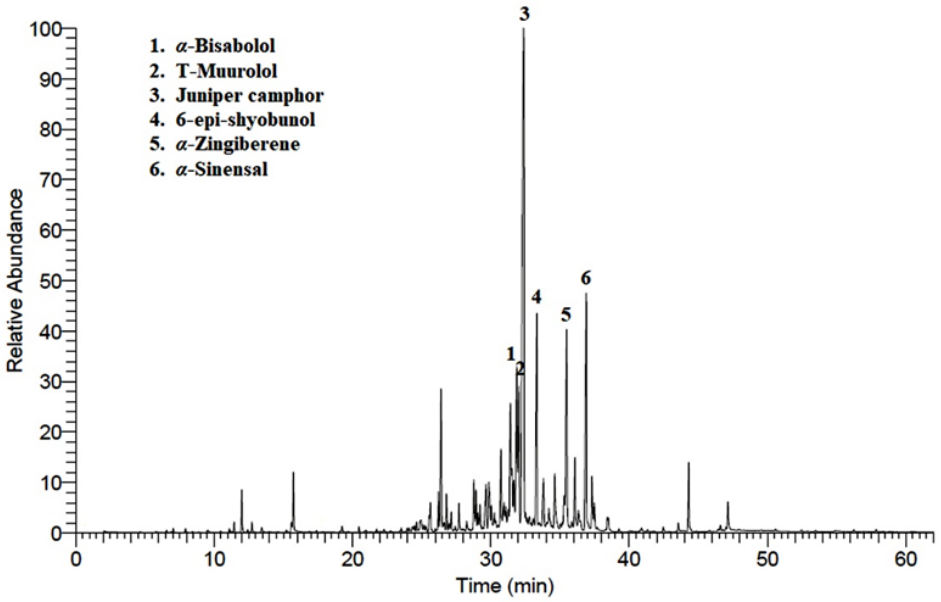

Figure 1. GC-MS chromatogram of the essential oil from the above-ground parts of *Pulicaria somalensis* (Shie). Peaks of the major compounds are numbered 1–6.

The sesquiterpene hydrocarbons characterized as a remarkable identified class with a concentration of 19.3%. α-Zingiberene (5.8%), α-cadinene (3.8%), and valencene (3.7%) were characterized as the principal components, while as trans-caryophyllene represented the minor one with a concentration of 0.1%.

Additionally, numerous reports describe that *Pulicaria* species have monoterpenes as main constituents, such as *P. undulata* [17], *P. mauritanica* [15], *P. jaubertii* [16], and *P. odora* [18]. Our findings exhibit that the monoterpenes are minor components involving oxygenated (3.7%) and non-oxygenated (1.0%) ones. Trans-chrysanthenyl acetate (1.3%) was found as a main compound of the oxygenated monoterpenes, while 1,8-cineole (0.2%) was the minor one. γ-Terpinene and *p*-cymene were the two identified oxygenated monoterpenes with concentrations of 0.1% and 0.9%, respectively.

In most of the cases, the EOs derived from the aromatic and the medicinal plants were poor resources of diterpenoids with some exceptions, such as *Lactuca serriola* L. (prickly lettuce), where the diterpene isocembrol was determined in high concentration (17.4%) [22]. This fact was achieved in our study by minor diterpene constituents. Only two diterpenoid components were identified, which includes the oxygenated one, geranyl linalool (1.8%) and the non-oxygenated one, geranyl-α-terpinene (0.7%). The previous studies of EOs of *Pulicaria* species deduced that these plants almost do not have diterpene components [16,18]. For example, the EO of *P. mauritanica* was described to have only one diterpenoid with a concentration of 0.2 of the total mass [15], while the EOs of *P. dysenterica* [13], *P. gnaphalodes* [14], *P. undulata* [17], and *P. jaubertii* [16] had no diterpenes.

Other components with low concentrations characterized in our study included hydrocarbons and volatile aromatic compounds. With minor concentration, the hydrocarbons comprised only two oxygenated compounds, γ-palmitolactone (0.8%) and *n*-octadecanal (0.1%), and two non-oxygenated, *n*-heneicosane (0.1%) and *n*-pentacosane (0.1%). Additionally, our results completely agree with previous studies of other *Pulicaria* species that indicate the minor hydrocarbons constituents [13–17].

Volatile aromatic and phenolic terpenoid compounds are very common in the EOs of *Pulicaria* species, especially cymene derivatives and isomers such as *p*-cymene, *m*-cymene, and *p*-cymen-8-ol [15,16,18]. In the same line, the EO of *P. somalensis* contained only one aromatic compound, *p*-cymene, with a low concentration (1.0%).

Table 1. Chemical constituents and concentration of the essential oil from the above-ground parts of *Pulicaria somalensis* (Shie).

No	Rt [a]	Compound	KI [b]	KI [c]	Conc. % [d]	Identification [e]
		Oxygenated Monoterpenes			**3.7**	
1	7.05	1,8-Cineole	1031	1047	0.2 ± 0.01	MS, KI
2	11.11	*trans*-Pinocarveol	1183	1180	0.3 ± 0.01	MS, KI
3	11.99	*cis*-Verbenol	1142	1144	0.9 ± 0.02	MS, KI
4	12.42	*endo*-Borneol	1139	1140	0.2 ± 0.01	MS, KI
5	12.73	4-Terpineol	1177	1179	0.2 ± 0.01	MS, KI
6	13.44	α Terpineol	1189	1188	0.1 ± 0.01	MS, KI
7	15.22	Pulegone	1237	1239	0.2 ± 0.01	MS, KI
8	15.59	Carvone	1242	1243	0.3 ± 0.01	MS, KI
9	15.73	trans-Chrysanthenyl acetate	1235	1234	1.3 ± 0.03	MS, KI
		Monoterpenes Hydrocarbons			**1.0**	
10	7.92	γ-Terpinene	1062	1060	0.1 ± 0.01	MS, KI
11	29.06	*p*-Cymene	1025	1025	0.9 ± 0.02	MS, KI
		Oxygenated Sesquiterpenes			**72.4**	
12	24.31	Germacrone	1693	1694	0.3 ± 0.01	MS, KI
13	25.18	Dihydro-β-agarofuran	1558	1557	0.7 ± 0.02	MS, KI
14	25.54	Cubedol	1580	1583	0.5 ± 0.02	MS, KI
15	25.99	Davana ether 1	1433	1433	0.7 ± 0.01	MS, KI
16	26.80	Spathulanol	1578	1580	0.9 ± 0.02	MS, KI
17	26.98	α-Acorenol	1629	1631	0.2 ± 0.01	MS, KI
18	27.70	Calarene epoxide	1588	1586	0.7 ± 0.02	MS, KI
19	27.96	Veridiflorol	1590	1592	0.1 ± 0.01	MS, KI
20	28.26	Nerolidol	1535	1535	0.3 ± 0.01	MS, KI
21	28.78	Germacrene D-4-ol	1511	1512	1.3 ± 0.04	MS, KI
22	28.94	*cis*-alpha-Santalol	1678	1677	1.2 ± 0.02	MS, KI
23	29.65	8-Cedren-13-ol	1689	1691	1.7 ± 0.03	MS, KI
24	29.86	Rosifoliol	1612	1612	1.7 ± 0.03	MS, KI
25	30.25	Agaruspirol	1631	1632	0.4 ± 0.02	MS, KI
26	30.35	Khusinol acetate	1827	1826	0.2 ± 0.01	MS, KI
27	30.95	γ-Eudesmol	1630	1633	0.7 ± 0.03	MS, KI
28	31.09	Fonenol	1627	1625	0.5 ± 0.02	MS, KI
29	31.27	Cubenol	1642	1643	0.3 ± 0.01	MS, KI
30	31.53	T-Cadinol	1640	1641	1.0 ± 0.04	MS, KI
31	31.67	Hinesol	1638	1638	0.9 ± 0.02	MS, KI
32	31.87	α-Bisabolol	1683	1682	5.3 ± 0.05	MS, KI
33	32.03	T-Muurolol	1608	1609	4.7 ± 0.07	MS, KI
34	32.38	Juniper camphor	1691	1691	24.7 ± 0.06	MS, KI
35	32.51	Humulane-1,6-dien-3-ol	1619	1618	0.1 ± 0.01	MS, KI
36	32.9	Isoaromadendrene epoxide	1579	1581	0.1 ± 0.01	MS, KI
37	33.07	Nerolidol-epoxyacetate	1638	1639	0.2 ± 0.01	MS, KI
38	33.33	6-epi-shyobunol	1517	1516	6.6 ± 0.05	MS, KI
39	33.55	Diepicedrene-1-oxide	1551	1551	1.8 ± 0.02	MS, KI
40	34.2	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydro-naphthalen-2-ol	1714	1715	0.8 ± 0.01	MS, KI
41	34.61	*cis*-α-Copaene-8-ol	1633	1634	1.6 ± 0.03	MS, KI
42	35.86	Aromadendrene oxide-(1)	1625	1628	0.4 ± 0.01	MS, KI
43	36.07	*Z*-α-trans-Bergamotol	1693	1693	2.4 ± 0.04	MS, KI
44	36.89	α-Sinensal	1752	1751	7.6 ± 0.06	MS, KI
45	37.31	*cis*-Lanceol	1753	1753	1.4 ± 0.03	MS, KI
46	37.49	*cis*-*Z*-α-Bisabolene epoxide	1814	1815	0.5 ± 0.01	MS, KI
47	38.42	Hexahydrofarnesyl acetone	1845	1842	0.3 ± 0.01	MS, KI
48	40.89	*E*-*cis*,epi-β-Santalol	1669	1668	0.1 ± 0.01	MS, KI
		Sesquiterpenes Hydrocarbons			**19.3**	
49	19.25	α-Cubebene	1351	1352	0.2 ± 0.01	MS, KI
50	20.47	α-Copaene	1376	1377	0.2 ± 0.01	MS, KI
51	21.74	Calarene	1427	1427	0.1 ± 0.01	MS, KI
52	22.28	*trans*-Caryophyllene	1428	1428	0.1 ± 0.01	MS, KI
53	23.53	γ-Muurolene	1477	1475	0.1 ± 0.01	MS, KI
54	23.94	Alloaromadendrene	1441	1442	0.1 ± 0.01	MS, KI

Table 1. *Cont.*

No	Rt [a]	Compound	KI [b]	KI [c]	Conc. % [d]	Identification [e]
55	24.49	delta-Cadinene	1524	1523	1.0 ± 0.01	MS, KI
56	24.64	α-Amorphene	1506	1504	0.7 ± 0.01	MS, KI
57	25.31	epi-Bicyclosesquiphellandrene	1482	1482	0.2 ± 0.01	MS, KI
58	25.63	α-Muurolene	1499	1498	0.8 ± 0.02	MS, KI
59	26.40	α-Cadinene	1538	1539	3.8 ± 0.05	MS, KI
60	26.56	*cis*-Calamenene	1521	1521	0.3 ± 0.01	MS, KI
61	27.43	α-Calacorene	1548	1549	0.7 ± 0.01	MS, KI
62	30.73	Di-epi-à-cedrene-(I)	1427	1428	2.1 ± 0.03	MS, KI
63	31.41	Valencene	1491	1492	3.7 ± 0.06	MS, KI
64	32.61	α-Guaiene	1439	1440	0.1 ± 0.01	MS, KI
65	35.48	α-Zingiberene	1495	1494	5.8 ± 0.06	MS, KI
		Oxygenated Diterpenes			**1.8**	
66	44.3	Geranyl linalool	2444	2443	1.8 ± 0.03	MS, KI
		Diterpenes Hydrocarbons			**0.7**	
67	36.34	Geranyl-α-terpinene	2142	2142	0.7 ± 0.02	MS, KI
		Oxygenated Hydrocarbons			**0.8**	
68	47.14	γ-Palmitolactone	2120	2119	0.8 ± 0.02	MS, KI
69	56.24	*n*-Octadecanal	1357	1357	0.1 ± 0.01	MS, KI
		Non-oxygenated Hydrocarbons			**0.2**	
70	46.6	*n*-Heneicosane	2300	2301	0.1 ± 0.02	MS, KI
71	57.85	*n*-Pentacosane	2500	2503	0.1 ± 0.01	MS, KI

[a] Rt: Retention time; [b] KI: Kovats retention index on DB-5 column with reference to *n*-alkanes; [c] experimental Kovats retention index; [d] values are average ± SD, and [e] the identification of essential oil (EO) components was performed based on the mass spectral data of compounds (MS) and Kovats indices (RI) with those of Wiley spectral library collection and NIST (National Institute of Standards and Technology) library database.

3.2. Correlation Between *P. somalensis* and other *Pulicaria* Species

The application of PCA on the concentration of various classes of the EO from different *Pulicaria* species revealed that *P. somalensis*, *P. stephanocarpa*, and *P. vulgaris* from Italy, *P. vulgaris* from Tunisia, *P. dysenterica* from Greece, *P. gnaphalodes* from Mashhad, Iran, and *P. glutinosa* were correlated with each other due to the similarity in the content of sesquiterpenes (Figure 2a). However, *P. dysenterica* from Iran was not correlated with other *Pulicaria* species, as it was characterized by the presence of diterpene. On the other hand, *P. undulata* from Egypt, *P. undulata* from Sudan, *P. vulgaris* from Iran, *P. odora*, *P. mauritanica* from Morocco, and *P. mauritanica* from Algeria, and *P. jaubertii* were correlated with each other, since these species have monoterpenes as the major class.

The application of AHC on the data of the major compounds (>5%) of the EO from different *Pulicaria* species showed that the *P. somalensis* was separated from other *Pulicaria* species (Figure 2b). These results reflected the characteristic pattern of the chemical composition of *P. somalensis*. Similarly, *P. odora*, *P. vulgaris* from Iran, *P. vulgaris* from Italy, *P. gnaphalodes* from Mashhad, Iran, *P. dysenterica* from Iran, *P. vulgaris* from Tunisia, *P. glutinosa*, and *P. incisa* from Algeria were also separated alone.

However, *P. undulata* from Sudan and Egypt were grouped together into one group, while *P. undulata* from Algeria, *P. jaubertii*, *P. incisa* from Egypt, *P. mauritanica* from Morocco, and *P. mauritanica* from Algeria were grouped together. *P. sicula*, *P. dysenterica* from Greece, and *P. stephanocarpa* were separated together, while *P. gnaphalodes* from Tehran, Iran and *P. vulgaris* from Iran were grouped together.

The obtained data from AHC showed that the same species varied in the chemical composition according to their geographical region. Abd El-Gawad et al. [41] reported that the chemical composition of the EO varied among different plant ecotypes due to variation in climate, soil, environmental variables, and the genetic pool. Our previous work on *Xanthium strumarium* L. (cocklebur), *Symphyotrichum squamatum* (Spreng.) Nesom (bushy starwort), and *Launaea* (Morrar) species indicated that a variation of the chemical composition of the EO was found to be strongly correlated with variation in the habitats [42,43].

The overall correlation analysis showed that *P. somalensis* has a specific chemical pattern of the EO, where it could be related to the genetic characteristics. These data of the EO chemical composition could be helpful for the chemotaxonomy of *Pulicaria* genus.

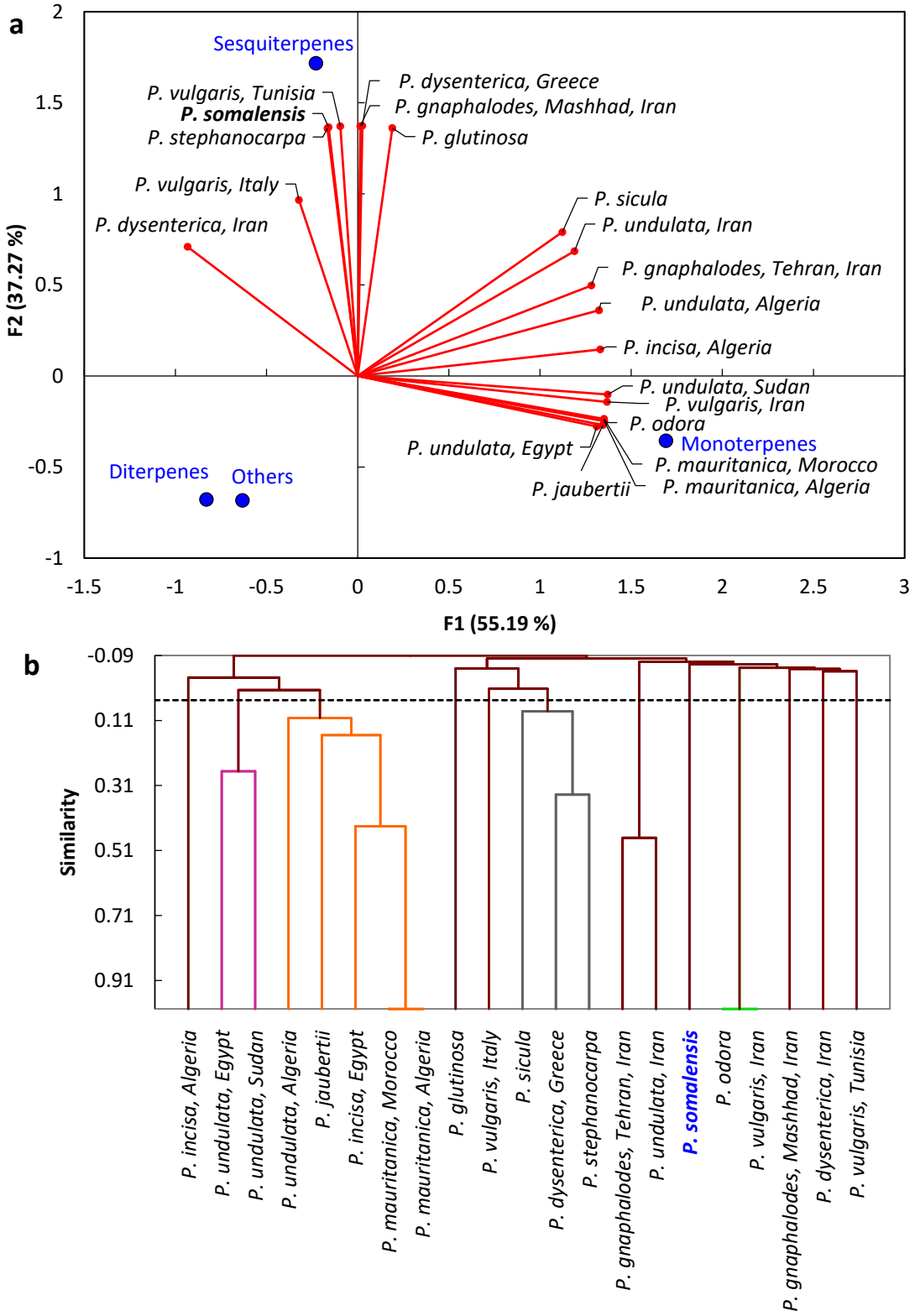

Figure 2. (**a**) Principal component analysis (PCA) based on the chemical classes of the essential oil, and (**b**) agglomerative hierarchical clustering (AHC) based on the major chemical compounds of the EO of *Pulicaria somalensis* (Shie) and other reported *Pulicaria* species.

3.3. Allelopathic Activity of the EO

The extracted EO from *P. somalensis* above-ground parts exhibited a significant allelopathic inhibitory activity on the germination and the seedling growth of the tested weeds (*B. pilosa* and *D. aegyptium*) in a dose-dependent manner (Figure 3). At a concentration of 1 mg mL^{-1} of the EO, germination, root growth, and shoot growth of *B. pilosa* were inhibited by 61.4%, 73.6%, and 55.4%, respectively.

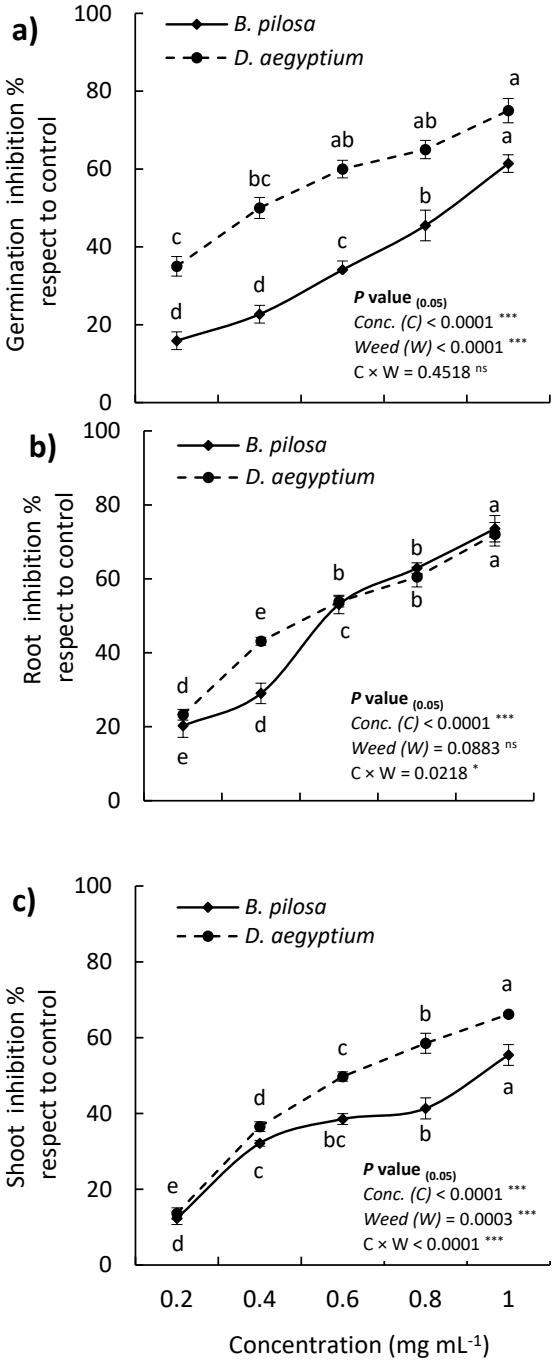

Figure 3. Allelopathic effect of the essential oil from the above-ground parts of *Pulicaria somalensis* (Shie) on (**a**) seed germination, (**b**) root growth, and (**c**) shoot growth of *Dactyloctenium aegyptium* (crowfoot grass) and *Bidens pilosa* (hairy beggarticks). Within each line, different letters indicate statistically significant differences at $p \leq 0.05$. ns: non-significant.

On the other hand, germination, root, and shoot growth of *D. aegyptium* were inhibited by 75.0%, 72.1%, and 66.2%, respectively. A highly significant difference in seed germination and shoot growth was observed between the two test weeds ($p < 0.0001$), while no significant difference ($p < 0.0883$) was observed based on the root growth (Figure 3). Usually, the root is more affected, as it is the first sprout organ and because it has direct contact with allelochemicals, as described in many studies [23,43–46].

According to the IC_{50}, the EO showed a more inhibitory effect against *D. aegyptium* (Figure 4). The IC_{50} value on the germination of *D. aegyptium* was doubled compared to *B. pilosa*. The IC_{50} values on the root growth of *B. pilosa* and *D. aegyptium* were comparable (0.6 mg mL^{-1}, each), while the IC_{50} values on the shoot growth were 1.0 and 0.7 mg mL^{-1}, respectively (Figure 4). Overall, the EO of *P. somalensis* showed more inhibitory activity against *D. aegyptium* than *B. pilosa*. This variation in the activity could be attributed to the genetic characteristics of the weeds [47].

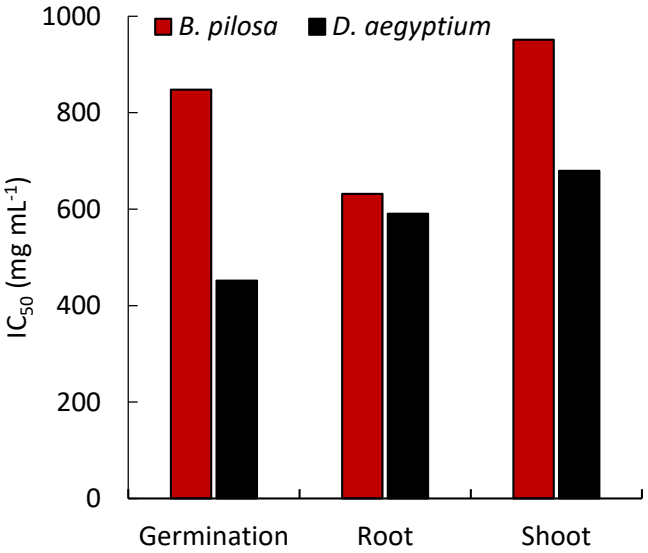

Figure 4. IC_{50} values of the essential oil extracts from the above-ground parts of *Pulicaria somalensis* (Shie) on germination, root, and shoot growth inhibition of *Dactyloctenium aegyptium* (crowfoot grass) and *Bidens pilosa* (hairy beggarticks).

The inhibitory activity of *P. somalensis* EO could be attributed to the high content of oxygenated terpenoid compounds, particularly sesquiterpenes (Table 1). Major compounds such as juniper camphor, α-sinensal, 6-epi-shyobunol, α-zingiberene, α-bisabolol, and T-muurolol could act either individually or synergistically as inhibitors for the germination and the growth of the *B. pilosa* weed. The oxygenated terpenoids usually have a significant role in biological activity compared to non-oxygenated compounds due to the reactivity of oxygen [41]. The EOs from *S. squamatum* and *L. serriola* have been reported to inhibit the germination and the seedling growth of the *B. pilosa* Abd-ElGawadm et al. [22] and Abd-ElGawad, et al. [42] due to the presence of sesquiterpenes as major components.

Juniper camphor has been reported as the main compound (15.5%) of antibacterial, antioxidant, and phytotoxic active EO from *Syzygium samarangense* Merr. & Berry (rose apple) Lawal, et al. [48]. Additionally, the EO from *Artemisia argyi* Levl et Vant (mugwort) has been reported to possess antifungal activity due to the high content of juniper camphor [49].

3.4. Antioxidant Activity of the EO

The antioxidant capacity of the EO from the above-ground parts of *P. somalensis* was tested by the ability to scavenge the DPPH and the ABTS. The results revealed that the scavenging activity was significantly increased by the increase of EO concentration (Table 2). The EO attained IC_{50} values of 81.2 mg mL^{-1} and 64.4 mg mL^{-1} based on DPPH and ABTS assays, compared to ascorbic acid with IC_{50} values of 21.7 mg mL^{-1} and 18.4 mg mL^{-1}, respectively. These data showed that the EO of

P. somalensis has meaningful antioxidant activity. This antioxidant activity could be ascribed to the major constituents of the EO, such as juniper camphor, α-sinensal, and 6-epi-shyobunol. These oxygenated sesquiterpenes might act individually or synergistically as antioxidants. The antioxidant role of the oxygenated compounds might be attributed to the free electrons due to the high oxygenation [42,43]. Although juniper camphor has been reported to have antifungal [49] and antibacterial activity and cytotoxicity [50], its antioxidant activity has still not been studied. Therefore, we recommend further studies to determine the biological activity, particularly the antioxidant activity, of the pure form of major compounds, especially juniper camphor.

Table 2. Percentage of scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) as well as the IC_{50} values of the essential oil (EO) from *Pulicaria somalensis* (Shie) compared with ascorbic acid.

Treatment	Concentration (µg mL^{-1})	DPPH		ABTS	
		Scavenging (%) *	IC_{50} (µg mL^{-1})	Scavenging (%)	IC_{50} (µg mL^{-1})
Pulicaria somalensis (EO)	100	59.1 ± 2.78^{A}	81.2	64.0 ± 1.54^{A}	64.4
	80	50.5 ± 0.73^{B}		54.3 ± 0.60^{B}	
	60	37.6 ± 0.35^{C}		51.7 ± 1.51^{C}	
	40	33.4 ± 0.96^{D}		38.7 ± 0.32^{D}	
	20	28.6 ± 0.80^{E}		33.3 ± 2.22^{E}	
	10	24.5 ± 1.20^{F}		28.1 ± 0.77^{F}	
Ascorbic acid			21.7		18.4

* values are average (n = 3) ± standard error, IC_{50}: the concentration of the sample that required to reduce the DPPH or ABTS absorbance by 50%. Different superscript letters within the column mean values significant variation at $p < 0.05$.

4. Conclusions

For the first time, the present study showed that the EO from *P. somalensis* has 71 compounds. Juniper camphor, α-sinensal, 6-epi-shyobunol, α-zingiberene, α-bisabolol, and T-muurolol were found as main constituents. The correlation analysis revealed that it has a specific EO chemical pattern via the absence of the correlation with other *Pulicaria* ecospecies. Biologically, EO showed significant allelopathic activity on the weeds (*B. pilosa* and *D. aegyptium*). Therefore, this EO could be integrated into the methods of the management of these weeds as an eco-friendly way, but after further study on the assessment of its activity, durability, and safety as bioherbicide at the field scale. Moreover, the EO reflected meaningful antioxidant activity compared to ascorbic acid. Because the biological activities of the pure form of the identified major compounds are still undetermined, a further study is recommended for the characterization of the pure major compounds, particularly juniper camphor.

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