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Analysis of Volatiles in *Senecio anteuphorbium* Essential Oil with a Focus on Its Allelopathic Effect by Means of Gas Chromatography

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Abstract: The present study aimed to investigate *Senecio anteuphorbium*, an endemic plant growing in West Morocco and widely used in local folk medicine. The essential oil (EO) extracted from the aerial parts was analyzed by gas chromatography and tested for allelopathic activity. The quantitation of the volatiles was carried out by means of GC-FID with response factors, which were validated through reliable calibration procedures, based on external and internal standardization. This analytical approach allowed to define the real concentration of each constituent (weight%, g/100 g) alongside the conventional relative percent. On the other hand, the identification process was supported by a dual matching based on both mass spectra and retention indices. The essential oil resulted in being rich in sesquiterpenes, with the predominant constituents being bicyclogermacrene (22.75 g/100 g), spathulenol (25.26 g/100 g), epi- γ -eudesmol (6.8 g/100 g), and selina-4,11-diene (5.08 g/100 g). The allelopathic effect was evaluated by studying the inhibition of the germination and growth of *Lactuca sativa* seeds. A potent allelopathic effect was recorded by the essential oil at a dose of 0.281 mg/mL, with almost a total inhibition of germination.

Keywords: *Senecio anteuphorbium*; response factors; true quantitation; allelopathy

1. Introduction

Nowadays, the use of natural resources has attracted the attention of many researchers due to their diversity, durability, bioactivity, and eco-friendly characteristics. Essential oils (EOs) are one of the richest sources of bioactive metabolites that justify their numerous biological activities, such as antibacterial, antiviral, insecticidal, and allelopathic activities [1–3]. Moreover, the use of essential oils as natural antioxidants attracts the interest of many scientists and researchers due to their utility in the prophylaxis and treatment of diseases related to oxidative stress [4].

Analytically speaking, an essential oil is usually regarded as a complex sample comprising a wide variety of volatile compounds strictly embedded with each other in a matrix. To unravel such an intricate composition, the high separation power of gas chromatography works as the most effective technique. After the widespread diffusion of essential oils in numerous different fields of current society, a detailed legislation on their global marketing has been issued. As a consequence, the need for reliable quantitative data has grown even more due to quality and safety requirements concerns. As well, an interest in essential oils' biological activity is continually growing [5]. The need for accurate quantitation in essential oils analysis is not only dictated by regulatory bodies (IFRA, EFSA, etc.) but becomes fundamental in novel characterization. Although relative percentage abundance is the most widespread approach to quantification by GC, this method is often improperly applied, e.g., for comparison of different essential oil samples from the same species [6]. In order to be compared, data first need to be standardized. The employment of calibration procedures with standards is even more important when true quantitation in physical units of concentration is the purpose of analysis. In the present study, response factors measured by means of external and internal methods were applied to true quantitation of the volatiles released by *Senecio anteuphorbium*. The genus *Senecio* comprises almost 1100 species distributed in Asia and Africa. In Morocco, *Senecio anteuphorbium* (syn. *Kleinia anteuphorbium* (L.) DC.), an endemic medicinal plant locally called "Achbartou", is commonly used in traditional medicine, mainly in the Sousse region, as a sedative for abdominal or back pain [7]. In fact, this fatty plant is also used as an anti-inflammatory, hemostatic, and in the treatment of rheumatism [8,9]. However, this plant species has received very little attention as regards its composition and biological activity. Beyond the chemistry, the scope of the study was to explore the allelopathic properties of the essential oil against *Lactuca sativa* germination and growth. In fact, a number of reports have previously highlighted the phytotoxic activity of the genus *Senecio* [10–15].

Allelopathy, a phenomenon that is gaining much interest in plant science, works via a complex mechanism. Plants contain several organic molecules that are biologically active. These molecules are associated with active roles in plant defense against pests, herbivores, and pathogens, as well as environmental stresses [16]. The production and concentration of secondary metabolites in plants are multifaceted processes that depend on the plant species, age, organs, and biotic and abiotic stresses they are subjected to [17]. Allelochemicals are liberated by plants into the outer environment and interact with close plants as well as other organisms corresponding to the suppression or stimulation of the growth, physiology, and development of the target species [18]. Briefly, allelopathy falls within the more recent and sustainable strategies applied to weed suppression in substitution of synthetic organic herbicides.

To the best of our knowledge, this work represents the first comprehensive investigation into the volatile constituents of *S. anteuphorbium*.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *S. anteuphorbium* were harvested during April 2019 (flowering stage) from Taghazout region (30°31'59" N, 9°42'00" W). The collected plant was air-dried in the shade for ten days and ground into a fine powder. A voucher specimen (No. 10119) was deposited in the Herbarium of Laboratory of Agri-Food, Biotechnologies and Valorization of Plant Bioresources, Faculty of Science, Marrakesh.

2.2. Extraction of the Essential Oil

The EO of the air-dried aerial parts of *S. anteuphorbium* (3 × 300 g) was extracted by hydro-distillation using a Clevenger-type apparatus (ENVEA, Casablanca, Morocco) for four hours by setting an initial temperature of 100 °C, decreased to a constant temperature of 70 °C after 30 min. The essential oil obtained was dried with sodium sulfate (Sigma-

Aldrich, St Louis, MO, USA) and stored at 4 °C until further analysis. The oil yield was calculated according to the following formula:

$$Y\% = \frac{V_{EO}}{DW} \times 100$$

where V_{EO} : volume of essential oil recovered (mL); and DW : amount of dry plant material used for extraction (g).

2.3. Gas Chromatography

2.3.1. GC–FID Analysis

Senecio anteuphorbium essential oils were injected into a Shimadzu GC-2010 system equipped with an AOC-20i autosampler and a split/splitless injector. The analytical column was a Zebron-5 ms, 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Phenomenex, Los Angeles, CA, USA). The oven program was: 50 °C, held for 1 min, at 3 °C/min to 250 °C, held 5 min. Injection temperature and volume were 250 °C and 1.0 μL, respectively. Samples were previously diluted 1:10 *v/v* in n-hexane. Injection mode: split, with a split ratio 1:50. Carrier gas was helium (u , 30 cm·s^{−1}; inlet pressure, 99.0 kPa). Detector temperature: 300 °C. Detector gases: H₂, 40 mL·min^{−1}; air, 400 mL·min^{−1}. Data were handled by means of GCsolution software (Shimadzu, Japan). For the calculation of response factors, the volatiles identified by GC–MS were grouped according to their structure (hydrocarbon, alcohol, ketone, etc.), and, for each group, a representative chemical was chosen and calibrated according to the following procedure. The reference standard (e.g., β-bisabolene for sesquiterpene hydrocarbons) was externally and internally calibrated by injecting 5 different levels of concentration within the linear range with the addition of a fixed amount of nonane as internal standard (final concentration 0.1 g/100 g). Each level of concentration was analyzed in triplicate (Table 1). The response factor was measured according to:

$$RF = \frac{[\text{Std}][i.s.]}{\text{Area std} : \text{Area } i.s.}$$

where RF , response factor; $[\text{Std}]$, concentration (g/100 g) of the standard to be calibrated; $[i.s.]$, concentration of the internal standard (g/100 g); Area std , FID peak area of the standard; $\text{Area } i.s.$, FID peak area of the internal standard (n-nonane). The concentration of each volatile component in essential oils real samples was then calculated:

$$[\text{VOC}] = \frac{(\text{Area}_{\text{VOC}} : \text{Area } i.s.) \cdot RF \cdot [i.s.] \cdot 100}{W_{oil}}$$

where Area_{VOC} , FID peak area of each volatile constituent; W_{oil} , weight (g) of the oil.

Table 1. Composition of *Senecio anteuphorbium* essential oil. Values are means of triplicate analyses.

Peak#	Group	Std.	Compound	RI _{exp}	RI _{db}	Area %	RF	Wt% (g/100 g)
1	H	✓	α-Pinene	933	933	0.16 ± 0.01	1.0	0.15 ± 0.01
2	A	✓	1-Octen-3-ol	978	978	0.32 ± 0.03	1.3	0.38 ± 0.03
3	A	✓	Linalool	1099	1101	0.26 ± 0.01	1.3	0.31 ± 0.01
4	K	✓	Isophorone	1122	1123	0.12 ± 0.01	1.3	0.14 ± 0.01
5	A		trans-Sabinol	1139	1140	0.32 ± 0.01	1.3	0.38 ± 0.01
6	A		trans-Verbenol	1142	1145	0.47 ± 0.02	1.3	0.56 ± 0.02
7	O	✓	trans-Linalool oxide	1173	1174	0.49 ± 0.04	1.5	0.67 ± 0.05
8	A	✓	p-Cymen-8-ol	1187	1189	0.62 ± 0.01	1.3	0.73 ± 0.01
9	A	✓	Myrtenol	1199	1202	0.37 ± 0.02	1.3	0.44 ± 0.02

Table 1. Cont.

Peak#	Group	Std.	Compound	RI _{exp}	RI _{db}	Area %	RF	Wt% (g/100 g)
10	K	✓	Verbenone	1206	1208	0.28 ± 0.01	1.3	0.33 ± 0.01
11	AL	✓	Safranal	1208	1201	0.28 ± 0.01	1.4	0.36 ± 0.01
12	K	✓	Pulegone	1242	1241	0.10 ± 0.02	1.3	0.12 ± 0.02
13	K	✓	Carvenone	1255	1257	0.29 ± 0.02	1.3	0.34 ± 0.02
14	A	✓	4-Vinylguaiaicol	1308	1309	0.77 ± 0.02	1.3	0.91 ± 0.02
15	H		Silphiperfol-5-ene	1329	1326	0.70 ± 0.02	1.0	0.64 ± 0.02
16	H	✓	δ-Elemene	1337	1335	0.28 ± 0.01	1.0	0.25 ± 0.01
17	H		Presilphiperfol-7-ene	1342	1339	0.39 ± 0.01	1.0	0.35 ± 0.01
18	A	✓	trans-p-Menth-6-en-2,8-diol	1375	1375	1.55 ± 0.04	1.3	1.83 ± 0.05
19	H		Silphiperfol-6-ene	1382	1380	0.22 ± 0.02	1.0	0.20 ± 0.02
20	H	✓	β-Patchoulene	1385	1383	0.66 ± 0.03	1.0	0.60 ± 0.02
21	H	✓	α-Copaene	1386	1385	1.72 ± 0.03	1.0	1.56 ± 0.03
22	H		Modhephene	1388	1384	0.71 ± 0.02	1.0	0.65 ± 0.01
23	H	✓	β-Elemene	1391	1389	0.71 ± 0.01	1.0	0.65 ± 0.01
24	H		α-Isocomene	1393	1387	0.36 ± 0.02	1.0	0.33 ± 0.02
25	H		α-Gurjunene	1409	1406	0.34 ± 0.01	1.0	0.31 ± 0.01
26	H		β-Isocomene	1411	1407	0.29 ± 0.01	1.0	0.26 ± 0.01
27	H	✓	(Z)-Caryophyllene	1415	1413	0.37 ± 0.02	1.0	0.34 ± 0.02
28	H	✓	Guaia-6,9-diene	1445	1444	0.21 ± 0.02	1.0	0.19 ± 0.01
29	H	✓	α-Humulene	1456	1454	0.41 ± 0.02	1.0	0.37 ± 0.02
30	H	✓	Alloaromadendrene	1460	1458	0.51 ± 0.02	1.0	0.46 ± 0.02
31	H		Selina-4,11-diene	1478	1476	5.59 ± 0.13	1.0	5.08 ± 0.11
32	H	✓	Germacrene D	1482	1480	1.87 ± 0.02	1.0	1.70 ± 0.02
33	H		Aristolochene	1491	1487	0.55 ± 0.02	1.0	0.50 ± 0.01
34	H	✓	Bicyclgermacrene	1501	1497	25.02 ± 0.09	1.0	22.75 ± 0.08
35	A		Cubebol	1520	1519	0.21 ± 0.02	1.3	0.25 ± 0.02
36	H	✓	δ-Cadinene	1523	1518	0.39 ± 0.03	1.0	0.35 ± 0.03
37	A		α-Elemol	1552	1546	0.12 ± 0.01	1.3	0.14 ± 0.01
38	A	✓	Spathulenol	1577	1576	21.37 ± 0.36	1.3	25.26 ± 0.42
39	A		Fokienol	1596	1596	4.96 ± 0.11	1.3	5.86 ± 0.13
40	A		1,10-di-epi-Cubenol	1616	1614	1.32 ± 0.10	1.3	1.56 ± 0.12
41	A		epi-γ-Eudesmol	1627	1624	5.75 ± 0.17	1.3	6.80 ± 0.20
42	A		T-muurolol	1652	1645	0.35 ± 0.04	1.3	0.41 ± 0.05
43	A		Cadin-4-en-10-ol	1661	1659	1.04 ± 0.04	1.3	1.23 ± 0.04
44	A		Shyobunol	1690	1686	1.74 ± 0.07	1.3	2.06 ± 0.08
45	A		β-Acoradienol	1763	1760	0.25 ± 0.02	1.3	0.30 ± 0.02
46	K		Aristolone	1765	1759	0.81 ± 0.02	1.3	0.96 ± 0.02
			HYDROCARBONS			41.46 ± 0.31		37.69 ± 0.38
			ALCOHOLS			41.79 ± 0.47		49.38 ± 0.37
			KETONES			1.60 ± 0.07		1.89 ± 0.08
			ALDEHYDES			0.28 ± 0.02		0.36 ± 0.03
			OXIDES			0.49 ± 0.02		0.67 ± 0.04

Group: H, hydrocarbon; A, alcohol; K, ketone; AL, aldehyde; O, oxide. Std.: co-injection of reference standard; RI_{exp}: retention index experimentally determined against a mixture of n-alkanes (C7-C40) on a Zebron-5 ms column. RI_{db}: retention index retrieved from FFNSC 2 and Adams 4th edition databases. RF: response factor.

The reference standards β -caryophyllene, caryophyllene oxide, farnesol, and citronellal, all supplied by Merck, were chosen as representative compounds of the different chemical groups.

2.3.2. GC–MS Analysis

Qualitative analyses were carried out on a GCMS-TQ8030 (Shimadzu) equipped with a Zebron-5ms column (30 m \times 0.25 mm i.d. \times 0.25 μ m film thickness). Carrier gas (He) parameters were the same as for GC–FID analysis. MS conditions: interface and source temperatures, 230 $^{\circ}$ C and 200 $^{\circ}$ C, respectively. Ionization mode: EI, 0.9 kV; acquisition mass range, 40–400 m/z ; scan speed, 1666 amu/s; scan interval, 0.25 s. Data were handled by means of GCMSsolution software. Identification was carried out by matching unknown spectra with two databases (FFNSC2, Adams 4th edn.). Moreover, retention indices were calculated by injecting a mix of saturated alkanes ranging from heptane to tetracontane (Merck, Darmstadt, Germany). Experimental retention indices were then compared with those listed in databases in order to restrict the list of candidates.

2.4. Allelopathic Activity

The allelopathic activity of *S. anteuophorbium* EO was evaluated against lettuce seeds according to Jalaei et al., with slight modifications [19]. In brief, commercial lettuce (*Lactuca sativa*) seeds were surface sterilized with 70% ethanol for 30 s, washed with sterile water to remove the ethanol, and then disinfected for 20 min by a 0.2% sodium hypochlorite solution, followed by three rinses of distilled water for 5 min each. After disinfection, 30 seeds were placed in glass Petri dishes lined with a filter paper Whatman No.1. The seeds were soaked with 10 mL of each EO concentration prepared in dimethyl sulfoxide (DMSO) (1%, v/v). The Petri dishes were sealed with parafilm and incubated in a climate room at 25 ± 1 $^{\circ}$ C with photoperiod 12:12 for 10 days [19]. The experience was performed in quadruplet, and a control containing DMSO 1% was added. Observations and measurements were made on the germination rate of seeds and the growth of lettuce seedlings. Plant growth in the incubation period was expressed by root length (cm), shoot length (cm), and seedling length (cm) at the end of the period. 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:

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

The germination and seedling growth were also evaluated by percentage of germination according to the following formula:

$$\text{Germination percentage} = (n/N) * 100$$

with n : number of germinated seeds in each concentration, and N : total number of seeds (30).

Moreover, mean germination time (MGT), germination rate (GR), and the vigor index (VI) were calculated.

3. Results and Discussion

3.1. Quantitative Analysis

The hydro-distillation extraction of the aerial parts of *S. anteuophorbium* from Taghazout region yielded 0.3% (v/w) of oil distillates. This EO was characterized by a dark brown color, an oily appearance, a density of 0.9 g/mL, and a freezing point above -21 $^{\circ}$ C. With regard to the yield, 0.15% was that obtained from *S. anteuophorbium* harvested in Essaouira region [20]. The higher yield of *S. anteuophorbium* from Taghazout (actual samples) can be explained by the different geographic provenance (different pedoclimatic conditions).

Figure 1 shows the GC–MS fingerprint of *S. anteuophorbium* EO, whereas its composition is reported in Table 1. The precision of the GC methodology was tested through the mea-

surement of standard deviations (reported in Table 1) and coefficients of variation, always lower than 5%. As can be seen, based on peak area normalization, the total identified and quantified fraction amounts to 85.62% of the whole EO. The volatiles were equally distributed between terpene hydrocarbons (41.46%) and alcohols (41.79%), with minor constituents being ketones, aldehydes, and oxides. *S. anteuphorbium* can be safely described as a sesquiterpene-rich oil, reporting as predominant components bicyclogermacrene (25.02%) and spathulenol (21.37%), a sesquiterpene alcohol. To confirm this, the chromatogram results are much more crowded in the second region, which is a typical elution zone of sesquiterpenes in essential oil analysis. Worth mentioning is the presence of characteristic components, such as silphiperfolene isomers, α - and β -isocomene, aristolochene, aristolone, and shyobunol. This chemical description matches roughly with that published by Elhidar et al. [20], the sole report on *S. anteuphorbium* found in the literature and totally focused on this species. However, the authors carried out only a tentative identification of the components by mass spectral matching with wide and generic collections (Wiley and NIST); in the present study, the mass spectral libraries used (FFNSC and Adams) were exclusively pertinent to flavor and fragrance compounds. Additionally, the identification procedure was boosted by the retention index matching [21] tool that was only mentioned but not implemented in Elhidar et al. [20]. Finally, an accurate calibration in absolute units (weight%, g/100 g) of single components was here carried out, enhancing the value of quantitative analysis [22]. There is evidence in the literature of a considerable number of papers on *Senecio* spp. Nonetheless, only a few publications are based on accuracy and reliability of both the data produced and methodologies used since, in many of them, the determination of artifacts (not naturally occurring compounds) is widely manifest [14,23,24]. For example, Irahai et al. have recently reported a GC–MS analysis of a variety of plant species, including *S. anteuphorbium* [24]. The GC data presented in that study show a lack of reliability in the GC–MS analytical protocol employed. Some compounds were artifacts (i.e., cyclotrisiloxane); many others were simply misidentified (i.e., β -maaliene, which is a sesquiterpene, notoriously eluting after monoterpenes); the mass spectral library used was only a NIST, capable of assigning an identity to a lower fraction of peaks (79%); finally, the source of retention indices was not specified. Nonetheless, a comparison between the two volatile fingerprints has been made, highlighting a substantial difference as regards the predominant constituents. Less than ten components were commonly found in the two compositions, among which selina-4,11-diene and shyobunol are worthy to be mentioned. Moreover, in many cases, these reports have been published in journals that do not enjoy scientific prestige. To further discuss the chemical constituents of the present *S. anteuphorbium* samples, it seems worthwhile to emphasize the similarity found with other *Senecio* spp. Spathulenol and germacrene B, which dominated the volatile fingerprint in *S. rowleyanus* and also showed powerful antioxidant and antimicrobial activities [25].

Rich in sesquiterpenes (i.e., germacrene D and A, γ - and δ -cadinene) were the essential oils from the roots and leaves of *S. rufinervis* [26]. The composition of *S. vernalis* was the most similar to that of *S. anteuphorbium*, bicyclogermacrene and spathulenol being the major volatiles, as here [27]. *S. trapezuntinus* appeared as a sesquiterpene rich oil, with (E)- β -farnesene as the predominant volatile, but the variety of minor sesquiterpenes was comparable to that reported by *S. anteuphorbium* [28]. Leaving aside the specific composition of *S. anteuphorbium*, it should be pointed out that numerous papers establish a close correlation between the sesquiterpene component and allelopathy [29,30]. Allelopathy, the natural phenomenon of plants interaction, represents great promise in crop science, with most allelochemicals being valid herbicides/pesticides in support of the sustainable development of agriculture. Among allelochemicals, spathulenol and bicyclogermacrene have been previously determined in a variety of plant species [31,32], confirming the present findings with regard to the allelopathic assays.

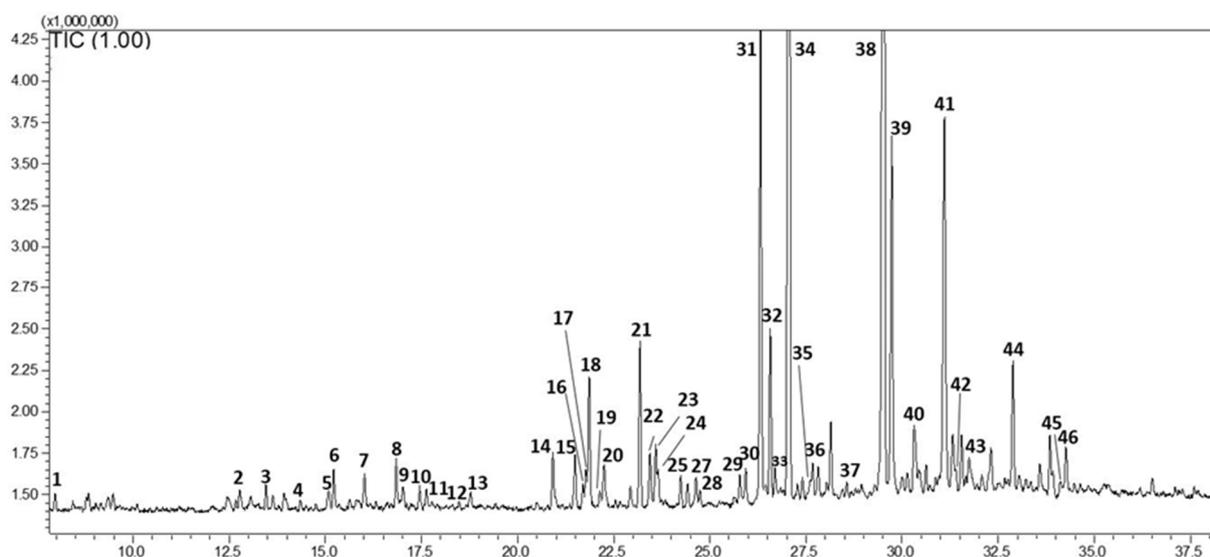


Figure 1. Total ion chromatogram obtained from GC–MS analysis of *S. anteuophorbium* essential oil. Peak top numbers refer to compounds listed in Table 1.

3.2. Allelopathic Activity

The allelopathic potential of the *S. anteuophorbium* EO was evaluated through germination percentage, root, shoot and seed lengths, and inhibitory growths percentage and factors on *Lactuca sativa* seeds. The results showed (Table 2, Figures 2 and 3) that the extracted EO from *S. anteuophorbium* exhibited a significant allelopathic inhibitory effect on the germination and the seedling growth of the tested seeds (*Lactuca sativa*) in a dose-dependent manner compared to the control.

Table 2. Effects of different concentrations of *S. anteuophorbium* essential oil on shoot, root, and seed germination indices and seedling growth factors of *L. sativa* at the end of incubation time. GP: germination percentage, MGT: mean germination time, GR: germination rate, VI: vigor index.

EO Concentration (mg/mL)	Shoot Growth (cm)	Root Growth (cm)	Seedling Growth (cm)	GP (%)	MGT (Day)	GR (Units)	VI (Units)
Control	2.34 ± 0.072 ^a	3.41 ± 0.121 ^a	5.61 ± 0.1 ^a	100 ^a	1.93 ± 0.14 ^a	8.91 ± 1.47 ^a	560.64 ± 12.47 ^a
0.018	2.33 ± 0.12 ^a	3.28 ± 0.21 ^a	5.76 ± 0.33 ^a	45.55 ± 6.94 ^b	2.01 ± 0.33 ^b	6.01 ± 0.57 ^b	260.95 ± 12.23 ^b
0.035	1.85 ± 0.12 ^b	2.7 ± 0.16 ^a	4.55 ± 0.21 ^b	15.55 ± 1.92 ^c	3.01 ± 0.52 ^c	1.7 ± 0.02 ^c	70.81 ± 7.17 ^c
0.07	1.06 ± 0.1 ^b	2.23 ± 0.21 ^b	3.29 ± 0.16 ^b	8.88 ± 1.92 ^{cd}	3 ± 0.64 ^c	1.05 ± 0.28 ^d	27.01 ± 13.37 ^{cd}
0.14	0.55 ± 0.02 ^c	1.28 ± 0.04 ^b	1.83 ± 0.03 ^c	7.77 ± 1.92 ^{de}	3.11 ± 0.51 ^c	0.92 ± 0.15 ^d	14.23 ± 1.89 ^d
0.28	0.29 ± 0.02 ^d	0.72 ± 0.01 ^c	1.01 ± 0.02 ^c	3.33 ^e	3.33 ± 0.8 ^c	0.4 ± 0.1 ^e	3.36 ± 0.76 ^e
IC ₅₀	0.12 ± 0.01	0.15 ± 0.02	-	-	-	-	-

IC₅₀ is the concentration at which 50% of growth inhibition was predicted to occur. Different letters indicate statistically significant differences at $p \leq 0.05$. Significance level: ^a $p > 0.05$, ^b $p < 0.05$, ^c $p < 0.01$, ^d $p < 0.001$, and ^e $p < 0.0001$.

According to the obtained results reported in Table 2 and Figure 3, at a high concentration of the EO (0.28 mg/mL), the growth of shoots and roots was reduced by 87.60% and 78.88%, respectively. The EO showed an IC₅₀ value of 0.12 mg/mL for shoot growth and 0.15 µg/mL for root growth, respectively, compared to the controls. As regards mean germination time (MGT), although the results show a statistically significant difference between the control and treated samples, MGT was the least affected parameter. The germination rate (GR) differed significantly between the different treatments, the highest being observed with the control (8.91 units) and the lowest being observed with *S. an-*

teuphorbium EO at 0.28 mg/mL with 0.4 units. Consequently, the GR factor was strictly concentration-dependent, as well as the vigor index (3.36 vs. 560.64, for 0.28 mg/mL EO and the control, respectively).

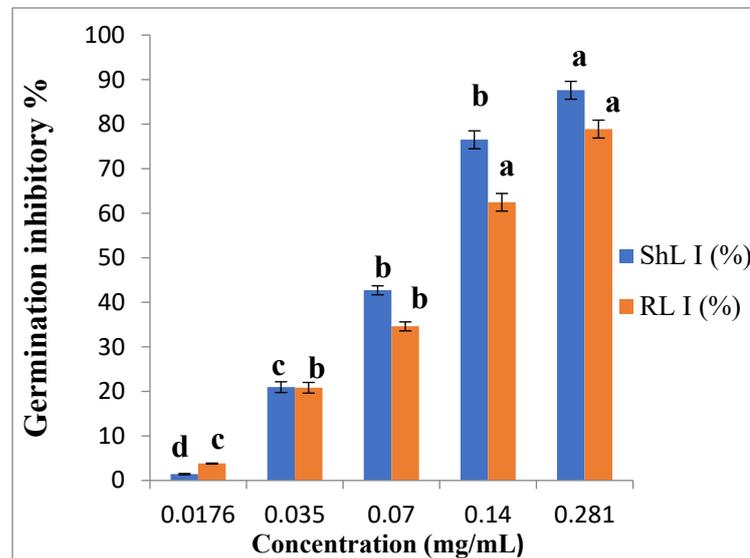


Figure 2. Allelopathic effect of the essential oil from *Senecio anteuphorbium* on root (RLI) and shoot (ShLI) growths of *Lactuca sativa* seeds. Different letters indicate statistically significant differences at $p \leq 0.05$.

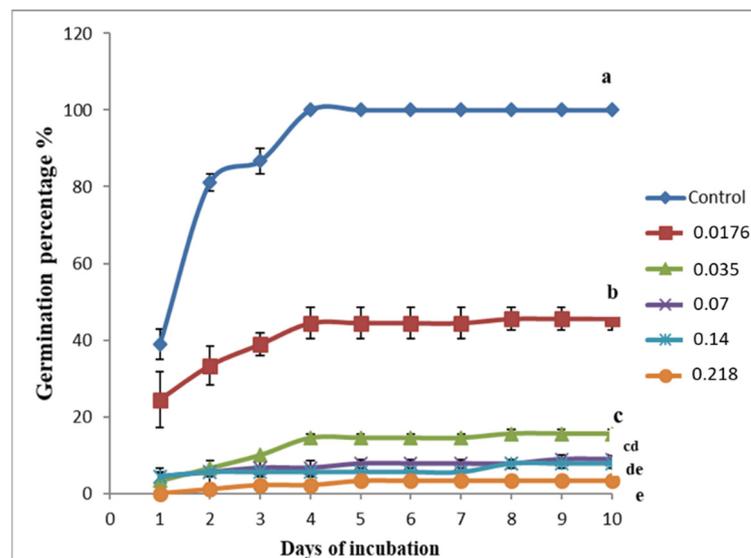


Figure 3. Cumulative germination observed in *L. sativa* seeds after treatment with different EO concentrations (mg/mL) of *Senecio anteuphorbium* at 1-day intervals.

The comprehensive observation of germination data after treatments at 1-day intervals (Figure 3) shows that all the EO concentrations inhibited germination compared with the control. The maximum germination percentage (100%) was recorded in the control and the minimum (3.33%) in the EO treatment (0.28 mg/mL) at the end of the incubation period. The genus *Senecio* has been widely reported in the literature due to the phyto-compounds found in extracts, with a potent allelopathic property. The phytotoxic effects of *S. westermanii* Dusén ethanol extracts and subfractions were evaluated on lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.) seeds. The results demonstrated an inhibitory

effect on the germination velocity index and growth. The plant also caused a change in respiration and photosynthesis [13]. Moreover, it was observed by Arancibia and his coauthors [14] that aqueous and ethanolic extracts, from *S. filaginoides*, significantly inhibited the germination of *Solanum lycopersicum* and *Lolium multiflorum* seeds in a dose-dependent manner. Cruz-Silva and his collaborators demonstrated that aqueous extracts of leaves and inflorescences of *S. brasiliensis* altered the germination and seedling development of *Lactuca sativa* L. (lettuce) [11]. The phytotoxic effectiveness of the *Senecio* genus has been poorly studied. To the best of our knowledge, the results obtained in this study are the first published data concerning the allelopathic activity of *Senecio anteuphorbium* essential oil. Compared to other species of the genus *Senecio*, the phytotoxic effects obtained in our study are higher than the allelopathic potential of EOs from the *Senecio* genus previously evaluated in other studies. Overall, it has been previously shown that the essential oil of *S. amplexicaulis* demonstrated remarkable and dose-dependent phytotoxic activity at the tested concentration and significant reduction in seed germination percentage of *Phalaris minor* and *Triticum aestivum* at 0.5 mg/mL; the oil inhibited germination of both *T. aestivum* ($65.00 \pm 1.00\%$) and *P. minor* ($58.34 \pm 1.52\%$) compared to the control [12]. In another study, the phytotoxic activity of the essential oil of *Senecio erucifolius* L. was evaluated against three weeds, namely *Medicago sativa* L., *Urtica cannabina* L., and *Amaranthus retroflexus* L. [15]. In general, the essential oil at low concentrations stimulated the growth of sprouts of the treated species; at high concentrations, it suppressed it. The length of the roots of *M. sativa*, *U. cannabina*, and *A. retroflexus* increased by 21.00, 10.46, and 2.53%, respectively, after treatment with oil at the lowest concentration (0.125 mg/mL) and decreased by 9.36, 23.00, and 19.53% after treatment at the highest concentration (4 mg/mL) [15]. In fact, the change in the parameters of germination indicates the changes in the metabolic reactions of the plants [33]. It might be speculated that the phytotoxic effect of *S. anteuphorbium* EO is due to the activity of the major compounds, bicyclogermacrene (25.02%) and spathulenol (21.37%) (Table 1). However, minor compounds, such as α -pinene and p-cymen-8-ol, have been reported as allelochemicals, and they could act either individually or synergistically as inhibitors on the germination and growth of the *Lactuca sativa* L. seeds [34]. Moreover, the oxygenated terpenoids usually have a significant role in biological activity compared to non-oxygenated compounds due to oxygen reactivity [29].

4. Conclusions

This article provides a dataset on the volatile quantitative composition and allelopathic properties of *S. anteuphorbium*, an endemic plant of Morocco used in traditional medicine. The EO composition was thoroughly investigated by GC techniques, applying true quantitation in order to overcome the limits of traditional approaches based on the sole relative abundance. The EO reported a predominant sesquiterpene component. A strong allelopathic effect against *Lactuca sativa* seeds was observed, suggesting an interesting application of the EO as an alternative bioherbicide. All the findings suggest the usefulness of further studies (i.e., investigation of the non-volatile fraction, antioxidant power, and mode of action) on this *Senecio* species.

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