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Assessment of the Allelopathic Effect, Antimicrobial Potential and Chemical Composition of *Senecio anteuphorbium* L. Essential Oil

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Abstract: The essential oil of *Senecio anteuphorbium* L (*Kleinia anteuphorbium* L.) was isolated by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). A total of 31 compounds were identified, with the dominant compounds being 1-nonene (53.7%), α -pinene (6%), 1-octene (5.9%), luguloxide (5.5%), and selina-4,11-diene (4.6%). The essential oil shows herbicidal activity against *Phalaris canariensis*, inhibiting seed germination and seedling growth at all concentrations tested (1-4 μ L/mL). It also exhibits antimicrobial activity against both gram-positive and gram-negative bacteria, with minimum inhibitory concentration (MIC) values of 6.9 and 13.8 μ L/mL, respectively. These results suggest that the essential oil of *S. anteuphorbium* has the potential to be used as a natural herbicidal and antimicrobial agent. This work aims to carry out phytochemical investigations on the herbicide properties of this natural product.

Keywords: *Senecio anteuphorbium* L.; essential oil; 1-nonene; antimicrobial activity; herbicidal activity. © 2024 ACG Publications. All rights reserved.

1. Introduction

Asteraceae (Compositae) is the largest family of flowering plants, with approximately 1,620 genera and more than 23,600 species [1,2]. The genus Senecio is one of the richest in species among the Asteraceae, with 3000 species [3]. It is an important genus because of its many phytochemical, pharmacological, botanical, and toxicological properties [5,4]. Pharmacological studies have shown that some Senecio species have activities such as antimicrobial [6,7], anti-fatigue, anti-inflammation, anticancer, and immunomodulation effects [8,9]. Previous studies of the genus Senecio have been concerned with alkamides [10] and flavonoids [11]. This group of plants is of interest because several species belonging to it have been used in folk medicine for their in vitro antifungal and antibacterial purposes [12]. As of today, no investigations have been performed specifically for the essential oils of this plant with the aim of revealing their biological activity. Hence, this plant species has become a subject of our scientific interest.

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In the present study, the essential oil chemical composition isolated from aerial parts of this important specie of the family Asteraceae, commonly used in folk medicine, was characterized by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analyses. Their respective antimicrobial activities against *Escherichia coli* ATCC25922, Proteus mirabilis ATCC35659, *Bacillus cereus* ATCC10876, *Aspergillus brasiliensis* ATCC16404, and *Candida albicans* ATCC10231 were investigated by the diffusion technique on solid media. The present investigation was undertaken to assess the phytotoxicity of *Senecio anteuphorbium* oil against weeds with a view to explore them as a bioherbicide for weed management. This research can be used to increase the quality and quantity of agricultural products, especially by decreasing the toxicological effects on environmental and living organisms, including humans.

2. Materials and Methods

2.1. Plant Material

The wild growing sample of the investigated plant was collected during the flowering stage from the Aourir region (South of Morocco), which is located at an altitude of 150 m. The plant *Senecio anteuphorbium* (*Klenia anteuphorbium*) were identified by Pr. Ahmed Ouhammou, Faculty of Sciences Semlalia, Cadi Ayyad University. A voucher specimen was deposited in herbarium under N° MARK 7634. The sample was dried in shadow at room temperature for 10 days.

2.2. Essential Oil Extraction and Analysis

2.2.1. Essential oil Extraction

The aerial parts of *Senecio anteuphorbium* (200 g) were submitted to hydrodistillation for 4 hours using a Clevenger type apparatus. The obtained essential oil was dried with anhydrous sodium sulphate and stored at 4°C before use. The yield was calculated based on the dry weight of the sample.

2.2.2. Essential Oil Analysis

The composition of the essential oil of species was determined by GC and GC/MS. GC/FID, the GC was carried out on a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector (FID) and a HPGC ChemStation Rev. A.05.04 data handling processor. A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm i.d., film thickness 0.20 μ m) and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μ m). The oven temperature was programmed at 70–220°C (3°C.min⁻¹), 220°C (15 min); injector temperature: 250°C; the carrier gas was helium, adjusted to a linear velocity of 30 cm.s⁻¹; the splitting ratio was 1:40, and the detectors temperature was 250°C.

GC/MS analysis was performed on a Hewlett-Packard 6890 gas chromatograph fitted with a HP1 fused silica column (polydimethylsiloxane 30 m \times 0.25 mm i.d., film thickness 0.25 μ m), which was directly coupled with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters are under the following conditions: interface temperature: 250°C; MS source temperature: 230°C; MS quadrupole temperature: 150°C; ionization energy: 70 eV; ionization current: 60 μ A; scan range: 35–350 units; and scans/s: 4.51.

2.3. Herbicidal Activity

Mature seeds of annual seeds of *Triticum durum* and *Phalaris canariensis* were sterilized with 15% sodium hypochlorite for 20 min⁻¹. They were then rinsed with distilled water. Empty and undeveloped seeds were discarded by floating in tap water, and the remaining seeds were used. Then, the oil was dissolved in tween-water solution (1%; v/v). The final concentrations of the treatments were 0 (control), 0.5, 1, 2, 3, 4, 5, and 6 μL/mL. The emulsions of 8 mL were transferred to a Petri dish placed on the bottom of two layers of filter paper. Afterward, 20 seeds of *P. canariensis* and *T. durum* were placed on the filter paper. The Petri dishes were closed with an adhesive tape to prevent the escaping of volatile compounds and were kept at 25°C on a growth chamber supply with 12 hours of fluorescent light [13]. The number of germinated seeds and seedling lengths were measured after 10 days, and all tests were arranged in a completely randomized design with three replications by treatment.

2.4. Antibacterial Activity

2.4.1. Bacterial Strains

The antimicrobial activity of the essential oil samples was tested toward five different microorganisms: two gram-negative bacteria, namely *Escherichia coli* ATCC25922 and *Proteus mirabilis* ATCC35659, one gram-positive bacteria, namely *Bacillus cereus* ATCC10876, and two fungi, namely *Aspergillus brasiliensis* ATCC16404 and *Candida albicans* ATCC10231.

2.4.2. Culture Medium and Inoculums

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 40°C. Inoculums was prepared by suspending a loop full of bacterial cultures in 10 mL of nutrient broth and was incubated at 37°C for 24 hours. The agar sterilized in a flask and cooled to 45–50°C was distributed by pipette (20 mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1 mL of bacterial suspension was taken and poured into Petri plates containing 20 mL of nutrient agar medium. Using the L-shaped sterile glass spreader, bacterial suspensions were spread to achieve a uniform lawn culture.

The bacterial suspensions were adjusted with sterile saline to a concentration of $1.0 \, \text{CFU/mL} \times 10^5 \, \text{CFU/mL}$. The inocula were prepared daily and stored at $+4^{\circ}\text{C}$ until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and check the validity of the inoculum.

2.4.3. Screening for Antibacterial activity

The agar diffusion method was used for the antimicrobial evaluations. Wells of 8 mm diameter were dug on the inoculated nutrient agar medium with a sterile cork borer, and 50 μ L of the bark essential oil (at various concentrations) were added in each well. The essential oil of required concentrations was prepared by dissolving the oils into appropriate quantities of DMSO, which did not influence the growth of bacteria used as a negative control. The plates were then incubated at 37°C overnight and examined for zone of inhibition. The diameter of the inhibition zone was measured in millimeters. The standard antibiotic drugs were also screened under similar conditions for comparison. All the assays were performed in triplicate and expressed as average values.

2.5. Antioxidant Activity

The assay was carried out by mixing 1.5 mL of methanolic solution of each essential oil with 2.0 mL of a 0.02 mM methanolic DPPH solution at three final concentrations (5, 25, and 100 mg essential oil/mL). The mixture was then incubated in the dark for 30 minutes at 25°C, and the absorbance at 517 nm was recorded as (A_{sample}) using a spectrophotometer. A blank experiment was also carried out, applying the same procedure to a solution without the test material, and the absorbance was

recorded as (A_{blank}). The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

% Inhibition =
$$100 \times (A_{blank} - A_{sample})/A_{blank}$$

2.6. Statistical Analysis

Data of germination and seedling growth assays were subjected to one-way analysis of variance (ANOVA) using the SPSS 13.0 software package. Differences between means were tested through Student–Newman–Keuls (SNK), and values of p < 0.05 were considered significantly different [14].

3. Results and Discussion

The essential oil, which was a pale-yellow color, was obtained by hydrodistillation in a Clevenger-type apparatus from whole plants of S. anteuphorbium L., with a yield of 0.2% (v/w) on dry weight basis. The essential oils of this plant were analyzed by GC as shown in Figure S1, and GC/MS with HP-5 column. The general chemical profile of the essential oils, the percentage content, and retention indices of the constituents are reported in Table 1. A total of 31 compounds were identified from the essential oil of S. anteuphorbium, which represented 91.1% of the oil extracted. The oil was dominated by aliphatic compound (59.7%), followed by sesquiterpene hydrocarbons (12.1%) and monoterpene hydrocarbons (11.4 %) (Table 1). The major components were 1-nonene (53.7%), αpinene (6%), 1-octene (5.9%), luguloxide (5.5%), and selina-4.11-diene (4.6%), while S. oreophyton essential oil contains p-mentha-1(7),8-diene (31%), S. pogonias and S. oreophyton, are characterized by a high content of α-phellandrene (22.0%), S. Graciliflorus. The chemical composition of the essential oil does not match that of other Senecio species reported in the literature; e.g., S. graciliflorus [15], S. pogonias and S. oreophyton [16], S. filaginoides aerial parts [17], S. cineraria DC leaves [18], and S. graveolens leaves [19] have dominance of monoterpene hydrocarbons. The oxygenated sesquiterpenes and monoterpenes as major chemical class of S. ambavilla (Bory) Pers [20] and S. tephrosioides Tucz essential oils, respectively [21].

Table 1. Kovats indices (KI) and percentage of chemical composition of *S. anteuphorbium* L essential oil.).

KI (a)	$\mathbf{KI}^{(\mathbf{b})}$	Compounds	%	
790	n.d.	1-Octene	5.9	
890	n.d.	1-Nonene	53.7	
915	n.d.	1,3-Nonadiene	t	
924	n.d.	α-Thujene	0.2	
931	1030	α-Pinene	6.0	
969	1117	β-Pinene	3.6	
982	1161	Myrcene	0.9	
999	1169	α -Phellandrene	0.0	
1006	1185	α-Terpinene	0.1	
1013	1274	<i>p</i> -Cymene	0.2	
1021	1214	β-Phellandrene	0.2	
1021	1217	1,8-Cineole	0.3	
1025	1233	Z-β-Ocimene	0.1	
1048	1248	γ-Terpinene	0.1	
1088	n.d.	1-Undecene	0.1	
1161	1594	Terpinene-4-ol	0.1	
1328	1467	δ-Elemene	0.9	
1367	1487	α-Copaene	1.3	
1380	1583	β-Elemene	0.1	
1399	1524	α-Gurjunene	0.2	
1407	1591	<i>E</i> -Caryophyllene	0.9	

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1439	1661	α-Humulene	0.5
1462	1667	Selina-4,11-diene	4.6
1465	1700	Germacrene D	2.0
1489	1748	Germacrene A	0.2
1496	1749	γ-Cadinene	0.6
1505	1749	δ-Cadinene	0.8
1511	1779	Liguloxide	5.5
1556	1973	Caryophyllene oxide	0.4
1608	2158	γ-Eudesmol	1.2
1631	2222	α -Cadinol	0.4
Monoterpe	11.4		
Oxygen co	0.4		
Sesquiterp	12.1		
Oxygen co	7.5		
Aliphatic c	59.7		
Total iden	91.1		

 $\mathbf{t} = \text{trace}; \mathbf{a}: \text{ apolar column}; \mathbf{b}: \text{ polar column}$

3.2. Herbicidal Activity of Essential Oil from Senecio anteuphorbium L.

The phytotoxic effects of essential oils obtained from the aerial parts of *Senecio anteuphorbium* L. were tested on germination and seedling growth of *P. canariensis*, which is a highly invasive weed in cultivated areas. According to the statistical analysis, the phytotoxic effects of the tested oil were significantly influenced by doses. The results show that the essential oil of *Senecio anteuphorbium* completely inhibits the emergence of weeds relative to the control (Table 2). In general, a dose–response relationship was observed, and the emergence declined with the increasing amount of *Senecio anteuphorbium* oil. At the doses of 1 and 2 μ L/mL, weed germination was reduced and was totally inhibited at 4 μ l/mL, while the germination of *Phalaris Canariensis* was totally inhibited by oil treatment at a dose of 4 μ L/mL. When germination was partially inhibited, not only emerged, even the seedling growth measured as roots and shoots lengths were significantly reduced; the reduction was greater with the increasing amount of oil. In the literature, the herbicidal effects of essential oils from the Asteraceae family against weeds have been previously reported [22-31]; nonetheless, no study has reported on the phytotoxic effects of *Senecio anteuphorbium*.

Furthermore, we reported that the major components of the oil have oxygenated mono and sesquiterpenes and their respective hydrocarbons. Based on previous reports, we can conclude that the phytotoxic effects of essential oils can be attributed to individual components, while synergism and antagonism do play an important role on the biological activity.

Nevertheless, it is interesting to note that, according to previous results, not only the monoterpene compounds may be responsible for germination inhibition, because previous assays with *Eucalyptus camaldulensis* essential oil, rich in the oxygenated sesquiterpene spathulenol (41.46 ± 3.94), showed that it also completely inhibited seed germination and seedling growth.

The exact mechanism by which germination and seedling growth are affected by *Senecio anteuphorbium* volatile oil is unknown and not prospected in our study. However, such inhibitory effects could be caused by allelochemicals interfering with physiological and biochemical processes in the target species [32-34]. Indeed, it has been reported that the inhibition of germination may be the consequence of the inhibition of water uptake, increased abscisic acid content, decreased indole-3-acetic acid and zeatin riboside contents, and disruption of the activity of metabolic enzymes that are involved in glycolysis and oxidative pentose phosphate pathway [35,36]. On the other hand, previous studies have shown that essential oils have phytotoxic effects that may cause anatomical and physiological changes in plant seedlings, leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such as mitochondria, possibly due to inhibition of DNA synthesis or disruption of membranes surrounding mitochondria and nuclei [37]. Muscolo et al. reported that the inhibition of seed germination in *Pinus laricio* was attributed to a disruption of the activity of metabolic enzymes that are involved in glycolysis and the oxidative pentose phosphate pathway [36]. Another

suggested mechanism for the inhibition of seed germination and radicle elongation is the disruption of dark or mitochondrial respiration. At this point, it has been shown that some volatile constituents strongly affect the respiratory activity by interfering with the electron flow in the cytochrome pathway, resulting in decreased adenosine triphosphate production and, hence, alteration of other cell processes, which are energy-demanding [38]. In contrast, due to the difficulties measuring the allelochemicals effects on mitochondrial respiration in intact plants because many of these effects are masked by photorespiration, it has been hypothesized that the ability of monoterpenes to act as allelochemicals on intact seeds is probably directly related to their ability to permeate intracellular compartments [38-40]. A work on natural herbicides is currently being developed. Recent work has confirmed that radulanine A is a natural herbicide origin, and a work to synthesize this molecule is currently being undertaken [41].

Table 2. Effects of Senecio anteuphorbium oil concentration and kinds of weeds on some factors*

Plants	Sample	Dose (µL/mL)	Germination (%)	Growth (cm)	
tested				Roots length	Aerial length
	Control	0	86.66±5.77a	8.43±0.51 ^a	11.33±0.57 ^a
	Huile essentielle	0.5	76.67±5.77 ^b	5.75±2.31 ^b	5.7±2.22 ^b
		1	53.33±5.77°	5 ± 1.4^{b}	5.6 ± 17^{b}
Phalaris		2	43.33 ± 5.77^d	3.5 ± 1.08^{c}	4.07 ± 0.83^{c}
Canariensis		4	00.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
		6	00.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	Control	0	100 ± 0.0^{a}	10.33±0.57 ^a	13.50±0.50a
		0.5	80±10.00b	8.25±2.47 ^b	4.5±0.70 ^b
Triticum	Essential oil	1	50.00±0.0°	5.03 ± 2.02^{c}	4.63 ± 3.60^{b}
durum		2	49.33±5.77°	5.00 ± 0.00^{c}	4.56 ± 0.00^{b}
		4	46.33±5.77°	2.23 ± 0.40^{d}	2.13±0.23°
		6	43.67±3.71°	2.10 ± 0.09^{d}	2.09 ± 0.12^{c}

^{*}Roots Length, aerial length and Germination percentage) according to Duncan's Multiple Range test in Petri dish Means in the same column by the same letter are not significantly different of the test Student-Newman-Keuls ($p \le 0.05$). (Mean of five replicates).

3.3. Antimicrobial Activity

The antimicrobial activity of *S. anteuphorbium* L. essential oil was qualitatively and quantitatively assessed by the presence or absence of inhibition zone diameters and minimum inhibitory concentration (MIC) values. Tables S3 and 4 report the inhibition zones and MIC values of the essential oil against two gram-negative and one gram-positive bacteria and one yeast and one mold using the diffusion technique on solid media. As reported in the literature, we consider an essential oil to have bacteriostatic action if the diameter of inhibition is greater than either 12 mm [42] or 15 mm [43,44]. The essential oils showed a wide antimicrobial spectrum: They were active against all tested strains, except *C. albicans*, by producing inhibition zone diameters varying from 55±2.5 mm to 23 mm; these diameters were sometimes wider than those obtained with usual antibiotics and antifungals (penicillin, pristinamycin, chloramphenicol, oxacillin, ceprofloxacine, gentamicin, polimyxine, colistin, ampicillin, riphampine, acid-naldaxique, cefazolin, rifampin, trimethoprim for bacteria, for *A. brasiliensis*, amphotericin, econazole miconazole, clotrimazole). Moreover, the results show a large variability in the bacteriostatic quality of the essential oil against the different strains. Finally, *E. coli* ATCC25922, *P.*

mirabilis ATCC35659, and B. cereus ATCC10876 were highly sensitive to the tested oil, producing average inhibition zones of 55 mm, 60 mm, and 40 mm, respectively. The essential oils were active against A. brasiliensis ATCC16404, whereas C. albicans ATCC10231 is a resistant strain to the tested oils

The MIC results of *S. anteuphorbium* L. essential oil obtained by the direct agar contact method are reported in Table S1 (see supporting information). MICs are inversely proportional to the diameters of the inhibition zones obtained with the antibio-aromatogramme [45]. Strains of *A. brasiliensis* ATCC16404 are the most resistant, only being inhibited at high concentrations of essential oil (MIC = 138 μ g/mL). The most sensitive bacteria were *E. coli* ATCC25922, *P. mirabilis* ATCC35659, and *B. cereus* ATCC10876, which were inhibited at MICs significantly lower than those obtained with other strains (between 6.9 μ g/mL and 13.8 μ g/mL).

In recent years, several researchers have reported that mono and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils of plant origin; these essential oils have enormous potential to strongly inhibit microbial pathogens [46,47]. Generally speaking, terpenes and flavonoids, which are phenolic in nature, are the active antimicrobial compounds of essential oils; it would seem reasonable that their antimicrobial or antibacterial mode of action might be related to that of other compounds.

The Senecio genus is rich in secondary metabolites like terpenes, flavonoids, and terpenoides. This class of secondary metabolites possesses antimicrobial activity [48-50]. Another event, which can explain its activity, is α-pinene, one of the major components of this plant, which has been found to have relatively strong antimicrobial properties [51]. This activity can be related to either trace or major components in the oil that could give rise to part of the antimicrobial activity. There are also possible synergistic and antagonistic interactions between the oil components [52]. It appears well that the essential oil of Senecio showed a good antibacterial activity and supports its medicinal uses and may find applications in cosmetics and natural food preservation for their broad spectrum of inhibitory activity.

Table 3. Antibacterial activity of *S. anteuphorbium* essential oils (MIC; μg/mL)

Bacterial strains	Minimum Inhibitory		
	Concentration (MIC) µg/mL		
Escherichia coli (ATCC 25922)	6.9		
Proteus mirabilis (ATCC 35659)	6.9		
Bacillus cereus (ATCC 10876)	13.8		
Candida albicans (ATCC 10231)	0.0		
Aspergillus brasiliensis (ATCC 16404)	13.8		

4. Chemotaxonomic Evaluation

In this study, 31 compounds were identified from the EO of *S. anteuphorbium*w, including the dominant compounds such as 1-nonene (53.7%), α -pinene (6%), 1-octene (5.9%), luguloxide (5.5%), and selina-4,11-diene (4.6%). Generally, this EO is characterized by the presence of aliphatic and sesquiterpenes as major compounds.

Sesquiterpenes are the main secondary metabolite of S. anteuphorbium which known by the antimicrobial activities as reported by many researchers. The exhibit antimicrobial activity against both Gram-positive (Bacillus cereus and Candida albicans) and Gram-negative bacteria (Escherichia coli and Proteus mirabilis), with the minimum inhibitory concentration (MIC) values of 6.9 and 13.8 μ L/mL, respectively.

Also, Table 2 display the Allelopathic effect of essential oil. This indicate that essential oil of *Senecio* anteuphorbium completely inhibited the emergence of weeds relative to the control.

In conclusion, our study focused on the correlation between the chemical composition and the effectiveness as herbicidal and antimicrobial agents of essential oil extracted from *Senecio anteuphorbium* L. The results of essential oil bioactivities show that *Senecio anteuphorbium* L. exhibits strong phytotoxic and antibacterial effects. Based on our preliminary results, the essential oil of *Senecio anteuphorbium* L. could be suggested as an alternative herbicide and antibiotic. However, further studies are needed to determine the cost, applicability, safety, and phytotoxicity against cultured plants of these agents as a potential bio-pesticide.

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Competing Interests

The authors declare that there are no competing interests exist.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products



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