

Communication

Chemical Composition and Antioxidant Activity of Essential Oil from *Daucus reboudii* Coss., an Endemic Plant of Algeria

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Abstract: Plants of the *Daucus* genus (Apiaceae) are used worldwide as traditional medicines or culinary ingredients. In this work, in order to explore a new chemotype, the essential oil obtained from the aerial parts of *Daucus reboudii* Coss., collected from the National Park of Gouraya (Bejaïa, Algeria), was analyzed by GC-MS. Twenty-eight compounds were identified, accounting for 96.6% of the total oil. (E)-anethol was the main constituent (59.4%), followed by estragol (21.2%) and dodecanal (4.4%). (E)-anethol is an uncommon constituent of *Daucus* genus, hence it could be considered as a marker that contributes to differentiating *D. reboudii* from other species. Metal chelating, ABTS^{•+} and DPPH[•] assays were performed to determine the antioxidant activity. The highest activity was revealed by the DPPH[•] method, where *D. reboudii* essential oil showed a significantly higher activity compared to the reference standard BHT at doses of 50 and 100 µg/mL. Results suggest that the essential oil from *D. reboudii* could have a potential use in the food industry as food preservative. Nevertheless, further studies are needed to assess its applicability, and to elucidate also the composition of non-volatile compounds of this plant.

Keywords: *Daucus reboudii* Coss.; essential oil; GC-MS; (E)-anethol; antioxidant



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1. Introduction

Because of their essential oils in particular, plants of the Apiaceae family have been used as spices or raw medicines since antiquity. Most of the species of the genus *Daucus* are encountered in Africa, Europe, West Asia and North America, and several species of this family (the carrot family) provide us with economically important food products. Among the others, *Daucus* genus is widely diffused, and *Daucus carota* is one of the most commonly used as a vegetable for human nutrition [1].

Many *Daucus* species are rich sources of bioactive compounds, such as essential oils, polyacetylenes and flavonoids [2–4], that are considered as the main contributors of their medicinal properties. These latter comprise stomachic, carminative and diuretic properties [5].

In Algeria there are about 25 *Daucus* species, nine of which are endemic: *D. alatus*, *D. gracilis*, *D. grandiflorus*, *D. jolensis*, *D. micranthus*, *D. paralias*, *D. serotinus*, *D. stenopterus* and *D. reboudii* [6]. *D. reboudii* Coss. (Figure 1), a species close to the world-diffused *D. carota*, is an endemic plant of Algeria and Tunisia. In Algeria, it grows exclusively in

the North-East of the country, in particular in Guelma and Kabylie regions [6]. *D. reboudii* grows spontaneously in cork oak forests [6,7], and it is characterized by a fennel savor. It is a perennial herbaceous plant, characterized by a small white down (1 mm in length) on the stems and along the petioles, and by 5–7 mm-long fruits [7].



Figure 1. Aerial parts of *Daucus reboudii* Coss. growing in Algeria.

The essential oil composition of many Apiaceae species has been already reported. Among *Daucus*, the essential oil from the species *carota* has been extensively studied, especially from the aerial parts [8–10] and seeds [11]. Nevertheless, to the best of our knowledge, only one study regarding the composition of essential oil from endemic *D. reboudii* is present in the literature, reporting a high content of monoterpenes hydrocarbons (69.3%) and oxygenated monoterpenes (14.9%) [7].

The present study aimed at investigating the composition of the essential oil obtained from the aerial parts of *D. reboudii* growing in Northern Algeria and its antioxidant properties, measured using different techniques, namely metal chelating, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH[•]) assays.

2. Materials and Methods

2.1. Plant Material and Extraction of Essential Oil

Aerial parts of *D. reboudii* were collected during the ripening stage near the National Park of Gouraya, Bejaïa, Algeria. Plants were identified by Dr. Khellaf Rebbas and a voucher sample was deposited at the Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, Algeria (*D. reboudii* voucher number ST/RK N° 05). The dried plant material (200 g) was coarsely cut and distilled in a Clevenger-like hydro-distillator for 2 h.

2.2. GC-MS Analysis

Gas chromatography–mass spectrometry (GC-MS) analyses were performed using a Varian CP-3800 GC equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) coupled with a Varian Saturn 2000 mass detector. Analytical conditions were as follows: injector and transfer line temperatures were set at 220 and 240 °C, respectively; the oven temperature was programmed from 60 °C to 240 °C at 3 °C/min, using helium at 1 mL/min as carrier gas; injection of 0.2 µL; split ratio 1:30.

2.3. Identification of Volatile Components

The volatile constituents were identified by comparing their retention times with those of pure samples, by comparison of their linear retention indices relative to the series of n-hydrocarbons, and by computer matching against commercial (NIST 2000 and ADAMS 2007) and homemade libraries of mass spectra built up from pure substances and MS literature data [12,13].

2.4. Determination of Antioxidant Activity

The free radical-scavenging activity of *D. reboudii* was determined spectrophotometrically by three different methods. The DPPH• assay was performed in accordance with a previously published protocol [14]. Briefly, 100 µL of essential oil dissolved in methanol was added to 1900 µL of a methanol DPPH solution. After vortexing, the solution was kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm, using a solution of DPPH in methanol as control. The scavenging activity on DPPH• was expressed as inhibition percentage using the equation reported in [14].

The ABTS•⁺ scavenging activity was tested according to Re et al. [15], and the results were compared with the absorbance values of the reference standards butylated hydroxytoluene (BHT) and α-tocopherol. Briefly, before use, the aqueous 7 mM ABTS•⁺ solution was incubated for 15 h in the dark at room temperature. Then, 100 µL of essential oil dissolved in methanol was added to 1900 µL of the ABTS•⁺ solution. After vortexing, the mixture was kept at room temperature for 10 min in the dark, after which the absorbance was measured at 734 nm, using an aqueous solution of ABTS•⁺ as control. The scavenging activity on ABTS•⁺ was expressed as inhibition percentage using the equation reported in [15].

The metal chelating activity by the ferrene-Fe²⁺ complexation assay was carried on following the method of Decker [16], using ethylenediaminetetraacetic acid (EDTA) as reference standard. Briefly, 40 µL of essential oil was added to 40 µL of a 0.2 mM FeCl₂ solution. The reaction was initiated after the addition of 80 µL of ferrene solution (0.5 mM). The mixture was vortexed and kept at room temperature for 10 min. The absorbance was recorded at 593 nm, and the inhibition percentage of ferrous ion chelating was calculated using the equation reported in [16].

Four different concentrations of *D. reboudii* essential oil were tested, namely 25, 50, 100 and 200 µg/mL.

2.5. Statistical Analysis

The antioxidant activities tests were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Student's *t*-test procedures were used for determination of significant differences between means, and *p*-values <0.05 were considered as statistically significant.

3. Results

3.1. Chemical Composition of the Essential Oil

The yield of the essential oil obtained from the aerial parts of *D. reboudii* was 0.9% (w/w). The chemical composition obtained by GC-MS is summarized in Table 1. Characteristic MS spectra of the identified compounds are reported in the Supplementary Materials.

Twenty-eight compounds were identified, representing 96.6% of the whole oil. A high content of oxygenated monoterpenes (82.1%) was observed, mainly because of the high percentage of (E)-anethol (59.4%) and estragol (21.2%). The second most representative chemical class of constituents was that of non-terpene derivatives (6.6%), mostly characterized by dodecanal (4.4%) and tetradecanal (0.7%), followed by monoterpene hydrocarbons (4.9%), largely represented by limonene (3.6%), myrcene (0.6%) and α-pinene (0.3%). Sesquiterpene hydrocarbons (1.7%) were another important class of volatiles in *D. reboudii* essential oil, represented mainly by β-caryophyllene (0.4%) and germacrene D (0.4%). Oxygenated sesquiterpenes were the less represented (1.3%).

Table 1. Chemical composition of the essential oil from the aerial parts of *Daucus reboudii*.

Compound	LRI *	Lit. RI	%
n-nonane	900	900	0.5
α -pinene	939	939	0.3
myrcene	991	991	0.6
p-cymene	1027	1027	0.3
limonene	1031	1031	3.6
γ -terpinene	1062	1062	0.1
n-undecane	1100	1099	0.2
estragole	1197	1195	21.2
N-decanal	1205	1204	0.2
trans-carveol	1219	1217	0.1
carvone	1244	1242	0.2
cis-chrysanthenyl acetate	1263	1262	1.0
(E)-anethole	1290	1289	59.4
carvacrol	1300	1298	0.2
undecanal	1305	1310	0.4
dodecanal	1409	1409	4.4
β -caryophyllene	1418	1418	0.4
α -humulene	1456	1456	0.3
(E)- β -farnesene	1460	1458	0.1
germacrene D	1480	1480	0.4
β -selinene	1485	1485	0.3
valencene	1493	1490	0.2
tridecanal	1509	1511	0.1
myristicin	1520	1520	0.8
caryophyllene oxide	1581	1581	0.4
tetradecanal	1612	1611	0.7
epi- α -bisabolol	1686	1685	0.1
hexadecanal	1824	1825	0.1
Grouped compounds [%]			
Monoterpene hydrocarbons			4.9
Oxygenated monoterpenes			82.1
Sesquiterpene hydrocarbons			1.7
Oxygenated sesquiterpenes			1.3
Non-terpene derivatives			6.6
Total identified			96.6

* LRI = Linear retention indices (HP-5 column).

In previous studies, the chemical composition of essential oils from different *Daucus* species has been largely investigated. These studies revealed the predominance of monoterpenes and/or sesquiterpenes, and sometimes phenylpropanoids, as major fractions. Indeed, the essential oils of *D. gingidium* ssp. *gingidium* and *D. carota* ssp. *carota* (from Italy, Lithuania and Poland) were dominated by the monoterpenes α -pinene and sabinene [5,17,18], whereas the main constituents of the oil of *D. carota* (from China) and *D. carota* var. *sativa* (from Egypt) were the sesquiterpenes carotol and daucol [19,20]. In the case of *D. sahariensis* and *D. carota* ssp. *hispanicus*, the major compound was the phenylpropanoid myristicin [21,22]. Moreover, in the oil of *D. carota* ssp. *maximus*, trans-methyl isoeugenol and β -bisabolene were identified as the main volatiles [8]. Nevertheless, in the aerial parts of *D. reboudii* growing in El Tarf, Algeria, Djarri et al. [7] found the monoterpenes α -pinene (39.7%) and sabinene (21.2%) as the main constituents. Our results showed that the chemical composition of the *D. reboudii* essential oil growing in Bejaïa, Algeria, was significantly different, mainly due to the presence of the oxygenated monoterpenes (E)-anethol and estragole. This result suggests that the *Daucus* species considered in this work could represent a new chemotype of *D. reboudii*, although further genetic and epigenetic investigations will be required.

3.2. Antioxidant Activity Evaluation

Table 2 shows the inhibition (%) of the free radical scavenging activities by DPPH[•], ABTS^{•+} and metal chelating assays. According to the results, in the DPPH[•] test the essential oil from *D. reboudii* showed an inhibition percentage comparable to those of the reference compounds BHT and α -tocopherol, and at the doses of 50 and 100 μ g/mL it was significantly ($p < 0.05$) higher than that of BHT. On the other hand, in the ABTS^{•+} assay, the essential oil demonstrated mild antioxidant activity compared to the same reference compounds, while in the metal chelating assay, the essential oil showed a very small inhibition percentage compared to reference EDTA at all the concentrations tested.

Table 2. Antioxidant activity of *Daucus reboudii* Coss. essential oil. Values are expressed as means \pm SD of three parallel measurements.

	% Inhibition			
	25 μ g/mL	50 μ g/mL	100 μ g/mL	200 μ g/mL
DPPH[•]				
<i>D. reboudii</i>	38.59 \pm 1.22	66.12 \pm 2.34 *	77.31 \pm 0.28 *	78.45 \pm 0.21
BHT	40.43 \pm 0.05	53.18 \pm 0.51	73.91 \pm 0.11	98.44 \pm 0.06
α -tocopherol	91.16 \pm 0.17	92.03 \pm 0.55	93.77 \pm 0.07	97.25 \pm 0.06
ABTS^{•+}				
<i>D. reboudii</i>	25.01 \pm 1.77	63.11 \pm 1.39	79.27 \pm 1.05	82.32 \pm 0.38
BHT	89.56 \pm 0.39	90.12 \pm 0.5	95.20 \pm 0.80	99.24 \pm 0.60
α -tocopherol	99.62 \pm 0.10	99.89 \pm 0.09	99.97 \pm 0.15	99.99 \pm 0.10
Metal chelating				
<i>D. reboudii</i>	3.91 \pm 1.14	15.66 \pm 0.02	33.61 \pm 0.30	38.77 \pm 1.31
EDTA	58.08 \pm 0.60	62.42 \pm 0.22	90.34 \pm 0.55	96.01 \pm 0.24

* $p < 0.05$ compared to BHT.

Although a correlation analysis was not performed, considering literature data we could propose that the activity of *D. reboudii* essential oil in the ABTS^{•+} assay could be related mainly to (E)-anethol, carvacrol and γ -terpinene. In fact, these compounds have been already reported to exert significant antioxidant activity in the ABTS^{•+} assay [23]. On the other hand, considering that both (E)-anethol and estragole, the main volatile constituents identified in *D. reboudii* essential oil, have been reported as scarcely active in the DPPH[•] assay [24], we can exclude their significant contribution to the activity observed in our study, which could be better associated to a synergism between the main compounds and the other phytoconstituents [24]. Nevertheless, further experimental studies on *D. reboudii* essential oil are needed.

4. Conclusions

This study reports the chemical composition and the antioxidant activity of the essential oil from the aerial parts of *D. reboudii* from Algeria. The results show significant differences between the chemical composition of the essential oil from *D. reboudii* compared to those from other *Daucus* species. The compounds that mostly characterized *D. reboudii* were the oxygenated monoterpenes (E)-anethol and estragole. Our study highlighted a possible new chemotype [(E)-anethol chemotype] in *D. reboudii* species, confirming the chemical polymorphism occurring either in the species or in the *Daucus* genus. The antioxidant capacity of the oil was evaluated using three complementary assays, namely metal chelating, ABTS^{•+} and DPPH[•] assays. The highest antioxidant activity was observed with DPPH[•] method, where *D. reboudii* showed a significantly higher activity compared to BHT at 50 and 100 μ g/mL. This result suggests that the essential oil from *D. reboudii* could have a potential use in the food industry as food preservative. Nevertheless, further studies are needed to explore other bioactivities of *D. reboudii* essential oil and to elucidate also the composition of non-volatile compounds.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2076-3417/11/4/1843/s1>.

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