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Essential oil and aroma composition of leaves, stalks and roots of celery *(Apium graveolens var. dulce)* from Tunisia

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Volatiles of leaves, stalks and roots of Tunisian celery (*Apium graveolens* var. *dulce*) were extracted either by hydrodistillation or by maceration in organic solvents. After concentration, essential oils and aroma extracts were studied by gas chromatography (GC) and GC–mass spectrometry (GC–MS). A high proportion of essential oils and aroma extracts consisted of phthalides with 74.6–76.6% in leaves, 56.8–74.1% in stalks and 57.7–79.7% in roots. The studied organs were particularly characterized by appreciable levels of terpene hydrocarbons (17.0–31.5%). Also, results showed that stalks were particularly rich in phenols with 8.7% of total volatiles. The main volatile constituents identified in the different extracts were (*Z*)-3-butylidenephthalide (27.8–38.4% in leaves, 30.5–38.9% in stalks, 30.5–52.0% in roots), 3-butyl-4,5-dihydrophthalide (34.2–41.0% in leaves, 24.1–27.8% in stalks, 12.3–13.2% in roots) and α-thujene (7.9–9.9% in leaves, 7.5–14.0% in stalks, 7.0–12.4% in roots). The three studied organs of *A. graveolens* (leaves, stalks and roots) could be considered good sources of phthalides known for their anti-inflammatory, anti-tumor and insecticidal properties. Nevertheless, the efficiency in the extraction of these bioactive compounds depends on both the organ and the method used.

Keywords: *Apium graveolens var. dulce*; Apiaceae; celery; essential oil; aroma compounds; phthalides

Introduction

Celery (*Apium graveolens* L.) is a member of the family Apiaceae (synonymous with Umbellifereae). The species is divided into two varieties *A. graveolens* var. *dulce* and *A. graveolens* var. *rapaceum*. The former is commonly known as 'celery' or 'stem celery', and is grown for its stems and leaves, which have culinary uses, particularly in salads and soups (1). The latter variety, known as 'celeriac', forms a large root tuber, resembling a bulb, which is cooked and eaten. The plants that form the basis of the current research are of the variety *dulce*.

Since antiquity, celery has been used as a popular aromatic herb and spice, and at various times both the whole plant and the seeds have been consumed as a medicine (2). In this way, the seeds have been used for medicinal purposes as a diuretic for bladder and kidney complaints, and an adjuvant in arthritic and rheumatic conditions (3). Celery seed tea is said to promote rest and sleep (4). Also it has been reported, in a clinical study, that celery stems possess anti-inflammatory properties, which may form a basis for the reputation of the plant as a medicinal treatment for rheumatic disease (5).

The volatiles of celery have been studied by several workers (1, 6–13). In all, about 165 components have been characterized, and notable features are the reported presence of several mono- and sesquiterpenes with limonene the major component, a number of alcohols and some carbonyl compounds including the phtalides. Berlingozzi and Mazzo (14) identified alkyl and alkylidenephthalides in celery volatiles and discussed the correlation between chemical structures and sensory properties. Indeed, four alkylidenephthalides, that is 3-(2-methylpropylidene) phthalide, 3-(3-methylbutylidene) phthalide, 3-(2-methyl-propylidine)-3a, 4-dihydrophthalide and 3-(3-methylbutylidene)-3a,4-dihydrophthalide, were detected in celery stem volatiles by Gold and Wilson (15), and found to possess a significant celery-like aroma. In those early works, however, the phthalides could not be successfully separated by gas chromatography (GC) due to their instabilities in GC columns (1). Using high-performance liquid chromatography (HPLC), Uhlig (16) separated 3-*n*-butylphthalide, sedanenolide (3-*n*-butyl-4,5-dihydrophthalide) and sedanolide (3-*n*-butyl-3a,4,5,6-tetrahydrophthalide) from eight cultivars of celery and three cultivars of celeriac. They concluded, through sensory evaluation, that sedanolide was the main contributor to the characteristic odor of celery. Studying aroma volatiles of a local variety of celery from Lybia, MacLeod et al. (8) unusually identified the predominance of three

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compounds that make up together *c*. 70% of total volatiles: apiole (23%), 3-butylphthalide (22%) and sedanolide (24%). In another study, MacLeod and Ames (9) reported that limonene and sedanenolide were the major components of celery through detailed analyses of the volatile components of celery using GC, GC–mass spectrometry (GC–MC) and GC–olfactometry (GC–O). Various physiological activities of the butylphthalides such as anti-inflammatory, anti-carcinogenic and insecticidal effects have recently been attracting interest (17, 18). Also, mosquitocidal, nematicidal and antifungal activities of sedanolide were reported by Momin and Nair (19).

In addition to the phthalides, Wilson (20–22) identified several mono- and sesquiterpene hydrocarbons, alcohols and carbonyl compounds in celery essential oil.

Despite these various studies, investigations on aroma composition of fresh celery from Tunisia have not yet been done. In addition, to the best of our knowledge, there is no data reporting aroma composition of the essential oil obtained from fresh celery roots. Also, according to bibliographic data, studies carried out on celery volatiles extracted from fresh material either by hydrodistillation or by maceration in organic solvents were not reported.

In the present work, aroma compounds of fresh Tunisian celery leaves, stems and roots extracted either by organic solvents or by hydrodistillation were investigated for the first time individually with GC and GC–MS.

Materials and methods

Chemicals

Solvents used in the experiments (diethyl ether, pentane) were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulfate (Na_2SO_4), 6-methyl-5-hepten-2-one used as internal standard and homologous series of C_8–C_{22} *n*-alkanes used for identification (by calculation of their retention indexes) were obtained from Sigma-Aldrich (Stein-Heim, Germany). Pure standards of *trans*-2-hexenal, *cis*-3-hexenol, α-pinene, β-pinene, myrcene, α-terpinene, limonene, (*E*)-β-ocimene, *trans*-linalool oxide, borneol, nerol, *cis*-carveol, geraniol, thymol, carvacrol, myrtenyl acetate, α-terpinyl acetate, neryl acetate, β-caryophyllene, α-humulene, germacrene-D, β-selinene, nonadecane, methyl eugenol, eicosane and phenol were purchased from Sigma-Aldrich (Stein-Heim, Germany) whereas, tricyclene, α-thujene, sabinene, 1,8-cineole, γ-terpinene, terpinolene, linalool, camphor, myrtenol, allo-aromadendrene and α-terpineol were obtained from Fluka (Ridel-de Haën, Switzeland).

Plant material

Cultivated celery (*Apium graveolens*) plants were collected from the region of Soliman in the Cap Bon (North East) of Tunisia in February 2008. The latitude, longitude and altitude of the region are respectively 36° 41' 47N, 10° 29' 30E and 26 m asl. The monthly temperature average was 17.8°C and the precipitation average was 458 mm/year.

The roots, leaves and stalks were harvested manually and were directly subjected to extraction procedures.

Determination of dry matter weight

After sampling, 15 g of every sample (leaves, stalks and roots) were weighed in order to determine their fresh matter weight and were dried at 100°C until constant weight was reached in order to evaluate their moisture contents and their dry matter weight.

Essential oils extraction

Essential oils were extracted by hydrodistillation over 180 minutes using 20 g of fresh sample (leaves or stalks or roots) in 0.5 L of distilled water. This time was fixed after a kinetic survey during 30, 60, 90, 120, 150, 180, 210 and 240 minutes. The distillate was extracted with diethyl ether and dried over anhydrous sodium sulfate. All experiments were conducted in triplicates and results were expressed on the basis of dry matter weight (DMW).

Extraction of aroma compounds by organic solvents

Five grams of each sample cut into small slices were extracted with 50 mL of the mixture diethyl ether/pentane (v/v) as a modified method inspired from the method described by Tonder et al. (23). The extraction time is 90 minutes under magnetic steering. This time was fixed after a kinetic survey during 30, 60, 90, 120 and 150 minutes.

Concentration of organic extracts

Organic extracts obtained by the two methods described above were concentrated under reflux in a piriform ball equipped with Vigreux column at 37°C after addition of the internal standard (6-methyl-5-hepten-2-one), which is used for quantification of total volatiles.

Gas chromatography (GC–FID)

Essential oils and aroma fractions were analyzed by gas chromatography (GC) using a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A HP-Innowax capillary column (polyethylene glycol:

30 m × 0.25 mm i.d × 0.25 mm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used; the flow of the carrier gas (N2, U) was 1.6 mL/minute and the split ratio 60:1. Analyses were performed using the following temperature program: oven isotherm at 35°C for 10 minutes, from 35° to 205°C at the rate of 3°C/minute and isotherm at 205°C for 10 minutes. Injector and detector temperatures were held, respectively, at 250° and 300°C.

Gas chromatography–mass spectrometry (GC–MS)

The GC–MS analyses were performed on a gas chromatograph HP 6890 interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, California, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm i.d × 0.25 mm film thickness) was used. The column temperature was programmed to rise from 40° to 280°C at a rate of 5°C/minute. The carrier gas was helium with a flow rate of 1.2 mL/minute. Scan time and mass range were 1 second and 50–550 m/z, respectively. The injected volume was 1 µL and the total run time was approximately 63 minutes.

Identification of aroma compounds

Identification of aroma compounds was based on the calculation of their retention indexes (RI) relative to (C_8–C_{22}) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching the mass spectral fragmentation patterns of different compounds with corresponding data (Wiley 275.L library) and other published mass spectra (24), as well as by comparison of their retention indexes with data from the Mass Spectral Library 'Terpenoids and Related Constituents of Essential Oils' (Dr Detley Hochmuth, Scientific Consulting, Hamburg, Germany) using the mass finder 3 software (www.massfinder.com). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak areas.

Statistical analysis

All data were reported as means ± standard deviation of three samples. Statistical analysis was performed with STATISTICA (Statsoft, 1998). Differences were tested for significance by using the ANOVA procedure, using a significance level of $p \leq 0.05$.

Results and discussion

The quantification of the total aroma fraction (as expressed by µg of total aroma per gram of dry matter weight), obtained from the three organs of celery either by hydrodistillation or by solvent extraction, showed that leaves are characterized by the best yield of total volatiles (Figure 1). Nevertheless, results obtained showed that solvent extraction is more effective in terms of total flavor extraction than hydrodistillation. In fact, in leaves, total aroma represent 0.6% (w/w) as obtained by hydrodistillation against 0.9% (w/w) as obtained by solvent extraction, 0.2% (w/w) against 0.7% (w/w) in stems and 0.1% (w/w) against 0.5% (w/w) in roots.

The analysis of essential oils obtained from celery organs by hydrodistillation showed a total of 14, 20 and 24 compounds established respectively in leaves, stalks and roots (Figure 2). These compounds account for about 99.29%, 99.24% and 99.17% of the total oils of *A. graveolens*, respectively. The components identified in the essential oils are listed in Table 1 in order of their experimental retention indices on the HP-5 column. All data were reported as means ± standard deviation of three repetitions. Results showed that phthalides represent the major class of the different essential oils. These compounds represent 76.6%, 56.8% and 57.7% of the total aroma of celery leaves, stalks and roots, respectively. The main phthalide identified in the three essential oils is (*Z*)-3-butylidenephtalide representing 38.4%, 30.5% and 30.5% of leaves, stalks and roots total aroma, respectively. 3-Butyl-4,5-dihydrophthalide (sedanenolide) is the second major phthalide of leaves and stalks with 34.2% and 24.1% of total aroma, respectively, whereas 3-*n*-butylphthalide is the second major phthalide of roots essential oil with a percentage of 14.8% of total aroma. This latter compound is also present in the two other organs with appreciable rates reaching 3.9% in leaves and 2.0% in stalks. On the other hand, the studied organs were also characterized by important levels of terpene hydrocar-

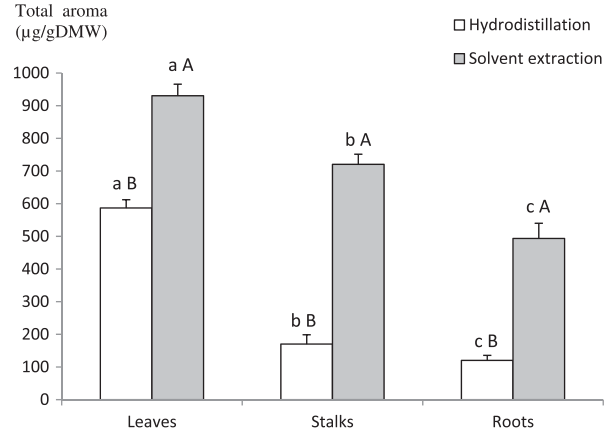

Figure 1. Variation of celery (*Apium graveolens*) total volatiles with organ and method of extraction. Variation between organs was significantly different at $p<0.05$ according to Duncan's test (small letters); Variation between extraction methods was significantly different at $p<0.05$ according to Duncan's test (capital letters).

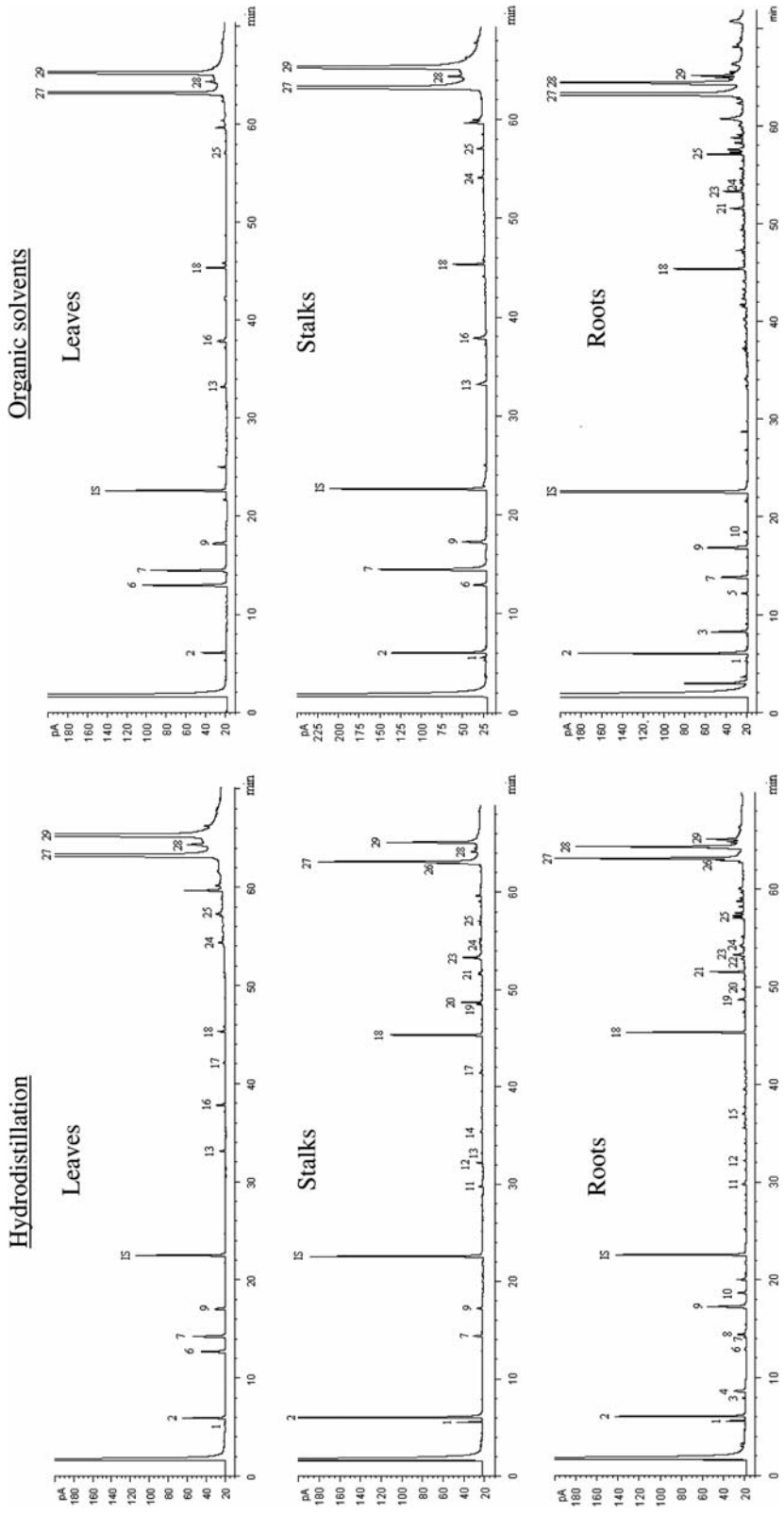

Figure 2. Gas chromatography–flame ionization detector (GC–FID) chromatograms of essential oil (hydrodistillation) and aroma (solvent extraction) from celery organs. **1**: α-pinene; **2**: α-thujene; **3**: β-pinene; **4**: sabinene; **5**: myrcene; **6**: α-terpinene; **7**: limonene; **8**: 1,8-cineole; **9**: γ-terpinene; **10**: terpinolene; **11**: camphor; **12**: linalool; **13**: β-caryophyllene; **14**: α-terpinyl acetate; **15**: myrtenyl acetate; **16**: borneol; **17**: geraniol; **18**: β-selinene; **19**: nonadecane; **20**: methyl eugenol; **21**: eicosane; **22**: spathulenol; **23**: eugenol; **24**: thymol; **25**: apiole; **26**: phenol; **27**: (*Z*)-3-*n*-butylidenephthalide; **28**: 3-*n*-butylphthalide; **29**: 3-butyl-4,5-dihydrophthalide; IS, internal standard. Compounds are eluted using a HP-Innowax column.

Table 1. Composition of the essential oils (w%) extracted from *Apium graveolens* organs by hydrodistillation.

Compound	RI[a]	RI[b]	RI[1]	RI[2]	Leaves	Stalks	Roots	Identification
α-Thujene	928	1035	929 (37)	1035 (38)	9.9[c]±0.7	14.0[a]±0.2	12.4[b]±0.2	MS, RI, CoI
α-Pinene	939	1032	938 (39)	1032 (39)	tr	0.9[a]±0.0	0.5[b]±0.0	MS, RI, CoI
Sabinene	975	1132	973 (39)	1132 (39)	–	–	1.0[a]±0.0	MS, RI, CoI
β-Pinene	980	1118	980 (39)	1118 (39)	–	–	0.3[a]±0.0	MS, RI, CoI
α-Terpinene	1018	1188	1017 (40)	1188 (38)	0.7[a]±0.0	–	0.1[b]±0.0	MS, RI, CoI
Limonene	1030	1203	1030 (39)	1203 (39)	3.6[a]±0.0	1.7[b]±0.0	0.9[c]±0.0	MS, RI, CoI
1,8-Cineole	1033	1213	1034 (39)	1213 (39)	–	–	2.2[a]±0.2	MS, RI, CoI
Phenol	1053	1921	1052 (45)	1920 (46)	–	4.9[a]±0.0	1.7[b]±0.0	MS, RI
γ-Terpinene	1062	1255	1059 (40)	1255 (38)	2.4[b]±0.0	1.6[c]±0.0	5.2[a]±0.1	MS, RI, CoI
Terpinolene	1092	1290	1092 (43)	1290 (38)	–	–	0.5[a]±0.0	MS, RI, CoI
Linalool	1098	1553	1098 (39)	1553 (39)	–	0.8[a]±0.0	0.1[b]±0.0	MS, RI, CoI
Camphor	1143	1531	1145 (39)	1532 (39)	–	0.6[a]±0.0	0.3[b]±0.0	MS, RI, CoI
Borneol	1165	1719	1167 (39)	1719 (39)	2.1[a]±0.0	–	–	MS, RI, CoI
Geraniol	1233	1857	1235 (39)	1857 (39)	0.3[b]±0.0	0.6[a]±0.0	–	MS, RI, CoI
Thymol	1295	2197	1293 (39)	2198 (39)	0.7[a]±0.0	0.7[a]±0.1	0.3[b]±0.1	MS, RI, CoI
Myrtenyl acetate	1321	1707	1323 (40)	1698 (39)	–	–	0.3[a]±0.1	MS, RI, CoI
α-Terpinyl acetate	1336	1389	1333 (39)	1352 (41)	–	0.1[a]±0.0	–	MS, RI, CoI
Eugenol	1356	2186	1353 (39)	2186 (39)	–	3.0[a]±1.1	1.1[a]±0.1	MS, RI, CoI
Methyl eugenol	1404	2000	1404 (42)	2005 (42)	–	3.3[a]±0.5	1.7[b]±0.5	MS, RI, CoI
β-Caryophyllene	1419	1612	1415 (39)	1612 (39)	1.4[a]±0.0	0.4[b]±0.1	–	MS, RI, CoI
β-Selinene	1481	1742	1475 (39)	1715 (39)	0.4[c]±0.0	7.5[b]±1.2	8.9[a]±0.8	MS, RI, CoI
Spathulenol	1576	2144	1578 (39)	2150 (39)	–	–	tr	MS, RI
3-*n*-Butylphthalide	1649	2520	1646 (42)		3.9[b]±0.2	2.0[c]±0.1	14.8[a]±1.2	MS, RI
(*Z*)-3-Butylidenephthalide	1665	2445	1667 (42)	2450 (47)	38.4[a]±2.3	30.5[b]±1.6	30.5[b]±1.8	MS, RI
Apiole	1670	–	1671 (48)		0.8[b]±0.0	0.7[c]±0.0	1.7[a]±0.1	MS, RI
3-Butyl-4,5-dihydrophthalide	1704	2562	1702 (9)		34.2[a]±6.9	24.1[b]±2.5	12.3[c]±0.5	MS
Nonadecane	1900	1900	1900 (49)	1900 (49)	–	0.3[a]±0.1	0.1[b]±0.0	MS, RI, CoI
Eicosane	2000	2000	2000 (49)	2000 (49)	–	0.5[b]±0.0	1.3[a]±0.2	MS, RI, CoI
Total identified					99.2	99.2	99.1	
Chemical classes								
Hydrocarbons					18.6[c]±1.0	27.3[b]±1.4	31.5[a]±1.5	
Alcohols					2.4[a]±0.5	1.5[b]±0.3	0.2[c]±0.0	
Aldehydes					–	0.1[b]±0.0	0.3[a]±0.0	
Phenols					0.7[c]±0.0	8.7[a]±0.2	3.2[b]±0.1	
Phthalides					76.6[a]±2.6	56.8[b]±2.0	57.7[b]±1.9	
Others					0.8[a]±0.2	0.7[b]±0.3	6.0[c]±0.9	

Notes: Components are listed in order of elution in apolar column (HP-5); RI[a], RI[b], retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax); RI[1], RI[2], retention indices according to bibliographic data on an apolar column (1) and polar column (2); MS, mass spectrometry; CoI, co-injection; tr, trace; volatile compound proportions were calculated from the chromatograms obtained on the HP-Innowax colun. values followed by the same small letter did not share significant differences at $p<0.05$ (Duncan test).

bons with 18.6%, 27.3% and 31.5% of total aroma of leaves, stalks and roots, respectively. This class of compounds is mainly represented by α-thujene with a percentage of 9.9% in leaves, 14.0% in stalks and 12.4% in roots. γ-Terpinene is another hydrocarbon compound present in the three organs with appreciable amounts reaching 2.4% in leaves, 1.6% in stalks and 5.2% in roots. Our results also showed that stalks and roots were particularly rich in β-selinene with 7.5% and 8.9% of total aroma, respectively. The essential oils obtained from celery stalks and roots were also characterized by high amounts of phenols representing together 8.7% and 3.2% of total volatiles, respectively. This class of compounds is constituted mainly of phenol (4.9% in stalks and 1.7% in roots) and eugenol (3.0% in stalks and 1.1% in roots), whereas in leaves it is only represented by thymol (0.7%). As for alcohols, they were mainly detected in leaves (2.4%) and stalks (1.5%) with borneol as major representative in leaves reaching 2.1% of total volatiles whereas linalool and geraniol were the main alcohols of stalks with 0.8% and 0.6% of total aroma, respectively. Finally, we noticed the identification in our samples of apiole, which is a characteristic compound of the Apiaceae family in a few amounts (0.8% in leaves, 0.7% in stalks and 1.7% in roots).

The analysis of volatiles obtained from celery organs by organic solvent extraction according to the method inspired from the work of Tonder et al. (23) revealed the presence of eleven, thirteen and fifteen identified compounds in leaves, stalks and roots, respectively (Figure 2). These compounds account for

about 98.9%, 99.2% and 98.6% of the total flavors, respectively (Table 2). Our results showed that phthalides represent the major class of celery extracts (74.6% for leaves, 74.1% for stalks, 79.7% for roots). The main phthalides identified were (*Z*)-3-butylidenephtalide (27.80% in leaves, 38.9% in stalks, 52.0% in roots), 3-butyl-4,5-dihydrophthalide (41.0% in leaves, 27.8% in stalks, 13.2% in roots) and 3-*n*-butylphtalide (5.8% in leaves, 7.4% in stalks, 14,4% in roots). On the other hand, results showed that the studied organs were also characterized by high proportions of terpene hydrocarbons, which account for 23.1%, 22.9% and 17.07 of total aroma respectively for leaves, stalks and roots. The main monoterpene hydrocarbons identified in the three organs were α-thujene (7.9% in leaves, 7.5% in stalks and 7.0% in roots) and limonene (6.5% in leaves, 7.9% in stalks, 1.7% in roots). As for terpene alcohols, they were only represented by borneol, which is detected only in leaves and stalks with appreciable amounts accounting, respectively, for 1.0% and 1.4% of total volatiles. Like for extractions obtained by hydrodistillation, the extracts obtained by organic solvents contained small amounts of apiole (0.4% in leaves, 0.9% in stalks and 1.3% in roots).

Based on the results reported above, it seems that phthalides (Lactones) are the highest contributors to the total volatiles whatever the organ and the method of extraction used are. These results agree with those of the most studies reported on the aroma of celery whatever the method of extraction (11, 25, 19). Nevertheless, our results showed that the percentage of phthalides depends on the organ studied; in fact, it seems that roots are the richest in these components. To the best of our knowledge, aroma composition of celery roots was carried out for the first time. On the other hand, it has been reported that phthalides are responsible for the typical flavor and aroma of celery (1,16) and that these compounds are the most significant bioactive compounds exhibiting many health benefits like protection against cancer, high blood pressure and hypercholesterolemia (12, 19). Other Apiaceae species were also reported to have high levels of phthalides in their essential oils such as the roots of a German specimen of *Meum athamanticum* (26).

3-*n*-Butylphthalide, (*Z*)-3-butylidene-phthalide and 3-butyl-4,5-dihydrophthalide (sedanenolide) are the only three phthalides identified in this work. These phthalides have been also identified by MacLeod and

Table 2. Composition of the volatiles (w%) extracted from *Apium graveolens* organs by organic solvents.

Compound	RI[a]	RI[b]	RI[1]	RI[2]	Leaves	Stalks	Roots	Identification
α-Thujene	928	1035	929 (37)	1035 (38)	7.9[a]±0.5	7.5[a]±0.4	7.0[b]±0.5	MS, RI, CoI
α-Pinene	939	1032	938 (39)	1032 (39)	–	1.0[a]±0.4	0.5[b]±0.1	MS, RI, CoI
β-Pinene	980	1118	980 (39)	1118 (39)	–	–	1.2[a]±0.0	
Myrcene	991	1174	993 (39)	1174 (39)	–	0.6[b]±0.0	0.9[a]±0.0	MS, RI, CoI
α-Terpinene	1018	1188	1017 (40)	1188 (38)	5.3[a]±0.6	0.5[b]±0.0	–	MS, RI, CoI
Limonene	1030	1203	1030 (39)	1203 (39)	6.5[b]±0.6	7.9[a]±1.0	1.7[b]±0.1	MS, RI, CoI
Terpinolene	1092	1290	1092 (43)	1290 (38)	–	–	0.5[a]±0.2	MS, RI, CoI
γ- Terpinene	1062	1255	1059 (40)	1255 (41)	1.1[b]±0.1	2.5[a]±0.5	2.2[a]±0.3	MS, RI, CoI
Borneol	1165	1719	1167 (39)	1719 (39)	1.0[b]±0.3	1.4[a]±0.2	–	MS, RI, CoI
Thymol	1295	2197	1293 (39)	2198 (39)	–	–	0.2[a]±0.0	MS, RI, CoI
Eugenol	1356	2186	1353 (39)	2186 (39)	–	–	0.7[a]±0.1	MS, RI, CoI
β-Caryophyllene	1419	1612	1415 (39)	1612 (39)	0.6[b]±0.0	1.1[a]±0.0	–	MS, RI, CoI
β-Selinene	1481	1742	1475 (39)	1715 (39)	1.5[b]±0.1	1.7[b]±0.0	2.3[a]±0.12	MS, RI, CoI
3-*n*-Butylphthalide	1649	2520	1646 (42)		5.8[c]±0.3	7.4[b]±0.6	14.4[a]±0.9	MS, RI
(*Z*)-3-Butylidenephthalide	1665	2445	1667 (42)	2450 (47)	27.8[c]±0.7	38.9[b]±1.6	52.0[a]±1.1	MS, RI
Apiole	1670	–	1671 (48)		0.4[c]±0.1	0.9[b]±0.0	1.3[a]±0.2	MS, RI
3-Butyl-4,5-dihydrophthalide	1745	2562	1702 (9)		41.0[a]±2.8	27.8[b]±1.3	13.2[c]±0.9	MS
Eicosane	2000	2000	2000 (49)	2000 (49)	–	–	0.5[a]±0.0	MS, RI, CoI
Total identified					98.9	99.2	98.6	
Chemical classes								
Hydrocarbons					23.1[a]±1.2	22.9[ab]±1.0	17.0[b]±0.9	
Alcohols					1.0[b]±0.4	1.4[a]±0.2	–	
Phenols					–	–	0.9[a]±0.1	
Phthalides					74.6[b]±2.0	74.1[ab]±2.2	79.7[a]±2.4	
Others					0.4[c]±0.0	0.9[b]±0.0	1.3[a]±0.1	
Total identified					99.2	99.4	99.1	

Notes: Components are listed in order of elution in apolar column (HP-5); RI[a], RI[b], retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax); RI[1], RI[2], retention indices according to bibliographic data on an apolar column (1) and polar column (2); MS, mass spectrometry; CoI, co-injection; RI, retention indice; volatile compound proportions were calculated from the chromatograms obtained on the HP-Innowax column. Values followed by the same small letter did not share significant differences at $p<0.05$ (Duncan test).

Ames (9) in the aerial parts of celery in percentages similar to our results whereas MacLeod et al. (8) identified only 3-*n*-butylphthalide together with sedanolide and sedanenolide. Unfortunately, in the present work, sedanolide has not been detected.

In addition to phthalides, celery aroma is also characterized by its appreciable content of terpenoid compounds, which is dependent on the organ studied and of the extraction method used. Comparing our results with bibliographic data, it seems that most of the terpenoids identified in this work were also reported in other studies carried out on celery essential oil. However, according to our results, limonene represented only 1.7–7.9% of the total volatiles, whereas MacLeod and Ames (9) reported that this compound constitute 35.5% of the celery volatiles. These results are in agreement with those of MacLeod et al. (8), who reported that this compound is present at a percentage of 2%. Another distinguishing feature is the absence of *p*-menthatriene in our samples, whereas MacLeod et al. (8) and MacLeod and Ames (9) have identified this compound in celery volatiles. Also, the presence of β-selinene in our samples is in agreement with previous works, which consider that this compound is estimated to contribute most to celery quality together with phtahlides (27).

Finally, the identification of apiole in our samples is in agreement with the work carried out by MacLeod et al. (8), who reported that this component is present at relatively high percentage reaching 23% of total volatiles. This compound is to some extent characteristic of the Apiaceae and has been identified previously in parsley (28–30).

As reported above, data on aroma fraction extracted from celery leaves, stalks and roots either by hydrodistillation or by organic solvents according to a modified method inspired from the work of Tonder et al. (23) are missing. In fact, in previous studies, authors reported other techniques of aroma extraction from celery materials. In this way, MacLeod et al. (8) proceeded to the extraction of aroma fraction from celery stems and leaves by steam distillation followed by an extraction with CH_2Cl_2. In another work, MacLeod and Ames (9) reported the extraction of volatile components from celery stems with a modified Likens and Nickerson apparatus using triply distilled 2-methylbutane. In more recent studies, other techniques were reported such as supercritical carbon dioxide extraction from roots, leaves and seeds (11). Studying the aroma quality of leaves and stalks in raw and boiled celery, Kurobayashi et al. (1) proceeded to the extraction of volatiles by distillation of the oil extracted from celery materials using a solvent assisted flavor evaporation (SAFE) apparatus at a temperature of 40°C and a pressure of 3×10^{-3} Pa using a diffusion pump.

Comparing our results with those obtained from other Apiaceae species, it seems that contents of aroma components in celery leaves were quite similar to those reported by Macleod et al. (29) in parsley leaves (0.7% w/w). On the other hand, total aroma contents of celery leaves are higher than those reported by Msaada et al. (31) in coriander leaves, stems and roots. In fact, according to these authors, the total volatiles represented 0.07% (w/w) in upper leaves, 0.007% (w/w) in basal leaves, 0.04% (w/w) in stems and 0.01% (w/w) in roots.

Finally, 3-*n*-butylphthalide and (*Z*)-3-butylidenephthalide have been identified in other Apiaceae species, such as the roots of *Angelica sinensis* (32) and *Levisticum officinalis* (33); these two phthalides have been also identified in the essential oil of *Cnidium officinale* Makino and where (*Z*)-3-butylidenephthalide represents the major component with a percentage of 43.3% followed by 3-*n*-butylphthalide with 21.1% of total volatiles (34). 3-Butylidenephthalide has been also extracted in high rates (over 30%) from Chinese *Angelica sinensis* (35). This bioactive compound has been reported by Tsai et al. (36) to have significant therapeutic anti-tumor efficacy on malignant brain tumors, which involved the induction of cell cycle rest and apoptosis.

Conclusion

This study concludes that the three studied organs of *A. graveolens* (leaves, stalks and roots) could be considered good sources of phthalides known for their anti-inflammatory, anti-tumor and insecticidal properties. Nevertheless, the efficiency in the extraction of these bioactive compounds depends on both the organ and the method used. In this perspective, it appears that roots are the richest organs and that extraction with organic solvents is the most efficient method for the extraction of phthalides.

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