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ARTICLE

Profiles of the Headspace Volatile Organic and Essential Oil Compounds from the Tunisian *Cardaria draba* (L.) Desv. and Its Leaf and Stem Epidermal Micromorphology

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ABSTRACT

In this work, we investigated aroma volatiles emanated by dry roots, stems, leaves, flowers, and fruits of *Cardaria draba* (L.) Desv. growing wild in Tunisia and its aerial part essential oils (EOs) composition. A total of 37 volatile organic compounds (96.7%–98.9%) were identified; 4 esters, 4 alcohols, 7 hydrocarbons, 12 aldehydes, 5 ketones, 1 lactone, 1 organosulfur compound, 2 organonitrogen compounds, and 1 acid. The hydrocarbons form the main group, representing 49.5%–84.6% of the total detected volatiles. The main constituent was 2,2,4,6,6-pentamethylheptane (44.5%–76.2%) reaching the highest relative percentages. Forty-two compounds were determined in the two fractions of EOs, representing 98.8% and 97.2% of the total oil composition, respectively. The principal components were hexadecanoic acid (34.6%), 6-methyl-5-hepten-2-one (18.3%), decanal (15.0%), 6,10,14-trimethyl-2-pentadecanone (13.2%), and *n*-pentacosane (13%). Micromorphological details of the leaf and stem epidermis using light microscopy revealed polygonal cells with sinuous walls in the adaxial and abaxial leaf surfaces and nearly rectangular and long ones with linear and thick walls for the stem epidermis. The stomata complexes were anisocytic in the leaf epidermis and mainly anisocytic and rarely paracytic in the stem epidermis. Non-glandular trichomes were unbranched and long with an acute apex or short with a convex apex. The glandular ones were identified for the first time in this species. They were short-stalked with a large secretory head. The highest stomatal index (17.02%) was recorded in the abaxial leaf surface. The identification of headspace volatiles and essential oil compounds can be used to characterize this species, and the various epidermis micromorphological features are very useful for biosystematics taxonomic studies within *Brassicaceae*.

KEYWORDS

Cardaria draba; aroma profile; essential oils; epidermis; trichomes

1 Introduction

Plants can produce and release natural fragrant aroma molecules widely used in food, pharmaceutical, agricultural, chemical, and cosmetic perfumery industries [1]. Currently, numerous wild species belonging to various families are used due to their richness in natural volatile compounds. Among them, the *Brassicaceae* Burnett is one of the prominent plant families of vegetal crops and medicinal plants having economic, agricultural, and scientific importance [2]. It comprises about 321 genera and more than 3660 species [3]. *Cardaria* Desv. is the largest genus including at least 250 species spread over the Americas, Africa, Asia, Europe, and Australia [2]. *Cardaria draba* (L.) Desv. (common name White top) [4,5] is indigenous to Eurasia and the Mediterranean area [6]. It is an herbaceous and spontaneous plant growing along roadsides and in sub-irrigated pastures [7]. Stems and leaves are covered by soft white trichomes [8]. Numerous fragrant white flowers are disposed of in a corymb inflorescence. Long fruiting pedicels carry indehiscent heart-shaped silicles [4].

White top is considered as an edible species of high nutritional value [9]. The plant decoction exhibits diuretic properties and previously, the fruits were employed as a condiment [10]. Young leaves have also forage value, given their higher content of protein [11]. Moreover, it is an allelopathic plant and is considered as a hyperaccumulator of heavy metals with a great phytoremediation potential [12–14]. Furthermore, *C. draba* is a high-value wild medicinal plant widely used by drug manufacturing given the richness of its extracts in alkaloid, phenol, flavonoid, terpenoid, and glucosinolate compounds with strong antioxidant, antibacterial, antimicrobial, antifungal, analgesic and antidiabetic activities [15–21].

Despite the numerous health benefits of essential oils (EOs) and volatile organic compounds (VOCs) in aroma therapy, medicinal uses, and food preservation due to their great biological potential, there is a lack of information concerning the qualitative and quantitative compositions of *C. draba* volatiles. Previous studies have been limited to *C. draba* particularly growing wild in Croatia, Iran, Turkey, and Algeria. The chemical composition of the volatiles was characterized by the predominance of 3-butenyl isothiocyanate, 4-methyl sulfinylbutyl isothiocyanate, 5-methylthio-pentanenitrile, bis (2-ethylhexyl) phthalate, 6,10,14-trimethyl-2-pentadecanone, and (E)-phytol [10,22–25].

To the best of our knowledge, no work has previously been done on Tunisian *C. draba*, particularly on its volatile profiles. Previous works focused on the fungal pathogens affecting this Tunisian species [26]. These volatile compounds are produced by specific secretory tissues known as glandular trichomes important for phylogenetic and biosystematic studies of many plants. Epiderm micromorphology studies of *C. draba* were focused only on Iraqi and Iranian species [27–29].

In this sense, this study aimed firstly to characterize the chemical compositions of the VOCs of roots, stems, leaves, flowers, and fruits, and of the EOs extracted from the aerial parts (leaves, stems, and flowers) of *C. draba*. They are important to precise the specificity of the Tunisian species in terms of aroma compounds commonly used in fragrance, cosmetic, pharmaceutical, and agri-food industries, as nutritional supplements. Secondly, we focused on the investigation of the micromorphological leaf and stem epidermal properties, to identify and describe the responsible secretory structures of these volatile compounds which are unidentified in this species and even in other species of the *Brassicaceae* family.

2 Materials and Methods

2.1 Plant Material

Plant samples of *Cardaria draba* (fresh leaves, stems, flowers, and roots) were collected in spring (March 2023) during the full flowering stage from populations growing wild in the Ksour-Essaf site (Latitude: 35°25′.02 Nord, Longitude: 10°59′.694 East, elevation: 15 m), located at 17 km from Mahdia; Center-East of Tunisia. Two months later (May 2023) fresh fruits were collected from the same population. Voucher specimens (N°124*Cd*1-5) authenticated by the botanist Pr. Fethia Harzallah-Skhiri was maintained in the herbarium of the Laboratory of Bioresources: Integrative Biology and Valorization

(LR14-ES06), High Institute of Biotechnology of Monastir, Tunisia. In the laboratory, the collected material was dried at room temperature in the dark after separating leaves, flowers, fruits, and roots from the stem, then, ground into a homogenous fine-grade powder using an electric grinder (Duronic CG 250 Premium 250 W) and weighed. The obtained powders were placed in glass jars until use. In addition, a part of the fresh organs (leaves and stems) was kept aside for micromorphological investigations of foliar and stem epidermis.

2.2 Essential Oil Extraction

Given that hydrodistillation of separate *C. draba* aerial parts did not yield essential oil, we mixed the dried powders of stems, leaves, and flowers. For each organ, we used 50 g. Hydrodistillation of 4 × 150 g from this mixture was conducted during 4 h with a Clevenger-type apparatus. The hydrodistillation afforded two separate and successive EO fractions. Fraction 1 (EOFr1) was obtained after 3 h of extraction, followed by the second one (Fraction 2; EOFr2), after 4 h. The two fractions were dried using anhydrous sodium sulfate (anh. Na_2SO_4) and subsequently weighed. The calculated essential oil fraction yield was expressed in % (v/w) concerning the dried weight. The obtained essential oil fractions were stored at 4°C in glass vials until analysis.

2.3 Headspace Solid-Phase Micro-Extraction (HS-SPME)

The Headspace analysis was used for screening of volatile organic compound (VOCs) profiles of 0.2 g of each root, stem, leaf, flower, and fruit dry powders with Solid Phase Micro-extraction (SPME) devices as described by Arbia et al. [30]. Briefly, after an equilibration period, the fiber was introduced to the headspace for 10 s then withdrawn into the needle, and then transferred to the GC/MS injector. Quantitative comparisons of relative peak areas were performed between the same chemicals in the different samples. Identification of the constituents was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and on computer matching against commercial and home-made library mass spectra built up from pure substances and components. GC/EI-MS analyses were performed with a Varian CP-3800 gas chromatograph using a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 um) and a Varian Saturn 2000 mass detector. Analytical conditions: injector and transfer line temperatures 220°C and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 ml/min; for essential oils: injection of 0.2 µl (10% hexane solution); split ratio 1:30; for SPME: injector temperature 250°C, splitless injection.

2.4 Preparing Epidermis Samples

To procure epidermal cells, a small incision of fresh stems and leaves where made with a scalpel, perpendicularly to the axis of the leaf and the stem. Thin and transparent pellicles of the epidermis layer from the stem and from both the abaxial and adaxial surfaces of the leaf were removed using fine forceps and then placed on a drop of water on a microscope glass slide. The obtained slides with the epidermis samples were observed under a light microscope from Leica Microsystems. The number, density, and distribution of stomata, epidermis cells, and trichomes are recorded in fifteen-unit areas, of 1 mm^2 of surface, randomly selected on the stem and on both leaf surfaces. Micrographs were taken from different regions of the sections using ×10, ×40, and ×100 magnifications.

2.5 Stomatal Index (SI)

To calculate the Stomatal index, the formula mentioned in Ditcher [31] was used. See Eq. (1).

$$SI = \frac{S}{S + E} \times 100 \quad (1)$$

S: stomata number per unit area (1 mm^2); E: epidermal cells number in the same unit area.

2.6 Statistical Analysis

The epidermal cell number, stomata, and trichomes of the stem and both adaxial and abaxial leaf surfaces were investigated using analysis of variance (ANOVA) with SPSS 13.0 for Windows (SPSS Inc. Chicago, II, USA). The results are expressed as the mean ± standard deviation (SD) in the lower and upper limits, followed by a mean value. The $p < 0.05$ was considered to indicate a significant statistical difference using Duncan's multiple range test.

3 Results and Discussion

3.1 Headspace Volatile Organic Compounds from Five Cardaria draba Organs

A total of 37 VOCs emanating from *C. draba* organs, representing 96.7%–98.9% of the total composition, were recorded (Table 1). Extracted volatile compounds belonged to several chemical groups that included 4 esters, 4 alcohols, 7 hydrocarbons, 12 aldehydes, 5 ketones, 1 lactone, 1 organosulfur compound, 2 organonitrogen compound, and 1 acid. The hydrocarbons form the main group, representing 49.5%–84.6% of total detected volatiles.

Table 1: Chemical composition in volatile organic compounds isolated from *Cardaria draba* organs powder

No.	Chemical classes	Compounds name	LRI[a]	Relative abundance[b] expressed in %				
				Root	Stem	Leaf	Flower	Fruit
1	Esters	Methyl valerate	827	–	–	1.4	**4.2**	1.9
2		Methyl hexanoate	928	–	–	–	1.4	–
3		Methyl nonanoate	1228	–	–	–	0.3	–
4		Dihydrolinalyl acetate	1276	–	–	0.6	–	–
		Total		0	0	2.0	5.9	1.9
5	Alcohols	1-Hexanol	869	0.4	0.3	–	–	–
6		Benzyl alcohol	1035	–	–	2.0	–	0.6
7		2,6-Dimethylcyclohexanol	1109	–	0.6	3.3	1.2	1.0
8		Phenylethyl alcohol	1111	–	–	–	3.2	1.5
		Total		0.4	0.9	5.3	4.4	3.1
9	Hydrocarbons	β-Pinene	982	–	0.3	–	–	–
10		2,2,4,6,6-Pentamethylheptane	993	**76.2**[c]	**63.5**	**46.2**	**44.5**	**58.0**
11		Limonene	1032	**7.5**	**7.4**	**4.6**	**4.3**	**5.4**
12		3,7-Dimethylnonane	1037	–	1.5	–	–	1.3
13		*n*-Undecane	1100	–	0.4	–	–	0.4
14		*n*-Dodecane	1200	0.4	0.9	–	–	–
15		*n*-Tetradecane	1400	0.5	0.5	–	0.7	–
		Total		84.6	74.5	50.8	49.5	65.1
16	Aldehydes	Hexanal	802	1.6	1.8	1.9	–	2.1
17		Heptanal	901	–	0.2	–	–	–
18		Benzaldehyde	963	–	0.8	2.5	1.3	1.5
19		Octanal	1001	–	0.4	–	–	1.0

(Continued)

Table 1 (continued)

No.	Chemical classes	Compounds name	LRI[a]	Relative abundance[b] expressed in %				
				Root	Stem	Leaf	Flower	Fruit
20		Nonanal	1102	2.3	**3.9**	2.3	2.4	**3.8**
21		Safranal	1198	–	–	1.4	1.0	1.1
22		Decanal	1206	1.1	1.8	–	–	1.0
23		β-Cyclocitral	1222	–	–	1.9	1.1	0.8
24		(E)-2-Decenal	1263	0.6	–	0.6	0.9	–
25		(E,Z)-2,4-Decadienal	1293	1.2	1.2	1.5	1.6	1.2
26		(E,E)-2,4-Decadienal	1318	**3.4**	**3.4**	**4.0**	**4.2**	**3.6**
27		(E)-2-Undecenal	1364	0.9	0.8	0.7	1.0	0.6
		Total		11.1	14.3	16.8	13.5	16.7
28	Ketones	6-Methyl-5-hepten-2-one	987	–	0.3	2.0	1.2	1.1
29		3,5-Octadien-2-one*(isomer1)	1073	0.3	1.6	3.3	1.2	1.7
30		3,5-Octadien-2-one*(isomer2)	1092	0.4	1.3	3.1	2.0	1.5
31		*(E)*-Geranylacetone	1456	0.4	0.5	1.3	1.3	0.9
32		*(E)*-β-Ionone	1488	–	1.0	**7.8**	**3.7**	2.5
		Total		1.1	4.7	17.5	9.4	7.7
33	Lactones	Dihydroactinidiolide	1536	–	0.6	**4.4**	2.6	2.8
		Total		0	0.6	4.4	2.6	2.8
34	Organosulfur compound	Dimethyl sulfoxide	826	–	1.4	1.2	**8.0**	1.6
		Total		0	1.4	1.2	8.0	1.6
35	Organonitrogen compound	2-Ethylpyridine	905	–	–	0.4	–	–
36		Benzyl nitrile	1138	0.4	0.3	–	–	–
		Total		0.4	0.3	0.4	0	0
37	Acids	Hexanoic acid	986	–	–	–	**4.2**	–
		Total		0	0	0	4.2	0
Total compounds identified				**97.6**	**96.7**	**98.4**	**97.5**	**98.9**

Note: [a] *LRI*, Linear retention index determined relative to the series of *n*-alkanes (C9–C28) on an *HP-5* capillary column. [b] Relative content determined on an *HP-5* capillary column. –, Not detected. *, exact isomer not determined. [c] Main compounds are in bold.

In the headspace of the root powder, 16 VOCs were isolated corresponding to 97.6% of the total volatiles. These compounds were detected in low to high proportions (0.3%–76.2%). They have been classified as 4 hydrocarbons (84.6%), 7 aldehydes (11.1%), 3 ketones (1.1%), 1 organonitrogen compound (0.4%), and 1 alcohol (0.4%). The major compound was identified as a hydrocarbon; 2,2,4,6,6-pentamethylheptane (No **10%**, 76.2%), followed by limonene (**11%**, 7.5%) and (E, E)-2,4-decadienal (**26%**, 3.4%). Fifteen VOCs were common with the stem, such as 1-hexanol (**5**), *n*-dodecane (**14**), and benzyl nitrile (**36**), and nine compounds were in common with the stem, leaf, flower, and fruit powder, no specific root VOCs were identified. For the stem headspace powder, 26 constituents were

detected (96.7%) identified as 7 hydrocarbons (74.5%), 9 aldehydes (14.3%), 5 ketones (4.7%), 1 organosulfur (1.4%), 2 alcohols (0.9%), 1 lactone (0.6%), and 1 organonitrogen (0.3%). As in root, 2,2,4,6,6-pentamethylheptane (**10%**, 63.5%), limonene (**11%**, 7.4%), nonanal (**20%**, 3.9%) and (E, E)-2,4-decadienal (**26%**, 3.4%) were the main component. Moreover, 2 trace volatile constituents were specific to the stem; β-pinene (**9%**, 0.3%) and heptanal (**17%**, 0.2%). On the other hand, 23 compounds (98.4%) were extracted in the leaf powder: 2 hydrocarbons (50.8%), 9 aldehydes (16.8%), 5 ketones (17.5%), 2 alcohols (5.3%), 1 lactone (4.4%), 2 esters (2.0%), 1 organosulfur compound (1.2%), and 1 organonitrogen compound (0.4%). Dihydrolinalyl acetate (ester, **4%**, 0.6%) and 2-ethylpyridine (**35%**, 0.4%) were specific to leaf powder. The major components were 2,2,4,6,6-pentamethylheptane (**10%**, 46.2%), (E)-β-ionone (**32%**, 7.8%), limonene (**11%**, 4.6%), dihydroactinidiolide (lactone, **33%**, 4.4%), and (E, E)-2,4-decadienal (**26%**, 4.0%). The 2,2,4,6,6-pentamethylheptane percentages (46.2%) were lower than those recorded in the root and stem powders. Methyl valerate (**1%**, 1.4%), safranal (**21%**,1.4%), and β-cyclocitral (**23%**, 1.9%) were only common with flower and fruit samples. However, benzyl alcohol (**6%**, 2.0%) was only common with fruit powder. Twenty-four compounds (97.5%) were identified in the dried flowers. Three were specific to this organ, particularly hexanoic acid (**37**) reaching 4.2%. The main constituent was 2,2,4,6,6-pentamethylheptane (**10%**; 44.5%) followed by dimethyl sulfoxide (**34%**; 8%). Among the 25 constituents in the fruit powder (98.9%), the main one was 2,2,4,6,6-pentamethylheptane (**10%**, 58%) as is the case for all the other samples. Limonene (**11**), nonanal (**20**), and (E, E)-2,4-decadienal (**26**) were detected at 5.4%, 3.8%, and 3.6%, respectively. None of the VOCs was specific to fruits.

In the headspace analyses for all the plant parts, esters were absent in root and stem VOCs, acids were present only in VOCs flowers, organosulfur compound, and lactone were absent in VOCs roots, and organonitrogen compounds were absent in leaves VOCs. The aliphatic hydrocarbon 2,2,4,6,6-pentamethylheptane (**10**), known as isododecane, largely contributed to the smell of *C. draba* organs and was the most plentiful constituent of VOCs extracted from roots (76.2%), stems (63.5%), and fruits (58.0%). According to Bell et al. [32], non-glucosinolate-derived compounds can contribute significantly to the *Brassicaceae* species aromas. The 2,2,4,6,6-pentamethylheptane component is known for its low odor and toxicity and it is a key ingredient in the cosmetics market as a fragrance agent [33]. Furthermore, this compound (**10**) has been reported previously belonging to the cattle and lamb meats' volatile profile having a characteristic odor [34]. In previous studies, this component has never been detected in the volatile emission profiles of *C. draba*. However, Bell et al. [32] determined volatile and odorant compounds in the headspace of four *Brassicaceae* species; *Eruca sativa* Mill., *Armoracia rusticana* G. Gaertn., *Eutrema japonicum* (Sieb.), and *Nasturtium officinale* L. Among the identified compounds, 2,2,4,6,6-pentamethylheptane was only detected in *N. officinale* but in very low content (0.69%). Besides, it was also detected among the highest contributing components and the source of fruity aromas of 9 types of Fenghuang Dancong tea, reaching 20.32% [35].

The second most important hydrocarbon in our samples is limonene (**11**), classified as a cyclic monoterpene. It is detected in all organ powders but mainly in roots and stems. According to Erasto et al. [36], it is among the frequently encountered volatile components of *Citrus*. Its occurrence could be attributed to its defensive role against herbivores. It possesses a pleasant lemon-like odor, which makes it extensively employed as a flavor additive in common food and cosmetics formulations. Its isomer D-Limonene occurs more commonly in 12 *Brassicaceae* vegetables such as Brussels sprouts, broccoli, radish, and cherry [37] and in *B. rapa* L. [38]. Limonene reached 21.1% of the VOCs identified in the floral scent of *B. oleracea* [39].

In the headspace of the different analyzed powders, except this from roots, a ketone (*E*)-β-ionone (**32**) was detected in moderate abundance (7.8%–1.0%). The name ionone is derived from the Greek term *iona* meaning violet odor. It is a natural plant component derived from the degradation of carotenoids [40]. In

recent years, according to Paparella et al. [41], β-ionone has antibacterial and fungicidal properties and an important potential as an anticancer treatment. It is also considered as an attractant or repellent of insects and is released following wounding by herbivores.

Dimethyl sulfoxide (**34**) was the only volatile sulfur compound detected in stems, leaves flowers, and fruit volatiles. It seems to have a moderate contribution, particularly, to the smell of *C. draba* flowers (8%). According to Zhou et al. [42], it could have originated from isothiocyanate responsible for its onion, cabbage, garlic, and sulfurous-pungent-like odors. This flavoring agent has different pharmaceutical applications and is an important contributor to the flavors in food industries [43]. Previously, it was detected by Nadaf et al. [24] in the volatiles of the Iranian *C. draba* aerial parts, but with a lower percentage (3.6%) than that recorded in our study.

According to Smart et al. [44], phenyl ethyl alcohol (**8**), and benzyl alcohol (**6**) which have been detected in flowers or fruit have a role of pollinator attraction in *Brassicaceae* species and characterize the flower fragrance of several other species. They are among the aroma compounds of three *B. juncea* L. accessions leaves [45]. Moreover, benzyl alcohol and phenyl ethyl alcohol are some of the volatiles identified in the fragrance of *B. nigra* (L.) Koch flowers (0.5%; 2.6%, respectively), *B. oleracea* L. (2.2%; 19.5%, respectively), *B. juncea* (0.3% 2.4%, respectively), *B. napus* (0.8%; 22.3%, respectively), and *B. carinata* A. Braun (1.3%; 11.6%, respectively) [39]. Nonanal (**20**) contributed also to the aroma of *C. draba* organs (2.3%–3.9%). It is known for its contribution to the nectar scents of certain flowers such as those of Orchids attracting mosquitoes [46].

3.2 Chemical Composition of Cardaria draba Aerial Parts Essential Oil

EOFr1 was colorless and had an intense and pungent aroma. Whilst, the EOFr2 showed a pale-yellow color with low odor strength and solidified when in contact with air. They appeared to contain both fragrant molecules and cuticular waxes. The yields of EOFr1 and EOFr2 were 0.031% and 0.010% (v/w), respectively.

3.2.1 Chemical Composition of the EO Fractions

A total of 42 chemical compounds (22 detected in the EOFr1 and 20 in the EOFr2) were identified. They account for 98.8% and 97.2% of the total EO composition, respectively. The identified EO compounds and their relative contents are presented in Table 2.

Table 2: Chemical composition of the two essential oil fractions extracted from the aerial parts (leaves, stems, and flowers) of *Cardaria draba* growing wild in Tunisia

No.	Compound name and class	*LRI*[a]	Relative content[b] expressed in %	
			EOFr1	EOFr2
1	Butyrolactone	918	**4.6**	–
2	6-Methyl-5-hepten-2-one	987	**18.3**[c]	–
3	3-Ethyl-1-hexanol	1031	1.2	–
4	Limonene	1032	2.8	–
5	1,8-Cineole	1034	1.6	–
6	3,5-Octadien-2-one*	1075	2.0	–
7	3,5-Octadien-2-one*	1093	1.8	–
8	Nonanal	1102	**9.8**	–

(Continued)

Table 2 (continued)

No.	Compound name and class	*LRI*[a]	Relative content[b] expressed in %	
			EOFr1	EOFr2
9	*(E)*-2-Nonenal	1163	2.0	–
10	Safranal	1198	1.6	–
11	Decanal	1204	**15.0**	–
12	*n*-Tetradecane	1400	1.7	–
13	β-Caryophyllene	1419	2.0	–
14	*(E)*-Geranyl acetone	1456	**7.7**	–
15	*(E)*-β-Farnesene	1459	1.9	–
16	*(E)*-β-Ionone	1489	1.4	–
17	Dihydroactinidiolide	1532	1.5	–
18	α-Bisabolol oxide B	1656	**4.1**	–
19	α-Bisabolone oxide A	1680	**5.2**	–
20	2-Pentadecanone	1697	1.9	–
21	Methyltetradecanoate	1727	–	0.2
22	α-Bisabolol oxide A	1746	**9.2**	–
23	Tetradecanoicacid	1761	–	0.4
24	Neophytadiene	1838	–	0.3
25	Hexahydrofarnesyl acetone (syn. 6,10,14-trimethyl-2-pentadecanone)	1845	1.5	**13.2**
26	Pentadecanoicacid	1866	–	0.4
27	*n*-Nonadecane	1900	–	0.7
28	*(E,E)*-Farnesyl acetone	1920	–	0.4
29	Methylhexadecanoate	1928	–	3.5
30	Isophytol	1949	–	0.3
31	Hexadecanoic acid	1967	–	**34.6**
32	Methyllinoleate	2096	–	1.4
33	*n*-Heneicosane	2100	–	**4.9**
34	Methyl *cis*-vaccenate	2102	–	0.7
35	Phytol	2115	–	2.4
36	Methyl octadecanoate	2129	–	0.3
37	9-Octadecenoic acid	2144	–	1.7
38	*n*-Docosane	2200	–	0.6
39	*n*-Tricosane	2300	–	**6.6**
40	Eicosanoic acid	2361	–	**9.6**
41	*n*-Tetracosane	2400	–	2.0

(Continued)

Table 2 (continued)

No.	Compound name and class	*LRI*[a]	Relative content[b] expressed in %	
			EOFr1	EOFr2
42	*n*-Pentacosane	2500	–	**13.0**
	Monoterpene hydrocarbons		2.8	–
	Oxygenated monoterpenes		1.6	–
	Sesquiterpene hydrocarbons		3.9	–
	Oxygenated sesquiterpenes		**18.5**	–
	Diterpenes		–	0.3
	Apocarotenes		**13.7**	**13.6**
	Non-terpene derivatives		**58.3**	**83.3**
	Total identified		**98.8**	**97.2**

Note: [a] *LR*, Linear retention index determined relative to the series of *n*-alkanes (C9–C28) on an *HP-5* capillary column. [b] Relative content determined on an *HP-5* capillary column. –Not detected*, exact isomer not determined. [c] Main compounds are reported in bold (>4%). EOFr1: Fraction 1 essential oil; EOFr2: Fraction 2 essential oil.

3.2.2 Chemical Composition of the EOFr1

Among the 22 compounds detected in the EOFr1 (Table 2), 6-methyl-5-hepten-2-one (**2**) followed by decanal (**11**) showed the highest percentages (18.3% and 15%, respectively). Three additional compounds were identified at moderate levels; nonanal (**8**), α-bisabolol oxide A (**22**), and *(E)*-geranyl acetone (**14**) (9.8%, 9.2%, and 7.7%, respectively). α-bisabolone oxide A (**19**), butyrolactone (**1**), and α-bisabolol oxide B (**18**) were represented in low relative contents in the EOFr1 (5.2%, 4.6%, and 4.1%, respectively). All the 14 other compounds were detected in very low relative abundances not exceeding 2.8%.

The main compound of the EOFr1; 6-methyl-5-hepten-2-one (syn. sulcatone) (**2**), is an unsaturated methylated ketone. It is a floral compound produced by over 400 plant species and characterized by its *Citrus* odor representing the principal component of Citronella, Lemon-grass, and Palma rosa EOs [47]. It acts as an anti-leukemic and induces apoptosis [48]. Decanal is a saturated fatty aldehyde with a pleasant sweet, waxy, floral, citrus, and pronounced odor [49]. It has a powerful role as an antifungal agent [50]. Nonanal is a saturated fatty aldehyde characterized by an orange-rose odor. It is a prevalent volatile compound found in at least 20 EOs, including Rose, *Citrus*, and Pine ones [51]. It is used in the perfume industry [52]. α-Bisabolol oxide A is a significant sesquiterpene alcohol. It is derived naturally from EOs of various ornamental and edible species [53]. It has a fruity nutty aroma close to coconut [54]. Its most important biological activities are its anti-inflammatory, anti-irritant, anti-bacterial, anti-cancer, and non-allergenic properties. It is also known for its neuro-, cardio-and nephro-protective and analgesic effects [54]. *(E)*-Geranyl acetone plays various roles in insect and plant interactions [55]. It is a flavoring agent having an apple-like fruity aroma [56]. Limonene (**4**) was detected in a low percentage (2.8%) confirming the results found by Bayan [22] where this component is present in the EO of *C. draba* aerial parts with percentages not exceeding 3.23%. However, the EO seed of *Brassica napus* L. was predominantly made up of limonene (90%) [57]. Another component, β-ionone (**16**), generally synthesized at low concentrations in plants, is present at a low percentage (1.4%). It was previously found in the EOs of the Algerian *C. draba* (2.01% and 2.57% in the aerial parts and leaves, respectively) [25] and of the Croatian species (1.5% in a mixture of leaves and flowering heads) [23].

3.2.3 Chemical Composition of the EOFr2

In EOFr2, 20 specific compounds were found. The 6,10,14-trimethyl-2-pentadecanone (**25**) (Table 2) common to the two fractions, was detected in high relative content (13.2% against 1.5% in EOFr1). The main constituent of the EOFr2 was hexadecanoic acid (**31%**; 34.6%), also called palmitic acid; the most common saturated long-chain fatty acid found in plants, animals, and microorganisms [58]. It is considered an important cuticular wax compound in plants [59]. It is used as an insecticide and an acaricide, and it has several benefits on skin health due to its anti-inflammatory properties [60]. It is followed by the 6,10,14-trimethyl pentadecan-2-one compound (**25%**; 13.2%) characterized by an odor and a tasting of Jasmin and celery woody [61]. It is the major constituent of numerous aromatic and medicinal plants EOs. It has antibacterial, anti-nociceptive, and anti-inflammation activities [62]. This latter compound is followed by *n*-pentacosane (**42%**; 13%) known for its anticancer, antimicrobial, antifungal, anti-inflammatory, antioxidant, and antiviral activities [63]. Moreover, eicosanoic acid (syn. arachidic acid) (**40**) was detected in moderate percentage (9.6%). It is a saturated long-chain fatty acid essential to brain development, repair, and maintenance, and neuron protection [37]. *n*-Tricosane was also detected in moderate percentage (**40**) (6.6%). The other components were present in low to very low relative abundances (0.2%–4.9%).

3.2.4 Chemical Classes Variation in the Two Cardaria draba Aerial Parts EO Fractions

The types and contents of the different chemical classes of the aerial parts EO components are reported in Table 2. Six classes were detected for the EOFr1 constituents, against 3 ones for those for EOFr2. The two fractions are rich in non-terpene derivative compounds, 83.3% for EOFr2, and 58.3% for EOFr1. Sesquiterpene hydrocarbons, monoterpene, and oxygenated monoterpenes and sesquiterpenes were specific to the EOFr1. Among the main oxygenated sesquiterpenes, compounds 18, 19, and 22 are particularly significant. However, the diterpenes chemical class was specific to the EOFr2 and represented by the isophytol (**30**). Percentages in apo carotenes constituents were almost identical in the two fractions represented mainly by compound **25** (13.2%) for EOFr2 and by particularly compound **14** for EOFr1. There was notable variation within the EO fraction. During the first hours of extraction, more volatile smaller compounds such as monoterpenes and sesquiterpenes are extracted, and in later periods larger fewer volatile compounds are extracted, such as alkanes.

Comparing our results with those of the published studies, qualitative and quantitative differences emerge. The EO composition exhibits significant variation depending on the geographical location and climate conditions [64]. The composition of the EOs of *C. draba* collected in Iran was different. Afsharypuor et al. [10] described a composition mainly dominated by 3-butenyl isothiocyanate (80.5%) for the EO derived from flowering aerial parts, the 4-methyl sulfinylbutyl isothiocyanate for the fruit and root EOs (72.1% and 30%, respectively), and hexadecanoic acid for the root EO (24.1%). All these compounds are not detected in Tunisian *C. draba*, except hexadecanoic acid (**31**) which has been detected at a slightly higher percentage (34.6%). The main volatile compounds isolated from the aerial parts of *C. draba* from Croatia, were 4-methylsulfanylbutyl isothiocyanate (28%), pentanenitrile (19.2%), hexadecanoic acid (10.8%), phytol (10.2%), and dibutyl phthalate (4.5%) [65]. Moreover, as specified by Kazemi et al. [66], the main components of the aerial and flowering parts EO of *C. draba* from Iran were Z-phytol (12.9%) and β-ionone (4.5%). In our study, we have also found these two compounds but in lower amounts (2.4% and 1.4%, respectively). For Nadaf et al. [24], the EO aerial parts collected at the flowering stage contain mainly bis2-ethylhexylphthalate (12.5%), dicyclohexyl-propanedinitrile (7.4%), dodecane (6.3%), 9,12,15-octadecatriene-1-ol (5.2%), tridecane (4.8%), and decahydro-1,6-dimethyl-naphthalene (4.6%). All these compounds are not identified in our study. The EOs of the air-dried leaves and the flowering parts of the Algerian Wite top exhibited the existence of two main constituents; 6,10,14-trimethyl-2-pentadecanone (11.08% and 20.61%, respectively) and *(E)*-phytol (39.67% and 11.38%, respectively) contributing to the high antioxidant capacity of those EOs [25]. In our work,

6,10,14-trimethyl-2-pentadecanone was also predominant in the EOFr2 (**25**; 13.2%). Phytol component (**35**) was identified in a lower percentage (2.4%). For the five compounds, 6-methyl-5-hepten-2-one (**2**), decanal (**11**), nonanal (**8**), *(E)*-gerany lacetone (**14**) and *(E)*-β-ionone (**16**) higher quantities were found in Algerian species [25]. The major constituents of the *C. draba* EO growing in Turkey were 5-methylthio-pentanenitrile (41.13%), decane (11.40%), and nonane (10.93%) [22]. These components were not detected in the specific EO profiles of the Tunisian species.

3.3 Micromorphological Features of Epidermal Cells, Stomatal Complexes, and Trichomes of C. draba Leaf and Stem

3.3.1 Leaf and Stem Epidermal Cells

Micromorphological features of epidermal cells in adaxial and abaxial leaf and stem surfaces are presented in Fig. 1. In the leaf, the epidermal cells have an irregular shape. On both surfaces, they were polygonal with sinuous walls. Walls of epidermal cells, on the leaf adaxial surface, seem to be slightly more undulating than those of the abaxial surface (Figs. 1a–1e). For Doaigey et al. [67], leaf epidermal cells of *C. draba* of Saudia Arabia are sinuous with thick anticlinal cell walls. For Iraqi *C. draba* leaf epidermal cells, Esmaeel et al. [27], described them as polygonal with feebly sinuous walls. Al-Saadi et al. [29] showed that the anatomical features of the leaf epidermal cells varied widely in 13 different *Brassicaceae* species. The cell walls can be thicker or thin, strongly undulate anticlinal or sinuous anticlinal according to the species. For *C. draba*, they are straight-sinuate anticlinal. Bibi et al. [68] studied the evolutionary and taxonomic potential of microscopic features in the leaf epidermis of 27 Pakistan *Brassicaceae* species. They noted the presence, often, of epidermal cells varied in form, encompassing polygonal, pentagonal, hexagonal, and tubular shapes. Leaf epidermal features have potential taxonomic significance. They help delimit the related taxa in *Brassicaceae* [69], particularly the sinuous character of epidermal cells [70].

On the contrary, the form of stem epidermal cells was nearly rectangular, long, and tapered with linear and thick walls. They were much longer than wide (Figs. 1f–1i). Mousavi et al. [28] previously described in cross-section of *C. draba* stem, rectangular epidermal cells. For Esmaeel et al. [27], the shape of the epidermal cells of the stem was oblong to longley with straight walls.

3.3.2 Leaf and Stem Stomatal Complexes

Leaf Stomatal Complex

In the examined *C. draba* leaf epidermis, the shape of the stomata was elliptic to elliptic-oblong. The stomatal complex, consisting of the stoma plus adjacent epidermal cells, was anisocytic on both adaxial and abaxial leaf surfaces of the Tunisian *C. draba*. It is characterized by three unequally sized subsidiary cells, easily distinct from other epidermal cells, accompanying the pair of guard cells. Among these three cells, one is comparatively smaller than the other two (Figs. 1c, 1e). Anisocytic stomata are created when the meristemoid divides two more times asymmetrically, forming surrounding subsidiary cells [71]. According to Al-Saadi et al. [29], the anisocytic type is dominant but other types can intermix in other *Brassicaceae* species. The stomatal complex variation type seems to be about the ecological environments [69]. Our result agreed with those of Esmaeel et al. [27] in the leaf epidermis of *C. draba* growing wild in Iraq, and in other *Cardaria* species [72]. For Bibi et al. [68], three various stomata types were identified in the leaf epidermis of Pakistan *Brassicaceae* species; anisocytic, anomocytic, and staurocytic. According to Doaigey et al. [67], the stomata are frequently anisocytic in the leaf epidermis of 34 *Brassicaceae* species but combined with anomocytic type in 21 species.

Stem Stomatal Complex

Microscopic investigation of the Tunisian *C. draba* stem epidermis revealed two different types of stomata complexes; mainly anisocytic (Fig. 1g) and rarely paracytic ones (Figs. 1h, 1i). As defined by van

Cotthem [73], the paracytic type includes side subsidiary cells oriented parallel to the guard cells. The sole previous work carried out by Esmaeel et al. [27] on the stem epidermis of *C. draba* in Iraq confirmed the occurrence of both anisocytic and paracytic stomata complexes in the stem epidermis of this species.

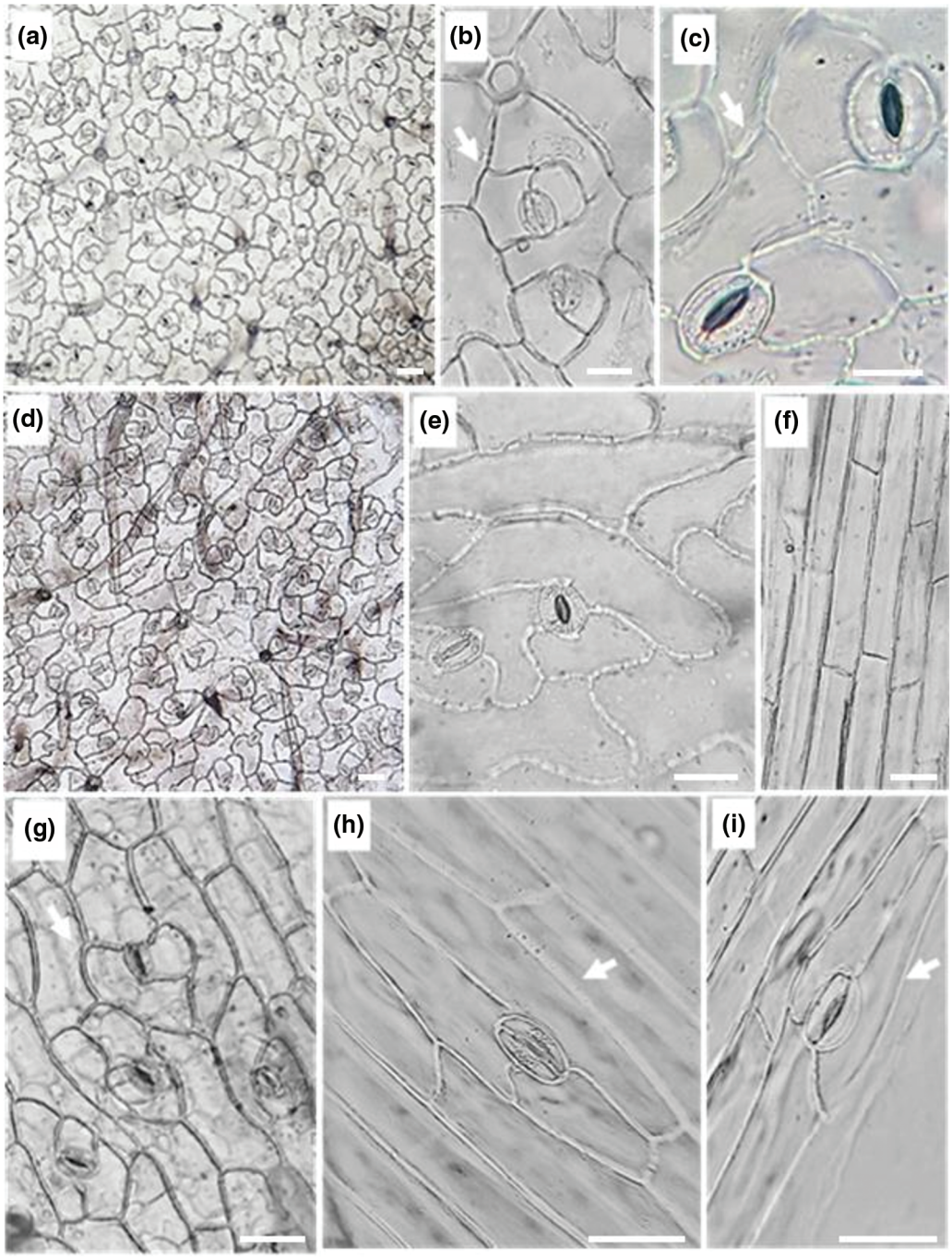

Figure 1: Epidermal characters in *C. draba* leaf and stem surfaces (Scale 50 μm). Epidermal cells and stomatal complexes with anisocytic stomatas on adaxial (a–c) and on abaxial (d, e) leaf surfaces; epidermal cells and stomatal complexes on stem surface (f–i); anisocytic stomatas (g) and paracytic stomatas (h, i) on stem epidermal surface. (a, d) ×40; (b, e, f, g) ×100; (c, h, i) ×400

3.3.3 Leaf and Stem Trichomes

In the present investigation non-glandular trichomes have been identified in each epidermis face of the leaf (Fig. 2) and that of the stem (Fig. 3). All trichomes are surrounded by thick walls. Two types of spaced unicellular and unbranched non-glandular trichomes were recorded. The first type was long with an acute apex (type 1) (Figs. 2a–2d, 2j–2l and 3a–3f) and the second one was short with a convex apex (type 2) (Figs. 2e–2g, 2m, 2n and 3g–3j).

Previous works revealed that trichomes in the leaf and stem epidermis of *C. draba* are non-glandular, short and long single-celled, conical, warty, and very dispersed [27,28]. They are backed with our results.

Glandular trichomes are identified in the leaf and stem epidermis. They are capitate type, short-stalked with a large secretory head (Figs. 2h, 2i, 2o, 2p and Fig. 3k). They are the site of production of specialized metabolites largely used as pesticides, fragrances, and pharmaceutical ingredients [74]. It is important to note that in the previous studies carried out on *C. draba*, glandular trichomes have never been reported contrary to our work. Ilyinska [75] recorded the occurrence of glandular structures in 14 species of *Brassicaceae* other than *C. draba*. The presence or absence of trichomes, the size, and the shape change depending on geographic location and climatic conditions have been considered important for the identification of *Brassica* species [76].

3.3.4 Density of Epidermal Cells, Stomata, and Trichomes in Leaf and Stem

The average numbers of the leaf epidermal cells are more important (996.0 cells/mm^2 against 967.4 cells/mm^2 in the adaxial and abaxial leaf surfaces, respectively) than that of the stem surface (755.2 cells/mm^2) (Table 3). These numbers are slightly elevated compared to those recorded by Al-Saadi et al. [29] on the leaf epidermis of *C. draba* (924.0 cells/mm^2 and 900.0 cells/mm^2 on the adaxial and the abaxial one, respectively). These authors reported that the epidermal cells number varies in 12 other *Brassicaceae* species. On the adaxial leaf epidermis, it ranged between 420.0 cells/mm^2 in *Lobularia maritima* (L.) Desv. and 1920.8 cells/mm^2 in *Nasturtium officinale* W.T.Aiton. On the abaxial epidermis, it varied from 361.0 cells/mm^2 in *L. maritima* to 1665.0 cells/mm^2 in *Sinapis alba* L.

Concerning the counted number of stomata, it was higher in the abaxial surface of the leaf epidermis (198.4/mm^2), followed by the adaxial one (182.2/mm^2) and then in the stem (42.6/mm^2) (Table 3). Al-Saadi et al. [29] also found that the stomata number of the abaxial leaf epidermis (186.23/mm^2) of *C. draba* was slightly higher than that of the adaxial one (184.52/mm^2).

In our study, the highest stomatal index percent (density of stomata relative to the epidermal cell count), was 17.02% recorded on the abaxial surface of the leaf, followed by 15.46% on the adaxial surface of the leaf and the lowest index (5.34%) was found in stem surface epidermis (Table 3). Comparable findings were reported by Al-Saadi et al. [29] for *C. draba*. The stomatal indexes of the abaxial and adaxial leaf faces were 17.14% and 16.64%, respectively. However, in previous work on *C. draba* epidermis, Esmaeel et al. [27] reported stomatal indexes higher than those found in our study. They reached 26.66%, 44.44%, and 16.66% in abaxial, adaxial leaf, and stem surfaces, respectively. These differences can be explained by the species-specific adaptation influenced by both genetic and environmental factors [77]. In addition, Segev et al. [78] found a correlation between photosynthesis and the stomatal index. An important value of the stomatal index correlates to a higher photosynthesis rate. According to Huma et al. [79], the stomata numbers, types, distribution, and frequencies show environmental conditions.

The trichome counting in both leaf surfaces, and stem epidermis, revealed that the average numbers of non-glandular trichomes are much higher than the glandular ones (113.2, 87.2, and 96.6/mm^2 against 6.4, 3.4, and 2.2/mm^2, respectively). The adaxial leaf surface epidermis was the richest in non-glandular and glandular trichomes (113.2 and 6.4, respectively). The non-glandular trichome results are in concordance with those recorded by Al-Saadi et al. [29] and Ilyinska [75] except for the glandular ones which have not been identified.

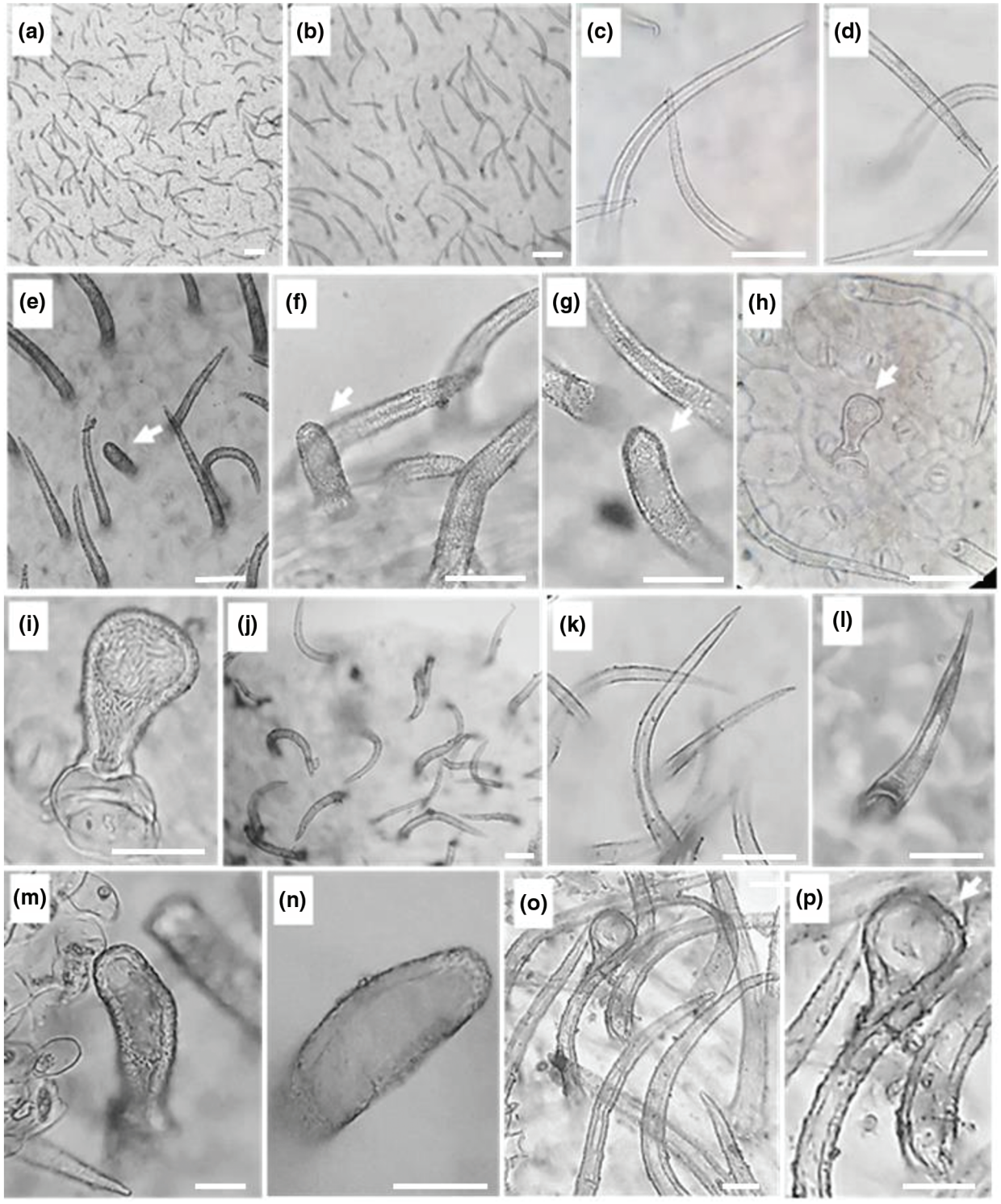

Figure 2: Leaf epidermal trichomes of *C. draba* (Scale 50 µm). Adaxial leaf surface non-glandular type 1 (a–d), non-glandular type 2 (e–g), and glandular (h, i) trichomes. Abaxial leaf surface non-glandular type 1 (j–l), non-glandular type 2 (m, n), and glandular (o, p) trichomes. (a) ×40; (b, e) ×100; (c, d, f, g, h, i, k, l, m, n, o, p) ×400

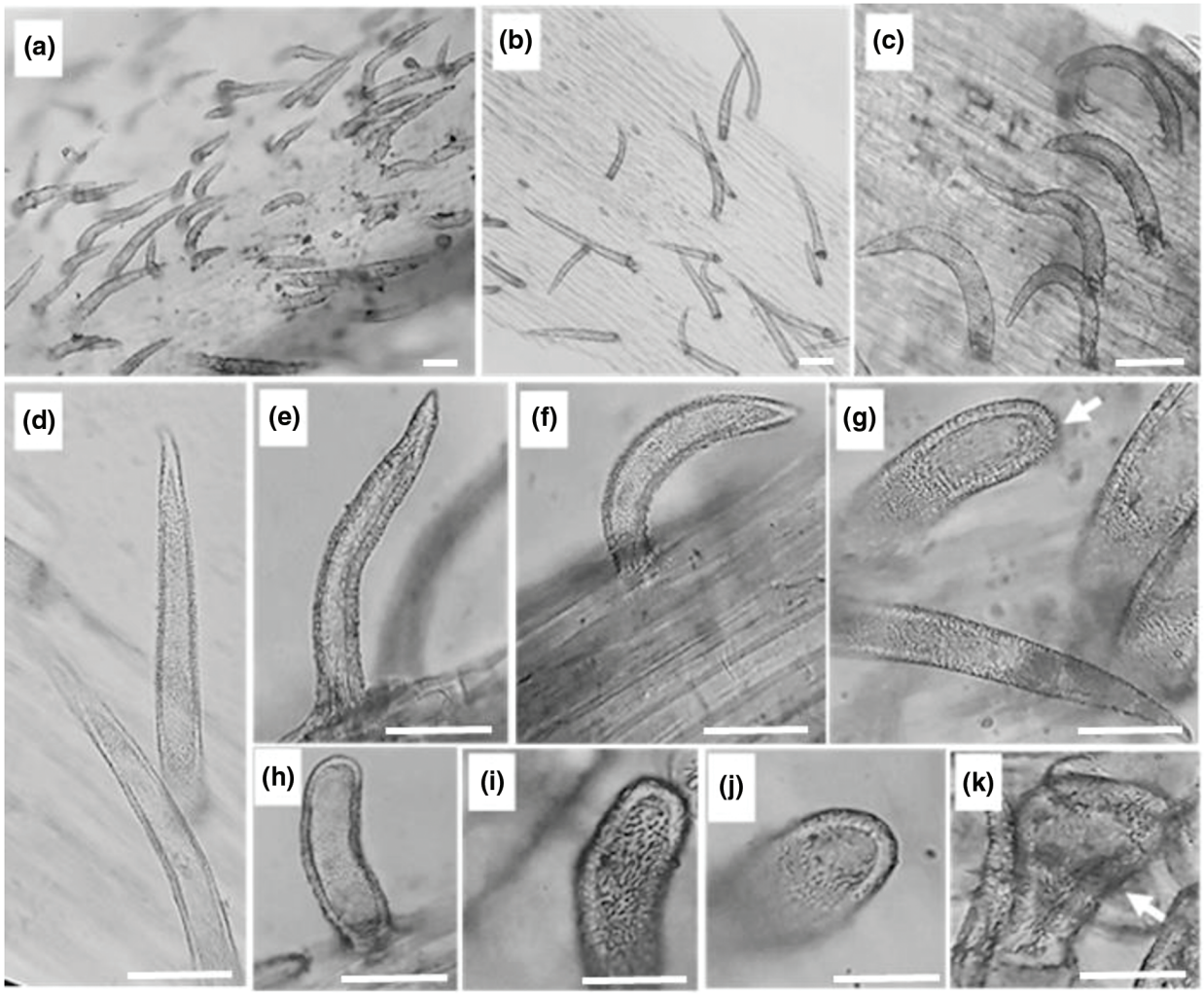

Figure 3: Stem epidermal trichomes of *C. draba* (Scale 50 μm). Non-glandular trichomes type 1 (a–f) and type 2 (g–j). Glandular trichome (k). (a, b) ×40; (c) ×100; (d–k) ×400

Table 3: Density of epidermal cells, stomata, and trichomes with corresponding stomatal index in leaf and stem epidermis surfaces of the Tunisian *Cardaria draba*

	Number/mm^2				Stomatal index (%)
	Epidermal cells	Stomata	Non-glandular trichomes (Types 1 and 2)	Glandular trichomes	
Adaxial leaf surface	(943–1023) 996.0 ± 0.1^a	(164–201) 182.2 ± 0.1^b	(97–142) 113.2 ± 0.1^a	(5–8) 6.4 ± 0.3^a	15.46
Abaxial leaf surface	(896–1009) 967.4 ± 0.3^b	(184–211) 198.4 ± 0.2^a	(78–94) 87.2 ± 0.2^c	(2–5) 3.4 ± 0.1^b	17.02
Stem surface	(704–802) 755.2 ± 0.1^c	(38–52) 42.6 ± 0.1^c	(87–104) 96.6 ± 0.2^b	(0–4) 2.2 ± 0.1^c	5.34

Note: Means ± SD with the same letter(s) in the same column are not significantly different ($p < 0.05$) (Duncan's multiple range test). Each pair of parentheses contains the least and most limits, followed by a mean value.

4 Conclusion

The present research work is the first report on the chemical composition of the headspace aroma and essential oils from several organs of the wild Tunisian *Cardaria draba*. It provides additional information about the selection of raw materials and odor markers in *C. draba* fragrant volatile compounds and essential oils, particularly root and stem VOCs. Several compounds in high quantities in essential oils were recorded. They have medicinal potential and could be introduced for applications in agri-food, cosmetic, and flavor factories. However, to fully use this species as a new source, large quantities of plant material are needed because of its low yield. Additionally, the study improves knowledge of *C. draba* leaf and stem epidermal characteristics which are useful anatomical tools and criteria to support taxonomic studies. Glandular trichomes are identified as secretory structures of volatiles for the first time in this *Brassicaceae* species.

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