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Introduction: Should clearly establish the objectives of the work and its relationship with other works in the same field

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Chemical Composition of the Volatile Components of *Tropaeolum majus* L. (Garden Nasturtium) from North Western Algeria

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Abstract Essential oil from *Tropaeolum majus* L. aerial parts, a plant native to North Western Algeria, was obtained by hydrodistillation. The oil volatile components were identified by a combination of gas chromatography/flame ionization detection (GC/FID), GC-mass spectrometry (GC-MS) techniques, and NMR spectroscopy. Nine components representing 92.0 % of the essential oil total (GC/FID chromatogram) were identified. The most abundant compounds were benzyl isothiocyanate (82.5 %), benzene acetonitrile (3.9 %) and 2-phenylethyl isovalerate (2.9 %). Higher content in nitrogen- and sulfur-containing compounds accounting to 86.4 % of the volatile fraction composition of *T. majus* were quantified.

Key Words: *Tropaeolaceae* ; *Tropaeolum majus* L.; Volatiles; GC; GC-MS; NMR

1. Introduction

T. majus (garden nasturtium, Great Indian cress) is a herbaceous annual plant of the *Tropaeolaceae* family. It is a native plant of the Andes in South America [1], but its adaptability to different climates has helped its dissemination throughout the world. The leaves of *T. majus* have been used in folk medicine against cardiovascular disorders, urinary tract infections, asthma, constipation [2-4]. Anticancer activity has been attributed to the extracts from the plant [5]. Recent studies have shown that the *T. majus* flowers are excellent sources of lutein, and the leaves contain both lutein and provitamin A and β -carotene [2,6,7]. Phytochemical studies detected the presence of fatty acids and flavonoids in seeds and leaves of *T. majus* [5,8,9]. Glucosinolates were isolated from leaves of this plant, as well as tetracyclic triterpenes [9,10]. This study evaluates the chemical composition of the volatiles components

of *T. majus*. As far as we know, this work is the first report on the chemical composition of the volatile fractions of *T. majus*.

2. Materials and Methods

2.1. Plant samples

The aerial parts (orange flowers, stems, leaves) of *T. majus* were collected at the flowering stage in May 2012 around Nedroma (Tlemcen, Algeria). Botanical identification of the plant was conducted by Prof. Noury BENABADJI and a voucher specimen of the plant was deposited in the Herbarium of the Laboratory of Biology, Abou Bekr Belkaïd University, Tlemcen, Algeria.

2.2. Sample preparation

Volatiles were obtained from the cooling of steam in the hydrodistillation process [11]. Practically, the volatile components were obtained by simple hydrodistillation (5 h) using about five-fold water to the fresh weight of the plant material. They were trapped with 1 mL of ether, dried over anhydrous sodium sulfate, concentrated under vacuum and stored at +4°C until analysis by GC-MS.

2.3. Oil Fractionation

Volatiles were submitted to flash chromatography [FC; silica gel 200–500 mm, elution with *n*-hexane]. The purified polar compound **6** was then analyzed by NMR (¹H and ¹³C) spectroscopy and compared to commercially available benzyl thiocyanate and benzyl isothiocyanate from Sigma-Aldrich.

2.4. GC analysis

GC analysis were carried out using a Perkin–Elmer (Waltham, MA, USA) Autosystem XL GC apparatus equipped with a dual flame ionization detection system and a fused-silica capillary columns (60 m x 0.22 mm I.D., film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50), using helium as the carrier gas (1 mL/min); the injection volume was 0.2 µL. Retention indices (RI) of the compounds were determined relative to the retention times of the series of *n*-alkanes (C₅–C₃₀) with linear interpolation, using the Van den Dool and Kratz equation [12] and software from Perkin–Elmer. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.5. GC-MS analysis

Samples were analyzed with a Perkin–Elmer Turbo mass detector (quadrupole), coupled to a Perkin–Elmer Autosystem XL, equipped with the fused-silica capillary columns Rtx-1 and Rtx-Wax (ion source temperature 150 °C; energy ionization 70 eV). EI mass spectra were acquired over the mass range 35–350 Da (scan time: 1 s). Other GC conditions were the same as described under GC except split 1/80.

2.6. Component identification

The identification of the constituents of the volatiles was performed through the comparison of their retention indices with the NIST database and literature data, as well as using the comparison of their mass spectra with the Wiley Mass spectral database and those described by Adams [13].

Quantitative data was calculated using electronic integration from the FID area data without the use of correction factors.

2.7. FTIR and NMR Analysis

Thin layer chromatography (TLC) was performed on Kieselgel 60 F254 plates from Merck with detection by UV light. FTIR spectra were recorded on a Cary 630 FTIR spectrometer from Agilent Technologies. NMR spectra (^1H and ^{13}C) of compound **6** was recorded in deuterated chloroform on a Bruker Avance III spectrometer operating at 400 MHz for ^1H and at 100 MHz for ^{13}C NMR. All shifts were referred to the internal standard tetramethylsilane (TMS).

3. Results

The volatile constituents of *T. majus* aerial parts were yellow with disagreeable odours. The total yield of volatiles through the hydrodistillation using a Clevenger apparatus was 0.01%. Table 1 shows the components, retention indices and percentage of composition. They are listed in the order of their elution from a capillary column. Nine compounds were identified in the volatile fraction representing 92.0 % of total volatiles. As shown in Table 1, the principal components of volatiles were benzyl thiocyanate (82.5 %), benzene acetonitrile (3.9 %), 2-phenylethyl isovalerate (2.9 %), and isocaryophyllene (1.0 %). The major parts of volatile constituents were no-terpenic compounds (86.4 %) and oxygenated no-terpenic compounds (3.3 %). These odorant compounds were released upon hydrolysis of aromatic glucosinolate by the endogenous enzyme myrosinase.

Table 1. Chemical composition of volatiles of *T. majus*.

No. ^a	Components	RI _{Lit} ^b	RI _a ^c	RI _p ^d	Volatiles(%) ^e	Identification ^f
1	α -Thujene	921	922	1023	0.6	RI, MS
2	Myrcene	974	976	1159	0.3	RI, MS
3	1,4-dimethyl-vinyl cyclohexene	1015	1016	-	0.2	RI, MS
4	Terpinene	1075	1075	-	0.2	RI, MS
5	Benzene acetonitrile	1089	1090	-	3.9	RI, MS
6	Benzyl isothiocyanate	-	1330	-	82.5	RMN ^1H , ^{13}C
7	Isocaryophyllene	1415	1407	1571	1.0	RI, MS
8	2-Phenyl ethyl isovalerate	1464	1465	1980	2.9	RI, MS
9	Phenoxy methyl benzene	1568	1543	-	0.4	RI, MS
10	NI	1722	-	-	1.0	
Total identification %					93.0	
% Monoterpene hydrocarbons					1.3	
% Sesquiterpene hydrocarbon					1.0	
% No-terpenic compounds					86.4	
% Oxygenated no-terpenic compounds					3.3	

^a Order of elution is given on apolar column (Rtx-1), ^b Retention indices of literature on the apolar column (RI_{Lit}), ^c Retention indices on the apolar Rtx-1 column (RI_a), ^d Retention indices on the polar Rtx-Wax column (RI_p), ^e Percentage are given on the apolar column, ^f RI: Retention Indices, MS: Mass Spectrometry in electronic impact mode. NI: Compounds not identified

4. Discussion

4.1. GC-MS analysis of volatile constituents

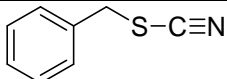
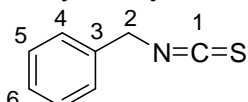
Volatiles come from the aerial parts of *T. majus* L. This plant belongs to the botanical order Brassicales and produces glucosinolates, which are important precursors of nitrogen- and sulfur-containing compounds. Those compounds often present low perception thresholds and various olfactive notes [9,10]. The samples were collected in May and the volatiles were obtained through hydrodistillation. Its chemical composition was analyzed using GC-MS. An analysis of the chemical profile of the isolated volatiles revealed the presence of nine compounds, mainly no-terpenic (86.4 %) and oxygenated no-terpenic (3.3 %). The principal components were benzyl isothiocyanate **6** (82.5 %) and benzene acetonitrile (3.9 %), two odorant nitrogen- and sulfur-containing compounds accounting to 86.4 % of the total volatiles. This result proves that *T. majus* extracts are one of the matrices of choice when the study of organonitrogen and organosulfur compounds is undertaken. These compounds are accompanied by monoterpene hydrocarbons (1.3 %) and sesquiterpene hydrocarbons (1 %). It should be noted that there are no earlier studies about the chemical composition of volatiles of the aerial parts of *T. majus* L. with which to compare our results.

4.2. Structure determination of **6**

Compound **6** was fully characterized by NMR and IR. To prove the exact structure of compound **6**, and more precisely to determine if this compound is the benzyl thiocyanate or the benzyl isothiocyanate, NMR spectroscopy was used. As shown in Table 2, protons of compound **6**, which appear as a singlet at 4.74 ppm, match perfectly with the benzylic protons of the benzyl isothiocyanate. Benzylic carbons of **6** match also with the benzylic carbon of the benzyl isothiocyanate (Table 2).

Additionally, TLC analysis confirms that the benzyl isothiocyanate (*R_f*: 0.2; hexanes) is the major component of the volatile fraction of *T. majus* L. (Garden Nasturtium) from North western Algeria.

Table 2. NMR (¹H, ¹³C) of benzylic position of **6**, benzyl thiocyanate, and benzyl isothiocyanate.

Compound	¹ H NMR (CH ₂) (ppm)	¹³ C NMR (CH ₂) (ppm)
 Benzyl thiocyanate	4.19	38.48
 Benzyl isothiocyanate	4.74	48.75
6	4.74	48.69

IR ν max (cm⁻¹) (film): 3069 (CH=); 2887; 2100 (N=C=S); 1608 (C=C); 1378; 1128; 1062; 763; 701

¹H NMR (400 MHz, CDCl₃, 25°C) δ_{ppm} : 4.74 (s, 2H, H-2); 7.28-7.44 (m, 5H, H-4, H-5 et H-6).

^{13}C NMR (100 MHz, CDCl_3 , 25°C) δ_{ppm} : 134.25 (C-1); 48.69 (C-2); 139.01 (C-3); 128.99 (C-4); 128.39 (C-5); 126.85 (C-6).

5. Conclusion

The profile in yellow-coloured volatile fraction of *Nasturtium* aerial parts (*T. majus*) was investigated. The nitrogen- and sulfur-containing compounds content were evaluated at 86.4 % and the benzyl isothiocyanate was identified as the major compound representing 82.5 % of the total volatiles. *T. majus* can be a potential source of natural organonitrogen and organosulfur compounds due to their wild year round availability and edible character. Therefore, it could be a source of bioactive phytochemicals for human health and the functional food industry.

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