

Volatile Constituents of Aerial Parts of *Capsella rubella* Reut.

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Abstract: Three different isolates from unexplored wild-growing Brassicaceae plant *Capsella rubella* were investigated in order to provide more complete insight of the composition and content of volatile constituents. The analysis of volatile compounds was performed by GC-FID/MS. *C. rubella* essential oil, that is isolate obtained by hydrodistillation, was characterized by a high percentage of 3,4-epithiobutanenitrile (67.8 %). The main volatile compounds in autolysate, *i.e.* isolate obtained by solvent extraction upon endogenous enzymatic hydrolysis, were 3,4-epithiobutanenitrile (44.1 %) and ethyl isothiocyanate (29.4 %). Same as in autolysate, the main volatile constituents of hydrolysate, *i.e.* isolate obtained by solvent extraction upon exogenous enzymatic hydrolysis, were 3,4-epithiobutanenitrile and ethyl isothiocyanate, but with ethyl isothiocyanate as the dominating compound (57.8 %) followed by 3,4-epithiobutanenitrile (15.6 %). The results showed that qualitative and quantitative composition of *C. rubella* volatile constituents depends on the isolation method. But, regardless of the isolation method sulfur- and nitrogen-containing volatile compounds were quantitatively dominating constituents in all isolates.

Keywords: *Capsella rubella*, essential oil, autolysate, hydrolysate, 3,4-epithiobutanenitrile, ethyl isothiocyanate.

INTRODUCTION

THE present paper represents the continuation of our research on chemistry of volatile compounds from Croatian wild-growing Brassicaceae plants.^[1–4] Brassicaceae plants are interesting as a source of sulfur-containing secondary metabolites, glucosinolates. During the extraction of these compounds their decomposition often occurs resulting with the isolation of volatile sulfur- and nitrogen-containing compounds which could be considered artifacts. So, volatile sulfur- and nitrogen-containing compounds are not naturally present in intact plant but are formed during extraction mostly by enzymatic breakdown or thermal degradation of naturally occurring sulfur-containing glucosinolates. These artifacts have been assigned for various biological properties such as anticancer and antimicrobial activities. For example, one class of volatile sulfur-containing compounds, known for its biological activity, are isothiocyanates.^[5]

In this paper we focused on *Capsella rubella* Reut., a plant widespread throughout Mediterranean region of

Croatia. *Capsella rubella* Reut. is the member of the small genus *Capsella* which includes only three species, *C. rubella*, *C. bursa-pastoris* and *C. grandiflora*.^[6] This genus is interesting because it is closely related to the *Arabidopsis thaliana*, a popular model organism in plant biology and genetics.^[7] Only two species of the genus *Capsella* grow in Croatia, *C. bursa-pastoris* and *C. rubella*.^[8] *C. rubella* is often replaced by mistake with *C. bursa-pastoris*, the most widespread *Capsella* specie throughout the world. Unlike *C. bursa-pastoris*, *C. rubella* is native to the Mediterranean. It is an annual ruderal crucifer which is considered a weed, at least in Croatia. It grows at arid soils and on localities exposed to sunlight and is differentiated from *C. bursa-pastoris* mostly for its flowers and siliques. The plants gathered for this research had whitish or reddish-tinged flowers and siliques had concave lateral margins. Same as *C. bursa-pastoris* or shepherd's purse, *C. rubella* or red shepherd's purse, has been used as edible vegetable by local people.^[8] It is known that *C. bursa-pastoris* has been used traditionally as anti-bleeding, anticancer, antithrombin, antioxidant and antidiabetes agent and for fever treatment in many

countries, but there are no records of medicinal properties of *C. rubella*.^[9,10]

There are some reports on chemical composition of volatile compounds of *C. bursa-pastoris* but, to our knowledge, there are no literature data about volatiles from *C. rubella*.^[9,11–14] The objective of this study was to investigate the chemical composition and content of volatile compounds from aerial parts of Croatian *C. rubella*. In order to provide more complete insight of composition and content of these compounds three different isolates of investigated plant, containing sulfur- and nitrogen-containing volatile compounds and other volatile compounds, were prepared and analysed by GC-FID/MS. Knowing that volatile sulfur- and nitrogen-containing compounds compose a portion of many essential oils, traditional methods which are commonly used for the essential oil isolation were used in this investigation too. One of these methods is hydrodistillation performed in Clevenger-type apparatus. Hydrodistillation enables simultaneous thermal degradation of sulfur-containing glucosinolates to volatile artifacts and their isolation together with other volatile compounds characteristic for the essential oils, such as monoterpenes, sesquiterpenes and phenylpropane derivatives. The volatile isolate obtained in this way is called essential oil. Another approach to the isolation of *C. rubella* volatiles used in this study was enzymatic hydrolysis of glucosinolates followed by solvent extraction of liberated volatiles (artifacts) and other volatiles. Enzymatic hydrolysis was performed with endogenous and exogenous myrosinase. Accordingly, two isolates obtained in this way are called autolysate (endogenous myrosinase) and hydrolysate (exogenous myrosinase).

EXPERIMENTAL

Material

Plant material of *Capsella rubella* was collected near Split (Dalmatia County, Mediterranean region of Croatia) during flowering in spring 2020 from wild-growing populations. The whole aerial parts of the plant (leaves, flowers and green siliques together) were used. The botanical identity of the plant material was confirmed by a local botanist and the voucher specimens are deposited. Three types of isolates were prepared, *i.e.* essential oil, autolysate and hydrolysate, and analysed by GC-FID/MS.

Isolation of Volatile Compounds

HYDRODISTILLATION

Essential oil was obtained by hydrodistillation of fresh plant material (200 g) in Clevenger-type apparatus for 3 hours using pentane for trapping. The hydrodistillation was performed by immersing minced plant material in a flask containing hot distilled water (~ 90 °C) and then boiled for

3 hours. After hydrodistillation pentane extract was separated, condenser and inner tube of Clevenger apparatus were washed with 5 mL of pentane two more times. The combined extracts were dried over anhydrous sodium sulfate and concentrated by careful fractional distillation to a final volume of *ca.* 0.5 mL. The obtained essential oil was stored at –18 °C until GC-FID/MS analysis.^[3,4,15,16]

ENDOGENOUS MYROSINASE HYDROLYSIS

In order to obtain another isolate namely autolysate, the fresh plant material (200 g) was chopped, mixed with 800 mL of water and allowed to autolyze, *i.e.* hydrolyzed by endogenous myrosinase, at approximately 27 °C for 24 hours. Upon hydrolysis, water solution was extracted with dichloromethane (100 mL) and dichloromethane extract was separated from the water layer by centrifugation at 3500 rpm. Dichloromethane extraction was repeated for two more times. Dichloromethane extracts were pooled together, dried over anhydrous sodium sulfate, concentrated to *ca.* 10 mL in a rotary evaporator (at 300 mm Hg approx. and 30 °C water bath temperature) and thereafter by careful fractional distillation to a final volume of *ca.* 0.5 mL.^[16,17] The concentrated extract (autolysate) was stored at –18 °C until further analysis.

EXOGENOUS MYROSINASE HYDROLYSIS

The plant material, used for exogenous myrosinase hydrolysis, was air-dried in a shady place at room temperature (~ 18–20 °C) for 10 days. Dried plant material (100 g) was chopped, put in boiled water (400 mL) and boiled for 5 minutes in order to inactivate plant enzymes. Water extract was left to cool to room temperature, 10 mg of exogenous myrosinase (thioglucosidase from *Sinapis alba* purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was added and allowed to hydrolyze at approximately 27 °C for 24 hours. After hydrolysis, extraction of water solution was performed as described above.^[1,2,16] The concentrated extract (hydrolysate) was stored at –18 °C until further analysis.

GC-FID/MS Analysis

The GC-FID analyses were performed on a Agilent Technologies (Palo Alto, USA) gas chromatograph model 7890A equipped with flame ionization detector and using non-polar HP-5MS column (5 % diphenyl and 95 % dimethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.2 µm). GC conditions were as follows: oven temperature was programmed from 70 °C isothermal for 2 min, then increased to 200 °C at a rate of 3 °C min⁻¹ and held isothermal for 20 min; injector temperature 250 °C. Injected volume was 1 µL and split ratio 1 : 50.

The GC-MS analyses were performed on a Agilent Technologies GC-MS system (GC model 7890A with a mass

selective detector model 5975C) using two columns with different polarity of stationary phases: polar HP-FFAP (polyethylene glycol-TPA modified; 50 m × 0.32 mm i.d., film thickness 0.52 μm) and non-polar HP-5MS column. GC operating conditions for polar column were: oven temperature was kept at 70 °C for 3.5 min, then programmed to 180 °C at a rate of 4 °C min⁻¹ and held isothermal for 18 min. For non-polar column GC operating conditions were as described above. Carrier gas was helium with flow rate 1 mL min⁻¹, injector temperature 250 °C, volume injected 1 μL, split ratio 1 : 50. MS conditions were: ionization voltage 70 eV, ion source temperature 280 °C, mass range 30–350 mass units.

Identification and Quantitative Determination of Components

The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C₈–C₂₂ *n*-alkanes. Compounds identification was accomplished by comparing their retention indices (*RI*) and mass spectra (*MS*) with those from our homemade library, as well as by comparing their mass spectra with spectra in the Wiley 275 and NIST02 library spectra databases or reported in the literature.^[1–4,17,18] These homemade *MS* and *RI* are determined from chromatographic data obtained for pure reference compounds obtained commercially and for the main components of many volatile isolates previously investigated in Department of Organic Chemistry, Faculty of Chemistry and Technology, University of Split. Mass spectras (*MS*) and retention indices (*RI*) on two columns from our homemade library were used for identification (denoted with (a) in Table 1). Some compounds were identified by comparing their *MS* with mass spectra stored in the Wiley 275 and NIST02-library spectra databases (denoted with (b) in Table 1). Furthermore, some compounds were identified comparing their mass spectra or retention indices with those found in literature (denoted with (c) in Table 1). The percentage composition of the samples was computed from the GC peak areas without using correction factors.

RESULTS AND DISCUSSION

Volatile sulfur- and nitrogen-containing compounds along with other volatile compounds obtained by hydrodistillation or enzymatic hydrolysis are given in Table 1. Twenty compounds were identified in both essential oil and autolysate, representing 98.2 % and 95.2 % of the total volatiles, respectively. On the other hand, eleven compounds identified in hydrolysate represented 89.7 % of the total volatiles.

Among volatile compounds found in the essential oil, ten compounds could be considered artifacts formed by

thermal degradation of naturally occurring glucosinolates and they composed the largest proportion of the total volatiles present in the essential oil (78.7 %). Seven of these compounds were sulfur-containing volatile compounds, namely isothiocyanates and three were both nitrogen- and sulfur-containing compounds, namely epithionitriles and nitriles. The dominating compound in the essential oil was epithionitrile, precisely 3,4-epithiobutanenitrile (67.8 %). This compound probably originated from degradation of glucosinolate with the trivial name sinigrin. Other quantitatively important constituent of the essential oil was allyl isothiocyanate (6.3%), also sinigrin degradation product. All other compounds belonging to the class of glucosinolate degradation products were present in the essential oil in much smaller amounts (< 1%). Two remaining nitriles, 10-(methylthio)decanenitrile and 11-(methylthio)undecanenitrile were identified both in the amount of 0.9 %. They probably originated from two glucosinolates, 9-(methylthio)nonyl glucosinolate and 10-(methylthio)decyl glucosinolate. One more sulfur-containing compound was identified in *C. rubella* essential oil, namely dimethyl trisulfide (2.3 %). Dimethyl trisulfide is not glucosinolate degradation product, but probably originated from *S*-methyl-L-cysteine sulphoxide. This amino acid derivative is known to be abundant and widespread in *Brassica* vegetables.^[15]

Regarding the essential oil constituents without sulfur or nitrogen the most abundant were aliphatic alcohols (8.1 %) and carbonyl compounds (5.1 %). The major compounds among them were (*Z*)-hex-3-en-1-ol, known as leaf alcohol (6.6 %), (*E*)-hex-2-enal (3.4 %), known as leaf aldehyde, and (*E*)-hex-2-en-1-ol (1.5 %). These compounds are considered common flavor and fragrance compounds and occur in the essential oils obtained from green parts of many plants but usually in small quantities.^[19] It is well known that these compounds are biosynthesized through lipoxygenase pathway from C₁₈-unsaturated fatty acids such as linolenic acid.^[20] In contrast to the majority of essential oils, which are mainly constituted of terpenes and phenylpropane derivatives, *C. rubella* essential oil contained only two terpene compounds, β-cyclocitral (0.3 %) and (*E*)-β-ionone (0.9 %).

In *C. rubella* autolysate only five sulfur- and nitrogen-containing volatile compounds were identified but they constituted 78.0 % of all volatiles. Four of these compounds were so-called artifacts, formed by glucosinolates hydrolysis. Same as in the essential oil, the main compound was 3,4-epithiobutanenitrile (44.1 %), glucosinolate sinigrin hydrolysis product. In contrast to the essential oil, another quantitatively important constituent of autolysate was ethyl isothiocyanate (29.4 %), hydrolysis product of glucosinolate glucolepidiin. The two remaining compounds probably originated from glucosinolates hydrolysis, presented in

Table 1. Chemical composition of *C. rubella* volatiles.

No	Compound name	RI ^(d)		Peak area ^(e) / %			No	Compound name	RI ^(d)		Peak area ^(e) / %		
		HP-5MS/ HP-FFAP		I	II	III			HP-5MS/ HP-FFAP		I	II	III
	Alkanes						20	4-methylpentyl isothiocyanate ^{(a),(c)}	1170 / 1678	0.5	–	–	
1	undecane ^{(b),(c)}	1103 / –	–	0.4	0.4		21	3-(methylthio)propyl isothiocyanate ^{(a),(c)}	1321 / 2183	0.5	–	–	
2	dodecane ^{(b),(c)}	1204 / –	–	0.4	–		22	9-(methylthio)nonyl isothiocyanate ^{(a),(c)}	1988 / –	0.2	–	–	
3	nonadecane ^{(b),(c)}	1900 / –	–	–	1.1		Nitriles and epithionitriles						
	Aliphatic alcohols and carbonyls						23	3,4-epithiobutanenitrile ^{(a),(c)}	“ / 1488	67.8	44.1	15.6	
4	(<i>E</i>)-hex-2-enal ^{(a),(b),(c)}	“ / 1338	3.4	1.7	–		24	10-(methylthio)decanenitrile ^{(a),(c)}	1748 / –	0.9	–	–	
5	(<i>Z</i>)-hex-3-en-1-ol ^{(a),(b),(c)}	“ / 1478	6.6	2.7	–		25	11-(methylthio)undecanenitrile ^{(a),(c)}	1854 / –	0.9	–	–	
6	(<i>E</i>)-hex-2-en-1-ol ^{(a),(b),(c)}	“ / 1478	1.5	–	–		Other sulfur-containing compounds						
7	(<i>E</i>)-hept-2-enal ^{(b),(c)}	961 / 1451	–	1.2	0.9	26	dimethyl trisulfide ^{(a),(b)}	978 / 1517	2.3	0.2	–		
8	(<i>E,E</i>)-hepta-2,4-dienal ^(b)	1018 / 1600	–	0.3	–		Terpenes						
9	phenylacetaldehyde ^{(a),(b)}	1053 / –	0.5	–	–	27	α -pinene ^{(a),(b)}	940 / 1127	–	3.2	–		
10	nonanal ^{(b),(c)}	1109 / –	0.9	0.3	–	28	camphene ^{(a),(b)}	956 / –	–	0.6	–		
11	(<i>E</i>)-dec-2-enal ^(b)	1270 / –	–	2.2	–	29	1,8-cineole ^{(a),(b)}	1038 / –	–	0.5	–		
12	(<i>E,Z</i>)-dec-2,4-dienal ^(b)	1300 / 1918	–	0.4	–	30	β -cyclocitral ^{(b),(c)}	1227 / 1771	0.3	–	–		
13	(<i>E,E</i>)-dec-2,4-dienal ^(b)	1325 / 1969	–	0.9	–	31	(<i>E</i>)- β -ionone ^{(a),(b)}	1492 / 2111	0.9	–	0.5		
14	tetradecanal ^{(b),(c)}	1724 / –	0.3	–	–		Other compounds						
	Isothiocyanates						32	1,3-di- <i>tert</i> -butylbenzene ^(b)	1260 / 1547	–	0.9	2.7	
15	ethyl isothiocyanate ^{(a),(c)}	“ / 1331	–	29.4	57.8	33	5,6,7,7a-tetrahydro-2-(4H)-benzofuranone ^(b)	1538 / –	–	–	3.6		
16	allyl isothiocyanate ^{(a),(b),(c)}	“ / –	6.3	4.0	2.4	34	1,2-diphenylethanedione ^(b)	1823 / –	–	–	4.4		
17	isobutyl isothiocyanate ^{(a),(c)}	959 / –	0.1	–	–	35	diisobutyl phthalate ^(b)	1878 / –	2.8	–	–		
18	but-3-enyl isothiocyanate ^{(a),(b),(c)}	989 / 1590	0.7	0.3	–	36	dibutyl phthalate ^(b)	1974 / –	–	1.5	0.3		
19	3-methylbutyl isothiocyanate ^{(a),(c)}	1066 / 1562	0.8	–	–		Total identified / %						
									98.2	95.2	89.7		

^(a) Compound identified by mass spectra and *RI* comparison with homemade library.

^(b) Compound identified by mass spectra comparison with Wiley 275 and NIST02 mass spectral libraries.

^(c) Compound identified by mass spectra or retention indices comparison with those found in literature.

^(d) Retention indices on HP-5MS and HP-FFAP columns

^(e) I = essential oil; II = autolysate; III = hydrolysate; – = not detected; “ = *RI* outside the limits of C₈-C₂₂ *n*-alkanes.

smaller amounts, were allyl isothiocyanate (4.0 %) and but-3-enyl isothiocyanate (0.3 %). But-3-enyl isothiocyanate is hydrolysis product of glucosinolate gluconapin. The fifth sulfur-containing volatile compound, dimethyl trisulfide, was the minor constituent of the autolysate (0.2 %).

Among fifteen compounds without sulfur or nitrogen identified in *C. rubella* autolysate, the majority were carbonyl compounds (7.0 %) and terpenes (4.3 %). The most abundant compound was monoterpene α -pinene (3.2 %), followed by leaf alcohol (*Z*)-hex-3-en-1-ol (2.7 %), the only one aliphatic alcohol identified in the autolysate. In contrast to the essential oil, the content of the compounds known as "green notes" is smaller, 4.4 % opposite to 11.5 %.

The hydrolysate is characterized by a smaller number of volatile compounds than the essential oil and autolysate, eleven opposite to twenty. But, same as in the essential oil and autolysate, the main constituents were sulfur- and nitrogen-containing compounds. Three of these compounds were found, namely ethyl isothiocyanate, 3,4-epithiobutanenitrile and allyl isothiocyanate, which represented 75.8 % of the total volatiles identified. The dominating compound was ethyl isothiocyanate (57.8 %), followed by 3,4-epithiobutanenitrile (15.6 %). These compounds were the main constituents in autolysate too, but with 3,4-epithiobutanenitrile as the main one. Both volatile isolates, autolysate and hydrolysate, were obtained by solvent extraction performed at room temperature upon hydrolysis, either by endogenous or exogenous

myrosinase. On contrary, ethyl isothiocyanate was not found in the essential oil. Essential oil is volatile isolate obtained by hydrodistillation, meaning at harsh conditions (plant material in boiling water for 3 hours). It is known from the literature that, in most cases, isothiocyanates are sensitive to thermal degradation in aqueous medium. The stability of some isothiocyanates, such as allyl isothiocyanate, methyl isothiocyanate, sulforaphane and benzylic-type isothiocyanates, in aqueous solution has been investigated.^[21–25] In all mentioned papers the liquid-liquid extraction of volatile degradation products from aqueous solution was performed, either with ether^[21] or dichloromethane. And, in case of dichloromethane extraction, the dichloromethane extracts were analyzed by GC-MS.^[22–25] Bridgart and Wilson reported the decomposition of methyl isothiocyanate, in aqueous solution even at room temperature within a ca. 1 month, and identified two decomposition products, *N*-methylthiocarbamate and 2,4-dimethyl-1,2,4-thiadiazolidine-3,5-dithione.^[26] As far as we know the stability of ethyl isothiocyanate under hydrodistillation conditions has never been investigated. However, taking into account all the facts about stability of related isothiocyanates, the lack of ethyl isothiocyanate in *C. rubella* essential oil could be ascribed to its thermal degradation and leaching of the possible degradation products into the boiling water.

Besides above mentioned compounds, other compounds identified in hydrolysate were present in smaller percentages, with carbonyl compounds as the main ones (8.9 %). Only one terpene compound was found, that is (*E*)- β -ionone (0.5 %). The compounds such as leaf alcohol and aldehyde were absent in this isolate probably as a result of plant material drying. It was expected knowing that these compounds are characteristic for fresh plant material and are even called "green notes".

The main similarity between volatile compounds found in three investigated isolates of *C. rubella* was that all isolates contained mostly sulfur- and nitrogen-containing compounds while the other compounds were present in much smaller amounts. All isolates contained two common sulfur- and nitrogen-containing compounds, namely 3,4-epithiobutanenitrile and allyl isothiocyanate. Regarding 3,4-epithiobutanenitrile, it is well-known that the formation of this compound is affected by the presence of epithiospecifier protein which causes the formation of epithionitriles instead of isothiocyanates during glucosinolate hydrolysis. The highest percentage of this epithionitrile in the essential oil was unexpected and even opposite to the findings of Matusheski *et al.* who reported that epithiospecifier protein is heat-sensitive.^[27] The main difference among volatile compounds in these isolates was the presence of ethyl isothiocyanate which was quantitatively important constituent of autolysate and the main constituent of hydrolysate, but was absent in the essential oil.

As was mentioned in Introduction section, as far as we know there are no reports regarding *C. rubella* volatile compounds. However, there are some reports on volatile compounds of closely related *C. bursa-pastoris*. Daxenbichler *et al.* identified four isothiocyanates in *C. bursa-pastoris* seeds from Turkey, erucin, arabin, camelinin and 9-methylsulphonylnonyl isothiocyanate. They, also, noted the presence of allyl isothiocyanate and iberberin which were previously reported in older papers.^[11] Vaughn and Berhow identified but-3-enyl isothiocyanate as the major constituent of autolysate obtained from commercially available seeds of *C. bursa-pastoris*.^[12] Kamali *et al.* investigated the essential oil from the aerial parts of Iranian *C. bursa-pastoris* in which they identified hydrocarbons as the most abundant constituents of the oil, followed by ethyl linoleate and palmitic acid. They identified only one sulfur-containing volatile compound, *i.e.* allyl isothiocyanate.^[9] In one older paper, Miyazawa *et al.* reported the essential oil composition of Japanese *C. bursa-pastoris* in which they did not identify any glucosinolate degradation products. The major constituent in the essential oil, either from aerial parts or root, was terpene compound camphor.^[14]

Different approaches to the volatile compounds isolation, *i.e.* hydrodistillation or solvent extraction upon enzymatic hydrolysis, ensures better conclusion about volatiles present in this wild-growing Brassicaceae plant. The results of this study showed that qualitative and quantitative composition of *C. rubella* volatile constituents depends on the isolation method. However, regardless of the isolation method sulfur- and nitrogen-containing volatile compounds were quantitatively dominating constituents in all isolates.

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