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Exudate Compounds of *Origanum* Species †

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Abstract: *Origanum* species are valuable medicinal and culinary herbs, more that their biocidal properties are very important for organic farming. The first substances involved in allelopathic interactions in nature are the exudate (surface) compounds. In the present study, acetone exudates of ten samples of *Origanum* species were comparatively analyzed by GC/MS and TLC. Plant material of *Origanum dictamnus* L., *Origanum vulgare* L. and *Origanum vulgare* subsp. *hirtum* as the latter taxa was represented by 8 patterns with different origin were studied. Flavonoid aglycones, terpenes, fatty acids and alcohols, triterpene acids and phenolic derivatives were identified. Methylated derivatives of flavones and non-methylated flavanones (naringenin and eriodictyol) were identified as the most common flavonoid aglycones. The most complex flavonoid profile was detected for *O. vulgare* ssp. *hirtum* samples. A few differences in the flavonoid profiles of *O. vulgare* ssp. *hirtum* from different origin of were found. Carvacrol was determined as main component of *O. vulgare* subsp. *hirtum* samples, whereas in *O. vulgaris* exudate long-chain fatty alcohol was found as abundant compound. The data obtained complement the knowledge of the distribution and role of exudate compounds.

Keywords: *Origanum vulgare* subsp. *hirtum*; *Origanum dictamnus*; Greek oregano; flavonoid aglycones; carvacrol; long-chain fatty alcohols

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1. Introduction

Exudate flavonoids and their distribution among the plant kingdom have been comprehensive studied by Prof. Eckhard Wollenweber and the followers of this approach for analysis [1–7]. In addition to being taxonomically important, these compounds have important ecological functions due to their location on the surface on plant. Exudate flavonoids often are defined as a surface, external and lipophilic [3,5]. Besides flavonoid aglycones in the exudate are contained also terpenes, fatty alcohols and acids, alkanes, phenolic compounds.

Origanum species are valuable medicinal and culinary herbs and their biocidal activity are very important for organic farmig in the last decades [8–10]. The results of the research on allelopathic interactions are scientific basis for selection of plant products with biocidal properties [11,12]. The first substances involved in these interactions in nature are the exudate compounds. Variability of production of metabolities in *Origanum* species have been reported not only during vegetation season [12] but also depending on the environmental conditions [13,14].

In the present study, acetone exudates of ten samples of *Origanum* species were comparatively analyzed by GC/MS and HPTLC. Plant material of *Origanum dictamnus*, *Origanum vulgare* and *Origanum vulgare* subsp. *hirtum* (Greek, oregano) as the latter taxa was represented by 8 patterns with different origin were examined.

2. Experiments

2.1. Plant Material

Aerial parts of studied samples were collected from plant collections of experimental fields at Institute of Roses, Essential and Medicinal Cultures Kazanluk and Institute of Biodiversity and Ecosystem Research (IBER) as well as from natural populations. The detailed information was presented at Table 1.

Table 1. Description of studied plant material.

No	Taxon	Description of Origin
Od	*O. dictamnus*	Plant collection Kazanlak, source material (seeds) purchased from seed plot https://zelena-prolet.com/
Ov	*O. vulgare*	Natural population, Trigrad, Bulgaria
Oh1	*O. vulgare* subsp. *hirtum*	Natural population, at the Struma valley Bulgaria
Oh2	*O. vulgare* subsp. *hirtum*	Plant collection IBER, source material (seeds) from natural population http://www.iber.bas.bg/sites/default/files/projects/plantscollection
Oh3	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, source material (seeds) purchased from Germany company https://www.pharmasaat.de
Oh4	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, source material from natural population, northern Greek
Oh5	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, Hebros variety
Oh6	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, candidate variety
Oh7	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, hybrid 1, seed progeny of *O. vulgare* subsp. *hirtum* obtained by free pollination of *O. vulgare* subsp. *hirtum* and *O. vulgare*
Oh8	*O. vulgare* subsp. *hirtum*	Plant collection Kazanlak, hybrid 2, seed progeny of *O. vulgare* subsp. *hirtum* obtained by free pollination of *O. vulgare* subsp. *hirtum* and *O. vulgare*

2.2. Preparation of Extracts

Plant exudates were prepared from 2 g air dried, not ground aerial parts by rinsing with 20 mL acetone for several minutes to dissolve compounds accumulated on the surface of plant tissue. The obtained extracts were concentrated for further GC/MS and TLC analysis.

2.3. TLC Analysis

Three TLC sorbents and mobile phases were used for the analysis of the flavonoid exudates. Toluene/dioxan/acetic acid (95:25:4, *v*/*v*/*v*) was used for the development of exudates on silica gel plates Kiselgel 60 F254. Toluene/methylethylketon/methanol (60:25:15, *v*/*v*/*v*) was used for Polyamid 11 F254 plates and water:acetic acid (60:40) for Celluluse F plates.. Chromatograms were viewed under UV light at 336 nm before and after spraying with "Naturstoffreagenz A", 1% solution of diphenylboric acideethanolamine complex in methanol. Flavonoid aglycones were identified by direct TLC comparison with markers available in E. Wollenweber's laboratory.

2.4. GC/MS Analysis

For GC/MS analysis 300 μL of each exudate was transferred to a vial and evaporated to dryness, then silylated with 50 μL of N, O-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) in 50 μL of pyridine for 2 h at 50 °C. The spectra were recorded on a Thermo Scientific Focus GC combined with a Termo Scientific DSQ mass detector as described previously [15].

3. Results

Ten exudates of *Origanum* samples listed at Table 1 were comparatively analyzed for determination of their main constituents. Flavonoid aglycones, fatty acids and alcohols,

terpenes and phenolic derivatives were identified. The flavonoid profiles of studied samples were determined by TLC. The results are presented at Table 2.

Table 2. Identified flavonoid aglycones in the studied samples by TLC. Od *O. dictamnus;* Ov *O. vulgare;* Oh1–Oh8 *O. vulgare* subsp. *hirtum* (details Table 1) Me—methyl ether.

Compounds	Od	Ov	Oh1	Oh2	Oh3	Oh4	Oh5	Oh6	Oh7	Oh8
Apigenin	●	●	●	●	●	●	●	●	●	●
Scutellarein 6,7-diMe	●			●			●		●	
Scutellarein 6,7,4' -triMe					●					
Scutellarein 6,7,8-triMe (*Xantomicrol*)	●						●	●		●
Luteolin	o	●	●	●	●	●	●	●	●	●
Naringenin			●	●	●	●	●	●	●	●
Eriodictyol		o	●	●	●	●	●	●	●	●

Methylated derivatives of flavones—apigenin and luteolin and non-methylated flavavanones: naringenin and eriodictyol were identified as the most common flavonoid aglycones. The flavonoid profile of *O. vulgare* was found to be the simplest. Only simple flavonoids—apigenin and luteolin as well as eridictyol in trace amount were established. The most complex flavonoid profile was detected for *O. vulgare* ssp. *hirtum* samples. A few differences in the flavonoid profiles of *O. vulgare* ssp. *hirtum* from different origin of were found. The exudates of Hebros variety, candidate variety and hybrid 2 contain xanthomicrol compound that was abundant in *O. dictamnus* exudate. Also scutellarein 6,7-dimethyl ether abundant of *O. dictamnus* profile was found in a few patterns of *O. vulgaris* ssp. *hirtum*—Hebros variety, hybrid 1 and plant material of IBER ex situ collection (Oh2).

The exudates of the studied samples were analysed for identification of other components by GC/MS analysis. The results are presented at Table 2. Monoterpene—carvacrol and long chain alcohol—1-hexacosanol were identified as the main component in the exudates. Differences on species level were found. *O. vulgaris* exudate contained about 60% of 1-hexacosanol but carvacrol was determined in low amount whereas in *O. vulgare* ssp. *hirtum* carvacrol was found as dominant component. Carvacrol content varied between different patterns of the last taxon in the range 5—49%. The sample of the natural locality from Bulgaria (Oh1) contained carvacrol in the largest amount. Exudates of hybrid 1, Hebros variety contained significant amount of carvacrol too. Hexacosanol except in *O. vulgare* profile was found in significant amount also in sample from natural Greek population (Oh4) and ex situ collection of IBER (Oh2). Triterpene acids and derivatives were identified also as the most common metabolite for studied samples. Ursolic acid was found in larger amount in exudates of *O. dictamnus, O. vulgare* and *O. vulgare* ssp. *hirtum* from natural population(Oh1). Oleanolic acid was determined only in *O. vulgare* exudate.

Table 3. Identified compounds of studied exudates of Origanum species; Od *O. dictamnus;* Ov *O. vulgare;* Oh1–Oh8 *O. vulgare* subsp. *hirtum* (details Table 1).The quantities are expressed in relative percentages (area %).

Compounds	Od	Ov	Oh1	Oh2	Oh3	Oh4	Oh5	Oh6	Oh7	Oh8
Carvacrol	**13.6**	**0.9**	**49.2**	**14.7**	**30.8**	**5.1**	**29.1**	**31.6**	**39.1**	**14.5**
Copaene	4.7									
Caryophyllene	1.4	0.8	3.3		0.8			1.3		
Hydroquinone derivative	3.4		19.6	0.5	0.4	0.4	5.6	3.2	7.6	1.6
Fatty acid	1.9									
Caryophyllene oxide	2.7	0.4	0.4		0.8				0.3	0.1
Tetradecanol							1.9			
Hexadecanol			1.1		0.2					
Hexadecanoic acid		1.1		0.8	0.4	0.3	0.6	0.1		0.1
Octadecatrienoic acid				0.7						0.1

Octadecanol						0.4		0.2		0.1
Polyunsaturated fatty acid					8.5	10.8	1.9			
Polyene					1.5	2.4	1.2			
Tetracosanol						1.7		0.6		1.2
Hexacosanol	**13.4**	**61.1**	**2.3**	**36.5**	**31.2**	**46.5**	**8.9**	**12.6**	**5.7**	**33.4**
Unsaturated fatty acid	1.8		1.2	1.1	8.5		22.5		0.6	9.8
ß-Sitosterol						1.4		0.2		0.6
Triterpene	2.1	0.5	4.6	1.1	0.3	2.9	2.2	1.2	1.1	0.6
Oleanolic acid		2.9								
Ursolic acid	8.2	6.9	10.4		0.2	0.8	1.3	0.2		

4. Discussion

First study on exudate flavonoids of *Origanum* species have been conducted by Tomás-Barberán et al. [16] but detailed summary, supplemented with new research have been reported by Skoula et al. [17]. The data obtained in the present study are consistent with the results received by Skoula et al., [17]. In addition to the flavonoids, data on the other components in the exudates was reported for the first time in the present study. Carvacrol and hexacosanol were established as the main components of the studied samples. Strong antifungal, antibacterial and phytotoxic properties have been proven for carvacrol [18]. Inhibitory affect on acetylcholinesterase and larvicidal activity have been demonstrated for hexacosanol [19]. This fatty alcohol has been reported for *Origanum vulgare* subsp. *virens* [20]. Methylated flavonoids such as xanthomicrol has been established to displayed acetylcholinesterase activity [21] that implies a manifestation of incecticidal action [22]. The presence of methylated flavonoids in certain patterns of *O. vulgare* ssp. *hirtum* (Oh2, Oh7, Oh8) can be interpreted that they are synthesized as a result of hybridization or as a protective response to stressors, because these compounds have established insecticidal and antimicrobial properties. Detected ursolic acid of the exudates posseses significant biological activity including antibacterial [23]. The presence of bioactive compounds on the surface of plants suggests their protective role against abiotic and biotic stress factors.

5. Conclusions

In the present study metabolite profiles of exudates of ten samples on three *Origanum* taxa were determined. Monoterpene phenol (carvacrol), long-chain primary fatty alcohol (hexacosanol), ursolic acid, methylated flavones and non-methylated flavanones were determined as main bioactive compounds. These are substances with proved strong biocidal activity that suggests their protective role for plants.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

IBER	Institute of Biodiversity and Ecosystem Research
GC/MS	Gas Chromatography/Mass Spectrometry
TLC	Thin layer chromatography

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