https://doi.org/10.31925/farmacia.2020.5.13 **ORIGINAL ARTICLE**

POTENTIALLY BIOACTIVE VOLATILE COMPOUNDS OF SOME *EPIPHYLLUM*, *HYLOCEREUS* AND *OPUNTIA* SPECIES (*CACTACEAE* FAMILY) CULTIVATED IN CENTRAL EUROPE AND THEIR HS-SPME GC-MS ANALYSIS

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Abstract

The aim of our work was to analyse volatile compounds obtained from the fruits of three plant genera – *Epiphyllum*, *Hylocereus* and *Opuntia* – using HS-SPME GC-MS (headspace solid phase microextraction gas chromatography - mass spectrometry) methods. In violet *Epiphyllum* fruits, 12 compounds were identified, the most abundant being 2,3-butane-diol and hexanoic acid. In pink *Epiphyllum* fruits, 13 compounds were identified, the most abundant being (*E*)-anethol and 1-nonadecene. In green *Epiphyllum* fruits, 10 compounds were identified, the most abundant being (*E*)-anethol and methyl eugenol. In white-fleshed *Hylocereus* fruits, 9 compounds were identified, the most abundant being 1-hexadecanol and nonadecane. In red-fleshed *Hylocereus* fruits, 11 compounds were identified, the most abundant being 1-hexadecanol and nonadecane. Analysis of violet *Opuntia* fruits yielded 12 compounds, the most abundant being nonanal and ethyl decanoate. Analysis of orange *Opuntia* fruits yielded 8 compounds, the most abundant being ethyl decanoate and (3*E*)-1,3-octadecadiene.

Rezumat

Scopul lucrării noastre a fost analiza compușilor volatili din fructele obținute de la trei genuri – *Epiphyllum*, *Hylocereus* și *Opuntia* – folosind o metodă HS-SPME GC-MS. În fructele violete de *Epiphyllum*, au fost identificați 12 compuși, cei mai abundenți fiind 2,3-butan-diolul și acidul hexanoic. În fructele de *Epiphyllum* roz, au fost identificați 13 compuși, cei mai abundenți fiind (*E*)-anetolul și 1-nonadecenul. În fructele de *Epiphyllum* verzi au fost identificați 10 compuși, cei mai abundenți fiind (*E*)-anetolul și metil eugenolul. În fructele de *Hylocereus* cu mezocarpul alb, au fost identificați 9 compuși, cei mai abundenți fiind 1-hexadecanolul și nonadecanul. În fructele de *Hylocereus* cu mezocarpul roșu, au fost identificați 11 compuși, cei mai abundenți fiind 1-hexadecanolul și nonadecanul. Prin analiza fructelor violete de *Opuntia* am obținut 12 compuși, cei mai abundenți fiind nonanalul și decanoatul de etil. Prin analiza fructelor portocalii de *Opuntia* am obținut 8 compuși, cei mai abundenți fiind decanoatul de etil și (3*E*)-1,3-octadecadiena.

Keywords: *Epiphyllum* ssp., *Hylocereus* ssp., *Opuntia* ssp., HS-SPME GC-MS

Introduction

Plant hybrids of the *Epiphyllum* Haw. genus are cultivated mostly as ornamental plants. Besides, fruits of *Epiphyllum* hybrids are edible. The genus *Hylocereus* (Berger) Britt. is famous for its interestingly looking edible fruits (pitaya/pitahaya, or dragon fruit). *Opuntia* ssp. Mill. fruits (tuna, cactus pears or prickly pears) and cladodes are widely consumed. The fruits can be used as natural colourants safe sources. Main classes of compounds present in this genus include betalains (red betacyanins and yellow betaxanthins), phenolic compounds, triterpenes, sterols, fatty acids, mucilage, vitamins, minerals and fibre [9, 11, 14, 15, 18, 28, 31, 32]. Current research indicates that these plants possess antioxidant, antiinflammatory, antiproliferative, hepatoprotective, antidiabetic, antihyperlipidaemic, antibacterial, anti-ulcerogenic, neuroprotective, hepato-protective and diuretic activity and wound healing activities [2, 9, 10, 13-18, 20, 22, 24, 26, 28, 30, 33].

The main objective of this study was to analyse the volatile compounds content and their composition in *Epiphyllum* ssp., *Opuntia* ssp. and *Hylocereus* ssp. fruits (*Cactaceae*) originating from plants cultivated in the Central Europe. The plant materials were collected from three gardens. This work is part of comprehensive pharmacognostic analyses. The efficient potent novel analytical method employed in this study used a combination of headspace solid phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry (GC-MS).

Materials and Methods

Plant material

Fruits of seven taxa of the *Cactaceae* family were analysed for the presence and quantity of volatiles. *Epiphyllum* ssp. Haw. (*Hylocereeae* tribe) hybrids (EE × EK – violet, EE × EE – pink and EK × EE – green fruits) from a private garden in Modra, SK [11]; *Hylocereus* (Berger) Britt. (*Hylocereeae* tribe; *H. costaricensis* – red-fleshed pitaya and *H. undatus* white-fleshed pitaya) from the Botanical Garden in Szeged, HU; *Opuntia* ssp. Mill. (*Opuntieae* tribe; *O. humifusa* – violet and *O. ficus-indica* – orange fruits) from the Comenius University Botanical Garden in Bratislava, SK. Ripe fruits were collected in September 2018. The *Epiphyllum* ssp. and *Opuntia* ssp. fruits originated from 5-years to 10-years old fruticose plants, and the pitaya/pitahaya (*Hylocereus*) from arboreous plants. Herbarium samples have been deposited at the Department of Pharmacognosy and Botany (FPHARM CU, SK). At the time of analysis, frozen (-18°C) fruits were cut into small pieces. They were placed in vials and directly used for HS-SPME GC-MS analysis.

Equipment

The composition of volatile compounds was measured by SPME GC-MS [AGILENT 6890/5973N GC/FID (Santa Clara, USA), CTC Combi PAL sampler (CTC Analytics AG, CH)]. Details of the analytical procedures have been published in a recent paper from our laboratory [8].

Results and Discussion

We determined the volatile compounds’ content in fruits of plants of the *Epiphyllum*, *Hylocereus* and *Opuntia* genera cultivated in Central Europe using the HS-SPME GC-MS technique. This method is a relatively new, very sensitive analytical method, particularly when used in pharmacognostic analyses/ control. The benefits of the HS-SPME method in pharmagnostic analysis are: small sample weight, direct handling of fresh, frozen or dry herbal drugs – without the need of hydrodistillation, the possibility to analyse volatile compounds of any plant – not restricted to typical essential oil bearing plants. The sensitivity of the GS-MS method is very good. Results of the HS-SPME GC-MS analyses are summarized in Table I.

Table I

Percentage compositions of volatile oil components of *Epiphyllum* ssp., *Hylocereus* ssp. and *Opuntia* ssp.

RT [min]	Essential oil compounds	KI	EV [%]	EP [%]	EG [%]	HW [%]	HR [%]	OV [%]	OO [%]
3.23	2,3-butanediol	773	27.1 ± 0.2	–	–	–	–	–	–
3.34	unknown	820	–	–	–	–	–	3.1 ± 0.2	–
7.31	ethyl hexanoate	998	–	–	–	–	–	2.0 ± 0.1	6.3 ± 0.5
7.43	1-octanol	1068	–	–	–	–	–	7.4 ± 0.6	–
9.55	nonanal	1100	–	–	–	–	–	43.5 ± 2.1	11.8 ± 1.0
9.62	*cis*-rose oxide	1108	2.1 ± 0.1	1.1 ± 0.1	9.0 ± 0.7	–	–	–	–
11.35	ethyl octanoate	1196	–	–	–	–	–	–	5.1 ± 0.4
11.46	α-terpineol	1186	1.9 ± 0.1	–	6.7 ± 0.5	–	–	–	–
12.06	neral	1238	–	1.6 ± 0.1	–	–	–	–	–
12.41	geraniol	1249	–	11.7 ± 0.1	–	–	–	–	–
12.71	orcinol dimethyl ether	1259	–	2.3 ± 0.2	–	–	–	–	–
14.61	(3*E*)-1,3-octadecadiene	1267	–	–	–	–	–	–	21.9 ± 2.0
13.09	(*E*)-anethol	1282	0.6 ± 0.0	28.1 ± 2.2	20.5 ± 2.0	–	–	–	–
14.86	ethyl decanoate	1395	–	–	–	–	–	12.1 ± 1.1	35.1 ± 3.2
15.88	unknown	1495	–	–	–	–	1.4 ± 0.1	–	–
16.61	methyl eugenol	1403	0.5 ± 0.0	–	14.7 ± 1.1	–	–	–	–
16.65	(*Z*)-methyl isoeugenol	1459	–	–	–	–	–	1.1 ± 0.1	–
17.95	dimethyl ionone	1565	–	–	–	–	1.1 ± 0.1	–	–
18.03	ethyl dodecanoate	1595	–	–	–	–	–	4.0 ± 0.3	10.6 ± 1.0
18.35	unknown	1609	–	–	–	–	–	3.3 ± 0.2	–
19.08	unknown	1620	–	–	–	1.7 ± 0.1	0.7 ± 0.0	–	–
19.16	1-heptadecene	1689	1.1 ± 0.1	2.0 ± 0.2	–	–	–	–	–
19.46	heptadecane	1700	–	2.3 ± 0.2	–	3.0 ± 0.3	3.6 ± 0.3	–	–
19.56	unknown	1638	–	–	–	–	–	3.1 ± 0.2	–
19.81	unknown	1356	–	–	–	0.8 ± 0.0	1.0 ± 0.0	–	–
20.74	unknown	1699	–	–	–	–	–	3.0 ± 0.1	–
21.43	3,4,5-trimethoxy benzoic acid	1771	–	6.5 ± 0.4	–	–	–	–	–

RT [min]	Essential oil compounds	KI	EV [%]	EP [%]	EG [%]	HW [%]	HR [%]	OV [%]	OO [%]
21.62	hexahydrofarnesyl acetone	1845	–	3.9 ± 0.3	–	–	–	–	–
21.93	1-hexadecanol	1874	–	–	–	64.2 ± 5.0	63.7 ± 5.3	–	–
22.07	1-nonadecene	1891	5.4 ± 0.5	14.0 ± 1.2	–	7.2 ± 0.6	6.8 ± 0.5	–	–
22.35	nonadecane	1900	–	3.8 ± 0.3	–	7.8 ± 0.6	8.0 ± 0.6	–	–
22.81	methyl hexadecanoate	1921	–	–	–	6.00 ± 0.5	6.3 ± 0.5	–	–
23.43	hexadecanoic acid	1959	15.2 ± 1.2	10.2 ± 1.0	7.0 ± 0.6	2.00 ± 0.2	1.6 ± 0.1	9.4 ± 0.8	3.9 ± 0.3
23.92	ethyl hexadecanoate	1993	10.4 ± 0.9	–	13.2 ± 1.2	–	–		
25.23	1-octadecanol	2077	–	–	–	–	–	–	–
25.35	heneicosane	2100	2.9 ± 0.2	3.4 ± 0.3	3.8 ± 0.3	–	3.5 ± 0.3	–	–
25.41	1-eicosanol	2276	–	–	–	4.2 ± 0.3	–	–	–
26.12	linoleic acid methyl ester	2092	–	–	–	–	–	4.2 ± 0.3	4.4 ± 0.3
26.22	linoleic acid ethyl ester	2155	8.4 ± 0.7	–	7.5 ± 0.6	–	–	–	–
26.34	ethyl oleate	2185	17.8 ± 1.5	–	12.7 ± 1.1	–	–	–	–
	Total identified		**93.4**	**9.9**	**95.1**	**96.9**	**97.4**	**96.2**	**98.3**

RT – retention time; KI – Kovats' retention index (obtained by GC-MS in this study using a DB-5MS column); EV – violet *Epiphylli fructus*; EP – pink *Epiphylli fructus*; EG – green *Epiphylli fructus*; HW – white-fleshed *Hylocerei fructus*, HR – red-fleshed *Hylocerei fructus*; OV – violet *Opuntiae fructus*; OO – orange *Opuntiae fructus*

Three *Epiphyllum* ssp. cactus hybrids were analysed – with violet, pink and green fruits. Volatile compounds analysis of the violet *Epiphylli fructus* (Figure 1) resulted in the identification of 12 constituents. Most abundant compounds were 2,3-butanediol, hexanoic acid, and ethyl hexadecanoate with a percentage of 27.1, 15.2 and 10.4, respectively. In the pink *Epiphylli fructus*, 13 compounds were identified. Most abundant were (*E*)-anethol, 1-nonadecene and geraniol, with a percentage of 28.1, 14.0 and 11.7, respectively. Nine compounds were identified in the green *Epiphylli fructus*. Most abundant compounds were (*E*)-anethol, methyl eugenol and ethyl hexadecanoate with a percentage of 20.5, 14.7 and 13.2, respectively. The comparison between three hybrids showed 4 compounds were present in all of them, namely cis-rose oxide, (*E*)-anethol, hexadecanoic acid, and heneicosane (Table I). There are very few scientific studies investigating the phytochemistry of *Epiphyllum* ssp. secondary metabolites. To our knowledge, none of these studies examined volatile compounds of the fruits of these plants.

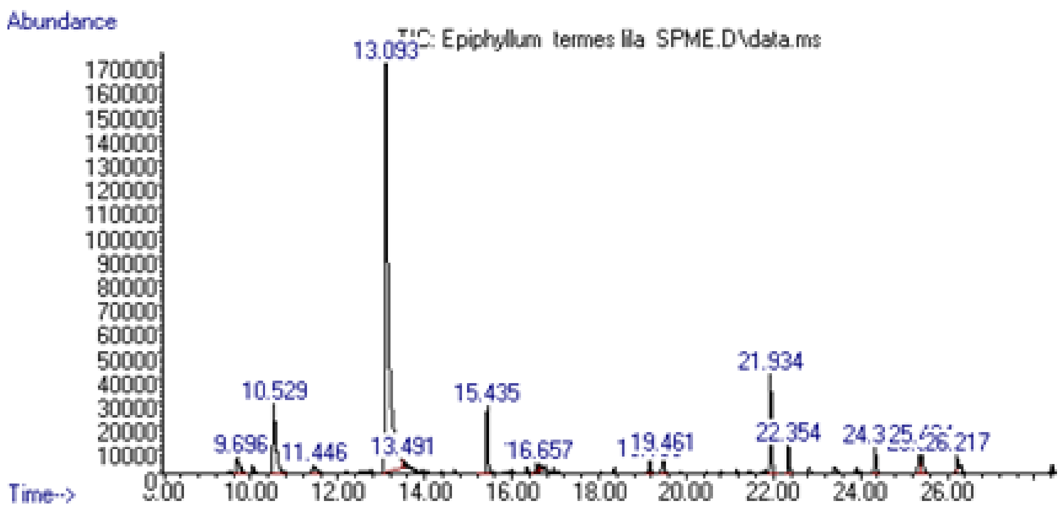

Figure 1.
GC chromatogram of *Epiphyllum* ssp. violet fruits

Two *Hylocereus* species, *H. undatus* (white-fleshed pitaya) and *H. costaricensis* (red-fleshed pitaya) were analysed separately. In *H. undatus* fruits, 9 volatile compounds were identified, namely 1-hexadecanol, with a percentage of 64.2, nonadecane, with a percentage of 7.8, 1 nonadecene, with a percentage of 7.2. Volatile compounds analysis of *H. costaricensis* fruits resulted in the identification of 11 constituents. Most abundant compounds were 1-hexadecanol, with a percentage of 63.7, nonadecane, with a percentage of 8.0, 1-nonadecene, with a percentage of 6.8. There are few studies addressing volatile compounds analysis in *Hylocereus* ssp. stems [25], flowers [27], peels [19], fruits [23], and seeds [4]. According to a literature search, only a few studies investigating volatile constituents of the *Hylocereus* genus have been published so far. Obenland *et al*. [21] studied the impact of storage conditions and variety on aromatic *Hylocereus spp.* volatiles by SPME GC-MS. Six varieties of pitahaya fruits (three *H. costarensis*, one *H. polyrhizus* × *H. undatus* and one *H. undatus* were examined in the study, and 34 aroma volatiles were identified. 2-Hexenal was the most prevalent aldehyde. Other identified compounds were hydrocarbons, alcohols, a ketone, an ester and a furan. We identified an alcohol, 1-hexadecanol, in our white-fleshed *Hylocerei fructus* (*H. undatus*) and red-fleshed *Hylocerei fructus* (*H. costarensis*) as the major compound – 64.2% and 63.7%, respectively, alongside the hydrocarbon nonadecane – 7.8% and 8.0% (Table I). Luo *et al*. [19] used SFE and GC-MS analysis to determine the chemical composition of *H. polyrhizus* and *H. undatus* fruit peel. 24 compounds were identified in *H. polyrhizus* peel extract, mostly triterpenoids (29.77%) and steroids (16.46%). The most abundant compounds were β-amyrin (15.87%), α-amyrin (13.90%) and octacosane (12.2%). As for *H. undatus* peel, 19 compounds were identified, mostly triterpenoids (23.39%) and steroids (19.32%) as well. Most abundant compounds were β-amyrin (23.39%), γ-sitosterol (19.32%) and octadecane (9.25%). Their analysis also showed 1-nonadecene with a percentage of 3.17 in the fruit peel of *H. polyrhizus*. According to our results, it was also present in *H. costaricensis*, with a percentage of 6.8 (Table I). Another study was conducted by Célis *et al*. [6] on *Hylocereus megalanthus*, yellow pitaya fruits. A total of 121 volatile constituents were identified. The most abundant compounds were 2-hexenal (5.1%) and γ-cadinene (4.7%). This study also revealed the presence of heptadecane and nonadecane, which were identified in our analyses as well (Table I), *H. undatus*: 3.0% and 7.8%, respectively, *H. costaricensis*: 3.6% and 8.0%). Analysis of volatile compounds in pitaya flowers undertaken by Shen *et al.* [27] revealed 49 volatile compounds by HS-SPME GC-MS methods, too. Most abundant were sesquiterpenes and their oxidized derivates (27.78%), alkanes (25.14%) and esters (14.40%).

The fruits of two *Opuntia* ssp. taxa were analysed – violet and orange. Analysis of the violet fruit resulted in the identification of 8 constituents. Most abundant compounds were nonanal, ethyl decanoate and hexadecanoic acid, with a percentage of 43.5, 12.1 and 9.4, respectively. Analysis of the orange fruit resulted in the identification of 8 constituents. Most abundant compounds were ethyl decanoate, (3*E*)-1,3-octadecadiene and nonanal with a percentage of 35.1, 21.1 and 11.8, respectively. The comparison between the two taxa showed that 6 compounds were present in both of them, namely ethyl hexanoate, nonanal, ethyl decanoate, ethyl dodecanoate, hexadecanoic acid and linoleic acid methyl ester. Volatile compounds composition has been studied in *Opuntia* ssp. cladodes, flowers, fruits and seeds [3, 7, 9, 12, 14, 30, 33]. Arena *et al.* [3] identified 16 volatile compounds of red, yellow and white prickly pear fruits using GC-MS and GC-FID methods, with 2-hexen-1-ol being the most abundant one with a percentage of 56.8, followed by hexan-1-ol with a percentage of 26.4. The most important aroma contributors among the identified compounds were (*E,Z*)-2,6-nonadien-l-ol and 2-methyl-butanoic acid methyl ester. The former aroma compound gives the typical odour of prickly pear, while the latter has a fresh fruity odour. The amount of 2-methylbutanoate was much higher than that of nonadienol in the white cultivar. Comparison of aroma values of the unripe and ripe fruits confirms these two compounds as the aroma quality factors at ripeness [3]. The complex aroma of the violet and red *Opuntiae fructus* analysed in our study is the result of several volatile odour types, e.g. apple peel (ethyl hexanoate), grape (ethyl decanoate), citrus (ethyl decanoate, 1-nonanal), fruits (ethyl hexanoate, ethyl octanoate), fat (1-nonanal, hexadecanoic acid) and green (1-nonanal) (Table I) [1]. An analysis of *O. vulgaris* fruit juice performed by Essa and Salama [12] resulted in the identification of 12 volatiles, with cyclopentanone and 2-propanol being the most abundant ones. Weckerle *et al*. [29] concluded that major compounds of the *O. ficus-indica* fruit were (*E*)-2-hexenal, 1-hexanol, (*E*)-2-hexen-1-ol, (*E*)-2-nonenol and (*E*)- and (Z)-2,6-nonadienol. They also identified nonanal and 1-octanol, which is in agreement with our results (Table I). Another study was conducted by Zito *et al.* [34] on fruits of two Sicilian cultivars of *O. ficus-indica*. Most abundant compounds in the red cultivar were heptacosane in the peel (5.5%, while comparably only 1.3% in the yellow peel), squalene in the pulp (27.2%, while only 5.3% in the yellow pulp) and hexadecenoic acid in the seeds (28.5%). As for the yellow cultivar, most abundant compounds were hexahydrofarnesyl acetone in the peel (19.1%, while only 5.4% in the red peel) and hexadecenoic acid in both the pulp (65.7%, while only 5.4% in the red pulp) and seeds (33.0%). The yellow fruits pulp also contained methyl linolenate (3.5%), present in both violet and orange fruits in our analyses (4.2% and 4.4%, respectively) (Table I). Bareh *et al.* [5] analysed volatiles of prickly pear fresh fruit, juice and jam. 14 compounds were identified, with (*E*)-3-hexen-1-ol and ethyl acetate being the major ones. Oumato *et al.* [23] studied volatile constituents of three *O. ficus-indica* varieties from Morocco. They identified 46 compounds, where the main constituents in all three varieties were 2-hexenal and 1-hexanol with percentages of 10.6%, 10.9% and 44.0% for the first compound and 10.3%, 5.9% and 18.7% for the second one. All three varieties of Moroccan *O. ficus-indica* fruits contained 1-octanol (1.0%, 0.8% and 0.2%) that was identified in our violet fruits (7.4%) and nonanal (1.4%, 2.8% and 1.1%) that was identified in both violet and orange fruits in our analyses (43.5% and 11.8%) (Table I) contributing to moss, nut, citrus, mushroom/fat and green odours [1].

Conclusions

We determined the volatile compounds' content in fruits of plants of the *Epiphyllum*, *Hylocereus* and *Opuntia* genera cultivated in Central Europe using the HS-SPME GC-MS technique. We identified and quantified 19 volatile compounds in *Epiphyllum* fruits, 14 volatile compounds in *Opuntia* fruits and 13 volatile compounds in *Hylocereus* fruits. Our results bring new data in this field of research and partially confirm data of similar studies published so far.

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Conflict of interest

The authors declare no conflict of interest.

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