

# CHEMICAL COMPOSITION OF THE ALKALOID EXTRACT ISOLATED FROM *HYLOTELEPHIUM SPECTABILE* (BOREAU) H. OHBA X *TELEPHIUM* (L.) H. OHBA AERIAL PARTS\*

Miloš G. Đukić\*\*, Jelena M. Jovanović, Gordana S. Stojanović, Snežana Č. Jovanović

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Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Niš, Serbia

There are many motives to study alkaloids within Crassulaceae family, such as chemotaxonomic consideration of alkaloid distribution and evaluation of their biological potential. Data on alkaloids from *Sedum telephium* (syn. *H. telephium*) aroused interest for further alkaloid investigation within the genus *Hylotelephium* H. Ohba. Thus, the aim of this study was to investigate and characterize other plant sources of alkaloids from the same genus. An alkaloid extract (AE-HS) was isolated from fresh aerial parts of a cultivated hybrid *H. spectabile* x *telephium*. The chemical composition of AE-HS was determined by GC-MS (gas chromatography – mass spectrometry) method. Eight components were identified, which made up 91.8% of the sample. The most present component of AE-HS was  $\gamma$ -crotonolactone (78.6%) followed by 3-methyl-2-butenal (8.6%) and (E)-coniferyl alcohol (2.8%). Nicotine was identified as the only alkaloid in the AE-HS (0.4%). Along with many non-alkaloid compounds, a previous study found six alkaloids in AE of *S. telephium* (~7%), suggesting differences from our results. Additionally, alkaloid distribution is limited within other genera of the family and belonging to the same genus does not mean a uniform qualitative composition. Further analyzes of AEs of *Hylotelephium* taxa would certainly shed light on the pattern of alkaloid distribution.

**Keywords:** *Hylotelephium spectabile* x *telephium*, alkaloids, GC-MS analysis

## Introduction

*Hylotelephium* H. Ohba is considered to belong to the genera of the Crassulaceae family. It had been classified as a section or subgenus of *Sedum*, until Hart (1995) redefined it as a separate genus. It contains about 33 species, spread in the temperate parts of East Asia, Europe, and North America. Some *Hylotelephium* species are consumed fresh (leaves) or cooked (leaves, roots). In traditional medicine various species of the genus *Hylotelephium* are used for skin diseases treatment, such as burns, pimples, wounds, as well as in anti-inflammatory and analgesic treatments [1-3]. The distribution of alkaloids is generally limited within the genus *Sedum* L. (*Sedum* acre-group); Several types of alkaloids have been identified - pyrrolidines and piperidines (2-monosubstituted and 2,6-disubstituted), which were considered in previous chemotaxonomic studies [4,5]. Phytochemical studies on the genus comprise several classes of secondary metabolites distributed in different plant organs: flavonoids, glucosides tannins, alkaloids, terpenes, lactones, organic acids, and polysaccharides [2,6-11]. According to published data, extracts of the studied *Hylotelephium* species showed several biological activities: high antioxidant and anti-inflammatory activity of *H. erythrostictum* aerial parts extract; antimicrobial and antioxidant activity of

leaf and flower extracts, fractions and hydrolysates of *H. spectabile* x *telephium*; anti-inflammatory and analgesic activity of the *H. kamtschaticum* methanol extract [1,12]. In addition to the listed biological activities, *H. spectabile* is characterized by the strong accumulation power of Cd without growth inhibition [1].

*H. spectabile* (Boreau) H. Ohba x *telephium* (L.) H. Ohba, a plant hybrid; parentage: *H. spectabile* (Boreau) H. Ohba and *H. telephium* (L.) H. Ohba. It is widespread in Serbian horticulture, grown as an ornamental plant and used in traditional medicine [2,9]. Previous studies on *H. spectabile* x *telephium* reported flavonoid profile of leaves and flowers characterized by different contents of flavonoid glucosides and flavonoid aglycones in methanol extracts, their hydrolysates and fractions [9] and profiles of volatile compounds isolated from root by headspace technique and hydrodistillation [2]. Although the distribution of alkaloids is generally limited to the acre-group members, the study by Gerelt-Od et al. (2015) found data on alkaloids from *Sedum telephium* (syn. *H. telephium*), which aroused interest in alkaloid investigation in the aerial parts of other *Hylotelephium* species [6]. The aim of this study was to investigate other plant sources of alkaloids from the same genus. Thus, the alkaloid extract was isolated

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\*\*Author address: Miloš G. Đukić, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Republic of Serbia, e-mail: milos.djukic@pmf.edu.rs

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from the aerial parts of *H. spectabile x telephium* (AE-HS) and characterized by GC-MS. The results obtained are discussed with respect to literature data.

## Experimental

### Plant material

The fresh aerial parts (stem with leaves) of cultivated plant *H. spectabile x telephium* were collected in October (2020) from garden pots. A voucher (No 6853) specimen was deposited at the Herbarium fund of the Faculty of Science and Mathematics, University of Niš (HMN).

### Extraction of the plant material

Prior to extraction, the collected aerial parts of the plant *H. spectabile x telephium* were cleaned and cut into small pieces. The weighed plant material (300 g) was placed in a dark laboratory flask, filled with 600 mL of methanol, and extracted by maceration for seven days. After extraction, the plant material was removed by filtration, and methanol was evaporated under reduced pressure. The alkaloid extract (AE-HS) was isolated from the residue according to the described procedure [6]. The obtained alkaloid extract (52 mg, yield 0.02%, w/w) was dissolved in dichloromethane (20 mg mL<sup>-1</sup>) for further GC-MS analysis.

### GC-MS analysis

The GC-MS analysis was performed on a 7890/7000B GC/MS/MS triple quadrupole mass spectrometer (Agilent Technologies, USA) with Combi PAL auto sampler. The separation of components was done on HP-5MS column (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness of stationary phase 0.25 μm). Carrier gas was helium (99.999%) with flow 1 mL min<sup>-1</sup>. Different temperature programs were used and the best separation of compounds was under the following conditions: a temperature program of 50 °C for 1 min, then 2 °C min<sup>-1</sup> to 300 °C; injection volume 2 μL, and split ratio 1:40. The contribution of the compounds was determined as the share of surface of every peak in the total surface of total ion chromatogram (TIC). Data processing was performed using AMDIS software (Automated Mass Spectral Deconvolution and Identification System, version 2.7) and NIST MS Search program (National Institute of Standards and Technology, version 2.0). The identification of the compounds was performed by comparing the retention indices of the compounds with the retention indices of C<sub>8</sub>-C<sub>40</sub> n-alkanes recorded on the same column and under the same GC-MS operating conditions, and their mass spectra with those listed in the mass spectrum libraries.

## Results and discussion

The results on chemical composition of the alkaloid extract of the species *H. spectabile x telephium* are summarized in Table 1.

**Tabela 1.** Chemical composition of the alkaloid extract isolated from the *H. spectabile x telephium* aerial parts

No.	RI	LI	MS data	MF	Compound	Composition (%)
1.	784	778	84(99.9), 55(59.6), 56(9.2), 85(4.6), 53(2.5), 54(2.3), 82(1.6) 83(0.7), 86(0.5), 57(0.4)	C <sub>8</sub> H <sub>16</sub> O	3-Methyl-2-butenal	8.6
2.	837	839*	59(99.9), 101(63.0), 58(37.0), 98(13.3), 83(9.3), 56(7.5), 55(5.0), 57(4.0), 60(3.5), 102(3.0)	C <sub>6</sub> H <sub>12</sub> O	4-Hydroxy-4-methyl-2-pentanone	0.3
3.	927	924*	55(99.9), 84(78.9), 54(19.2), 53(4.6), 85 (3.7), 56(3.5), 83(2.6), 82(0.9), 52(0.5), 57 (0.3)	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	γ-Crotonolactone	78.6
4.	1112	1107	91(99.9), 92(57.2), 122(31.0), 65(11.8), 77(4.9), 93(4.3), 78(3.8), 51(3.6), 89(3.5), 63(3.2)	C <sub>8</sub> H <sub>10</sub> O	Phenethyl alcohol	0.4
5.	1343	1342	84(99.9), 133(30.3), 162(20.5), 161 (18.9), 119(8.1), 85(6.3), 92(5.8), 118(5.4), 82(4.8), 130(4.5)	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub>	Nicotine	0.4
6.	1455	1459*	121(99.9), 152(38.2), 93(18.4), 65(11.1), 122(6.7), 153(3.3), 63(2.7), 151(2.3), 64(2.2), 92(2.1)	C <sub>8</sub> H <sub>16</sub> O <sub>3</sub>	Methylparaben	0.4
7.	1522	1513	119(99.9), 91(96.3), 79(69.2), 107(54.9), 105(41.5), 77(36.8),	C <sub>10</sub> H <sub>14</sub> O	2,4,5-Trimethylbenzenemethanol	0.3
8.	1733	1733	137(99.9), 180(78.3), 162(51.3), 124(50.2), 91(49.1), 147(40.3), 119(34.5), 103(21.4), 131 (20.6), 77 (20.1)	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	(E)-Coniferyl alcohol	2.8
9.	2111	-	71(99.9), 82(72.8), 95(71.5), 81(66.6), 123(58.7), 69(54.2), 68(52.3), 57(51.2), 83(46.4), 55(43.2)	-	Phytol ester	0.4
10.	2399	2398*	129(99.9), 112(42.0), 147(30.7), 57(27.8), 71(22.7), 70(21.6), 111(20.2), 113(19.6), 55(17.2), 101(10.6)	C <sub>22</sub> H <sub>42</sub> O <sub>4</sub>	Bis(2-ethylhexyl) adipate	0.3
11.	2472	-	55(99.9), 129(76.5), 69(73.7), 83(64.3), 81(53.2), 97(51.7), 98(49.1), 67(46.6), 95(42.7), 57(42.1)	-	Unidentified compound	0.7
					<b>Total identified</b>	<b>93.2</b>

RI - experimentally determined indices by co-injection of a homologous series of n-alkanes C<sub>8</sub>-C<sub>40</sub> on HP-5MS column, LI - Adams' retention indices [13] and \* - retention indices from NIST Chemistry WebBook [14].

In the studied alkaloid extract eleven components were identified, which make up 93.2% of the sample. The major components were lactone γ-crotonolactone

(78.6%), followed by 3-methyl-2-butenal (8.6%), and (*E*)-coniferyl alcohol (2.8%). Other components were represented by less than 1%. By comparing the obtained results with the published data on the alkaloid extract of *H. telephium* [6], there are differences in the qualitative and quantitative composition of the extracts.

The principal compound in each is lactone, but it originates from different acids:  $\gamma$ -crotonolactone in the studied sample, and dehydromevalonic lactone (5.99%) in *H. telephium* sample. With regard to alkaloid compounds, only nicotine (0.4%) was identified in the studied AE-HS sample, while Gerelt-Od et al. (2015) identified 6 alkaloids (~7%): 3-methyl-2-carbethoxyindole (4.7%), 2-(2-hydroxyphenyl)benzothiazole (1.6%), N,4,5-trimethylphenyl-1,2-diamine (1.2%), 2-pyrrolidinecarboxylic acid-5-oxo-, ethyl ester (0.9%), 4-methyl-3,6-diisopropyl-2,5-diketo-morpholine (1.6%) and 4-amino-3,5-diethylpyridine (1.1%), but not nicotine [6]. Regardless of the origin of the studied hybrid (one of the parent species is *H. telephium*), it is obvious that the chemical composition of these two alkaloid extracts is quite different, both qualitatively and quantitatively. The members of the genus *Hylotelephium* have not been sufficiently investigated regarding alkaloids. The distribution on piperidine and pyrrolidine alkaloids within Crassulaceae is limited to the *Sedum acre*-group [4,15]. Among many other alkaloids, nicotine has been identified in the well-known stonecrop species - *Sedum acre* [16,17]. Certainly, it is necessary to do a more extensive analysis with numerous samples to determine a certain pattern of distribution of alkaloids within the genus *Hylotelephium*.

### Conclusion

Data on the chemical composition of alkaloid extract of hybrid *H. spectabile* x *telephium* aerial parts were revealed for the first time. Also, the profiles of two alkaloid extracts were compared: the studied hybrid *H. spectabile* x *telephium* from the Republic of Serbia vs. the parent species *H. telephium* from Mongolia. The extract of *H. spectabile* x *telephium*, among many non-alkaloid compounds, contains 0.4% of nicotine (piperidine type alkaloids), while six alkaloids (~7%) were identified in parent species *H. telephium* from Mongolia [6]. Lastly, the alkaloid content in the studied sample is not significant and does not represent a confident scientific data by which we would claim that the representatives of this genus contain alkaloids. Further analysis alkaloid extracts of *Hylotelephium* taxa would shed light on the alkaloid profile and distribution.

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## Izvod

# HEMIJSKI SASTAV ALKALOIDNOG EKSTRAKTA IZOLOVANOG IZ NADZEMNOG DELA BILJKE *HYLOTELEPHIUM SPECTABILE* (BOREAU) *H. OHBA* X *TELEPHIUM* (L.) *H. OHBA*

Miloš G. Đukić, Jelena M. Jovanović, Gordana S. Stojanović, Snežana Č. Jovanović

(ORIGINALNI NAUČNI RAD)  
UDK 582.711.16:547.94  
DOI 10.5937/savteh2102005D

Departman za Hemiju, Prirodno matematički fakultet, Univerzitet u Nišu, Niš, Srbija

Postoji mnogo motiva za proučavanje alkaloida unutar porodice Crassulaceae, poput hemotaksonomskog razmatranja distribucije alkaloida i procene njihovog biološkog potencijala. Podaci o alkaloidima *Sedum telephium* (sin. *H. telephium*) pobudili su interesovanje za dalja istraživanja alkaloida u okviru roda *Hylotelephium* H. Ohba. Cilj ove studije bio je ispitati druge biljne izvore alkaloida iz istog roda i okarakterisati hemijski sastav alkaloidnog ekstrakta. Alkaloidni ekstrakt (AE-HS) izolovan je iz svežih nadzemnih delova kultivisanog hibrida *H. spectabile* x *telephium*. Hemijski sastav AE-HS određen je metodom GC-MS (gasna hromatografija-masena spektrometrija). Identifikovano je osam komponenti (91,8%). Najzastupljenija komponenta AE-HS je  $\gamma$ -krotonlakton (78,6%), zatim 3-metil-2-butenal (8,6%) i (*E*)-koniferil alkohol (2,8%). Nikotin je identifikovan kao jedini alkaloid AE-HS (0,4%). Pored mnogih nealkaloidnih jedinjenja, prethodna studija otkrila je šest alkaloida u AE *S. telephium* (~7%), što nagoveštava razlike u odnosu na naše rezultate. Pripadnost istom rodu ne znači ujednačen kvalitativni sastav. Svakako dalje analize AE taksona *Hylotelephium* rasvetlile bi obrazac distribucije alkaloida.

**Ključne reči:** *Hylotelephium spectabile* x *telephium*, alkaloidi, GC-MS analiza