ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Amaryllidaceae alkaloids GC/MS analysis in *Galanthus woronowii* and *Galanthus nivalis* of Russian origin.

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ABSTRACT

Galanthus woronowii and *Galanthus nivalis* contain various Amaryllidaceae alkaloids and are used in traditional medicine and homeopathy for treatment of nervous system disorders. Determination of Amaryllidaceae alkaloid profile of *G. woronowii* and *G. nivalis*, growing in the Botanical Garden of I.M. Sechenov First Moscow State Medical University (Moscow, Russia). Alkaloids were isolated from plant material by conventional procedures, utilizing acid-base extraction. The capillary gas chromatography-mass spectrometry (GC-MS) analyses were used to determine isolated compounds. Characterization of Amaryllidaceae alkaloids was performed by matching to the MS library and comparisons of their mass spectra (NIST05 database). Five Amaryllidaceae alkaloids were detected in *G. woronowii*, among them galanthamine, tazettine, galanthine, lycorine, (1α,2β)-9,10-dimethoxy-3,12-didehydro-galanthan-1,2-diol and ten compounds from *G. nivalis* including trisphaeridine, ismine, michelalbine, 6a-desoxy-6-methoxy-criwelline, tazettine, 11-hydroxy-vittatine, dihydrohemanthidine, dihydroepinatalensine, lycorine, 3-epimacronine.

Keywords: Galanthus woronowii, Galanthus nivalis, Amaryllidaceae alkaloids, GC/MS

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ISSN: 0975-8585

INTRODUCTION

The family *Amaryllidaceae* includes about 85 genera and 1100 species which are distributed throughout the warm temperate and tropical regions of the world. [1] It is widely thought that the genus *Galanthus* includes 19 species, six varieties and two natural interspecies hybrids (World Cheklist of Selected Plant Families).[2] *Galanthus woronowii* Losinsk. (Woronow's snowdrop), *Galanthus nivalis* L. (common snow drop) are an early-spring flowering bulbous plants species cultivated for its ornamental qualities in gardens. Plants of the genus *Galanthus* L. contain bioactive Amaryllidaceae alkaloids. These alkaloids exhibit a wide range of biological activities including acetylcholinesterase inhibition, antiviral, antitumor, and antiprotozoal activity.[3], [4], [5], [6] Amongst the Amaryllidaceae alkaloids, there are galanthamine and lycorine, which halides – lycorine hydrochloride (broncholytic) and galanthamine hydrobromide (acetylcholinesterase inhibitor) – are used in medicine.[7] Today pharmacologic treatments for Alzheimer's disease around the world include galanthamine hydrobromide.[8], [9] Mother tinctures produced from both *Galanthus* species are used in preparation of homeopathic drugs.[10]

Previous phytochemical investigations on *G. woronowii* Amaryllidaceae alkaloids resulted in the isolation of lycorine, galanthamine, galanthine, galanthusine, demethylhomolycorine and tazettine.[11], [12] In addition, studies of G. woronowii collected from Turkey, previously known improperly as *G. ikariae* Baker, indicated the presence of tazettine, lycorine, galanthamine, crinine, 2-demethoxymontanine and 3 epihydroxybulbispermine.[13], [14] In last phytochemical investigation of *G. woronowii* (Caykara, Trabzon, Turkey) several compounds were isolated and identified as galanthine, galanthamine, 2-O-(3'-hydroxybutanoyl)lycorine, narwedine, O-methylleucotamine, sternbergine, lycorine, sanguinine and salsoline.[15]

Six Amaryllidaceae alkaloids – galanthamine, lycorine, nivalidine, narwedine, tazettine, hyppeastrine – were isolated by TLC from *G. nivalis* of Ukrainian origin (Lviv).[16] Phytochemical studies on *G. nivalis* in Bulgaria resulted in the identification of five compounds: 11-O-(3'-hydroxybutanoyl)hamayne, 3,11-O-(3',3''-dihydroxybutanoyl)hamayne, 3,11,3''-O-(3',3'',3'''-trihydroxybutanoyl)hamayne, 3-O-(2''-butenoyl)-11-O-(3'-hydroxybutanoyl)hamayne, and 2-O-(3'-acetoxybutanoyl)lycorine, together with five known Amaryllidaceae alkaloids: lycorine, ungeremine, tazettine, ismine, hamayne.[17]

The aim of this study is to analyze Amaryllidaceae alkaloids composition of *G. nivalis* and *G. woronowii*, growing in the Botanical Garden of I.M. Sechenov First Moscow State Medical University (Moscow, Russia).

EXPERIMENTAL

The plant material was collected at blooming period from the Botanical Garden of I.M. Sechenov First Moscow State Medical University in April 2016. The whole plants – aerial parts (flowers, leaves) and the bulbs with roots – of *G. woronowii* and *G. nivalis* were air-dried and powdered.

Extract preparation

One gram of plant material was extracted for three times with methanol (10 mL) in an ultrasonic bath at room temperature (25 °C) for 40 min. Then solvent was evaporated, the obtained residues were dissolved in 15 mL of 3% sulfuric acid, and then neutral compounds were removed with diethyl ether (3 × 15 mL). Basification of the acidic aqueous phases was done by adding 25% ammonium hydroxide to pH 9–10 and following alkaloid extraction with chloroform (3 × 15 mL) was performed. Then chloroform extracts were combined, dried over anhydrous sodium sulfate and filtered. The organic solvent was removed under vacuum to obtain the final alkaloid extract. The extract was dissolved in methanol (10 mg of extract in 500 µL) and subjected to GC-MS analysis.

GC-MS analysis

The capillary gas chromatography-mass spectrometry (GC-MS) analyses were performed on an Agilent 7000 Series Triple Quadrupole GC-MS System equipped with the Agilent 7890A Series gas chromatograph (Agilent Technologies, USA) and Agilent Technologies MassHunter Workstation Software Qualitative Analysis ver. B.06.00 Build 6.0.633.0. The mass spectrometer was operated in electron impact mode (EI) at 70 eV and

ISSN: 0975-8585

scanned from 40 to 400 Da. The temperature program was: 1 min hold at 80 °C, 80–220 °C at 30 °C min^{-1} and 1 min hold at 220 °C, 220–280 °C at 5°C min^{-1} and 30 min hold at 280°C. The injector temperature was 280°C. The flow rate of the carrier gas (helium) was 2 mL min^{-1}. The split ratio was 1:30. An HP-5 MS column (30 m × 250 µm × 0.25 µm) was used. 1 µl of the methanol solution was injected. The Amaryllidaceae alkaloids were identified by comparing the mass spectral fragmentation pattern of the compound with standard reference spectra from NIST 05 database (NIST Mass Spectral Database, ver. 5.0). The total ion current (TIC) percentage for each alkaloid is given in Table 1. The relative alkaloid content (RAC) in the alkaloid fraction was calculated by using the single compound peak area related to the total peak areas of all compounds (100% method). The GC-MS peaks area depends on: the related compounds concentration, the intensity of related compounds mass spectral fragmentation. This implies that the data in Table 1 does not appropriate for absolute quantifications but affords ground for comparing alkaloid profile in test samples.

RESULTS AND DISCUSSION

Based on the GC-MS analysis, five alkaloids were detected in extract prepared from *G. woronowii* and ten from *G. nivalis* (Table 1). Chromatograms of alkaloid extracts from 2 snowdrop species are presented at Figure 1 and Figure 2. The identified compounds in *G. woronowii* possessed various Amaryllidaceae alkaloid skeleton types including galanthamine, tazettine, lycorine; skeleton types of tyramine, narciclasine, haemanthamine, tazettine, lycorine were presented in *G. nivalis*.

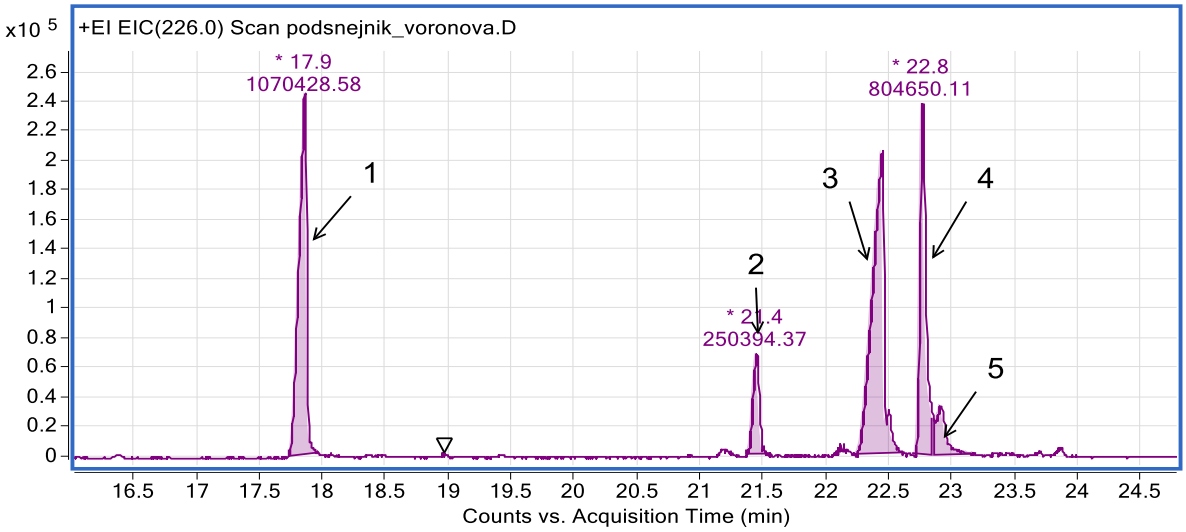

Figure 1: Chromatogram of *G. woronowii* extract. 1: galanthamine, 2: tazettine, 3: galanthine, 4: lycorine, 5: (1α,2β)-9,10-dimethoxy-3,12-didehydro-galanthan-1,2-diol.

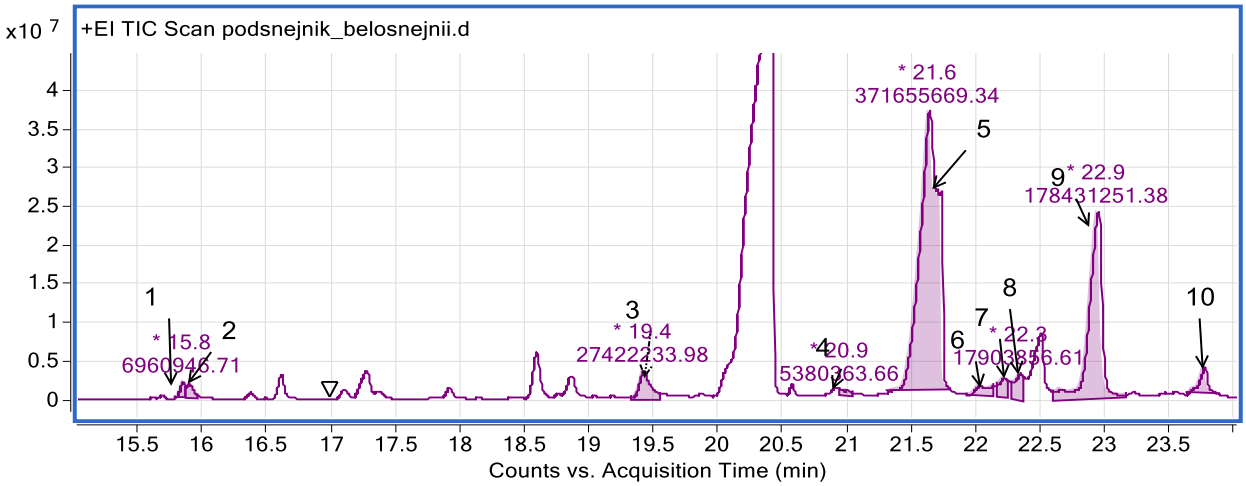

Figure 2: Chromatogram of *G. nivalis* extract: 1: trisphaeridine, 2: ismine, 3: michelalbine, 4: 6a-desoxy-6-methoxy-criwelline, 5: tazettine, 6: 11-hydroxy-vittatine, 7: dihydrohemanthidine, 8: dihydroepinatalensine, 9: lycorine, 10: 3-epimacronine.

ISSN: 0975-8585

Table 1. GC-MS data of the alkaloids in *G. woronowii* and *G. nivalis* extracts

Compound		RT, min	m/z	Relative alkaloid content (RAC), %	
				G. woronowii	*G. nivalis*
1.	Trisphaeridine	15.8	999 (223), 269 (222), 169 (138), 224 (155), 111 (110), 164 (110), 225 (15), 226 (1)	–	1.05
2.	Ismine	15.9	999 (238), 449 (257), 239 (163), 168 (149), 196 (139), 110 (19), 258 (78), 211 (70), 226 (70), 240 (18)	–	1.20
3.	Galanthamine	17.9	999 (287), 905 (286), 606 (42), 414 (216), 379 (174), 305 (115), 301 (44), 275 (244), 210 (43), 288 (179)	28.98	–
4.	Michelalbine	19.4	999 (281), 929 (280), 379 (252), 299 (251), 269 (262), 263 (219), 282 (161), 253 (57), 264 (31), 283 (20)	–	4.15
5.	Criwelline, 6a-desoxy-6-methoxy	20.9	999 (70), 775 (42), 666 (75), 350 (314), 341 (44), 330 (82), 261 (258), 211 (45), 181 (113), 313 (173),	–	1.40
6.	Tazettine	21.4	999 (247), 891 (42), 812 (44), 808 (70), 620 (45), 618 (71), 331 (297), 291 (115), 201 (188), 181 (167)	6.78	
		21.6			56.20
7.	Vittatine, 11-hydroxy-	22.1	999 (181), 910 (269), 860 (227), 834 (115), 806 (287), 720 (243), 648 (43), 643 (225), 555 (42), 555 (44)	–	2.15
8.	Dihydrohemanthidine	22.2	999 (42), 944 (319), 788 (56), 694 (149), 574 (211), 529 (71), 506 (77), 476 (43), 452 (258), 451 (256)	–	1.93
9.	Dihydroepinatalensine	22.3	999 (303), 464 (186), 367 (211), 367 (259), 305 (44), 301 (226), 293 (115), 282 (43), 275 (258), 270 (201)	–	2.71
10.	Galanthine (Galanthan-1-ol, 3,12-didehydro-2,9,10-trimethoxy-, (1α,2β)-)	22.4	999 (242), 982 (243), 349 (317), 244 (200), 184 (125), 268 (174), 316 (143), 162 (140), 127 (41), 123 (45)	37.38	–
11.	Lycorine	22.8	999 (226), 780 (227), 358 (44), 280 (18), 242 (28), 287 (212), 186 (147), 268 (162), 155 (119), 144 (111)	21.78	
		22.9			26.59
12.	3-Epimacronine	23.8	999 (44), 852 (42), 831 (201), 651 (245), 463 (70), 278 (139), 232 (128), 228 (115), 202 (43), 199 (127)	–	2.62
13.	Galanthan-1,2-diol, 3,12-didehydro-9,10-dimethoxy-, (1α,2β)-	22.9	999 (242), 887 (243), 404 (303), 284 (199), 302 (186), 244 (148), 228 (85), 304 (80), 286 (70), 268 (68)	5.08	–

Generally, galanthamine, lycorine and tazettine type alkaloids were major components in the tested samples. Galanthamine (28.98%), galanthine (37.38%) and lycorine (21.78%) were found to be the main alkaloids in the extract of *G. woronowii* (88.14% in total). The extract obtained from *G.nivalis* contained tazettine (56.20%) and lycorine (26.59%) in very high amounts (82.79% in total). Tazettine and lycorine were found to be present in both snowdrop species.

ACKNOWLEDGEMENTS

We thank our colleague Malinkin A.D. from "Federal research center of nutrition and biotechnology' who provided insight and expertise that greatly assisted the research. We would also like to show our gratitude to the Bessonov V.V. for sharing his pearls of wisdom with us during the research.

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