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RESEARCH ARTICLE

Alkaloid profiling of *Galanthus woronowii* Losinsk. by GC-MS and evaluation of its biological activity

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ABSTRACT

The alkaloid profiles of the aerial parts and the bulbs of *Galanthus woronowii* Losinsk. were analyzed by means of Gas Chromatography-Mass Spectrometry (GC-MS). Totally, twenty-eight compounds were detected. Galanthamine and galanthine were found to be the main alkaloids both in the extracts of the aerial parts and the bulbs. Most of the identified compounds were lycorine and galanthamine-type Amaryllidaceae alkaloids. In addition, the acetylcholinesterase (AChE) inhibitor potentials of the extracts prepared from the aerial parts and bulbs were determined by *in vitro* Ellman's method and both of the extracts were found to exhibit significant activity (aerial parts: IC_{50}= 0.027 µg/mL and bulbs: IC_{50}= 0.084 µg/mL).

Keywords: *Galanthus woronowii*; Amaryllidaceae; GC-MS; alkaloids.

1. Introduction

The genus *Galanthus* L. belonging to the family Amaryllidaceae, is represented by 14 taxa and one hybrid in Turkey. Among these taxa, *Galanthus woronowii* Losinsk., is found in north-eastern part of Turkey [1, 2]. Previous phytochemical investigations have revealed that the genus *Galanthus* L. (Amaryllidaceae) is a very rich source of chemically diverse alkaloids, a quite number of them reported to be new compounds [3, 4].

G. woronowii is also distributed in Caucasus, Transcaucasus, Southern Russia and Georgia. Moreover, *G. woronowii* with broad green leaves is an attractive plant used in gardening and also for decorative purposes [1, 2]. Previously, *G. woronowii* has been a subject of several phytochemical studies such as isolation of galanthamine, lycorine, tazettine, galanthine and sanguinine [5-7]. In the present study, the alkaloid profile of *G. woronowii* was investigated by GC-MS. To the best of our knowledge, this is the first report of a GC-MS study on the alkaloids of *G. woronowii* of Turkish origin. Moreover, AChE inhibitory activity potentials of the extracts prepared from *G. woronowii* were examined spectrophotometrically by using a micro-plate assay modified from *in vitro* Ellman's method with 96-well micro-plate reader [8].

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2. Materials and Methods

2.1. Plant Material

Galanthus woronowii Losinsk. was collected in 2009 from Derepazarı-Rize north-east of Turkey. The plant was identified by Prof. M. Ali Önür (Ege University). A voucher specimen (No: 1417) has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

2.2. Extract Preparation

The extracts were prepared from the aerial parts and the bulbs of *G. woronowii*. Air-dried, powdered plant material (500 mg) was separately extracted 3 times with methanol (5 mL) at room temperature. The solvent was evaporated under reduced pressure, the residues dissolved in 10 mL 2 % sulfuric acid and the neutral compounds were removed with diethyl ether (3x10 mL). The acidic aqueous phases were basified with 25 % ammonia to pH 9-10 and the alkaloids were extracted with chloroform (3x10 mL). The combined chloroform extracts were then dried over anhydrous sodium sulphate, filtered and the organic solvent was distilled in *vacuo* to afford the alkaloidal extract [9]. Prior to the GC/MS analysis, the extracts were dissolved in CH_3OH (5 mg extract in 250 µL CH_3OH).

2.3. Gas Chromatography-mass spectrometry (GC/MS)

The alkaloids were detected by capillary gas chromatography-mass spectrometry (GC/MS) on a Hewlett-Packard 6890+MSD 5975, (Hewlett-Packard, Palo Alto, CA, USA) operating in the electron impact mode (EI, 70 eV). The temperature conditions worked with the following program: 100-180°C at 15°C min^{-1}, 180-300°C at 5°C min^{-1} and a 10 min hold at 300°C. Injector temperature was 250°C. Helium was used as a carrier gas at a flow rate of 0.8 mL min^{-1}. The split ratio was 1:20. A HP–5 MS column (30m x 0.25mm x 0.25µm) was used.

The spectra of co-eluting chromatographic peaks were determined by the use of AMDIS 2.64 (NIST, National Institute of Standardization and Technology, Gaithersburg, MD, USA). The alkaloids were identified by comparing the mass spectral fragmentation with standard reference spectra from the NIST 05 database (NIST Mass Spectral Database, PC-Version 5.0 (2005), National Institute of Standardization and Technology, Gaithersburg, MD) or applying co-chromatography with previously isolated authentic standards and in comparison with data obtained from the literature. The percentage of the total ion current (TIC) and the Kovats retention indices (RI) for each compound were given in Table 1. The area of the GC-MS peaks depends both on the concentration of the corresponding compound and on the intensity of their mass spectral fragmentation. The data given in Table 1 do not reveal a real quantification. However, they can be used for a relative comparison of the alkaloids.

2.4. Acetylcholinesterase (AChE) Inhibitory Activity

AChE inhibitory activity was assessed spectrophotometrically by using a micro-plate assay modified from Ellman's method with 96-well micro-plate reader, as previously described [8, 10]. The dilutions of each sample were prepared to get final concentrations ranging between 75-7.5x10^{-5} µg/mL. The inhibitory concentration of 50% (IC_{50}) was determined by a GraphPad Prism V3.0 software (GraphPad Software, San Diego, CA, USA). Galanthamine was used as a positive control.

3. Results and Discussion

Totally, twenty-eight compounds were detected by GC/MS (Table 1). The structures of the alkaloids were given in Figure 1. The identified alkaloids belonged to galanthamine-, lycorine-, tazettine-, and phenanthridine-type Amaryllidaceae alkaloids plus two other bases tyramine (**1**) and hordenine (**2**) were reported. The results of the chromatographic determination indicated that *G. woronowii* is a rich source of lycorine- and galanthamine-type Amaryllidaceae alkaloids. Galanthamine, an anti-Alzheimer drug [11] (**4**) and galanthine (**15**) were found as major alkaloids in both of the extracts. Also, lycorine (**18**) an alkaloid with different biological activities [12], methylleucotamine (**23**) and narwedine (**8**) were detected. In addition to these findings, one tazettine-type alkaloid, deoxytazettine (**11**) and one phenanthridine-type alkaloid, 5,6-dihydrobicolorine (**3**) were determined. Interestingly, two compounds (GW-1 and GW-2) (**14, 21**) showing mass spectral fragmentation of lycorine-type alkaloids were recorded by GC-MS in the alkaloidal extracts.

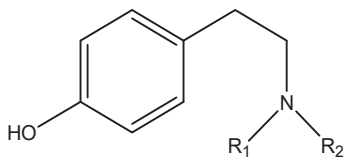
Previous karyological research on Turkish *Galanthus* populations indicated a polymorphism existed among different karyotypes and SAT –chromosomes which might be important in the alkaloidal variation [13-15]. Up to date, phytochemical studies carried on various *Galanthus* species revealed that they were an intruguing source for alkaloids

Table 1. Relative composition of the alkaloids in *Galanthus woronowii*

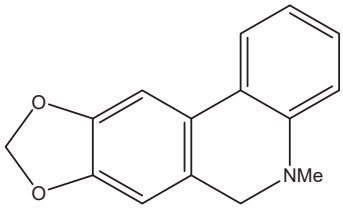
				Content*		
Compound	**RT**	**RI**	***m/z* (relative intensity,%)**	**Aerial Parts**	**Bulbs**	**References**
Tyramine[a] **(1)**	7.21	1414	137(M^+,16), 108(100), 107(60), 91(6), 77(24), 65(3)	t	t	[17]
Hordenine[a] **(2)**	12.72	1467	165(M^+,1), 121(2), 107(5), 91(3), 77(6), 58(100)	-	t	[18]
5,6-Dihydrobicolorine[b] **(3)**	20.82	2321	239(M^+,42) 238(100), 180(8), 152(5), 118(5), 90(3)	t	t	[19]
Galanthamine[c] **(4)**	21.41	2405	287(M^+,89), 286(100), 270(13), 244(26), 230(13), 216(32), 174(26), 128(9), 115(13)	36.2	36.4	NIST05, [20], S
Sanguinine[c] **(5)**	21.56	2413	273(M^+,100), 272(86), 256(22), 230(20), 225(17), 216(46), 212(24), 211(34), 202(37)	0.1	-	[17]
N-Demethylgalanthamine[c] **(6)**	21.90	2442	273(M^+,99), 272(100), 230(36), 202(27), 174(14)	0.3	1.2	[21]
Epigalanthamine[c] **(7)**	22.07	2454	287(M^+,86), 286(100), 244(20), 230(15), 216(62), 174(25)	t	t	[22]
Narwedine[c] **(8)**	22.47	2482	285(M^+,85), 284(100), 242(20), 228(11), 216(20), 199(18), 174(29)	1.6	1.1	[17]
Anhydrolycorine[d] **(9)**	22.91	2502	251(M^+,45), 250(100), 220(2), 192(11), 191(10), 165(3), 124(6)	0.4	0.1	NIST05, [21]
O-acetylgalanthamine[c] **(10)**	23.35	2534	329(M^+,37), 328(31), 270(100), 226(11), 216(25), 174(11)	0.1	-	[17], [23]
Deoxytazettine[e] **(11)**	23.47	2541	315(M^+,21), 300(16), 250(8), 231(100), 211(16), 169(6), 141(9)	-	0.1	[21]
Assoanine[d] **(12)**	23.98	2579	267(M^+,50), 266(100), 250(29), 222(8), 205(3), 193(7), 180(7)	0.1	0.1	[11], S
11,12-Didehydroanhydrolycorine **(13)**	24.42	2602	249(M^+,59), 248(100), 190(26), 163(8), 95(12)	t	t	[24]
GW-1[d] **(14)**	25.37	2668	265(M^+,85), 264(100), 248(17), 191(13), 178(18)	t	t	-
Galanthine[d] **(15)**	25.99	2709	317(M^+,26), 316(18), 298(11), 284(14), 268(19), 266(15), 244(15), 243(93), 242(100), 228(9)	50.5	56.2	[18]
1-*O*-acetyl-9-*O*-methylpseudolycorine[d] **(16)**	26.36	2729	345(M^+,34), 284(27), 243(77), 242(100), 228 (9)	t	0.5	[7]
Incartine[d] **(17)**	26.49	2739	333(M^+,43), 332(100), 296(28), 259(71), 258(86)	t	t	[17]
Lycorine[d] **(18)**	26.59	2743	287(M^+,30), 268(24), 250(32), 227(68), 226(100), 211(5), 147(9)	4.3	3.5	[21]
9-*O*-methylpseudolycorine[d] **(19)**	26.87	2788	303(M^+,35), 284(24), 243(86), 242(100)	0.1	0.5	[25]
Sternbergine[d] **(20)**	27.09	2801	331(M^+,36), 270(27), 254(10), 252(14), 229(67), 228(100), 43(9)	-	0.1	[25], S
GW-2[d] **(21)**	27.52	2841	297(M^+,62), 296(100), 280(17), 266(4), 252(5), 224(3), 149(6)	2.7	-	-
2-*O*-acetyllycorine[d] **(22)**	27.78	2879	329(M^+,18), 268(76), 250(100), 240(12), 226(23), 192(11)	0.2	-	[26,27]
Methylleucotamine[c] **(23)**	28.45	2956	373(M^+,100), 330(2), 270(100), 216(18), 165(9)	1.5	0.1	[28]
3-*O*-(3'-acetoxybutanoyl) galanthamine[c] **(24)**	29.92	3070	415(M^+,19), 384(9), 286(2), 270(100), 216(18)	0.2	-	[29]
2-*O*-2'-butenoyllycorine[d] **(25)**	30.95	3125	355(M^+,21), 269(66), 268(83), 250(100), 226(35), 192(6), 147(11)	t	-	[18, 30]
2-*O*-2'-butenoyllycorine isomer[d] **(26)**	31.54	3156	355(M^+,21), 269(64), 268(88), 250(100), 226(31), 192(10), 147(9)	0.4	t	[18, 30]
2-*O*-(3'-hydroxybutanoyl)lycorine[d] **(27)**	32.83	3226	373(M^+,13), 372(9), 269(51), 268(70), 251(38), 250(100), 226(32), 147(7)	0.9	0.1	[29]
2-*O*-(3'-Acetoxybutanoyl)lycorine[d] **(28)**	34.05	3336	415(M^+,13), 414(6), 269(57), 268(70), 250(100), 227(19), 226(28), 192(11)	0.5	-	[18]

a:other type b: phenanthridine-type c: galanthamine-type d: lycorine-type e: tazettine-type S: Standard t:trace < 0.1

*The area of the GC/MS peaks depends not only on the concentration of the related compounds, but also on the intensity of their mass spectral fragmentation.



1 R_1=R_2=H
2 R_1=R_2=CH_3



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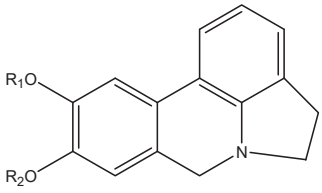
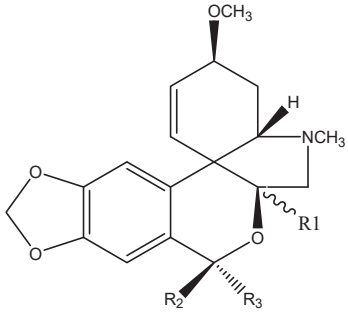
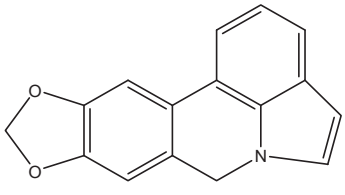


9 R_1+R_2= CH_2
12 R_1= R_2 = CH_3



11 R_1= *α*H R_2=R_3=H



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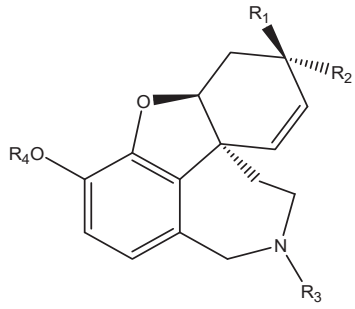
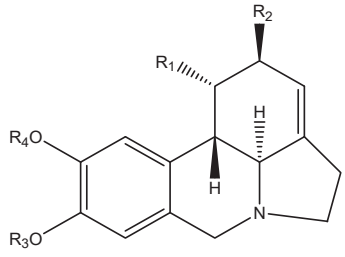


4 R_1= OH, R_2=H, R_3=R_4= CH_3
5 R_1= OH, R_2=H, R_3=CH_3, R_4= H
6 R_1= OH, R_2= R_3=H, R_4= CH_3
7 R_1= H, R_2=OH, R_3=R_4= CH_3
8 R_1+R_2= O, R_3=R_4= CH_3
10 R_1= OAc, R_2=H, R_3=R_4= CH_3
23 R_1= $OCOCH_2CHOHCH_3$ R_2=H, R_3=R_4= CH_3
24 R_1= $OCOCH_2CH(OCOCH_3)CH_3$ R_2=H, R_3=R_4= CH_3



15 R_1= OH, R_2 = OCH_3, R_3=R_4= CH_3
16 R_1= OAc, R_2 = OH, R_3=R_4= CH_3
18 R_1= R_2 =OH, R_3+R_4= CH_2
19 R_1= R_2 =OH, R_3=R_4= CH_3
20 R_1= OAc, R_2= OH, R_3= H, R_4= CH_3
22 R_1= OH, R_2= OAc, R_3+R_4= CH_2
25 R_1= OH, R_2= $OCOC_3H_5$, R_3+R_4= CH_2
27 R_1= OH, R_2= $OCOC_3H_6OH$, R_3+R_4= CH_2
28 R_1= OH, R_2= $OCOCH_2CH(OCOCH_3)CH_3$, R_3+R_4= CH_2

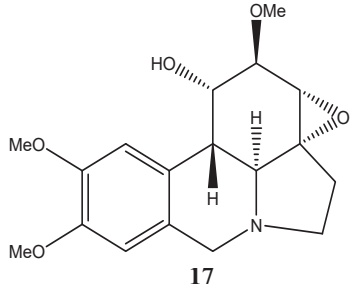


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Figure 1. Structures of alkaloids identified in *G. woronowii*: Tyramine (**1**), hordenine (**2**), 5,6-dihydrobicolorine (**3**), galanthamine (**4**), sanguinine (**5**), *N*-demethylgalanthamine (**6**), epigalanthamine (**7**), narwedine (**8**), anhydrolycorine (**9**), *O*-acetylgalanthamine **(10),** deoxytazettine **(11),** assoanine **(12),** 11,12-didehydroanhydrolycorine **(13),** galanthine **(15),** 1-*O*-acetyl-9-*O*-methylpseudolycorine **(16),** incartine **(17),** lycorine **(18),** 9-*O*-methylpseudolycorine **(19),** sternbergine **(20),** 2-*O*-acetyllycorine **(22)**, methylleucotamine **(23),** 3-*O*-(3'-acetoxybutanoyl) galanthamine **(24),** 2-*O*-2'-butenoyllycorine **(25),** 2-*O*-(3'-hydroxybutanoyl) lycorine **(27),** 2-*O*-(3'-Acetoxybutanoyl) lycorine **(28)**

with diverse chemical structures [3, 4, 7, 9]. This may be due to several factors including polymorphism and exogenous influences such as collection site and seasonal growth. Moreover, in support of this idea, our previous investigations on different specimens of *Galanthus woronowii* collected from different localities revealed that the quantities of bioactive alkaloids lycorine and galanthamine ranged between 0.008-0.364 % and 0.003-0.506 % [16].

In conclusion, it is evident that the qualitative and quantitative alkaloid composition in *G. woronowii* specimens shows significant variation and GC-MS is proved to be a fast and relieable technique allowing identification of alkaloids in these plants.

Both of the extracts showed potent AChE activity (aerial parts:IC_{50}= 0.027 µg /mL and bulbs: IC_{50}= 0.084 µg/mL) which may be due to the presence of galanthamine- or lycorine-type Amaryllidaceae alkaloids. Especially, the presence of galanthamine (IC_{50}= 0.15 µM) and galanthine (IC_{50}= 7.75 µM) in both of the extracts may well contribute to the AChE inhibitory activity of the extracts [7, 11].

Acknowledgments

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