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Original Article

Alkaloid profiling in *Galanthus gracilis* Celak. from western Turkey by GC/MS

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ABSTRACT

Galanthus L. is generally acknowledged as a valuable biological source due to its Amaryllidaceae alkaloids with unusual chemical structures and a wide variety of biological activities. Among these alkaloids, galanthamine is the first example of their practical application in medicine as a potent and selective inhibitor of the enzyme acetylcholinesterase, and already present on the market as a therapeutic agent for Alzheimer's disease. Lycorine is the main Amaryllidaceae alkaloid deeply investigated for its biological activity for many years. In this study the alkaloidal profile of *G. gracilis* collected from western Aegean (Alankıyı/Bayındır) will be illustrated for the first time. GC-MS profiling was carried out on the crude alkaloidal extract obtained from dried and powdered plant material. 11 alkaloids were detected by GC-MS, including graciline, demethylhomolycorine and tazettine as the major ones.

Keywords: *Galanthus gracilis*, Amaryllidaceae, graciline, demethylhomolycorine, tazettine

INTRODUCTION

Amaryllidaceae is a monocotyledon family which is formed by about 85 genera and 1100 species comprising mostly tropical or subtropical plants. *Galanthus* L. is a very interesting genus belonging to the family Amaryllidaceae considered as a very large family with 85 genera and 1100 species (Evans et al. 2002). The genus *Galanthus* L. (Amaryllidaceae) is represented by 14 species (15 taxa) in Turkey (Davis 2006). This genus has been very well known as producers of numerous alkaloids with interesting chemical structures and biological activities such as antitumor, antiviral and acetylcholinesterase inhibitory activity. Previous reports on the alkaloids of this genus reveal that *Galanthus* species possess Amaryllidaceae alkaloids with potentials to be models for new synthetic therapeutic compounds. The most reputed Amaryllidaceae alkaloid galanthamine is a drug prescribed for Alzheimer's disease and it is widely used all around the world (Gabrielsen et al. 1992; Lopez et al. 2002).

Within the course of some ongoing phytochemical studies on Turkish *Galanthus* taxa, *G. gracilis* Celak.has been collected from different localities and afforded number of new and known alkaloids. The most remarkable results of these researches were the presence of a unique subgroup of the Amaryllidaceae alkaloids called gracilines and the isolation of an unusual pentacyclic dinitrogenous alkaloid called gracilamine (Noyan et al. 1998; Unver et al. 1999; UnverKaya 2005; Bozkurt-Sarıkaya et al. 2014). Based on previous findings, *G. gracilis* is a valuable source for alkaloids with interesting chemical structures and a wide range of bioactivities. In the present study, we aimed to investigate the alkaloidal composition of *G. gracilis* collected from a different localisation considering the possible differences depending on climatic and geographical factors. The results have been compared with the ones of previous findings.

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MATERIAL AND METHODS

Plant material

G. gracilis was collected from Alankıyı/Bayındır in the province of İzmir and identified by Prof. Dr. Mustafa Ali Onur from Ege University, Faculty of Pharmacy, Department of Pharmacognosy.

Extraction

The extract was prepared from the bulbs of *G. gracilis*. Briefly, air-dried and powdered plant material (500 mg) was extracted three times using methanol (5 mL) in an ultrasonic bath for 30 minutes at room temperature. After the extraction procedure, solvent was evaporated under *vacuo*. The residue was dissolved in 10 mL 2% H_2SO_4, and the neutral compounds were removed with petroleum ether (3 ×10 mL). The acidic aqueous phases were basified with 25% NH_4OH up to pH 9–10 and extracted with $CHCl_3$ (3×10 mL). The combined chloroform extracts were then dried over anhydrous Na_2SO_4 and filtered through filter paper, and the organic solvent was distilled under *vacuo* to obtain the alkaloidal extract. The extract was dissolved in methanol (1 mg extract in 500 µL CH_3OH) prior to GC/MS Analysis.

GC-MS analysis

Thermo GC-Trace Ultra Ver: 2.0., Thermo MS DSQ II (Thermo Fisher Scientific, San Jose, CA, USA) was used to carry out GC-MS analysis in the electron impact mode (EI, 70 eV).

The oven temperature was programmed as: 80˚C for 1 min, 80-250˚C (10 ˚C X min^{-1}), 250˚C for 2 min, 250-300˚C (10 ˚C X min^{-1}) and 300˚C for 10 min. The injections were run at 250˚C, in the splitless mode. A TR-5 MS column (30 m × 0.25 mm × 0.25 µm) and helium (at a flow rate of 0.8 mL min^{-1}) were used as a stationary phase and carrier gas respectively.1 mg extract was dissolved in 500 µL methanol. Mass spectra of chromatographic peaks were analysed and evaluated using software Xcalibur (version 2.07; Thermo Fisher Scientific San Jose, CA, USA). Identification of the compounds were followed out checking the mass spectral fragmentations against the standard reference spectra from NIST MS Search 2.0 (National Institute of Standards and Technology, Gaithersburg, MD, USA) or the spectra of the authentic standards (S) which were previously isolated by our working team or spectral data procured from the literature. The percentage of the total ion current (TIC) for all the compounds were calculated and given as can be seen in Table 1. The area under the GC-MS peaks points out not only the concentration but also the intensity of mass spectral fragmentation of detected compounds. The ratio of each compound in the extract was exhibited as the percentage of the total alkaloid content in Table 1 and Figure 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.

RESULTS AND DISCUSSION

As previous reports shows, *Galanthus gracilis* collected from Yılanlıdağ/Muğla is hosting plant for 20 alkaloids of 7 Amaryllidaceae skeleton types which are phenanthridine, homolycorine, galanthamine, crinine, indole, tazettine and lycorine types. Also these previous findings show that; homolycorine and 8-O-demethylhomolycorine are the main alkaloids for the aerial parts and bulbs of the plant respectively (Bozkurt-Sarıkaya et al. 2014).

Table 1. Chemical composition of the *G. gracilis* bulb methanolic extracts on GC/MS analysis (collected from Alankıyı/Bayındır).

Compound Name	Retention Time (min)	[M+]	m/z (rel.%)	Bulbs (%)
Graciline type alkaloid-1	13.29	-	225 (100), 167 (10), 139 (31), 84 (7)	0.63
Graciline (Sarıkaya et al. 2013)	15.26	283 (10)	282 (7), 264 (4),254 (10),240 (8), 227 (6),226 (21), 225 (100), 139 (7)	73.2
Graciline type alkaloid-2	15.85	-	283 (84), 268 (25), 254 (43), 240 (78), 225 (100), 215 (35), 197 (29), 139 (45)	1.63
Graciline type alkaloid-3	16.32	-	283 (100), 254 (26), 242 (38), 240 (23), 228 (68), 225 (23), 139 (23)	0.49
Galanthindole (Bozkurt-Sarikaya et al. 2014)	16.68	281 (100)	264 (15), 263 (20), 262 (22), 252 (16), 204 (10), 191 (17), 132 (15), 107 (12)	4.98
6-O-Methylpretazettine (Bozkurt-Sarikaya et al. 2014)	17.31	345 (14)	330 (16), 261 (100), 239 (29), 230 (15), 201 (21)	0.9
6-O-Methylpretazettine isomer (Bozkurt-Sarikaya et al. 2014)	17.46	345 (10)	330 (12), 261 (100), 239 (24), 230 (19), 201 (25)	<0.01
Tazettine (Berkov et al. 2009)	17.77	331 (14)	316 (10), 298 (17), 247 (100), 227 (13), 211 (13), 201 (19), 181 (19), 152 (13), 115 (17)	5.76
Hippamine (Berkov et al. 2008)	18.33	301 (80)	300 (9), 227 (90), 226 (100), 227 (15)	2.41
Homolycorine (Bozkurt-Sarikaya et al. 2014)	18.60	315 (-)	109 (100), 108 (22), 94 (3)	2.76
Demethylhomolycorine (Bozkurt-Sarikaya et al. 2014)	19.24	301 (-)	109 (100), 108 (2), 94(2)	7.24

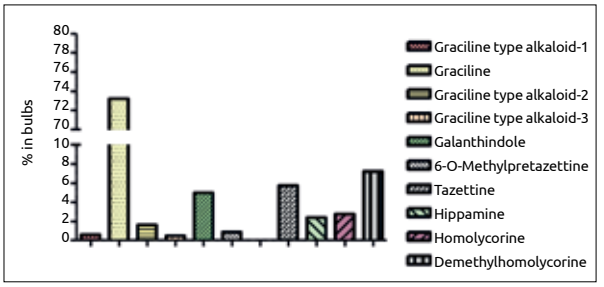

Figure 1. Graphical presentation of total amount percent of each of alkaloids in bulbs for *Galanthus gracilis*

GC/MS analysis on the bulbs methanolic extract of *G. gracilis* collected from Alankıyı/İzmir revealed that, this plant is a good source of bioactive compounds with a total of 11 alkaloids as shown in Table 1. The detected alkaloids possessed different skeleton types such as; graciline, indole, tazettine, lycorine and homolycorine. Generally, graciline, homolycorine and tazettine type alkaloids are major alkaloids in the tested material (Table 1). The extract of the bulbs of *G. gracilis* contain graciline as the main alkaloid (73.2%). Similarly, this extract comprises demethylhomolycorine (7.24%) and tazettine (5.76%) in considerable percentage. In addition an indole type alkaloid, galanthindole, is also found in significant rate (4.98%).

Aside from *Galanthus gracilis*; *G. elwesii* Hook., *G. xvalentinei*, *G. woronowi* Losinsk., *G. rizehensis* and *G. reginae-olgae* subsp. *vernalis* were already investigated by GC-MS (Berkov et al. 2004; Bozkurt et al. 2017; Conforti et al. 2010; Sarıkaya et al. 2013; Sarikaya et al. 2013). In terms of alkaloidal content, only *Galanthus gracilis* and *G. xvalentinei* nothosubsp. *subplicatus* are similar by the fact that they contain graciline type alkaloids. Among these interesting two species, *Galanthus gracilis* seems to have higher relative percentage of graciline types than *G. xvalentinei* nothosubsp. *subplicatus*.

CONCLUSION

It can be concluded that this plant is a valuable source of alkaloids with diverse chemical structures including graciline type alkaloids. The alkaloidal profile of *G. gracilis* naturally growing in Alankıyı/Bayındır differs from the other samples collected from Kemalpasa/Izmir and Yilanlidag/Mugla (Bozkurt-Sarıkaya et al. 2014; Sarıkaya et al. 2013). *G. gracilis* has a great value because of its extraordinary alkaloidal profile. Even this plant has completely different compounds such as graciline type alkaloids. The bioactivity of the graciline-type alkaloids is not well studied. Therefore the detection of gracilines in *G. gracilis* may also encourage chemists to synthesize these alkaloids for the purpose of investigating various bioactivities to be used in drug development studies.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author has no conflict of interest to declare.

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