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Short Communication

Quantification of galantamine in *Narcissus tazetta* and *Galanthus nivalis* (Amaryllidaceae) populations growing wild in Iran

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Abstract

Galantamine (GAL), a morphine-like alkaloid produced by some members of the Amaryllidaceae plant family, is a possible therapeutic agent in Alzheimer's disease because of its central cholinergic effects. GAL has been extracted from the plant sources or produced synthetically for pharmaceutical use. Limited supply of the natural source and high cost of synthetic production has led to a search for alternative sources of this valuable compound. In the present study, a total of six *Galanthus nivalis* populations (GNPs) and 11 *Narcissus tazetta* populations (NTPs) were collected across different regions of Iran and were then subjected to the high-performance liquid chromatography analysis for their GAL quantification. The GAL content ranged from 0.05 to 0.36 mg/g dry weight (DW) in the bulbs of GNPs, and from 0.03 to 0.33 mg/g DW in the bulbs of NTPs. Maximum content of GAL (0.36 and 0.33 mg/g DW) was measured in the Zirab population of *G. nivalis* and Ghaemshahr population of *N. tazetta*, respectively. Our results provided a suitable material for further agronomical and biotechnological strategies for enhanced production of valuable GAL compound on a large scale.

Keywords: acetylcholinesterase inhibitor, alkaloid, Alzheimer's disease, Amaryllidaceae, HPLC

Introduction

Amaryllidaceae alkaloids represent a kind of phenylalanine and tyrosine derivatives restricted to the only Amaryllidaceae plant family (Zhong, 2005). Galantamine (GAL, Fig. 1), a benzazepine alkaloid, was first isolated from the bulbs and flowers of *Galanthus caucasicus* and *Galanthus woronowii* by Bulgarian scientists in the mid-20th century (Heinrich and Teoh, 2004). This has been also obtained from the other related genera such as *Narcissus, Leucojum, Lycoris* (Bastida *et al.*, 1990; Moraes-Cerdeira *et al.*, 1997; Cherkasov and Tolkachev, 2002). GAL is a long acting, selective, reversible and competitive inhibitor of the acetylcholinesterase (AChE) enzyme (Thomsen *et al.*, 1998), which is marketed as a hydrobromide salt under the name of Razadyne® (formerly Reminyl®) and Nivalin® for the treatment of Alzheimer's disease (AD), poliomyelitis and other neurological diseases (Heinrich and Teoh, 2004). Recently the more widespread licensing of GAL throughout the world has caused a need for alternative sources. Synthetic methods have been developed and used to produce GAL for the pharmaceutical industry (Tiffen, 1997), but high costs and increasing demand

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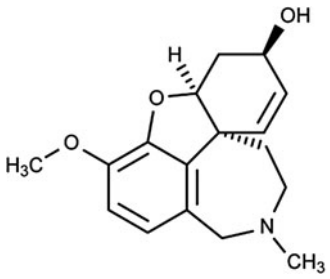

Fig. 1. Chemical structure of GAL.

make extraction from plant sources an attractive option. A potential source for large-scale extraction of the alkaloid is members of Amaryllidaceae family especially *Narcissus* and *Galanthus* species (Lubbe *et al.*, 2013). As a part of our ongoing studies on the natural source of GAL and due to the importance of this compound in clinical medicine (Jiang *et al.*, 2015; Naharci *et al.*, 2015), we report here for the first time quantification of this alkaloid in some wild populations of *G. nivalis* and *N. tazetta*. Additionally, our findings provide valuable information for the selection of GAL-rich genotypes.

Experimental

A total of six *G. nivalis* populations (*GNP1–GNP6*) and 11 *N. tazetta* populations (*NTP1–NTP11*) were collected across different geographical regions from the north to the southwest of Iran. The bulbs of wild *G. nivalis* and *N. tazetta* populations were collected at fruiting set stage in March and April 2014 from their natural habitats, respectively (Table 1). Air-dried bulbs (300 mg) of all collected *GNPs* and *NTPs* were powdered in a mortar and extracted three times with methanol (3 × 5 ml) by sonication at room temperature as described previously (Georgieva *et al.*, 2007). After filtration, the plant residues were rinsed with methanol (2 × 5 ml) and the combined methanol extract was evaporated under vacuum. The dry extract was redissolved in 3% H_2SO_4 (4 ml) and defatted with diethyl ether (3 × 5 ml). After basification to pH 9–10 with 25% ammonia, the GAL was extracted with chloroform (3 × 5 ml). The organic solvent was dried under reduced pressure in a rotary evaporator at 40°C (Heidolph Instruments GmbH, Schwabach, Germany). The extracts were dissolved in high-performance liquid chromatography (HPLC) grade methanol (1 ml), filtered through a Millipore filter (0.45 mm) and stored in a refrigerator until analysis. HPLC and liquid chromatography-mass spectrometry (LC-MS) analyses were performed as described in the Supplementary Material (online only).

Discussion

One of the most used HPLC method for the quantitative determination of GAL and some other Amaryllidaceae

Table 1. Geographic location of the studied *Galanthus nivalis* (GN) and *Narcissus tazetta* (NT) populations from Iran

			Geographic location		
Plant species	Population name	Population code	Latitude (N)	Longitude (E)	Altitude (m)
GN	Zirab	*GNP1*	36°10′62″	52°58′00″	462
	Sangedeh	*GNP2*	36°03′27″	53°14′78″	1537
	Gorgan	*GNP3*	36°47′01″	54°26′58″	370
	Poonel	*GNP4*	37°33′00″	49°06′45″	32
	Noor	*GNP5*	36°28′56″	51°54′55″	514
	Sari	*GNP6*	36°08′00″	53°18′16″	780
NT	Yazd	*NTP1*	31°51′37.20″	54°24′53.77″	1233
	Kazeroon	*NTP2*	29°14′43.02″	51°59′02.69″	793
	Shiraz	*NTP3*	30°00′24.95″	52°37′56.32″	1608
	Booshehr	*NTP4*	27°49′51.77″	52°19′45.83″	636
	Shahrekord	*NTP5*	32°04′09.41″	50°53′33.65″	2688
	Behbahan	*NTP6*	30°36′58.20″	50°16′25.43″	323
	Tabas	*NTP7*	33°36′07.15″	56°56′10.97″	687
	Arak	*NTP8*	33°54′57.48″	50°26′45.47″	1802
	Isfahan	*NTP9*	32°38′42.07″	51°30′44.12″	1605
	Ghaemshahr	*NTP10*	36°25′20.90″	52°51′39.36″	106
	Noor-Abad	*NTP11*	30°06′30.43″	51°32′01.58″	963

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alkaloids was developed by Sellés *et al.* (1999). Recently, different HPLC systems for analysis of GAL in various species of Amaryllidaceae family have also been reported (Petruczynik *et al.*, 2016; Svinyarov *et al.*, 2016). Although, the technique has been previously used for estimation of GAL in some members of Amaryllidaceae family such as *Narcissus confusus* (Sellés *et al.*, 1999), *Leucojum aestivum* (Schumann *et al.*, 2012), *Galanthus trojanus, Galanthus cilicicus, Galanthus elwesii* (Kaya *et al.*, 2004, 2014), *Zephyranthes rosea* and *Clivia miniata* (Petruczynik *et al.*, 2016), but no reports are available regarding quantitative estimation of GAL in wild growing populations of *G. nivalis* and *N. tazetta* from Iran. The identification and quantitative determination of GAL in all collected *GNPs* and *NTPs* was established by comparison of the retention time, MS spectra and peak area with the standard. According to Sellés *et al.* (1999), a mobile phase consisting of acetonitrile gave symmetrical and sharp peak of GAL at a RT (retention time) of 20.1 min. Figure 2 show LC-MC total ion chromatogram of the extract. All calibration curves were linear over the concentration ranges with correlation coefficients (r) higher than 0.9990. The GAL content ranged from 0.05 to 0.36 mg/g dry weight (DW) in the bulbs of *GNPs*, and from 0.03 to 0.33 mg/g DW in the bulbs of *NTPs* (Table 2). Maximum content of GAL (0.36 and 0.33 mg/g DW) was measured in the Zirab population of *G. nivalis* and Ghaemshahr population of *N. tazetta*, respectively. Studies have reported that different geographical regions and cultural practices affected the chemical compositions of the plants (Hadian *et al.*, 2011; Aghaei *et al.*, 2013; Khadivi-Khub *et al.*, 2014). Georgieva *et al.* (2007) studied GAL distribution in various wild populations of *L. aestivum* by GC-MS (gas chromatography-mass spectrometry) and found that the GAL content ranged from 28 to 2104 µg/g DW in the bulbs, and from traces to 454 µg/g DW in the shoot-clumps. Due to the importance of GAL as natural compound with potent of the AChE inhibition (Jiang *et al.*, 2015; Naharci *et al.*, 2015), there is a need for further investigations on the variations of the compounds within and among wild populations of both plant species. The variations in GAL content in the studied *GNPs* and *NTPs* suggest that the genetic factor plays an important role in the biosynthesis of this compound. The chemical variation can be also attributed to environmental factors. Hanover (1992) provides evidence that terpene biosynthesis are strongly controlled by genetic factors, he also reported instances of environmental variation in terpene expression under extreme habitat conditions. In this respect, an intensive selection in wild plants is necessary to obtain GAL-rich cultivars for the extraction of this pharmaceutically interesting compound. In conclusion, our results show that *G. nivalis* populations especially Zirab population (*GNP1*) and Noor population (*GNP5*) are valuable source of GAL. It can provide an ample

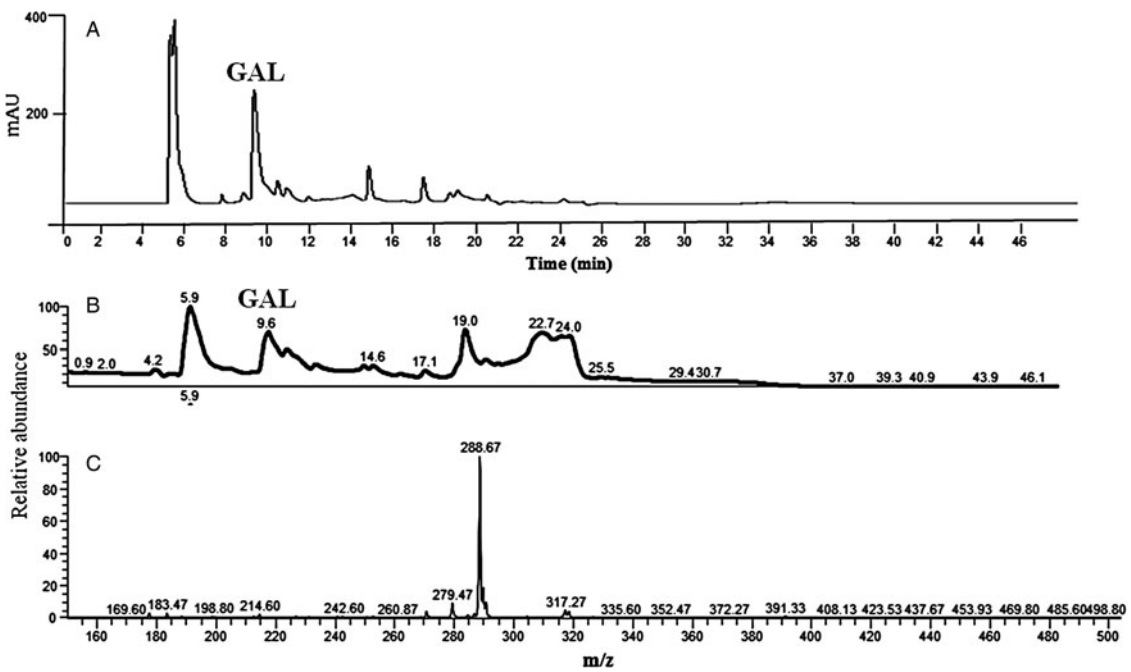

Fig. 2. HPLC-UV chromatogram of the methanolic extract of *Narcissus tazetta* recorded at 280 nm (A), total ion chromatogram (TIC) related to LC-MS analysis of the same extract (B), and mass spectrum of GAL in which 288.6 m/z (C) could be assigned to its proton adduct ion. (Both chromatographic and mass spectrometric condition of analyses are explained in the experimental section.)

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Table 2. Content of galantamine (GAL) in the bulb of studied *Galanthus nivalis* (GN) and *Narcissus tazetta* (NT) populations from Iran

Plant species	Population name	Population code	GAL content (μg/g DW)
GN	Zirab	*GNP1*	354
	Sangedeh	*GNP2*	232
	Gorgan	*GNP3*	219
	Poonel	*GNP4*	254
	Noor	*GNP5*	351
	Sari	*GNP6*	49
NT	Yazd	*NTP1*	77
	Kazeroon	*NTP2*	123
	Shiraz	*NTP3*	35
	Booshehr	*NTP4*	180
	Shahrekord	*NTP5*	140
	Behbahan	*NTP6*	37
	Tabas	*NTP7*	208
	Arak	*NTP8*	33
	Isfahan	*NTP9*	99
	Ghaemshahr	*NTP10*	326
	Noor-Abad	*NTP11*	97

opportunity to take this plant for extensive research for mass cultivation on plants and enhanced anti-AD compound production through different breeding and biotechnological strategies such as cell suspension cultures and large-scale cultivation in bioreactor system.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262117000107.

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