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Original article

Nerinine and homolycorine, amaryllidaceae alkaloids from the bulbs of *Galanthus transcaucasicus* Fomin

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Abstract

Background and objectives: Many members of the Amaryllidaceae are regarded as toxic. The toxic constituents that occur in the whole family are referred to as the Amaryllidaceae alkaloids. The main aim of this study was the identification of alkaloid compounds from *Galanthus transcaucasicus* Fomin, a medicinal plant from Amaryllidaceae. **Methods:** Planar and column chromatography techniques were used for isolation of alkaloid components. GC/MS analysis was carried out for the identification of alkaloid compounds. **Results:** Silica gel column chromatography of the alkaloidal extract of *G. transcaucasicus* bulbs afforded seven fractions. Preparative thin layer chromatography of these fractions led to the isolation of compounds **1** (nerinine) and **2** (homolycorine). Galantamine was not detected in any of these fractions. **Conclusion:** Our findings showed that *G. transcaucasicus* could be a new source of bioactive alkaloids for possible applications in pharmaceutical industries.

Keywords: alkaloid, Amaryllidaceae, *Galanthus*, GC/MS

Introduction

Amaryllidaceae family possesses about 85 genera and more than 1100 species which are distributed all around the world especially throughout warm regions and tropical areas [1]. These plants are well-known for their ornamental and traditional uses. They are also famous for their wide pharmacologically active alkaloids [2].

The genus *Galanthus*, produces biologically active alkaloids called Amaryllidaceae alkaloids [3]. These alkaloids have been found to possess significant bioactivities such as antiviral, antiprotozoal, antitumor and cholinesterase inhibitory activities [4-6]. Acetylcholinesterase (AChE) inhibitors are currently used for the symptomatic treatment of Alzheimer's disease (AD). Galantamine is an Amaryllidaceae alkaloid which possesses dominant and selective AChE inhibitory effect and shows long acting, reversible and competitive activity [7]. It has been shown that other Amaryllidaceae alkaloids exhibited similar or stronger AChE inhibitory activity compared to galantamine [8]. The genus

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Galanthus has been represented in the flora of Iran by two endemic species: *G. transcaucasicus* Fomin and *G. alpines* Sosn [9]. However, some sources have refered to several other species that do not have the certainty of identification. *G. transcaucasicus* is an endemic species distributed in the Caucasia and north-west (Azerbaijan and Ardabil provinces) and north of Iran [9,10].

There are many reports about the phytoconstituents of the genus *Galanthus* but we could find just one study on *G. transcaucasicus* in the literature review. Several classes of Amaryllidaceae alkaloids including galanthamine, lycorine, tazettine, narwedine and caranine have been isolated from the bulbs of *G. transcaucasicus* by Salehi-Sourmaghi *et al*. [11]. In the present study, we have reported the identification of homolycorine and nerinine alkaloids from the bulbs of *Galanthus transcaucasicus* Fomin.

Experimental

Plant material

Galanthus transcaucasicus Fomin bulbs were collected in flowering stage in May 2014, from Oak *(Quercus* spp.*)* woodland edges in Andabil (37° 38' N, 48° 33' E; the central district of Khalkhal region), Ardebil province, Iran. The plant was identified by Prof. Nazemiyeh (Tabriz University of Medical Sciences) and a voucher specimen (Tbz-FPh 761) was deposited at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

Chemicals

Solvents were obtained from Merck (Germany) or Scharlau (Spain) companies and other chemicals and reagents were purchased from Merck (Germany).

Extraction and Isolation

Plant bulbs were air dried at room temperature and powdered. The powdered samples (200 g) were dampened by 1% HCl and extracted with 96% ethanol (3×500 mL; 24 h each at room temp.). The extracts were combined and evaporated to yield alcohol free aqueous residue (50 mL). The aqueous residue was filtered and extracted by petroleum ether to remove lipids and pigments and then its pH was adjusted to 10 by ammonia solution (25%). The basic solution was extracted with chloroform (3×50 mL) to afford the crude alkaloidal extract (0.9 g). The extract was subjected to silica gel 60 F_{254} (mesh 70-230) column chromatography (2.5×57 cm) using gradient elution of $CHCl_3$/MeOH (100:0 to 0:100) to obtain 67 fractions. TLC technique (pre-coated silica gel 60 F_{254} plates, Merck (Germany); $CHCl_3$/MeOH/NH_4OH 9:0.5:0.5; Dragendorff's reagent) was used to monitor the fractions and similar fractions were combined to afford 7 final major fractions:**1** (37 mg); **2** (42 mg); **3** (70 mg); **4** (51 mg); **5** (67 mg); **6** (115 mg); **7** (375 mg).

Further purification of fractions **1-3** was done by preparative thin layer chromatography (pre-coated silica gel 60 F_{254} plates, 2mm, Merck (Germany), $CHCl_3$/MeOH/NH_4OH 9:0.5:0.5) and the obtained subfractions were tested for the presence of galantamine. Finally, two alkaloid rich subfractions were dissolved in $CHCl_3$ and kept in a fridge for a week to render pale yellow sediment. The solutions of sediments in $CHCl_3$ were exposed to GC/MS analysis and compounds **1** and **2** were determined (figure 1).

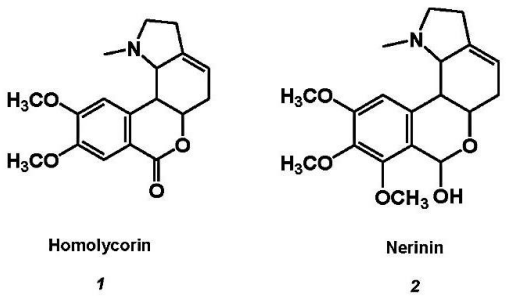

Figure 1. Structures of the isolated compounds from *Galanthus transcaucasicus* Fomin

GC/MS analysis

Alkaloid compounds were identified using gas chromatography coupled with mass spectrometer detector (Shimadzu, Japan, QP-5050 A). The GC/MS apparatus was equipped with DB-1 capillary column (60 m length, 0.25 mm i.d., 0.25 µm stationary thickness). An electron impact (EI) ionization system, with ionization energy of 70

eV and solvent delay 5.0 min was employed for detection of components. Helium (99.99%) was used as the carrier gas at constant flow rate of 0.7 mL/min, and split ratio was 1:19. Temperature program for the column included as follows: the initial oven temperature was kept at 50 °C for 2 min, then temperature was raised from 50 °C to 310 ºC at rate 2.5 °C/min and was maintained for 25 min. The temperature of the injector was 280 °C. Identification of compounds was achieved by the Wiley 229, Nist 107, Nist 21 Libraries comparing as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literatures [12-14].

Results and Discussion

Galanthus L. species (Amaryllidaceae), are known to produce Amaryllidaceae alkaloids with diverse structures and interesting biological activities [2]. A great number of alkaloids that are found in the Amaryllidaceous plants has been separated effectively and identified by GC/MS [15-18]. It is claimed that GC/MS technique has many applications in building a relationship between phylogeny and phytochemical diversity of Amaryllidaceae family [19,20].

In this study, the extract was fractionated using column chromatography and visualization of alkaloid compounds in the fractions was carried out on the TLC plates using Dragendorff's reagent. Preliminary analysis using TLC showed that there is a small amount of galantamine in plant samples but it was not detected in any of fractions. In continuation, further studies of fractions using TLC and GC/MS analysis revealed 11 Amaryllidaceae alkaloids while two of them were identified (figure 1).

The GC/MS analysis of compound **1**, led to the identification of an alkaloid compound homolycorine (9,10-dimethoxy-1-methyl-lycorenan-7-one) with molecular formula of $C_{18}H_{21}NO_4$. Its molecular weight was found to be 315.3 g/mol. MS profile of **1** showed the base peak at m/z 109 followed by m/z 110 and 108 as the other main peaks (table 1, figure 2). To the best of our knowledge, homolycorine has not been previously isolated from *G. transcaucasicus*.

Available GC/MS data proposed the structure of nerinine (8,9,10-trimethoxy-1-methyllycorenan-7-ol; MW 347.4 g/mol) with the molecular formula $C_{19}H_{25}NO_5$ for compound **3** (table 1). It deems that the fragments 195, 207 and 221 could be due to the presence of a 3,4,5 tri-methoxy benzene ring in structure of the compound which in turn can confirm the presence of nerinine (figure 3). Our literature review revealed that nerinine and homolycorine are rare in the genus *Galanthus*. Both compounds homolycorine and nerinine are classified as homolycorine-type Amaryllidaceae alkaloids. Although homolycorine has been isolated from some members of the genus *Galanthus*, this is the first report on the isolation of nerinine from this genus. Nerinine has been previously reported from some species belonging to Amaryllidaceae family such as *Hippeastrum morelianum* [20].

There is just one report on *Galanthus transcaucasicus* Fomin in the literature. In that work five isoquinoline type alkaloids namely galanthamine, narwedine, lycorine, caranine and tazettine were isolated from the bulbs of *Galanthus transcaucasicus* Fomin [11]. Our literature review revealed that nerinine and homolycorin are rare in the genus *Galanthus*. Also the alkaloid patterns of two *Galanthus transcaucasicus* population from Ardabil and Alborz mountains [11] were compared and our finding showed that their alkaloid patterns were different.

Lack of congruence between earlier phytochemical studies, specialized GC/MS analysis and other group findings may have been caused by several different phenomena. One contributing factor is the difference between the plant specimens collection area.

It is noteworthy that some of the observed differences between alkaloid patterns may have

Table 1. GC/MS profile of alkaloid rich fractions obtained from hydro-ethanol extract of *Galanthus transcaucasicus* bulbs

Fractions	Compound	RT (min)	$[M]^+$	*m/z* (rel. int. %)	MS Ref.
3-6	Homolycorine **(1)**	35.15	315	206(≈1), 178(3), 150(≈1), 109(100), 108(48), 94(3), 82(3)	13-15
9-11	Nerinine **(2)**	36.58	347	221(1), 207(2), 195 (≈1), 151(1), 110(62), 109(100), 108(22), 94(4)	14

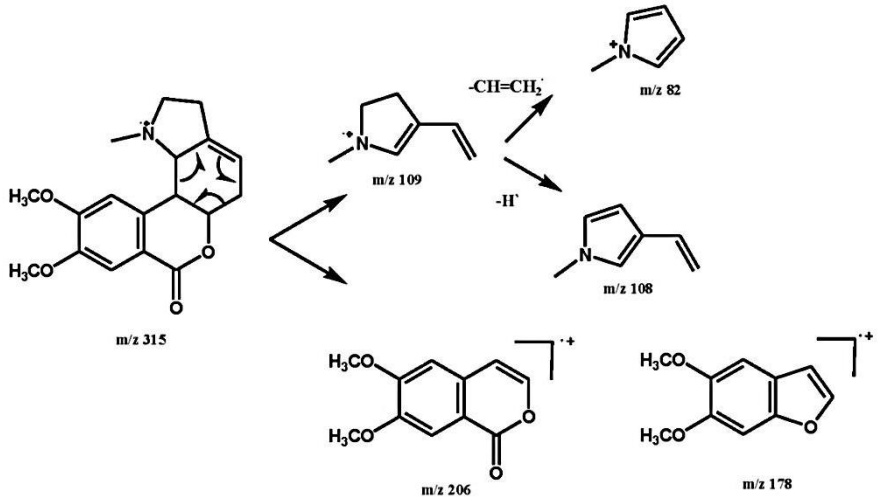

Figure 2. Proposed fragmentation pattern of homolycorine

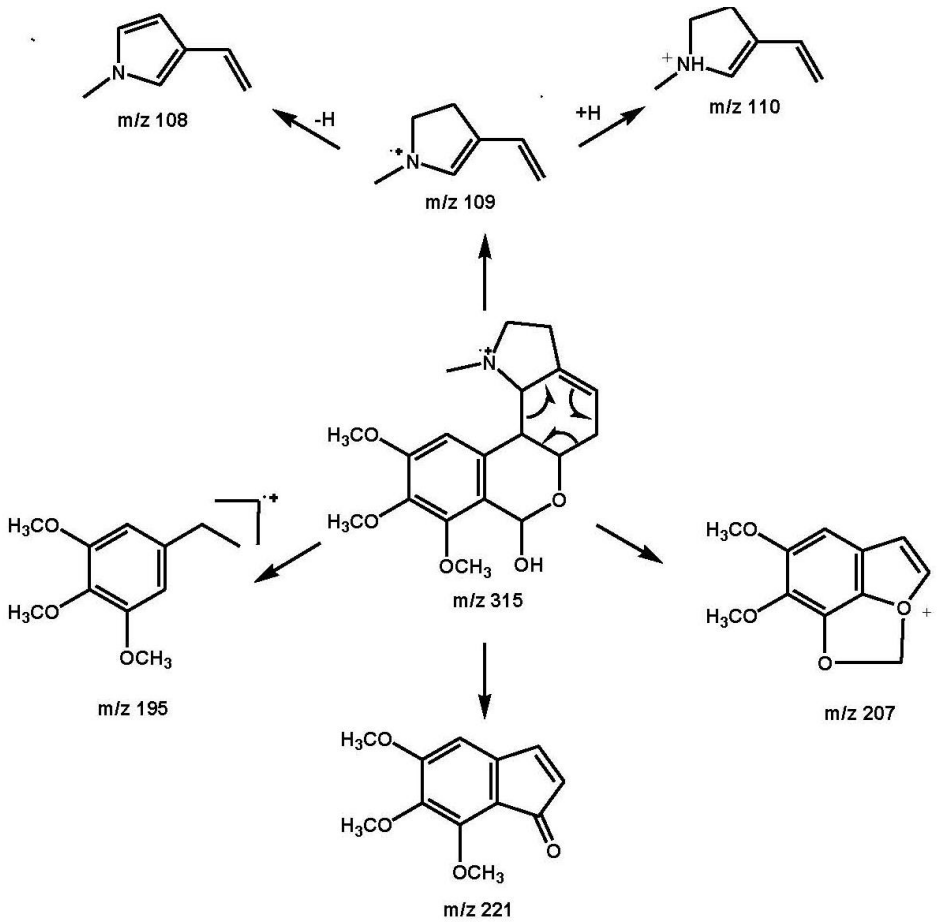

Figure 3. Proposed fragmentation pattern of nerinine

been caused by a combination of changes in the plant population [epigenetic background, chemotypes and environmental factors (temperature, soil composition, rainfall, humidity, *etc*.)] [21-23]. Previous studies of Berkov *et al*. on 25 *G. elwesii* and 7 *G. nivalis* populations in Bulgaria have revealed that some *G. elwesii* populations have different chemotypes, though factors determining alkaloid diversity remain unclear. On this basis, both *G. elwesii* and *G. nivalis* displayed intraspecies variability in their alkaloid profiles and the populations showed a wide variation in the number of alkaloidal constituents [3,24]. In addition, there are some evidence which confirms the involvement of environmental factors on genetic variability resulting in alkaloid diversity [25,26]. On the Other hand, we found that some Amaryllidaceae alkaloids are unstable and undergo structural changes over time and during the extraction process. It should also be noted that similar results have been reported previously by Salehi-Sourmaghi *et al*. [11].

The alkaloid patterns of several parts of *Galanthus nivalis* and *Galanthus elwesii* have been studied by GC/MS [16]. Thirty-seven alkaloids were detected, 25 for *G. nivalis* and 17 for *G. elwesii*. The predominant alkaloids in the roots of *G. nivalis* species were found to belong to the lycorine and tazettine structural types; bulbs were dominated by tazettine, leaves by lycorine and flowers by haemanthamine type alkaloids. Also, the predominant alkaloids in *G. elwesii* roots, bulbs and leaves were those of homolycorine type. But homolycorine (**2**) was found just in leaves of *G. elwesii*. A general feature of the homolycorine type alkaloids with a double bound $\Delta^{3,4}$ was the low intensity of their molecular ions [22]. These alkaloids have intensive fragments in the low mass range representing the pyrrolidine ring fragments, and less intensive ion fragments in the middle mass range which encompasses the aromatic lactone or hemiacetal moiety.

Herein, we reported the isolation and identification of two alkaloid compounds from the bulbs of *G. transcaucasicus*. This is the first report of the isolation and purification of homolycorine and nerinine from *G. transcaucasicus*.

Our findings indicated that, this species could be a new source of alkaloid compounds for possible uses in pharmaceutical industries. The identified Amaryllidaceae alkaloids in this study belong to homolycorine type alkaloids. Our findings together with previous studies could be used for chemotaxonomical studies of genus *Galanthus*. The use of secondary metabolites, and Amaryllidaceae alkaloids in particular, as chemotaxonomic markers should be very precocious because of the variations found in the alkaloid patterns between the populations. Also, further studies for determination of phytochemical and pharmacological properties of *G. transcaucasicus* are warranted.

Acknowledgements

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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