The first phytochemical report of *Galanthus transcaucasicus* Fomin

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**ABSTRACT**

**Background and the purpose of study:** *Galanthus transcaucasicus* Fomin (Amaryllidaceae) is an endemic species to the Caucasus and Alborz mountains in Iran which locally named “Gol-e-Barfi”. While there are many reports on pharmacological activities of *Galanthus* species’ alkaloids, there is no report on *G. transcaucasicus* and this article is the first phytochemical study on this species.

**Methods:** Extracted alkaloids from *G. transcaucasicus* bulbs were isolated using different chromatographic methods and the structures of the components were determined by physical and spectroscopic data.

**Results:** Five isoquinoline type alkaloids namely galanthamine(8.04%), narwedine(6.90%), lycorine(19.48%), caranine(3.45%) and tazettine(5.75%) of total alkaloid extract were isolated from the bulbs of *Galanthus transcaucasicus* Fomin.

**Major conclusion:** Because of the presence of biologically active alkaloids especially galanthamine and the major alkaloid lycorine in Gol-e-Barfi, the plant may be used as a natural source for pharmaceutical purposes.

**Keywords:** *Galanthus transcaucasicus*, Galanthamine, Lycorine, Tazettine, Isoquinoline alkaloids

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**INTRODUCTION**

*Galanthus* is an important genus of the Amaryllidaceae family and the species are native to many parts of Europe including Bulgaria, the eastern parts of Turkey, the Caucasus Mountain and Iran (1, 2). They are bulbous plants with narrow grassy leaves, erect flowering stalks and white flowers (3). The name *Galanthus* is derived from two Greek words “gala” for milk and “anthos” for flower, which are descriptive of the snow white blossoms of this genus (4). The majority of alkaloids especially isoquinoline type like as galanthamine, lycorine, caranine, narciclasine, tazettine, narwedine and montanine which have a wide range of acetylcholinesterase inhibitory, antitumor, antiviral, immunostimulatory and antimalarial activities, have been isolated from this genus species (5). *Galanthus transcaucasicus* Fomin (snowdrop) which is locally named “Gol-e-Barfi” is an endemic species of the Caucasus and the Alborz mountains in Iran (2, 6). A thorough literature survey revealed that there is no report on this species and this article describes, identification of alkaloids especially galanthamine and lycorine from *Galanthus transcaucasicus* Fomin.

**MATERIALS AND METHODS**

**General**

Melting points were determined on a Reichert-Jung apparatus and are uncorrected. FT-IR spectra were recorded using a Nicolet 550-A spectrometer (KBr disks). EIMS spectra were recorded by a Hewlett Packard 5973 Mass Spectrometer at 70 eV. NMR spectra were acquired at 500 MHz for 1H and 125 MHz for 13C, on a Bruker Avance 500 spectrometer, using CDCl3 and CD3OD as solvents and TMS as internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. TLC aluminium sheets (silica gel 60 F254 20 × 20), silica gel 60GF254 and silica gel (70-230 mesh) were used for analytical and preparative TLC and column chromatography, respectively. Sephadex LH-20 was used for gel filtration. With exception of Sephadex LH-20 and galanthamine standard which were from Pharmacia and Sigma-Aldrich companies, respectively, all other chemicals were provided by Merck company. Spots on chromatograms were detected under UV light (254 and 366 nm) and Dragendorff’s reagent.

**Plant material**

*Galanthus transcaucasicus* Fomin specimens were collected in April 2008 from the west (Rostam Abad of Rood Bar) and east (Sang Deh of Sari) of Alborz Mountain in Iran. Voucher specimens were identified by Dr. Gholamreza Amin and are deposited under No.
Extraction and isolation
The air-dried and powdered bulbs of *Galanthus transcaucasicus* from the west and east of Alborz mountains (2 and 1 kg respectively) were extracted by percolation method with 96% EtOH (17.6 and 12.1 liter. respectively) at room temperature. The ethanol extracts were evaporated under reduced pressure to give brown gummy extracts (236.2 and 57.5 g, yield: 11.81% and 5.75% respectively) (7).

The crude extracts were dissolved in 2%aq. HCl separately. The acidic solution was filtrated and basified to pH 9 -10 by 25%aq. NaOH and then extracted successively with CHCl3. The organic solvents were dried over anhydrous Na2SO4 and evaporated to give the crude alkaloid extracts. The basic crude alkaloid extract was then fractionated by column chromatography on silica gel by gradient elution with petroleum ether (40-60°C), petroleum ether- chloroform, chloroform- methanol and finally, methanol. Forty and eight fractions (50 ml each) were collected and monitored by TLC using CHCl3-MeOH (7:3) and CHCl3-MeOH (3:4) as solvent systems. Fractions obtained by 5% to 80% MeOH-CHCl3, contained alkaloids. By mixing similar fractions 4 sub-fractions are obtained. Further purification was carried out by successive preparative TLC, preparative column chromatography and Sephadex LH-20. Since during this investigation it was found that the compounds are unstable, following prompt method was used for isolation and purification of alkaloids.

The crude alkaloid extract was dissolved in MeOH from which lycorine (III), the major alkaloid, crystallized directly (8). The solution was concentrated and subjected to preparative TLC using CHCl3-MeOH (7:3) as mobile phase to afford galanthamine (I) and caranine (IV) (Rf : 0.58 & 0.72 ). The spot with Rf 0.50 - 0.54 was subjected to further fractionation and purification by preparative TLC using CH2Cl2-MeOH (7:1) as the solvent system to yield narwedine (II) (Rf: 0.44) and tazettine (V) (Rf: 0.33) (8). The compounds were separated from the layers with MeOH.

Galanthamine (I)
White needles, m.p. 124 - 126°C. FT-IR νmax cm⁻¹ : 3300, 1620, 1595, 1515, 1420, 1280, 1040. EIMS 70 eV, m/z (rel. int.): 287 [M⁺] (92), 286 (100), 244 (26), 230 (15), 226 (18), 216 (33), 174 (28). 1H-NMR (500MHz,CDCl3): δ 1.60 (1H, dd, J = 13.5, 2.3 Hz, H-11β), 2.03(1H,ddd, J = 15.7, 5.0, 2.5 Hz, H-2a), 2.11 (1H, dd, J = 13.5, 3.2 Hz, H-11α), 2.42(3H, s, NMe), 2.71(1H, dt, J= 15.7, 1.8 Hz, H-2β), 3.08(1H, br d, J = 14.6 Hz, H-12α), 3.30(1H, br t, J = 14.6 Hz, H-12β), 3.71 (1H, d, J = 15.2 Hz, H-6a), 3.85(3H, s, OMe), 3.89(1H, s, 3-OMe), 4.12(1H, d, J = 15.2 Hz, H-6β), 4.16(1H, t, J = 4.5 Hz, H-3), 4.63(1H,br s, H-1), 6.02(1H, dd, J = 10.2, 4.9 Hz, H-4), 6.08(1H, d, J = 10.2 Hz, H-4a), 6.64(1H, d, J = 8.2 Hz, H-7), 6.68 (1H, d, J = 8.2 Hz, H-8) (9).

Narwedine (II)
White amorphous powder, m.p. 192-193°C. EIMS 70 eV, m/z (rel. int.): 285 [M⁺] (93), 284 (100), 242 (27), 216 (28), 199 (27), 174 (37). 1H-NMR (500 MHz, CD3OD and CDCl3): δ 2.14 – 2.24 (2H, m, H-11α and H-11β), 2.45 (3H, s, NMe), 2.72 (1H, dd, J = 17.5, 4.0 Hz, H-2α), 3.08 (1H, dd, J = 17.5, 1.5 Hz, H-2β), 3.14 – 3.27(2H, covered by solvent peak, H-12α and H-12β), 3.78 (3H, s, OMe), 3.81 (1H, d, J = 12.0 Hz, H-6), 4.20 (1H, br d, J = 12.0 Hz, H-6), 4.69 (1H,br s, H-11), 6.56 (1H, d, J = 10.4 Hz, H-4), 6.64 (1H, d, J = 8.4 Hz, H-7), 6.67 (1H, d, J = 8.4 Hz, H-8), 6.87 (1H,d,J = 10.8 Hz, H-4a) (10).

Caranine (IV)
White needles, m.p. 270°C. FT-IR νmax cm⁻¹: 3324, 2865, 1503, 1486, 1356, 1312, 1262, 1238, 1038, 1001. EIMS 70 eV, m/z (rel. int.): 287 [M⁺] (41), 286 (22), 268 (27), 250 (12), 228 (14), 227 (79), 226 (100), 147 (11). 1H-NMR (500MHz, CD3OD): δ 2.44 (1H, dd, J = 14.8, 9.0 Hz, H-12α), 2.56 – 2.73 (3H, m, H-11α, β and H-10b), 2.88 (1H, d, J = 10.5 Hz, H-4a), 3.55(1H, dd, J = 14.4, 7.5 Hz, H-12β), 3.55(1H, dd, J = 14.2, 1.2 Hz, H-6αa), 4.13(1H, d, J = 14.2 Hz, H-6β), 4.18 (1H, br s, H-2α), 4.48 (1H, s, H-1), 4.61 (2H, br s, 1-OH and 2-OH), 5.56 (1H, br s, H-3), 5.92 (2H, s, OCHO), 6.65 (1H, s, H-7), 6.88 (1H, s, H-10). 13C-NMR (125 MHz, CD3OD): δ 29.3 (C-11), 41.4 (C-10b), 54.7 (C-12), 57.8 (C-6), 62.4 (C-4a), 71.9 (C-1), 73.2 (C-2), 102.3 (OCHO), 106.0 (C-10), 108.2 (C-7), 119.1 (C-3), 129.8 (C-10a), 130.4 (C-6a), 143.8 (C-4), 147.7 (C-8), 148.3 (C-9) (11).

Tazettine (V)
White crystalline powder, m.p. 195-198°C. FT-IR
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v_max cm⁻¹: 3196, 1475, 1453, 1380, 1127, 883, 706. EIMS 70 eV, m/z (rel. int.): 331 [M⁺] (30), 316(15), 298 (22), 247 (100), 201 (15), 181 (12), 152 (10). 1H-NMR (500 MHz, CD3OD and CDCl3): δ 1.63 (1H, ddd, J = 15.8, 5.2, 6.7 Hz, H-4), 2.23 (1H, dm, J = 15.8 Hz, H-4a), 2.58 (3H, s, OMe), 2.71 (1H, d, J = 10.8 Hz, H-12), 3.17 (1H, d, J = 10.8 Hz, H-12a). 3.49 (3H, s, OMe), 3.84 (1H, br s, 11-OH), 4.06 – 4.20 (1H, m, H-3), 2.42 (3H, s, NMe), 2.44 (1H, dd, J = 14.6, 9.0 Hz, H-12). 4.16 (1H, m, H-2), 5.78 and 5.79 (2H, 2x, OCH2O), 5.92 – 5.97 (1H, m, H-2), 6.37 (1H, s, H-7), 6.72 (1H, s, H-10) (12).

RESULTS AND DISCUSSION

The alkaloid content of the total extract of bulbs of Galanthus transcaucasicus Fomin from the east and west of Alborz Mountains were 5.50% and 4.27%, respectively and this variability may be justified by alkality of the soil of the east compared to the west regions. A reported articles on the other species of Galanthus showed that the alkaloid contents in bulbs of G. nivalis, G. gracilis and G. plicatus growing in Turkey were 0.52%, 1.41% and 2.42% of total extracts (13-15) and for G. nivalis and G. elwesi growing in Bulgaria were 0.04% and 0.088% respectively (16, 17). The major alkaloids of the reported species were tazettine (38.3%) and 8-Odemethylhomolycorine (31.8%) (8,16,17). The basic chloroform extract of the bulbs of Galanthus transcaucasicus Fomin afforded the isoquinoline type alkaloids such as galanthamine (8.04%), narwedine (6.90%), lycorine (the major alkaloid 19.48%), caranine (3.45%) and tazettine (5.75%) (Fig.1).

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Figure 1. Structures of compounds I-V.
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