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

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 $^1$H and 125 MHz for $^{13}$C, on a Bruker Avance 500 spectrometer, using CDCl$_3$ and CD$_3$OD as solvents and TMS as internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. TLC aluminum sheets (silica gel 60 F$_{254}$ 20 × 20), silica gel 60GF$_{254}$ 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 MeOH (2\% aq. HCl) and finally, methanol. Forty and eight fractions (50 ml each) were collected and monitored by TLC using CHCl\(_3\) – MeOH (7:1) as the solvent system to yield narwedine (II) (93), 287 (M\(^+\)) (41), 286 (22), 268 (27), 250 (12), 228 (14), 227 (79), 226 (100), 147 (11). \(^1\)H-NMR (500MHz, CD\(_3\)OD and CDCl\(_3\)): δ 2.14 – 2.24 (2H, m, H-11α and H-11β), 2.45 (3H, s, OMe), 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), 6.00 (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).

Lycorine (III)

White needles, m.p. 270°C. FT-IR v\(_{max}\) cm\(^{-1}\): 3324, 2865, 1503, 1486, 1356, 1312, 1263, 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). \(^1\)H-NMR (500MHz, CD\(_3\)OD): δ 2.44 (1H, dd, J = 14.8, 9.0 Hz, H-12a), 2.56 – 2.73 (3H, m, H-11α, β and H-10b), 2.88 (1H, d, J = 10.5 Hz, H-4a), 3.35(1H, d, J = 14.4, 7.5 Hz, H-12b), 3.55(1H, dd, J = 14.2, 1.2 Hz, H-6a), 4.13 (1H, d, J = 14.2 Hz, H-6b), 4.18 (1H, br s, H-2a), 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, OCH\(_2\)), 6.65 (1H, s, H-7), 6.88 (1H, s, H-10). \(^1\)C-NMR (125 MHz, CD\(_3\)OD): 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 (OCH\(_2\)), 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).

Caranine (IV)

White amorphous powder, m.p. 179-180°C. \(^1\)H-NMR (500MHz, CD\(_3\)OD and CDCl\(_3\)): δ 2.13 (1H, t, J = 18.6 Hz, H-12α), 2.28 (1H, d, J = 7.7 Hz, H-10b), 2.42-2.57 (3H, m, H-2α, β and H-11), 2.92 – 2.95 (1H, m, H-4a), 3.18-3.23 (1H, m, H-12), 3.67 (1H, dd, J = 19.2, 3.5 Hz, H-6a), 3.96 (1H, d, J = 19.5 Hz, H-6b), 4.29 (1H, br s, 1-OH), 4.60 - 4.64 (1H, m, H-1), 5.41 – 5.64 (1H, m, H-3), 5.73 and 5.74 (2H, 2d, J = 1.4 Hz, OCH\(_2\)), 6.43 (1H, s, H-7), 6.71 (1H, s, H-10). \(^1\)C-NMR (125 MHz, CD\(_3\)OD and CDCl\(_3\)): δ 28.8 (C-11), 32.2 (C-2), 39.4 (C-10b), 54.1 (C-12), 56.2 (C-6), 61.5 (C-4a), 71.9 (C-1), 101.5 (OCH\(_2\)), 105.4 (C-10), 107.7 (C-7), 119.4 (C-3), 130.4 (C-10a), 130.9 (C-6a), 142.0 (C-4), 146.8 (C-8), 147.6 (C-9) (11).

Tazettine (V)

White crystalline powder, m.p. 195-198°C. FT-IR...
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 alkalinity 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).

Galanthamine, the most important alkaloid, is a long acting, competitive and reversible acetylcholinesterase inhibitor for mid-to-moderate Alzheimer’s disease (18). Narwedine, the biogenetic precursor of galanthamine, has been used as a respiratory stimulant (5). It inhibits the action of narcotics and hypnotics and enhances the analgesic activity of morphine as well as the pharmacological effects of caffeine, carbazole, arecoline and nicotine (19). Lycorine, has appreciable AChE inhibitory activity. It is an analgesic, like aspirin, and has hypotensive activity. Additionally, lycorine has antiviral, antitumor, antimalarial, anti-inflammatory, antiplatelet, emetic and cytotoxic activities (11). Caranine, another lycorine type alkaloid, is a hypotensive which also has shown AChE activity (11). Tazettine is mildly active against certain tumor cell lines, it also displays weak hypotensive and antimalarial activities and it has been reported as an extraction artifact from pretazettine (11).

On the basis of the total alkaloid content of Galanthus transcaucasicus Fomin especially its major alkaloid lycorine, this species will be subjected to further investigation.

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REFERENCES


