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Short communication

Quantitative Determination of Galanthamine and Lycorine in *Galanthus elwesii* by HPLC-DAD

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In this study, aerial parts and bulbs of *Galanthus elwesii* Hook. (Amaryllidaceae), collected from three different localities in Southern Turkey, were quantitatively analyzed for their content of galanthamine and lycorine, by using High Performance Liquid Chromatography (HPLC). The chromatographic separation was performed using an isocratic system with a mobile phase of trifluoroacetic acid-water-acetonitrile (0.01:90:10) applied at a flow rate 1 mL/min using diode array detector. The content of galanthamine in the aerial parts and bulbs of *G. elwesii* collected from Cimi village (Antalya) was determined as 0.346 and 0.042 %, respectively. The aerial parts of *G. elwesii* collected from Ibradi (Antalya) was found to contain 0.287 % galanthamine, whereas the bulbs contained 0.095 % of this alkaloid. Galanthamine was not detected in the samples of *G. elwesii* growing in Kayrak village (Mersin). Among the tested specimens, lycorine was only found in the bulbs of *G. elwesii* collected from Ibradi (Antalya) and Kayrak village (Mersin) as 0.005 and 0.015 %, respectively.

Key words: *Galanthus elwesii*, Amaryllidaceae, Galanthamine, Lycorine, HPLC-DAD.

Galanthus elwesii Üzerinde YBSK-DAD Yöntemi ile Galantamin ve Likorin Miktar Tayini

Bu çalışmada, Türkiye'nin güneyinde üç farklı lokaliteden toplanan *Galanthus elwesii* Hook. (Amaryllidaceae) bitkisinin toprak üstü kısımları ve soğanlarındaki galantamin ve likorin içeriği Yüksek Basınçlı Sıvı Kromatografisi (YBSK) kullanılarak, kantitatif olarak analiz edilmiştir. Kromatografik ayırım, trifloroasetik asit-su-asetonitril mobil fazının (0.01:90:10) akış hızı 1 mL/dk olacak şekilde uygulandığı izokratik bir sistemden yararlanılarak ve DAD detektörü kullanılmak suretiyle gerçekleştirilmiştir. Cimi köyünden (Antalya) toplanan *G. elwesii* bitkisinin toprak üstü kısımları ve soğanlarındaki galantamin içeriği sırasıyla % 0.346 ve 0.042 olarak tayin edilmiştir. İbradi (Antalya)'den toplanan *G. elwesii* bitkisinin toprak üstü kısımlarının % 0.287 galantamin içerdiği tespit edilirken, soğanların bu alkaloidi % 0.095 oranında içerdiği saptanmıştır. Kayrak köyünden (Mersin) toplanan *G. elwesii* örneklerinde ise galantamin tespit edilmemiştir. Çalışılan örnekler arasında likorin, sadece Ibradi (Antalya) ve Kayrak köyünden (Mersin) toplanan *G. elwesii* soğanlarında sırasıyla % 0.005 ve 0.015 oranlarında bulunmuştur.

Anahtar kelimeler: *Galanthus elwesii*, Amaryllidaceae, Galantamin, Likorin, YBSK-DAD.

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INTRODUCTION

The family Amaryllidaceae, consisting of about 1100 species in 85 genera (1), is well known for its alkaloids with diverse chemical structures and biological activities (2). Galanthamine (Figure 1), an important alkaloid found in Amaryllidaceae species, is a long acting, selective, reversible and competitive acetylcholinesterase inhibitor. It is used for the treatment of mild and moderate cases of Alzheimer's disease (3). Lycorine (Figure 2), a common alkaloid in Amaryllidaceae family, has been proven to have a wide spectrum of biological activities including antiviral (4), antitumor (5), antimalarial (6) and antiinflammatory activities (7). Due to their important medicinal properties, it has been of great interest to determine the content of these alkaloids in the plants of Amaryllidaceae family. Up to date, various methods have been described concerning the quantification of galanthamine and lycorine (8-11).

Among the Amaryllidaceae genera in Turkey, *Galanthus* L. is represented by 14 taxa and one hybrid (12). Of these taxa, *Galanthus elwesii* Hook. has a wide natural distribution and can be found in Bulgaria, northeastern Greece, the eastern Aegean Islands, southern Ukraine and Turkey. Within Turkey, this species has the widest distribution among others and naturally grows in northwestern, western and southern Anatolia (13).

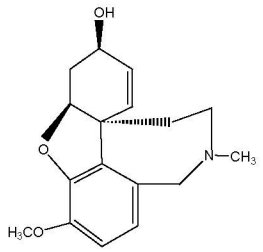

Figure 1. Galanthamine

In the present study, aerial parts and bulbs of *G. elwesii*, collected from three different localities in southern Turkey, were quantitatively analyzed for their content of galanthamine and lycorine, by using High Performance Liquid Chromatography coupled with diode array detector (HPLC-DAD).

EXPERIMENTAL

Plant material

G. elwesii was collected from Cimi village and Ibradi (both in Antalya) and from Kayrak village (Mersin). The plants were identified by Prof. M. Ali Onur from the Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir (Turkey). Voucher samples of *G. elwesii* (No's 1404, 1405, 1408) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Chemicals

The standard galanthamine and lycorine were previously isolated from several *Galanthus* species in our laboratory and authenticated by spectral analyses (UV, IR, NMR and MS). HPLC grade acetonitrile (Lab Scan Analytical Sciences), TFA (Trifluoroacetic acid) (Merck) and chromatographic grade double-distilled water were used for the preparation of the mobile phase. Other chemicals used in the assay were of analytical grade.

HPLC instrument

HPLC analysis was carried out using a liquid chromatographic system (Agilent 1100 series), equipped with a DAD (Agilent 1200 series), a quaternary pump system (Agilent 1100 series G1311A), a vacuum degasser (Agilent 1100 series G1322A), a thermostatted column compartment (Agilent 1100 series G1316A), a manual injector with 20 µL loop (Agilent

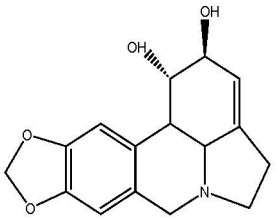

Figure 2. Lycorine

1100 series G1328A Rheodyne 7725i). Data analysis was carried out with "Agilent Chem Station Software". The chromatographic assay was performed on a Hichrom C_{18} column (5µ, 250 mm, 4.6 mm) at 290.4 nm. The mobile phase consisted of TFA:water:acetonitrile (0.01:90:10, v/v/v) applied at a flow rate of

Table 1. Galanthamine and lycorine contents of each plant extract.

Plant	Locality	Specimen	Galanthamine % (mean values ±SD)	lycorine % (mean values ±SD)
Galanthus elwesii	Cimi Village (Antalya)	Bulbus	0.042 ±0.001	ND
		Herba	0.346 ±0.025	ND
	Ibradi (Antalya)	Bulbus	0.095 ± 0.002	0.005 ± 0.0002
		Herba	0.287 ± 0.005	ND
	Kayrak (Mersin)	Bulbus	ND	0.015 ± 0.001
		Herba	ND	ND

SD:Standard Deviation; ND:Not Dedected

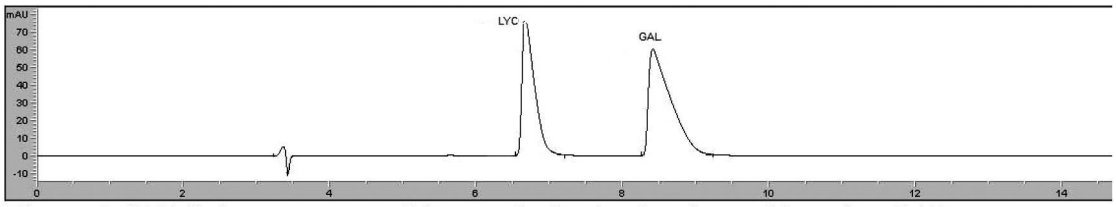

Figure 3. HPLC chromatogram of the standard galanthamine and lycorine **GAL:** Galanthamine **LYC:** Lycorine.

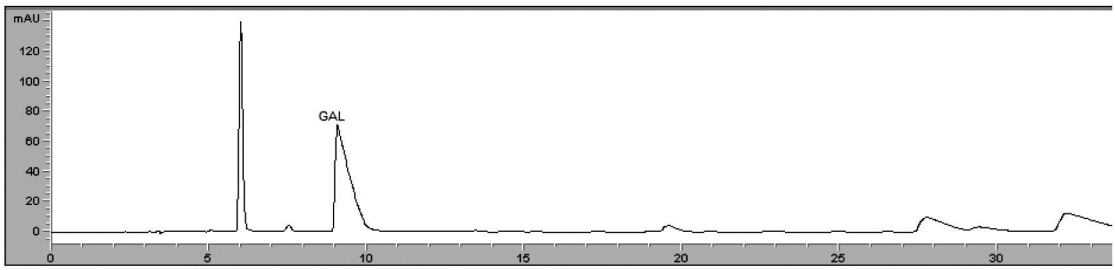

Figure 4. HPLC chromatogram of the total alkaloidal extract of Herba Galanthi prepared from *G. elwesii* (Cimi, Antalya) **GAL:** Galanthamine.

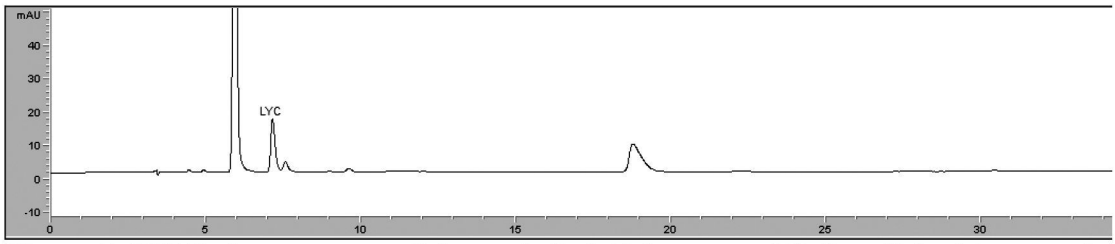

Figure 5. HPLC chromatogram of the total alkaloidal extract of Bulbus Galanthi prepared from *G. elwesii* (Kayrak, Mersin) **LYC:** Lycorine.

1mL/min (11,14). The analysis was performed at 25°C. The quantitative determination of galanthamine and lycorine was carried out by the external standard method based on peak areas.

Preparation of standard solutions

For the preparation of the calibration curves of galanthamine and lycorine, 2 mg of each alkaloid was dissolved in 5 mL 0.1 % TFA and filtered (Sem Concept Syringe 100 x 13 mm, 0.45 µm). By diluting the stock solutions, nine

different concentrations of galanthamine and lycorine were prepared within the ranges of 1.0-400 µg/mL and 1.0-300 µg/mL respectively. Each standard solution was injected into the column in triplicate with a volume of 20 µL and the regression equation of the calibration curve was obtained as y=11.451763 x - 2.40657 (r^2= 0.99993) for galanthamine and as y= 15.8423032 x-1.339608 (r^2=0.99985) for lycorine.

Extract preparation

The extraction procedure was carried out as previously, described. Prepacked columns based on diatomaceous earth for liquid-liquid sample purification has been used for the rapid preparation of the extracts (14,15). Briefly, about 200 mg of powdered plant material was macerated with 5 mL of 2 % hydrochloric acid for 5 hours in an ultrasonic bath at 40°C and then the extract was made alkaline with 1 mL of 26 % ammonium hydroxide and the volume was adjusted to 10 mL in a volumetric flask with distilled water. The basic solution centrifuged at 5000 rpm for 10 min and aliquots of 3 mL were applied on the Extrelut® (Merck) columns. The alkaloids were eluted with chloroform (3 x 5 mL) for 10 min. The organic solvent was distilled in *vacuo* to afford the alkaloidal extract. The extract was dissolved in 1 mL 0.1 % TFA and filtered. 20 µL was injected into the HPLC column. Each analysis was carried out in triplicate.

RESULTS AND DISCUSSION

The identification and quantitative determination of galanthamine and lycorine in *G. elwesii* collected from different localities in southern Turkey, was established by comparison of the retention times and peak areas with those of standards. The HPLC chromatogram of the standards is given in Figure 3. Detection by DAD increased the sensitivity of the HPLC method. Galanthamine was found in the specimens of *G. elwesii* growing in Cimi village and Ibradi (both in Antalya). Of these specimens, the aerial parts of *G. elwesii* collected from Cimi village, was found to contain the highest content of this alkaloid (Figure 4). Galanthamine was not detected in the samples of *G. elwesii* growing in Kayrak village (Mersin). Lycorine, was only found in the bulbs of the specimens of *G. elwesii* growing in Kayrak village (Mersin) and Ibradi (Antalya). Of these specimens, the bulbs of *G. elwesii* collected from Kayrak village (Mersin), was found to contain the highest content of lycorine (Figure 5) (Table 1).

Although, *G. elwesii* has been a subject of several phytochemical studies (16-18), there is scanty data on the quantification of alkaloids of this species. To the best of our knowledge, quantitative analysis of the alkaloids has been carried out only in *Galanthus elwesii* of Turkish origin. The results of previous investigations revealed that *G. elwesii* specimens collected from Karaburun, Izmir contained both galanthamine (0.007-0.026 %) and lycorine (0.004-0.013 %) (19). In another study, lycorine content was reported as 0.011 % in *G. elwesii* specimens collected from Antalya, Akseki (20). Moreover, in a previous study, *G. elwesii* growing in Yamanlar, Izmir was not found to contain galanthamine and lycorine (10,21).

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