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ORIGINAL ARTICLE

Quantification of Galantamine in *Sternbergia* Species by High Performance Liquid Chromatography

Sternbergia Türlerinde Yüksek Performanslı Sıvı Kromatografisi ile Galantamin Miktar Tayini

Özlem Bahadır ACIKARA, Betül Sever YILMAZ*, Dilhun YAZGAN, Gülçin Saltan İŞCAN

Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey

ABSTRACT

Objectives: This study describes the qualitative and quantitative analysis of galantamine in *Sternbergia* species growing in Turkey.

Materials and Methods: Galantamine was isolated from *Sternbergia fischeriana* bulbs and the structure of the compound elucidated by spectroscopic methods. The qualitative and quantitative analysis of galantamine was investigated in *Sternbergia lutea* subsp. *lutea*, *S. lutea* subsp. *sicula*, *Sternbergia candida*, *S. fischeriana*, and *Sternbergia clusiana* using a specially developed and validated high performance liquid chromatography (HPLC) method.

Results: *S. lutea* subsp. *sicula* had the highest content of galantamine, i.e., 0.0165±0.0002 g/100 g. The limits of detection and quantification were 7.5 µg and 25 µg, respectively.

Conclusion: Isolation of galantamine from *S. fischeriana* growing in Turkey is reported for the first time. An HPLC method was developed for identification and quantification of galantamine in *Sternbergia species*.

Key words: Galantamine, HPLC, *Sternbergia* spp.

ÖZ

Amaç: Bu çalışmada Türkiye'de yetişen *Sternbergia* türlerinin galantamin içeriklerinin kalitatif ve kantitatif analizi amaçlanmıştır.

Gereç ve Yöntemler: Galantamin *Sternbergia fischeriana* yumrularından izole edilmiş ve yapısı spektroskopik yöntemler kullanılarak aydınlatılmıştır. *Sternbergia lutea* subsp. *lutea*, *S. lutea* subsp. *sicula*, *Sternbergia candida, S. fischeriana* ve *Sternbergia clusiana* türlerinin galantamin içeriği kalitatif ve kantitatif olarak yeni geliştirilen ve valide edilmiş bir yüksek performanslı sıvı kromatografisi (HPLC) yöntemi kullanılarak yapılmıştır.

Bulgular: *S. lutea* subsp. *sicula* türünün 0.0165±0.0002 g/100 g olarak en yüksek galantamin içeriğine sahip olduğu belirlenmiştir. Galantamin için saptama ve kantifikasyon sınırı değerleri sırasıyla 7.5 µg ve 25 µg olarak belirlenmiştir.

Sonuç: Türkiye'de yetişen *S. fischeriana* türünden galantamin izolasyonu ilk kez bu çalışma ile rapor edilmiştir. Ayrıca bu çalışma ile *Sternbergia* türlerinin galantamin içeriğinin tespit edilmesi ve miktar tayini için yeni bir HPLC metodu geliştirilmiştir.

Anahtar kelimeler: Galantamin, HPLC, *Sternbergia* spp.

***Correspondence: E-mail:** sever@pharmacy.ankara.edu.tr, **Phone:** +90 532 300 69 94 **ORCID-ID:** orcid.org/0000-0003-2084-9514
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INTRODUCTION

Galantamine is approved by the Food and Drug Administration for the treatment of mild to moderate Alzheimer's disease.[1] Razadyne® (formerly Reminyl®) and Nivalin® are licensed drugs of galantamine, currently available on the market.[2] This drug inhibits acetylcholinesterase enzyme reversibly and acts as an allosteric modulator of the nicotinic cholinergic receptor. Interaction potentiates cholinergic nicotinic neurotransmission by modulating ion channel activity in the presence of acetylcholine.[1-4] This drug provides the requisite cholinergic stimulation without producing desensitization. Furthermore, galantamine appears to be a more powerful elevator of frontal cortical dopamine levels compared to other cholinesterase inhibitors such as donepezil.[1] Galantamine exerts neuroprotection on neuronal cell cultures subjected to oxidative stress or amyloid beta stress. Neuroprotection in rat hippocampal slices subjected to oxygen and glucose deprivation followed by a reoxygenation period was also demonstrated by galantamine. Galantamine also acts as a neuroprotective agent in an *in vivo* model of global cerebral ischemia, even when given after the ischemic insult.[5,6]

Galantamine was firstly isolated from the snowdrop, *Galanthus woronowii*. Generally Amaryllidaceae plants including *Narcissus*, *Galanthus*, *Lycoris*, and *Leucojum* species are used for extraction of galantamine. *Leucojum aestivum* is known as the main source of this compound. *Narcissus* species also contain galantamine in varying amounts from trace amounts to as much as 2.5% of dry weight. Synthetic methods for production of galantamine have been developed; however due to their high cost, plants are still the main sources for galantamine production.[7]

The galantamine content of Amaryllidaceae plants was investigated using different high performance liquid chromatography (HPLC) methods.[7] An isocratic solvent system consisting of an acetonitrile:methanol:water (containing 7.5 mM triethanolamine, pH 6.9) mixture as mobile phase was used for detecting galantamine on an RP-C8 column in *L. aestivum*.[8] In another study conducted on *L. aestivum*, an acetonitrile:methanol:buffer pH 4.5 (10:10:80) mixture was used for elution on an RP-C18 column to determine galantamine amount.[9] Lubbe et al.[10] also reported HPLC analysis of galantamine in *Narcissus pseudonarcissus* on a C18 column using 10% (v/v) acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) as mobile phase. *Galanthus elwesii* was also analyzed for its galantamine content by using a mobile phase comprising a TFA:water:ACN (0.01:90:10) mixture on an RP-C18 column.[11] Petruczynik et al.[12] analyzed galantamine on an RP-C18 column with a mobile phase containing 5% MeCN, 20% acetate buffer at pH 3.5, and 0.025 mL^{-1} diethylamine as well as on an SCX column using an 8% MeCN and phosphate buffer at pH 2.5 mixture as mobile phase in *L. aestivum*, *Leucojum vernum* var. *carpaticum*, *Galanthus nivalis*, *Zepyranthes rosea*, and *Clivia minata*.The genus *Sternbergia* Waldst. and Kit. (Amaryllidaceae) is represented by eight species and they are widely distributed from the East Mediterranean to Caucasia. In Turkey six taxa of this genus grow naturally.[13] *Sternbergia* species are well known due to their alkaloid contents, i.e., lycorine and galantamine, with interesting pharmacological properties.[14,15] Alkaloids including lycorine, homolycorine, haemanthidine, haemanthamine, 6α- and 6β-hydroxy-haemanthamine, and tazettine have been isolated from *Sternbergia* species.[14-18] It has been reported that *Sternbergia* species contain especially crinine-type and lycorine-type Amaryllidaceae alkaloids.[13]

In order to investigate new sources for galantamine, *Sternbergia* species were investigated using HPLC in the current study. *Sternbergia lutea*, *Sternbergia sicula*, *Sternbergia fischeriana*, *Sternbergia clusiana*, and *Sternbergia colchiciflora*, which were collected from different locations of Anatolia, were analyzed using HPLC. An isocratic system was developed and used for HPLC analysis. Galantamine, which was isolated from *S. fischeriana* bulbs previously, was used for quantification of the galantamine contents of *S. lutea*, *S. sicula*, *S. fischeriana*, *S. clusiana*, and *S. colchiciflora*.

EXPERIMENTAL

Plant materials

Sternbergia species were collected from different parts of Anatolia as shown in Table 1. Voucher specimens are kept at the Herbarium of Ankara University, Faculty of Pharmacy with their herbarium numbers (Table 1).

Isolation of galantamine

Galantamine was isolated from *S. fischeriana* bulbs. The dried bulbs (500 g) were extracted with ethanol (5 L) by percolation. Ethanolic extract was filtered and concentrated under vacuum at 50°C by evaporation. The pH of the extract was adjusted to 3 by addition of 5% HCl. After filtration $CHCl_3$ was used for liquid–liquid extraction. The chloroform part was concentrated under vacuum by evaporation to obtain extract A (13.7538 g), which contained lycorine and tazettine. The remaining acidic-water part was extracted with $CHCl_3$ after addition of alkali solution

Table 1. Plant materials, collected places, and herbarium numbers

Species	Herbarium numbers	Collection sites
Sternbergia candida Mathew & T.Baytop	AEF 23794	Muğla-Fethiye
Sternbergia clusiana (Ker Gawl.) Ker Gawl. ex Sprengel	AEF 23697	Kahramanmaraş-Göksun
Sternbergia fischeriana (Herbert) Rupr.	AEF 23793	Antakya-Yayladağ
Sternbergia lutea subsp. *lutea* Waldst. & Kit.	AEF 23694	İzmir-Torbalı
Sternbergia lutea subsp. *sicula* Tineo ex Guss.	AEF 23695	Muğla-Marmaris

(NH_4OH 25% to obtain pH 8). The concentrated chloroform part gave extract B (1.9712 g). Extract B was separated by Chromatotron on aluminum oxide GF Gypsum (Merck 1092) plates. Elution was performed with a $CHCl_3$:MeOH (9:1) mixture. Fractions 1-4 were subjected to further separation by preparative TLC on precoated TLC sheets (Merck 5744) eluting with $CHCl_3$:MeOH (85:15) to obtain galantamine (5.04 mg).[19] The structure of the isolated compound was elucidated by ^{1}H-NMR and comparison of these data with the literature.[19-23]

Galantamine: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 6.63 (1H, d, *J*=8 Hz, H-12); 6.53 (1H, d, *J*=8 Hz, H-11); 6.02 (1H, d, *J*=10.3 Hz, H-4); 6.00 (1H, d, *J*=10.3 Hz, H-3); 4.47 (1H, brs, H-16); 4.10 (1H, m, H-2α); 4.06 (1H, d, *J*=15.2 Hz, H-9β); 3.98 (1H, d, *J*=15.2 Hz, H-9α); 3.05 (1H, m, H-7α); 2.95 (1H, m, H-7β); 2.56 (1H, m, H-1α), 2.45 (1H, m, H-1β); 1.50 (1H, m, H-6α); 1.47 (1H, m, H-β), 3.72 (s, O-CH_3), 2.51 (s, N-CH_3).

HPLC analysis

HPLC analyses were carried out using an Agilent LC 1100 model chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA). The diode-array detector was set at wavelength 292 nm and peak areas were integrated automatically by computer using Agilent software. The chromatograms were plotted and processed using the above-mentioned software. Separation was carried out using a SUPELCOSIL LC-18 column (250×4.6 mm i.d.; 5 µm; Supelco, Bellefonte, PA, USA). The mobile phase was made up of ammonium carbonate (Laboratory BDH Reagent, Poole, UK) water solution (purified water was obtained by using Milli-Q Plus System (Millipore Corp., Molsheim, France) and acetonitrile (HPLC grade 99.93 % purity, Sigma-Aldrich 270717) (85:15 v/v) applied at a flow rate of 1 mL/min, column temperature 24˚C, and 20 µL portions were injected into the liquid chromatography system.

Preparation of standard and sample solutions

Standard stock solution was prepared in 1 mg/mL concentration. First 10 mg of galantamine was weighed in a 10 mL volumetric flask and then it was dissolved in 10 mL of 1% H_2SO_4. Different concentration levels (0.025 mg/mL, 0.05 mg/mL, 0.075 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL) were prepared by diluting the stock solution.

Sample solutions were prepared by extraction of dried and powdered bulbs (10 g) of each plant with 1% H_2SO_4 by rinsing at room temperature for 7 days. The extraction procedure was tested with Mayer's reagent to be sure all alkaloids were extracted. Each extract was filtered through a 0.45-mm membrane filter and adjusted to a final volume of 500 mL with acidic solution.

Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established at a signal to noise ratio (S/N) of 3 and 10, respectively. LOD and LOQ concentrations were experimentally verified by six injections of galantamine. The precision of the method (intra-day variations of replicate determinations) was checked by injecting galantamine nine times at the LOQ level.

RESULTS AND DISCUSSION

To date, any *Sternbergia* species growing in Turkey has been reported that does not contain galantamine. In addition, galantamine has not been determined in any *Sternbergia* species growing in Turkey. However in the present study galantamin was detected and quantified in *Sternbergia* species.[13,24] This study led to the isolation of this compound from the bulbs of *S. fischeriana* collected from Yayladağ in Antakya Province. Additionally, the current study describes the development of a method for identifying and quantifying galantamine in *Sternbergia* species. Good separation and determination of this compound were achieved using a mobile phase consisting of ammonium carbonate and acetonitrile (85:15 v/v) on a SUPELCOSIL LC-18 column (250×4.6 mm×5 µm) at wavelength 292 nm as shown in Figures 1 and 2.

LOD and LOQ values were 7.5 µg and 25 µg, respectively. Table 2 shows the wavelength measured, the calculated calibration curve, and the LOD and LOQ results for this compound. The precision of the method is expressed as the relative standard

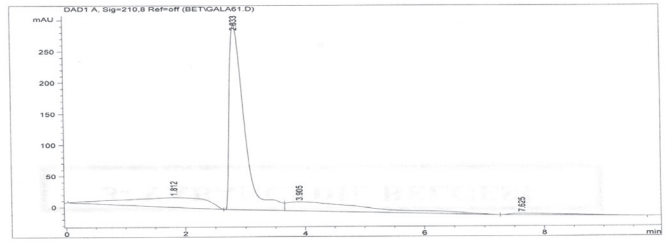

Figure 1. HPLC chromatogram of galantamine

HPLC: High performance liquid chromatography, DAD: Diod-array detector

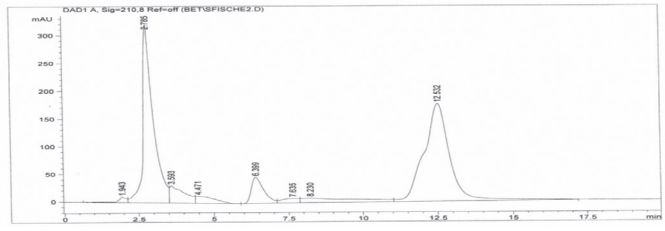

Figure 2. HPLC chromatogram of *Sternbergia fischeriana*

HPLC: High performance liquid chromatography, DAD: Diod-array detector

Table 2. Linearity results, LOQ, and LOD

Compound	λ	Equation	r^2	Slope RSD %	Intercept RSD %	LOQ (µg)	LOD (µg)
Galantamine	292	Y=118484.33X + 448.2	0.995	2.0594	4.4493	25	7.5

LOQ: Limit of quantification, LOD: Limit of detection, RSD: Relative standard deviation

Table 3. Galantamine contents of *Sternbergia* species

Species	Galantamine % (n=3, mean ± standard deviation)
Sternbergia candida	0.0092±0.0005
Sternbergia clusiana	0.0077±0.0001
Sternbergia fischeriana	0.0069±0.0006
Sternbergia lutea subsp. *lutea*	0.0100±0.0005
Sternbergia lutea subsp. *sicula*	0.0165±0.0002

deviation at the LOQ level.

The presence of galantamine (Figure 3) in *S. lutea* subsp. *lutea*, *S. lutea* subsp. *sicula*, *S. candida*, *S. fischeriana*, and *S. clusiana* was analyzed quantitatively and qualitatively by HPLC. The current study results, as shown in Table 3, revealed that all plant samples contain galantamine and the highest content was determined in *S. lutea* subsp. *sicula* (0.0165±0.0002% dw) followed by *S. lutea* subsp. *lutea* (0.0100±0.0005% dw). According to previous studies, *G. woronowii* and *L. aestivum* contain 0.003-0.506% and 0.0028-0.2104% galantamine, respectively.[25,26] The galantamine content ranged from 0.05 to 0.36 mg/g dw in the bulbs of *G. nivalis* and from 0.3 to 0.033 mg/g dw in the bulbs of *Narcissus tazetta* samples collected from different locations in Iran. According to the results, geographical regions and cultural practices affected the chemical composition of the plants. The chemical variations can be attributed to environmental factors.[7] *L. aestivum* plants collected during different periods of vegetation were analyzed for their galantamine contents and the amounts were determined as 0.13% and 0.14%, respectively, for the plant in bloom and fructification, respectively.[9] According to Petruczynik et al.[12], different extraction procedures such as maceration, extraction in an ultrasonic bath, and extraction in an ultrasonic bath following maceration yielded different amounts of galantamine. Galantamine content in *L. aestivum* was determined as 0.0196 mg/mL, 0.0273 mg/mL, and 0.0949 mg/mL, respectively, by the mentioned extraction procedures. Ultrasonic bath extraction following maceration induced a relatively high amount of galantamine extraction. In the same study the highest amount of galantamine was determined in *L. aestivum* roots with 2.3524 mg/g dw, followed by leaves with 1.6611 mg/g dw. *Z. rosea* bulbs and *C. minata* leaves as well as roots were found to contain 0.8384 mg/g dw and 0.1489 mg/g dw, and 0.0284 mg/g dw galantamine, respectively. In all parts of *G. nivalis* galantamine content varied from 0.0003 mg/g dw to 0.0178 mg/g dw. *G. elwesii* samples collected from two different locations in Turkey, İzmir and Karaburun, contained 0.026% and 0.007% galantamine, respectively.[11] The amount of galantamine also varied from 2.36 mg/g dw to 3.32 mg/g dw in *N. pseudonarcissus* bulbs collected from the Netherlands.[10] According to our results, galantamine content of the *Sternbergia* species was lower than that of *L. aestivum* when they were compared. The current study's results revealed that *Sternbergia* species are not valuable sources for galantamine extraction. Differences in galantamine content among all the investigated species could be explained by the existence of chemotype. Furthermore, a number of factors such as temperature, season, stages of maturity, geographical origin, climatic conditions, and soil can affect the phytochemical content of plants.[27,28] Plants cultivated under different conditions exhibit an alteration in the quantity of phytochemicals and therefore display varied therapeutic effects.[29,30]

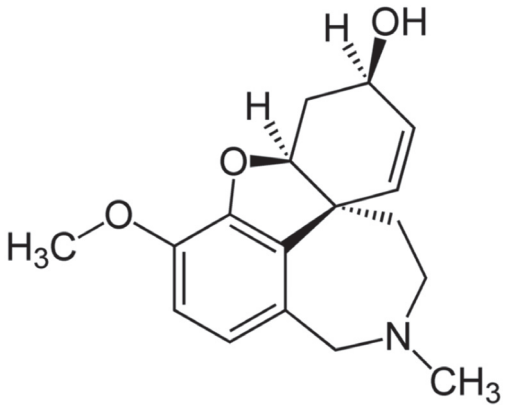

Figure 3. Structure of galantamine

CONCLUSIONS

The present study is the first report of galantamine isolation from *Sternbergia* species growing in Turkey. An HPLC method was developed for identification and quantification of galantamine in the genus *Sternbergia*. The presence of galantamine could be related to growing conditions such as temperature, season, climatic conditions, soil, or stages of maturity as well as geographical origin. Chemotype of the mentioned species could be also the reason for the presence of galantamine in *Sternbergia* species. Therefore, further studies will be planned to investigate *Sternbergia* species collected from different locations in Turkey for their galantamine contents.

Conflict of Interest: No conflict of interest was declared by the authors.

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