ORIGINAL RESEARCH

Quality control evaluation and acetylcholinesterase inhibitory activity of *Galanthus woronowii* Losinsk.

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ABSTRACT: Aerial and underground parts of *Galanthus woronowii* Losinsk., a wild growing species in north-eastern Anatolia, were collected during flowering period. Quality control and acetylcholinesterase inhibitory activity determinations were carried out on Bulbus and Herba Galanthi prepared from plants collected from two different localities. In the context of quality control studies, contents of humidity, total ash, sulphated ash, acid-insoluble ash and total alkaloids of the drug specimens were determined and found to range between 8.463-9.343 %, 6.950-14.947 %, 9.743-17.930 %, 1.102-3.565 % and 0.247-0.499 %, respectively. Additionally, acetylcholinesterase inhibitory activity of the alkaloidal extracts prepared from the drug specimens were determined by using Thin Layer Chromatography (TLC) combined with a bioautographic assay based on *in vitro* Ellman method. All of the alkaloidal extracts displayed acetylcholinesterase inhibitory activity.

KEY WORDS: *Galanthus woronowii*, Amaryllidaceae, Quality control determination, Acetylcholinesterase inhibitory activity

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INTRODUCTION

Galanthus woronowii Losinsk., is one of the fourteen species (fifteen taxa) of *Galanthus* L. growing naturally in Turkey (1,2). It is distributed in Caucasus, Transcaucasus, southern Russia, Georgia and northeastern Turkey (3,4). This species is a low-to mid altitude species, growing at altitudes from 20 to 1500 m however more usually occurring from 200 to 600 m (4). *G. woronowii* with broad green leaves is an attractive plant for gardening (4). Among the *Galanthus* species growing in Turkey, together with the bulbs of *G. elwesii* Hook., bulbs of *G. woronowii* are exported (5, 6).

Previous investigations on *G. woronowii* resulted in the isolation of several Amaryllidaceae alkaloids (7-10). This group of alkaloids are known to exhibit interesting biological properties such as antiviral (11), antitumor (12), antimalarial (13) and acetylcholinesterase inhibitory activities (14). Among these alkaloids galanthamine is an acetylcholinesterase inhibitor and therefore, it is used in the treatment of Alzheimer's disease (15).

Comprising a part of our ongoing studies on Turkish *Galanthus* species, the present study was undertaken to determine the quality standarts of drugs prepared from the aerial parts and bulbs of *G. woronowii* collected during flowering period. The gravimetric determinations of humidity, total ash, sulphated ash, acid-insoluble ash and also titrimetric determinations of the total alkaloidal content were carried out according to European Pharmacopeia (16). Moreover, with the aim of determining the acetylcholinesterase inhibitor potential of this plant, alkaloidal extracts prepared from the aerial parts and bulbs of *G. woronowii*, were screened for their acetylcholinesterase inhibitory activity by using Thin Layer Chromatography (TLC) combined with a bioautographic assay based on *in vitro* Ellman method (17).

MATERIALS AND METHODS

Plant Material

Galanthus woronowii Losinsk. samples were collected during flowering period, from Çaykara, Trabzon in March 2006 and from Derepazarı, Rize in March 2009. The plants were identified by one of us (M. A. Önür). Voucher samples (No's 1358 and 1417) are deposited in the Herbarium of

TABLE 1. Quality control determination results of *G. woronowii*

Specimen#	Humidity (%)*	Total Ash (%)*	Sulphated Ash (%)*	Hydrochloric Acid– Insoluble Ash (%)*	Total Alkaloids (%)*
1	9.263 ± 0.012	8.857 ± 0.050	13.567 ± 0.101	1.102 ± 0.032	0.412 ± 0.005
2	9.343 ± 0.021	14.460 ± 0.046	17.930 ± 0.118	3.565 ± 0.112	0.499 ± 0.006
3	8.472 ± 0.010	6.950 ± 0.078	9.743 ± 0.015	2.303 ± 0.114	0.250 ± 0.006
4	8.463 ± 0.025	14.947 ± 0.031	11.190 ± 0.053	1.722 ± 0.161	0.247 ± 0.006

#1: Bulbus/Derepazarı,Rize; 2: Herba/Derepazarı,Rize; 3: Bulbus/Çaykara,Trabzon; 4: Herba/Çaykara,Trabzon
*n=3, Mean Results ± Standard Deviations

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Chemicals

Acetylthiocholine iodide (ATCI), acetylcholinesterase enzyme (AchE) type VI-S from electric eel, 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from Sigma. Tris-HCl was obtained from Merck. Galanthamine was isolated from various Amaryllidaceae plants in our laboratory and identified by spectroscopic analysis (UV, IR, MS, NMR) (18). The other chemicals were of analytical purity.

Quality Control Determinations

European Pharmacopeia was referred to for the gravimetric assays of humidity, total ash, sulphated ash acid-insoluble ash. The total alkaloidal content of each drug specimen was evaluated by using a titrimetric method cited in European Pharmacopeia for different alkaloid-containing drugs (16) and alkaloid extraction was carried out as described, previously (19).

FIGURE 1: AChE inhibitory activity of alkaloidal extracts of *G. woronowii*. **G:** Galanthamine, **1:** Bulbus/Derepazarı,Rize; **2:** Herba/Derepazarı,Rize; **3:** Bulbus/Çaykara,Trabzon; **4:** Herba/Çaykara,Trabzon.White spots indicate inhibition.

Acetylcholinesterase Inhibitory Activity Determination

The method used for the determination of acetylcholinesterase inhibitory activity was modified from a previous study (17). 20 microliters of each plant extract (10 mg/ml) and 10 microliters of galanthamine (1.5 mg/ml) dissolved in chloroform-methanol (8:2), were spotted on a TLC plate (Silica gel F_{254}, 0.2 mm, Aluminium sheet, Merck). Chloroform-methanol (8:2) mixture was used as the mobile phase for the development of the TLC plate. The plate was allowed to dry at room temperature, then it was sprayed with 1mM ATCI and 1mM DTNB in Tris-HCl, pH:8. After 3-5 minutes drying, the plate was sprayed with 3 Unit/ml AChE in Tris-HCl, pH:8. 20 minutes later, a yellow background appeared; occurrence of white spots marked positive reaction.

RESULTS AND DISCUSSION

The results obtained from the humidity, total ash, sulphated ash, acid-insoluble ash and total alkaloid determination assays are reported in Table 1. During the course of our ongoing studies on Turkish *Galanthus* species, previously Herba and Bulbus Galanthi drugs prepared from *G. elwesii* Hook., *G. gracilis* Ćelak., *G. trojanus* A.P. Davis & N. Özhatay and *G. plicatus* Bieb. subsp. *byzantinus* (Baker) D. A. Webb were investigated for their contents of humidity, total ash, sulphated ash and total alkaloids (19-22). Previous data and the results of the present study, may be utilized for the determination of the standard values for the elaboration of prospective monographs on Herba and Bulbus Galanthi.

The total alkaloidal content is an important criterion for the evaluation of the quality of Herba and Bulbus Galanthi. In our study, the total alkaloids ranged between 0.247-0.499 %. Aerial parts of *G. woronowii* collected from Derepazarı, Rize were found to contain the maximal amount of total alkaloids. The minimal amount of total alkaloids was detected in the aerial parts of *G. woronowii* collected from Çaykara, Trabzon (Table 1). Previously, the contents of individiual alkaloids and the quantity of the total bases obtained during isolation studies have been reported for this plant (10,23,24). However, to the best of our knowledge, this is the first report on the quantification of the total alkaloids in *G. woronowii*.

In addition to the quality control determinations, acetylcholinesterase inhibitor potentials of the alkaloidal extracts were screened by TLC in combination with bioactivity staining based on Ellman's method. The active extracts were

Galanthus woronowii Losinsk.'in kalite kontrol açısından değerlendirilmesi ve asetilkolinesteraz inhibitör aktivitesi

ÖZET: Kuzeydoğu Anadolu'da doğal olarak yetişen *Galanthus woronowii* Losinsk.'in toprak üstü ve toprak altı kısımları bitki çiçekli dönemde iken toplanmıştır. İki farklı lokaliteden toplanan bitkilerden hazırlanan Bulbus ve Herba Galanthi drogları üzerinde kalite kontrol ve asetilkolinesteraz inhibitör aktivite tayinleri gerçekleştirilmiştir. Kalite kontrol çalışmaları kapsamında drog örneklerinin nem, total kül, sülfat külü, hidroklorik asitte çözünmeyen kül ve total alkaloit içerikleri saptanmış ve bunların sırasıyla % 8.463-9.343, % 6.950-14.947, % 9.743-17.930, % 1.102-3.565 ve % 0.247-0.499 arasında değiştiği bulunmuştur. İlaveten, drog örneklerinden hazırlanan alkaloit ekstrelerinin asetilkolinesteraz inhibitör aktiviteleri *in vitro* Ellman yöntemine dayalı biyootografik bir deney ile kombine edilen İnce Tabaka Kromatografisinden (İTK) yararlanılarak tespit edilmiştir. Bütün alkaloit ekstreleri asetilkolinesteraz inhibitör aktivite göstermiştir.

ANAHTAR KELİMELER: *Galanthus woronowii*, Amaryllidaceae, Kalite kontrol tayini, Asetilkolinesteraz inhibitör aktivite

detected by the formation of white spots after spraying the substrate, dye and enzyme which gave a yellow background (17). All of the alkaloidal extracts contained galanthamine and also other alkaloids with AchE inhibitory activity (Figure 1). It is already known that AchE inhibitory activity is related mainly with galanthamine- and lycorine-type Amaryllidaceae alkaloids (14,25,26). Our ongoing phytochemical studies on this species, revealed that *G. woronowii* contained galanthamine, lycorine and other alkaloids belonging to the galanthamine- or lycorine-type of Amaryllidaceae alkaloids (18,27,28) which have been previously shown to possess AchE inhibitory activity (14,25,27).

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