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QUANTITATIVE ANALYSIS OF LYCORINE IN *Sternbergia* SPECIES GROWING IN TURKEY

Gulcin Saltan Citoglu*, Betul Sever Yilmaz, and Ozlem Bahadir

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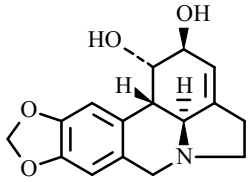
Plants of the family Amaryllidaceae are well known not only for their ornamental value but also for the alkaloids they produce. Some of these alkaloids exhibit interesting pharmacological and/or biological properties. However, the most extensively studied effects are those of non-specific inhibition, such as antiviral and antitumour activities [1]. *Sternbergia* species, a member of this family, was found to contain lycorine as a major alkaloid. *Sternbergia* is represented by 6 taxa in Turkey [2].



In our previous studies, six alkaloids (lycorine, homolycorine, galanthamine, haemanthamine, haemanthidine, and tazettine) were isolated, chemically characterized, and analyzed by HPLC in different species of *Sternbergia* [3–5]. The analgesic, antiinflammatory, antimicrobial, and antioxidant activities of some S*ternbergia* species and lycorine were also investigated by us [3, 5–7].

This paper is a part of our ongoing studies [3–7] on this genus in which we attempt to quantify of lycorine from five species of the genus *Sternbergia*.

On reviewing the literature regarding the analysis of lycorine, we found a lack of HPLC systems for studying lycorine. Furthermore, an HPLC procedure for the separation and quantification of lycorine from the acidic extract of *Sternbergia lutea* has also been reported by Evidente et al. [8].

Good separation and determination of *Sternbergia lutea* ssp. *lutea* in bulbs was performed by using the mobile phase consisting of ammonium carbonate and acetonitrile (85:15 v/v) and a Supelcosil LC-18 column (250×4.6 mm i.d., 5 μm Supelco, Belleforte, PA, USA) at a flow rate of 1 mL/min and column temperature 24°C. Chromatograms were plotted by a Diod-Array Detector (DAD) at the wavelength 292 nm.

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. Limits of quantification (LOQ) were experimentally verified by six injections of lycorine at the LOD and LOQ concentrations. The LOD was calculated to be 2.5 μg/mL and the LOQ was calculated to be 7.5 μg/mL for lycorine (Table 1).

The precision of the method (intra-day variations of replicate determinations) was checked by injecting lycorine nine times at the LOQ level. The precision of the method, expressed as the RSD % at the level, was 0.646 % for lycorine (Table 2).

Quantitative determination of lycorine in the bulbs of *Sternbergia* species was carried out by RP-HPLC using the external standard method.

The assay results of *Sternbergia* species are shown in Table 3. Standard solutions of lycorine were added to the plant extracts and injected at each time. The area of peaks corresponding to the standards were increased to prove the presence of these compounds. Their percent mean and standard deviation values are summarized in the same Table 3.

Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100, Ankara, Turkey, fax: +90 312 213 10 81, e-mail: gulcin.saltan@pharmacy.ankara.edu.tr. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 665-666, November-December, 2008. Original article submitted March 1, 2007.

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TABLE 1. Linearity Results, Limit of Detection (LOD), and Limit of Quantification (LOQ)

Compound	λ	Equation	r^2	Slope (RSD %)	Intercept (RSD %)	LOQ, μg/mL	LOD, μg/mL
Lycorine	292	Y = 14.962X + 45.244	0.9994	0.057	4.022	7.5	2.5

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X = concentration (μg/mL); Y = area.
RSD % = (Standard Deviation/Mean)×100.

TABLE 2. Precision of the Developed Method at the LOQ Level (n = 9)

Compound	λ	Peak Area (mean)	RSD %
Lycorine	292	115.67	0.646

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*RSD % values are given in parentheses.
RSD % = (Standard Deviation/Mean)×100.

TABLE 3. Contents of Lycorine in *Stenbergia* Species

Species	Lycorine % (n = 3, mean) Mean±SD	Species	Lycorine % (n = 3, mean) Mean±SD
S. candida	0.43±0.0045	*S. lutea* ssp. *lutea*	1.069±0.0136
S. clusiana	0.429±0.0061	*S. lutea* ssp. *sicula*	1.007±0.0167
S. fischeriana	0.543±0.0022		

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SD: standard deviation.

The linearity of the HPLC method was checked by injecting six mixtures of standard acidic solutions (n = 6) containing 0.025, 0.05, 0.075, 0.1, 0.2, and 0.4 μg/mL of lycorine.

Triplicate 20 μL injections were made for the standard solution to check the reproducibility of the detector response at each concentration level. The peak area of each solution was plotted against the concentration to obtain the calibration curve. The linear regression was found as Y = 14962X + 45.244. The calibration curve showed excellent linearity, with correlation coefficients higher than 0.9994.

After standardization with pure sample, the method was applied to crude acid extracts of dried bulbs of the *Sternbergia* species mentioned.

The content of lycorine in the extracts was calculated from the calibration curve using the formula Y = 14962X + 45.244. The lycorine content of each plant extract is shown in Table 3.

These results are similar to those of Evidente et al. [8]. They reported that the lycorine level in the extracts of the bulbs of *Sternbergia lutea* was about twice that in the leaves (2.107 and 0.910 %, respectively).

This HPLC method is considered very suitable for the quantitative determination of lycorine in plant extracts of alkaloid-bearing Amaryllidaceae species. The described process also shows some advantages that make it recommendable: very small amounts of plant material are required (10 g) and the procedure involves relatively low cost of the reagents. Thus, this modified method can be applied for the determination of the other Amaryllidaceae alkaloids. This is the first report of quantification of lycorine in *Sternbergia lutea* ssp. *sicula*, *S. candida*, *S. fischeriana* and *S. clusiana*.

HPLC grade acetonitrile and sulfuric acid were obtained from Merck KGaA, Darmstad, Germany. Ammonium carbonate were provided by Sigma-Aldrich (Steinheim, Germany). Lycorine was previously isolated and identified in our laboratory from *S. clusiana* and *S. fischeriana* by standard procedures [3–5].

Plant Material. Bulbs of *Sternbergia lutea* ssp. *lutea* Walts. A. Kit., *Sternbergia lutea* ssp. *sicula* Tineo ex Guss., *Sternbergia candida* Mathew & T. Baytop, *Sternbergia fischeriana* (Herbert) Rupr., and *Sternbergia clusiana* (Ker Gawl.) Ker Gawl. Ex Sprengel were collected from the Izmir-Torbali, Mugla-Marmaris, Mugla-Fethiye, Antakya-Yayladag, and Kahramanmaras-Goksun, respectively. Voucher specimens are kept at the Herbarium of Ankara University, Faculty of Pharmacy (AEF 23694, AEF 23695, AEF 23794, AEF 23793, AEF 23697, respectively).

Extraction Procedure. Dried and powdered bulbs (10 g) of each plant were extracted with 1% H_2SO_4 by maceration at room temperature for 7 days. Each extract was filtered through a 0.45 mm membrane filter and adjusted to a final volume of 500 mL with acidic solution.

Lycorine was isolated as previously described elsewhere [3]. Their structure elucidations were performed using spectroscopic techniques.

A standard solution was prepared by dissolving 10 mg of lycorine in 100 mL of 1% sulfuric acid.

HPLC analysis was carried out using Agilent LC 1100 model chromatograph (Agilent Technologies, Inc., California, USA). The Diod-Array Detector (DAD) was set at wave length, 292 nm, and peak areas were integrated automatically by computer using Agilent software. The chromatograms were plotted and processed by using the above-mentioned software. Separation was carried out using a Supelcosil LC-18 column (250 ×4.6 mm i.d.; 5 μm; Supelco, Belleforte, PA, USA). The mobile phase was made up of ammonium carbonate and acetonitrile (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 chromatograph.

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