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Isolation and identification of alkaloids and anthocyanins from flower and bulb of *Lycoris radiata* using HPLC and LC-ESI-MS

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ABSTRACT

Three anthocyanins (cyanidin 3-diglucoside, cyanidin 3-sambubioside and cyanidin 3-glucoside) together with eleven known alkaloids (lycoricidine, hipppeastrine, *O*-Demethyllycoramin, lycoricidinol, galanthine, lycorine, lycorenine, lycoramine, galanthamine, homolycorine and pretazettine) were identified in the flower and bulb of *Lycoris radiata* using high-performance liquid chromatography coupled with UV detection and electrospray ionization mass spectrometry. Anthocyanins play a major role in protecting plant's DNA from the UV spectrum of sunlight and also in attracting insects for the purpose of pollination. Thus, knowledge on the contents and types of anthocyanins of *L. radiata* will help to evaluate the adaptive evolution of flowers and provide useful information for the ornamental breeding.

Keywords: *Lycoris radiata*; Anthocyanins; Alkaloids; Flower; Bulb

1. INTRODUCTION

Plants of the family *Amaryllidaceae* are well known for their ornamental values and also for their alkaloids they produce. Some of these alkaloids exhibit interesting pharmacological and biological activities. *Lycoris radiata* belongs to *Amaryllidaceae* family of perennial plants, and 20 different species are distributed in East Asia including China, Japan, Korea and Nepal. The Korean peninsula has seven *Lycoris* species such as *L. flavescens*, *L. chinensis* var. *sinuolata*, *L. uydoensis*, *L. sanguinea* var. *koreana*, *L. chejuensi*, *L. radiata* and *L. squamigera* respectively [1]. Since 2000, several Korean researches have reported identification of metabolites from *Lycoris* species [2,3]. *L. radiata* has ornamental and medical values and therefore the interest of plant breeding is high. Floral colors of *L. radiata* are due to biological pigments of different kinds of molecules, such as porphyrins, carotenoids, anthocyanins and betalains. Anthocyanins occur only in terrestrial plants and are thought to be evolutionarily adaptive for several different plants biological processes. Four anthocyanin components, pelargonidin-3-glucosides, pelargonidin-3-xylosylglucosides, cyanidin-3-glucosides, and cyanidin-3-xylosylglucosides were identified in *L. radiata* [4]. To our best knowledge, cyanidin 3-diglucoside and cyanidin 3-sambubioside have not been reported in the flower of *L. radiata*, but they were found to be an important determinant of floral colors in *L. radiata*.

2. MATERIALS AND METHODS

2.1. Plant Materials

Flowers and bulbs of *L. radiata* harvested in 2011 were provided by the Yeonggwang Agricultural Technology Center (Yeonggwang-gun, Korea).

2.2. Extraction of Alkaloids and Anthocyanins

For alkaloids, a 100 mg of lyophilized powder was transferred to 2 mL eppendorf tube and mixed with 1 mL of water/sulfuric acid solution (99:1, v/v). The solution was mixed thoroughly by vortexing for 2 min and then allowed to stand at room temperature for 24 h without disturbance. In the case of anthocynins, a 100 mg portion of lyophilized powder was transferred to a 2 mL eppendorf tube, and 2 mL of water/formic acid solution (95:5,

v/v) was added. The solution was vigorously mixed for 5 min, sonicated for 20 min, and centrifuged at 8000 rpm for 30 min. The extract was filtered through a 0.45 μm PTFE syringe filter (Advantec DISMIC-13HP, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate was analyzed by HPLC and LC-ESI-MS.

2.3. LC-ESI-MS Analysis of Alkaloids and Anthocyanins

HPLC analyses were carried out with a flow rate of 1.0 mL/min at column oven temperature of 40˚C and detector wavelength of 292 nm. The solvent system employed was (A) 10 mM ammonium carbonate and (B) 10% 10 mM ammonium carbonate in acetonitrile. The solvent program used is as follows: 0 min solvent B 0%, 13 min solvent B 7%, then kept constant at solvent B 7% till 18 min, 23 min solvent B 10%, then kept constant at solvent B 10% till 28 min, 35 min solvent B 20%, 50 min solvent B 70%, then kept constant at solvent B 70% till 55 min, rapidly reduce solvent B to 0% at 55.1 min, and then kept constant at solvent B 0% till total 60 min. For the identification of individual alkaloids, the MS analysis was carried out with an ESI interface operating in the positive ion mode $[M+H]^+$. The MS operating conditions were as follows: ion spray voltage, 5.5 kV; curtain gas (N_2), 20 psi; nebulizing gas and heating gas (N_2), 50 psi; heating gas temperature, 550˚C; spectra range, m/z 100 - 500 (scan time, 4.8 sec).

For anthocynins, an API 4000 Q TRAP tandem mass spectrometer (Applied Biosystems, Foster City, CA), equipped with an Agilent 1200 series HPLC system and an electrospray ionization-tandem mass spectrometry (ESI-MS) source in positive ion mode was used. The HPLC conditions were the same as those described above, but the mobile phase was changed to (A) water/formic acid (99:1, v/v) and (B) acetonitrile/formic acid (99:9, v/v) because of low pH of less than 2.0 required.

3. RESULTS AND DISCUSSION

The MS spectral data by comparing with data of a previous report [5,6] led to identification of eleven known alkaloids (lycoricidine (peak no. 4), hipppeastrine (6), *O*-demethyllycoramine (7), lycoricidinol (8), galanthine (9), lycorine (10), lycorenine (12), lycoramine (13), galanthamine (14), homolycorine (16), and pretazettine (18). Seven unknown compounds were also observed. The peak numbers are the elution order in the HPLC chromatogram in **Table 1**. LC-ESI-MS spectra of lycorine and cyanidin 3-sambubioside (anthocyanin) were showed in **Figure 1** and **Figure 2(a)**. Identification of anthocyanins was primarily based on the comparison of their retention time and elution order together with mass spectrometric data with standards and isolation methods described previously [7,8]. Separation of identified anthocyanins on HPLC with their retention time and elution order together with mass spectrometric data were shown in **Table 1**, **Figure 2(b)** and **Figure 3**. LC-ESI-MS spectra of three anthocyanins were compared with the published reports of other anthocyanins derivatives, which coincide in fragmentation patterns and further confirmed that the identified anthocyanins were cyanidin 3-diglucoside, cyanidin 3-sambubioside and cyanidin 3-glu-coside respectively. HPLC chromatogram of peaks 1 and 3 had exhibited similar MS profiles but showed different retention times (23.8 and 26.0 min). The LC-ESI-MS analysis indicated that peaks 1, 2 and 3 contained aglycone cyanidin (MW, molecular weight, 287) as a common backbone with varied monosaccharide glucose (MW, 180) and peaks 2 had monosaccharide with the combination of D-xylose and D-glucose (MW, 312). Cyanidin 3-diglucoside and cyanidin 3-glucoside exhibited molecular ions at m/z 287, respectively, whereas cyanidin 3-sambubioside showed at m/z 294 because this compound produced only one major fragment [9,10]. Peaks 1 and 3 had the same aglycone (cyanidin) as peak 3 but

Table 1. Alkaloids identified in the bulb of *Lycoris radiate.*

No.[a]	RT[b]	Trivial names	$[M+H]^+$(m/z)	Fragment ion (m/z)
1	12.13	Unknown	279	290/ 326
2	12.85	Unknown	301	279/ 264
3	14.99	Unknown	268	278/ 286
4	25.44	Lycoricidine	292	279/ 228
5	29.56	Unknown	290	272/ 254
6	30.72	Hipppeastrine	316	334/ 288
7	33.11	*O*-Demethyllycoramine	276	290/ 219
8	35.79	Lycoricidinol	308	290/ 276
9	39.10	Galanthine	318	279/ 276
10	39.59	Lycorine	288	332/ 270
11	45.98	Unknown	480	428/ 279
12	46.70	Lycorenine	318	300/ 288
13	46.99	Lycoramine	290	233/ 215
14	48.09	Galanthamine	288	213/ 231
15	48.46	Unknown	302	284/ 255
16	48.74	Homolycorine	316	302/ 298
17	51.11	Unknown	256	210/ 137
18	53.87	Pretazettine	332	272/ 240

[a]The elution order of alkaloids in HPLC chromatogram (**Figure 1**); [b] Retention time (min).

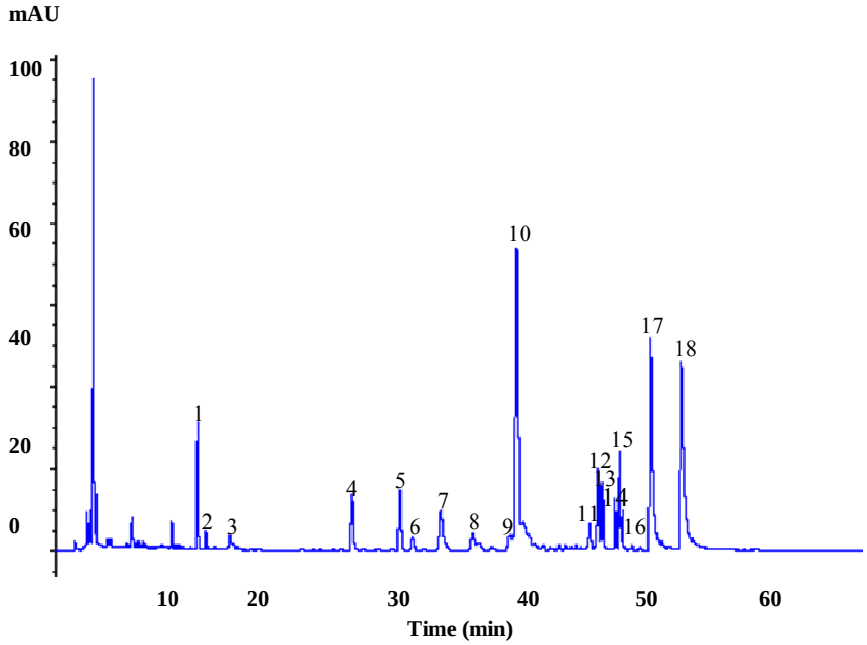
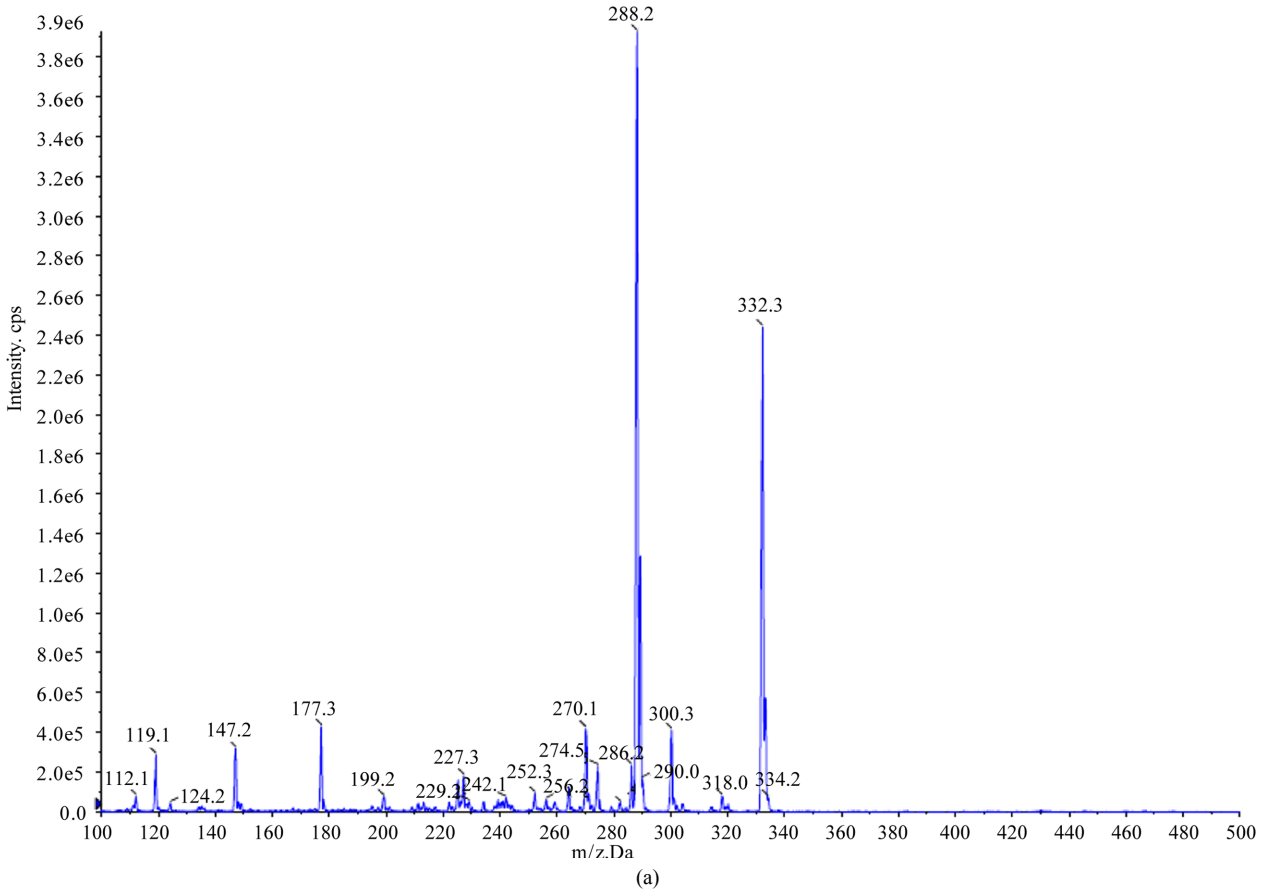


Figure 1. HPLC profiles of alkaloids isolated from the bulb of *Lycoris radiata*. Peak numbers refer to the alkaloids listed in **Table 1**: 1, unknown; 2, unknown; 3, unknown; 4, lycoricidine; 5, unknown; 6, hipppeastrine; 7, *O*-demethyllycoramine; 8, lycoricidinol; 9, galanthine; 10, lycorine; 11, unknown; 12, lycorenine; 13, lycoramine; 14, galanthamine, 15, unknown, 16, homolycorine; 17, unknown; 18, pretazettine.



(a)

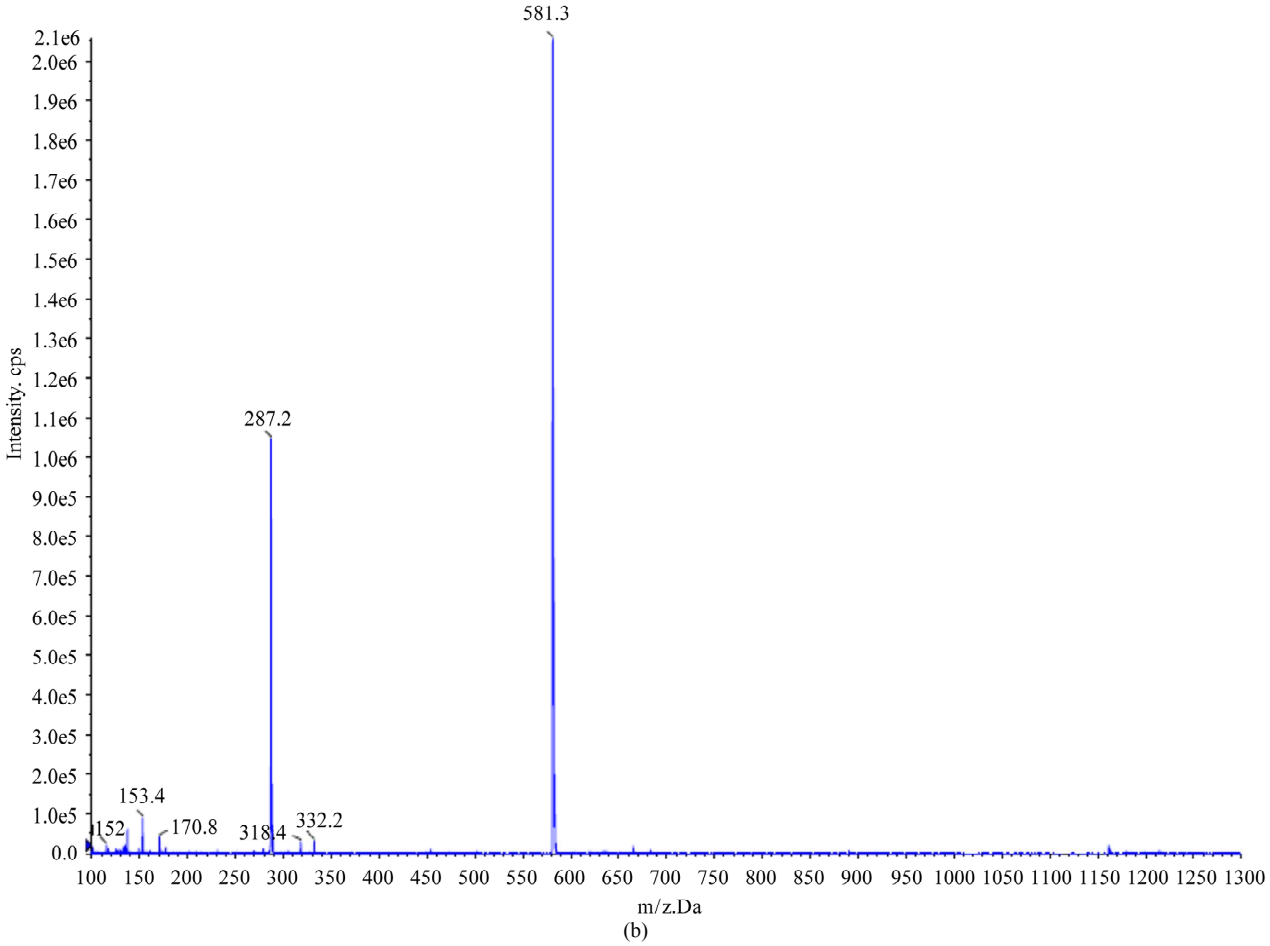


Figure 2. LC-ESI-MS spectra of (a) lycorine (alkaloid) and (b) cyanidin 3-sambubioside (anthocyanin) from *Lycoris radiata*.

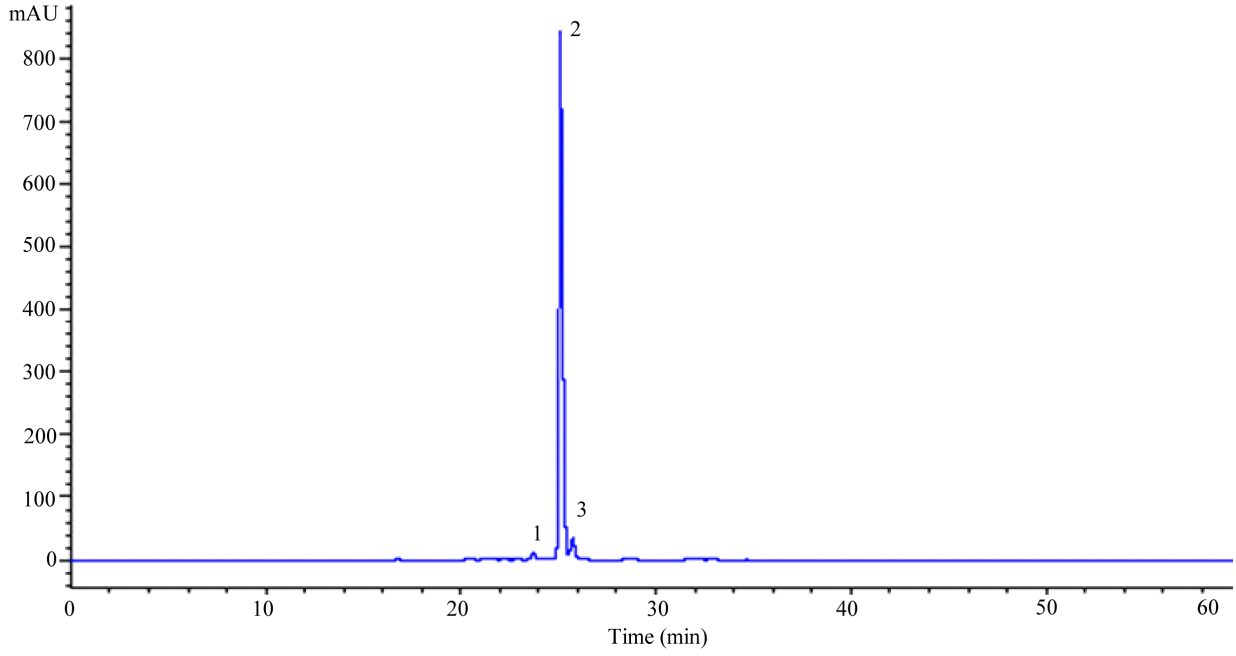


Figure 3. HPLC chromatogram of anthocyanins isolated from the flower of *Lycoris radiata*. Peak numbers refer to the anthocyanins listed in **Table 2**: 1, cyanidin 3-diglucoside; 2, cyanidin 3-sambubioside; 3, cyanidin 3-glucoside.

Table 2. Anthocyanins identified in the flower of *Lycoris radiata*.

No.[a]	RT[b]	Trivial names	$[M+H]^+$ (m/z)
1	23.8	cyanidin 3-diglucoside	318/287
2	25.1	cyanidin 3-sambubioside	581/287
3	26.0	cyanidin 3-glucoside	449/287

[a]The elution order of anthocyanins in HPLC chromatogram (**Figure 3**). [b]Retention time (min).

showed different retention times (23.8 vs 26.0 min). Similar types of anthocyanins were found in red leaf lettuce, pistachio, small red bean, red cabbage and red onion respectively [8].

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