



Evaluation of Chemical Compositions of *Lycoris radiata* at Different Development Stages and Cultivation Sites in South Korea

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Alkaloid contents of *Lycoris radiata* at different cultivation places and time periods were investigated. Eleven known alkaloid compounds such as lycoricidine, hipppeastrine, O-demethyllycoramine, lycoricidinol, galanthine, lycorine, lycorenine, lycoramine, galanthamine, homolycorine and pretazettine and seven unknown compounds were identified and quantified from the bulbs of *L. radiata*. Total alkaloid contents ranged from 1.58 to 5.55 mg/g dry weight (DW) in one year old and 1.64 to 4.79 mg/g DW in two year old *L. radiata*. Lycorine was noticed as the highest concentration of the identified alkaloid compound (1.43 mg/g DW). Moreover 25 % of the total alkaloid contents were contributed by lycorine in each time period.

Key Words: *L. radiata*, Different harvesting time, HPLC, LC-ESI-MS, Alkaloids, Lycorine.

INTRODUCTION

Lycoris radiata belong to Amaryllidaceae family in perennial plants and 20 different species distributed in East Asia including China, Japan, Korea and Nepal. It is speculated that this plant originated from southern parts of China and introduced into Japan 3000 years ago. Japanese ancestors used these bulbs, which contain 30 % starch as food before rice became their main food. In addition, it is used as an emergency food in practice¹. Korean Peninsula has seven *Lycoris* species such as *L. flavescens*, *L. chinensis* var. *sinuolata*, *L. uydoensis*, *L. sanguinea* var. *koreana*, *L. chejuensis*, *L. radiata* and *L. squamigera*, respectively². The bulbs of *Lycoris* species grow around temples have been traditionally used for the altar portrait. It is a traditional ground cover plant in the levee of paddy fields, protecting the levee from soil erosion and suppressing weeds³⁻⁵. Alkaloids from Amaryllidaceae attracted great interest of synthetic organic chemists because of their several biological activities and prospective diversity in pharmacology⁶. So far, more than 100 alkaloids have been isolated

from Amaryllidaceae plants⁷, which has diverse bioactivities such as antiviral, antifeedant, antineoplastic and acetylcholinesterase inhibitory activities². Some alkaloids extracted have been shown to exhibit various natural antitumor activities. Plants in the genus *Lycoris* produce a wide array of biologically active constituents; of particular interest is the species, *L. radiata*, which produces lycorine and other active alkaloids which is used as an antimicrobial agents^{8,9}. The alkaloid compositions of *L. radiata* have been actively studied in 1960' in Japan and the compositions are identified as lycoricidine, lycorine, lycorenine, lycoramine, galanthamine, etc.^{9,10}. Different functions of alkaloids have been reported as lycoricidine for plant growth regulator, lycoramine and galanthamine for cholinesterase inhibition and growth-inhibiting activity on *Avena coleoptile*¹¹. Lycorine is also known to be a powerful inhibitor of growth of higher plants, red algae. Antihypertensive effect of lycorenine was investigated by animal experimentation and yeast *in vitro*¹². Lycorine has been demonstrated to suppress cell growth of the human leukemia cell line HL-60 and multiple myeloma cell line KM3 by arresting the cell cycle, subsequently

inducing apoptosis of tumor cells^{13,14}. It has been found that lycorine-mediated apoptosis was triggered through the cytochrome C-mediated and caspase-dependent pathway¹⁴.

In Korea, since 2000 many researches are related to of *Lycoris* species¹⁵⁻¹⁷. Because, *L. radiata* are widely distributed 170 ha in around Bulgap-Mountain (Yeonggwang, Korea), it started flowering for two weeks in the middle of September. In the present study, the alkaloid contents of *L. radiata* at different ages were investigated according to (i) harvest time (from March to August) and cultivation sites (Yeonggwang A, B, and C). In addition, the alkaloid contents of the methanol extracts were compared with seven *Lycoris* species.

EXPERIMENTAL

The bulbs of *L. radiata* were provided from the Yeonggwang Agricultural Technology Center, where it was cultivated and harvested at different time period from 14 March 2011 to 27 February 2012. Plant materials were freeze-dried and finely powdered by mortar and pestle. They were cultivated in Mangok-ri 181-59, Gunseo-myeon, Yeonggwang-gun, Jeollanamdo, Korea) by local famers (referred as 'A'). In addition, they were collected from two cultivating sites, Moak-ri 8, Bulgap-myeon (referred as 'B') and Giryong-ri 2, Baeksu-eup (referred as 'C') from 11 July to 1 August 2011.

Extraction of alkaloids by various solvents: The bulbs of *L. radiata* were shade dried at room temperature and ground in a manual mill. The powder was mixed with methanol for a period of 1 h for five times. The extract was filtered through a Buchner funnel with Whatman No. 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40°C. Crude methanol extract was suspended in water and partitioned with *n*-hexane directed to hexane extract and aqueous layer. The aqueous layer was partitioned with dichloromethane, ethyl acetate and *n*-butanol individually (Fig. 1). Furthermore, to compare alkaloid contents in different species, seven *Lycoris* sp. (*L. radiata*, *L. flavescens*, *L. sanguinea* var.

koreana, *L. squamigera*, *L. uydoensis*, *L. chinensis* var. *sinuolata* and *L. chejuensis*) which are widely distributed in the Korean Peninsula were collected and their bulbs were extracted by methanol solvent. All the crude extracts were filtered through a 0.45 µm PTFE syringe filter before evaluating individual alkaloid components.

Extraction of alkaloids: 100 mg of lyophilized powder were transferred to 2 mL Eppendorf tube and mixed with 1 mL solution containing water/sulfuric acid (99/1, v/v). The solution was mixed thoroughly by vortexing for 2 min and kept at room temperature for 24 h without disturbance. After centrifugation (13,000 rpm, 15 min, 4 °C) the supernatant were filtered through 0.45 µm PTFE syringe filter.

Separation and quantification of alkaloids: Alkaloids were analyzed by 1200 series HPLC system (Agilent Technologies, CA, USA) equipped with an Capcell Pak C₁₈ column (250 mm × 4.6 mm i.d., particle size 5 µm) (Shiseido, Tokyo, Japan). HPLC analysis was carried out with a flow rate of 1.0 mL/min at column oven temperature of 40 °C and a 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 was used as follows: 0 min solvent B 0 %, 13 min solvent B 7 %, then kept constant at solvent B 7 % by 18 min, 23 min solvent B 10 %, then kept constant at solvent B 10 % by 28 min, 35 min solvent B 20 %, 50 min solvent B 70 %, then kept constant at solvent B 70 % by 55 min, suddenly drops down to solvent B 0 % at 55.1 min and then kept constant at solvent B 0 % for 4.9 min (total 1 h). The individual alkaloids were quantified by comparing the area of external standard (lycorine) using HPLC. For the identification of the individual alkaloids, the MS analysis was carried out with an ESI interface operated in positive ion mode. The MS operating conditions were as follows: ion spray voltage, 5.5 kV; curtain gas (N₂), 20 psi; nebulizing gas and heating gas (N₂), 50 psi; heating gas temperature, 550 °C; spectra range, *m/z* 100-500 (scan time, 4.8 s)¹⁸.

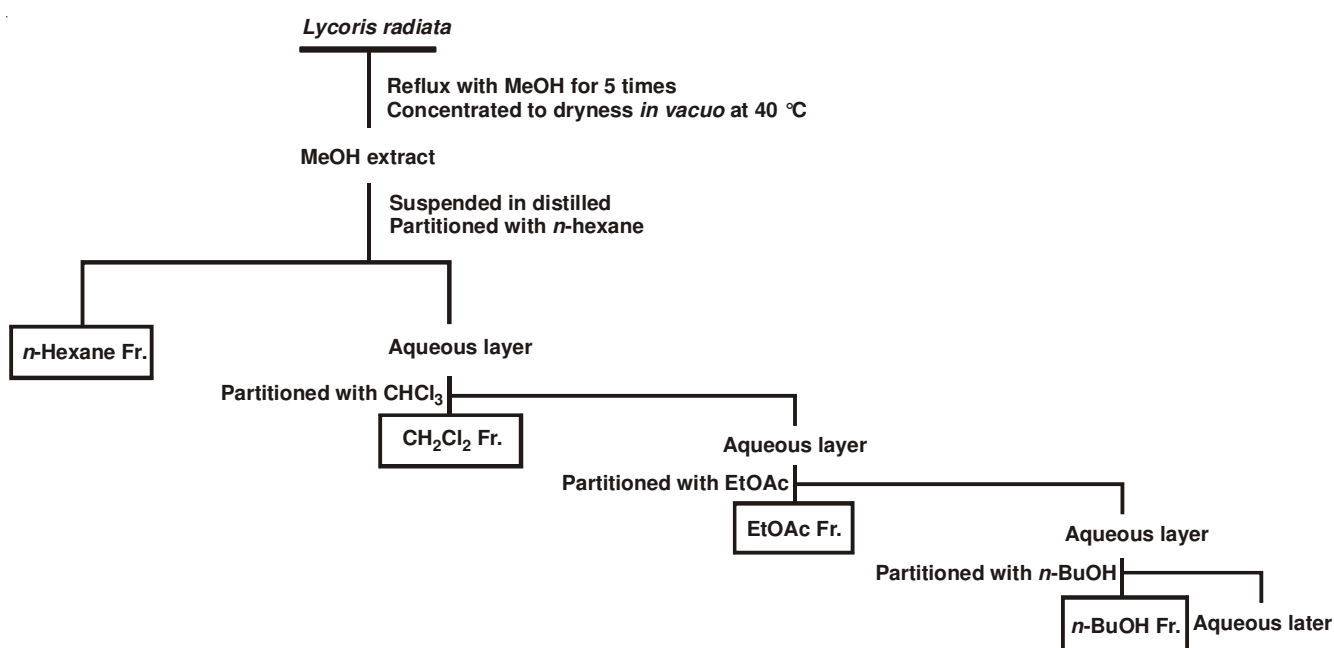


Fig. 1. Schematic flow chart for extraction and fractionation of *Lycoris radiata* by various solvents

RESULTS AND DISCUSSION

Variation of alkaloid contents: The content of total alkaloid present in *L. radiata* was quantified in three different places at different time periods were expressed in mg/g dry weight (DW). Total alkaloid contents ranged from 1.58 to 5.55 mg/g DW and 1.64 to 4.79 mg/g DW, respectively in 1 (under 10 g) and 2 years (over 10 g) old *L. radiata* from place A (Tables 1, 2 and Fig. 2). The average content of alkaloid in two year old plant was 10 % higher than that of 1 year old. The concentration of total alkaloid of 1 year old plant was nearly 1.2-fold ranging maximum of 5.55 mg/g DW and minimum 1.58 mg/g DW, whereas 2 years old plant exhibited maximum of 4.79 mg/g DW and lower range 1.64 mg/g DW, respectively. Intermediate levels (between 1.5-2.5 mg/g DW) of total alkaloid contents noticed in plant harvested at April (04-25), May (05-23) and June (06-20). As shown in Tables 1 and 2, 1 year old *L. radiata* harvested in different time point showed lycorine as the highest

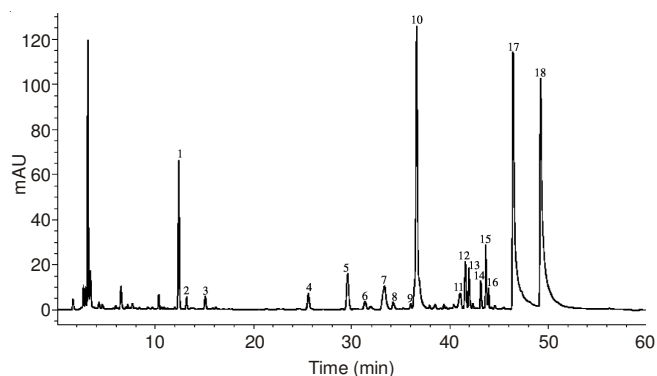


Fig. 2. HPLC profiles of alkaloids isolated from *L. radiata*. Peak numbers refer to the saponins listed in Table-1. Peak No. (1) unknown, (2) unknown, (3) unknown, (4) lycoricidine, (5) unknown, (6) hipppeastrine, (7) O-demethyllycoramine, (8) Lycoricidinol, (9) galanthine, (10) lycorine, (11) unknown, (12) lycorenine, (13) lycoramine, (14) galanthamine, (15) Unknown, (16) homolycorine, (17) unknown and (18) pretazettine

TABLE-1
ALKALOID CONTENTS IN THE BULB (1 YEAR OLD, UNDER 10 g) OF *Lycoris radiata* (mg/g DW)

No. ^a	Trivial name	Harvest time (month-day)										
		1 (03-14)	2 (03-28)	3 (04-11)	4 (04-25)	5 (05-09)	6 (05-23)	7 (06-06)	8 (06-20)	9 (07-04)	10 (07-18)	11 (08-01)
1	Unknown	0.54±0.07	0.61±0.02	0.65±0.05	0.03±0.00	0.02±0.03	0.07±0.06	0.21±0.28	0.14±0.13	0.11±0.08	0.21±0.33	0.12±0.09
2	Unknown	0.03±0.01	ND ^b	ND	ND	ND	ND	0.02±0.01	ND	ND	ND	ND
3	Unknown	0.05±0.01	0.02±0.00	0.07±0.04	0.16±0.10	0.13±0.11	0.12±0.04	0.13±0.06	0.06±0.06	0.16±0.06	0.19±0.06	0.22±0.06
4	Lycoricidine	0.08±0.02	0.06±0.01	0.06±0.00	0.18±0.04	0.23±0.16	0.15±0.05	0.17±0.07	0.17±0.09	0.27±0.09	0.30±0.07	0.31±0.06
5	Unknown	0.17±0.05	0.15±0.01	0.16±0.00	0.14±0.12	0.03±0.04	0.08±0.04	0.08±0.06	0.06±0.06	0.12±0.04	0.25±0.07	0.16±0.04
6	Hippeastrine	0.05±0.01	0.05±0.01	0.06±0.01	0.05±0.03	0.02±0.03	0.04±0.03	0.04±0.03	0.03±0.04	0.06±0.02	0.08±0.02	0.16±0.04
7	O-Demethyllycoramine	0.18±0.08	0.09±0.01	0.14±0.04	0.17±0.06	0.17±0.13	0.13±0.09	0.18±0.09	0.17±0.09	0.25±0.07	0.27±0.08	0.28±0.06
8	Lycoricidinol	0.05±0.01	0.05±0.00	0.07±0.01	ND	ND	ND	ND	ND	ND	ND	ND
9	Galanthine	0.01±0.02	0.01±0.01	0.10±0.01	0.08±0.17	ND	0.01±0.02	ND	ND	0.05±0.04	0.05±0.05	ND
10	Lycorine	1.43±0.21	1.11±0.09	0.97±0.02	0.78±0.25	0.42±0.36	0.87±0.42	0.85±0.36	0.91±0.58	1.20±0.36	1.23±0.32	1.38±0.30
11	Unknown	0.12±0.01	0.11±0.01	0.11±0.01	0.01±0.01	0.01±0.02	0.02±0.01	0.00±0.01	0.01±0.01	0.01±0.02	0.02±0.01	0.02±0.01
12	Lycorenine	0.19±0.03	0.18±0.01	0.20±0.01	0.25±0.08	0.22±0.18	0.32±0.16	0.29±0.14	0.37±0.19	0.37±0.11	0.38±0.08	0.41±0.09
13	Lycoramine	0.14±0.02	0.12±0.01	0.14±0.01	0.06±0.01	0.01±0.02	0.03±0.03	0.03±0.02	0.01±0.01	0.03±0.02	0.06±0.01	0.05±0.01
14	Galanthamine	0.08±0.02	0.06±0.00	0.08±0.01	0.07±0.05	0.08±0.06	0.11±0.05	0.11±0.05	0.10±0.06	0.12±0.04	0.12±0.05	0.09±0.03
15	Unknown	0.18±0.04	0.15±0.02	0.18±0.05	0.14±0.07	0.10±0.08	0.14±0.07	0.15±0.08	0.14±0.11	0.27±0.09	0.29±0.07	0.34±0.07
16	Homolycorine	0.07±0.01	0.06±0.01	0.08±0.02	0.06±0.03	0.04±0.04	0.06±0.03	0.07±0.04	0.06±0.05	0.15±0.0	0.16±0.04	0.22±0.07
17	Unknown	0.98±0.66	0.12±0.02	0.14±0.02	ND	0.02±0.02	0.02±0.03	0.02±0.01	0.04±0.04	0.04±0.02	0.04±0.01	0.08±0.03
18	Pretazettine	1.18±1.05	0.04±0.01	0.04±0.01	ND	ND	0.02±0.03	0.01±0.02	ND	ND	ND	ND
	Total	5.55±2.12	2.97±0.18	3.25±0.16	2.26±0.92	1.58±1.04	2.36±1.18	2.55±0.80	2.31±1.55	3.37±1.02	3.62±1.15	3.85±0.91

^aNo.: the elution order of HPLC analysis; ^bND: not detected.

TABLE-2
ALKALOID CONTENTS IN THE BULB (2 YEAR OLD, OVER 10 g) OF *Lycoris radiata* (mg/g DW)

No. ^a	Trivial name	Harvest time (month-day)										
		1 (03-14)	2 (03-28)	3 (04-11)	4 (04-25)	5 (05-09)	6 (05-23)	7 (06-06)	8 (06-20)	9 (07-04)	10 (07-18)	11 (08-01)
1	Unknown	0.55±0.01	0.63±0.01	0.62±0.02	0.79±1.18	0.29±0.17	0.12±0.15	0.22±0.18	0.63±0.47	0.77±0.52	0.30±0.03	0.44±0.04
2	Unknown	0.03±0.00	ND ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Unknown	0.06±0.01	0.03±0.01	0.06±0.02	0.07±0.03	0.08±0.06	0.10±0.08	0.16±0.03	0.06±0.03	0.18±0.04	0.20±0.05	0.20±0.03
4	Lycoricidine	0.09±0.01	0.07±0.01	0.07±0.01	0.15±0.03	0.11±0.08	0.10±0.06	0.16±0.07	0.11±0.02	0.17±0.04	0.18±0.02	0.18±0.01
5	Unknown	0.15±0.01	0.16±0.01	0.16±0.02	0.24±0.09	0.13±0.10	0.10±0.09	0.14±0.04	0.18±0.05	0.15±0.01	0.13±0.02	0.14±0.02
6	Hippeastrine	0.05±0.01	0.06±0.01	0.07±0.01	0.05±0.04	0.02±0.02	0.03±0.04	0.07±0.03	0.05±0.02	0.08±0.02	0.18±0.04	0.08±0.00
7	O-Demethyllycoramine	0.12±0.01	0.11±0.02	0.16±0.02	0.11±0.03	0.08±0.06	0.06±0.06	0.13±0.05	0.14±0.03	0.18±0.01	0.18±0.02	0.20±0.11
8	Lycoricidinol	0.05±0.01	0.05±0.01	0.09±0.01	ND	ND	ND	ND	ND	ND	ND	0.08±0.02
9	Galanthine	0.02±0.00	0.02±0.00	0.10±0.01	0.04±0.04	0.03±0.03	0.03±0.04	0.05±0.04	0.07±0.04	0.04±0.03	ND	ND
10	Lycorine	1.23±0.15	1.05±0.10	1.08±0.14	0.89±0.30	0.65±0.48	0.56±0.48	0.97±0.23	1.30±0.23	1.44±0.11	1.26±0.10	1.53±0.12
11	Unknown	0.12±0.02	0.12±0.02	0.14±0.01	ND	0.01±0.02	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.01±0.01	0.12±0.01
12	Lycorenine	0.19±0.01	0.21±0.02	0.24±0.03	0.29±0.11	0.07±0.13	0.19±0.17	0.33±0.09	0.37±0.07	0.46±0.05	0.40±0.03	0.36±0.02
13	Lycoramine	0.14±0.01	0.13±0.02	0.18±0.02	0.03±0.03	0.01±0.02	0.01±0.02	0.02±0.01	0.04±0.01	0.04±0.01	0.04±0.00	0.04±0.01
14	Galanthamine	0.07±0.00	0.07±0.01	0.09±0.01	0.07±0.04	0.07±0.05	0.05±0.04	0.08±0.02	0.11±0.03	0.10±0.01	0.08±0.02	0.09±0.01
15	Unknown	0.18±0.03	0.18±0.03	0.20±0.02	0.16±0.06	0.09±0.07	0.10±0.10	0.18±0.04	0.17±0.05	0.30±0.04	0.32±0.07	0.33±0.05
16	Homolycorine	0.08±0.01	0.07±0.01	0.09±0.01	0.06±0.02	0.04±0.03	0.04±0.05	0.08±0.02	0.10±0.03	0.19±0.04	0.20±0.06	0.20±0.05
17	Unknown	0.85±0.98	0.13±0.01	0.16±0.03	0.12±0.07	0.10±0.09	0.06±0.09	0.08±0.06	0.12±0.04	0.14±0.03	0.23±0.19	0.13±0.01
18	Pretazettine	0.82±1.02	0.03±0.00	0.05±0.01	ND	ND	ND	ND	ND	ND	ND	ND
	Total	4.79±2.03	3.13±0.27	3.55±0.33	3.08±1.38	1.88±1.19	1.64±1.53	2.80±0.65	3.56±0.91	4.40±0.65	3.71±0.33	4.18±0.29

^aNo.: the elution order of HPLC analysis; ^bND: not detected.

concentration of the identified compound especially plant harvested at March from 'A' region documented maximum amount (1.43 mg/g DW), followed by August (1.38 mg/g DW) and July (1.23 mg/g DW), respectively, whereas in the case of 2 year old category maximum lycorine observed (1.53 mg/g DW) when harvested at August followed by July (1.44 mg/g DW). However in other harvested periods, all identified alkaloids other than lycorine noticed less than 1.00 mg/g DW; moreover pretazettine showed 1.18 mg/g DW when cultivated at March. Galanthine, galanthamine, lycoricidinol and hippeastrine were noticed in all the time period ranged from 0.01 to 0.31 mg/g DW. About 25 % of the total alkaloid contents were contributed by lycorine in each time period.

Alkaloid contents of *L. radiata* harvested in different places and different time period were shown in Tables 3 and 4. Results indicated that total alkaloid contents ranged from 2.86 to 4.45 and 2.35 to 4.42 mg/g DW were observed in place

'B' and 'C', respectively. Maximum total alkaloid (4.45 mg/g DW) observed in place 'B', when harvested at August. Lycorine is grouped in main phenanthridine in Amaryllidaceae alkaloid, reported significant antitumor activity was detected in three cultivation places and concentrations were dominated whereas the other alkaloids contents comparatively less¹⁴. As shown in Tables 4 and 5, one and two year-old plant collected at different place and different time periods showed lycorine as the highest concentration of the identified compound which was obtained from place B where collected in August (1.66 mg/g DW), followed by place C where collected in July (1.57 mg/g DW) and place A, collected in August (1.53 mg/g DW), respectively. However, lycoricidine, hippeastrine, O-demethyl lycoramine, lycorenine, lycoramine, galanthamine and homolycorine were notice in all the time periods. Lycoricidine, a main compound of growth-inhibiting activity, was found very little amounts as 0.3 mg/g DW (8.3 % of the total) and 0.23

TABLE-3
ALKALOID CONTENTS IN THE BULB (1 YEAR OLD, UNDER 10 g) OF *Lycoris radiata* (mg/g DW)
HARVESTED AT DIFFERENT REGION AND TIME PERIODS

No.	Trivial name	Harvest time (month-day, sites)											
		1 (07-11, A)	2 (07-11, B)	3 (07-11, C)	4 (07-18, A)	5 (07-18, B)	6 (07-18, C)	7 (07-25, A)	8 (07-25, B)	9 (07-25, C)	10 (08-01, A)	11 (08-01, B)	12 (08-01, C)
1	Unknown	0.38±0.42	0.06±0.08	0.03±0.04	0.21±0.33	0.05±0.03	0.04±0.03	0.38±0.64	0.19±0.18	0.15±0.11	0.12±0.09	0.81±1.39	0.14±0.18
2	Unknown	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Unknown	0.21±0.03	0.15±0.04	0.13±0.10	0.19±0.06	0.19±0.12	0.15±0.09	0.20±0.06	0.18±0.09	0.19±0.08	0.22±0.06	0.16±0.05	0.16±0.07
4	Lycoricidine	0.27±0.07	0.24±0.08	0.23±0.14	0.30±0.07	0.27±0.13	0.25±0.08	0.28±0.07	0.26±0.06	0.26±0.08	0.31±0.06	0.27±0.08	0.26±0.09
5	Unknown	0.15±0.03	0.21±0.09	0.16±0.12	0.25±0.07	0.23±0.10	0.12±0.05	0.13±0.03	0.14±0.05	0.14±0.04	0.16±0.04	0.15±0.02	0.11±0.05
6	Hippeastrine	0.08±0.01	0.07±0.02	0.04±0.05	0.08±0.02	0.07±0.05	0.05±0.04	0.07±0.02	0.06±0.04	0.07±0.02	0.16±0.04	0.13±0.05	0.11±0.05
7	O-Demethyllycoramine	0.25±0.04	0.22±0.10	0.19±0.11	0.27±0.08	0.25±0.11	0.27±0.11	0.26±0.06	0.24±0.08	0.18±0.09	0.28±0.06	0.26±0.07	0.21±0.05
8	Lycoricidinol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	Galanthine	0.03±0.06	0.05±0.05	0.04±0.04	0.05±0.05	0.04±0.05	0.09±0.07	0.02±0.03	0.02±0.03	ND	ND	0.09±0.10	0.18±0.05
10	Lycorine	1.33±0.48	0.99±0.37	0.84±0.58	1.23±0.32	1.09±0.45	1.08±0.39	1.22±0.31	1.10±0.35	1.17±0.34	1.38±0.30	1.26±0.26	1.08±0.39
11	Unknown	0.02±0.02	ND	0.01±0.01	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	ND
12	Lycorenine	0.42±0.13	0.31±0.11	0.25±0.17	0.38±0.08	0.34±0.14	0.32±0.12	0.37±0.09	0.32±0.10	0.37±0.10	0.41±0.09	0.40±0.10	0.31±0.11
13	Lycoramine	0.07±0.02	0.05±0.01	0.03±0.03	0.06±0.01	0.05±0.02	0.04±0.01	0.05±0.01	0.04±0.01	0.04±0.01	0.05±0.01	0.07±0.02	0.06±0.02
14	Galanthamine	0.12±0.06	0.09±0.04	0.08±0.06	0.12±0.05	0.11±0.05	0.08±0.03	0.08±0.03	0.08±0.03	0.08±0.03	0.09±0.03	0.09±0.02	0.07±0.03
15	Unknown	0.32±0.04	0.25±0.09	0.18±0.13	0.29±0.07	0.30±0.15	0.25±0.12	0.30±0.07	0.26±0.11	0.30±0.08	0.34±0.07	0.30±0.09	0.21±0.07
16	Homolycorine	0.19±0.01	0.15±0.04	0.11±0.09	0.16±0.04	0.20±0.11	0.14±0.06	0.17±0.05	0.16±0.06	0.19±0.06	0.22±0.07	0.18±0.06	0.13±0.04
17	Unknown	0.03±0.03	0.02±0.02	0.02±0.03	0.04±0.01	0.04±0.02	0.03±0.03	0.06±0.03	0.05±0.03	0.08±0.03	0.08±0.03	0.07±0.02	0.08±0.04
18	Pretazettine	ND	ND	ND	ND	ND	0.01±0.03	ND	ND	ND	ND	0.18±0.36	ND
	Total	4.01±1.29	2.86±1.10	2.35±1.67	3.62±1.15	3.24±1.46	3.05±1.21	3.72±1.15	3.23±1.24	3.37±0.95	3.85±0.91	4.45±1.43	3.11±1.19

*ND: Not detected.

TABLE-4
ALKALOID CONTENTS IN THE BULB (2 YEAR OLD, OVER 10 g) OF *Lycoris radiata* (mg/g DW)
HARVESTED AT DIFFERENT REGION AND DIFFERENT TIME PERIODS

No.	Trivial name	Harvest time (month-day, sites)											
		1 (07-11, A)	2 (07-11, B)	3 (07-11, C)	4 (07-18, A)	5 (07-18, B)	6 (07-18, C)	7 (07-25, A)	8 (07-25, B)	9 (07-25, C)	10 (08-01, A)	11 (08-01, B)	12 (08-01, C)
1	Unknown	0.25±0.11	0.26±0.06	0.31±0.13	0.30±0.03	0.31±0.00	0.32±0.06	0.32±0.09	0.29±0.02	0.34±0.02	0.44±0.04	0.39±0.02	0.37±0.13
2	Unknown	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Unknown	0.17±0.02	0.15±0.02	0.23±0.02	0.20±0.05	0.17±0.2	0.15±0.04	0.18±0.03	0.13±0.06	0.18±0.03	0.20±0.03	0.21±0.03	0.13±0.06
4	Lycoricidine	0.23±0.06	0.21±0.01	0.17±0.04	0.18±0.02	0.20±0.01	0.21±0.02	0.19±0.05	0.18±0.06	0.20±0.02	0.18±0.01	0.21±0.03	0.13±0.06
5	Unknown	0.15±0.00	0.15±0.02	0.13±0.01	0.13±0.02	0.15±0.01	0.16±0.01	0.17±0.01	0.13±0.05	0.16±0.02	0.14±0.02	0.17±0.02	0.11±0.06
6	Hippeastrine	0.13±0.03	0.14±0.04	0.18±0.04	0.18±0.04	0.21±0.04	0.21±0.02	0.06±0.01	0.04±0.03	0.07±0.00	0.08±0.00	0.06±0.01	0.04±0.04
7	O-Demethyllycoramine	0.21±0.02	0.22±0.03	0.17±0.03	0.18±0.02	0.22±0.01	0.27±0.03	0.17±0.05	0.12±0.05	0.32±0.09	0.28±0.11	0.22±0.02	0.14±0.08
8	Lycoricidinol	ND	ND	ND	ND	ND	ND	0.27±0.03	0.24±0.10	0.10±0.06	0.08±0.02	0.19±0.07	0.15±0.08
9	Galanthine	ND	ND	ND	ND	0.13±0.01	0.08±0.01	0.20±0.05	0.13±0.09	0.15±0.05	ND	0.07±0.05	0.01±0.01
10	Lycorine	1.30±0.06	1.29±0.09	1.22±0.10	1.26±0.10	1.45±0.05	1.44±0.09	1.48±0.09	1.22±0.49	1.57±0.12	1.53±0.12	1.66±0.10	1.14±0.61
11	Unknown	0.02±0.01	0.02±0.03	0.09±0.16	0.01±0.01	0.04±0.02	0.05±0.08	0.03±0.04	0.07±0.03	0.11±0.01	0.12±0.01	0.11±0.01	0.04±0.05
12	Lycorenine	0.41±0.04	0.40±0.03	0.37±0.04	0.40±0.03	0.45±0.01	0.43±0.04	0.36±0.06	0.26±0.10	0.35±0.04	0.36±0.02	0.35±0.03	0.21±0.14
13	Lycoramine	0.04±0.01	0.04±0.01	0.04±0.00	0.04±0.00	0.05±0.00	0.04±0.01	0.03±0.01	0.04±0.02	0.03±0.00	0.04±0.01	0.05±0.01	0.05±0.03
14	Galanthamine	0.09±0.01	0.10±0.02	0.08±0.01	0.08±0.02	0.10±0.01	0.10±0.02	0.09±0.03	0.09±0.04	0.09±0.00	0.09±0.01	0.11±0.02	0.07±0.04
15	Unknown	0.24±0.04	0.27±0.07	0.31±0.05	0.32±0.07	0.31±0.02	0.26±0.04	0.29±0.07	0.22±0.09	0.32±0.06	0.33±0.05	0.27±0.03	0.20±0.10
16	Homolycorine	0.15±0.03	0.17±0.04	0.22±0.04	0.20±0.06	0.20±0.03	0.14±0.02	0.17±0.04	0.14±0.07	0.19±0.04	0.20±0.05	0.15±0.02	0.11±0.05
17	Unknown	0.20±0.11	0.15±0.06	0.50±0.077	0.23±0.19	0.17±0.02	0.27±0.11	0.23±0.11	0.12±0.02	0.24±0.16	0.13±0.01	0.13±0.00	0.09±0.03
18	Pretazettine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Total	3.58±0.17	3.58±0.19	4.01±0.78	3.71±0.33	4.15±0.17	4.12±0.23	4.24±0.39	3.42±1.27	4.42±0.38	4.18±0.29	4.35±0.30	2.97±1.39

*ND: Not detected.

TABLE-6
AMOUNT OF ALKALOID PRESENT IN CRUDE METHANOL EXTRACTS OF DIFFERENT *Lycoris* sp. (mg/mL)

No.	Trivial name	<i>L. flavescens</i>	<i>L. chinensis</i> var. <i>sinuolata</i>	<i>L. uydoensis</i>	<i>L. sanguinea</i> var. <i>koreana</i>	<i>L. chejuensis</i>	<i>L. radiata</i>	<i>L. squamigera</i>
1	Unknown	3.00	2.31	2.43	0.63	0.80	1.34	0.47
2	Unknown	0.26	0.24	0.38	0.26	0.41	0.69	0.08
3	Unknown	2.16	1.12	2.11	3.22	1.84	3.08	0.83
4	Lycoricidine	5.85	3.05	3.50	9.54	0.71	1.18	2.60
5	Unknown	0.49	0.57	0.74	1.52	0.20	0.33	0.21
6	Hippeastrine	0.90	0.76	0.97	2.36	0.46	0.76	0.44
7	O-Demethyllycoramine	0.84	1.87	1.00	2.18	1.14	1.91	2.49
8	Lycoricidinol	3.71	1.80	2.69	2.92	1.41	2.36	0.66
9	Galanthine	1.80	0.41	0.50	4.00	1.18	1.98	0.90
10	Lycorine	5.64	5.22	8.39	5.00	5.19	8.67	8.05
11	Unknown	0.58	0.76	0.52	1.74	0.29	0.48	0.22
12	Lycorenine	0.58	0.40	1.09	0.98	0.65	1.09	2.48
13	Lycoramine	0.91	0.54	0.53	1.52	0.96	1.60	1.75
14	Galanthamine	0.55	0.52	0.50	1.97	1.22	2.05	0.75
15	Unknown	2.50	1.96	1.58	2.78	0.39	0.65	0.32
16	Homolycorine	2.11	4.82	3.08	3.39	0.49	0.83	0.51
17	Unknown	0.10	0.09	0.16	0.39	0.13	0.21	0.33
18	Pretazettine	0.26	0.17	0.08	0.23	0.14	0.23	0.18
Total		32.24	26.60	30.26	44.61	17.61	29.45	23.28

(6.4 %) mg/g DW in 1- and 2-year-old plants, respectively and only detected limited collecting places¹¹. Literature study showed that Amaryllidaceae plant family provides a great diversity of alkaloids which have been extensively studied due to their challenging chemistry and promising activities⁷. The relative concentration of the individual alkaloids between the cultivated areas was widely varied, thereby indicating that the assessment of alkaloid concentration of *L. radiata* would be effective.

The contents of total alkaloid present in *L. radiata* was quantified by extracting with organic solvents depending upon their polarity such as *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol (Table-5). Total alkaloid contents extracted in *n*-butanol and ethyl acetate (188.87 and 68.33 mg/g DW, respectively) were marginally higher than the extracts of

dichloromethane. The main alkaloid with contents of 34 % of the total alkaloid in hexane and butanol extract was lycorine, however the major alkaloid found in methylene chloride and ethyl acetate was lycorenine (18.57 mg/g DW) and lycoramine (7.69 mg/g DW), respectively. Among the different organic solvents, *n*-butanol was identified as the best for extraction of alkaloid since the promising alkaloid lycorine obtained from this solvent was significantly higher. Distribution of individual and total alkaloid contents of *L. flavescens*, *L. chinensis* var. *sinuolata*, *L. uydoensis*, *L. sanguinea* var. *koreana*, *L. chejuensis*, *L. radiata* and *L. squamigera* were extracted by organic solvent methanol (Table-6). Among the different *Lycoris* species *L. uydoensis* showed the highest (44.61 mg/mL) total alkaloids followed by *L. flavescens* (32.24 mg/mL). Lycorine, lycoricidine, lycoricidinol and galanthine were the dominant alkaloids in all the species.

Conclusion

This study attempted to identify the bioactive substances associated with medicinal values were quantified in *L. radiata* cultivated in different region and different cultivation periods. These results insisted that the cultivating place, time and the organic solvent used for extraction from *L. radiata* is very important to retain and extract the entire alkaloid.

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TABLE-5

ALKALOID CONTENTS IN VARIOUS SOLVENT EXTRACTS OF *Lycoris radiata* (mg/g DW)

No.	Trivial name	<i>n</i> -Hexane	Dichloro-methane	Ethyl acetate	<i>n</i> -Butanol
1	Unknown	0.12	1.08	1.93	0.99
2	Unknown	0.07	0.16	0.66	0.20
3	Unknown	0.06	1.67	1.05	0.95
4	Lycoricidine	0.65	0.40	16.47	28.11
5	Unknown	ND	ND	0.21	5.95
6	Hippeastrine	ND	0.17	0.54	5.68
7	O-Demethyllycoramine	1.40	0.84	15.92	12.07
8	Lycoricidinol	0.34	0.15	0.87	19.42
9	Galanthine	1.25	0.34	1.09	ND
10	Lycorine	4.93	1.63	5.69	67.28
11	Unknown	0.26	0.44	1.67	12.70
12	Lycorenine	1.91	18.57	3.06	4.12
13	Lycoramine	0.68	6.40	7.69	13.84
14	Galanthamine	0.27	3.01	3.98	6.93
15	Unknown	1.49	0.79	3.14	6.68
16	Homolycorine	0.62	6.58	3.14	1.30
17	Unknown	0.11	0.36	0.91	2.31
18	Pretazettine	0.21	0.22	0.31	0.34
Total		14.37	42.81	68.33	188.87

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