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Identification for Flavones in Different Parts of *Cirsium japonicum*

Su-Jeong Kim and Gun-Hee Kim†

Department of Food and Nutrition, Duksung Women's University, Seoul 132-714, Korea

Abstract

***Cirsium japonicum* is a herbaceous perennial plant grown worldwide, which has been used as a folklore medicine due to its anti-inflammatory properties. A few studies have reported its functional properties, but analytical methods that more confidently and reproductively analyze the flavonoids are required. To establish analytical methods for the detection of flavones in *Cirsium japonicum*, the potential of HPLC and LC/MS were investigated. For this, the plants were separated into 4 parts; the root, stem, leaves, and flowers. The flavones in each part of the dried materials were analyzed by HPLC. Identification of flavones was performed by LC/MS. The leaves and flowers of *Cirsium japonicum* gave the optimum peaks, which were not detected by HPLC in the other parts of plants. Using LC/MS, three kinds of flavones were tentatively identified from the leaves, which were thought to be luteolin (5,7,3′,4′-tetrahydroxy-flavone), apigenin (4′,5,7-trihydroxyflavone), and hispidulin (4′,5,7-trihydroxy-6-methoxyflavone). Two flavones were detected from the flowers, which were been assumed to be apigenin and luteolin.**

Key words: *Cirsium japonicum*, flavones, HPLC, LC/MS

INTRODUCTION

Cirsium species grow widely in Asian and Western countries, such as Korea, China, Japan, USA, Canada, and Australia as they grow very well anywhere and breed rapidly. *Cirsium* species belong to the *composite* family, and are used as medicinal plants all over the world (1), and have been known as a traditional home and folk remedy source in Asian countries including Korea. The dried root of *Cirsium* species is one of the botanical origins of the crude drug "Wazokudan", and is used for the treatment of neuralgia and rheumatism in Japan. It is also an important herb, which has been used as an anti- agent for hemorrhage, rheumatoid arthritis, urination and a treatment for scabies, and for many abdominal & intestinal disorders in oriental medicine (2). However, *Cirsium* species are classified into weeds in Western countries, but are also known for their biological control against insects (3).

It has been reported that the root of *Cirsium japonicum* contained 307.4 mg of Fe ion which was 4 times higher level than the other medicinal herbs (4). *Cirsium japonicum* also contains many medicinal components such as pectolinarins, acacetins, rhamnoglucosides, ciryneols A-E, and heptadecenes and so on. The polyacetylene compounds, cirnocols A-C and heptadeca-1-ene-11, 13-diyne-8,9,10-triol were isolated from *Cirsium japonicum* (5). A few reports have identified flavones in the leaves of *Cirsium* species, such as cirsimaritin-4′-*O*-glucoside (6), cirsimaritin-4′-*O*-rutinoside (7), pectolinarin, cirsitakaoside (5,7-dihydroxy-8,4′-dimethoxy-flavone-7-*O*-β-D-glucoside), cirsitakaogenin (5,7-dihydroxy-8,4′-dimethoxy- flavone) (8), kaempferol-3-*O*-glucoside, quercetin-3-*O*- glucoside, quercetin-3-galactoside, apigenin-7-*O*-diglucoside, genkwanin-4′-*O*-glucoside (9), hispidulin 4′-glucoside, nepetin 4′-gluciside, and hispidulin 7,4′-glycoside (10).

Several studies have reported on flavonoid components in *Cirsium* species, but more confident and reproductive methods are still required for flavonoids research on plant resources.

The objective of this study was to investigate the flavone contents in different parts of *Cirsium japonicum* using high pressure liquid chromatography and liquid chromatography / mass spectrometry, to establish research methodologies for functional foods and neutraceuticals materials.

MATERIALS AND METHODS

Sample preparation

Cirsium japonicum was collected from Daegwallyeong, Gangwon-do, Korea on July 16, 2001. These plants were separated into 4 parts; the root, stem, leaves, and flowers. Each part of the dried root, stem, leaves, and flowers was crushed in a blender and then was extracted.

†Corresponding author. E-mail: ghkim@duksung.ac.kr
Phone: +82-2-901-8496, Fax: +82-2-901-8372

Extraction

Each part of the dried material (0.5 g) was extracted with 50 mL of 80% aqueous methanol (Fronine Pty Ltd.). A simple filtration was conducted using a funnel and filter paper (Whatmann No.114, 12.5 cm) after samples of the dried materials had been mixed, using a magnetic stirrer, with the aqueous methanol for 30 min.

Separation

High pressure liquid chromatography: The extracted samples were analyzed using a Shimazu SPD-10A series (Kyoto, Japan). The HPLC system consisted of an SIL-$10A_{XL}$ auto injector, a CTO-10A column oven, SPD-M10A photodiode array detector, and SPD-10A UV-VIS detectors linked with Class-10A and LC-10AT pumps to the column (Supelco C_{18} 15 cm×2.1 mm, 5 micron) fitted with a C_{18} guard column (Alltech, Deerfield) at 40 °C. The HPLC water was purified using by Millipore Milli Q plus filtration system. 1% of 0.1 M formic acid in acetonitrile (HPLC grade, Allied Signal, UN) (A) and 1% of 0.1 M formic acid in water (B) were used as the eluents. The gradient was started at 17~40% (A) for 10 min, remained at 40% until 15 min, followed by 90% for 3 min and ramped back down to 17% over 2 min. After 22 min, the run was stopped with a flow-rate of 1 mL/min. As double channels were used, it is possible to observe different of UV spectra at the same time. UV-spectra were recorded at 270 and 345 nm.

Liquid chromatography/mass spectrometry: The LC/MS was conducted on a Waters (Alliance, USA), which consisted of a Waters 2690 separation module, a Waters 996 photodiode array detector, a Waters integrity^{1M} system and a Thermabeam^{TM} mass detector. The column was a discovery C_{18} 15 cm×2.1 mm, 5 micron (Supelco, Bellfonte, PA, USA) fitted with a C_{18} guard column (Alltech, Deerfield) at 40°C. The eluents were 1% 0.1 M formic acid in acetonitrile (HPLC grade, Allied Signal, UN) (A), and 1% 0.1 M formic acid in water (B), at a flow-rate of 0.3 mL/min. The gradient was started at 17~40% (A) for 10 min remained at 40% until 15 min, followed by 90% for 3 min and ramped back down to 17% over 2 min and then stop 22 min. The source temperature was set at 200°C and the nebulizer temperature at 85°C, in TMD. The LC-Scan Limits were from 70 to 450 a.m.u. at a scan rate of 1.0 scan/s. The MS was linked by a Millenium^{32}.

RESULTS AND DISCUSSION

High pressure liquid chromatography analysis

The methodologically, in much of the literatures has been reported that UV, TLC and NMR as those mainly used to analyze flavonoids in *Cirsium* species (6,11-14). Recently, structures can be more accurately and easily determined due to the continuing improvements in equipment and techniques or the spectroscopic characterization (3). For the determination of analytical method there is the need to standardize protocols for the extraction and chromatographic separation for analyzing flavonoids of *Cirsium* species. Pervious study has shown that about 12 flavonoid compounds present in *Cirsium* species, including apigenine, chrysoeriol, diosmetin, hispidulin, kaempferol, luteolin, narnigenin, quercetin, rutin, and tricin (3).

In this study, the flavones in the root, stem, leaves, and flowers of *Cirsium japonicum* were to analyze using HPLC. With HPLC, the general flavone spectrum required extraction, which was conducted with methanol and ethanol. The two absorption maxima used for flavones are usually at 240~285 and 300~550 nm (15). The chromatogram was able to be confirmed with dual channels, at 270 and 345 nm. Comparatively, the best separation was achieved using a Supelco discovery C_{18} 250 mm×4.6 mm, 5 micron column at a flow rate of 0.3 mL/min.

As a result, the root and stem did not show any peaks at 270 and 345 nm. These chromatograms are shown to Fig. 1 and 2. Whereas the leaves and flowers showed intensive peaks, these are shown to Fig. 3 and 4.

The leaves and flowers were selected for further study. In addition, three kinds of flavones were assumed to have been detected from the leaves and two from the flower by the HPLC methods.

Identification by liquid chromatography / mass spectrometry

Three peaks were detected from the leaves by HPLC (Fig. 5). One of the peaks, with the UV λ_{max} of luteolin

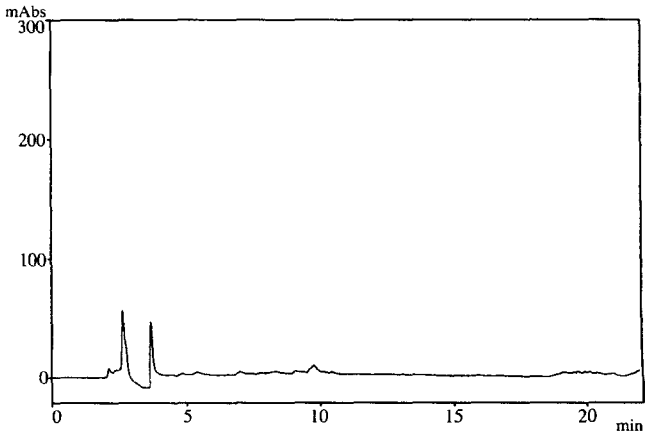

Fig. 1. HPLC chromatogram from the root of *Cirsium japonicum* at 270 nm.

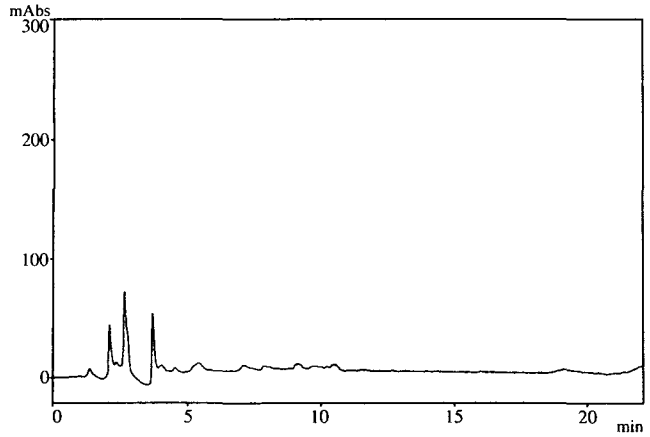

Fig. 2. HPLC chromatogram from the stem of *Cirsium japonicum* at 270 nm.

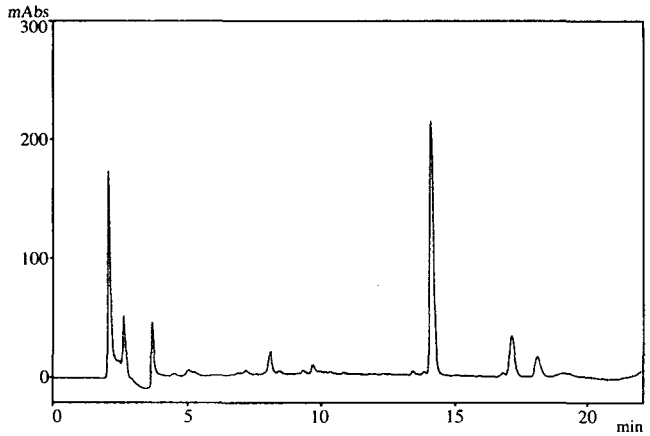

Fig. 3. HPLC chromatogram from the leaves of *Cirsium japonicum* at 270 nm.

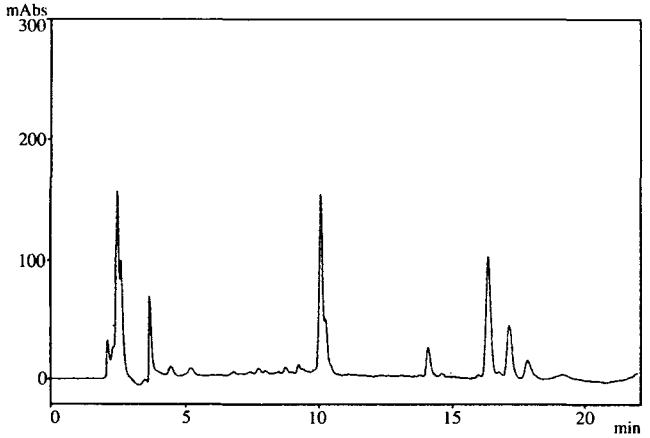

Fig. 4. HPLC chromatogram from the flowers of *Cirsium japonicum* at 270 nm.

was shown in the leaves at 209.9, 253.4 and 337.6 nm, with a retention time (RT) of 11.44 min using the photodiode array detector from the LC/MS. Many researchers have investigated that the separation of luteolin from *Cirsium* species (16-19). The mass spectrum of luteolin gave a molecular ion at m/z 286 (Fig. 5).

The second peak found in the leaves sample was assumed to be apigenin. The UV λ_{max} was detected at 267.6 and 337.6 nm, and as m/z of 270 at an RT of 12.83 min (Fig. 6). The UV λ_{max} was found to be in accordance with that of Markham (13), who showed an apigenin UV λ_{max} at 267, 296 shoulder, and 337 nm. The mass spectrum of apigenin gave a molecular ion at m/z 270 (Fig. 7).

The last peak was shown at retention time of 13.23 min with UV λ_{max} at 207.5, 251.0, 267.0 and 347.2 nm. The mass spectrum showed an ion at m/z 300 in the leaves sample. Therefore, the last peak was considered as hispidulin (Fig. 8). In the past, hispidulin had been separated from leaves of *Cirsium oligophyllum* using NMR, FAB-MS, and UV (6). In addition, Park et al. (11) identified hispidulin-7-*O*-neohesperidoside from leaves of *Cirsium japonicum* var. *ussuriense*.

In the flowers, many peaks were detected by HPLC, but only two were accurately identified with LC/MS (Fig. 9). One, with UV λ_{max} was at 207.5, 253.4 and 348.4 nm and a mass spectrum molecular ion at m/z 286 and an RT of 11.27 min, was considered to be luteolin (Fig. 10). The other was considered as apigenin, with UV λ_{max} was at 267.6 and 337.6 nm, a mass spectrum molecular ion at m/z 270 and an RT of 12.66 min (Fig. 11). All compounds were matched over 80% correspondence to the library linked with Millennium[32].

Several reports have analyzed the flavones in *Cirsium* species, which were based on the methods of Markham (13). This study developed methods for the analyses of flavones by HPLC and LC/MS. The methods established in this study can be used to analyze various flavonoids in food plants even though these methods were developed using *Cirsium japonicum* as a model. The hydrolysis step was skipped in the methods, making the extraction procedure and separation very simple and fast.

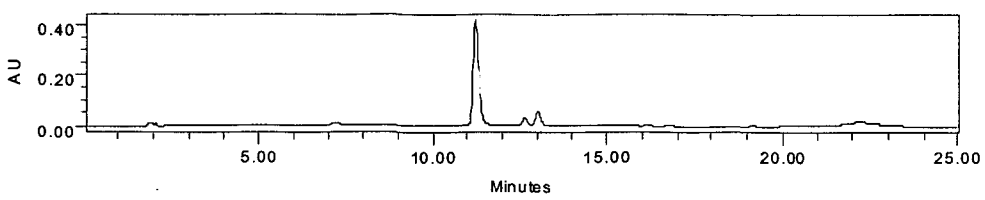

Fig. 5. LC/MS chromatogram from the leaves of *Cirsium japonicum*

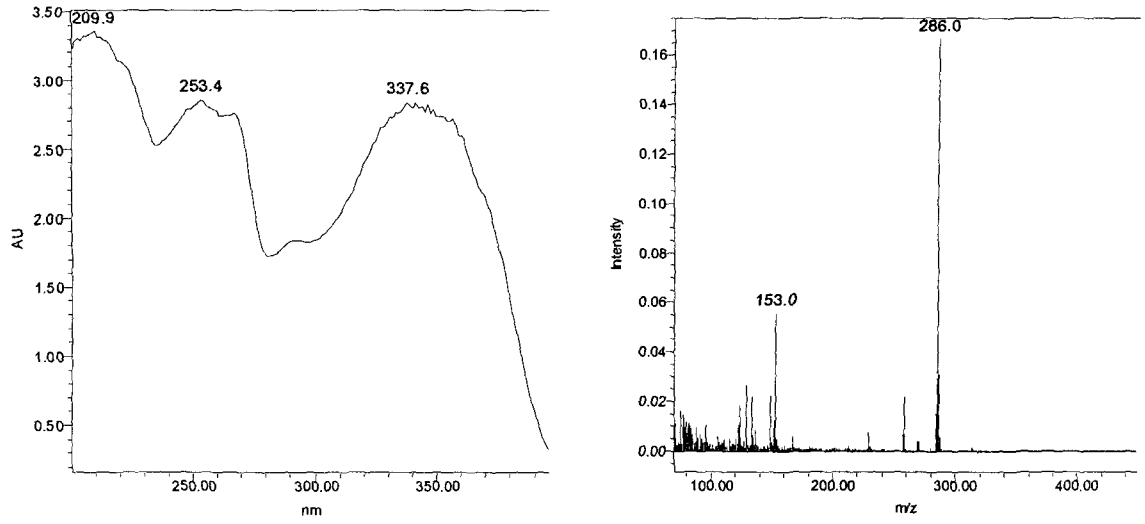

Fig. 6. UV and Mass spectra of LC/MS from the leaves of *Cirsium japonicum* at R.T=11.44 min.

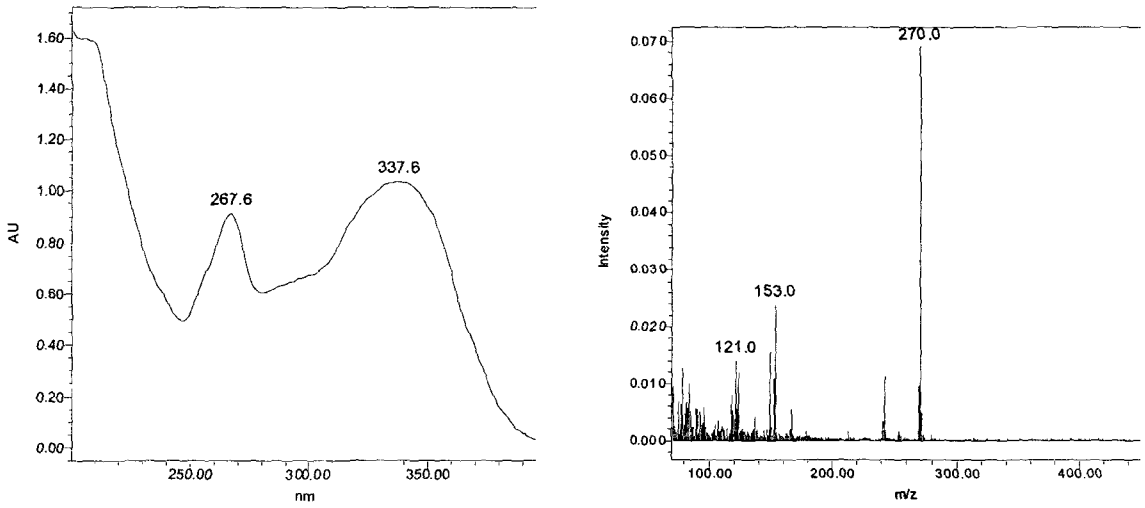

Fig. 7. UV and Mass spectra of LC/MS from the leaves of *Cirsium japonicum* at R.T=12.83 min.

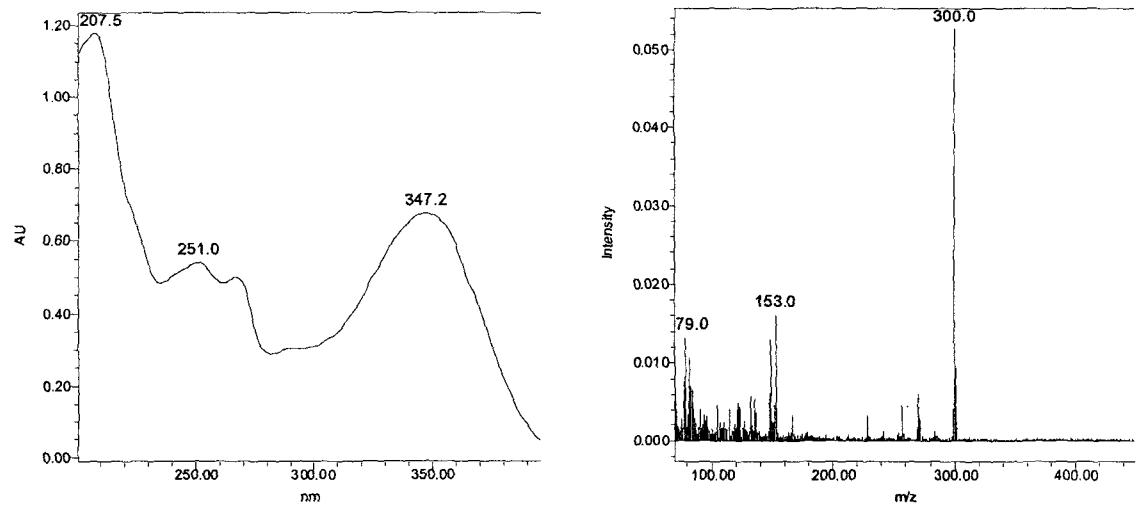

Fig. 8. UV and Mass spectra of LC/MS from the leaves of *Cirsium japonicum* at R.T=13.23 min.

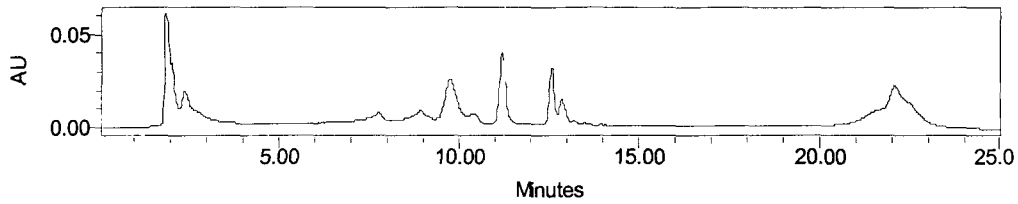

Fig. 9. LC/MS chromatogram from the flowers of *Cirsium japonicum*.

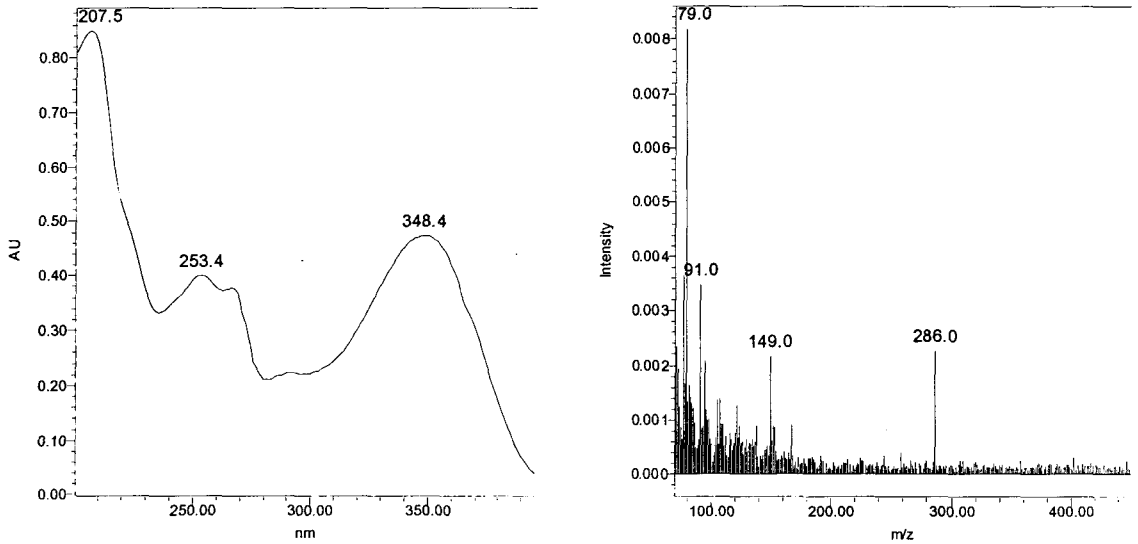

Fig. 10. UV and Mass spectra of LC/MS from the flowers of *Cirsium japonicum* at R.T=11.23 min.

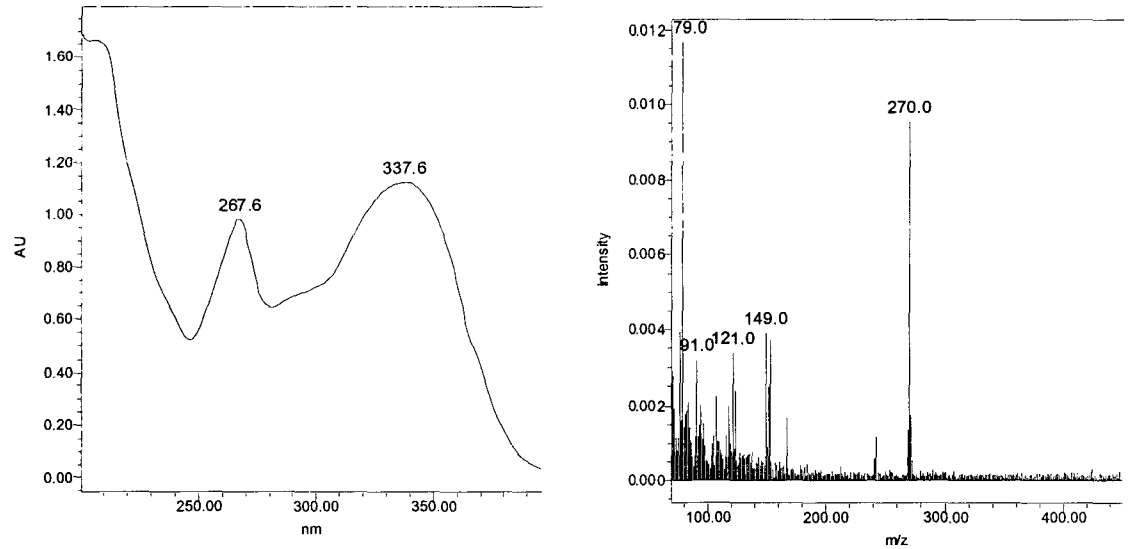

Fig. 11. UV and Mass spectra of LC/MS from the flowers of *Cirsium japonicum* at R.T=12.66 min.

ACKNOWLEDGEMENTS

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REFERENCES

1. Lee CB. 1980. *Daehan a Plant Picture Book*. Kyongmun Publications, Seoul. p 767-768.
2. Akamasu E. 1970. *Modern Oriental Drugs*. Yishiyakusha, Tokyo. p 23.
3. Jordon-Thaden JE, Svata ML. 2003. Chemistry of *Cirsium* and *Carduus*: a role in ecological risk assessment for biological control of weeds? *Biochemical Systematics and Ecology* 31: 1353-1396.
4. Whang JB. 1997. Survey for Approximate Composition

and Minerals Content of medicinal Herbs.
5. Takaishi Y, Okuyama T, Masuda A, Nakano K, Murakami N. 1991. Absolute configuration of a triolacetylene from *Cirsium japonicum*. *Phytochemistry* 30: 2321-2324.
6. Iwashina T, Kamenosono K, Ueno T. 1999. Hispidulin and nepetin-4′-glucosides from *Cirsium oligophyllum*. *Phytochemistry* 51: 1109-1111.
7. Morita N, Shimizu M. 1973. Flavonoids in *Cirsium*. *Yakugaku Zasshi* 83: 615-617.
8. Lin CN, Arisawa M, Shimizu M, Morita N. 1978. The constituents of *Cirsium japonicum* DC var. takaoense Kitamura: Isolation of two flavonoids, cirsitakaoside and cirsitakaogenin. *Chem Pharm Bull* 26: 2036-2039.
9. Wallace JW, Bohm BA. 1971. Cirsimaritin-4′-*O*-rutinoside, a new flavone glycoside from *Cirsium brevistylum*. *Phytochemistry* 10: 452-454.
10. Lin CN, Arisawa M, Shimizu M, Morita N. 1978. The constituents of *Cirsium japonicum* D.C. var. *takaoense* Kitamura: Isolation of two flavonoids, cirsitakaoside and cirsitakaogenin. *Chem Pharm Bull* 26: 2036-2039.
11. Park JC, Lee JH, Choi JS. 1995. A flavone diglycoside from *Cirsium japonicum* var. *ussuriense*. *Phytochemistry* 39: 261-262.
12. Wallace JW, Bohm BA. 1971. Cirsimartin-4′-*O*-rutinoside, a new flavone glycoside from *Cirsium brevistylum*. *Phytochemistry* 10: 452-454.
13. Markham KR. 1983. Revised structures for the flavones cirsitakaoside and cirsitakaogein. *Phytochemistry* 22: 316-317.
14. Youn HY, Chang IM. 1978. Separation and identification of cirsimartin from *Cirsium pendulum* Fisch. *Kor J Pharmacol* 9: 145-147.
15. Markham KR. 1982. Techniques of flavonoid identification. Academic press, New York. p 39.
16. Wu TS, Kuoh CS, Jen SI. 1981. Constituents of Formosan folk medicine. *Taiwan Yaoxue Zazhi* 32: 88-90.
17. Rasulov FA, Serkerov SV, Ismailov NM, Novruzov EN. 1989. Chemical investigation of *Cirsium echinus*. *Biologicheskikh Nauk* 6: 14-16.
18. Kim JS, Kwon CS, Son KH. 2000. Inhibition of a glucosidase and mylase by luteolin, a flavonoid. *Biosci Biotechnol Biochem* 64: 2458-2461.
19. Elliger CA, Chan BC, Waiss AC. 1980. Flavonoids as larval growth inhibitors. Structural factors governing toxicity. *Naturwissenschaften* 67: 358-360.

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