



Determination of Silybin B in the Different Parts of *Silybum marianum* using HPLC-UV

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Abstract – Silymarin is the standardized extract from *Silybum marianum* which consists mainly of flavonoids and polyphenols. It is highly regarded for its hepatoprotective ability. Silybin B is a flavonolignan and one of the active components of silymarin. The content of silybin B in various parts of *S. marianum* was analyzed by HPLC-UV. Results show that the extract of seeds contain the highest amount of silybin B (7.434 mg/g DW). The petioles of *S. marianum* showed a low content of silybin B. This study revealed that seeds of *S. marianum* contain high amount of silybin B and could be a good source of the compound.

Keywords – HPLC-UV, *Silybum marianum*, silybin B, silymarin

Introduction

Milk thistle [*Silybum marianum* L. (Asteraceae)] is an annual to biennial plant that natively grows in Africa, Europe, and Asia. The plant possesses an erect ridged stem that reaches approximately 20 - 150 cm in height and has deeply lobed leaves with white patches along the veins. Each stem has solitary composite flower heads, about 2 inches in diameter with purple disc florets. It grows in warm, dry soil, and blooms in July-August.^{1,2} *S. marianum* is widely regarded as one of the most popular herbs worldwide and its usage could be traced back since ancient times to treat liver and gallbladder-related diseases.³ Recently, it has been used as a demulcent, tonic, and an antidepressant.⁴

Milk thistle is a commercially important plant in which it is listed as one of the top-selling herbal supplements in the United States.⁵ It is widely regarded for its antioxidant and hepatoprotective benefits. The active component of this plant is silymarin- a standardized extract obtained from *S. marianum*.^{6,7} Further phytochemical studies on silymarin have suggested that approximately 70-80% are flavonolignans such as silybin, isosilybin, silydianin, and

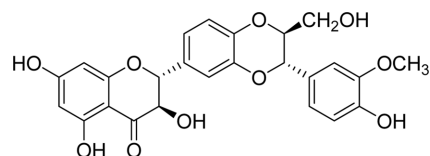


Fig. 1. Structure of silybin B.

silychristin, in which silybin is considered as its major bioactive component.⁸

Silybins have been reported to have antioxidant properties by scavenging free radicals and inhibiting lipid peroxidation.⁹ Moreover, they showed protective effects against genomic injury, and are able to increase protein synthesis of hepatocytes, decrease the activity of tumor promoters, and slow calcium metabolism. They are also involved in stabilization of mast cells and chelation of iron.^{10,11} With its growing popularity and increasing commercial demand, content analysis to identify potential sources of silybin is deemed important.

In the present study, extracts from the leaves, petioles, flowers, stems, seeds, and germinated seeds of *S. marianum* were subjected for a content determination of silybin B using HPLC-UV (Fig. 1). The results of this study serve as basis for the quantitative determination of silybin B in *S. marianum* for future reference.

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Experimental

Plant materials – *S. marianum* were supplied by Imsil Herbal Medicine Association, Imsil 55955, Korea. A voucher specimen (No. LEE 2017-02) was kept at the Herbarium of Department of Integrated Plant Science, Chung-Ang University, Korea.

Instrumentation and chemicals – Chromatographic analysis was performed using HPLC system equipped with a pump (Waters 1525 Binary HPLC Pump, Miami, USA), an auto-sampler, and UV-Vis detector (Waters 2489 UV/VIS detector (Miami, USA). Silybin B was obtained from Sigma Aldrich Co. (St. Louis, MO, USA). Ethanol (EtOH) and HPLC grade solvents (water, MeOH and acetonitrile) were purchased from J. T. Baker Chemicals (Parkway Center Valley, PA, USA).

Sample preparation – The different parts (each 15 g) of *S. marianum* were separated, dried, and finely powdered. They were extracted with ethanol (300 mL) for 3 h (3×) under reflux (65 - 75 °C) and concentrated *in vacuo*. Each extract (20 mg) was dissolved in MeOH. The solution was filtered using Whatman 0.45 µm syringe filter (Piscataway, NJ, USA) prior to HPLC analysis.

HPLC conditions – Each sample was dissolved in HPLC methanol (20 mg/mL). The samples were analyzed using an INNO C₁₈ (4.6 × 250 mm, 5 µm) column. All chromatographic analysis was recorded at 287 nm. The solvent system used a gradient elution of two solvents: 0.5% acetic acid in water (A) and acetonitrile (B). The elution started with 70% (A) (0 - 10 min); 70 - 20% (A) (10 - 25 min); 20 - 0% (A) (25 - 30 min) and extended until 35 min; 0 - 45% (A) (35 - 45 min) and maintained until 50 min. The flow rate was 1 mL/min and the injection volume was 10 µL. The column temperature was maintained at 30 °C.

Limits of detection (LOD) and limits of quantification (LOQ) – For the efficiency of the analytical method for silybin B, LOD and LOQ were done. The LOD and LOQ were calculated based on linear regression equation, and the values were determined separately at a signal to noise ratios of 3 and 10, respectively.

Calibration curve – For the preparation of the calibration curve, 1 mg/mL of silybin B was dissolved in MeOH and was subjected to serial dilution. The peak area of the

individual compound was compared with those of a standard curve prepared from the corresponding standard. The peak area (Y), concentration (X, mg/mL) and mean values (n = 5) of the calibration functions of the compounds were calculated.

Result and Discussion

S. marianum has been regarded as an effective antioxidant and hepatoprotectant. There have been a lot of references on the effectiveness of silymarin in a worldwide usage. In particular, seeds of *S. marianum* have been used as hepatoprotectant for more than 2000 years.⁴

In this study, content analysis was performed to determine the amount of silybin B from the various parts of *S. marianum* by HPLC-UV analysis. The linear calibration equation of silybin B was $Y = 2,000,000X - 163,298$. The correlation coefficient (r^2) was 0.9993 as shown in Table 1. The retention time of silybin B was 16.73 min. The LOD and LOQ were 1.16 and 3.51 mg/mL, respectively (Table 1). Results show that the seeds and germinated seedlings of *S. marianum* contained the highest contents of silybin B at 7.434 and 0.852 mg/g DW, respectively (Table 2). The petioles of *S. marianum* showed low content of silybin B. The chromatographic results for all samples were shown in Fig. 2. For better separation of silybin B in the chromatogram of seeds in Fig. 2F., the elution gradient was modified using 0.5% acetic acid in water and acetonitrile (70:30 to 60:40 in 30 min) as shown in Fig. 3B.

Silybins constitutes of two diastereoisomers, silybin A and silybin B. They comprise about 50 - 70% of the silymarin extract. In pharmaceutical products, they compose about 20 - 40%.¹² Recent pharmacological studies have

Table 2. Silybin B content of *S. marianum*

Sample	Content (mg/g DW)
Leaves	0.113 ± 0.004
Petioles	0.043 ± 0.002
Flowers	0.083 ± 0.001
Stems	0.064 ± 0.001
Seeds	7.434 ± 0.041
Germinated Seeds	0.852 ± 0.002

Table 1. Calibration curve of silybin B

Compound	t _R	Calibration equation ^a	r ² ^b	LOD (mg/mL)	LOQ (mg/mL)
Silybin B	16.73	Y = 2,000,000X + 163,298	0.9993	1.16	3.51

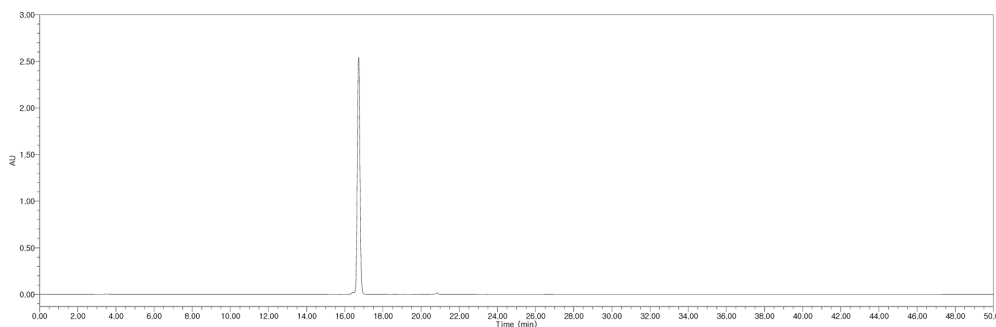
^a Y = peak area, X = concentration of standards (mg/mL).

^b r² = correlation coefficient for five data points in the calibration (n = 5)

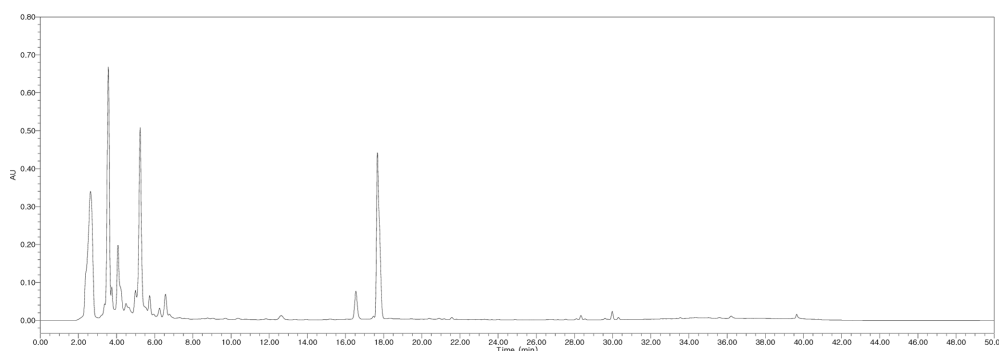
reported that silybins can act as an antioxidant agent in which they inhibit radical formation, binds to radical species, interferes with lipid peroxidation, and increases the intracellular content of scavengers.¹³ Trappoliere *et al.* (2009) has also suggested that it can promote anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and anti-fibrogenic actions by reducing the pro-fibrogenic potential of hepatic stellate

cells.¹⁴ Silybin is also reported to have antiproliferative activity against cancer cell lines and antibacterial effect on gram-positive bacteria by inhibiting RNA and protein synthesis.¹⁵⁻¹⁸

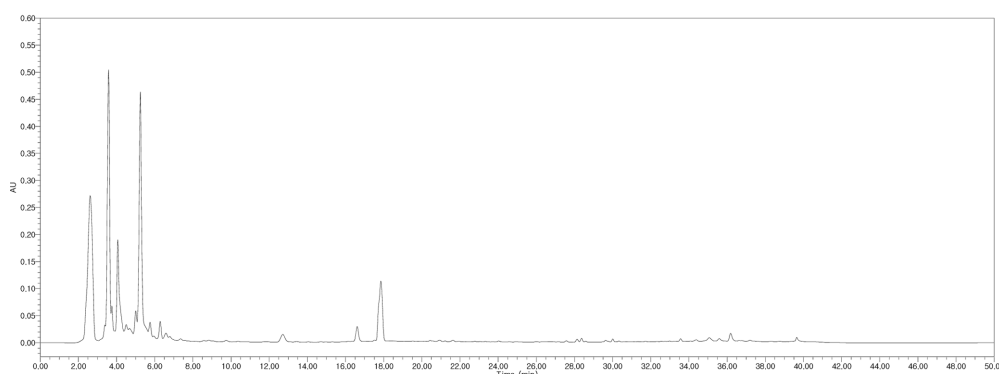
Several studies have analyzed the content of flavonolignans present in *S. marianum* and silymarin. In a study conducted by AbouZid *et al.* (2016), they have suggested that seeds from *S. marianum* that are mature;



(A)

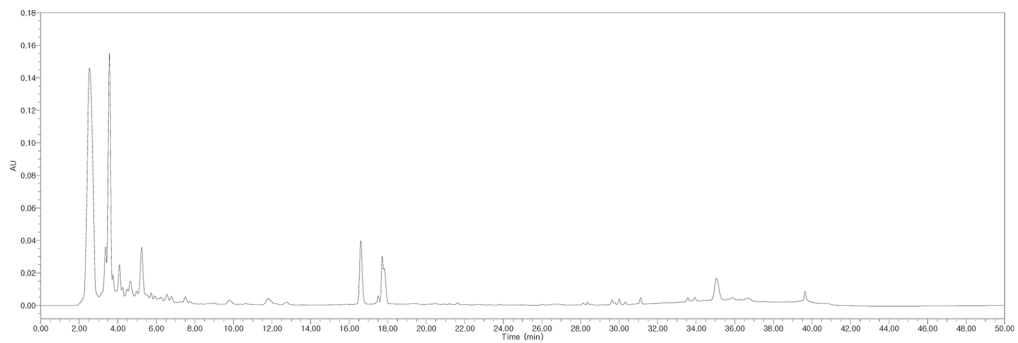


(B)

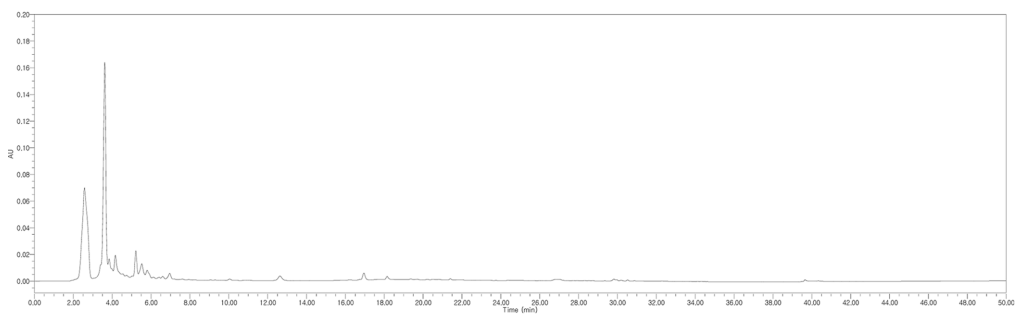


(C)

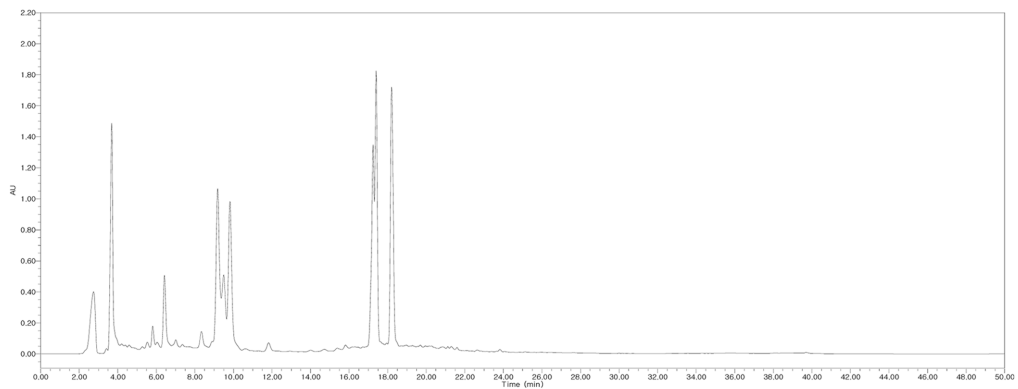
Fig. 2. HPLC chromatograms of silybin B (A) and the EtOH extracts of leaves (B), petioles (C), flowers (D), stems (E), seeds (F), and germinated seeds (G) of *S. marianum*.



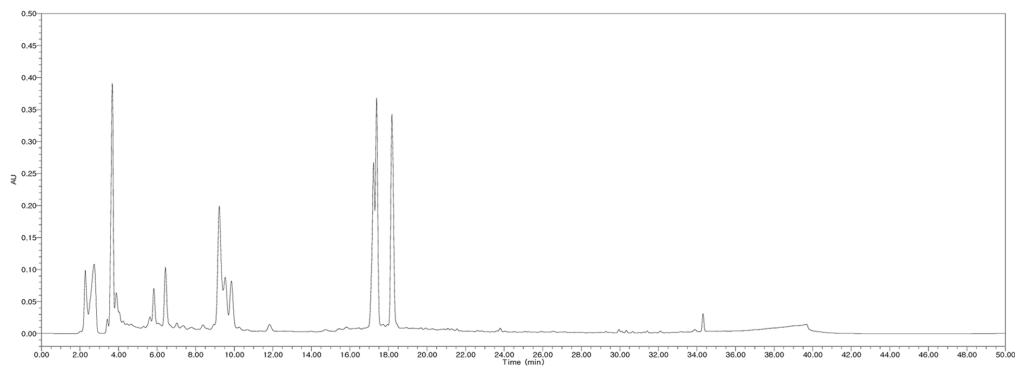
(D)



(E)

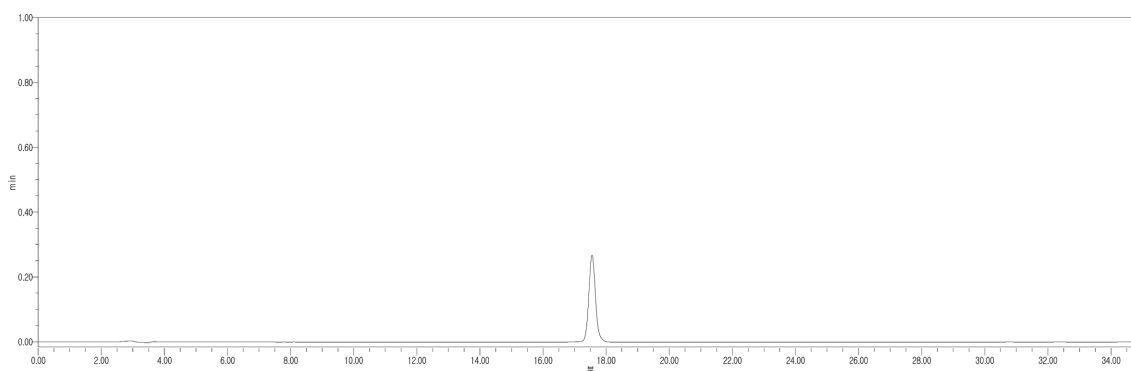


(F)

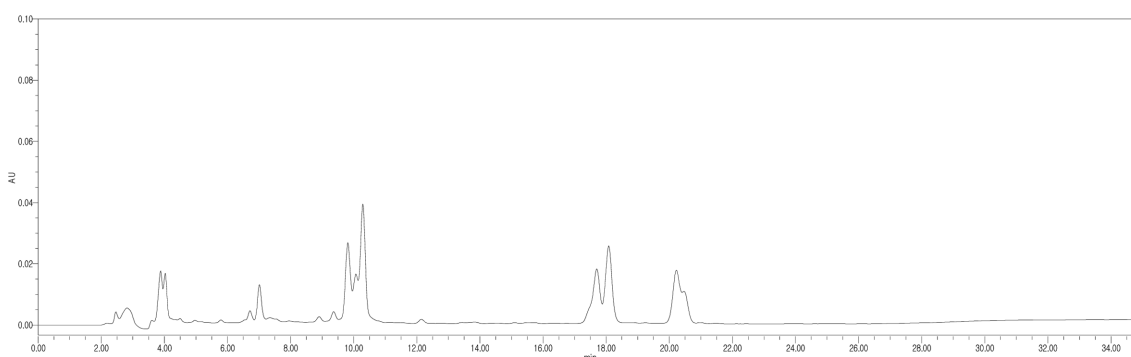


(G)

Fig. 2. continued



(A)



(B)

Fig. 3. HPLC chromatograms of silybin B (A) and the EtOH extracts of seeds (B) of *S. marianum*.

and black fruits contain more silymarin.² This result could be important in the selection of seeds for silymarin production. As reported by Cappelletti and Caniato (1984), flavanolignans are localized in the outer portion of the pericarp of the seed.¹⁹ This could support the results of this study in which seeds showed the highest content among the parts examined. However, there are only few studies that surveyed the content of silymarin and its components from the different parts of *S. marianum*. This study could be used as a guide in choosing a preferable source for the isolation of silymarin and its constituents.

In conclusion, our study revealed that seeds of *S. marianum* contain higher amounts of silybin B than germinated seeds and other parts of *S. marianum*. The HPLC-UV conditions used for the analysis of silybin B could be used for future reference. Furthermore, our results demonstrated that *S. marianum* seed is a good source of silybin B.

Acknowledgements

This work was supported by the grant from Imsil Herbal Medicine Association (2017), Imsil, Korea.

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Received September 8, 2017

Revised November 10, 2017

Accepted December 22, 2017