

## "Research Note"

### ANALYSIS OF SILYMARIN COMPONENTS IN THE SEED EXTRACTS OF SOME MILK THISTLE ECOTYPES FROM IRAN BY HPLC\*

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**Abstract** – Silymarin is a mixture of flavonolignans from the seeds of *Silybum marianum* (L.) Gaertner, containing silybinin, isosilybinin, silydianin, silychristin and the dihydroflavonol of taxifolin. Flavonolignan components are largely responsible for the medical benefits attributed to silymarin. The aim of this research was to study the variations in composition and content of flavonolignans of silymarin samples from seeds of some native milk thistle ecotypes of Iran, along with a foreign cultivar. Silymarin was extracted by a two-step (defatting and extraction) process using n-hexane, ethyl acetate and methanol in a Soxhlet extraction from the seeds. The content and composition of the main components in different silymarin samples were analyzed by the HPLC method against external standard of silybinin. The HPLC method allowed a good separation between flavonoid components, especially diastereomers of silybinin A and B. The qualitative and quantitative data obtained by HPLC clearly showed that silymarin samples were the same with respect to their flavonolignan composition and they were mainly different based on the content of their components. Silymarin samples from native ecotypes had lower quantities of silybinin as compared to that of silymarin from cultivated ones, but they had higher amounts of other compounds such as silychristin, silydianin and isosilybinin.

**Keywords** – *Silybum marianum*, silymarin, flavonolignans, high performance liquid chromatography

## 1. INTRODUCTION

Milk thistle (*Silybum marianum* (L.) Gaertner, Asteraceae) is an herbaceous annual or biennial plant native to the Mediterranean area, but it has become naturalized by cultivation in the hot, dry areas of southern Europe, Africa, China, Australia, South America, and in many parts of North America as well as west of Asia [1, 2]. It grows as wild populations in open fields of many northern and western parts of Iran.

Generally, the seed extract of the *Silybum marianum* is expressed as total silymarin [3]. Silymarin consists of some flavonolignans (Fig. 1), including silybinin (SBN<sub>A</sub> and SBN<sub>B</sub>), isosilybinin (ISBN<sub>A</sub> and ISBN<sub>B</sub>), silydianin (SDN), silychristin (SCN) and taxifolin (TXF) [4].

Silymarin corresponds to the sum of SBN, ISBN, SDN and SCN concentrations [4]. Dried extracts of milk thistle seeds contain approximately 60% silymarin. Silymarin consists of four flavonolignans of silybinin (~ 50 to 60%), isosilybinin (~ 5%), silychristin (~ 20%) and silydianin (~ 10%) [5].

Up to now, the main components of silymarin have been extracted, separated and analyzed by TLC, HPLC, UV-Vis spectrophotometry, electrochemical or MS detection and capillary electrophoresis [3, 6-8].

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In this paper, the main flavonolignan components of silymarin samples from four milk thistle ecotypes from Iran have been studied by the HPLC method and the results compared quantitatively and qualitatively with those of silymarin from a foreign cultivar.

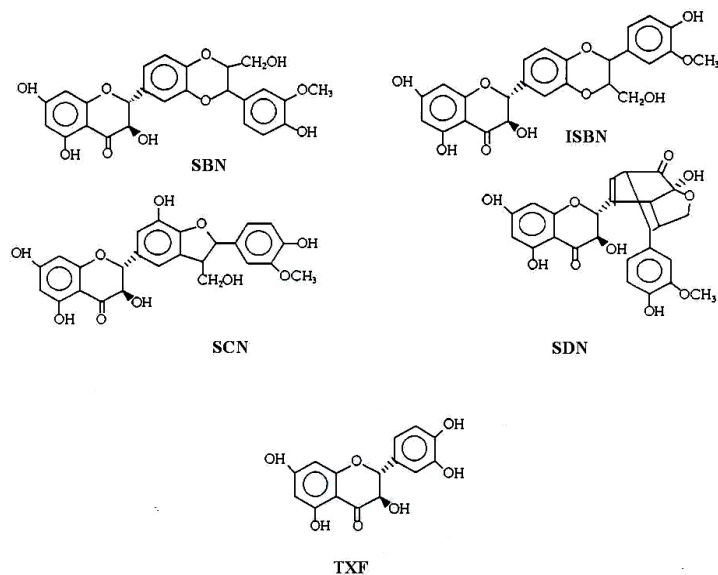


Fig. 1. Structure of main silymarin components (3)

## 2. MATERIALS AND METHODS

### a) Plant materials

Seeds of cultivated *Silybum marianum* (a Chinese product, Hungarian accession, Plantarum Medicinarum Hortus Botanicum Institute) were collected from the field (Karadj City, Iran) after cultivation for one year (August 2005). Seeds of four native ecotypes of milk thistle plants were harvested from different Iranian geographical locations in the north, west and central regions during the flowering season (from June to August 2005) (Table 1).

Table 1. Characteristics of the analyzed native ecotypes of milk thistle from Iran

Ecotype	Locality
No.1	Prov. Isfahan, foothills near Isfahan, 1400 m above sea level, extremely dry climate, average annual rainfall 116mm, loamy clay soil
No.2	Prov. Kermanshah, foothills near Kermanshah, 840 m above sea level, mountainous moderate climate, average annual rainfall 300- 500 mm, clay loam soil
No.3 (population 1)	Prov. Guilan, Rasht, Chapar-Pard- Zaman, Ziba Kenar, km 16, - 7m above sea level, moderate Caspian climate (humid subtropical), average annual rainfall 1200 mm, sandy clay soil
No.3 (population 2)	Prov. Guilan, Rasht, Khoshk Bidjar, Ziba Kenar, km 5, -22 m above sea level, moderate Caspian climate (humid subtropical), average annual rainfall 1200 mm, sandy clay soil
No.4 (population 1)	Prov. Mazandaran, Marzan Abad, km 1, 700-800 m above sea level, Mediterranean climate, average annual rainfall 650 mm, light and moisture forest soil
No.4 (population 2)	Prov. Mazandaran, Marzan Abad, km 4, 700-800 m above sea level, Mediterranean climate, average annual rainfall 650 mm, light and moisture forest soil

### **b) Extraction procedure from the seeds**

Silymarin extraction was a two-step process in which powdered seeds were first defatted. In order to defat the seeds, about 10g of finely powdered seeds of different samples were accurately weighed ( $\pm 0.1$  mg) and were first extracted with n-hexane for 4 hours and then with ethyl acetate for 8 hours in a Soxhlet extractor. Ethyl acetate solution was evaporated under reduced pressure on a rotary evaporator instrument at a temperature not exceeding 50°C and extracted silymarins were obtained as soft yellow powder. Methanolic solutions (1.2 mg/mL) of silymarin samples were used for HPLC analysis after filtration [9].

### **c) Reference solutions**

An accurately weighed quantity of standard silybinin (Sigma, USA) was dissolved in methanol and diluted with methanol to obtain solutions with known concentrations (0.004, 0.02 and 0.2 mg/mL). The calibration curve was plotted based on the areas of the sum of the silybinin A and silybinin B peaks versus the concentration of silybinin solution, and a regression line was obtained for calibration (correlation coefficient=1). The percentage of each relevant component of silymarin samples were calculated as silybinin and external standard method was used [9].

### **d) Silymarin standard solution**

Methanolic solution of standard silymarin (Sigma, USA) (0.7 mg/mL) was used to understand the chromatographic behavior of the flavonolignan components of silymarin in analytical conditions.

### **e) HPLC assay**

The analysis of silymarin samples was carried out using a Knauer K2600A liquid chromatograph (Germany), equipped with a Nucleosil C18 (150 × 4.6 mm I.D, 5  $\mu$ m) column. A mixture of methanol-water (50:50, v/v) served as the mobile phase. The elution has been made in an isocratic mode at a flow-rate of 1mL/min and the detection made at 288 nm. One analysis requires 20 min.

## **3. RESULTS**

Figure 2 shows the chromatographic profile of methanolic solutions of two silymarin samples as compared to that of standard silymarin. As can be seen in chromatograms, six principal peaks were observed, with each peak identified as one of the flavonolignan constituents of the silymarin samples.

The quantitative data obtained from the samples analysis by HPLC have been reported in Table 2. Quantitative analyses showed that the amount of total silymarin varied from 23.98 to 45.46% for different ecotypes. The highest amount of silybinin for silymarin samples from two populations of the ecotype number of 4 were calculated 24.86 and 19.74%, respectively. An interesting finding was that, the highest amounts of other components such as silychristin, silydianin, and isosilybinin were obtained 20.17, 19.59, and 10.44 %, respectively, for silymarin samples from native ecotype numbers of 2, 3 (population 1), and 2, and they were better than the foreign variety.

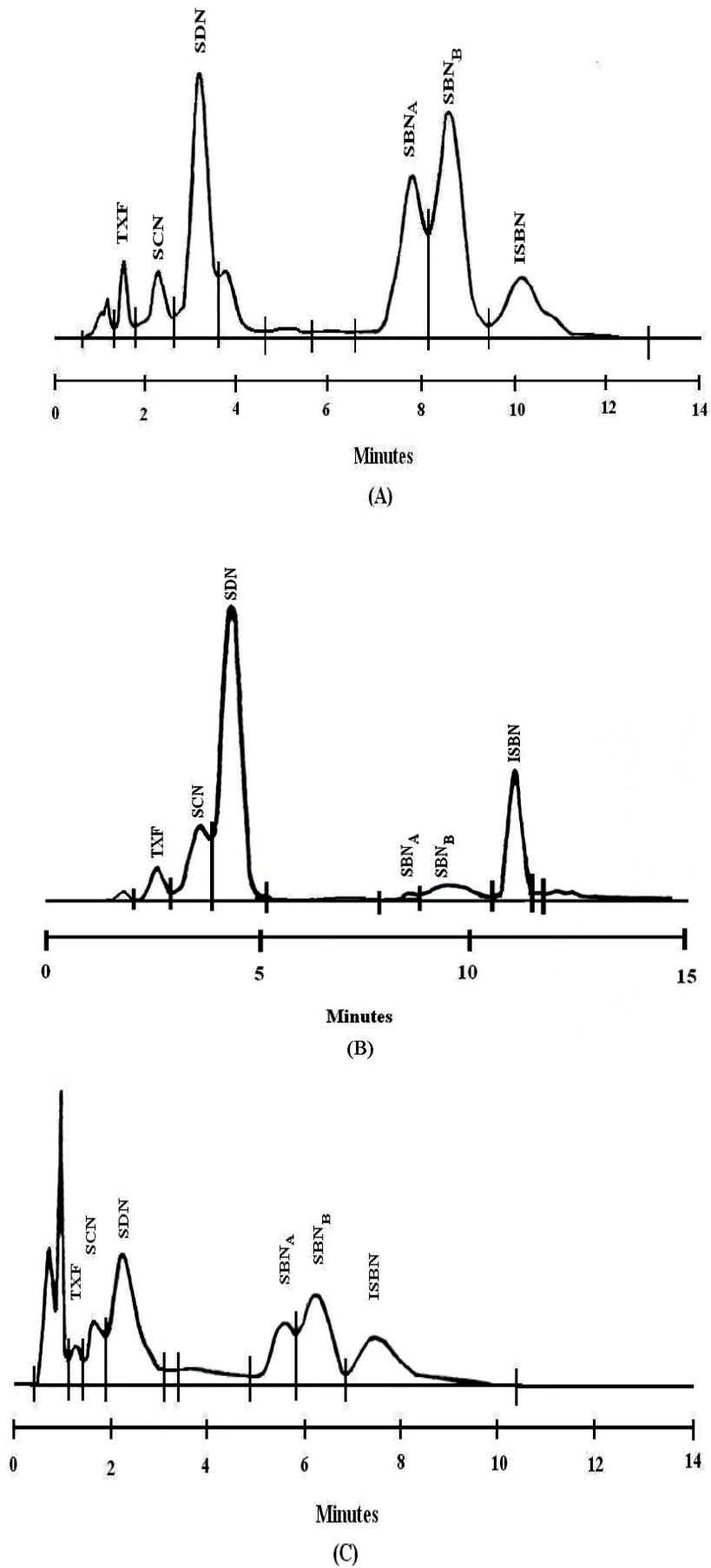


Fig. 2. Chromatograms of standard silymarin (A) and silymarin samples from ecotypes of 3 (B) and 4 (C)

Table 2. Results of HPLC analysis of silymarin samples

Silymarin samples	Content (g/100g DW)					
	Taxifolin	Silychristin	Silydianin	Silybinin <sup>a</sup>	Isosilybinin <sup>b</sup>	Total Silymarin
SM-S	3.14 ± 0.35	5.31 ± 1.65	16.76 ± 0.31	40.94 ± 1.5	3.67 ± 0.60	69.82
SM-C	0.59 ± 0.19	11.47 ± 1.83	2.78 ± 1.09	38.68 ± 2.61	4.26 ± 1.87	57.78
SM-E1	0.92 ± 0.2	7.43 ± 1.31	2.20 ± 0.75	8.45 ± 2.51	4.98 ± 1.18	23.98
SM-E2	1.88 ± 0.61	20.17 ± 2.25	3.65 ± 1.08	7.24 ± 2.76	10.44 ± 1.9	43.38
SM-E3 (population 1)	2.10 ± 0.81	9.43 ± 2.88	19.59 ± 1.60	4.13 ± 1.95	5.41 ± 2.63	42.66
SM-E3 (population 2)	3.19 ± 0.54	15.2 ± 2.45	10.02 ± 1.65	3.86 ± 1.06	3.95 ± 2.90	36.33
SM-E4 (population 1)	1.54 ± 1.16	4.56 ± 1.59	10.22 ± 1.98	24.86 ± 1.89	2.10 ± 1.91	43.28
SM-E4 (population 2)	3.22 ± 1.29	5.25 ± 2.95	11.18 ± 2.12	19.74 ± 2.86	6.07 ± 2.05	45.46

<sup>a</sup>Sum of silybinin diastereomers

<sup>b</sup>Sum of isosilybinin diastereomers

Abbreviations: SM-S, standard silymarin; SM-C, silymarin from cultivated plants; SM-E1-4; silymarin samples from native ecotype numbers 1 to 4. Values are expressed as means ± SD.

#### 4. DISCUSSION

The results of chemical analyses showed that all silymarin samples were the same in view of their components, but were different based on their flavonolignans content. Silymarin samples from wild ecotypes had lower quantities of silybinin as compared to that of cultivated ones, but they had higher amounts of other components such as silychristin, silydianin and isosilybinin. It seems that they are good samples for further studies.

Although, the main active compound is believed to be silybinin, there are some reports that show other constituents of silymarin may be responsible for its pharmacological activities [10]. Since milk thistle is very adaptable to many different habitats, this suggests that some ecotypes with more favorable amounts of flavonolignans are suitable for domestication and intended purposes. Despite the economical and pharmaceutical values of milk thistle, efforts on domestication and breeding of this plant have been low [11].

There is evidence of genetic differences between ecotypes of milk thistle with regard to the content of silymarin and its constituents. Different environmental conditions (rainfall, temperature, soil texture, altitude from the sea and etc.) also effect silymarin products [12].

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