



Research Paper

A comparison of some phytochemical characteristics of five populations of *Silybum marianum* seeds from different parts of Iran.

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ABSTRACT

Silybum marianum seeds has traditional uses as reconstitute, stimulant, diuretic and expectorant. The aim of this study was to determine the important phytochemical characteristics of *Silybum marianum* seeds collected from different parts of Iran. Important characteristics of this plant include essential oil content, total phenol and flavonoid content and various radical scavenging activities. Based on obtained results, *S. marianum* grown in Naghadeh showed the highest oil content (20.0%) percentage, followed by Urmia (19.36 %), Saghez (19.17%), Khoy (18.85%), Marivan (18.56%) and Jolfa (18.34%), respectively. Total phenolic content of *S. marianum* was of the order: Marivan (29.58 mg GAE g⁻¹ DW), Naghadeh (28.75 mg GAE g⁻¹ DW), Khoy (28.64 mg GAE g⁻¹ DW), Saghez (28.33 mg GAE g⁻¹ DW), Jolfa (27.87 mg GAE g⁻¹ DW), and Urmia (27.03 mg GAE g⁻¹ DW). The highest (3.11 mg QE g⁻¹ DW) and the lowest (2.31 mg QE g⁻¹ DW) total flavonoid content of *S. marianum* seeds were observed in Marivan and Urmia, respectively. The plants grown in Naghadeh area showed the highest radical scavenging activities, including DPPH (58.31%) and nitric oxide (29.76%), whereas the highest super oxide radical scavenging activity was obtained in Saghez (92.27%). According to results, no noticeable differences were observed in total phenol and flavonoid contents in various studied sites. Whereas, oil content, DPPH, super oxide and nitric oxide radical scavenging activities varied in the different studied sites. Therefore, different climate changes significantly affect the phytochemical composition of *S. marianum* and as such, should be the focus of medical studies.

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INTRODUCTION

The botanical name for milk thistle is *Silybum marianum* (L.) Gaertn, a member of the plant family Asteraceae which is an annual or biennial plant (Tamayo et al., 2007; Nazir et al., 2018). This fairly typical thistle has red to purple flowers and shiny pale green leaves with white veins (Bosisio et al., 1992). Originally a native of Southern Europe through to Asia, and now distributed throughout the world. Milk thistle is available in the United States as a dietary supplement (Gordon et al., 2006). The fruit and seeds of the milk thistle plant have been used for more than 2000 years as treatment for liver and biliary disorders (Davis-Searles

et al., 2005; Momenkiaei and Raofie, 2018). The seeds are the medicinal part of the plant, which is indigenous to Europe but also can be found in the United States and South America. The active constituent of milk thistle is silymarin, a mixture of flavonolignans comprising 4 isomers: silibinin, isosilibinin, silichristin, and silidianin. Most supplements are standardized according to their silibinin (often called silybin) content (Wilasrusmee et al., 2002; Le et al., 2018). In turn, silibinin and isosilibinin are both mixtures of 2 diastereomers, silibinin A and B and isosilibinins A and B, respectively. Because of the milk thistle's lipophilic nature,



Figure 1: Different regions of study area.

it is usually administered in capsule or tablet form rather than as an herbal tea (Bosisio et al., 1992). Special formulations of silibinin have been developed to enhance the bioavailability of the herbal product; these forms are sold as a dietary supplement under the names Legalon, Silipide, and Siliphos (Gordon et al., 2006). As a supplement, milk thistle is regulated as a food and has not been approved by the US Food and Drug Administration as treatment for cancer or for any other medical condition (Torres et al., 2013; Zhang et al., 2020). The available evidence suggests that *S. marianum* extracts have an important hepatoprotective as well as anticancer, antidiabetic, and cardio protective effect (Shaker et al., 2010; Qin et al., 2017). However, high-quality clinical studies are limited, and very few have rigorously evaluated the purported anticancer and other pharmacological activities of this interesting herb (Tamayo et al., 2007; Kalthoff and Strassburg, 2019). Despite negative reports, *S. marianum* seems to have some effect in chronic liver diseases, particularly alcohol related liver disease, toxin-induced liver disease, and viral liver disease (Wilasrusmee et al., 2002; AbouZid et al., 2017). The beneficial effect of *S. marianum* in drug interactions is also noteworthy. The whole herb and its constituents may prevent nephrotoxicity associated with the use of acetaminophen, cisplatin, vincristine, and cyclosporine, as well as radiotherapy (Torres et al., 2013; Hosseini et al., 2018). A prospective clinical study on the protective effect of Legalon *S. marianum* extract in workers exposed to organic solvents documented a significant improvement in liver function test for those taking Legalon as compared with those receiving no treatment (Gurley et al., 2006; Drouet et al., 2019).

MATERIALS AND METHODS

The seeds used in this study were obtained from the local market in different regions of Iran (Figure 1). The samples

were cleaned manually to get rid of all foreign matter, broken and immature seeds. The initial moisture content of seeds was determined by oven drying at $105 \pm 1^\circ\text{C}$ for 24 h (Selvi et al., 2006). The seeds at the different moisture levels were prepared by adding calculated quantity of water, mixed thoroughly and then sealed in separate polyethylene bags. The seeds were kept at 5°C in a refrigerator for a week to allow the moisture to distribute uniformly throughout the sample. Before each test, the required quantities of the samples were taken out of the refrigerator and allowed to warm up to room temperature. Laboratory experiments were performed in agriculture department of Urmia University.

Oil content

The extraction with ether method was used for measuring total oil content (Fokina et al., 2018). One gram of each sample was transferred in test tubes and 10 ml ether were added again. Tubes were placed in 40°C for 12 h and the above solutions were transferred in balanced tubes. Tubes were placed in 40°C oven for 4 h for evaporation. Weight difference of tubes before and after experience was used for oil content.

Total phenolic content (TPC)

Measuring of total phenolic compounds in flowers was performed using Folin-Ciocalteu method adapted from Singleton et al. (1999). In details, 10 μl of methanolic extracts and 1600 μl of distilled water were mixed together and then 200 μl of Folin-Ciocalteu reagent (10% V/V prepared in distilled water) was added and left at 25°C for 5 min. Thereafter, 200 μl of sodium carbonate (7.5%) was added and kept for 30 min (at 25°C in dark place). The absorbance of the solution was determined at 760 nm using a spectrophotometer (DB-20/DB-20S UV/Visible Spectrophotometer, USA) for quantitative analysis of TPC,

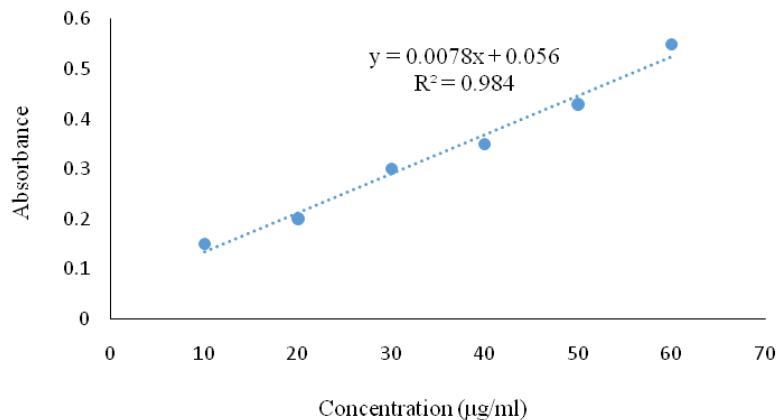


Figure 2: Calibration curve for standard gallic acid.

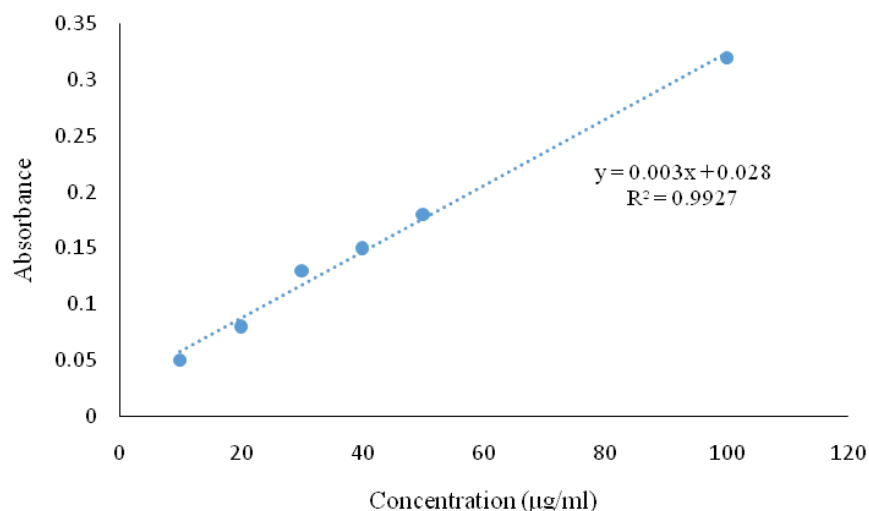


Figure 3: Calibration curve for standard Quercetin acid.

the gallic acid was used as an external standard, and TPC was expressed as mg gallic acid g⁻¹ DW (Figure 2).

Total flavonoid content (TFC)

The analysis of total flavonoid content in flower extracts was carried out using aluminum chloride colorimetric method. Briefly, 30 µl of the extract was mixed with 150 µl of sodium nitrate (5% W/V) and was allowed to stand for 5 min, followed by the addition of 3 ml of Aluminum chloride hexahydrate (10% W/V) and incubated for 5 min. Then, 1 ml of NaOH (1.0 M) was added and the mixture was diluted to the mark with distilled water. After incubation at 25°C in the dark for 30 min, the absorbance of the solution was measured at 510 nm by spectrophotometer. For the quantification of TFC, the Quercetin (QE) was used as an external standard, and TFC was expressed as mg QE g⁻¹ DW

(Figure 3) (Chantiratikul et al., 2009).

The radical scavenging activity of the extracts was evaluated using the colorimetric method described by Brand-Williams et al (1995). Briefly, 15 µl of methanolic extract was mixed with 2.0 ml of the DPPH solution and the mixture was incubated in the dark at 20°C for 30 min. Thereafter, the absorbance of the solution was measured at 517 nm. The following equation was used to calculate the DPPH inhibition (Khalighi-Sigaroodi et al., 2012):

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} and A_{sample} are the absorbance of the control and the sample, respectively.

Also, Seeds mucilage percentage was determined by standard method after immersing in water (Shafagh et al., 2006).

Table 1: Average of some phytochemical properties of *Silybum marianum*.

Study area	Oil content (%)	Total phenol (mg GAE g ⁻¹ DW)	Total flavonoid (mg QE g ⁻¹ DW)
Naghadeh	20.0	28.75	2.59
Marivan	18.56	29.58	3.11
Jolfa	18.34	27.87	2.44
Saghez	19.17	28.33	2.51
Khoy	18.85	28.64	2.50
Urmia	19.36	27.03	2.31

Table 2. Radical scavenging activity of *Silybum marianum*

Study area	DPPH Radical scavenging (%)	Nitric oxide Radical scavenging (%)	Super oxide Radical scavenging (%)
Naghadeh	58.31	29.76	69.08
Marivan	55.71	28.95	66.79
Jolfa	57.57	28.80	69.69
Saghez	47.01	28.67	92.27
Khoy	48.97	26.18	88.61
Urmia	53.10	26.56	90.03

RESULTS AND DISCUSSION

The phytochemical properties of the studied *S. marianum* from different regions of Iran are shown in Tables 1 and 2. The results obtained in the present study showed that there was no much difference between various varieties of *Silybum marianum* in different phytochemical properties. The oil content, total phenol and flavonoid content were analysed in seeds of *S. marianum* collected from various parts of western Iran. According to our results in Table 1, the *S. marianum* grown in Naghadeh showed the highest oil content percentage (20.0%), followed by Urmia (19.36%), Saghez (19.17%), Khoy (18.85%), Marivan (18.56%) and Jolfa (18.34%), respectively. This can be attributed to the effect of different climate conditions of Iran. Increasing the area under cultivation of common oilseeds, and identifying and cultivating new sources are necessary steps to provide oil. Oil and fat are the main components of food, with a gram of about 9.2 kcal of energy producing a good taste in the body (Ram et al., 2005). Recently, with the growth of public knowledge, people's demand for oils that are useful in addition to providing energy and creating a healthier taste has increased (Carrier et al., 2003). The seeds of *S. marianum* contain betaine, trimethylglycine, and a large amount of oil that has been implicated in the anti-inflammatory and anti-hepatitis effects of the extract (Kim et al., 2009). Previous researches have reported the anti-hepatitis effect of *S. marianum* seeds oil. Also, oil from marigold seed contains high levels of certain nutrients such as phospholipid and E supply (Capasso et al., 2009; Doehmer et al., 2011). Reports indicate that this oil

contains essential and unnecessary fatty acids such as linoleic acid, oleic acid, linolenic acid, stearic acid, palmitic acid and compounds such as tocopherols and phytosterols that can be used as edible oil. So we decided to analyze the oil content of *S. marianum* seeds. According to Figure 1, phytochemical components in Iranian *S. marianum* varied from one country to another, which can be attributed to different climatic conditions.

However, different trends about total phenolic and flavonoid content among various varieties were found. The *S. marianum* grown in Marivan showed the highest amount of total phenol and flavonoid content (29.58 and 3.11 mg g⁻¹, respectively), followed by Marivan, Naghadeh, Khoy, Saghez, and Urmia (Table 1). Silymarin is a combination of flavonoids extracted from the phenolic extract of dried *S. marianum* seeds (5–9%) that have gained attentions since many years ago. Flavonoids and other phenolic compounds are widely distributed in plants, and their diverse biological activity including antioxidant, antimicrobial, and anti-inflammatory have been reported in numerous studies (Tůmová et al., 2006). Various studies have shown that silymarin is widely used to combat oxidative stress due to its antioxidant and protective properties against a variety of free radical species (Khan et al., 2009; Doehmer et al., 2011). Phenolic compounds with antioxidant and antiradical properties can play an important role in preserving food products and maintaining human health (Capasso et al., 2009). Due to the native nature of marigold in Iran and its easy and inexpensive access to food and medicinal use of this plant from time immemorial in Iran, the study of phenolic and flavonoid compounds could be a



Figure 4: Scheme of studied *Silybum marianum*.

prelude to the practical use of this plant extract as an antioxidant in industry; being food and medicinal so as to enable both a convenient and affordable source of food and to promote health and food security (Ram et al., 2005).

Comparison of our results with other studies in Turkey showed that there is 2 times higher amounts of total phenolic and flavonoid contents in Iranian *S. marianum* plants (Figure 4) than other species (Davis-Searles et al., 2005). Genetic background and growth conditions may be responsible for phenolic compounds changes in different species (Bosisio et al., 1992). Environmental factors (such as soil composition, temperature, rainfall, and ultraviolet radiation) are the most effective factors on the phenolic content (Gordon et al., 2006).

Based on our results in Table 2, the *Silybum marianum* grown in Naghadeh and Saghez showed the highest and lowest radical scavenging activity relative to others, respectively. Active oxygen radicals can attack the best cellular constituents such as fatty acids, proteins, nucleic acids and pigments (Capasso et al., 2009). Oxygen radicals are capable of destroying cell membrane lipids, proteins, and hereditary substances (Khan et al., 2009). It is well known today that oxidative degradation caused by the activity of these molecules causes and promotes a number

of chronic diseases such as cardiovascular disease, cancer disease (Carrier et al., 2003). Antioxidant compounds are needed to counteract the toxic effect of oxygen free radicals. Plant cells usually use enzymatic antioxidant systems such as super oxidase dismutase, catalase, antioxidant metabolites, phenol, etc. to solve this problem (Carrier et al., 2003; Kim et al., 2009). Oxidative stress is caused by the overproduction of free radicals and reactive oxygen species and the weakening of the antioxidant system due to the low production of endogenous antioxidants (Ram et al., 2005). Variation of phytochemical properties in the studied sites is shown in Figure 5. According to our results, no noticeable differences were observed in total phenol and flavonoid contents in various studied sites. Whereas, oil content, DPPH, super oxide and nitric oxide radical scavenging activity were variable in the different studied sites.

The principal component analysis was performed to understand the relationships between different variables. In all samples, total phenol and flavonoids had close relationship and affects each other, so classified in one group, as well as nitric oxide and DPPH radical scavenging showed close relationship. Whereas, superoxide radical scavenging and oil content did not show significant relationship with other properties (Figure 6).

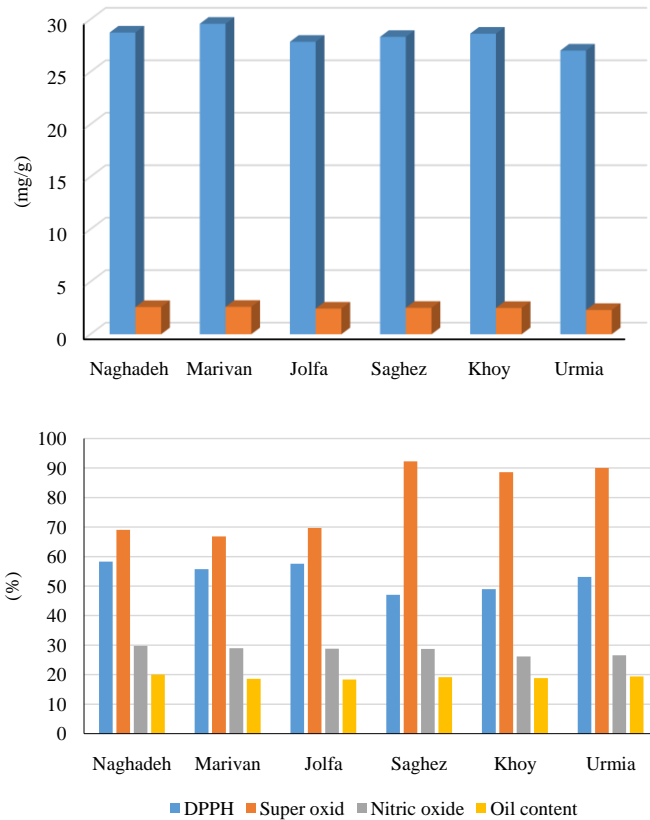


Figure 5: Comparison of different phytochemical properties in the various studied sites.

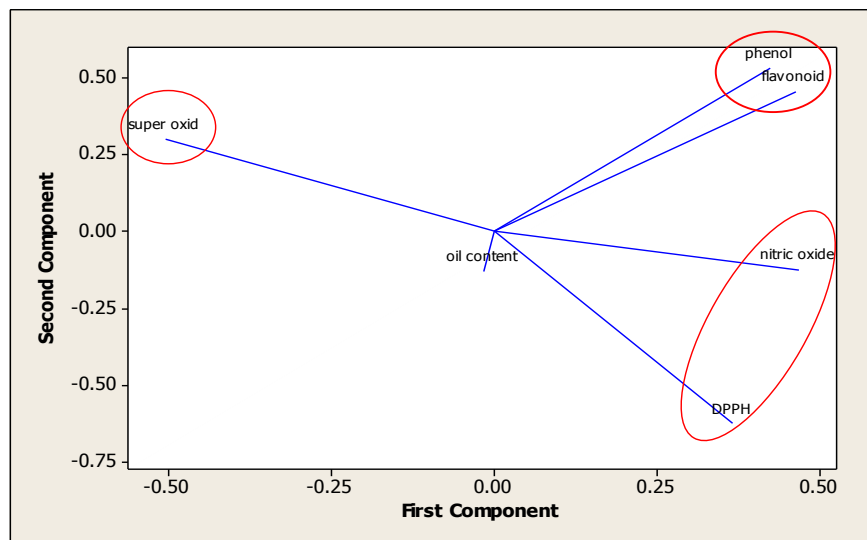


Figure 6: Statistical relationship between different phytochemical parameters.

Conclusion

Some phytochemical properties of five population *S. marianum* from different parts of Iran were evaluated. According to our results the *Silybum marianum* grown in

Marivan showed the highest amount of total phenol and flavonoid content, followed by Marivan, Naghadeh, Khoy, Saghez, and Urmia. Also, the plants grown in Naghadeh and Saghez areas showed the highest and lowest radical scavenging activity relative to others, respectively. In

comparison with other researches of other countries, it can be concluded that different climate changes significantly affect the phytochemical composition of *S. marianum* that should be the focus of medical studies.

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