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Original Article

Study on *Silybum marianum* Seed through Fatty Acids Comparison, Peroxide Tests, Refractive Index and Oil Percentage

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ABSTRACT
In this study, *milk thistle* seeds were collected from four regions of Iran (Ahvaz, Lorestan, Kazeroon and Zarghan). The oil extracted from seeds of plant using n-hexane solvent extraction soxhlet. The extracted oil was analyzed by gas chromatography and fatty acids were identified in all four samples. In all four samples, the oil refractive index of *Milk thistle* oil fatty acids *vis*. Linoleic acid, Oleic acid, Palmitic acid was evaluated. The Peroxide test and determination of oil percentage was performed in *Milk thistle* seed oil.

Key words: Milk Thistle, Oil, Extraction, Peroxide, Fatty Acids

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INTRODUCTION

Fatty acids play an important role in human nutrition. Particularly, unsaturated fatty acids are associated with a reduced risk of developing cardiovascular disease, inflammatory and autoimmune diseases such as asthma, Crohn's disease, arthritis, and certain cancers, including colon, breast and prostate cancers.[1] Nutritional deficiencies or disturbances can depend on different factors and cause a variety of diseases.[2]

Silybum marianum (family: Compositae) is an annual or biennial plant, native to the Mediterranean area and now it known to have spread to other warm and dry regions.[3] This plant also grows in many regions in Iran. Extracts from the mature milk thistle seeds are used as medical remedies for liver disease, liver cirrhosis for prevent liver cancer .[4,5]

This particular plant which can reach heights of two meters has dark shiny green leaves with spiny scalloped edges, purple to reddish purple flowers, and an indeterminate growth habit that results in staggered flowering and maturity.[6] Milk thistle is a serious weed in many areas of North and South America, Africa, Australasia, and the Middle East .[7]

The pharmaceutical compound of milk thistle is derived from its fruits, which are achene's (Fructus silybi mariani). In their dry pericarp and seed coat the plant accumulates a group of flavonolignans commonly referred to as silymarin.[8] Milk thistle (*Silybum marianum*) is an herb that is increasingly used in oncology research and treatment settings. Historically, it has been used to treat liver and biliary disorders and has been used in detoxification and cleansing protocols.[9] The aim of this study was investigation on *Silybum marianum* oil plant.

MATERIALS AND METHODS

Materials

Milk thistle seed were collected from four regions in Iran, (Ahvaz, Lorestan, Kazeroon and Zarghan (Fars- Iran) in July 2015. After collecting the seeds of milk thistle the seeds were dried and prepared for extraction of oil (Figure 1).

Oleic acid composition of oil was identified by gas chromatography, also measure the refractive index, determining the percentage of oil, determining the peroxide value; *Milk thistle* seed for oils was performed.

Oil extraction

The seeds oil was extracted using Soxhlet.[10,11] The seeds were dried from the *Milk thistle* plant. 30 grams of milk thistle seed were powder and then the powder, cartouche putinto Soxhlet, special balloons solvent and 500 ml of n-hexane solvent added into Soxhlet in 70 ° a period of 8 hours. Then solvent flask containing the milk thistle seed oil was separated by Rotary and the weight of oil was determined. Grams of oil obtained from the dried plants are used for oil extraction subtraction. Methyl esters are identified using gas chromatography.

Analysis of fatty acid methyl esters by GC

The FAMES were analyzed by GC according to the method described by Azadmard-Damirchi and Dutta.[12] The GC instrument was equipped with a flame ionization detector and a split/split less injector. A film thickness fused-silica capillary column BPX70 (SGE, Austin, TX, USA) was used for analysis. Injector and detector temperatures were 230 and

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Figure 1: Milk thistle plant

250 °C, respectively. Oven conditions were 158 °C increased to 220 °C at a rate of 2 °C/min and maintained for 5 minutes.

Helium was used as the carrier gas and nitrogen as the make-up gas at a flow rate of 30 ml/min. The FAMES were identified by comparison of their retention times with standard FAMES and the peak areas reported as a percentage of the total fatty acids.[13]

Determination of oil peroxide

Peroxide value (PV) was determined according to the AOAC method.[14] About 5 g of oil was weighed into a 250 mL flask. Previously prepared acetic acid/chloroform (CHCl3) solution (30 mL), saturated potassium iodide (0.5 mL) and distilled water (30 mL) were added with occasional shaking. The mixture was titrated with 0.05 mol L−1 Na2S2O3 with vigorous shaking; 0.5 mL of 10 mL L−1 starch solution was added a dtitration was continued with vigorous shaking, to release all iodine from the CHCl3 layer, until the blue color just disappeared. PV was calculated using this equation.[15]

$$PV\ (meq\ O2\ kg{-}1\ sample) = (S \times 2M \times 1000)/m$$

Where S (mL) is the volume of Na2S2O3 consumed (blank corrected), M (mol L−1) is molarity of Na2S2O3 and m (g) is the mass of the test sample.[15]

Measure the refractive index

A Metrohm 743 Rancimat (Metrohm AG, Herisau, Switzerland) was used for analysis of the oxidative stability index (OSI). The tests were done with 3 g oil sample at a 120 °C temperature in airflow rate of 15 L h−1.[16]

Results and discussions

The oil content and the fatty acid composition found in our experiments correspond well with previously published values.[17-20] The climatic conditions and varieties grown greatly influence the oil and fatty acids content.[20] Oil obtained from the seeds of *milk thistle* were analyzed with gas chromatography, and the composition of fatty acids in all four samples of milk thistle oil from the areas was prepared and identified (Table 1). The composition of fatty acids in plant oils contains *Milk thistle* was included C18:2, C18:1, C16:0. The Linoleic acid achieved from *Milk thistle* which collected from Lorestan and Ahvaz exhibited more amount compared with Kazeroon and Zarghan. Fatty acid composition of plants collected from Ahvaz, Lorestan, Kazeroon and Zarghan is shown in figure 1 to 4, respectively.

Table 1: *Milk thistle* seed oil fatty acid composition

Fatty acid composition		Oil sample (Ahvaz)	Oil sample (Lorestan)	Oil sample (Kazeroon)	Oil sample (Zarghan)
C16:0	*Palmitic acid*	8.55	8.36	7.99	9.26
C16:1	*Palmitoleic acid*	---	---	---	---
C17:1	*margaric acid*	---	---	---	---
C18:0	*Stearic acid*	5.609	7.72	5.607	5.01
C18:1	*Oleic acid*	28.68	35.85	28.54	30.42
C18:2	*Linoleic acid*	54.65	43.57	54.71	52.78
C18;3	*Linolenic acid*	2.50	4.48	3.13	2.51
C20:0	*arashidic acid*	---	---	---	---
C22:0	*Behenic acid*	---	---	---	---

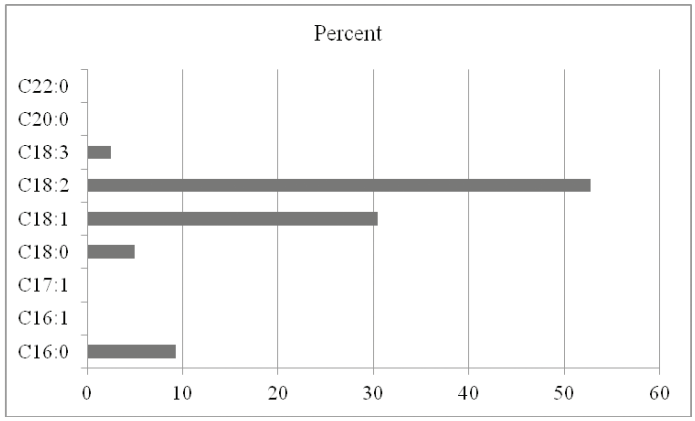

Figure 1: *Milk thistle* oil fatty acid composition extracted from Ahvaz

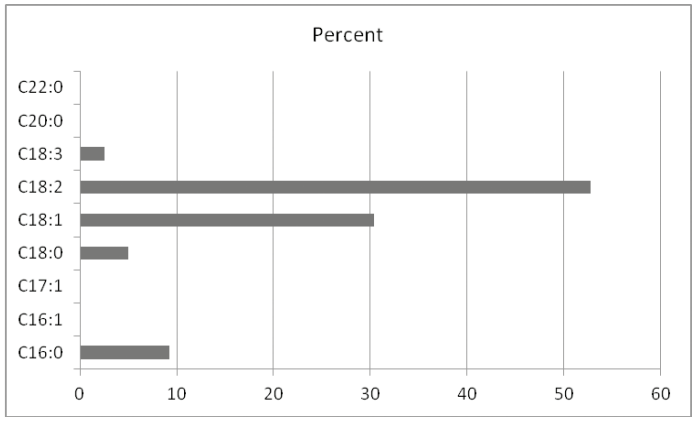

Figure 2: *Milk thistle* oil fatty acid composition extracted from Lorestan

Oxidative stability

Oxidative stability of oil for each four sample was performed. The results are shown in table 2. Results in this experiment showed a significant effect of weather conditions on the oxidation of the oil sample.

Determine percentage of oil

Percentage of oil was determined for each sample and the results are shown in table 3. The highest percent of oil content exhibited from Ahvaz.

Refractive index

Rancimat device used to measure the refractive index of the oil and the results are presented in bellow (Table 4). The less number of double bonds in samples revealed less refractive index and the samples with higher refractive index showed lower melting point and saturation.

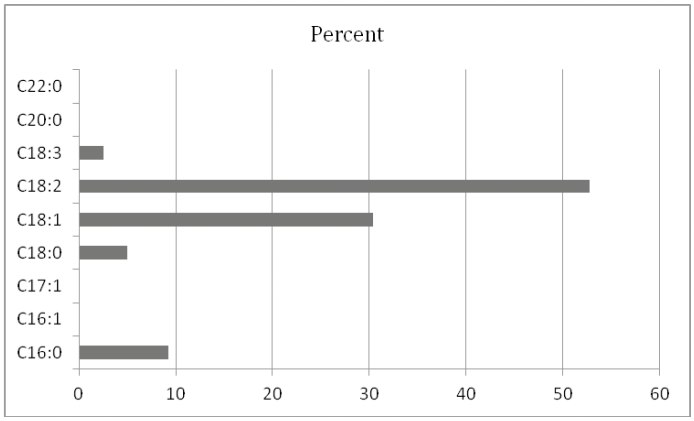

Figure 3: *Milk thistle* oil fatty acid composition extracted from Kazeroon

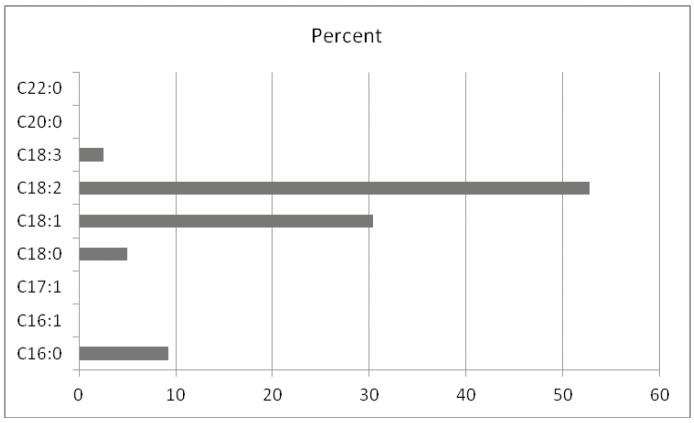

Figure 4: *Milk thistle* oil fatty acid composition extracted from Zarghan

Table 2: The oxidative value for *Milk thistle* seed oil

Sample oil	Oxidation value (pv)
Oil sample (Ahvaz)	0.51
Oil sample (Lorestan)	0.69
Oil sample (Kazeroon)	0.68
Oil sample (Zarghan)	0.57

Table 3: Percentage of oil in samples

Sample oil	percentage of oil
Oil sample (Ahvaz)	25.61%
Oil sample (Lorestan)	24.02%
Oil sample (Kazeroon)	25.32%
Oil sample (Zarghan)	21.28%

Table 4: Refractive index of the oil samples

Sample oil	Refractive index
Oil sample (Ahvaz)	1.4656
Oil sample (Lorestan)	1.46482
Oil sample (Kazeroon)	1.4656
Oil sample (Zarghan)	1.4651

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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