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FATTY ACID COMPOSITION OF *Silybum marianum* L. SEEDS AND ANTIMICROBIAL ACTIVITY OF SEED OIL AND SILYMARIN EXTRACT

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ABSTRACT

In this study, it was aimed to determine fatty acid profile and the antimicrobial activity of the seed oil obtained from the seed of *Silybum marianum* L. and silymarin which was extracted through methanol extraction of *Silybum marianum* L. seed. The antimicrobial activity of extracts was determined by testing against the microorganisms of *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus plantarum, Lactobacillus pentosus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa,* and *Candida albicans*. Gas Chromatography-Mass Spectrometry was used to determine the fatty acid profile of the seed oil. The main components of the seed oil were detected as octadecadienoic acid (44.06 %), octadecenoic acid (20.18 %), hexadecenoic acid (14.63 %) and octadecanoic acid (7.75 %). Seed oil revealed antimicrobial effect on *P. mirabilis*, *P. aeruginosa* and *L. pentosus* ELB41 and silymarin had antimicrobial effect on *L. plantarum* ELB75, *L. pentosus* ELB37 and *B. subtilis*.

Keywords: *Silybum marianum* L., milk thistle, fatty acid composition, silymarin, antimicrobial activity

Silybum marianum L. TOHUMLARININ YAĞ ASİDİ KOMPOZİSYONU İLE TOHUM YAĞI VE SİLYMARİN EKSTRAKTININ ANTİMİKROBİYAL ETKİSİ

ÖZ

Bu çalışmada Meryemana dikeni (*Silybum marianum* L.) tohumlarından elde edilen tohum sabit yağı ile metanol ekstraksiyonuyla elde edilen silmarin etken maddesinin antimikrobiyal aktivitesinin belirlenmesi amaçlanmıştır. Ekstrelerin; *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus plantarum*, *Lactobacillus pentosus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* ve *Candida albicans* mikroorganizmalarına karşı antimikrobiyal aktiviteleri belirlenmiştir. Bununla birlikte bitki tohumlarından elde edilen yağın, yağ asitleri kompozisyonunun belirlenmesi için Gaz

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Kromatografisi-Kütle Spektrometresi (GC-MS) yöntemi kullanılmıştır. Yağın ana bileşenleri; oktadekadienoik asit (% 44.06), oktadekenoik asit (% 20.18), hekzadekanoik asit (% 14.63) ve oktadekanoik asit (% 7.75) olarak saptanmıştır. Çalışmamızda; tohum yağı ekstraktının *P. mirabilis, P. aeruginosa* ve *Lactobacillus pentosus* ELB41 üzerine; silmarinin ise *Lactobacillus plantarum* ELB75, *Lactobacillus pentosus* ELB37 ve *B. subtilis*' e karşı antimikrobiyal etkisinin olduğu saptanmıştır.
Anahtar kelimeler: *Silybum marianum* L., meryemana dikeni, yağ asidi kompozisyonu, silymarin, antimikrobiyal aktivite

INTRODUCTION

The medical and aromatic plants are rich in terms of phytochemical materials like tannins, terpenoids, alkaloids and flavonoids (Dorman and Deans, 2000) and their usage in the treatment of the disease dates back to old times. World health organization reports 20 thousand plants that are used as a spice and other medical purposes, and 25 percent of the pharmaceutical medicines used today are produced from the medical plant (Acıbuca and Bostan Budak, 2018). Around the world, there is about 900 medical and aromatic plant that is cultured. In recent years, there is an increase in usage of the medical plants for health protection against the increase of the diseases, side effects of chemical medicines, and health concerns. According to FAO, the 30 percent of the medicines sold around the world consist of components produced through plant material (FAO, 2005; Acıbuca and Bostan Budak, 2018).

It is reported that antioxidant, anti-inflammatory, immunomodulatory, anti-lipid, and hepaprotective effects of *Silybum marianum* L. which is a member of *Compositae* family and is widely used for the medical purpose since the ancient ages as. The composition of *Silybum marianum* L. seed contains 1.5-3 % of flavonolignan which is a therapeutic agent (Bijak, 2017). Flavonolignans consist of 70-80 % of silymarin flavonolignans, 20-30 % of them are formed through polymeric and oxide polyphenolic components which cannot be chemically identified fractions (Sanchez-Sampedro et al., 2007). Silymarin is a plant secondary metabolite and composed of a mixture of four different flavonolignans isomers as silybin (60-70%), silychristin (20%), silydianin (10%) and isosilybin (5%) (Shaarawy et al., 2009; Rasool et al., 2014). Silymarin is a lipophilic extract that is obtained from seeds after removing the seed oil through the extraction (Ramasamy and Agarwal, 2008). Silymarin concentration of seed is higher than the content of leaf and fruit (Duke, 1999). Dried seed extracts contain 60% silymarin (50-60% silybin, 5% isosilybin, 20% silychristin and 10% silydianin) (Burgess, 2003). Silybin which is found of high amounts in silymarin, is known as the most active phytochemical in it and it is said that it is the most important component which provides the health benefit of silymarin (Dixit et al., 2007). It is reported that *Silybum marianum* L. seeds contain simple flavonoids like taxifolin, quercetin, and dihydrokaempherol besides flavonolignans (Montebianco et al., 2010). It is reported that the aboveground parts of the plant have apigenin, kaempherol, apigenin 7-glycoside, β-sitosterol, β-sitosterol 3-glycosides (Meriçli, 1984). The oil content of seeds is 20-30 of whole seed and it consists of 60% of linoleic, 30% of oleic and 9% of palmitic acids (Wagner et al., 1968). Seeds contain betain, trimetilglisin and essential fatty acids and it is known that these components contribute to the hepaprotective and anti-inflammatory effects of silymarin (Dixit et al., 2007; Ghosh et al., 2010). In different literatures, it is reported that *Silybum marianum* L. has an antimicrobial effect on bacteria (Lee et al., 2012), fungus (Rakelly de Oliveira et al., 2015**)**, virus (McClure et al., 2012) and behaves as a stimulant (Alidoost et al., 2006) on the immune system.

In this study, it was aimed to determine the antimicrobial activity of seed oil and silymarin extract of *Silybum marianum* L. collected from Tekirdağ province (Turkey) on *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300*, Bacillus subtilis* NRRL NRS-744, *Lactobacillus plantarum* ELB75, *Lactobacillus pentosus* ELB41, *Lactobacillus pentosus* ELB37, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. In addition to antimicrobial activity, the fatty acid profile of the seed oil was determined.

MATERIAL AND METHOD

Plant material

Seeds of *Silybum marianum* L. was harvested at Tekirdağ location (40°58'52.0"N 27°33'58.6"E) when the harvest time comes and was dried by having cleaned after the foreign matter was separated for 5 hours at 50±1 °C. Dried seeds have waited under controlled conditions until the analysis.

Seed oil extraction

Due to the high lipid content in *Silybum marianum* L., the European Pharmacopoeia **(**European Pharmacopoeia, 2010**)** recommends a two-step process of its extraction. First, the seeds were defatted using *n*-hexane; second, silymarin was extracted with methanol. The cleaned seeds were dried for 5 hours at drying-oven (EN 400, Nüve, Turkey). After it was grinded until total homogeny, 5 g of powdered material were weighed in each Soxhlet cartouche and 250 ml of *n*-hexane added into each Soxhlet balloons. The oil of seed was extracted for 8 hours at Soxhlet extractor. Then the solvent flask containing the seed oil and *n*-hexane was separated by rotary evaporator (Heidolph Hei-Vap Precision). Afterward, the seed oil was kept at the oven (FN 400, Nüve, Turkey) for 1 hour at 105±1°C in order to separate from residual *n*-hexane. It was weighted after cooled at ambient temperature at desiccator. The obtained oil was kept under controlled conditions until the analysis.

Silymarin extraction

After drying the oil-free cake, it was transferred back to the Soxhlet extractor for silymarin extraction (Wilanowska and Wisniewski, 2015). The oil-free cake was subjected to methanol extraction in an automatic extractor at 175±1 °C for silymarin extraction. After 30 min of immersion and 180 min of washing, respectively, recovery was performed for 15 min. In this way, methanol was recovered. The obtained silymarin extract was kept in a moisture-free condition at 4±1 °C until analysis.

Determination of antimicrobial activity

In order to determine the antimicrobial activity of seed oil and silymarin extract, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 43300), *Bacillus subtilis* (NRRL NRS-744) as gram-positive bacteria; *Lactobacillus plantarum* (ELB75), *Lactobacillus pentosus* (ELB41) and *Lactobacillus pentosus* (ELB37) as lactic acid bacteria; *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453), *Pseudomonas aeruginosa* (ATCC 27853) as gram-negative bacteria and *Candida albicans* (ATCC 10231) were used as yeast strain.

Antimicrobial activity of extracts was studied by modifying of agar well diffusion method. The stock culture of bacteria was inoculated into Nutrient Agar and MRS agar (for *Lactobacillus* spp.) and incubated at 37 ± 1 °C for 24 h. Yeast stock culture was inoculated into Sabouraud 2% Dextrose Agar and incubated at 30 ± 1 °C for 48 h. After incubation, colonies growing on the medium were transferred into Mueller Hinton Broth, MRS Broth and Sabouraud 2% Dextrose Broth. Bacteria and yeast suspensions were prepared as 0.5 McFarland which are approximately 10^8 CFU/ml and 1-5×10^6 cell, respectively and densitometer (Den 18, McFarland Densitometer) was used for adjustment. Prepared suspensions were inoculated into Mueller Hinton Agar, MRS Agar and Sabouraud 2% Dextrose Agar using sterile ecuvion sticks. 10 µl of extracts were inoculated into well in agar medium. Inoculated petri dishes for bacteria and yeast were incubated at 37 ± 1 °C for 24 h and at 30 ± 1 °C for 48 h, respectively. Ciprofloxacin (CIP, 5 µg/disc) and methanol were used as negative and positive controls, respectively. After incubation zone diameters were measured to determine the microbial growth and expressed as mm (CLSI, 2012).

Gas Chromatography-Mass Spectrometry (GC-MS)

The fatty acid composition of *Silybum marianum* L. seeds was performed using Gas chromatography-mass spectrometry (GC-MS, Hewlett Packard 6890) at Central Research Laboratory in Tekirdağ Namık Kemal University (Tekirdağ, Turkey). The samples were diluted at a ratio of 1:100 to analyse with *n*-hexane. An HP-5MS column (coated with (5 %-phenyl)-methylpolysiloxane phase) (30 m × 0.25 mm, 0.25-µm film thicknesses) was used as the stationary phase. Helium was used as carrier gas at a flow rate of 0.8 ml/min. The samples were

injected with a splitting 40:1 to 1 µl. The injector temperature was 250 °C. The GC-MS interface temperatures were maintained at 250°C. The oven temperature was programmed from 40 °C (5 min), 10 °C/min ramp rate to 280 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 30 to 450. While providing the components the data of WILEY and OIL ADAMS libraries were based. Considering the results, the percentages of components were obtained using of FID detector and the identification of the components were carried out with MS detector.

RESULTS AND DISCUSSION

Fatty acid profile by using Gas Chromatography

The fatty acid composition of oil extracted from seeds of *Silybum marianum* L. was performed by using Gas Chromatography-Mass Spectrometry (GC-MS). The fatty acid composition of extracted oil was shown in Figure 1 and Table 1.

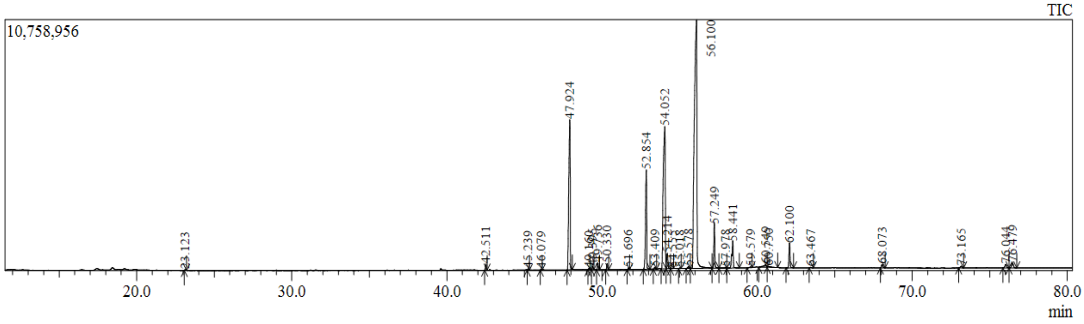

Figure 1. Fatty acid composition of *Silybum marianum* L. seed oil

Table 1. Fatty acid composition of the seed oil of *Silybum marianum* L.

	Fatty acids		Area %	R. time*
1	$C_{18:2}$	Linoleic acid	44.06	56.100
2	$C_{18:1}$	Oleic acid	20.18	54.052
3	$C_{16:0}$	Palmitic acid	14.63	47.924
4	$C_{18:0}$	Stearic acid	7.75	52.854
5	$C_{20:0}$	Arachidic acid	3.04	57.249
6	$C_{20:1}$	Gondoic acid	2.64	58.441
7	$C_{22:0}$	Behenic acid	1.80	62.100
8	$C_{18:1}$	Vaccenic acid	1.37	54.214
9	$C_{16:2}$	Conjugated Linoleic acid	0.60	76.479
10	$C_{20:2}$	Eicosadienoic acid	0.52	60.549
11	$C_{18:2}$	7,10-Octadecadienoic acid	0.42	76.044
12	$C_{17:0}$	Margaric acid	0.40	50.330
13	$C_{16:1}$	Palmitelaidic acid	0.39	49.736
14	$C_{14:0}$	Myristic acid	0.33	42.511
15	$C_{24:0}$	Lignoceric acid	0.31	68.073
16	$C_{16:1}$	Lycopodic acid	0.19	49.395
17	$C_{19:1}$	9-Oxononanoic acid	0.12	53.409
18	$C_{16:1}$	Hypogeic acid	0.07	49.160
19	$C_{15:0}$	Pentadecanoic acid	0.07	45.239
20	$C_{22:1}$	Erucic acid	0.05	63.467
21	$C_{21:0}$	Heneicosylic acid	0.05	59.579
22	$C_{8:0}$	Caprylic acid	0.04	23.123
23	$C_{19:0}$	Nonadecyclic acid	0.04	55.018

* R. time, retention time.

30 different compounds were detected in the results of the fatty acid composition. Fatty acids which were found in high amounts were shown in Table 1. It was found that 67 % of fatty acids were unsaturated. 45.32 % of them formed polyunsaturated and 21.59 % of the composition formed monounsaturated fatty acids. Major fatty acids of *S. marianum* seed oil was found as octadecadienoic acid (44.06 %, $C_{18:2}$, linoelic acid), octadecenoic acid (20.18 %, $C_{18:1}$, oleic acid), hexadecanoic acid (14.63%, $C_{16:0}$, palmitic acid) and octadecanoic acid (7.75%, $C_{18:0}$, stearic acid). Others were eicosadienoic acid (3.04 %, $C_{20:0}$, arachidic acid), 11-eicosenoic acid (2.64 %, $C_{20:1}$, gondoic acid) and docosanoic acid (1.80 %, $C_{22:0}$, behenic acid). Similar to our results **(**Mhamdi et al., 2016**)** it was reported that the predominance of linoleic (50.5 %) and oleic (30.2 %) acids in dry matter of extracted oil from *S. marianum* seeds. In addition to this, they also detected the presence of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), arachidic acid ($C_{20:0}$), γ-linolenic acid ($C_{18:3}$, n-6) and Cis-11-eicosenoic acid ($C_{20:1}$, n-9). It has been shown that (Hasanloo et al., 2008) the main fatty acids detected in the Iranian *S. marianum* seeds were: linoleic acid (45.36%) and oleic acid (31.58%). Similarly, it was reported that **(**Nasrollahi et al., 2016**)** linoleic (43.57-54.78 %), oleic (28.68-35.85 %) and palmitic acid (7.99-9.26 %) were detected in the oil of *S. marianum* which were collected from four regions of Iran (Ahvaz, Lorestan, Kazeroon and Zarghan). It was also reported that fatty acid compositions of different varieties remained the same, but the amount of fatty acid compositions differed according to regions. Similar to our results, it was reported (Çelik and Kan, 2013) that arachidic, stearic, and palmitic acid as saturated and linoleic acid as major unsaturated fatty acid of *S. marianum* seed oil. In addition to this, a lower amount of different fatty acids was also found in seed oil.

In summary, the fatty acid profile of seed oil includes pentadecanoic acid ($C_{15:0}$) which is found rare in nature and found a rate of 1.2 % in cow milk, vaccenic acid and hypogeic acid which were found in human milk and ruminant milk. In addition to this, oleamide which accumulates cerebrospinal fluid and has been searching its potential for sleeping disorders treatment was detected in the seed oil of *S. marianum*.

Determination of antimicrobial activity in seed oil and silymarin extract

It was determined that the extracts obtained from the seeds of *Silybum marianum* L. has an antimicrobial effect against different types of microorganisms (Table 2). Antimicrobial effect of the *Silybum marianum* L. seed oil was detected on *P. mirabilis (*inhibition zone, 11 mm), *P. aeruginosa* (inhibition zone, 12 mm) and *L. pentosus* (ELB41) (inhibition zone, 13 mm). However, the effect on the other microorganisms could not be determined. The methanol extract of silymarin was found to be more effective on *L. plantarum* (inhibition zone, 14 mm) and *L. pentosus* (ELB37) (inhibition zone, 13 mm) species. Besides, it was detected that there was antimicrobial effect against *B. subtilis* (inhibition zone, 12 mm). It was detected that silymarin extract has no antibacterial effect against Gr (-) bacteria. Both seed oil and silymarin extract were found to ineffective against *C. albicans,* therefore antifungal activity was not detected in our study. This result was probably due to the cell structure of the fungus, mainly the chitin cell walls of *C. albicans* (Rakelly de Oliveira et al., 2015).

In a study (Shah et al., 2011), inhibition zone (i.z.) of 17, 15 and 21 mm diameters were detected for the seed oil of blue capitulum of *Silybum marianum* plant against *B. subtilis, P. vulgaris* and *S. aureus*, respectively. However, the inhibition zone of 22, 13 and 19 mm were detected for the seed oil of white capitulum of *Silybum marianum* plant against *B. subtilis, P. vulgaris* and *S. aureus*, respectively. Similarly, the results of the antimicrobial activity of silymarin correlated with the findings of Mojgan and Roya (2016), who reported no significant effect on Gr (-) bacteria. Mohammed et al. (2019) reported that antibacterial effects of S. *marianum* extracts were higher than antifungal effects and ethanol extracts have higher antimicrobial activity than methanol extracts. Unlike the results of our study, plant extracts have a higher antimicrobial effect against Gr (-) bacteria **(**Mohammed et al., 2019**)**. Similarly, neither silymarin nor seed oil has antifungal

activity. It was reported that *n*-hexane extract of *S. marianum* seed had antimicrobial effect on both Gr (+) and Gr (-) bacteria (Yaldız, 2017). It was reported that (Zaouia et al., 2010) antimicrobial activity of water and methanol extracts of *S. marianum,* which was collected from Algeria, was detected against *E. coli* (i.z. 12 mm, 8 mm), *P. aeruginosa* (i.z. 12 mm, 11 mm), *S. aureus* (i.z. 11 mm, 10 mm) and *C. albicans* (i.z. 9 mm, 11 mm), respectively. In our study, similarly, antibacterial effect of seed oil was observed and detected as it was effective on *P. mirabilis*, *P. aeruginosa* and *L. pentosus*. The inactivation activity of *S. marianum* seed oil could be related to its content of phenolics and tocopherols (Andrzejewska et al. 2015). Radhika et al. (2017) reported that silymarin had no antibacterial effect on *S. aureus* and *E. feacalis* which is parallel to findings in our study. The inactivation activity of *S. marianum* could be related to antimicrobial action of silymarin flavonolignans and polymeric flavonoids which contain hydroxyl phenolic groups that interfere with the bacterial synthetic processes by enzyme inhibition (Avila et al., 2008; Li et al., 2012). It is also believed that phenolic compounds possess the capacity to form complexes with extracellular soluble proteins that bind to bacterial cell wall (Tsuchiya et al., 1996; Rakelly de Oliveira et al., 2015). Although not noted as seed oil, in a study where the extraction and agar diffusion methods overlapped (Kareem and Ali, 2005), it was reported that seed oil did not have any antibacterial activity on the studied strains.

Table 2. The antimicrobial activity (zone of inhibition) of different extracts of *S. marianum* L.

	Zone of inhibition (mm) of different extracts		
Organisms	Seed oil (10 µL)	Silymarin (10 µL)	*Ciprofloxacin* (5 µg)
E. faecalis (ATCC 29212)	NZ*	NZ	17
S. aureus (ATCC 43300)	NZ	NZ	20
B. subtilis (NRRL NRS-744)	NZ	12	35
L. plantarum (ELB75)	NZ	14	9
L. pentosus (ELB41)	13	NZ	8
L. pentosus (ELB37)	NZ	13	10
E. coli (ATCC 25922)	NZ	NZ	25
P. mirabilis (ATCC 12453)	11	NZ	27
P. aeruginosa (ATCC 27853)	12	NZ	27
C. albicans (ATCC 10231)	NZ	NZ	ND**

*NZ: No zone detected; **ND: Not detected.

According to the result of the studies, it was observed that antibacterial activities were different from one another. It was thought that the difference caused different antibacterial effect is based on different extraction solvents, the harvest territory of *S. marianum,* the usage of the plants flowers, seeds, or all parts at extraction.

CONCLUSION

The antimicrobial activity of the seed oil and methanol extract of silymarin obtained from the seeds of the milk thistle (*Silybum marianum* L.) collected at Tekirdağ province was investigated against different types of microorganisms and extracts were found to be effective against some of the microorganisms. Seed oil was found to be effective on *P. mirabilis*, *P. aeruginosa* and *L. pentosus* while the antibacterial activity of silymarin extract was detected on *L. plantarum*, *L. pentosus* and *B. subtilis*. Infections caused by pathogens have a high prevalence, where they are responsible for the increase in worldwide morbi-mortality. The antimicrobial activity of natural products such as oil or extracts of seeds like *Silybum marianum* L. has

been conducted with the aim of generalizing the spectrum of antimicrobial therapy. Popularity of use of plants and their derivatives for infections caused by various microorganisms has been increasing in recent years. *Silybum marianum* L. has been traditionally used in treating diseases for many years and silymarin is an active component of the plant which has a major component called silybin. In recent years, studies investigating the pharmacological effects associated with these compounds have increased.

Results show that *Silybum marianum* L. has a potential to be used as food preservatives and pharmaceutical treatments, as a natural antibacterial agent, together with the fact that the use of silymarin and silybin is considered safe. In addition to this, it was also possible to enrich the fatty acid content of food in terms of polyunsaturated and monounsaturated fatty acids which is known with health benefits. It was thought that the higher concentration levels of seed oil and silymarin extracts could be further studied in order to detect the antimicrobial activity of different concentrations. *Silybum marianum* L. seed oil and silymarin extract might be a potential alternative to the food applications in order to reduce using synthetic chemicals in food industry. The authenticity of the results is important in terms of no other study with the indigenous *Silybum marianum* L. of Tekirdağ province was encountered in literature.

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CONFLICT OF INTEREST

The authors express no conflict of interest associated with this work.

AUTHORS' CONTRIBUTIONS

Sıla Barut Gök was responsible for experimental design. Mine Aydın Kurç and Sıla Barut Gök performed the microbiological analysis. Elif Ceren Pehlivan, Yasemin Erdoğdu and Sıla Barut Gök were responsible for extracting of silymarin and seed oil. Yasemin Erdoğdu and Sıla Barut Gök were evaluated on fatty acid composition of seed oil. All authors were responsible for interpretation and discussion of the results. All authors approved the submitted version.

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