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THE EFFECTS OF EXTRACTION TECHNIQUES ON THE ANTIOXIDANT POTENTIAL OF EXTRACTS OF DIFFERENT PARTS OF MILK THISTLE (*SILYBUM MARIANUM* L.)

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ABSTRACT

Background. Extracts of milk thistle, particularly from seeds, are used as a valuable source of natural antioxidants in different industries, for example pharmaceutical and cosmetic. The leaves and flowers are also known to be a source of biologically active compounds, as well as those with an antioxidant capacity. The selection of the extraction parameters, such as type and concentration of extractant, and extraction time, have an impact on the antioxidant capacity of the obtained extracts. The aim of this study was to evaluate the antioxidant activities of extracts obtained using different parts of raw material. The impact of different parameters of extraction on antioxidant capacity was also assessed.

Materials and methods. The seeds, flowers and leaves were extracted using a Soxhlet apparatus, ultrasound and shaking. 96% (v/v) and 70% (v/v) ethanol, concentrated methanol, acetone and petroleum ether were applied as solvents. The impact of the extraction time was also evaluated. The extracts were evaluated using DPPH, ABTS, FRAP and Folin-Ciocalteu techniques.

Results. The obtained extracts, except for the samples in petroleum ether, showed the antioxidant capacity. Soxhlet extraction, especially that which uses ethanol, methanol and acetone, seems to be a valuable extraction method.

Conclusion. To sum up, many factors could affect the antioxidant capacity and the total polyphenol content of *Silybum marianum* L. extracts. The solvent and an appropriately selected extraction method seem to be important factors in the effective isolation of active substances and could lead to the more effective application of this valuable plant material in different industries.

Keyword: milk thistle, antioxidant capacity, ultrasound-assisted extraction, Soxhlet apparatus, shaking

INTRODUCTION

Milk thistle (*Silybum marianum* L.) is a plant belonging to the Asteraceae family. This plant is rich in phenolics, including flavonolignans (silibinin, isosilybin, silydianin and silychristin) and taxifolin. These compounds are of pharmacological importance due to their hepatoprotective properties (Škottová et al., 2004). The antioxidant capacity of milk thistle has been confirmed by many authors, however, most studies have only been based on the evaluation of its fruits extracts (Chambers et al., 2017; Lucini et al., 2016; Malinowska, 2017). Extracts of various parts of the plant, such as the leaf and the flower, also show

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radical scavenging abilities, but their application as therapeutics is rather limited (Ahmad et al., 2012; Andrzejewska et al., 2015). The antioxidant capacity of raw material may depend on the chemical structure of biologically active compounds in plants, as well as on the scheme of extract preparation, i.e. the method of extraction including applied extractants (Pawlak and Sielicka, 2016; Rababah et al., 2010; Wang and Weller, 2006). The chemical composition and polarity of the solvent are important factors in a determination of the antioxidant capacity because they can affect the mechanism of single electron (SET) or hydrogen atom transfer (HAT) (Pawlak and Sielicka, 2016). In the methods of the SET mechanism, the working solution consisted of oxidant color changes during the reduction initiated by the addition of antioxidants. The method of determining the oxygen radical absorbance capacity is based on the HAT mechanism (Cybul and Nowak, 2008). The extraction of raw material can be carried out using both classic and recently developed methods (Wang and Weller, 2006). The Soxhlet extraction is a standard technique used for decades and is frequently applied as a reference to assess the effectiveness of other methods. It is considered to be efficient, however, its disadvantages are its relatively large solvent consumption and rather long extraction time (Wang and Weller, 2006). Another method to obtain valuable plant extracts is continuous mixing of the raw material with a solvent, for example using a magnetic stirrer. This procedure allows the entire content of the vessel to be mixed to increase the contact area to break the mechanical barrier, i.e. the cell wall, and then to isolate the active substances (Kumar et al., 2017). Another, relatively modern method to obtain plant extracts, classified as the so-called green extraction technique, is ultrasound-assisted extraction. Due to its economical, ecological and efficiency characteristics, it is often used in the cosmetic and pharmaceutical industries to obtain valuable plant extracts. The extracts are obtained faster (Chemat et al., 2012; Drouet et al., 2019) and the method is considered to be less polluting due to the saving of solvents and energy (Wang et al., 2008). The aim of this study was to evaluate the antioxidant capacity of extracts of different parts of milk thistle obtained with different extraction methods and solvents applied as extractants.

MATERIALS AND METHODS

Reagents

2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich, USA; iron(III) chloride hexahydrate, Folin-Ciocalteu phenol reagent – from Merck, Darmstadt, Germany; 99.5% acetic acid, sodium acetate anhydrous, potassium persulfate, 36% hydrochloric acid, sodium carbonate anhydrous, ascorbic acid and applied solvents, i.e. ethanol, methanol, acetone and petroleum ether, were from Chempur, Piekary Śląskie, Poland. All reagents were of analytical grade.

Plant material

The raw material consisted of the fresh leaves, flowers and seeds of milk thistle, which were harvested from July to September in 2015 from a natural state, i.e. an agricultural wasteland near the city (53°40'39.6"N, 14°49'44.2"E). The plants were collected during the flowering and fructification periods.

The raw material was identified by the first author (doctor of agricultural sciences, specialist in plant physiology). The harvested material was dried at room temperature until it was a constant weight.

Sample preparation

The dry material was extracted using three methods: ultrasound-assisted extraction at a frequency of 40 kHz for 15, 30 or 60 min with a temperature set between 30–35°C (ultrasound bath – Sonic-2, Polsonic Palczyński); extraction in a Soxhlet apparatus of five full cycles; and being shaken on a reciprocating platform (Chem-Land SK-0330-PRO) for 240 min at 500 rpm. Ethanol (96% and 70% (v/v)), methanol, acetone and petroleum ether were applied as extractants. The 5% (w/v) extracts of the plants in each of the abovementioned solvents were prepared and stored at +4°C until analysis. Three individual samples were made from each extract.

Determination of DPPH, ABTS, FRAP and total polyphenol content

To evaluate the antioxidant capacity, the DPPH, FRAP and ABTS methods were applied, whereas the total polyphenol content was measured using the Folin-

-Ciocalteu technique. The antioxidant capacity of the extract was evaluated using the DPPH method as previously described (Nowak et al., 2017; Zielonka-Brzezicka et al., 2018). In this method, an aliquot of 150 µl of the tested extract was mixed with 2850 µl of properly diluted DPPH solution in 96% (v/v) ethanol (absorbance of the working solution at 517 nm in 1 cm cuvettes was 1.00 ±0.02). After 10 min incubation at room temperature, the absorbance was measured in 1 cm glass cuvettes at a wavelength of 517 nm using a Hitachi U-5100 spectrophotometer. The activity of scavenging ABTS radicals was evaluated according to the previously described procedure (Nowak et al., 2018). In this method, a stock solution was prepared by dissolving 7 mM 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in 2.45 mM potassium persulfate aqueous solution, followed by incubation in the dark at room temperature for 24 hours. To get the working solution, the stock solution was diluted with 50% (v/v) methanol to obtain an absorbance of 1.00 ±0.02 at 734 nm. 25 µl of the examined extract was added to 2500 µl of the working solution, thoroughly mixed and, after 6 min incubation at room temperature, the absorbance was measured at 734 nm. The ferric ion reducing power (FRAP) was determined as previously described (Florkowska et al., 2018). The working solution was prepared by mixing 1 volume of 10 mM TPTZ (in 40 mM HCl), 1 volume of 20 mM $FeCl_3$ and 10 volumes of acetate buffer (pH 3.6). 2320 µl of working solution was mixed with 80 µl of extract. The absorbance of the extracts was measured at a wavelength of 593 nm. The total polyphenol content (TPC) was determined as described previously (Nowak et al., 2017). In this method, to 150 µl of the extract, 150 µl of tenfold diluted Folin-Ciocalteu reagent, 1350 µl 0.01 M sodium carbonate solution and 1350 µl of water were added and thoroughly mixed. The absorbance was measured at 750 nm.

For the DPPH, ABTS and FRAP methods, ascorbic acid was used as a reference, and the activity was expressed as VCEAC (vitamin C equivalent antioxidant capacity) in mg of vitamin C/g of raw material. For the Folin-Ciocalteu method, gallic acid was applied as a reference and the results were expressed as gallic acid equivalents (GAE) in mg gallic acid/g raw material.

Statistical analysis

The results are presented as arithmetic means ±standard deviations (SD). The statistical analysis of the results was carried out using a one-way analysis of ANOVA, with the significance level $p < 0.05$. Differences between the used solvents were determined by Tukey's test ($n = 3$). The significance of differences between the extraction methods was evaluated with the Wilcoxon test ($\alpha < 0.05$). Pearson's linear correlation between the results obtained by individual methods was also determined. All calculations were done using Statistica 12PL software (StatSoft).

RESULTS

Tables 1–3 present the mean (±SD) antioxidant capacity of the extracts of milk thistle leaves, flowers and seeds evaluated using three methods: DPPH, FRAP and ABTS, while the total polyphenol content was evaluated using the Folin-Ciocalteu technique.

The antioxidant activity of the leaf extracts evaluated using the DPPH method was higher for the samples obtained by Soxhlet extraction in 70% (v/v) ethanol than for the extracts in 96% (v/v) ethanol, methanol and acetone. Also, high levels of activity were found for the extracts prepared in 96% (v/v) ethanol using ultrasounds for 60 min. The extracts of *Silybium marianum* leaves showed high ferric ion reducing power when assessed with the FRAP method. In this case, the highest results were found for the extracts prepared by Soxhlet extraction in 70% ethanol. The highest antioxidant capacity of leaf extracts evaluated using the ABTS method were found for the extracts in 70% ethanol prepared using a Soxhlet apparatus – Table 1. Similarly, the highest polyphenol content was observed for leaf extracts in 70% (v/v) ethanol obtained by Soxhlet extraction (Table 1).

The mean (±SD) antioxidant capacity of the milk thistle flower extracts are summarized in Table 2. The highest activity obtained using the DPPH and FRAP methods, was found for extracts prepared in acetone, methanol and 96% ethanol, using one-hour ultrasound-assisted extraction. In the case of the FRAP method, the highest antioxidant capacity of flowers was also found for their acetonic extract after a one-hour application of ultrasound. The antioxidant capacity of the flower extracts measured using the ABTS method,

Table 1. Antioxidant capacity of milk thistle leaf extracts (mean ±SD)

Solvent	Extraction time min	VCEAC (vitamin C equivalent antioxidant capacity) mg of vitamin C/g of raw material			GA mg of gallic acid/g of raw material
		DPPH	FRAP	ABTS	F-C
Soxhlet extraction					
96% (v/v) ethanol	–	1.84 ±0.27 b	85.48 ±0.39 b	9.76 ±0.20 b	1.89 ±0.142 b
70% (v/v) ethanol	–	2.62 ±0.02 a	127.52 ±0.49 a	10.37 ±0.20 a	4.14 ±0.08 a
Methanol	–	1.70 ±0.16 b	80.06 ±0.40 c	4.43 ±0.15 c	1.72 ±0.109 b
Acetone	–	1.32 ±0.39 b f	64.83 ±0.39 d	1.43 ±0.19 d	0.95 ±0.075 c
Petroleum ether	–	n.a.	6.18 ±0.27 e	n.a.	n.a.
Shaking					
96% (v/v) ethanol	240	0.77 ±0.06 b	71.68 ±0.53 b	3.39 ±0.29 b	2.20 ±0.02 a
70% (v/v) ethanol	240	0.16 ±0.02 c	43.51 ±0.40 c	4.22 ±0.19 a	0.94 ±0.10 c
Methanol	240	0.81 ±0.05 c	83.38 ±0.53 c	4.21 ±0.34 a	2.22 ±0.45 a
Acetone	240	1.07 ±0.03 a	74.28 ±0.27 a	4.64 ±0.20 a	1.76 ±0.02 b
Petroleum ether	240	n.a.	6.86 ±0.32 d	n.a.	n.a.
Ultrasound-assisted extraction					
96% (v/v) ethanol	15	0.57 ±0.06 ab	26.33 ±0.39 ee	1.66 ±0.34 e	0.61 ±0.02 cd
	30	0.42 ±0.03 bc	22.51 ±0.38 ef	1.80 ±0.26 ef	0.59 ±0.09 cd
	60	1.34 ±0.01 a	56.21 ±0.27 b	3.49 ±0.23 c	1.75 ±0.04 a
70% (v/v) ethanol	15	0.11 ±0.00 d	18.20 ±0.39 f	2.43 ±0.15 de	0.54 ±0.02 de
	30	0.32 ±0.02 cd	31.32 ±0.38 d	2.54 ±0.11 d	0.58 ±0.02 de
	60	0.92 ±0.04 ab	60.70 ±0.33 a	4.22 ±0.19 ab	1.68 ±0.103 a
Methanol	15	0.60 ±0.03 bc	48.75 ±0.48 bc	3.83 ±0.63 bc	1.25 ±0.10 b
	30	0.79 ±0.4 ab	50.00 ±0.53 bc	4.57 ±0.13 a	1.25 ±0.76 b
	60	0.97 ±0.01 a	69.36 ±0.61 a	4.67 ±0.13 a	1.82 ±0.87 a
Acetone	15	0.60 ±0.09 a	44.15 ±0.73 a	1.14 ±0.23 f	0.76 ±0.57 c
	30	0.44 ±0.06 bc	42.30 ±0.27 bc	1.18 ±0.18 f	0.67 ±0.78 cd
	60	0.85 ±0.09 bc	65.83 ±0.38 a	3.51 ±0.19 bc	1.36 ±0.11 cd
Petroleum ether	15	n.a.	3.18 ±0.43 i	n.a.	n.a.
	30	n.a.	10.21 ±0.31 g	n.a.	n.a.
	60	n.a.	7.21 ±1.24 h	n.a.	n.a.

The values marked with different letters differ significantly between the used solvents ($p < 0.05$, $n = 3$).
n.a. – no antioxidant capacity.

Table 2. Antioxidant capacity of milk thistle flower extracts (mean ±SD)

Solvent	Extraction time min	VCEAC (vitamin C equivalent antioxidant capacity) mg of vitamin C/g of raw material			GA mg of gallic acid/g of raw material
		DPPH	FRAP	ABTS	F-C
Soxhlet extraction					
96% (v/v) ethanol	–	2.09 ±0.03 b	147.70 ±0.43 a	14.17 ±0.23 a	2.84 ±0.43 a
70% (v/v) ethanol	–	2.27 ±0.14 ab	97.39 ±0.43 c	11.16 ±0.26 c	2.39 ±0.04 b
Methanol	–	2.34 ±0.05 a	110.62 ±0.32 b	12.48 ±0.10 b	2.20 ±0.10 c
Acetone	–	1.21 ±0.01 c	94.39 ±0.59 d	10.88 ±0.20 d	1.52 ±0.10 d
Petroleum ether	–	n.a.	7.28 ±0.37 e	2.25 ±0.14 e	n.a.
Shaking					
96% (v/v) ethanol	240	2.28 ±0.08 a	80.99 ±0.32 b	3.53 ±0.28 c	0.85 ±0.08 c
70% (v/v) ethanol	240	0.23 ±0.00 c	15.34 ±0.31 c	2.00 ±0.16 d	0.50 ±0.04 d
Methanol	240	2.31 ±0.05 a	82.56 ±0.31 b	4.67 ±0.27 b	1.23 ±0.09 b
Acetone	240	1.68 ±0.05 b	92.29 ±0.38 a	5.29 ±0.30 a	1.45 ±0.06 a
Petroleum ether	240	n.a.	7.00 ±0.27 d	n.a.	n.a.
Ultrasound-assisted extraction					
96% (v/v) ethanol	15	1.82 ±0.02 c	71.50 ±0.45 d	7.27 ±0.18 c	1.14 ±0.05 d
	30	1.12 ±0.00 d	45.33 ±0.34 e	8.35 ±0.33 b	0.65 ±0.04 f
	60	2.71 ±0.06 a	110.15 ±0.43 b	8.67 ±0.20 b	2.25 ±0.11 b
70% (v/v) ethanol	15	0.15 ±0.01 f	16.52 ±0.22 h	0.61 ±0.21 h	0.54 ±0.02 g
	30	0.47 ±0.01 e	27.54 ±0.5 f	0.75 ±0.11 h	0.76 ±0.01 e
	60	0.41 ±0.03 e	23.62 ±0.43 g	1.07 ±0.12 g	0.99 ±0.03 de
Methanol	15	2.15 ±0.11 e	72.68 ±0.34 d	3.86 ±0.18 f	1.57 ±0.03 c
	30	2.14 ±0.01 b	78.31 ±0.39	4.18 ±0.16 e	1.51 ±0.10 c
	60	2.75 ±0.10 b	105.27 ±0.43 d	7.20 ±0.24 c	2.28 ±0.11 b
Acetone	15	1.93 ±0.04 a	99.03 ±0.33 b	5.24 ±0.26 d	1.65 ±0.08 c
	30	1.86 ±0.08 c	94.07 ±0.54 c	5.41 ±0.11 d	1.57 ±0.14 c
	60	2.77 ±0.13 a	177.44 ±0.63 a	9.49 ±0.16 a	3.48 ±0.01 a
Petroleum ether	15	n.a.	4.19 ±0.60 i	n.a.	n.a.
	30	n.a.	4.93 ±0.39 i	n.a.	n.a.
	60	n.a.	6.68 ±0.40 i	n.a.	n.a.

The values marked with different letters differ significantly between the used solvents ($p < 0.05$, $n = 3$).
n.a. – no antioxidant capacity.

Table 3. Antioxidant capacity of milk thistle seed extracts (mean ±SD)

Solvent	Extraction time min	VCEAC (vitamin C equivalent antioxidant capacity) mg of vitamin C/g of raw material			GA mg of gallic acid/g of raw material
		DPPH	FRAP	ABTS	F-C
Soxhlet extraction					
96% (v/v) ethanol	–	0.85 ±0.02 c	149.02 ±0.39 b	17.13 ±0.15 b	4.38 ±0.23 a
70% (v/v) ethanol	–	2.04 ±0.14 a	195.59 ±6.28 a	18.70 ±0.17 a	4.75 ±0.08 a
Methanol	–	1.20 ±0.07 b	143.39 ±0.38 b	15.01 ±0.38 c	3.54 ±0.03 b
Acetone	–	0.26 ±0.05 d	91.33 ±0.27 d	8.34 ±0.18 d	2.67 ±0.09 c
Petroleum ether	–	n.a.	12.45 ±0.45 d	0.58 ±0.20 e	n.a.
Shaking					
96% (v/v) ethanol	240	–	58.52 ±0.39 b	3.12 ±0.15 c	2.80 ±0.11 c
70% (v/v) ethanol	240	0.94 ±0.05 a	99.35 ±1.56 a	13.14 ±0.22 a	2.98 ±0.103 b
Methanol	240	0.42 ±0.03 b	92.43 ±0.49 a	10.83 ±0.21 b	2.78 ±0.076 b
Acetone	240	0.07 ±0.01 c	101.77 ±0.54 a	1.33 ±0.07 d	3.13 ±0.26 a
Petroleum ether	240	n.a.	10.24 ±0.48 c	n.a.	n.a.
Ultrasound-assisted extraction					
96% (v/v) ethanol	15	n.a.	30.35 ±0.40 g	3.67 ±0.08 cd	1.01 ±0.13 g
	30	n.a.	38.73 ±0.60 f	5.10 ±0.11 c	1.44 ±0.05 h
	60	n.a.	55.35 ±0.65 d	5.60 ±0.17 d	2.50 ±0.10 d
70% (v/v) ethanol	15	0.14 ±0.03 c	47.97 ±0.65 e	3.49 ±0.18 d	1.11 ±0.45 g
	30	0.30 ±0.03 b	62.67 ±2.69 c	5.45 ±0.26 bc	2.64 ±0.08 d
	60	0.74 ±0.03 a	86.73 ±0.27 a	7.74 ±0.21 a	3.71 ±0.06 b
Methanol	15	0.08 ±0.04 d	51.43 ±0.54 d	3.17 ±0.19 e	1.76 ±0.13 e
	30	0.35 ±0.05 b	78.78 ±0.22 b	5.35 ±0.18 bc	2.65 ±0.08 d
	60	0.20 ±0.08 bc	89.58 ±0.40 a	5.81 ±0.21 e	3.17 ±0.11 c
Acetone	15	0.15 ±0.09 c	65.16 ±0.91 c	1.07 ±0.21 h	2.70 ±0.103 d
	30	0.18 ±0.09 bc	80.99 ±0.21 b	2.23 ±0.12 g	3.18 ±0.09 c
	60	0.19 ±0.12 bc	88.97 ±0.55 a	2.74 ±0.18 f	3.28 ±0.15 a
Petroleum ether	15	n.a.	11.67 ±0.49 i	n.a.	n.a.
	30	n.a.	15.16 ±0.65 h	n.a.	n.a.
	60	n.a.	15.31 ±0.21 h	n.a.	n.a.

The values marked with different letters differ significantly between the used solvents ($p < 0.05$, $n = 3$).
n.a. – no antioxidant capacity.

was the highest for the samples prepared by Soxhlet extraction in 96% ethanol, methanol, 70% ethanol and acetone. The highest polyphenol content in the flower extracts was observed for the extracts in acetone prepared using one-hour ultrasound-assisted extraction (Table 2).

The antioxidant capacity of milk thistle seeds evaluated using the DPPH technique, was the highest for extracts prepared in 70% ethanol and methanol by Soxhlet extraction. The antioxidant capacity of the extracts prepared using other extraction methods was lower, below 1.00 VCEAC, while that of the seed extract obtained by shaking and using ultrasound-assisted extraction in concentrated ethanol and petroleum ether was negligible. The highest total polyphenol content in the seed extracts was observed for the extracts in 70% (v/v) ethanol obtained by Soxhlet extraction (Table 3).

The Pearson correlation coefficients between the capacity of the extracts of all parts of the milk thistle obtained using different applied methods are presented in Table 4. In most cases, highly significant relationships were found between the antioxidant capacities determined using different methods (Table 4).

Statistical analysis of the differences between the antioxidant capacities of the extracts obtained using the studied extraction methods is presented in Table 5. In most cases, statistically significant differences were found between the Soxhlet and two other extraction methods applied (Table 5).

Table 4. Correlation coefficients between the activities determined with the applied methods

	Leaves		Flowers		Seeds	
	r	*p*	*r*	*p*	*r*	*p*
DPPH/FRAP	0.874	$p < 0.0001$	0.837	$p < 0.0001$	0.862	$p < 0.0001$
DPPH/ABTS	0.758	$p < 0.001$	0.578	$p < 0.01$	0.885	$p < 0.0001$
DPPH/F-C	0.578	$p < 0.05$	0.160	NS	0.604	$p < 0.05$
FRAP/ABTS	0.779	$p < 0.0001$	0.767	$p < 0.0001$	0.680	$p < 0.0001$
FRAP/F-C	0.419	$p < 0.05$	0.176	NS	0.583	$p < 0.001$
ABTS/F-C	0.731	$p < 0.001$	0.743	$p < 0.001$	0.569	$p < 0.05$

r – correlation coefficient, *p* – probability value.
NS – not statistically significant ($p > 0.05$).

Table 5. Differences of antioxidant capacity between Soxhlet and two other extraction methods evaluated using the Wilcoxon test

Method of antioxidant capacity determination	Soxhlet vs. ultrasound assisted extraction		Soxhlet vs. shaking	
	z	*p*	*z*	*p*
DPPH	1.647	$p = 0.099$ (NS)	2.431	$p = 0.015$
ABTS	2.745	$p = 0.006$	2.902	$p = 0.003$
FRAP	2.196	$p = 0.028$	2.353	$p = 0.018$
Folin-Ciocalteu	0.862	$p = 0.388$ (NS)	0.356	$p = 0.721$ (NS)

NS – non-significant differences.

DISCUSSION

Silybum marianum L. is a plant with a known antioxidant capacity used as an anti-inflammatory and hepatoprotective agent (Chambers et al., 2017; Shaker et al., 2010). In our study, all the extracts of the studied parts of the milk thistle showed an antioxidant capacity, however, this parameter depended on the solvent and the extraction method applied as well as the part of the plant used. In the case of the DPPH method, the radical scavenging activity was usually higher in the flowers than in the leaves and seeds. Ahmad et al. (2012) also observed a higher antioxidant capacity for milk thistle flower extracts compared to leaves when evaluated with the DPPH method. Milk thistle seeds are known to be a source of antioxidants (Harrabi et al., 2018; Lucini et al., 2016). In our study, the antioxidant capacity of this raw material, measured using the DPPH method, was the highest for extracts in 70% ethanol obtained by Soxhlet extraction. Harrabi et al. (2018) also reported a high antioxidant capacity in milk thistle seed oil measured using the DPPH method. The authors stated that the antioxidant capacity of this plant depended on the maturity of the raw material, where the highest level was observed in the immature seeds, then the intermediate ones, while the lowest was observed in those with full maturity. Polyphenols are plant components of high antioxidant capacity (Klensporf-Pawlik and Przybylski, 2015) and could play an important role in hepatoprotective activity (Madani et al., 2008). In addition, the concentration of polyphenols can highly correlate with the antioxidant capacity, as demonstrated in *Silybum marianum* fruits and seeds (Lucini et al., 2016; Malinowska, 2017). In our study, in most cases, highly significant relationships were found between the antioxidant capacity determined using different methods. No correlation between DPPH and F-C or between FRAP and F-C methods for flower extracts may be due to the different antioxidant compositions of this plant part, other than polyphenols. The DPPH and FRAP methods are used for total antioxidant potential analysis whereas the F-C method is based on polyphenol antioxidants. In our study, the total polyphenol content differed in the individual extracts, which might depend, among other things, on the extraction solvent. The highest concentrations were observed in seeds extracted in acetone using a shaker. The extraction method, the used solvent and its polarity in particular, can affect the effectiveness of the extraction of active substances from plants and, as a consequence, their antioxidant capacity (Drouet et al., 2019; Gawlik-Dziki and Kowalczyk, 2007; Morales et al., 2020; Wianowska and Wiśniewska, 2015). This observation was confirmed by Pawlak and Sielicka (2016). They suggested that the differences in antioxidant capacity of extracts prepared using various extractants are related to their different polarities. These authors found higher contents of phenolic compounds in chokeberry extracts in diluted solvents than in concentrated ones. The most effective extractant in their study was an acetone:water mixture (50:50, v/v). According to Wianowska and Wiśniewska (2015), acetone used to extract silymarin from seeds by Soxhlet extraction was more effective than methanol and ethyl acetate. They highlighted that a significant content of lipids in seeds could be an obstacle to extraction of some active substances. In the present study, a high antioxidant capacity was found in extracts prepared in all the solvents used, except for the extracts in nonpolar petroleum ether, which had a negligible antioxidant capacity. Gawlik-Dziki and Kowalczyk (2007) reported a high content of phenolic compounds in wheat germ extracts in 50% methanol. A higher total polyphenol content in oregano and thyme extracts prepared in undiluted methanol, compared to 70% ethanol and water, was found by Rababah et al. (2010). In their study, extraction was performed at two different temperatures (20°C and 60°C). The higher temperature was more effective for all the solvents used. The other factor which could affect antioxidant capacity is the extraction method. In our study, Soxhlet extraction was found to be the most optimal method in the majority of cases. Such extracts showed a higher antioxidant capacity and total polyphenol content compared to those obtained using other techniques. High results were also observed in some samples prepared by ultrasound assisted extraction, however, a longer extraction time had to be applied and optimization of this parameter seems to be of importance. In our study, the length of the ultrasound-assisted extraction was 15, 30 or 60 min. The highest antioxidant capacity was usually found in a long-lasting process (60 min). However, Drouet et al. (2019) stated that the most favorable extraction time for isolation of active substances from

milk thistle is 45 min, while a longer extraction could lead to degradation of active substances in the raw material. According to Wianowska and Wiśniewski (2015), the content of silymarin in the milk thistle seeds decreased when the extraction time was prolonged from 5 to 20 min. Wang et al. (2008) found that the optimum ultrasound-assisted extraction time to obtain the highest total polyphenol content in wheat bran extracts was 25 min if 64% ethanol was applied at a temperature of 60°C. In our study, different results were obtained. In most cases the higher total polyphenol content was found during the longest extraction (60 min) regardless of the solvent used. However, in our study, extraction was performed at 40°C and this parameter could also have had an impact on the isolation of active ingredients.

CONCLUSION

To sum up, many factors could have an influence on the antioxidant capacity and the total polyphenol content of *Silybum marianum* L. extracts. A properly selected extraction method and the applied solvent seem to be important factors in the effective isolation of active substances and could lead to the more effective application of this valuable plant material.

REFERENCES

Ahmad, N., Fazal, H., Abbasi, B. H., Anwar, S., Basir, A. (2012). DPPH free radical scavenging activity and phenotypic difference in hepatoprotective plant (*Silybum marianum* L.). Toxicol. Ind. Health., 29(5), 460–467. https://doi.org/10.1177/0748233712436637

Andrzejewska, J., Martinelli, T., Sadowska, K. (2015). *Silybum marianum*: non-medical exploitation of the species. Ann. Appl. Biol., 167, 285–297. https://doi.org/10.1111/aab.12232

Chambers, C. S., Holečková, V., Petrásková, L., Biedermann, D., Valentová, K. Buchta, M., Křen, V. (2017). The silymarin composition… and why does it matter??? Food Res. Int., 100(3), 339–353. https://doi.org/10.1016/j.foodres.2017.07.017

Chemat, F., Vian, M. A., Cravotto, G. (2012). Green extraction of natural products: concept and principles. Int. J. Mol. Sci., 13(7), 8615–27. https://doi.org/10.3390/ijms13078615

Cybul, M., Nowak, R. (2008). Przegląd metod stosowanych w analizie właściwości antyoksydacyjnych wyciągów roślinnych [Review of the methods applied to measuring of antioxidant activity of plant extracts]. Herba Pol., 54(1), 68–78 [in Polish].

Drouet, S., Leclerc, E., Garros, L., Tungmunnithum, D., Kabra, A., Abbasi, B. H., …, Hano, Ch. (2019). Green ultrasound-assisted extraction optimization of the natural antioxidant and anti-aging flavonolignans from milk thistle *Silybum marianum* (L.) Gaertn. fruits for cosmetic applications. Antioxidants, 8, 304A. https://doi.org/10.3390/antiox8080304

Florkowska, K., Duchnik, W., Nowak, A., Klimowicz, A. (2018). Właściwości antyoksydacyjne papryki ostrej odmiany Hungarian yellow [The antioxidant activity of Hungarian yellow hot pepper]. Pomeranian J. Life Sci., 64(3), 126–131 [in Polish]. https://doi.org/10.21164/pomjlifesci.455

Gawlik-Dziki, U., Kowalczyk, D. (2007). Wpływ warunków ekstrakcji na aktywność przeciwutleniającą ekstraktów z kiełków rzodkiewki [Influence of extraction conditions on antioxidant activity of extracts from radish sprouts]. Żywn. Nauka Techn. Jakość, 1(50), 132–139 [in Polish].

Harrabi, S., Ferchichi, A., Bacheli, A., Fellach, H. (2018). Policosanol composition, antioxidant and anti-arthritic activities of milk thistle (*Silybium marianum* L.) oil at different seed maturity stages. Lipids Health Dis., 17(1), 82. https://doi.org/10.1186/s12944-018-0682-z

Klensporf-Pawlik, D., Przybylski, R. (2015). Antioxidant activity of selected wild Canadian prairie fruits. Acta Sci. Pol. Technol. Aliment., 14(4), 357–366. https://doi.org/10.17306/J.AFS.2015.4.35

Kumar, S. P. J., Prasad, S. R., Banerjee, R., Agarwal, D. K., Kulkarni, K. S., Ramesh, K. V. (2017). Green solvent and technologies for oil extraction from oilseeds. Chem. Cent. J., 11, 9. https://doi.org/10.1186/s13065-017-0238-8

Lucini, L., Kane, D., Pellizzoni, M., Ferrari, A., Trevisi, E., Ruzickova, G., Arslan, D. (2016). Phenolic profile and *in vitro* antioxidant power of different milk thistle [*Silybum marianum* (L.) Gaertn.] cultivars. Ind. Crop. Prod., 83, 11–16. https://doi.org/10.1016/j.indcrop.2015.12.023

Madani, H., Telebolhosseini, M., Asgary, S., Naderi, G. H. (2008). Hepatoprotective activity of *Silybum marianum* and *Cichorium intybus* against thioacetamide in rat. Pakistan J. Nutr., 7(1), 172–176. https://doi.org/10.3923/pjn.2008.172.176

Malinowska, P. (2017). Antioxidant activity of extracts from plant seeds used in cosmetics products. Tow. Prob. Jakości, 1, 146–153. https://dx.doi.org/10.19202/j.cs.2017.01.14

Morales, M., Zapata, K., Sagaste, C. A., Angulo, A. A., Rojano, B. (2020). Optimization of the ultrasound-assisted extraction of polyphenol, mangiferin, and its antioxidant expression in mango peel (*Mangifera indica*) using response surface methodology. Acta Sci. Pol. Technol. Aliment. 19(1), 5–14. https://doi.org/10.17306/J.AFS.2020.0733

Nowak, A., Maciejewska, A., Duchnik, W., Florkowska, K., Klimowicz, A. (2018). Wpływ rozpuszczalnika na właściwości antyoksydacyjne ekstraktów z zielonej herbaty (*Camellia sinensis* L.) [The effect of a solvent on the antioxidant properties of green tea (*Camellia sinensis* L.) extracts]. Probl. Hig. Epidemiol., 99(3), 245–258 [in Polish].

Nowak, A., Zielonka-Brzezicka, J., Pechaiko, D., Tkacz, M., Klimowicz, A. (2017). Ocena właściwości antyoksydacyjnych liści *Ginkgo biloba* L. po zakończeniu wegetacji [The evaluation of the antioxidant properties of *Ginkgo biloba* L. leaves after the end of the growing season]. Pomeranian J. Life Sci., 63(1), 9–15 [in Polish].

Pawlak, S., Sielicka, M. (2016). Wpływ rozpuszczalnika zastosowanego do ekstrakcji na oznaczoną zawartość związków fenolowych i aktywność przeciwutleniającą wytłoków z aronii [Influence of the solvent used for extraction on the determined content of phenolic compounds and antioxidant activity of chokeberry pomace]. In D. Górecka, E. Pospiech (Eds), Zagospodarowanie ubocznych produktów przemysłu spożywczego (pp. 25–35). Poznań: Wydawnictwo Uniwersytetu Przyrodniczego [in Polish].

Rababah, T., Banat, F., Rababah, A., Ereifej, K., Yang, W. (2010). Optimization of extraction conditions of total phenolics, antioxidant activities, and anthocyanin of oregano, thyme, terebinth, and pomegranate. J. Food Sci., 75(7), 626–632. https://doi.org/10.1111/j.1750-3841.2010.01756.x

Shaker, E., Mahmoud, H., Mnaa, S. (2010). Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. Food Chem. Toxicol., 48, 803–806. https://doi.org/10.1016/j.fct.2009.12.011

Škottová, N., Kazdováb, L., Oliyarnyk, O., Vecera L., Sobolová, L., Ulrichová, J. (2004). Phenolics-rich extracts from *Silybum marianum* and *Prunella vulgaris* reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. Pharmacol Res., 50, 123–130. https://doi.org/10.1016/j.phrs.2003.12.013

Wang, J., Sun, B., Cao, Y., Tian, Y., Li, X. (2008). Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chem., 106, 804–810. http://doi.org/10.1016/j.foodchem.2007.06.062

Wang, L., Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol., 17, 300–312. https://doi.org/10.1016/j.tifs.2005.12.004

Wianowska, D., Wiśniewski, M. (2015). Simplified procedure of silymarin extraction from *Silybum marianum* L. Gaertner. J. Chrom. Sci., 53(2), 366–372. https://doi.org/10.1093/chromsci/bmu049

Zielonka-Brzezicka, J., Nowak, A., Klimowicz, A., Wira, D., Grzesiak, K., Rędzikowska, E., …, Bilska, J. (2018). Aktinidia chińska jako źródło prozdrowotnych antyoksydantów [*Actinidia chinensis* as a source of health-promoting antioxidants]. Probl. Hig. Epidemiol., 99(3), 238–244 [in Polish].