INFLUENCE OF MACERATION, SOLVENT TYPES, AND EXTRACTION DURATIONS ON THE YIELD OF MILK THISTLE SEEDS *(SILYBUM MARIANUM)* EXTRACTION

ORIGINAL SCIENTIFIC PAPER

Halid Makić [1]✉, Samira Hotić [1], Elvisa Hodžić [1], Nenad Stojanović [2], Jasmina Ibrahimpašić [1], Samira Dedić [1], Emina Ćehajić Gradinović [3]

DOI: 10.51558/2232-7568.2023.17.1.67

RECEIVED 2024-06-09 **ACCEPTED** 2024-06-25

[1] Biotechnical Faculty, University of Bihać, Bihać, Bosnia and Herzegovina a
[2] Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina
[3] International Organization for Migration, Bihać, Bosnia and Herzegovina

✉ halidmakicbtf@gmail.com

ABSTRACT:
The study investigated the extraction yield of defatted Silybum marianum seed samples using maceration as the sole extraction technique. Different solvent types (methanol, ethanol, and water) and extraction durations were tested. Prior to extraction, the samples were ground and defatted with n-hexane. For each combination of solvent type, and extraction duration, the extracted mass (g of extract/g of defatted sample) was determined. The impact of each parameter on the yield was analyzed, revealing significant effects. Results showed that water-based maceration for 4 hours yielded the highest average mass of dry extract, followed by shorter durations at 2 hours. Ethanol occasionally outperformed methanol, particularly at the 2-hour mark, but methanol consistently produced lower yields across longer extraction durations. These findings emphasize the need for careful optimization of solvent type and extraction duration to maximize extraction yield. Subsequent analysis using Tukey's HSD test revealed significant differences in dry extract mass among solvents. Water yielded the highest at 2 and 4 hours, ethanol at 4 hours, and methanol at 4 hours as well.

KEYWORDS: Silybum marianum; maceration; solvent types; plant extraction, yield analysis

INTRODUCTION

Classical techniques for solvent extraction of plant compound matrices usually involve choosing a solvent and utilizing agitation, and/or heat. Traditional methods like maceration, Soxhlet extraction, and percolation are common but come with drawbacks such as being time-consuming, requiring large quantities of solvent, and potentially causing degradation of active compounds [1]. The selection of extraction methods varies based on factors such as the plant species or botanical characteristics [2]. For example, maceration is a cost-effective method where plant material is immersed in solvent for extraction [3].

Additionally, different solvents are employed to extract compounds from plants, with extraction outcomes influenced by solvent types [4; 5]. Achieving complete extraction without chemical alteration is essential [6]. Commonly used solvents include water and aqueous blends of ethanol, methanol, and acetone [7]. Studies indicate that aqueous ethanol is more effective than methanol and acetone, while, conversely, water surpasses 80% methanol or 70% ethanol in extracting certain compounds from tea [8;9].

In this study, the extraction dynamics of milk thistle seeds *(Silybum marianum)* seed samples was meticulously analyzed. Focus was placed on conventional maceration using methanol, ethanol, and water as solvents. With varying extraction durations, the aim was to optimize yield and quality. Preceding extraction, samples were ground and defatted with n-hexane. The goal was to discern the optimal combination of parameters for maximum extraction efficiency and quality..

MATERIALS AND METHODS

Milk thistle seeds (*Silybum marianum*) were ground into a fine powder with a particle diameter of 0.4 mm using a blender. The extraction process was conducted in two steps, starting with defatting. The ground sample was weighed and extracted with n-

hexane for 4 hours at room temperature, using a sample-to-solvent ratio of 20 grams of sample to 200 ml of petroleum ether. The samples were then filtered and dried to remove any remaining petroleum ether. For maceration extraction, 10 grams of defatted sample it was weighed into glasses and solvent was added. The samples were extracted with 200 ml of solvent for 2, 4, 6 and 8 hours. The solvents used were ethanol and methanol. After extraction, the solvent was removed using a rotary evaporator (BUCHI Rotavapor R-215) under the following conditions: bath temperature of 60°C, boiling point of 40°C and cooling water temperature of 20°C. The extract was then dried at 40°C to constant weight, and the extraction yield was calculated gravimetrically.

RESULTS AND DISCUSSION

After conducting the measurements, the yield results obtained are presented in Table 1. The highest average yield of milk thistle seeds (*Silybum marianum*) for extraction via maceration in five repetitions using water as a solvent was measured with an exposure time of 4 hours. The yield value was Me=1.0468 grams (g), with a standard deviation of SD=0.0045 g and a standard error of SE=0.0020 g. The 95% confidence interval (CI) for the mean ranged from 1.0411 g to 1.0524 g, with a value range from a minimum of 1.0392 g to a maximum of 1.0511 g. The lowest average yield for this solvent was recorded with an 8-hour exposure, yielding Me=0.510340 g, with SD=0.0182 g, an SE of 0.0081 g, a 95% CI from 0.4876 g to 0.5330 g, and a value range from a minimum of 0.5008 g to a maximum of 0.5430 g (Table 1).

For the ethanol (EtOH) solvent, the highest average yield was achieved with a 4-hour exposure, amounting to Me=0.687200 g with SD=0.0060 g and SE=0.0026 g. The 95% CI for the mean ranged from 0.6797 g to 0.6946 g, with a measured value range in five repetitions from a minimum of 0.6769 g to a maximum of 0.6916 g. The lowest average yield of dry milk thistle seed extract (*Silybum marianum*) using EtOH as a solvent was measured with an 8-hour exposure. The average yield was Me=0.397000 g, with SD=0.0327 g and SE=0.0146 g, while the 95% CI ranged from 0.3563 g to 0.4376 g, with a yield range from a minimum of 0.3635 g to a maximum of 0.4492 g (Table 1).

For maceration extraction using methanol (MtOH) as a solvent, the highest average yield in five repetitions was achieved with a 4-hour exposure. The average yield value was Me=0.8360 g with SD=0.0029 g and SE=0.0013 g, while the 95% CI ranged from 0.8322 g to 0.8397 g, with a value range from a minimum of 0.8312 g to a maximum of 0.8394 g (Table 1). The lowest average yield in five repetitions for this case was measured with a 2-hour extraction, yielding Me=0.4274 g with SD=0.0045 g and SE=0.0020 g, while the 95% CI for the mean was from 0.4217 g to 0.4330 g, with a value range from a minimum of 0.4198 g to a maximum of 0.4308 g (Table 1).

Table 1. Structure of milk thistle seed yields (*Silybum marianum*) during maceration extraction in water, EtOH, and MeOH at exposure times of 2, 4, 6, and 8 hours.

S	ET h	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
						LB	UB		
Water	**2**	5	1.0351	0.0007	0.0003	1.0342	1.0359	1.0344	1.0363
	4	5	1.0468	0.0045	0.0020	1.0411	1.0524	1.0392	1.0511
	6	5	0.9526	0.0136	0.0060	0.9356	0.9695	0.9382	0.9702
	8	5	0.5103	0.0183	0.0081	0.4876	0.5330	0.5008	0.5430
	T	20	0.8862	0.2259	0.0505	0.7804	0.9919	0.5008	1.0511
EtOH	**2**	5	0.6376	0.0023	0.0010	0.6347	0.6405	0.6342	0.6403
	4	5	0.6872	0.0060	0.0026	0.6797	0.6946	0.6769	0.6916
	6	5	0.6467	0.0113	0.0050	0.6326	0.6607	0.6311	0.6621
	8	5	0.3970	0.0327	0.0146	0.3563	0.4376	0.3635	0.4492
	T	20	0.5921	0.1182	0.0264	0.5367	0.6475	0.3635	0.6916
MeOH	**2**	5	0.4274	0.0045	0.0020	0.4217	0.4330	0.4198	0.4308
	4	5	0.8360	0.0029	0.0013	0.8322	0.8397	0.8312	0.8394
	6	5	0.7421	0.0084	0.0037	0.7316	0.7526	0.7317	0.7539
	8	5	0.5490	0.0145	0.0065	0.5309	0.5671	0.5342	0.5650
	T	20	0.6386	0.1642	0.0367	0.5617	0.7155	0.4198	0.8394

Legend: S = solvent, ET = extraction time, h = hours, T = Total, Mean = (Me), Std. Deviation = (SD), Std. Error = (SE), LB = Lower Bound, UB = Upper Bound, Minimum = Min., Maximum = Max.

To evaluate the influence of solvent type and extraction time on the yield of dry mass of milk thistle (*Silybum marianum*) seed extract as the dependent variable, a two-way analysis of variance (ANOVA) of different groups was applied to examine the individual and combined effects of extraction time and solvent type as independent variables on the mean values of dry mass extract as the dependent variable. Preliminary analysis examined the conditions for the application of two-way ANOVA. The assumption of normal distribution was not significantly violated, and Levene's Test of Equality of Error Variances showed statistical significance: F (11, 48) =2.934, Sig.=0.005, indicating a violation of the assumption of variance homogeneity, thus requiring a stricter significance level of p = 0.05. A significance level of p = 0.01 is used for further analysis. The impact of solvent selection and extraction time on yield value was examined using two-way ANOVA of different groups, with extraction time in four time intervals: 2, 4, 6, and 8 hours (Table 2).

During extraction, a statistically significant main effect of solvent selection on the average value of dry extract mass was found: F (2, 48) = 2860.18, p = 0.000, with an effect size indicator of eta squared of 0.992, characterized as a strong effect. The main effect of extraction time on the average value of dry extract mass also proved to be statistically significant at the significance level of p = 0.01; F(3, 48) = 2201.28, Sig = 0.000, with an effect size that can be classified as large, partial eta squared is 0.993 (Table 2).

Table 2. Results of the application of two-way analysis of variance of different groups.

Source	SS	DF	MS	F	Sig.	Partial Eta Squared
CM	2.740[a]	11	0.249	1425.71	0.000	0.997
I	29.87	1	29.878	171004.60	0.000	1.000
OT	0.999	**2**	0.500	**2860.18**	**0.000**	**0.992**
VR	1.154	**3**	0.385	**2201.28**	**0.000**	**0.993**
OT * VR	0.587	**6**	0.098	**559.77**	**0.000**	**0.986**
Error	0.008	**48**	0.000			
a). R Squared = 0.997 (Adjusted R Squared =0 .996)						

Legend: SS = Sum of Squares CM = Corrected Model, I = Intercept, DF = degrees of freedom, MS = Mean Square, F-test, Sig. = p-value, Partial Eta Squared = Partial eta squared, Adjusted R Squared = Adjusted R squared.

The interaction of solvent type and extraction time was statistically significant; F (6, 48) = 559.77, p = 0.000. (Table 2) As the statistical significance of the interaction between solvent type, extraction time, and the average value of dry extract mass was determined, further exploration through subsequent tests is warranted. Therefore, we further conducted an analysis of simple effects by dividing the sample into groups and considering the dependence of the extracted mass of dry extract on the type of solvent used in:

- Group **g1**-when extraction was performed after 2 hours;
- Group **g2**-when extraction was performed after 4 hours;
- Group **g3**-when extraction was performed after 6 hours;
- Group **g4**-when extraction was performed after 8 hours.

[1]Cohen, J. W. (1988). Statistical power analysis for the behavioral sciences (2nd edn). Hillsdale, N: Lawrence Erlbaum Associates. According to Cohen's criteria, if the partial eta squared (r) is: (r = 0.1), the effect is small; (r = 0.3), the effect is medium; (r = 0.5) or higher, the effect is large.

The conditions for the application of one-way analysis were not violated, so a statistically significant difference was found, at a significance level of p=0.01, in the average value of the mass of extracted dry extract between solvents (water, EtOH, and MeOH). Subsequent comparisons in group g1 - extraction by maceration after 2 hours using the Tukey's HSD test showed that the actual differences in average mass of extracted dry extract, extracted after 2 hours, when water was used as the solvent (Me=1.0351, SD=0.0007), significantly differed from the average mass of dry extract when EtOH was the extraction solvent (Me=0.6376 g, SD=0.0023 g), with a mean difference, R=0.3974 g, Sig=0.000, as well as when MeOH was the solvent (Me=0.4274 g, SD=0.0045 g) with a mean difference R=0.6077 g, Sig=0.000, at a significance level of p = 0.01. (Table 3, Table 4, Figure 2).

Furthermore, the Tukey's HSD test showed that the average value of dry extract mass obtained using ethanol (Me=0.6376 g, SD=0.0023 g) significantly differed from the average mass of extracted dry extract

when methanol was used as the solvent, (Me=0.4274 g, SD=0.0045 g), with a mean difference R=0.2102 g, Sig. = 0.000, at a significance level of p = 0.01 in extraction conducted by maceration (Table 3, Table 4, Figure 1).

Table 3. Descriptive statistics of extracted dry extract mass after 2 hours of maceration extraction.

S	N	Me	SD	SE	95% CI Interval for Mean		Min.	Max.
					LB	UB		
Water	5	1.0351	0.0007	0.0003	1.0342	1.0359	1.0344	1.0363
EtOH	5	0.6376	0.0023	0.0010	0.6347	0.6405	0.6342	0.6403
MeOH	5	0.4274	0.0045	0.0020	0.4217	0.4330	0.4198	0.4308
Total	15	0.7000	0.2608	0.0673	0.5556	0.8445	0.4198	1.0363
ANOVA				**F(2,14)=54255,24,**			**Sig.=0.000**	

Legend: S = Solvent, Me = Mean, SD = Standard Deviation, SE = Standard Error, 95% Confidence Interval for Mean = 95% CI, Min = Minimum, Max = Maximum

Table 4. Results of post-hoc comparison of average mass of dry extract using Tukey's HSD test for actual differences in extraction after 2 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
Water	EtOH	0.3974*	0.0018	0.000	0.3907	0.4041
	MeOH	0.6077*	0.0018	0.000	0.6010	0.6143
EtOH	Water	-0.3974*	0.0018	0.000	-0.4041	-0.3907
	MeOH	0.2102*	0.0018	0.000	0.2035	0.2169
MeOH	Water	-0.6077*	0.0018	0.000	-0.6143	-0.6010
	EtOH	-0.2102*	0.0018	0.000	-0.2169	-0.2035
***. The mean difference is significant at the p=0.01 level.**						

Legend: S = solvent, MD = Mean Difference, Average Difference, SE = Standard Error, Sig. = Significance, 99% CI = 99% Confidence Interval, 99% Confidence Interval for Mean Difference.

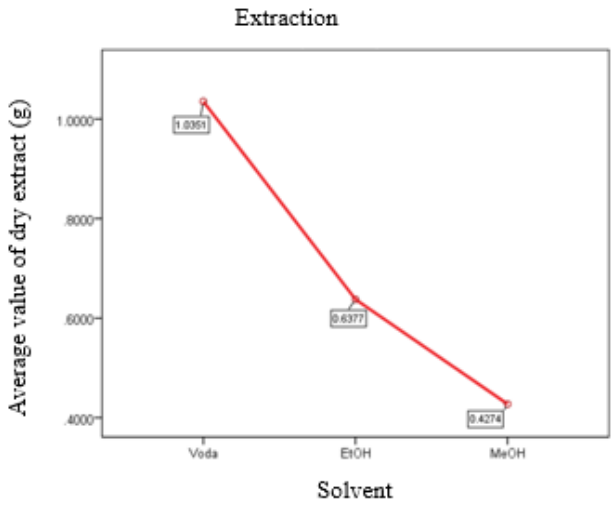

Fig. 1. Average values of extracted dry mass obtained through maceration extraction relative to the type of extraction solvent implemented after 2 hours.

Subsequent comparison within group g2 - maceration extraction after 4 hours using Tukey's HSD test for real differences showed that the average value of extracted dry mass when water was used as the extraction solvent (Mean=1.0468, SD=0.0045) significantly differs from the average dry mass value when ethanol (EtOH) was used as the solvent; (Mean=0.6872 g, SD=0.0060), with an average difference, R=0.3596 g, Sig.=0.000 and when methanol (MeOH) was the solvent; (Mean= 0.8360 g, SD=0.0029) with an average difference R= 0.2108 g, Sig=0.000, at a significance level of p = 0.01. (Table 5, Table 6, Figure 3).

Furthermore, subsequent comparison of the average values of extracted dry mass obtained using ethanol (Mean= 0.6872 g, SD=0.0060) significantly differs from the average value of extracted dry mass obtained using methanol as the extraction solvent (Mean= 0.8360 g, SD=0.0029) with an average difference R=-0.1488 g, Sig.=0.000, at a significance level of p = 0.01 in the maceration extraction method (Table 5, Table 6, Figure 2).

Table 5. Descriptive indicators of extracted dry mass of extract after 4 hours of maceration extraction.

S	N	Me	SD	SE	95% CI Interval for Mean		Min.	Max.
					LB	UB		
Water	5	1.0468	0.0045	0.0020	1.0411	1.0524	1.0392	1.0511
EtOH	5	0.6872	0.0060	0.0026	0.6797	0.6946	0.6769	0.6916
MeOH	5	0.8360	0.0029	0.0013	0.8322	0.8397	0.8312	0.8394
Total	15	0.8566	0.1527	0.0394	0.7720	0.9412	0.6769	1.0511
ANOVA				**F(2,14)= 7441.507,**		**Sig.=0.000**		

Legend: S = solvent, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI = 95% Confidence Interval for Mean, Min. = Minimum, Max. = Maximum

Table 6. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction after 4 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% CI Confidence Interval	
					LB	UB
Water	EtOH	0.3596*	0.0029	0.000	0.3490	0.3701
	MeOH	0.2108*	0.0029	0.000	0.2002	0.2213
EtOH	Water	-0.3596*	0.0029	0.000	-0.3701	-0.3490
	MeOH	-0.1488*	0.0029	0.000	-0.1593	-0.1382
MeOH	Water	-0.2108*	0.0029	0.000	-0.2213	-0.2002
	EtOH	0.1488*	0.0029	0.000	0.1382	0.1593
***. The mean difference is significant at the 0.01 level.**						

Legend: S = solvent, MD = Mean Difference, Average difference SE = Std. Error, Standard Error Sig. = Significance 99% CI = 99% Confidence Interval.

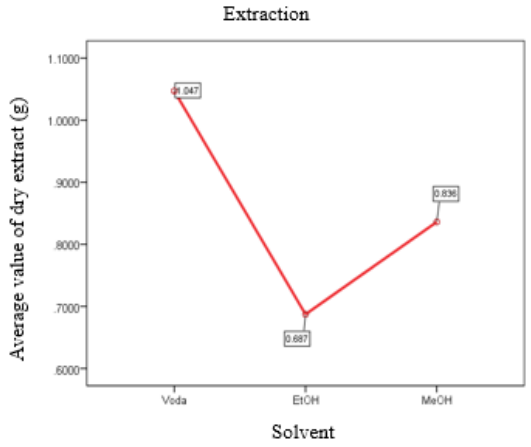

Fig. 2. Average values of extracted dry mass through maceration extraction relative to the type of extraction solvent implemented after 4 hours.

Subsequent comparison within group g3 - maceration extraction after 6 hours using Tukey's HSD test for real differences in the average values of extracted dry mass showed that the average dry mass value extracted by soaking in water (Mean=0.9526, SD=0) significantly differs from the average dry mass value extracted by ethanol (Me=0.6467, SD=0.0113), with an average difference, R=0.3059 g, Sig.=0.000, as well as from the average dry mass value extracted by methanol (Me= 0.7421, SD=0.0084) with an average difference R= 0.2104 g, Sig=0.000, at a significance level of p=0.01. (Table 7, Table 8, Figure 4). Furthermore, subsequent comparison of the average values of extracted dry mass obtained using ethanol (Me= 0.6467, SD=0.01133) significantly differs from the average value of extracted dry mass obtained using methanol as the extraction solvent (Me= 0.7421, SD=0.0084) with an average difference R=-0.0954800 grams, Sig.=0.000, at a significance level of p = 0.01 in the maceration extraction method (Table 7, Table 8, Figure 3).

Table 7. Descriptive indicators of extracted dry mass of extract after 6 hours of maceration extraction method.

E	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					LB	UB		
Water	5	0.9526	0.0136	0.0060	0.9356	0.9695	0.9382	0.9702
EtOH	5	0.6467	0.0113	0.0050	0.6326	0.6607	0.6311	0.6621
MeOH	5	0.7421	0.0084	0.0037	0.7316	0.7526	0.7317	0.7539
Total	15	0.7804	0.1326	0.0342	0.7070	0.8539	0.6311	0.9702
ANOVA			**F(2,14)= 952.649,**		**Sig.=0.000**			

Legend: E - Extraction, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI - 95% Confidence Interval for Mean, Min - Minimum, Max - Maximum

Table 8. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction method after 6 hours.

(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
1 Water	2 EtOH	0.3059*	0.0071	0.000	0.2803	0.3314
	3 MeOH	0.2104*	0.0071	0.000	0.1848	0.2360
2 EtOH	1 Water	-0.3059*	0.0071	0.000	-0.3314	-0.2803
	3 MeOH	-0.0954*	0.0071	0.000	-0.1210	-0.0698
3 MeOH	1 Water	-0.2104*	0.0071	0.000	-0.2360	-0.1848
	2 EtOH	0.0954*	0.0071	0.000	0.0698	0.1210

***. The mean difference is significant at the 0.01 level.**

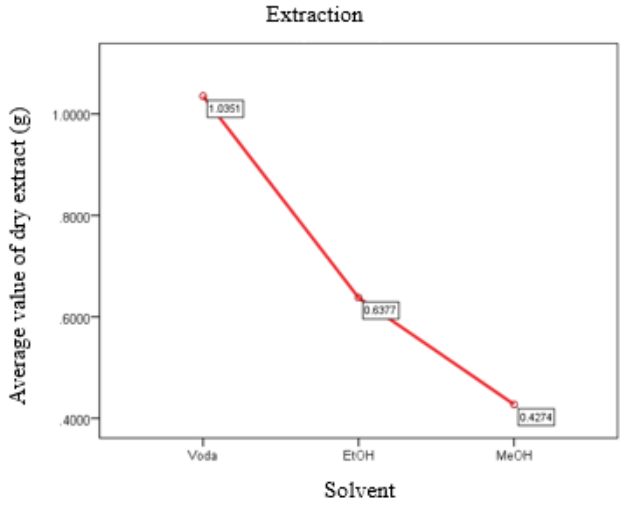

Fig. 3. Average values of extracted dry mass in relation to the type of extraction solvent used after 6 hours.

Subsequent comparison within group g4 - maceration extraction after 8 hours using Tukey's HSD test for real differences showed that the average dry mass value of Milk thistle extract extracted after 8 hours of soaking in water (Me= 0.5103, SD=0.0182) significantly differs from the average dry mass value extracted by ethanol (Me= 0.397000, SD=0.0327) with an average difference, R=0.1133, Sig.= 0.000, at a significance level of p = 0.01, **but does not significantly differ** from the average dry mass extracted by methanol (Me= 0.5490, SD=0.0145) (Table 9, Table 10, Figure 5). Furthermore, the average dry mass of extract obtained by soaking in ethanol (Me= 0.3970, SD=0.0327) significantly differs from the average dry mass obtained by soaking in methanol (Me= 0.5490, SD=0.0145), with an average difference R= 0.1520, Sig.= 0.000, at a significance level of p = 0.01 (Table 9, Table 10, Figure 4).

Table 9. Descriptive indicators of extracted dry mass of extract after 8 hours of maceration extraction.

E	N	Me	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					LB	UB		
Water	5	0.5103	0.0182	0.0081	0.4876	0.5330	0.5008	0.5430
EtOH	5	0.3970	0.0327	0.0146	0.3563	0.4376	0.3635	0.4492
MeOH	5	0.5490	0.0145	0.0065	0.5309	0.5671	0.5342	0.5650
Total	15	0.4854	0.0701	0.0181	0.4466	0.5243	0.3635	0.5650
ANOVA					**F(2,14)= 57.850,**		**Sig.=0.000**	

Legend: E - Extraction, Me - Mean, SD - Standard Deviation, SE - Standard Error, 95% CI - 95% Confidence Interval for Mean, Min - Minimum, Max - Maximum

Table 10. Results of subsequent comparison of the average dry mass of extract using Tukey's HSD test for real differences in maceration extraction after 8 hours.

Tukey HSD						
(I) S	(J) S	MD (I-J)	SE	Sig.	99% Confidence Interval	
					LB	UB
Water	EtOH	0.1133*	0.0146	0.000	0.0609	0.1657
	MeOH	-0.0387	0.0146	0.053	-0.0911	0.0137
EtOH	Water	-0.1133*	0.0146	0.000	-0.1657	-0.0609
	MeOH	-0.1520*	0.0146	0.000	-0.2044	-0.0996
MeOH	Water	0.0387	0.0146	0.053	-0.0137	0.0911
	EtOH	0.1520*	0.0146	0.000	0.0996	0.2044
*. The mean difference is significant at the 0.01 level.						

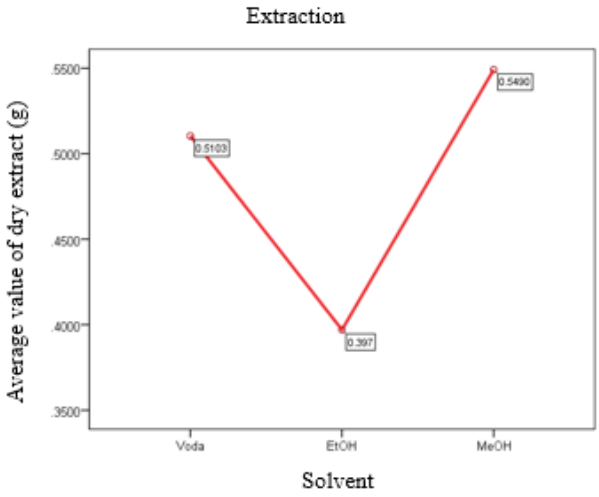

Fig. 4. The average values of extracted dry mass through maceration extraction relative to the type of extraction solvent used after 8 hours.

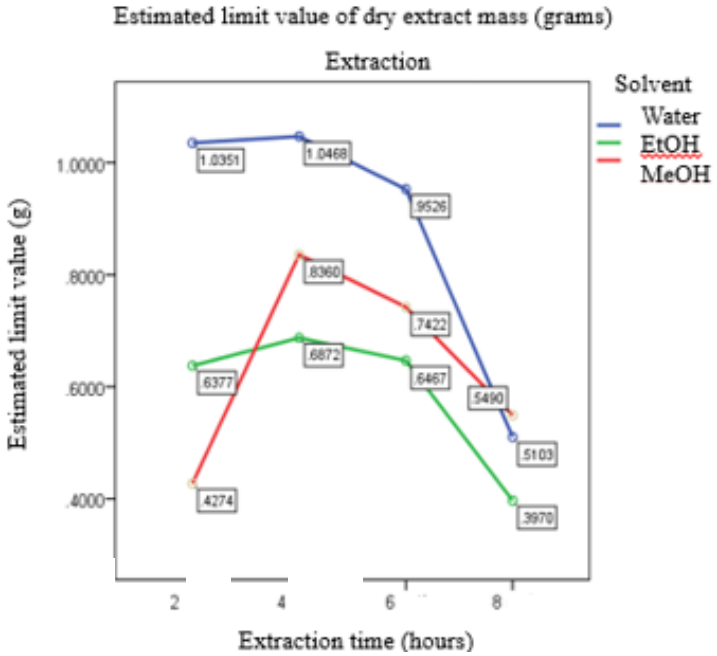

Figure 5. Average values of extracted dry mass of extract according to the type of solvent in relation to the extraction time.

Table 11. Results of applying ANOVA in assessing the influence of extraction time on the average extracted dry mass of Milk thistle in relation to the type of solvent used in the maceration extraction method.

Extraction Time	Solvent Type		
	G1: (Water)	G2:(EtOH)	G3: (MeOH)
2 hours			
4 hours	F(3,19)= 2383.36	F(3,19)= 279.94	F(3,19)= 2181.61
6 hours	Sig.=0.000	Sig.=0.000	Sig.=0.000

Table 12. Average values of extracted dry mass of homogeneous subsets grouped by subsequent comparison using Tukey's HSD test for real differences, isolated when the extraction was conducted using the maceration method and water and ethanol were used as solvents, at a significance level of p=0.01.

ET	N	Water			ET	Ethanol (EtOH)		
		Subset for alpha = 0.01				Subset for alpha = 0.01		
		1	2	1		1	2	3
8	5	**0.5103**			8	0.3970		
6	5		**0.9526**		2		0.6376	
2	5			**1.0351**	6		0.6467	0.6467
4	5			**1.0468**	4			0.6872
S		1.000	1.000	0.412	S	1.000	0.849	0.011

Legend: ET-Extraction Time, S = Sig.,

Table 13. Average values of extracted dry mass of homogeneous subsets grouped by subsequent comparison using Tukey's HSD test for real differences, isolated when the extraction was conducted using the maceration method and methanol was used as the solvent, at a significance level of p=0.01.

Extraction Time hours	Methanol (MeOH)			
	Subset for alpha = 0.01			
	1	2	3	4
2 hours	**0.4274**			4
8 hours		0.5490		
6 hours			0.7421	
4 hours				0.8360
Sig.	**1.000**	1.000	1.000	1.000

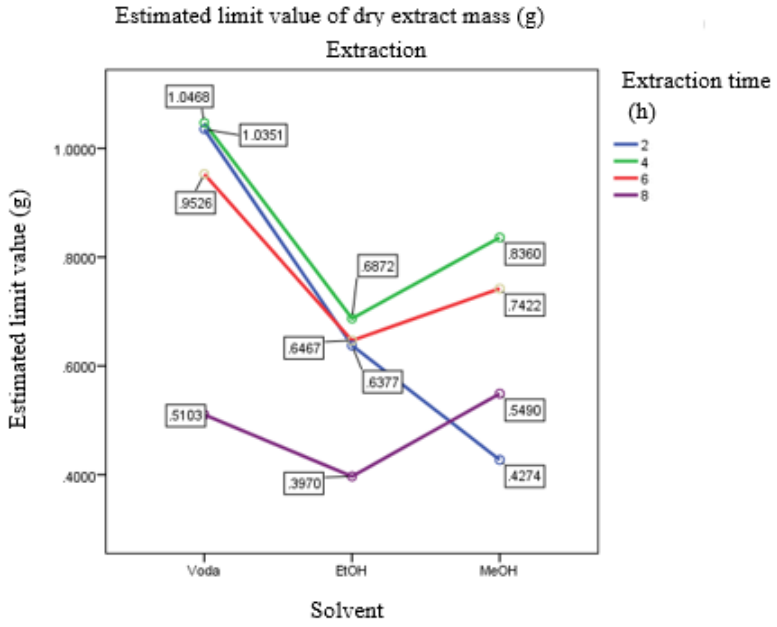

Fig. 6. Average values of extracted dry mass of Milk thistle extract according to extraction time in relation to the extraction solvent.

Table 14. Results of subsequent comparison of the average dry mass of extractusing Tukey's HSD test for real differences in maceration extraction according to the type of solvent and exposure time.

S	(I) ET h	(J) ET h	MD (I-J)	SE	Sig.	99% Confidence Interval	
						LB	UB
Water	2	**4**	**-0.0117**	**0.0073**	**0.412**	-0.0387	0.0153
		6	0.0825*	0.0073	0.000	0.0554	0.1095
		8	0.5247*	0.0073	0.000	0.4977	0.5517
	4	**2**	**0.0117**	**0.0073**	**0.412**	-0.0153	0.0387
		6	0.0942*	0.0073	.000	0.0671	0.1212
		8	0.5364*	0.0073	0.000	0.5094	0.5634
	6	2	-0.0825*	0.0073	0.000	-0.1095	-0.0554
		4	-0.0942*	0.0073	0.000	-0.1212	-0.0671
		8	0.4422*	0.0073	0.000	0.4152	0.4692
	8	2	-0.5247*	0.0073	0.000	-0.5517	-0.4977
		4	-0.5364*	0.0073	0.000	-0.5634	-0.5094
		6	-0.4422*	0.0073	0.000	-0.4692	-0.4152
EtOH	2	4	-0.0495*	0.0111	0.002	-0.0904	-0.0086
		6	**-0.0090**	**0.0111**	**0.849**	-0.0499	0.0318
		8	0.2406*	0.0111	0.000	0.1997	0.2815
	4	2	0.0495*	0.0111	0.002	0.0086	0.0904
		6	**0.0405**	**0.0111**	**0.011**	-0.0004	0.0814
		8	0.2902*	0.0111	0.000	0.2492	0.3311
	6	**2**	**0.0090**	**0.0111**	**0.849**	-0.0318	0.0499
		4	-0.040	0.0111	0.011	-0.0814	0.0004
		8	0.2497*	0.0111	0.000	0.2087	0.2906
	8	2	-0.2406*	0.0111	0.000	-0.2815	-0.1997
		4	-0.2902*	0.0111	0.000	-0.3311	-0.2492
		6	-0.2497*	0.0111	0.000	-0.2906	-0.2087
MeOH	2	4	-0.4086*	0.0055	0.000	-0.4291	-0.3880
		6	-0.3147*	0.0055	0.000	-0.3353	-0.2942
		8	-0.1216*	0.0055	0.000	-0.1421	-0.1011
	4	2	0.4086*	0.0055	0.000	.03880	0.4291
		6	0.0938*	0.0055	0.000	0.0732	0.1143
		8	0.2869*	0.0055	0.000	0.2664	0.3074
	6	2	0.3147*	0.0055	0.000	0.2942	0.3353
		4	-0.0938*	0.0055	0.000	-0.1143	-0.0732
		8	0.1931*	0.0055	0.000	0.1726	0.2136
	8	2	0.1216*	0.0055	0.000	0.1011	0.1421
		4	-0.2869*	0.0055	0.000	-0.3074	-0.2664
		6	-0.1931*	0.0055	0.000	-0.2136	-0.1726

Legend: S = solvent, ET = Extraction Time, h = hours,

CONCLUSION

Based on the conducted comparisons, we conclude that the highest average mass of dry extract extracted by **maceration** was achieved by **soaking in water**, with an extracted mass in five repetitions after **4 hours** averaging Me=1.0468 **g**, followed by extraction after 2 hours with an average extracted mass of **Me=1.0351 g**, and extraction after 6 hours with an average extracted mass of Me=0.9526 g (Table 1, Figure 5).
When extraction was performed after 8 hours, the average mass of dry extract extracted in five repetitions with **methanol** as the solvent, Me=0.5490, does not significantly differ from the average mass of dry extract extracted by soaking in **water**, Me=0.5103 (Table 1, Figure 5). In maceration extraction performed after 2 hours, the average mass of dry extract of milk thistle seeds (*Silybum marianum*) extracted using ethanol, Me=0.6377, was higher than that extracted by methanol, Me=0.4274 g, while the lowest yield by maceration occurred using methanol for extraction after 4, 6, and 8 hours, with average yield values ranging from Me=0.6872 g to Me=0.3970 g (Table 1, Figure 5).

Subsequent comparison using Tukey's HSD test revealed that the average values of dry extract mass extracted in five repetitions using the following solvents:

- *water* significantly differ from each other and can be grouped into three homogeneous groups. One group consists of the extracted mass Me=0.5103 g after 8 hours, the second group consists of the average mass of Me=0.9526 g extracted after 6 hours, and the third group consists of the average values of Me=1.0351 grams extracted after 2 hours and Me=1.0468 grams extracted after 4 hours, where the highest average yield was achieved, at the level of statistical significance p=0.01 (Table 12, Table 14, Figure 6).
- *ethanol* significantly differ from each other and can be grouped into three homogeneous groups. One group consists of the average mass of dry extract extracted after 8 hours: Me=0.3970 g, the second group consists of the extracted dry extract masses of milk thistle after 2 and 6 hours: Me=0.6376 and Me=0.6467 g, and the third group consists of the average values of dry extract mass extracted after 6 hours: Me=0.6467 g and 4 hours: Me=0.6872 g, where the highest average value for ethanol was obtained, at the significance level p=0.001 (Table 12, Table 14, Figure 6).
- *methanol* significantly differ from each other and can be grouped into four homogeneous groups. Each group consists of the average mass of dry extract of milk thistle extracted in the following order: after 2 hours Me=0.4274, after 8 hours: Me=0.5490 g, after 6 hours: Me=0.7421, and after 4 hours, Me=0.8360 g where the highest yield was achieved (Table 13, Table 14, Figure 6).

REFERENCES

[1] M. D. L. de Castro, L. E. Garcia-Ayuso, „Soxhlet extraction of solid materials: an outdated technique with a promising innovative future", Anal. Chim. Acta 369, 1-10, 1998.

[2] M. Patrascu, M. Radoiu, „Rose Essential Oil Extraction from Fresh Petals Using Synergetic Microwave & Ultrasound Energy: Chemical Composition and Antioxidant Activity", Assessment Journal of Chemistry and Chemical Engineering, 10, 136-142, 2016.

[3] M. I. N. Njila, E. Mahdi, D. M. Lembe, Z. Nde, D. Nyonseu, „Review on Extraction and Isolation of Plant Secondary Metabolites", 7th International Conference on Agricultural, Chemical, Biological and Environmental Sciences, Kuala Lumpur (Malaysia), 67-72, 2017.

[4] M. Pinelo, M. Rubilar, J. Sineiro, M. J. Nunez, „Extraction of antioxidant phenolics from almond hulls (Prunus amygdalus) and pine sawdust (Pinus pinaster)", Food Chemistry, 85, 267-273, 2004.

[5] A. H. Goli, M. Barzegar, M. A. Sahari, „Antioxidant activity and total phenolic compounds of pistachio (Pistachia vera) hull extracts", Food Chemistry, 92, 521-525, 2004.

[6] Y. Zuo., H. Chen, Y. Deng, „Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-erh teas using HPLC with photodiode array detector", Talanta, 57, 307-316, 2002.

[7] T. Sun, C. Ho, „Antioxidant activities of buckwheat extracts", Food Chemistry, 90, 743-749, 2005.

[8] H. Wang, K. Helliwell, „Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography", Food Research International, 34, 223-227, 2001.

[9] S. Khokhar, S. G. M. Magnusdottı´, „Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom", Journal of Agricultural and Food Chemistry, 50, 565-570, 2002.