Optimization of a Green Ultrasound-Assisted Extraction of the Natural Antioxidant and Anti-aging Flavonolignans from Milk Thistle *Silybum marianum* (L.) Fruits with Potent Cosmetic Applications

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Supplementary Materials

Figure S1 Biplot representation of the linear relation between predicted *vs* measured SILM contents in the 27 sample extracts. Light blue contours represented $p = 0.05$.

Figure S2 3D plots from the model predicted TAX extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Figure S3 3D plots from the model predicted SILC extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Figure S4 3D plots from the model predicted SILD extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Figure S5 3D plots from the model predicted SILA extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Figure S6 3D plots from the model predicted SILB extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Figure S7 3D plots from the model predicted ISILA extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.
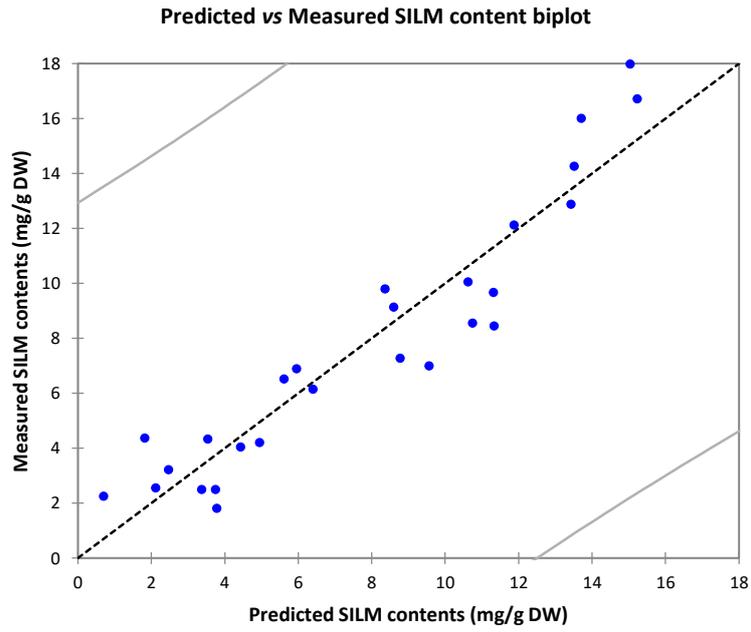
Figure S8 3D plots from the model predicted ISILB extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.

Table S1 Results of full factorial design experiments for the extraction of TAX, SILC, SILD, SILA, SILB, ISILA and ISILB from mature fruits of *Silybum marianum*.

Table S2 Values, standard deviations and statistical analysis of the regression coefficients for the TAX, SILC, SILD, SILA, SILB, ISILA and ISILB extraction yield from mature fruits of *Silybum marianum* as a function of the 3 different variables (X1: ethanol concentration, X2: ultrasound frequency and X3: extraction duration).

Table S3 ANOVA results of the TAX, SILC, SILD, SILA, SILB, ISILA and ISILB extraction models.

Table S4 Individual antioxidant and anti-aging activities of the 27 US extract samples.

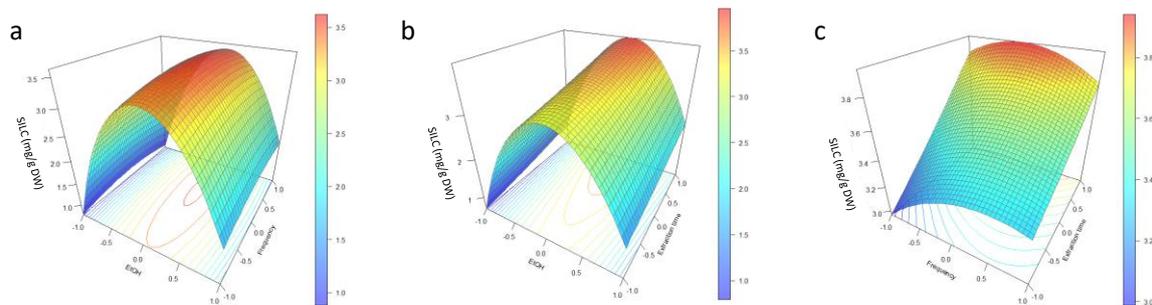**Predicted *vs* Measured SILM content biplot**

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53   **Figure S1** Biplot representation of the linear relation between predicted *vs* measured SILM contents in the 27 sample
54   extracts. Light blue contours represented *p* = 0.05.
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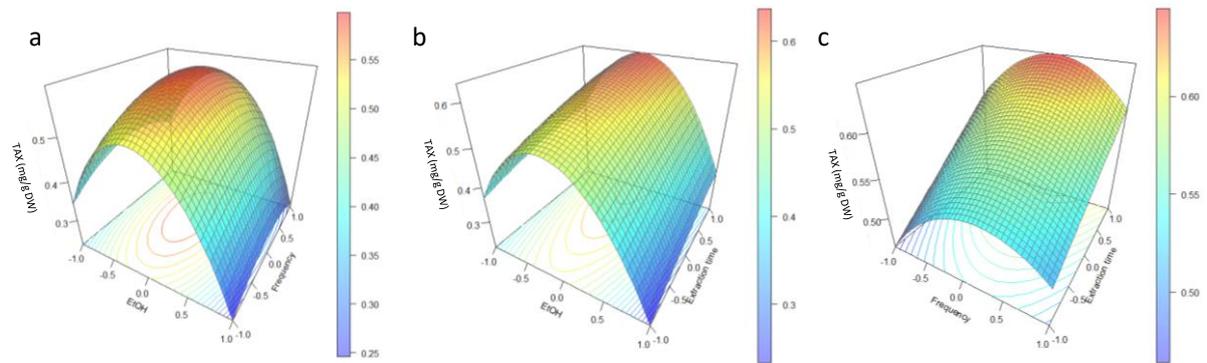
Figure S1 3D plots from the model predicted TAX extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.
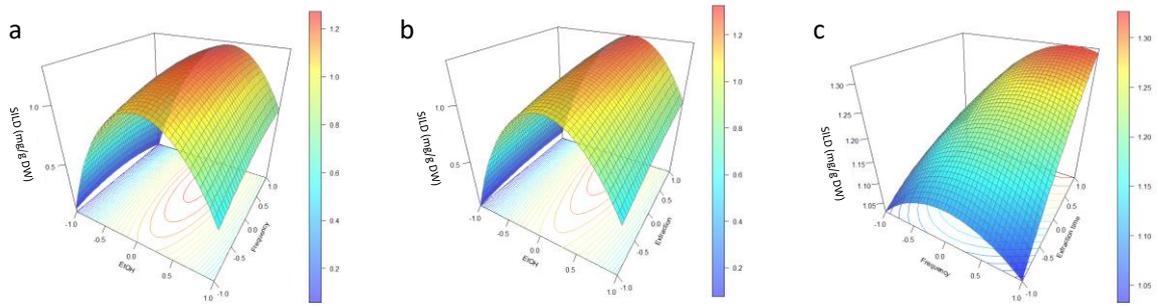
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Figure S3 3D plots from the model predicted SILC extracted quantities from mature fruits of *Silybum marianum* as
a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration,
and (c) ultrasound frequency and extraction duration.

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67 **Figure S4** 3D plots from the model predicted SILD extracted quantities from mature fruits of *Silybum marianum* as
68 a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration,
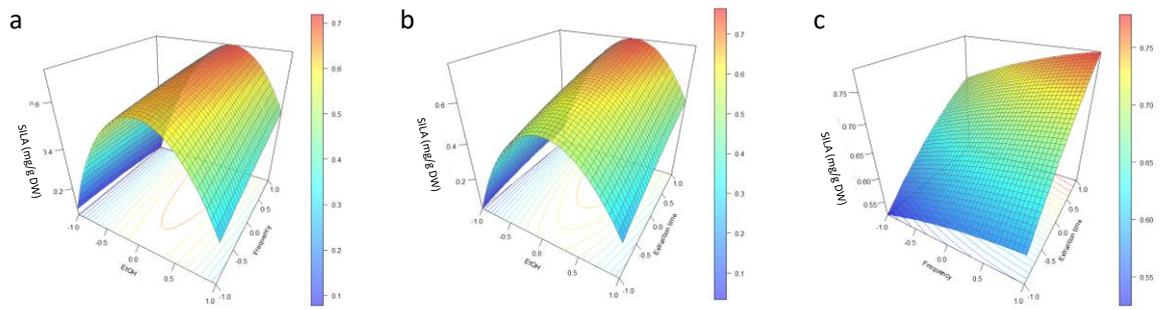69 and (c) ultrasound frequency and extraction duration.
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Figure S5 3D plots from the model predicted SILA extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.
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Figure S6 3D plots from the model predicted SILB extracted quantities from mature fruits of *Silybum marianum* as
a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration,
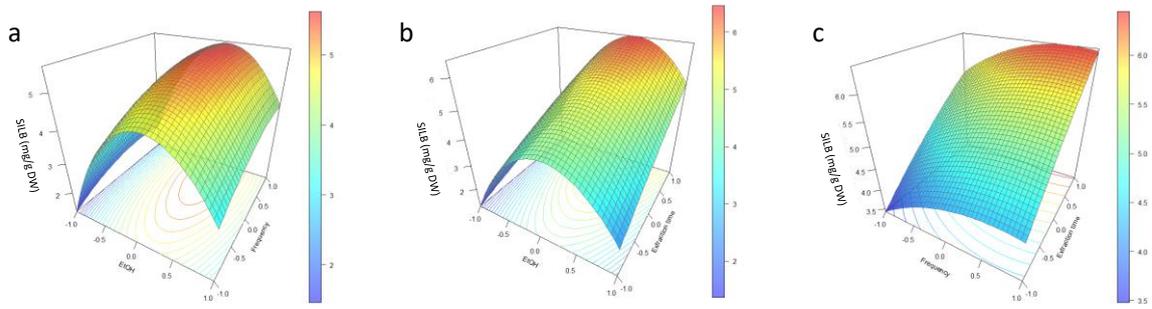and (c) ultrasound frequency and extraction duration.
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Figure S7 3D plots from the model predicted ISILA extracted quantities from mature fruits of *Silybum marianum* as a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration, and (c) ultrasound frequency and extraction duration.
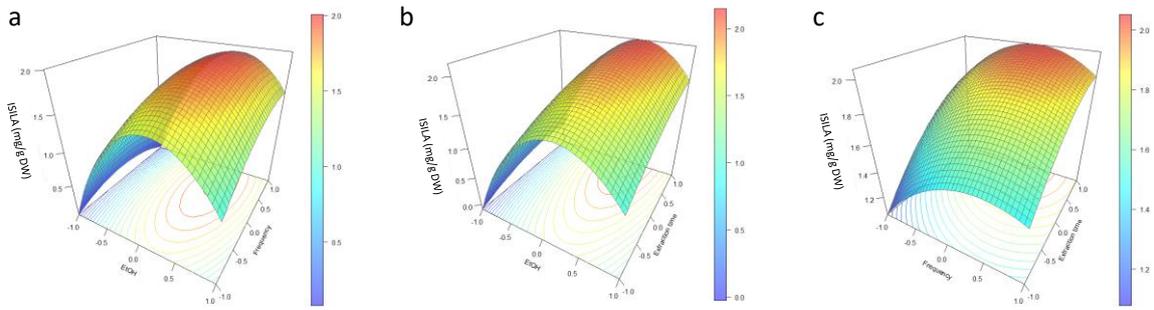
85
86 **Figure S8** 3D plots from the model predicted ISILB extracted quantities from mature fruits of *Silybum marianum* as
87 a function of (a) ethanol concentration and ultrasound frequency, (b) ethanol concentration and extraction duration,
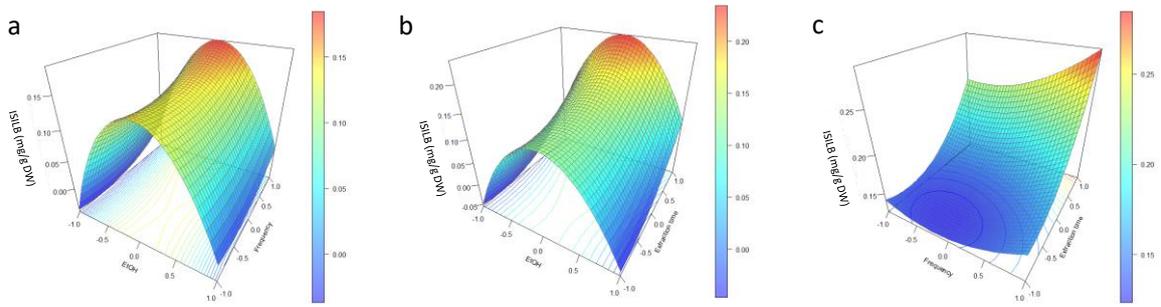88 and (c) ultrasound frequency and extraction duration.

89 **Table S1** Results of full factorial design experiments for the extraction of TAX, SILC, SILD, SILA, SILB, ISILA and
90 ISILB from mature fruits of *Silybum marianum*
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Run ID	TAX	SILC	SILD	SILA	SILB	ISILA	ISILB
Run ID#1	0.24	0.06	0.46	0.19	1.29	nd	nd
Run ID#2	0.39	1.03	2.43	0.33	2.20	0.61	nd
Run ID#3	0.16	0.39	1.36	0.16	1.80	0.45	nd
Run ID#4	0.43	0.05	0.62	nd	1.46	nd	nd
Run ID#5	0.51	1.05	2.69	0.42	3.65	1.16	0.17
Run ID#6	0.21	0.59	1.59	0.30	2.66	1.16	nd
Run ID#7	0.40	nd	1.71	0.12	2.14	nd	nd
Run ID#8	0.42	0.74	2.97	0.44	2.79	1.05	0.04
Run ID#9	0.25	0.55	1.43	0.33	3.16	1.14	0.03
Run ID#10	0.46	0.05	1.07	0.07	1.57	nd	nd
Run ID#11	0.56	0.93	3.77	0.64	4.56	1.53	0.14
Run ID#12	0.25	0.42	1.51	0.27	2.87	0.81	nd
Run ID#13	0.30	0.04	0.40	0.01	1.06	nd	nd
Run ID#14	0.66	1.20	4.06	0.69	5.79	1.86	nd
Run ID#15	0.26	0.84	2.24	0.47	4.33	1.66	nd
Run ID#16	0.42	nd	nd	nd	2.07	nd	nd
Run ID#17	0.43	1.39	3.23	0.65	5.35	1.62	0.20
Run ID#18	0.26	0.73	1.75	0.49	4.08	1.79	0.04
Run ID#19	0.27	0.01	0.83	0.04	1.35	nd	nd
Run ID#20	0.64	1.31	4.21	0.85	6.59	2.14	0.25
Run ID#21	0.25	0.46	1.47	0.28	3.66	1.04	0.10
Run ID#22	0.54	nd	1.51	0.12	2.03	nd	nd
Run ID#23	0.65	1.24	4.06	0.75	7.27	2.43	0.31
Run ID#24	0.30	0.88	1.94	0.48	4.87	1.59	nd
Run ID#25	0.38	nd	1.06	nd	2.60	nd	nd
Run ID#26	0.68	1.52	4.14	1.09	7.52	2.49	0.55
Run ID#27	0.27	0.72	1.96	0.37	4.21	1.01	nd

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93 Values are the means of 3 independent replicates expressed in mg/g DW. nd: not detected.

94 **Table S2** Values, standard deviations and statistical analysis of the regression coefficients for the TAX, SILC, SILD,
95 SILA, SILB, ISILA and ISILB extraction yield from mature fruits of *Silybum marianum* as a function of the 3 different
96 variables (X1: ethanol concentration, X2: ultrasound frequency and X3: extraction duration).
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Source	TAX	SILC	SILD	SILA	SILB	ISILA	ISILB
Constant	0.59^{***}	1.23^{***}	3.60^{***}	0.67^{***}	5.40^{***}	1.89^{***}	0.14^{*}
X_1	-0.068^{**}	0.30^{***}	0.42^{*}	0.14^{***}	0.89^{***}	0.59^{***}	0.01^{ns}
X_2	0.015^{ns}	0.05^{ns}	0.06^{ns}	0.04^{ns}	0.45^{*}	0.14^{ns}	0.02^{ns}
X_3	0.054^{*}	0.09^{*}	0.33^{*}	0.09^{*}	1.05^{***}	0.28^{*}	0.05^{ns}
X_1^2	-0.233^{***}	-0.83^{***}	-2.23^{***}	-0.45^{***}	-2.46^{***}	-1.06^{***}	-0.18^{*}
X_2^2	-0.056^{ns}	-0.08^{ns}	-0.16^{ns}	-0.01^{ns}	-0.36^{ns}	-0.23^{ns}	0.02^{ns}
X_3^2	-0.011^{ns}	-0.03^{ns}	0.02^{ns}	-0.02^{ns}	-0.12^{ns}	-0.13^{ns}	0.04^{ns}
X_1X_2	-0.010^{ns}	0.07^{ns}	0.03^{ns}	0.05^{ns}	0.04^{ns}	0.14^{ns}	$nd7^{ns}$
X_1X_3	$nd08^{ns}$	0.05^{ns}	0.03^{ns}	0.04^{ns}	0.33^{ns}	0.07^{ns}	$nd1^{ns}$
X_2X_3	$-nd8^{ns}$	0.05^{ns}	-0.10^{ns}	0.01^{ns}	$nd5^{ns}$	-0.07^{ns}	$nd1^{ns}$

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99 *** for $p < nd1$; ** for $p < 0.01$; * for $p < 0.05$; ns not significant.
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Table S3 ANOVA results of the TAX, SILC, SILD, SILA, SILB, ISILA and ISILB extraction models

Source	TAX	SILC	SILD	SILA	SILB	ISILA	ISILB
Model F-value	8.03***	32.85***	14.73***	10.12***	8.48***	13.33***	2.70*
Lack of fit F-value	0.12ns	0.027ns	0.068 ns	0.087 ns	0.085 ns	0.062 ns	0.34 ns
R^2	0.810	0.946	0.886	0.843	0.852	0.876	0.589
adj R^2	0.709	0.917	0.826	0.759	0.774	0.810	0.371
CV %	0.415	0.342	0.118	0.342	0.322	0.270	0.895

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105   R^2: determination coefficient; R^2 adj: adjusted R^2; CV variation coefficient value; *** significant $p <$ nd1; **

106   significant $p < 0.01$; * significant $p < 0.05$; ns not significant.

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Table S1 Individual antioxidant and antimicrobial activities vs RA contents in the 27 US extract samples.

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Run ID#	CUPRAC[a]	AGE[b]	COLA[b]	ELA[b]
1	51.88	8.59	12.18	8.28
2	92.49	25.18	20.20	10.80
3	98.73	16.50	11.62	10.04
4	59.83	9.31	6.17	10.69
5	111.61	41.06	27.47	11.38
6	103.80	27.11	16.28	8.56
7	83.81	15.78	12.07	9.63
8	99.07	33.72	28.73	12.36
9	97.65	27.98	16.39	8.46
10	51.33	11.47	8.53	7.87
11	122.82	48.72	34.37	16.71
12	128.12	25.43	15.14	7.69
13	64.47	6.64	11.25	6.84
14	152.73	58.23	37.16	15.16
15	110.01	39.62	23.91	12.50
16	100.01	10.13	4.20	7.51
17	146.98	55.62	37.72	17.27
18	131.23	37.82	21.36	11.13
19	46.02	9.14	10.49	8.96
20	173.26	68.15	44.67	21.05
21	120.16	29.77	16.91	8.60
22	65.86	15.06	11.36	7.15
23	172.09	67.02	47.25	21.30
24	105.13	45.98	23.52	12.17
25	143.49	15.40	9.60	8.30
26	183.80	74.32	49.13	22.93
27	101.26	34.02	20.75	10.64

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111 Values are means of 3 independent replicates; colours represent the relative activities or contents, from blue (for
112 relative low activities or contents) to red (for relative high activities or contents); [1] extraction conditions are
113 described in Table 2; Two antioxidant assays were conducted: CUPRAC (expressed as ascorbic acid equivalent
114 antioxidant capacity (AEAC, in μM AEAC)) and the inhibition of advanced glycation end product (AGE) formation
115 (expressed in % of inhibition relative to a control obtained by measuring the activity of the corresponding extraction
116 solvent). Two anti-aging assays were conducted by determining the inhibition activity of each extracts toward
117 collagenase (COL) and elastase (ELA) enzymes (expressed in % of inhibition relative to a control obtained by
118 measuring the activity of the corresponding extraction solvent).