

Antioxidant Activity of Hepatoprotective Silymarin and *Silybum marianum* L. Extract

D.I. Hadaruga* and N.G. Hadaruga**

* Department of Applied Chemistry and Organic-Natural Compounds Engineering, "Politehnica" University of Timisoara, Faculty of Industrial Chemistry and Environmental Engineering, 300006-Timisoara, P-ta Victoriei 2, Romania
Phone: +40 256 404224, Fax: +40 256 403060, E-Mail: daniel.hadaruga@chim.upt.ro

** Department of Food Control, Banat's University of Agricultural Sciences and Veterinary Medicine – Timisoara, Faculty of Food Products Technologies, 300645-Timisoara, Calea Aradului 119, Romania
Phone: +40 256 277373, Fax: +40 256 277326, E-Mail: nico_hadaruga@yahoo.com

Abstract: The paper presents the antioxidant activity evaluation of *Silybum marianum* L. extract in comparison with the commercial silymarin standard solution (2 mg/mL) and the time-dependent antioxidant capacity by calculating the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) reaction rate in the presence of these biosystems. The best result was obtained in the case of *S. marianum* L. extract, with a double DPPH reaction rate after 1200s in comparison with the commercial silymarin standard solution.

Keywords: silymarin, silybin, *Silybum marianum* L., hepatoprotection, antioxidant activity, DPPH method

1. Introduction

Silymarin is the bioactive extract from *Silybum marianum* L. seeds (Asteraceae) and contains 65-85% flavonolignans like silychristin, isosilychristin, silydianin, silybin A and B, isosilybin A and B, Figure 1), and also 20-35% fatty acids, flavonoids, and other polyphenolics. The major source of silymarin is fruits and seeds from this plant, but traces of these compounds can occur in all plant parts [1].

The natural compound, silybin, is a mixture of two diastereomers (in approximately 1:1 ratio): silybin A/B, (2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*R*/2*S*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-2,3-dihydro-1,4-benzodioxin-6-yl]chroman-4-one. The separation of these two isomers can be achieved by flash chromatography of glycosylated diastereomers, followed by enzymatic or acid hydrolysis, and also by semipreparative HPLC [1,2].

Silybum extracts were used from centuries for the treatment of liver diseases (hepatitis, cyrosis, and icterus), against the intoxication with *Amanita* species, and also in the case of peoples with alcoholic problems. The hepatoprotective effect of these extracts (against hepatotoxicity of carbon tetrachloride, paracetamol, or D-galactosamine) are due to the antioxidant properties of flavonoids, to the inhibition of the synthesis of phosphatidylcholine, and stimulating of hepatic synthesis of RNA proteins; silybin also stimulate the RNA polymerase I, and further the ribosomal RNA and the protein synthesis. Silymarin and *Silybum* species have also antiinflammatory and anticancerigenic activity [1,2].

The aim of this study is to continue the series of studies of our team on the extraction, purification, and antioxidant activity evaluation of biocompounds and biosystems from natural sources [3-5] with the evaluation of antioxidant activity of *Silybum marianum* L. extract in comparison with the commercial silymarin.

2. Experimental

Materials. The *S. marianum* L. seeds were collected in the autumn of year 2008 from the West side of Romania (Arad). Silymarin (Sigma) was used for comparison of the antioxidant activity. In order to obtain the *S. marianum* extracts and to evaluate the antioxidant activity of extracts and commercial silymarin, 96 % ethanol (v/v, reagent grade, Chimopar București) was used. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) reagent grade, used for the antioxidative activity evaluation, was obtained from Sigma.

***Silybum marianum* L. extracts.** The main biocompounds from *S. marianum* L. (especially flavonolignans) were separated by solid-liquid extraction by

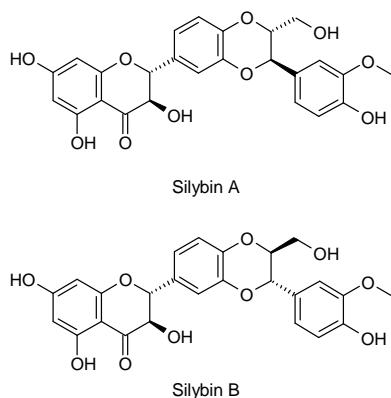


Figure 1. Structures of the main compounds from *S. marianum* L.

using a 100 mL extractor. ~5 g Finely grounded *S. marianum* seeds and 25 mL 96% ethanol were added to the extraction flask, the extraction being achieved at room temperature under inert atmosphere with continuous stirring for 30 minutes. The mixture was decanted and the residue was extracted for the second time in the same manner for another 30 minutes. The extracts were combined and 95 mL *S. marianum* L. extract was obtained.

Antioxidative activity. *S. marianum* L. extract and commercial silymarin were analyzed from the antioxidative point of view by using DPPH method. The reaction of DPPH was spectrophotometrically measured for 1800 s at 517 nm on a CamSpec M501 spectrophotometer and the data were analyzed using the corresponding UV-Vis Analyst ver. 4.67 acquisition&data handling soft. The raw *S. marianum* extract and the undiluted (2 mg silymarin/mL in ethanol 96%) and diluted standard silymarin solutions (tenth fold diluted samples) were mixed with 1 mM DPPH ethanolic solution in 96% ethanol and the decrease of the absorbance at 517 nm was measured (the ratio of *S. marianum* extract or silymarin solution : DPPH solution : ethanol were 1 : 1 : 13). The antioxidative activity of the extract and silymarin solutions was evaluated as relative absorbance of the sample in the presence of DPPH solution after the constance of the absorbance at 517 nm ($A\% = A(t)/A(t=0) \cdot 100$).

Rate of the DPPH consumption. The mean rate of the DPPH consumption in the presence of extract and silymarin solution samples was evaluated for 0-40 s, 40-200 s, 200-600 s, 600-1200 s, and 1200-1800 range intervals (*i1-5*), by using the formula:

$$\bar{v} = -\frac{\Delta C_{DPPH}}{\Delta t} \quad (\mu M/s)$$

where \bar{v} is the DPPH consumption rate ($\mu M/s$), $\Delta C_{DPPH} / \Delta t$ – is the variation of the DPPH concentration in time.

3. Results and Discussion

Silymarin is a mixture of flavonolignans, resulted by radicalic coupling of coniferyl acid with various flavonoids (Figure 2).

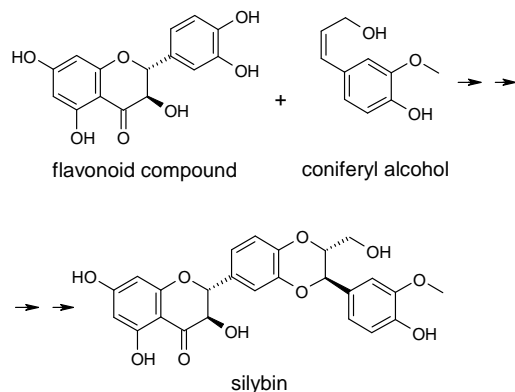


Figure 2. Biosynthetic way for silybin

The presence of phenolic hydroxyl groups on the silybin structure (as well as on the flavonoid and coniferyl alcohol starting structures) confer to the silymarin (where silybin is the main compound) and *Silybum marianum* L. extract antioxidative effect. Silybin can be in equilibrium with the tautomeric forms (enolic forms), which have also antioxidative activity (enhanced due to the presence of the extra enolic hydroxyl group from 4-position, Figure 3).

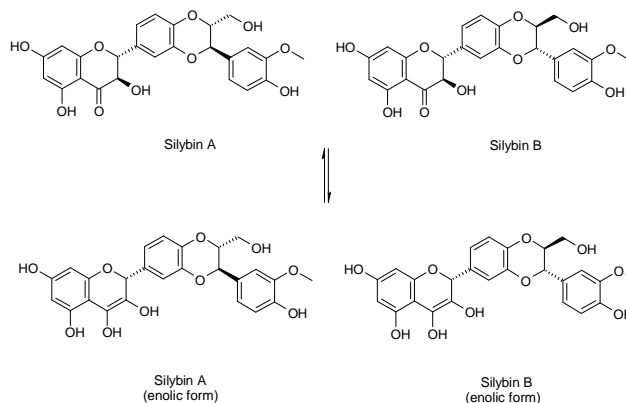


Figure 3. Tautomerism of silybin

The standard silymarin solution, the tenth fold diluted silymarin solution and the *Silybum marianum* L. extract were evaluated for antioxidant activity capacity by using the DPPH method. Thus, the monitoring of the DPPH absorbance at 517 nm in the presence of these silybin-containing systems revealed that the concentration of DPPH decrease even after 30 minutes. In order to evaluate the overall antioxidant activity the percent of the residual absorbance (the ratio of the absorbance of silybin-containing system in the presence of DPPH and the absorbance of a blank DPPH solution, as percent), which expressed the capacity of the silybin-containing sample to react with the DPPH radical; the lower value of the percent of residual absorbance indicate a higher antioxidant activity.

For the case of silymarin solutions the antioxidant activity was higher, the percent of residual absorbance being 9 % in the case of silymarin standard solution and 74 % for the tenth fold diluted silymarin silution (Figures 4 and 5).

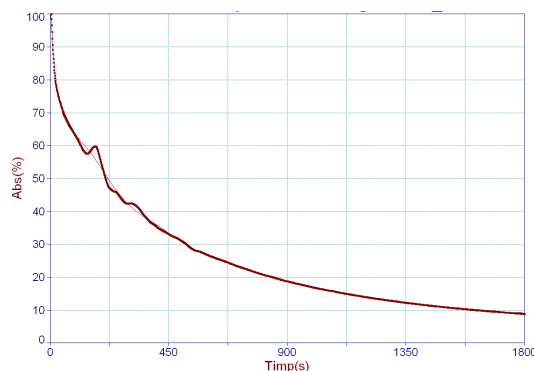


Figure 4. Antioxidant activity of silymarin standard solution

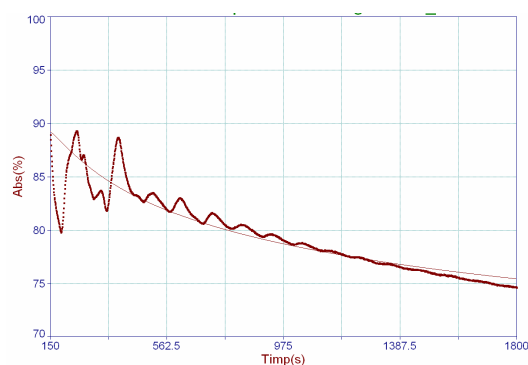
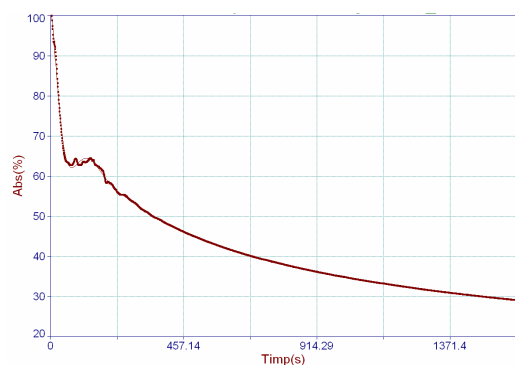
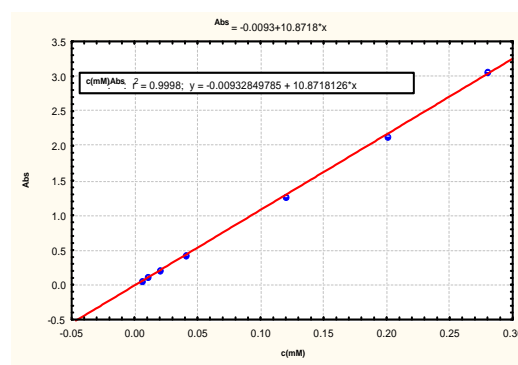
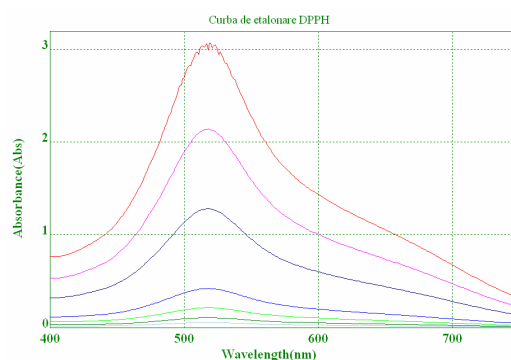


Figure 5. Antioxidant activity of tenth fold diluted silymarin solution

For the *Silybum marianum* L. extract the antioxidant activity was approximately 28% (up to 1400 s, Figure 6), in the range of antioxidant activity of standard and tenth fold diluted silymarin solution.

Figure 6. Antioxidant activity of *Silybum marianum* L. extract

The rate of DPPH consumption in the presence of silybin-containing systems is an important tool in order to evaluate the profile of the antioxidant activity: if the rate of DPPH consumption is non-nule even after a long time (e.g. 30 minutes) the antioxidant activity exists, but in the case of higher reaction rate in the first interval and a insignificant rate after this interval, the overall antioxidant activity is lower and the biosystem evaluated is improper to use. The DPPH consumption rate can be evaluated by using the variation of DPPH absorbance (at 517 nm) in the presence of silybin-containing systems in time. The superimposed spectra and the calibration curve of the DPPH calibration solutions are presented in Figure 7.

Figure 7. Superimposed visible spectra (up) and the $Abs = f(c, mM)$ calibration curve (down) for DPPH

The overall antioxidant activity is not sufficient for the antioxidant quality of biosystems; the overall antioxidant activity can be higher, but only for the short time period and after that this activity can dissappear. This activity is important to be non-nule even after a long time and the relevant parameter for this fact is the rate of DPPH (or other radicalic compound) consumption. From this point of view, five time intervals were considered to be relevant: $i1$ 0-40s, $i2$ 40-200s; $i3$ 200-600s, $i4$ 600-1200s, $i5$ 1200-1800s. In the case of *S. marianum* L. extract, the DPPH reaction rate was little bit higher than in the case of silymarin standard solution, but in both cases the reaction rate has significant values, even after 20 minutes (0.01-0.02 $\mu\text{M/s}$, Table 1). The allure of the variation of DPPH concentration in time are presented in Figures 8 and 9.

TABLE 1. The DPPH mean reaction rate for five time ranges* in the presence of silymarin standard solution and *S. marianum* extract

No	Sample	\bar{v}_{i1} ($\mu\text{M/s}$)	\bar{v}_{i2} ($\mu\text{M/s}$)	\bar{v}_{i3} ($\mu\text{M/s}$)	\bar{v}_{i4} ($\mu\text{M/s}$)	\bar{v}_{i5} ($\mu\text{M/s}$)
1	Silymarin standard solution	0.9	0.1	0.074	0.026	0.011
2	<i>S. marianum</i> L. extract	2.3	0.079	0.1	0.043	0.024

* $i1$ – represent the time interval 0-40s, $i2$ – 40-200s, $i3$ – 200-600s, $i4$ – 600-1200s, $i5$ – 1200-1800s

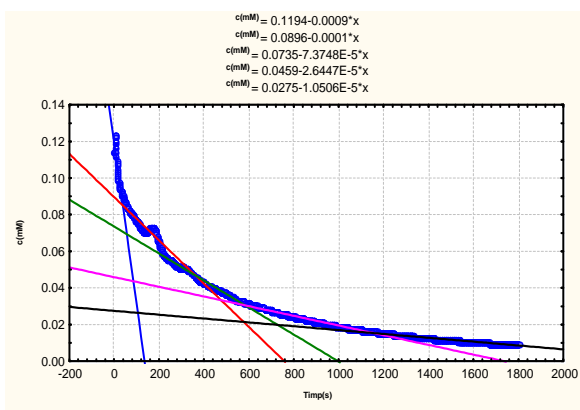


Figure 8. The variation of DPPH concentration (mM) in time in the case of silymarin standard solution

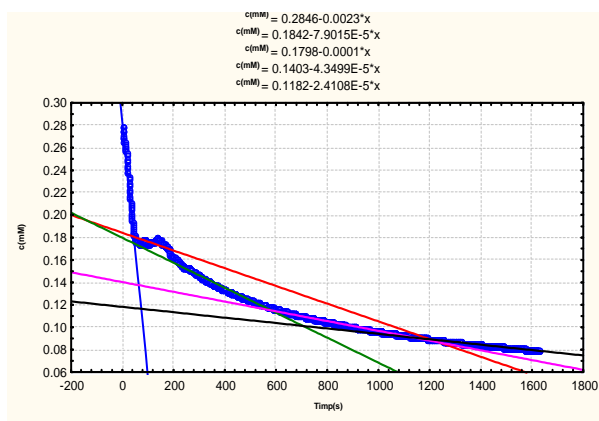


Figure 9. The variation of DPPH concentration (mM) in time in the case of *S. marianum* L. extract

4. Conclusions

The following conclusions can be drawn among to these antioxidative activity evaluations of silymarin and *Silybum marianum* L. extracts:

(1) both commercial silymarin and *Silybum marianum* L. extract have good antioxidant activity, evaluated by DPPH method, the last natural extract having approximately 33% of the antioxidant activity of silymarin standard solution (2 mg/mL); (2) even the overall antioxidant activity of commercial silymarin is higher the DPPH consumption rate and furthermore the antioxidant capacity of *Silybum marianum* L. extract is better.

ACKNOWLEDGEMENTS

The authors want to thanks to Dr. Anca Hermenean ("Vasile Goldis" West University of Arad) for providing the *S. marianum* L. samples and to V. Damşa ("Politehnica" University of Timisoara) for the help in obtaining of the *S. marianum* L. extract. This work was supported by Ministry of Education and Research from Romania, PN2 62072/2008.

REFERENCES

1. Ramasamy K., and Agarwal R., *Cancer Letters*, 269, **2008**, 352-362.
2. Bosisio E., Benelli C., and Pirola O., *Pharmacological Research*, 25, **1992**, 147-165.
3. Coneac G., Gafițanu E., Hadaruga D.I., Hadaruga N.G., Pînzaru I.A., Bandur G., Urșica L., Paunescu V., and Gruia A., *Chem. Bull. "Politehnica" Univ. (Timișoara)*, 53(1-2), **2008**, 56-60.
4. Costescu C.I., Hadaruga N.G., Hadaruga D.I., Lupea A.X., Ravis A., and Pârnu D., *Journal of Agroalimentary Processes and Technologies*, 14(2), **2008**, pp. 417-432.
5. Dumbrava D.G., Hadaruga N.G., Hadaruga D.I., Gruia A., Tatu C., Paunescu V., and Lupea A.X., *Journal of Agroalimentary Processes and Technologies*, 14(2), **2008**, pp. 433-441

Received: 29 August 2009

Accepted: 30 September 2009