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MDPI

SUPPLEMENTARY MATERIALS

Chitosan Elicitation Impacts Flavonolignan Biosynthesis in *Silybum marianum* (L.) Gaertn Cell Suspension and Enhances Antioxidant and Anti-inflammatory Activities of Cell Extracts

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Supplementary Materials List:

Figure S1. Aspects of cell suspension cultures of *S. marianum* submitted to different concentrations of chitosan.

Figure S2: Loading scores of the first (PC1) and second (PC2) axis of the principal component analysis of the parameters measured in extract of cell suspension cultures of *S. marianum* in response to chitosan elicitation.

Table S1: Actual values for PCC (Pearson correlation coefficient) presented in Figure 4 showing the relation between the main phytochemicals and the biological activities (antioxidant and anti-inflammatory) of extracts of cell suspension cultures of *S. marianum* in response to chitosan elicitation.

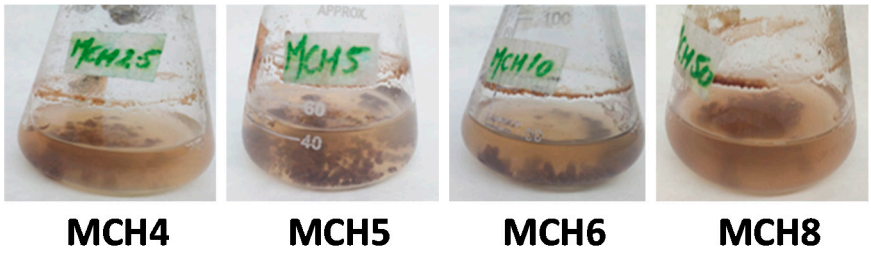

Figure S1. Aspects of cell suspension cultures of *S. marianum* submitted to different concentrations of chitosan (MCH4: 2.5 mg/L chitosan; MCH5: 5.0 mg/L chitosan; MCH6: 10.0 mg/L chitosan; MCH8: 50.0 mg/L chitosan).

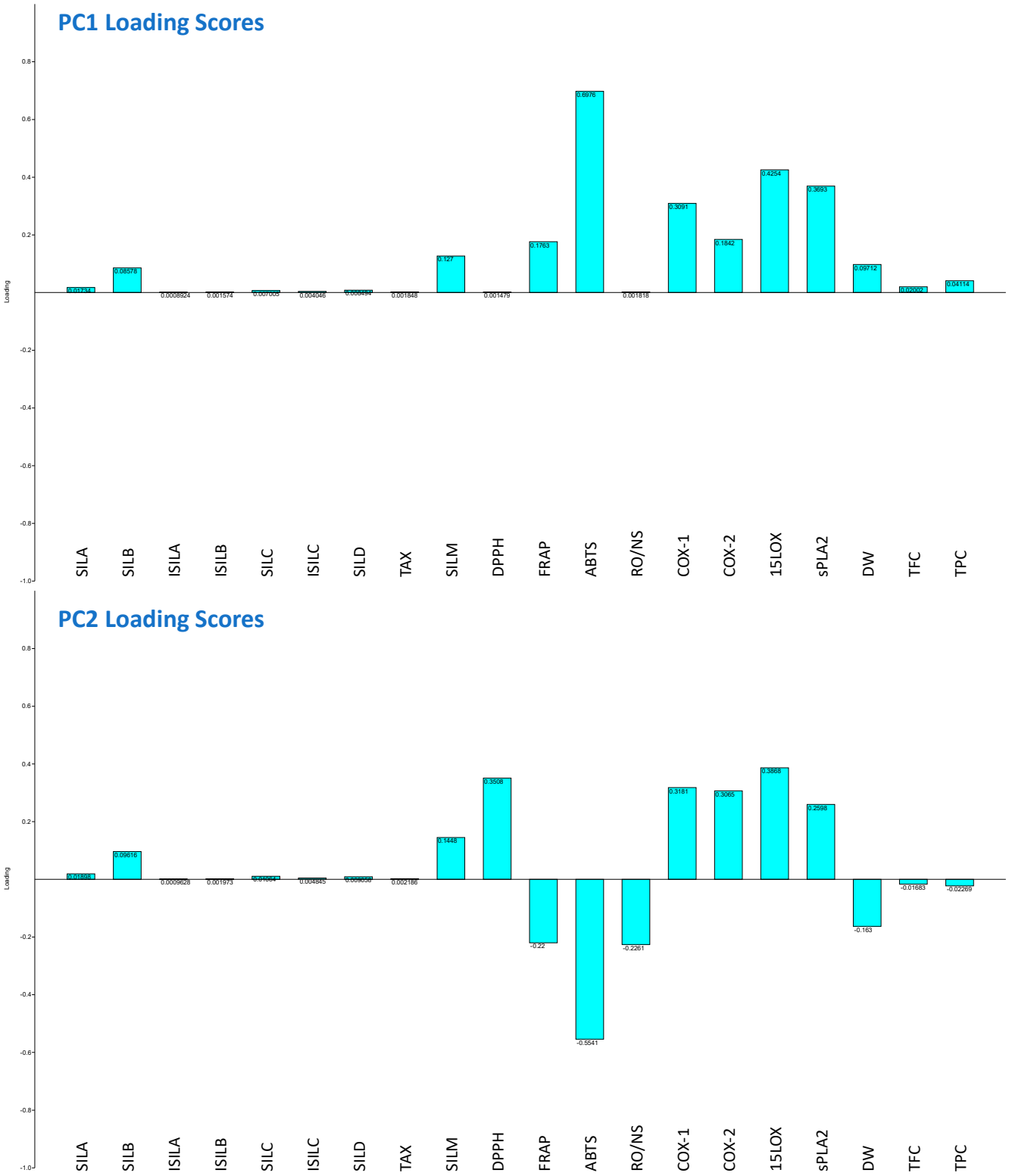

Figure S2: Loading scores of the first (PC1) and second (PC2) axis of the principal component analysis of the parameters measured in extract of cell suspension cultures of *S. marianum* in response to chitosan elicitation.

Phytochemicals: SILA: silybin A; SILB: silybin B; ISILA: isosilybin A; ISILB: isosilybin B; SILC: silychristin; ISILC: isosilychristin; SILD: silydianin; TAX: taxifolin; SILM: silymarin; TFC: total flavonoid content; TPC: total phenolic content. **Antioxidants assays:** DPPH: 2,2-diphenyl-1-picrylhydrazyl *in vitro* antioxidant assay; FRAP: ferric reducing antioxidant power *in vitro* antioxidant assay. ABTS: 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid *in vitro* antioxidant assay; RO/NS: cellular antioxidant assay (reactive of oxygen and nitrogen species). Anti-inflammatory: COX-1: cyclooxygenase 1 inhibition; COX-2: cyclooxygenase 2 inhibition; 15LOX: 15-lipoxygenase inhibition; sPLA2: secretory phospholipase A2 inhibition. **Biomass:** DW: dry weight.

Table S1: Actual values for PCC (Pearson correlation coefficient) presented in Figure 4 showing the relation between the main phytochemicals and the biological activities (antioxidant and anti-inflammatory) of extracts of cell suspension cultures of *S. marianum* in response to chitosan elicitation.

	SILA	SILB	ISILA	ISILB	SILC	ISILC	SILD	TAX	SILM	**TPC**	**TFC**
DPPH	*0.365*	*0.330*	*0.357*	*0.289*	*0.356*	*0.307*	*0.302*	*0.415*	*0.335*	*-0.101*	*-0.022*
	ns	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
FRAP	*0.287*	*0.301*	*0.266*	*0.236*	*0.213*	*0.313*	*0.267*	*0.253*	*0.291*	*0.344*	*0.756*
	ns	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
ABTS	*0.565*	*0.558*	*0.565*	*0.482*	*0.418*	*0.535*	*0.571*	*0.555*	*0.551*	0.782	0.815
	ns	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*	*
ROS/RNS	*-0.152*	*-0.183*	*-0.097*	*-0.139*	*-0.237*	*-0.254*	*-0.140*	*-0.228*	*-0.181*	*0.452*	*0.268*
	ns	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
COX1	0.960	0.950	0.970	0.913	0.918	0.945	0.955	0.959	0.952	*0.392*	*0.464*
	*	*	*	*	*	*	*	*	*	*ns*	*ns*
COX2	0.802	0.810	0.805	0.781	0.770	0.801	0.825	0.828	0.809	*0.437*	*0.298*
	***	***	***	***	***	***	***	***	***	*ns*	*ns*
15-LOX	0.927	0.942	0.897	0.882	0.878	0.946	0.926	0.937	0.936	*0.490*	*0.660*
	***	***	**	**	**	***	***	***	***	*ns*	*ns*
SPLA2	0.853	0.877	0.814	0.815	0.808	0.886	0.850	0.851	0.868	*0.473*	*0.751*
	**	**	*	*	*	**	**	**	**	*ns*	*ns*

Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
Phytochemicals: SILA: silybin A; SILB: silybin B; ISILA: isosilybin A; ISILB: isosilybin B; SILC: silychristin; ISILC: isosilychristin; SILD: silydianin; TAX: taxifolin; SILM: silymarin; TFC: total flavonoid content; TPC: total phenolic content. **Antioxidants assays:** DPPH: 2,2-diphenyl-1-picrylhydrazyl *in vitro* antioxidant assay; FRAP: ferric reducing antioxidant power *in vitro* antioxidant assay. ABTS: 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid *in vitro* antioxidant assay; RO/NS: cellular antioxidant assay (reactive of oxygen and nitrogen species). Anti-inflammatory: COX-1: cyclooxygenase 1 inhibition; COX-2: cyclooxygenase 2 inhibition; 15LOX: 15-lipoxygenase inhibition; sPLA2: secretory phospholipase A2 inhibition. **Biomass:** DW: dry weight.