

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

Muzamil Shah¹, Hasnain Jan¹, Samantha Drouet², Duangjai Tungmunnithum³, Jafir Hussain Shirazi⁴, Christophe Hano^{2*}, Bilal Haider Abbasi^{1*}

¹ Department of Biotechnology, Quaid-i-Azam University, Islamabad-45320, Pakistan; mshah@bs.qau.edu.pk (M.S.); rhasnain849@gmail.com (H.J.); bhabbasi@qau.edu.pk (B.H.A.)

² University of Orleans, Laboratoire de Biologie des Ligneux et des Grandes Cultures (LBLGC), INRAE USC1328, F28000 Chartres, France; samanta.drouet@univ-orleans.fr (S.D.); hano@univ-orleans.fr (C.H.)

³ Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajathevi, Bangkok 10400, Thailand; duangjai.tun@mahidol.ac.th (D.T.)

⁴ Islamia University of Bahawalpur, Bahawalpur, Pakistan; jafir.shirazi@iub.edu.pk (J.H.S.)

* Correspondence: bhabbasi@qau.edu.pk (B.H.A.); hano@univ-orleans.fr (C.H.) Tel: +33-77-698-41-48 (B.H.A.); +33-237-309-753 (C.H.)

Supplementary Materials List:

Figure S1: 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.

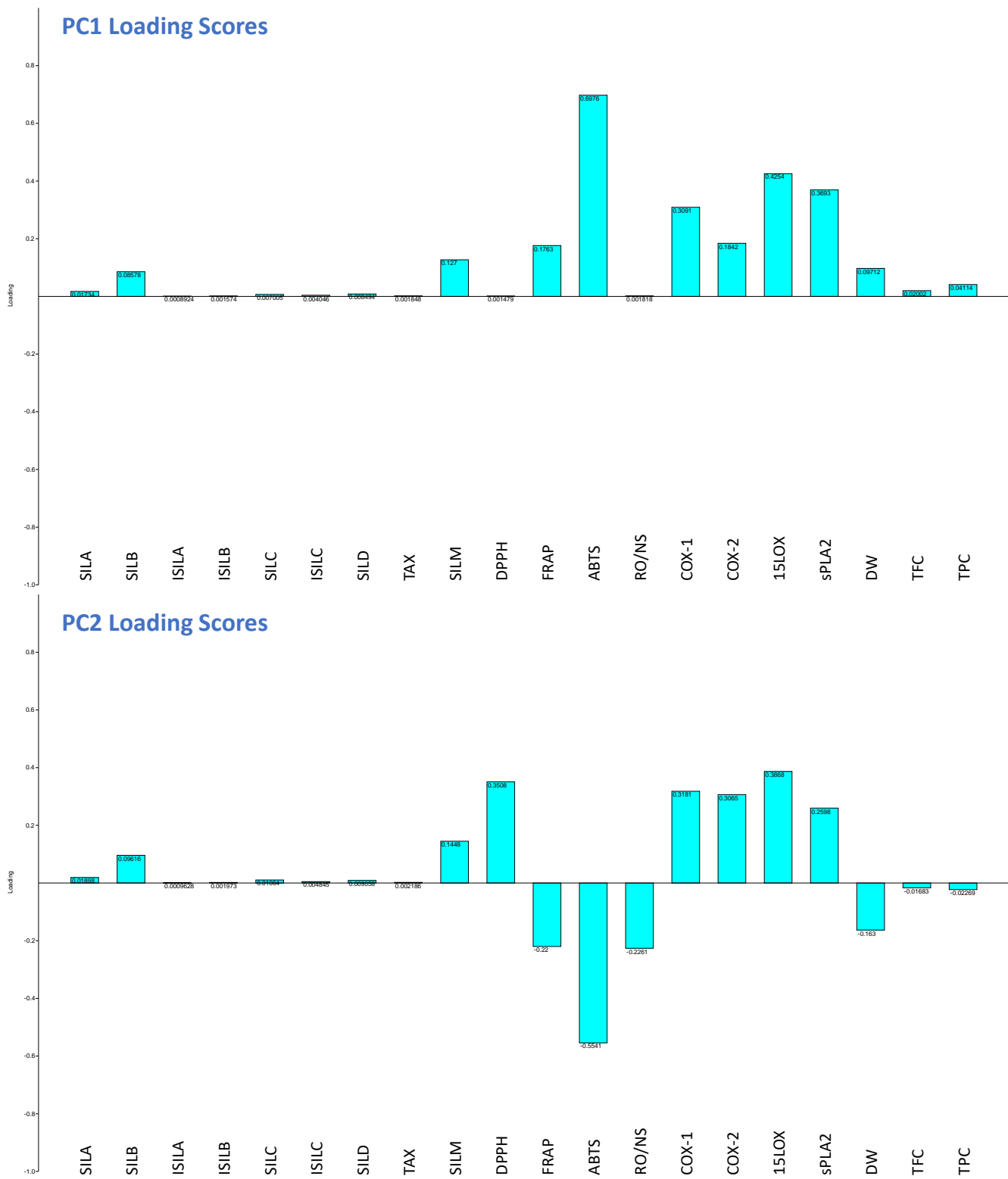


Figure S1: Loading scores of the first (PC1) and second (PC2) axis of the principal component analysis of the parameters measured in extract of cell suspension cultures of *S. marianum* in response to chitosan elicitation.

Phytochemicals: SILA: silybin A; SILB: silybin B; ISILA: isosilybin A; ISILB: isosilybin B; SILC: silychristin; 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-azino-bis(3-ethylbenzothiazoline-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
	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
FRAP	0.287	0.301	0.266	0.236	0.213	0.313	0.267	0.253	0.291	0.344	0.756
	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
ABTS	0.565	0.558	0.565	0.482	0.418	0.535	0.571	0.555	0.551	0.782	0.815
	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	*
ROS/RNS	-0.152	-0.183	-0.097	-0.139	-0.237	-0.254	-0.140	-0.228	-0.181	0.452	0.268
	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
COX1	0.960	0.950	0.970	0.913	0.918	0.945	0.955	0.959	0.952	0.392	0.464
	*	*	*	*	*	*	*	*	*	<i>ns</i>	<i>ns</i>
COX2	0.802	0.810	0.805	0.781	0.770	0.801	0.825	0.828	0.809	0.437	0.298
	***	***	***	***	***	***	***	***	***	<i>ns</i>	<i>ns</i>
15-LOX	0.927	0.942	0.897	0.882	0.878	0.946	0.926	0.937	0.936	0.490	0.660
	***	***	**	**	**	***	***	***	***	<i>ns</i>	<i>ns</i>
SPLA2	0.853	0.877	0.814	0.815	0.808	0.886	0.850	0.851	0.868	0.473	0.751
	**	**	*	*	*	**	**	**	**	<i>ns</i>	<i>ns</i>

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-azino-bis(3-ethylbenzothiazoline-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.

40
41
42
4344
45
46
47
48
49
50
51
52