

Supplementary Material for:

# Polyphenol-Enriched Extracts of *Prunus spinosa* Fruits: Anti-Inflammatory and Antioxidant Effects in Human Immune Cells Ex Vivo in Relation to Phytochemical Profile

Anna Magiera <sup>1,\*</sup>, Monika Ewa Czerwińska<sup>2,3</sup>, Aleksandra Owczarek<sup>1</sup>, Anna Marchelak<sup>1</sup>, Sebastian Granica<sup>4</sup> and Monika Anna Olszewska<sup>1</sup>

<sup>1</sup> Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Lodz, 1 Muszynskiego St., 90-151 Lodz, Poland

<sup>2</sup> Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, 1 Banacha St., 02-097 Warsaw, Poland

<sup>3</sup> Centre for Preclinical Research, Medical University of Warsaw, 1B Banacha St., 02-097 Warsaw, Poland

<sup>4</sup> Microbiota Lab, Centre for Preclinical Studies, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, 1 Banacha St., 02-097 Warsaw, Poland

\* Correspondence: anna.magiera@umed.lodz.pl (A. Magiera); Tel.: +48 503316997

## Supplementary Material

### Table of Contents

#### S.1. Results

**Table S1.** UHPLC-PDA-ESI-MS<sup>3</sup> identification data of compounds detected in the dry extracts from fresh and dried fruits of *P. spinosa*.

**Figure S1.** Structures of the dominant polyphenolic compounds of *P. spinosa* fruits extracts.

**Table S2.** Quantitative profile of the *P. spinosa* fruit (mg/g fw).

**Figure S2.** Effect of the fruit extracts and pure compounds on viability (membrane integrity) of human immune cells.

**Table S3.** Correlation (*r*) coefficients and probability (*p*) values of linear relationships between antioxidant and anti-inflammatory activity parameters and phenolic contents of *P. spinosa* fruits extracts.

#### S.2. Reference Standards

#### S.3. Plant Material

**Figure S3.** Representative image of a sample of fresh *P. spinosa* fruits.

## References

## S.1. Results

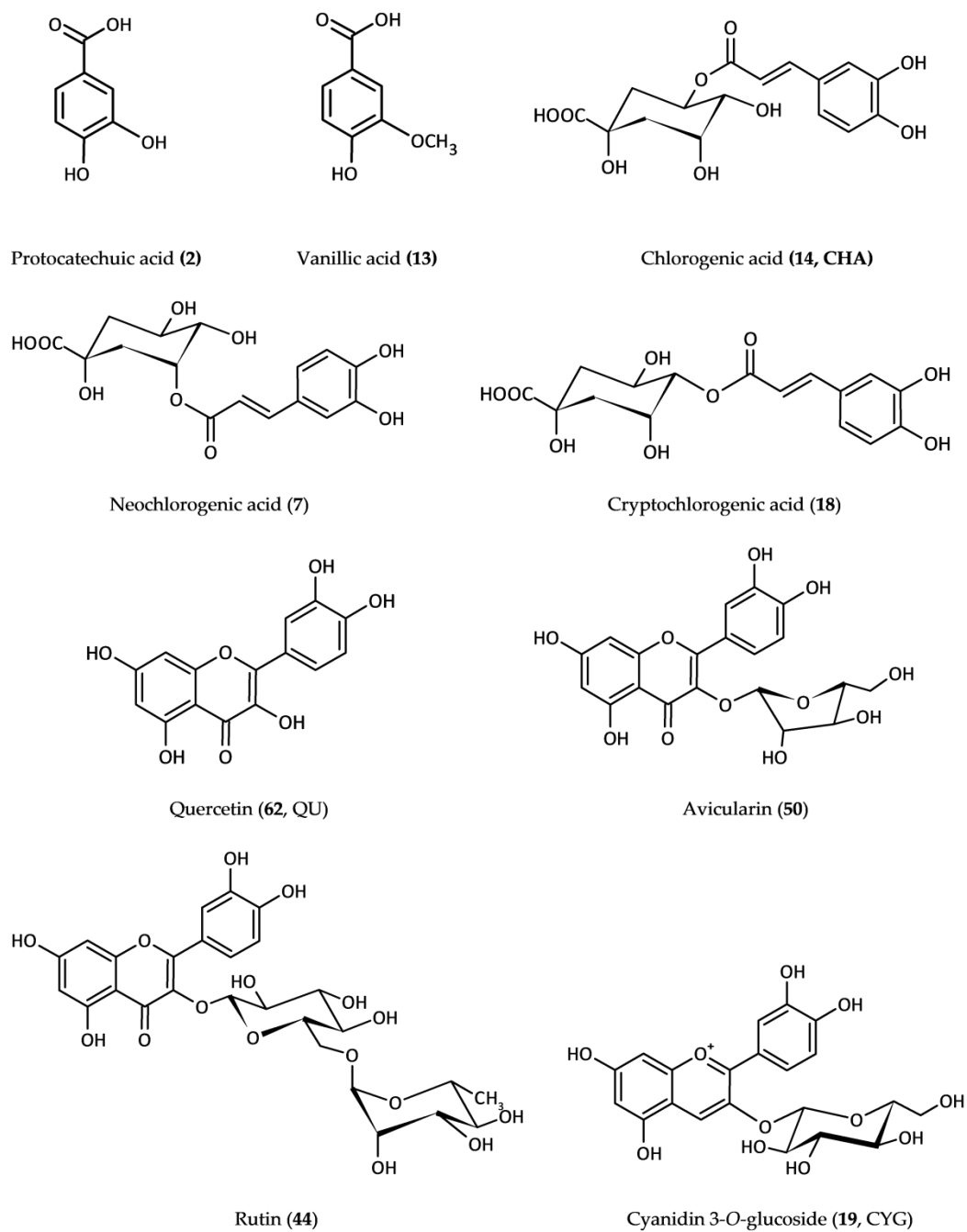
**Table S1.** UHPLC-PDA-ESI-MS<sup>3</sup> identification data of compounds detected in the extracts from fresh fruits of *P. spinosa*.

Peak	Analyte	R <sub>t</sub> (min)	UV λ <sub>max</sub> (nm)	[M–H] <sup>–</sup> (m/z)	Fragmentary ions	[M–H] <sup>+</sup> (m/z)	Fragmentary ions	Fruit extracts	References
1	Vanillic acid <i>O</i> -hexoside	3.9	250 290	329	167	-	-	BFF, EAFF, MEF, WRF	-
2	Protocatechuic acid <sup>a,b</sup>	4.6	260 295	153	153	155	155	DEFF, EAFF	-
3	Unidentified	5.2	325	517	335	-	-	BFF, MEF	-
4	Unidentified	5.2	275	315	179, 161	-	-	WRF	-
5	cis-3- <i>O</i> -Caffeoylquinic acid <sup>b</sup>	5.7	322	353	191, 179	-	-	EAFF, MEF, WRF	[1]
6	3- <i>O</i> -Caffeoylquinic acid hexoside <sup>b</sup>	6.2	325 295	515	353 (MS <sup>3</sup> : 191, 179), 335, 341, 179			BFF	[2]
7	3- <i>O</i> -Caffeoylquinic acid (neochlorogenic acid) <sup>a</sup>	6.7	325 265 211	353	191, 179, 135	-	-	BFF, DEFF, EAFF, MEF, WRF	[3]
8	<i>p</i> -Hydroxybenzoic acid <sup>a</sup>	7.1	267 290	137	-	-	-	DEFF, EAFF	-
9	3- <i>O</i> -Caffeoylquinic acid hexoside <sup>b</sup>	7.8	210 325 270	515	353 (MS <sup>3</sup> : 191, 179), 191, 341	-	-	BFF, EAFF	[2]
10	Vanilloyl malate hexoside <sup>b</sup>	8.9	260	445	329, 167	-	-	EAFF	-
11	3- <i>O-p</i> -Coumaroylquinic acid	9.2	310	337	163, 191	-	-	BFF, DEFF, EAFF, MEF, WRF	[1]
12	Caffeic acid 3/4- <i>O</i> -hexoside	9.5	317 285	341	179, 135	-	-	BFF	-
13	Vanillic acid <sup>a</sup>	10.1	216 260 290	167	167	169	169	DEFF	-
14	5- <i>O</i> -Caffeoylquinic acid (chlorogenic acid, CHA) <sup>a</sup>	10.6	325	353	191, 179	-	-	BFF, DEFF, EAFF, MEF	[3]
15	cis-3- <i>O</i> -Feruloylquinic acid <sup>b</sup>	11.0	215 325 293	367	193	-	-	DEFF, EAFF	[1]
16	Caffeic acid <sup>a</sup>	11.2	323 214 286	179	-	-	-	DEFF	-
17	Unidentified	11.2	300 225	415	293	-	-	BFF	-
18	4- <i>O</i> -Caffeoylquinic acid (cryptochlorogenic acid) <sup>a</sup>	11.5	325 215	353	173, 191, 179	-	-	BFF, DEFF, EAFF, MEF	[3]
19	Cyanidin 3- <i>O</i> -glucoside (CYG) <sup>a</sup>	11.6	515 280	447	285, 401	449	287	BFF, MEF, WRF	-

20	Caffeic acid 3/4- <i>O</i> -hexoside	12.1	320	341	179, 135	-	-	EAFF	-
21	4- <i>O</i> - <i>p</i> -Coumaroylquinic acid	12.3	310	337	173	-	-	DEFF	[1]
22	3- <i>O</i> -Feruloylquinic acid	12.5	322	367	193	-	-	DEFF, EAFF	[1]
23	Cyanidin 3- <i>O</i> -rutinose <sup>a</sup>	12.6	515 280	593	285, 467	595	287	BFF, MEF, WRF	-
24	Vanillin <sup>a,b</sup>	13.3	285	-	-	153	153	DEFF, EAFF	-
25	Unidentified	14.1	216 262	295	251	-	-	DEFF	-
26	Peonidin 3- <i>O</i> -glucoside <sup>a</sup>	14.4	515 280	607	299	-	301	BFF, MEF	[4]
27	<i>cis</i> -3- <i>O</i> - <i>p</i> -Coumaroylquinic acid <sup>b</sup>	14.6	305	337	163	-	-	DEFF, EAFF	[1]
28	Peonidin 3- <i>O</i> -rutinose	15.0	515 280	607	299	-	301	BFF, MEF, WRF	[4]
29	4- <i>O</i> -Caffeoylshikimic acid <sup>b</sup>	15.2	325	335	179, 135	-	-	DEFF, EAFF	[1]
30	<i>p</i> -Coumaric acid <sup>a</sup>	15.8	310	163	-	-	-	DEFF	-
31	4- <i>O</i> -Feruloylquinic acid <sup>b</sup>	16.5	320 217	367	176	-	-	EAFF	[1]
32	Sinapoyl malate hexoside <sup>b</sup>	16.5	280 313	501	223	-	-	DEFF	-
33	Caffeoylshikimic acid <sup>b</sup>	17.4	280	335	161, 135, 179	-	-	BFF, DEFF, EAFF, MEF, WRF	[1]
34	Sinapoyl malate hexoside <sup>b</sup>	18.5	275	501	223, 339	-	-	DEFF	[5]
35	Caffeoylshikimic acid <sup>b</sup>	18.9	215	335	161, 179	-	-	DEFF, EAFF	[1]
36	Quercetin dirhamnoside-hexoside <sup>b</sup>	19.7	350	755	301, 609	-	303	BFF	-
37	Unidentified	20.9	280	317	287	-	-	DEFF	-
38	Kaempferol dihexoside <sup>b</sup>	23.3	280 330	609	447, 285	-	287	MEF	-
39	<i>p</i> -Coumaroylshikimic acid <sup>b</sup>	23.8	300 205	319	119, 145, 275, 163, 257	-	-	DEFF, EAFF	[6]
40	Quercetin hexoside-pentoside	24.7	354	595	301, 433	-	303	BFF, MEF	-
41	Quercetin rhamnoside-hexoside	25.6	355	609	301	-	303	BFF, MEFF	-
42	Quercetin 3- <i>O</i> - $\beta$ -D-galactoside (hyperoside) <sup>a</sup>	26.2	264 355	463	301	-	303	DEFF, EAFF, MEF	-
43	Kaempferol rhamoside-dihexoside	26.3	280 350	755	593, 285	-	287	WRF	-
44	Quercetin 3- <i>O</i> -(6''- <i>O</i> - $\alpha$ -L-rhamnopyranosyl)- $\beta$ -D-glucopyranoside (rutin) <sup>a</sup>	26.6	265 350	609	301	-	303	BFF, DEFF, EAFF, MEF, WRF	-
45	Quercetin 3- <i>O</i> - $\beta$ -D-glucopyranoside (isoquercitrin) <sup>a</sup>	27.7	260 355	463	301	-	303	BFF, DEFF, EAFF, MEF	-
46	Quercetin 3- <i>O</i> -(2''- <i>O</i> - $\beta$ -D-glucopyranosyl)- $\alpha$ -L-arabinofuranoside <sup>a,b</sup>	28.7	255 355	595	433, 301	-	303	BFF, MEF, WRF	-
47	Quercetin 3- <i>O</i> - $\alpha$ -D-xylopyranoside (reinutrin) <sup>a,b</sup>	30.1	256 356	433	301	435	303	DEFF, EAFF	-
48	Quercetin 3- <i>O</i> - $\alpha$ -L-arabinopyranoside (guaiaiverin) <sup>a,b</sup>	30.4	255 355	433	301	435	303	DEFF, EAFF	-

49	Quercetin acetyl-dihexoside <sup>b</sup>	31.5	266 350	667	301	-	303	DEFF	-
50	Quercetin 3-O- $\alpha$ -L-arabinofuranoside (avicularin) <sup>a,b</sup>	35.1	255 355	433	301	435	303	BFF, DEFF, EAFF, MEF	-
51	Quercetin 3-O-(4''-O- $\beta$ -D-glucopyranosyl)- $\alpha$ -L-rhamnopyranoside (multinoside A) <sup>a,b</sup>	35.8	254 355	609	447, 301	611	449, 303	BFF, EAFF, MEF	-
52	Quercetin 3-O- $\alpha$ -L-rhamnopyranoside (quercitrin) <sup>a</sup>	36.5	255 355	447	301	449	303	BFF, DEFF, EAFF, MEF	-
53	Isorhamnetin rhamnoside-hexoside (Isorhamnetin rutinoside)	37.2	260 350	623	315	-	-	BFF, MEF	-
54	Isorhamnetin rhamnoside <sup>b</sup>	37.2	350	461	315	-	-	DEFF	-
55	Quercetin malyl-pentoside <sup>b</sup>	38.0	254 350	549	433, 301	-	435, 303	DEFF, EAFF	-
56	Quercetin acetyl-hexoside	38.4	250 350	505	301	-	303	DEFF, MEF	-
57	Quercetin malyl-pentoside <sup>b</sup>	39.3	255 355	549	433, 301	-	435, 303	DEFF	-
58	Quercetin pentoside	43.7	255 350	433	301	435	303	DEFF	-
59	Caffeoylquinic acid butyl ester <sup>b</sup>	44.2	325 292	409	161, 135, 191	-	-	BFF	[7]
60	Kaempferol hexoside <sup>b</sup>	44.4	255 355	447	285	-	287	DEFF	-
61	Quercetin acetyl-hexoside-rhamoside	46.5	254 350	651	609, 301	-	303	EAFF, MEF	-
62	Quercetin (QU) <sup>a</sup>	49.2	255 356	301	-	-	303	DEFF, EAFF	-
63	Unidentified	50.9	350	337	322	-	-	DEFF	-

<sup>a</sup> Identified with authentic standards. <sup>b</sup> Detected in *P. spinosa* fruits for the first time. *Rt*, retention times. UV  $\lambda_{\text{max}}$ , absorbance maxima in UV-Vis spectra.  $[\text{M}-\text{H}]^-$ , deprotonated molecular ions in MS spectra recorded in a negative ion mode.  $[\text{M}+\text{H}]^+$ , protonated molecular ions in MS spectra recorded in a positive ion mode. Nomenclature of quinic acid esters including chlorogenic acid isomers is according to IUPAC rules adopted by Clifford et al. [3,8].

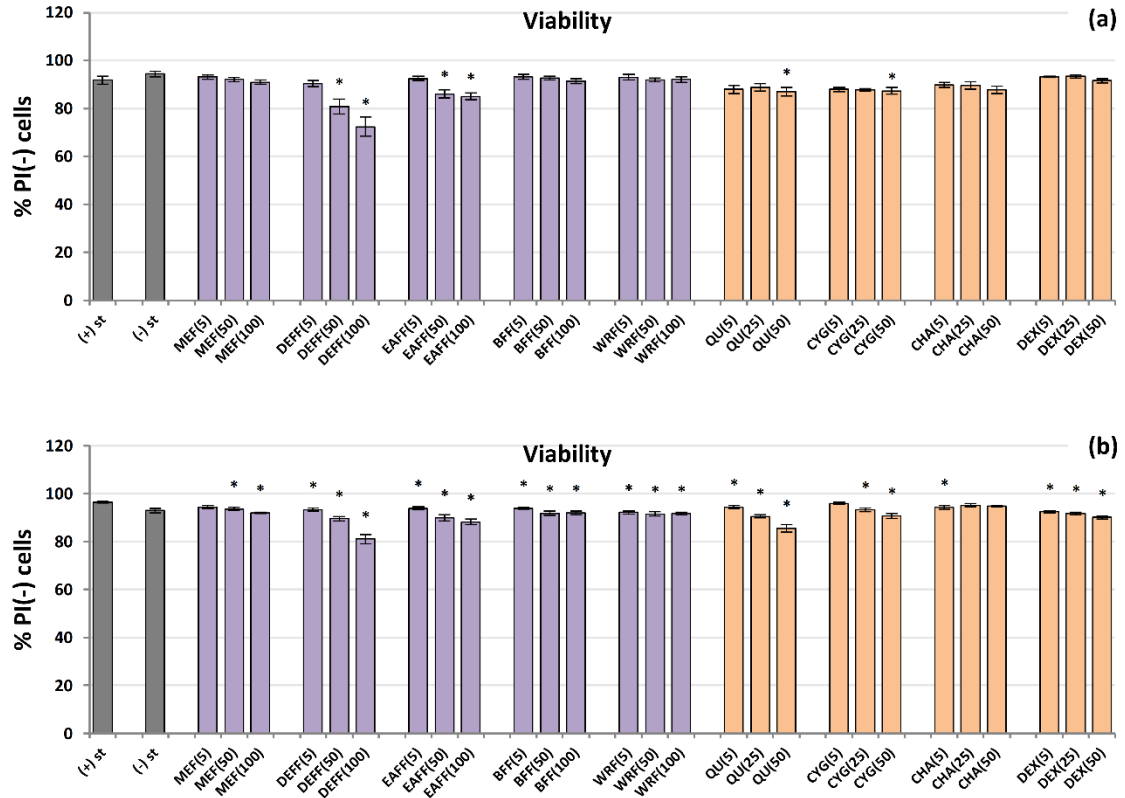


**Figure S1.** Structures of the dominant polyphenolic compounds of *P. spinosa* fruits extracts. Numbers in parentheses (bold font) refer to those in Figure 1 (main article) and supplementary Table S1.

**Table S2.** Quantitative profile of the *P. spinosa* fruit (mg/g fw).

Total contents	
TPC (GAE)	13.69 ± 0.55
TPH	4.46 ± 0.09
TPA	3.07 ± 0.05
TAC	0.72 ± 0.02
TFL	0.66 ± 0.03
TTC (PB2)	6.96 ± 0.30
Individual compounds	
Avicularin ( <b>50</b> )	0.21 ± 0.02
Hyperoside ( <b>42</b> )	0.03 ± 0.0007
Isoquercitrin ( <b>45</b> )	0.01 ± 0.0006
Rutin ( <b>44</b> )	0.25 ± 0.003
Quercitrin ( <b>52</b> )	0.02 ± 0.001
Cyanidin 3-O-glucoside ( <b>19</b> , CYG)	0.31 ± 0.02
Cyanidin 3-O-rutinoside ( <b>23</b> )	0.22 ± 0.01
Peonidin-3-O-glucoside ( <b>26</b> )	0.15 ± 0.01
Neochlorogenic acid ( <b>7</b> )	2.43 ± 0.05
Chlorogenic acid ( <b>14</b> , CHA)	0.15 ± 0.003
Cryptochlorogenic acid ( <b>18</b> )	0.24 ± 0.01

Results are presented as means ± SD ( $n = 3$ ). Numbers in parentheses (first column, in bold) refer to those in Figure 1 (main article) and supplementary Table S1. TPC, total phenolic contents in gallic acid equivalents (GAE); TPH, total contents of low-molecular-weight phenols determined by HPLC-PDA; TPA, total phenolic acids; TAC, total anthocyanins; TFL, total flavonoids; TTC, total tannins in procyanidin (PB2) B2 equivalents.



**Figure S2.** Effect of fruit extracts/fractions (5-100  $\mu\text{g/mL}$ ) and standards (5-50  $\mu\text{M}$ ) on viability (membrane integrity) of human immune cells expressed as a percentage of PI(-) cells (propidium iodide-negative): **(a)** effect on viability of neutrophils after 24 h incubation; **(b)** effect on viability of PBMCs after 48 h incubation. Standards: DEX, dexamethasone; QU, quercetin; CYG, cyanidin 3-*O*-glucoside; CHA, chlorogenic acid; Positive control: Triton X-100 solution (98.6% of PI(+) cells). Data expressed as means  $\pm$  SD of five independent experiments performed with cells isolated from five independent donors. Statistical significance in Dunnett's test: \* $p < 0.05$  compared with the stimulated control.

**Table S3.** Correlation (*r*) coefficients and probability (*p*) values of linear relationships between biological activity parameters and phenolic contents of *P. spinosa* fruits extracts.

<b>Neutrophils</b>				
<b>r (<i>p</i>) for</b>	<b>ROS level</b>	<b>TNF-<math>\alpha</math> secretion</b>	<b>ELA-2 secretion</b>	<b>IL-8 secretion</b>
TPC	-0.7455 (0.000)*	-0.7463 (0.001)*	-0.4777 (0.029)*	-0.4545 (0.089)
TPH+TTC	-0.7398 (0.000)*	-0.6504 (0.009)*	-0.4481 (0.042)*	-0.3927 (0.148)
<b>PBMCs</b>				
<b>r (<i>p</i>) for</b>		<b>TNF-<math>\alpha</math> secretion</b>	<b>IL-10 secretion</b>	<b>IL-6 secretion</b>
TPC		-0.8689 (0.000)*	0.5787 (0.024)*	-0.6200 (0.014)*
TPH+TTC		-0.8761 (0.000)*	0.6116 (0.015)*	-0.6070 (0.016)*

Values calculated using activity and concentration parameters reported in Table 1 and Figures 3-5. TPC, total phenolic contents in gallic acid equivalents (GAE); TPH, total contents of low-molecular-weight phenols determined by HPLC-PDA; TTC, total tannins in procyanidin (PB2) B2 equivalents. Asterisks mean statistical significance of the linear relationships ( $p < 0.05$ ).



## S.2. Reference Standards

All standards used for phytochemical profiling and biological activity tests were of HPLC grade (purity at least 95%). The standards of dexamethasone, protocatechuic acid, *p*-hydroxybenzoic acid, 3-*O*-caffeoylquinic acid (neochlorogenic acid), 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), 5-*O*-caffeoylquinic acid (chlorogenic acid, CHA), cynarin, *p*-coumaric acid, caffeic acid, vanillic acid, vanillin, quercetin (QU), quercetin 3-*O*- $\alpha$ -L-arabinofuranoside (avicularin), quercetin 3-*O*- $\beta$ -D-galactopyranoside (hyperoside), quercetin 3-*O*- $\beta$ -D-glucopyranoside (isoquercitrin), quercetin 3-*O*-(6''-*O*- $\alpha$ -L-rhamnopyranosyl)- $\beta$ -D-glucopyranoside (rutin) and (-)-epicatechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The standards of cyanidin 3-*O*- $\beta$ -D-glucopyranoside (CYG), cyanidin 3-*O*-rutinoside and peonidin 3-*O*- $\beta$ -D-glucopyranoside were purchased from PhytoLab (Vestenbergsgreuth, Germany). All other standards, such as quercetin 3-*O*- $\alpha$ -L-arabinopyranoside (guaiaverin), quercetin 3-*O*- $\beta$ -D-xylopyranoside (reinutrin), quercetin 3-*O*- $\alpha$ -L-rhamnofuranoside (quercitrin), quercetin 3-*O*-(4''-*O*- $\beta$ -D-glucopyranosyl)- $\alpha$ -L-rhamnopyranoside and quercetin 3-*O*-(2''-*O*- $\beta$ -D-glucopyranosyl)- $\alpha$ -L-arabinofuranoside were isolated previously in our laboratory from flowers and leaves of *P. spinosa* with at least 95% HPLC purity [9,10].

## S.3. Plant Material



**Figure S3.** Representative image of a sample of fresh *P. spinosa* fruits.

---

## References

- 1 Jaiswal, R.; Sovdat, T.; Vivan, F.; Kuhnert, N. Profiling and characterization by LC-MS<sup>n</sup> of the chlorogenic acids and hydroxycinnamoylshikimate esters in maté (*Ilex paraguariensis*). *J. Agri. Food Chem.* **2010**, *58*, 5471-5481.
- 2 Jaiswal, R.; Müller, H.; Müller, A.; Karar, M. G.; Kuhnert, N. Identification and characterization of chlorogenic acids, chlorogenic acid glycosides and flavonoids from *Lonicera henryi* L. (Caprifoliaceae) leaves by LC-MS<sup>n</sup>. *Phytochemistry* **2014**, *108*, 252-263.
- 3 Clifford, M.N.; Johnston, K.L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MS<sup>n</sup> identification of chlorogenic acids. *J. Agri. Food Chem.* **2003**, *51*, 2900-2911.
- 4 Colombo, F.; Di Lorenzo, C.; Regazzoni, L.; Fumagalli, M.; Sangiovanni, E.; de Sousa, L.P.; Bavaresco, L.; Tomasi, D.; Bosso, A.; Aldini, G.; Restani, P.; Dell'Agli, M. Phenolic profiles and anti-inflammatory activities of sixteen table grape (*Vitis vinifera* L.) varieties. *Food Funct.* **2019**, *10*, 1797.
- 5 Morreel, K.; Saeys, Y.; Dima, O.; Lu, F.; Van de Peer, Y.; Vanholme, R.; Ralph, J.; Vanholme, B.; Boerjan, W. Systematic structural characterization of metabolites in *Arabidopsis* via candidate substrate-product pair networks. *Plant Cell* **2014**, *26*, 929-945.
- 6 Ben Said, R.; Hamed, A.I.; Mahalel, U.A.; Al-Ayed, A.S.; Kowalczyk, M.; Moldoch, J.; Oleszek, W.; Stochmal, A. Tentative Characterization of Polyphenolic Compounds in the Male Flowers of *Phoenix dactylifera* by Liquid Chromatography Coupled with Mass Spectrometry and DFT. *Int. J. Mol. Sci.* **2017**, *18*, E512.
- 7 Wu, Z.J.; Ma, X.L.; Fang, D.M.; Qi, H.Y.; Ren, W.J.; Zhang, G.L. Analysis of caffeic acid derivatives from *Osmanthus yunnanensis* using electrospray ionization quadrupole time-of-flight mass spectrometry. *Eur. J. Mass Spectrom.* **2009**, *15*, 415-429.
- 8 Clifford, M.N.; Knight, S.; Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS<sup>n</sup>. *J. Agri. Food Chem.* **2005**, *53*, 3821-3832.
- 9 Olszewska, M.; Wolbiś, M. Further flavonoids from the flowers of *Prunus spinosa* L. *Acta Pol. Pharm.* **2002a**, *59*, 133-137.
- 10 Olszewska, M.; Wolbiś, M. Flavonoids from the leaves of *Prunus spinosa* L. *Pol. J. Chem.* **2002b**, *76*, 967-974.