

## Supplementary Materials

A



B



**Figure S1.** Blackthorn fruits, *Prunus spinosa* **A** - Crni vrh, central Serbia; **B** – Ljig, western Serbia

**Table S1.** Bacterial and yeast strains used in experiments.

<b>Pathogenic microorganisms</b>
<b>Gram-positive bacteria</b>
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Rosenbach ATCC 6538
<i>Staphylococcus epidermidis</i> ATCC 12228
<i>Enterococcus faecalis</i> ATCC 29212
<i>Bacillus subtilis</i> ATCC 6633
<b>Gram-negative bacteria</b>
<i>Escherichia coli</i> ATCC 25922
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> NCIMB 8267
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony NCTC 6017
<i>Pseudomonas aeruginosa</i> ATCC 27853,
<b>Yeast</b>
<i>Candida albicans</i> ATCC 24433
<b>Probiotic strains</b>
<b>Gram-positive bacteria</b>
<i>Lactobacillus plantarum</i> Lp 299v
<i>Limosilactobacillus reuteri</i> Protectis (DSM 17938) (formerly <i>Lactobacillus reuteri</i> )
<i>Lactobacillus rhamnosus</i> GG (LGG)
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> ST-21
<b>Yeast</b>
<i>Saccharomyces boulardii</i> strain 1
<i>Saccharomyces boulardii</i> strain 2
<b>Mixtures of probiotic strains</b>
<b>MIX 1</b> <i>Lactobacillus helveticus</i> Rosell-52, <i>L. rhamnosus</i> Rosell-11, <i>Bifidobacterium longum</i> Rosell-175
<b>MIX 2</b> <i>Lactobacillus helveticus</i> Rosell-52, <i>L. rhamnosus</i> Rosell-11, <i>Bifidobacterium longum</i> Rosell-175,
<i>Saccharomyces boulardii</i>

**Table S2.** Identified compounds in blackthorn fruit extracts

<i>t<sub>R</sub></i> (min)	UV $\lambda_{\text{max}}$ (nm)	ESI-MS data ( <i>m/z</i> )	Assignment
10.1	254, 290	329, 167	Vanillic acid hexoside
11.1	236, 296sh, 324	353, 191, 179, 135	Caffeoylquinic acid 1
17.2	242, 296sh, 324	367, 193, 134	Feruloylquinic acid
17.8	240, 296sh, 328	353, 191, 179, 173, 135	Caffeoylquinic acid 2
21.0	280, 518	595, 449, 287	Cyanidin hexoside + cyanidin deoxyhexoside-hexoside
24.5	280, 518	609, 463, 301	Peonidin hexoside + peonidin deoxyhexoside-hexoside
26.6	234, 296sh, 326	335, 161, 133	Caffeoylshikimic acid
30.9	256, 266sh, 352	595, 300	Quercetin pentoside-hexoside 1
31.7	256, 266sh, 354	609, 300	Quercetin deoxyhexoside-hexoside 1
32.7	256, 266sh, 354	463, 300	Quercetin hexoside 1
32.9	256, 266sh, 352	595, 300	Quercetin pentoside-hexoside 2
33.2	256, 266sh, 354	463, 300	Quercetin hexoside 2
35.0	256, 266sh, 356	433, 300	Quercetin pentoside 1
35.8	256, 266sh, 356	433, 300	Quercetin pentoside 2
36.4	254, 266sh, 352	623, 609, 315, 300	Methylquercetin deoxyhexoside-hexoside + quercetin deoxyhexoside-hexoside 2
37.0	256, 266sh, 352	433, 300	Quercetin pentoside 3
37.4	256, 264sh, 350	447, 300	Quercetin deoxyhexoside
40.5	254, 266sh, 356	447, 314	Methylquercetin pentoside 1
41.3	254, 266sh, 356	447, 314	Methylquercetin pentoside 2
42.3	262, 344	431, 284	Kaempferol deoxyhexoside
42.7	254, 264sh, 354	447, 314	Methylquercetin pentoside 3
43.6	256, 266sh, 352	651, 301	Quercetin acetyl-(deoxyhexoside-hexoside)
44.4	256, 266sh, 354	519, 314	Methylquercetin acetylhexoside
46.0	256, 374	301	Quercetin

*t<sub>R</sub>* – retention time. Mass spectra of phenolic acids and flavonoids were generated in the negative mode, whereas mass spectra of anthocyanins were acquired using positive mode.

**Table S3.** Correlations between total phenolic (TPC), total flavonoid (TFC) and total anthocyanin (TAC) content and antioxidant properties of blackthorn fruit extracts.

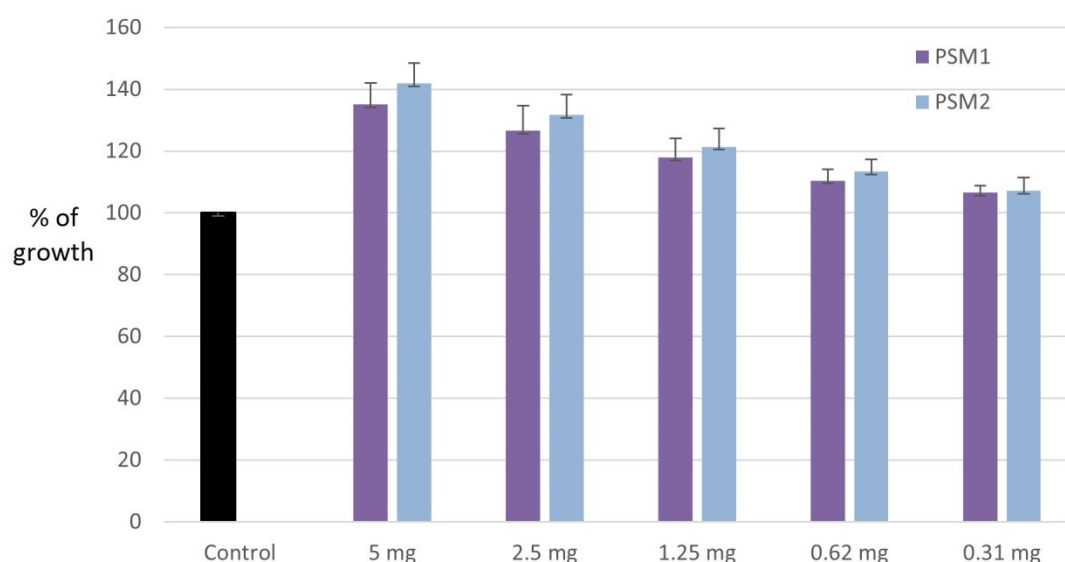
	DPPH	ABTS	FRAP	$\beta$ -carotene bleaching inhibition	ACI
<b>PSM1</b>					
TPC	0.983***	0.985***	0.943***	0.808***	0.981***
TFC	0.211	0.325	0.383	-0.287	0.138
TAC	0.722	0.449	0.643	0.962**	0.773
<b>PSM2</b>					
TPC	0.942***	0.333	0.766*	0.924***	0.928***
TFC	-0.099	0.822**	0.348	-0.336	0.123
TAC	0.897*	-0.099	0.459	0.942**	0.734

PSM1 – methanol extract of *P. spinosa* fruit from the location in western Serbia; PSM2 – methanol extract of *P. spinosa* fruit from the location in central Serbia; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

**Table S4.** Prebiotic effect of blackthorn fruit methanol extracts.

Probiotic strain	PSM1 (mg/mL)					
	5	2.5	1.25	0.625	0.313	0
<i>L. plantarum</i>	132.5 Aa	124.0 Aab	117.1 Aab	104.8 Ab	105.5 Ab	100 b
<i>L. reuteri</i>	133.7 Aa	125.7 Aab	114.2 Aab	108.4 Aab	104.6 Aab	100 b
<i>L. rhamnosus</i>	127.2 Aa	117.0 Aab	113.4 Aabc	109.4 Abc	107.1 Abc	100 c
<i>S. thermophilus</i>	136.2 Aa	124.2 Ab	114.4 Abc	111.4 Ac	104.7 Acd	100 d
<i>S. boulardii</i> 1	139.7 Aa	126.4 Aab	117.3 Abc	111.2 Abcd	105.1 Acd	100 d
<i>S. boulardii</i> 2	148.9 Aa	143.9 Aa	129.8 Ab	111.1 Ac	110.5 Ac	100 d
<i>L. helveticus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> (Mix 1)	127.5 Aa	120.9 Aab	111.8 Abc	110.0 Bbcd	108.1 Acd	100 d
<i>L. helveticus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> , <i>S. boulardii</i> (Mix 2)	135.0 Aa	130.4 Aab	124.9 Aab	117.4 Abc	107.6 Acd	100 d
Mean $\pm$ SD	135.1 $\pm$ 7.0	126.6 $\pm$ 8.1	117.9 $\pm$ 6.3	110.5 $\pm$ 3.5	106.6 $\pm$ 2.1	100.0 $\pm$ 0.0
Probiotic strain	PSM2 (mg/mL)					
	5	2.5	1.25	0.625	0.313	0
<i>L. plantarum</i>	137.3 Aa	128.1 Aab	118.2 Aabc	110.6 Abc	104.8 Abc	100 c
<i>L. reuteri</i>	138.1 Aa	123.8 Aab	112.7 Aab	106.5 Aab	104.0 Ab	100 b
<i>L. rhamnosus</i>	136.6 Aa	129.0 Aab	121.9 Aabc	114.7 Abcd	110.7 Acd	100 d
<i>S. thermophilus</i>	140.3 Aa	128.5 Ab	114.4 Ac	110.4 Acd	99.3 Ad	100 d
<i>S. boulardii</i> 1	151.2 Aa	134.6 Ab	126.4 Abc	114.9 Acd	110.5 Acd	100 d
<i>S. boulardii</i> 2	152.8 Aa	145.2 Aa	129.8 Ab	118.4 Ac	111.5 Ac	100 d
<i>L. helveticus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> (Mix 1)	141.8 Aa	129.4 Ab	122.7 Abc	114.4 Acd	109.8 Ade	100 e
<i>L. helveticus</i> , <i>L. rhamnosus</i> , <i>B. longum</i> , <i>S. boulardii</i> (Mix 2)	137.5 Aa	135.0 Aa	125.2 Aab	116.7 Abc	106.9 Acd	100 d
Mean $\pm$ SD	142.0 $\pm$ 6.4	131.7 $\pm$ 6.5	121.4 $\pm$ 5.9	113.3 $\pm$ 3.9	107.2 $\pm$ 4.2	100.0 $\pm$ 0.0

PSM1 – methanol extract of *P. spinosa* fruit from the location in western Serbia; PSM2 – methanol extract of *P. spinosa* fruit from the location in central Serbia; Results are presented as percent of growth compared to the positive control of each probiotic strain (100 %). Different letters a–e indicate significant differences ( $p < 0.05$ ) between the growths of probiotic species/strain treated with the increasing concentrations of same extract, whereas letters A–B indicate significant differences ( $p < 0.05$ ) between the growths of probiotics species/strain treated with the same concentration of different extract (PSM1 and PSM2).



**Figure S2.** Stimulation of probiotic growth with blackthorn fruit methanol extracts.

PSM1 – methanol extract of *P. spinosa* fruit from the location in western Serbia; PSM2 – methanol extract of *P. spinosa* fruit from the location in central Serbia; Results are expressed as mean percent of growth stimulation  $\pm$  SD of all tested probiotic strains (listed in Table S4) cultivated with PSM1 or PSM2 compared the growth of positive control (expressed as 100% of growth).