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# Prebiotic and antioxidant effects of the extracts from fruits and flowers of *Cereus hildmannianus*

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The authors are fully responsible for both the content and the formal aspects of the Electronic Supplementary Material. No editorial adjustments were made.

**Electronic Supplementary Material**

Supplementary Tables S1–S5

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Table S1. Lactobacilli and bifidobacterial strains used in the study

| Strain | Species | Origin |
|---|---|---|
| CCDM 213 | *Lactobacillus paracasei* ssp. *paracasei* | human faeces |
| CCDM 146 | *Lactobacillus rhamnosus* | human faeces |
| CCDM 466 | *Lactobacillus helveticus* | grasslands, Czech Republic |
| CCDM 364 | *Lactobacillus delbrueckii* ssp. *bulgaricus* | yoghurt |
| CCDM 318 | *Bifidobacterium dentium* | dental caries |
| BB-12 | *Bifidobacterium animalis* ssp. *lactis* | original culture |
| CCDM 775 | *Bifidobacterium longum* ssp. *longum* | human faeces |
| CCDM 559 | *Bifidobacterium bifidum* | child faeces |

CCDM – Culture Collection of Dairy Microorganisms Laktoflora®

Original paper

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Table S2. Compounds putatively identified by UHPLC-ESI-QToF-MS/MS in extracts of flowers (PE and SE) and fruits (EE and ME) from *Cereus hildmannianus*

| Compound | No. | Mf | $M_T$ ($m/z$) | Precursor ion ($m/z$) | Error (ppm) | $t_R$ (min) | Fragment ions | Sample | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Succinic acid | 1 | $C_4H_6O_4$ | 118.0266 | 117.0188 [M-H]$^-$ | –1.70 | 0.95 | 73; 99 | PE | HMDB0000254 |
| | | | | 117.0188 [M-H]$^-$ | –1.70 | 0.99 | | SE | |
| | | | | 117.0184 [M-H]$^-$ | 1.70 | 0.95 | | EE | |
| | | | | 117.0184 [M-H]$^-$ | 1.70 | 0.96 | | ME | |
| Benzoic acid | 2 | $C_7H_6O_2$ | 122.0368 | 121.0288 [M-H]$^-$ | –1.65 | 4.55 | 77; 91; 92; 93 | PE | HMDB0001870 |
| | | | | 121.0288 [M-H]$^-$ | –1.65 | 4.56 | | SE | |
| | | | | 121.0290 [M-H]$^-$ | –3.30 | 4.56 | | EE | |
| | | | | 121.0285 [M-H]$^-$ | 0.82 | 4.56 | | ME | |
| Malic acid | 3 | $C_4H_6O_5$ | 134.0215 | 115.0032 [M-H]$^-$ | –2.88 | 1.12 | 71; 72; 87; 89; 115 | PE | HMDB0000744 |
| | | | | 115.0033 [M-H]$^-$ | 0.86 | 0.84 | | SE | |
| | | | | 115.0029 [M-H]$^-$ | 4.34 | 0.84 | | EE | |
| | | | | 115.0029 [M-H]$^-$ | 4.34 | | | ME | |
| Salicylic acid | 4 | $C_7H_6O_3$ | 138.0336 | 137.0230 [M-H]$^-$ | 3.64 | 4.03 | 93; 94 | PE | HMDB0001895 |
| | | | | 137.0236 [M-H]$^-$ | –0.72 | 4.06 | | SE | |
| | | | | 137.0230 [M-H]$^-$ | 3.64 | 6.68 | | EE | |
| | | | | 137.0235 [M-H]$^-$ | 0.00 | 6.67 | | ME | |
| Vanillin | 5 | $C_8H_8O_3$ | 152.0473 | 151.0387 [M-H]$^-$ | 1.98 | 5.16 | 95; 108; 136; 137 | SE | HMDB0012308 |
| Gallic acid | 6 | $C_7H_6O_5$ | 170.0215 | 169.0130 [M-H]$^-$ | 1.77 | 5.93 | 93; 97; 107; 111; 123; 125; 139; 151 | PE | HMDB0005807 |
| | | | | 169.0132 [M-H]$^-$ | 0.59 | 5.92 | | SE | |
| | | | | 169.0134 [M-H]$^-$ | –0.59 | 5.93 | | EE | |
| 2-isopropylmalic acid | 7 | $C_7H_{12}O_5$ | 176.0684 | 175.0601 [M-H]$^-$ | 0.00 | 4.31 | 83; 113; 123; 139 | PE | NIST 1052052 |
| | | | | 175.0605 [M-H]$^-$ | –2.28 | 3.76 | | SE | |
| | | | | 175.0609 [M-H]$^-$ | –4.45 | 4.35 | | EE | |
| | | | | 175.0601 [M-H]$^-$ | 0.00 | 4.38 | | ME | |
| Azelaic acid | 8 | $C_9H_{16}O_4$ | 188.1049 | 187.0965 [M-H]$^-$ | 0.00 | 4.86 | 97; 123; 125; 169 | SE | HMDB0000784 |
| | | | | 187.0965 [M-H]$^-$ | 0.00 | 4.87 | | EE | |
| | | | | 187.0962 [M-H]$^-$ | 1.60 | 4.89 | | ME | |
| Citric acid | 9 | $C_6H_8O_7$ | 192.0270 | 191.0194 [M-H]$^-$ | –4.18 | 1.44 | 85; 87; 111; 129; 147 | PE | HMDB0000094 |
| | | | | 191.0191 [M-H]$^-$ | –2.61 | 1.38 | | SE | |
| | | | | 191.0194 [M-H]$^-$ | –4.18 | 0.86 | | EE | |
| | | | | 191.0186 [M-H]$^-$ | 0.00 | 1.53 | | ME | |

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Table S2. To be continued

| Compound | No. | Mf | $M_T$ ($m/z$) | Precursor ion ($m/z$) | Error (ppm) | $t_R$ (min) | Fragment ions | Sample | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Ferulic acid | 10 | $C_{10}H_{10}O_4$ | 194.0579 | 193.0498 $[M-H]^-$ | –2.59 | 4.72 | 85; 89; 95; 131; 133; 149; 161 | PE | HMDB0000954 |
| | | | | 193.0493 $[M-H]^-$ | 0.00 | 6.09 | | SE | |
| | | | | 193.0496 $[M-H]^-$ | –1.55 | 6.09 | | EE | |
| | | | | 215.0321 $[M-H]^-$ | 0.46 | 1.62 | | ME | |
| Glucoheptonic acid | 11 | $C_7H_{14}O_8$ | 208.0583 | 225.0609 $[M-H]^-$ | –1.77 | 0.71 | 87; 89; 119; 161; 179 | PE | Santos et al. (2022) |
| | | | | 225.0611 $[M-H]^-$ | –2.66 | 0.74 | | SE | |
| | | | | 225.0616 $[M-H]^-$ | –4.88 | 0.76 | | EE | |
| | | | | 225.0607 $[M-H]^-$ | –0.88 | 0.76 | | ME | |
| Piscidic acid | 12 | $C_{11}H_{12}O_7$ | 256.0583 | 237.0398 $[M-H_2O-H]^-$ | –4.21 | 5.86 | 133; 193; 121 | PE | HMDB0030809 |
| | | | | 237.0395 $[M-H_2O-H]^-$ | –2.95 | 5.86 | | SE | |
| | | | | 237.0397 $[M-H_2O-H]^-$ | –3.79 | 5.85 | | EE | |
| | | | | 237.0389 $[M-H_2O-H]^-$ | –0.42 | 3.99 | | ME | |
| Hydroxybenzoic acid-hexose | 13 | $C_{13}H_{16}O_8$ | 300.0845 | 299.0759 $[M-H]^-$ | –4.01 | 3.80 | 93; 94. 137; 138; 209; 261 | ME | Santos et al. (2022) |
| Tianshic acid | 14 | $C_{18}H_{34}O_5$ | 330.2406 | 329.2322 $[M-H]^-$ | –3.03 | 5.53 | 171; 211; 229 | PE | Santos et al. (2022) |
| | | | | 329.2320 $[M-H]^-$ | –2.42 | 5.51 | | SE | |
| | | | | 329.2328 $[M-H]^-$ | –4.85 | 5.55 | | EE | |
| | | | | 329.2326 $[M-H]^-$ | –4.25 | 5.50 | | ME | |
| Caffeoyl-glucose | 15 | $C_{15}H_{18}O_9$ | 342.0950 | 341.0865 $[M-H]^-$ | –2.34 | 3.68 | 119; 135; 143; 161; 179 | PE | Santos et al. (2022) |
| | | | | 341.0869 $[M-H]^-$ | –3.51 | 4.22 | | SE | |
| | | | | 341.0865 $[M-H]^-$ | –2.34 | 4.00 | | ME | |
| Ferulic acid-hexose | 16 | $C_{16}H_{20}O_9$ | 356.1107 | 355.1025 $[M-H]^-$ | –2.81 | 4.18 | 134; 149; 175; 160; 178; 193 | PE | Santos et al. (2022) |
| | | | | 355.1029 $[M-H]^-$ | –3.94 | 4.17 | | SE | |
| | | | | 355.1032 $[M-H]^-$ | –4.78 | 4.24 | | EE | |
| | | | | 355.1020 $[M-H]^-$ | –1.40 | 4.87 | | ME | |
| Sinapic acid-hexoside | 17 | $C_{17}H_{22}O_{10}$ | 386.1212 | 385.1138 $[M-H]^-$ | –5.97 | 4.93 | 164; 179; 190; 205; 208; 223 | EE | Santos et al. (2022) |
| | | | | 385.1125 $[M-H]^-$ | –2.59 | 4.97 | | ME | |
| Dihydroxybenzoic acid-O-dipentoside | 18 | $C_{17}H_{22}O_{12}$ | 418.1111 | 417.1027 $[M-H]^-$ | –4.07 | 4.46 | 152; 163; 179; 241; 285 | ME | Santos et al. (2022) |
| Benzyl alcohol-dihexose | 19 | $C_{19}H_{28}O_{11}$ | 432.1631 | 431.1550 $[M-H]^-$ | –0.69 | 4.04 | 101; 135; 149; 164; 179; 208; 223 | EE | Santos et al. (2022) |

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Table S2. To be continued

| Compound | No. | Mf | $M_T$ ($m/z$) | Precursor ion ($m/z$) | Error (ppm) | $t_R$ (min) | Fragment ions | Sample | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Lucuminic acid | 20 | $C_{19}H_{26}O_{12}$ | 446.1424 | 445.1335 $[M\text{-}H]^-$ | –2.24 | 3.74 | 75; 103; 119;<br>131; 161; 401 | ME | HMDB0035007 |
| Kaempferol<br>3-O-rutinoside | 21 | $C_{27}H_{30}O_{15}$ | 594.1585 | 593.1508 $[M\text{-}H]^-$<br>593.1506 $[M\text{-}H]^-$<br>593.1509 $[M\text{-}H]^-$<br>593.1493 $[M\text{-}H]^-$ | –3.87<br>–3.54<br>–4.04<br>–1.34 | 4.44<br>4.43<br>4.26<br>4.47 | 235; 255; 267;<br>285; 286 | PE<br>SE<br>EE<br>ME | Santos et al.<br>(2022) |
| Quercetin<br>3-O-rutinoside | 22 | $C_{27}H_{30}O_{16}$ | 610.1533 | 609.1450 $[M\text{-}H]^-$<br>609.1455 $[M\text{-}H]^-$<br>609.1463 $[M\text{-}H]^-$<br>609.1440 $[M\text{-}H]^-$ | –3.44<br>–4.26<br>–5.58<br>–1.80 | 4.35<br>4.38<br>4.42<br>4.40 | 178; 241; 242;<br>285; 297 | PE<br>SE<br>EE<br>ME | HMDB0003249 |
| Narcissin | 23 | $C_{28}H_{32}O_{16}$ | 624.1690 | 623.1610 $[M\text{-}H]^-$<br>623.1616 $[M\text{-}H]^-$<br>623.1611 $[M\text{-}H]^-$ | –4.81<br>–5.77<br>–4.97 | 4.50<br>4.48<br>4.49 | 165; 225; 242;<br>243; 286 | PE<br>SE<br>EE | Santos et al.<br>(2022) |
| Isorhamnetin<br>3-O-sophoroside-<br>7-O-rhamnoside | 24 | $C_{34}H_{42}O_{21}$ | 786.2218 | 785.2119 $[M\text{-}H]^-$<br>785.2133 $[M\text{-}H]^-$<br>785.2140 $[M\text{-}H]^-$ | –3.18<br>–4.96<br>–5.85 | 4.34<br>4.36<br>4.39 | 179; 357; 623 | PE<br>SE<br>EE | Santos et al.<br>(2022) |

UHPLC-ESI-QToF/MS/MS – ultra-high performance liquid chromatography-coupled with electrospray ionisation/quadrupole-time-of-flight/mass spectrometry/mass spectrometry; Mf – molecular formula; $M_T$ – theoretical exact mass; $m/z$ – mass-to-charge ratio; PE – petal extract; SE – sepal extract; EE – epicarp extract; ME – mesocarp extract

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Table S3. Putative identification of compounds by UHPLC-ESI-QToF-MS/MS in extracts of fruits (EE and ME) and flowers (PE and SE) from *Cereus hildmannianus*

| Compound | No. | Mf | $M_T$ ($m/z$) | Fruits | | Flowers | | Class |
|---|---|---|---|---|---|---|---|---|
| | | | | EE | ME | PE | SE | |
| Succinic acid | 1 | $C_4H_6O_4$ | 118.0266 | √ | – | √ | √ | carboxylic acid |
| Benzoic acid | 2 | $C_7H_6O_2$ | 122.0368 | √ | √ | √ | √ | carboxylic acid |
| Malic acid | 3 | $C_4H_6O_5$ | 134.0215 | √ | √ | √ | √ | carboxylic acid |
| Salicylic acid | 4 | $C_7H_6O_3$ | 138.0336 | √ | √ | √ | √ | carboxylic acid |
| Vanillin | 5 | $C_8H_8O_3$ | 152.0473 | – | – | – | √ | phenolic aldehyde |
| Gallic acid | 6 | $C_7H_6O_5$ | 170.0215 | √ | – | √ | √ | phenolic acid |
| 2-isopropylmalic acid | 7 | $C_7H_{12}O_5$ | 176.0684 | √ | √ | √ | √ | carboxylic acid |
| Azelaic acid | 8 | $C_9H_{16}O_4$ | 188.1049 | √ | √ | – | √ | phenolic acid |
| Citric acid | 9 | $C_6H_8O_7$ | 192.0270 | √ | √ | √ | √ | phenolic acid |
| Ferulic acid | 10 | $C_{10}H_{10}O_4$ | 194.0579 | √ | √ | √ | √ | phenolic acid |
| Glucoheptonic acid | 11 | $C_7H_{14}O_8$ | 208.0583 | √ | √ | √ | √ | phenolic acid |
| Piscidic acid | 12 | $C_{11}H_{12}O_7$ | 256.0583 | √ | √ | √ | √ | phenolic acid |
| Hydroxybenzoic acid-hexose | 13 | $C_{13}H_{16}O_8$ | 300.0845 | – | √ | – | – | phenolic acid |
| Tianshic acid | 14 | $C_{18}H_{34}O_5$ | 330.2406 | √ | √ | √ | √ | phenolic acid |
| Caffeoyl-glucose | 15 | $C_{15}H_{18}O_9$ | 342.0950 | – | √ | √ | √ | phenolic acid |
| Ferulic acid-hexose | 16 | $C_{16}H_{20}O_9$ | 356.1107 | √ | √ | √ | √ | phenolic acid |
| Sinapic acid-hexoside | 17 | $C_{17}H_{22}O_{10}$ | 386.1212 | √ | √ | – | – | phenolic acid |
| Dihydroxybenzoic acid-O-dipentoside | 18 | $C_{17}H_{22}O_{12}$ | 418.1111 | – | √ | – | – | phenolic acid |
| Benzyl alcohol-dihexose | 19 | $C_{19}H_{28}O_{11}$ | 432.1631 | √ | – | – | – | aromatic alcohol |
| Lucuminic acid | 20 | $C_{19}H_{26}O_{12}$ | 446.1424 | – | √ | – | – | phenolic acid |
| Kaempferol 3-O-rutinoside | 21 | $C_{27}H_{30}O_{15}$ | 594.1585 | √ | √ | √ | √ | flavonol |
| Quercetin 3-O-rutinoside | 22 | $C_{27}H_{30}O_{16}$ | 610.1533 | √ | √ | √ | √ | flavonol |
| Narcissin | 23 | $C_{28}H_{32}O_{16}$ | 624.1690 | √ | – | √ | √ | flavonol |
| Isorhamnetin 3-O-sophoroside-7-O-rhamnoside | 24 | $C_{34}H_{42}O_{21}$ | 786.2218 | √ | – | √ | √ | flavonol |
| Number of phenolic compounds, organic acids and derivatives | | | | 15 | 17 | 13 | 15 | 20 |
| Number of flavonoids | | | | 4 | 2 | 4 | 4 | 4 |
| Total number of phenolic compounds | | | | 19 | 19 | 17 | 19 | 24 |

UHPLC-ESI-QToF/MS/MS – ultra-high performance liquid chromatography-coupled with electrospray ionisation/quadrupole-time-of-flight/mass spectrometry/mass spectrometry; Mf – molecular formula; $M_T$ – theoretical exact mass; $m/z$ – mass-to-charge ratio; EE – epicarp extract; ME – mesocarp extract; PE – petal extract; SE – sepal extract; √ – identified in sample

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Table S4. Measurement of the pH of extracts from *Cereus hildmannianus* stems by bifidobacteria and lactobacilli strains, the values are presented as a change of pH after 24 h of cultivation (ΔpH)

| Bacterial strains | BM | WCH | GR | Fruit | | Flower | |
|---|---|---|---|---|---|---|---|
| | | | | EE | ME | SE | PE |
| *Lacticaseibacillus paracasei* ssp. *paracasei* CCDM 213 | 0.63 | 2.14 | 2.46 | 1.87 | 3.48 | 1.65 | 1.57 |
| *Lacticaseibacillus rhamnosus* CCDM 146 | 0.78 | 2.20 | 1.35 | 1.87 | 2.24 | 1.67 | 1.66 |
| *Lacticaseibacillus helveticus* CCDM 466 | 0.71 | 2.31 | 1.68 | 1.96 | 2.36 | 1.78 | 1.74 |
| *Lacticaseibacillus delbrueckii* ssp. *bulgaricus* CCDM 364 | 0.66 | 2.20 | 1.06 | 1.93 | 2.32 | 1.73 | 1.71 |
| *Bifidobacterium dentium* CCDM 318 | 1.23 | 2.25 | 1.78 | 2.08 | 2.40 | 2.03 | 2.02 |
| *Bifidobacterium longum* ssp. *longum* CCDM 775 | 1.05 | 2.09 | 0.95 | 2.76 | 2.38 | 1.50 | 1.74 |
| *Bifidobacterium bifidum* CCDM 559 | 1.43 | 2.24 | 1.00 | 2.02 | 2.39 | 1.82 | 1.80 |
| *Bifidobacterium animalis* ssp. *lactis* BB-12® | 1.22 | 2.37 | 1.33 | 2.10 | 2.43 | 2.03 | 2.03 |

CCDM – Culture Collection of Dairy Microorganisms; BM – basal medium with no sugar (negative control); WCH – Wilkins-Chalgren anaerobic broth (growth control); GR – BM with Orafti® GR chicory inulin (positive control); EE – epicarp extract; ME – mesocarp extract; SE – sepal extract; PE – petal extract

Table S5. Fermentability of extracts from *Cereus hildmannianus* by *Bifidobacterium* and *Lactobacillus* species, given as a change in absorbance (at 620 nm) after 24 h of cultivation ($\Delta A_{620\ \mathrm{nm}}$)

| Bacterial species/strains | BM | WCH | GR | Fruit | | Flower | |
|---|---|---|---|---|---|---|---|
| | | | | EE | ME | SE | PE |
| *Lacticaseibacillus paracasei* ssp. *paracasei* CCDM 213 | 0.32 ± 0.12$^{a}$ | 0.73 ± 0.10$^{d}$ | 0.86 ± 0.07$^{e}$ | 0.61 ± 0.04$^{c}$ | 0.81 ± 0.06$^{de}$ | 0.48 ± 0.11$^{b}$ | 0.53 ± 0.07$^{bc}$ |
| *Lacticaseibacillus rhamnosus* CCDM 146 | 0.36 ± 0.11$^{a}$ | 0.85 ± 0.09$^{d}$ | 0.48 ± 0.15$^{b}$ | 0.70 ± 0.07$^{c}$ | 0.93 ± 0.08$^{d}$ | 0.67 ± 0.05$^{c}$ | 0.66 ± 0.09$^{c}$ |
| *Lacticaseibacillus helveticus* CCDM 466 | 0.24 ± 0.04$^{a}$ | 0.79 ± 0.23$^{d}$ | 0.35 ± 0.03$^{ab}$ | 0.49 ± 0.06$^{bc}$ | 0.75 ± 0.22$^{d}$ | 0.53 ± 0.07$^{c}$ | 0.50 ± 0.14$^{bc}$ |
| *Lacticaseibacillus delbrueckii* ssp. *bulgaricus* CCDM 364 | 0.27 ± 0.05$^{a}$ | 0.80 ± 0.16$^{c}$ | 0.38 ± 0.17$^{ab}$ | 0.45 ± 0.16$^{ab}$ | 0.78 ± 0.23$^{c}$ | 0.51 ± 0.18$^{b}$ | 0.50 ± 0.23$^{b}$ |
| *Bifidobacterium dentium* CCDM 318 | 0.44 ± 0.14$^{a}$ | 0.86 ± 0.09$^{d}$ | 0.54 ± 0.12$^{b}$ | 0.78 ± 0.06$^{cd}$ | 1.02 ± 0.05$^{e}$ | 0.79 ± 0.04$^{cd}$ | 0.71 ± 0.02$^{c}$ |
| *Bifidobacterium longum* ssp. *longum* CCDM 775 | 0.27 ± 0.05$^{a}$ | 0.78 ± 0.13$^{c}$ | 0.36 ± 0.14$^{ab}$ | 0.41 ± 0.23$^{ab}$ | 0.83 ± 0.24$^{c}$ | 0.49 ± 0.10$^{b}$ | 0.47 ± 0.08$^{b}$ |
| *Bifidobacterium bifidum* CCDM 559 | 0.27 ± 0.09$^{a}$ | 0.80 ± 0.09$^{c}$ | 0.34 ± 0.08$^{a}$ | 0.63 ± 0.07$^{b}$ | 0.83 ± 0.10$^{c}$ | 0.66 ± 0.04$^{b}$ | 0.62 ± 0.07$^{b}$ |
| *Bifidobacterium animalis* ssp. *lactis* BB-12® | 0.34 ± 0.03$^{a}$ | 0.91 ± 0.07$^{d}$ | 0.42 ± 0.13$^{a}$ | 0.71 ± 0.07$^{bc}$ | 0.98 ± 0.11$^{d}$ | 0.72 ± 0.06$^{c}$ | 0.61 ± 0.11$^{b}$ |

$^{a-e}$Different superscript letters indicate statistically significant differences ($P < 0.05$) by the multiple comparison (LSD) test; values are mean ± SD (standard deviation); CCDM – Culture Collection of Dairy Microorganisms; BM – basal medium with no sugar (negative control); WCH – Wilkins-Chalgren anaerobic broth (growth control); GR – BM with Orafti® GR chicory inulin (positive control); EE – epicarp extract; ME – mesocarp extract; SE – sepal extract; PE – petal extract