https://doi.org/10.17221/128/2024-CJFS

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

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Citation: Santos É.S., Krausová G., Hyršlová I., Machado M.F.P.S., de Oliveira A.J.B., Özlük G., Gonçalves R.A.C. (2024): Prebiotic and antioxidant effects of the extracts from fruits and flowers of *Cereus hildmannianus*. Czech J. Food Sci., 42: 415–422.

Abstract: *Cereus hildmannianus* is a species with various nutritional and medicinal properties; however, the fruits and flowers have scarcely been explored. In this regard, the study investigated the bioproduction of total sugar content (TSC), total phenolic content (TPC), and total flavonoid content (TFC), antioxidant [DPPH – 2,2-diphenyl-1-picrylhydrazyl and ABTS – 2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonic acid)], iron chelation, and prebiotic activities of methanolic extracts from fruits (epicarps – EE, mesocarps – ME) and flowers (sepals – SE, petals – PE) of *C. hildmannianus*. The chemical profiles of the extracts were determined by ultra-high-performance liquid chromatography coupled with mass spectrometry. The highest accumulations of TSC were observed in the ME (64%), while the SE also had a high TFC (17 µg QE per mg DW; QE – quercetin equivalents, DW – dry weight) and the EE had a high TPC (646 µg GAE per mg DW; GAE – gallic acid equivalents). A total of 24 compounds (phenolic and organic acids; and glycosylated flavonoids) were putatively identified. The greatest antioxidant activities were obtained with the PE (DPPH: 199 µmol Trolox per mg DW; and ABTS: 59 µmol Trolox per mg DW), while the highest prebiotic effect was obtained with ME and EE regarding both fermentability and production of lactic and acetic acids by multiple species of *Lactobacillus* and *Bifidobacterium*. These promising results of bioactive compounds in the fruits and flowers of *C. hildmannianus* have potential applications for food and pharmaceutical purposes.

Keywords: cactus; phytochemicals; food; phenolic compounds; prebiotic activity

Natural substances derived from plants hold great potential for use in the pharmaceutical, cosmetic, and food industries due to their beneficial properties, biological activities, and non-toxic nature (Yang et al. 2019). The demand for functional foods containing prebiotic ingredients is also steadily increasing.

Supported by the National Council for Scientific and Technological Development, Coordination for the Improvement of Higher Education Personnel, and Fundação Araucária. The study was also supported by the Ministry of Agriculture of the Czech Republic – Institutional support MZE-RO1424.

https://doi.org/10.17221/128/2024-CJFS

The prebiotic effect of plants is gaining increasing attention in human and animal nutrition and health. Prebiotics are defined as 'substrates selectively utilised by host microorganisms, providing health benefits' (Gibson et al. 2017). Current research is focused on discovering new prebiotic compounds that stimulate a wider range of beneficial microorganisms. While traditionally carbohydrate-based, such as inulin, these compounds may also include non-carbohydrate substances like phenolic compounds, fatty acids, and micronutrients (Cunningham et al. 2021).

Cereus hildmannianus K. Schum. (Cactaceae), native to southern Brazil, is cultivated for its medicinal and nutraceutical properties. Cactaceae species are known for producing significant amounts of fibre, fermentable carbohydrates, and phenolic compounds, including phenolic acids, glycosides, and flavonols, along with other bioactive compounds (Carpena et al. 2023). However, research has been limited to a few species, underscoring the need for further studies to explore fermentation as a broader valorisation strategy.

The current study hypothesises that the cactus species *C. hildmannianus* has a great potential to satisfy the demand for functional foods and prebiotic ingredients from their exotic fruits and/or flowers. Thus, this study aimed to chemically characterise the fruits (epicarp and mesocarp) and flowers (petals and sepals) by ultra-high performance liquid chromatography-coupled with electrospray ionisation/quadrupole-time-of-flight/mass spectrometry/mass spectrometry (UHPLC-ESI-QToF/MS/MS). The phenolic and sugar contents of the extracts were quantified by UV-Vis methods, as well as the iron ion chelating capacity, antioxidant and prebiotic activities were evaluated.

MATERIAL AND METHODS

Plant material. The fruits and flowers were collected from plants of *C. hildmannianus* at the State University of Maringá, Paraná, Brazil, latitude 23°40'15"S, longitude 51°56'22"W, altitude 517 m above sea level, from January to March (summer) in 2020. The species was identified by Dr. Daniela Cristina Zappi from the Instituto Tecnológico Vale (ITV) and a voucher (No. HUEM 36127) and register (No. A05B398) were added to the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen). After collection, the materials were separated: the fruits into epicarp and mesocarp, and the flowers into sepals and petals.

Extraction procedure. Five grams of lyophilised samples (fruits: epicarp and mesocarp) and (flowers: sepals and petals) were powdered and exhaustively extracted (three extractions every 24 h) with methanol (MeOH) (1 : 20, *w*/*v*) at room temperature. The solid parts were separated by filtration using a filter paper, subsequently, the solvent was removed using a rotavapor (R-100; Büchi, Switzerland) at 35 °C, generating the following crudes extracts: epicarp (EE), mesocarp (ME), petals (PE), and sepals (SE). Then the extracts were lyophilised at –30 °C and vacuum of 120 µm Hg (L101; Liobras, Brazil) and stored at –20 °C.

Chemical characterisation. The total sugar content (TSC) in the extracts (50 $\mu g \cdot mL^{-1}$) was determined by the phenol-sulfuric acid method according to DuBois et al. (1956) with D-fructose (Sigma-Aldrich, Germany) as a standard. The results are expressed as percentages in relation to the fructose standard.

The total phenolic content (TPC) was determined by the Folin-Ciocalteau method and total flavonoid content (TFC) was measured using an aluminium chloride ($AlCl_3$) method, described by Santos et al. (2022b).

Ultra-high performance liquid chromatography (UHPLC-ESI-QToF-MS/MS) was done as follows: extracts (5 mg) were resuspended in 1 mL of methanol (99.9%) and 3 µL were analysed by ultra-high performance liquid chromatography (UHPLC; Nexera X2; Shimadzu, Japan) coupled with HRMS-QToF (Impact II, Bruker Corporation, USA) equipped with an ESI source, the methodology was described by Santos et al. (2022b).

Biological properties. Experimental data on antioxidant activity were obtained by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and ABTS [2,2'-azino-bis-(-3-ethylbenzothiazoline-6-sulfonicacid), diammonium salt, ~98%] method (Thaipong et al. 2006).

The iron ion chelating capacity of the extracts was determined by the method of Santos et al. (2022a). The solution of extracts at an amount of 0.8 mL (1 $mg \cdot mL^{-1}$) was mixed with 0.4 mL of iron (II) sulphate (Fe^{2+}) and iron (III) chloride (Fe^{3+}) at a concentration of 600 $\mu g \cdot mL^{-1}$, then it was incubated for 10 min at room temperature. Acetate buffer (1.5 mL; pH 5.6) coupled with gallic acid (1.5 mL; 1%; Sigma, USA) was then added, and the samples were left to shake at room temperature for 10 min. Ethylenediaminetetraacetic acid (EDTA) was used as a control. The absorbances were obtained in the visible spectrum at 570 nm. The obtained data were used to plot linearity curves so that the regression equa-

https://doi.org/10.17221/128/2024-CJFS

tion and correlation coefficient equation could be determined. The chelating capacity (%) was calculated using Equation 1.

$$Chelating\ capacity\ (\%) = \frac{AC_{570} - AS_{570}}{AC_{570}} \times 100 \qquad (1)$$

where: AC_{570} – the control absorbance (iron ions + H_2O) at 570 nm; AS_{570} – the absorbance of the samples (iron ions + EDTA or extracts) at 570 nm.

Four species of *Lactobacillus* (*Lacticaseibacillus paracasei* ssp. *parabases, Lacticaseibacillus rhamnosus, L. helveticus*, and *L. delbrueckii* ssp. *bulgaricus*) and four species of *Bifidobacterium* (*B. dentium, B. animalis* ssp. *lactis, B. longum* ssp. *longum,* and *B. bifidum*) were tested in this study, see Electronic Supplementary Material (ESM), Table S1. The strains originated from the Culture Collection of Dairy Microorganisms Laktoflora® (CCDM, Czech Republic), and the commercial probiotic strain, *Bifidobacterium animalis* ssp. *lactis* BB-12®, was obtained from Chr. Hansen (Denmark). Prior to each analysis, bacterial cells were transferred twice to fresh De Man-Rogosa-Sharpe (MRS; pH 6.2) broth (Merck KGaA, Germany) with L-cysteine hydrochloride for the *Bifidobacterium* spp. or fresh MRS (pH 5.7) for the *Lactobacillus* spp. and cultivated under anaerobic conditions at 37 °C for 18 h using the Oxoid (UK) anaerobic system with an anaerobic jar and gas-generating system.

To assess bacterial growth in the assays, the following media were used: basal medium (BM; 10 g tryptone, 10 g peptone, 5 g yeast extract, 1 mL Tween 80, 0.5 g L-cysteine hydrochloride, 1 L distilled water, pH 6.5), BM containing commercial prebiotic formula Orafti® GR chicory inulin (2 $g \cdot L^{-1}$; Beneo, Belgium), BM containing *C. hildmannianus* extracts (EE, ME, SE, and PE; 2 $g \cdot L^{-1}$), and Wilkins-Chalgren anaerobic broth (WCH; Oxoid, UK) as growth control. Media for *Bifidobacterium* spp. were supplemented with 0.5 $g \cdot L^{-1}$ L-cysteine hydrochloride. Briefly, fresh bacterial suspensions in the exponential growth phase were centrifuged (6 000 × g, 7 min), washed twice with sterile physiological saline solution, and then resuspended at a final concentration of 10^3–10^4 $CFU \cdot mL^{-1}$ (CFU – colony-forming units). Media were inoculated with a fresh overnight grown culture of each strain, 1.0% (*v*/*v*) of bacterial suspension in triplicate into the respective media and cultivated anaerobically at 37 °C for 24 h. The growth of bacterial cells was evaluated as the change in absorbance at a wavelength of 620 nm (ONDA V-10 Plus; Giorgio Bormac, Italy) and by measuring the pH (sensION 1; HACH, USA) of the medium.

In order to determine the bacterial metabolites, concentrations of lactic and acetic acids were measured using the isotachophoresis (ITP) method by IONOSEP 2003 (Recman, Czech Republic). Before analysis, the separated samples were diluted with 150 volumes of deionised water and then purified using the Puradisc FP 30 filter with a pore size of 0.2 μm (Whatman, Germany). A solution containing 10 mM HCl, 22 mM aminocaproic acid, and 0.1% 2-hydroxyethyl cellulose (pH 4.5) was used as the leading electrolyte (LE). The used trailing electrolyte (TE) was 5 mM caproic acid. All chemicals were sourced from Sigma-Aldrich (Germany). The used initial and final stream values were 80 A and 30 A, respectively.

Statistical analysis. The assays were performed in triplicate (independent experiments), and the results are presented as means ± standard deviation (SD). The data were analysed using Statistica software (version 10). Analysis of variance (ANOVA) and post-hoc Tukey's and multiple comparison (LSD) tests were used to evaluate significant differences in the experiments. The results were considered statistically significant for values of $P < 0.05$.

RESULTS AND DISCUSSION

Extractive yield and phenolic compounds. Dry fruits (epicarp and mesocarp tissue) and flowers (tissue of sepals and petals) were exhaustively extracted with methanol. The highest extract yields were obtained from the mesocarp (ME; 81.5 ± 2.1%; $P < 0.05$), followed by the epicarp (EE; 56.4 ± 2.3%), both of which showed a higher mass yield than those from the sepals (SE) and petals (PE), Table 1. The highest TSC was obtained from the ME (64.0 ± 1.4%; $P < 0.05$), followed by the EE (35.5 ± 5.1%) and the SE (28.8 ± 2.9%), Table 1. The extracts from the fruits of *C. hildmannianus* presented 2.06 g per 100 g of TSC in their constitution (Pereira et al. 2013).

The highest TPC was found in the EE (646.7 ± 7.5 μg GAE per mg DW; $P < 0.05$; GAE – gallic acid equivalents, DW – dry weight), followed by the PE (632.6 ± 5.2 μg GAE per mg DW) and the SE (535.9 ± 4.5 μg GAE per mg DW), Table 1. The highest TFC was found in the SE (17.5 ± 1.1 μg QE per mg DW; QE – quercetin equivalents), followed by the EE (14.7 ± 0.8 μg QE per mg DW) and the PE (7.4 ± 0.15 μg QE per mg DW), Table 1.

https://doi.org/10.17221/128/2024-CJFS

Table 1. Extractive yield (%), total sugar content (TSC), total phenolic content (TPC), and total flavonoid content (TFC) of extracts of the fruits and flowers from *Cereus hildmannianus*

Extracts	Yield (%)	TSC (%)	TPC (µg GAE per mg DW)	TFC (µg QE per mg DW)
EE	56.4 ± 2.3[b]	35.5 ± 5.1[b]	639.4 ± 7.2[a]	14.6 ± 0.8[b]
ME	81.5 ± 2.0[a]	64.0 ± 1.4[a]	55.5 ± 0.5[c]	n.d.
SE	4.2 ± 0.5[c]	28.8 ± 2.9[bc]	535.8 ± 4.5[b]	17.3 ± 1.1[a]
PE	5.3 ± 2.1[c]	22.5 ± 5.8[c]	632.6 ± 5.2[a]	7.3 ± 0.1[c]

[a–c]Different letters indicate statistically significant differences by Tukey's test ($P < 0.05$); EE – epicarp extract; ME – mesocarp extract; PE – petal extract; SE – sepal extract; n.d. – not detected; GAE – gallic acid equivalents; DW – dry weight; QE – quercetin equivalents

In previous studies on cladodes of *C. hildmannianus*, significant levels of phenolic compounds (756 µg GAE per mg DW) and flavonoids (90 µg QE per mg DW) were reported (Santos et al. 2022b). Meanwhile, *C. peruvianus* cladode extracts showed TPCs of 11 mg GAE per g and 15 mg GAE per g in two different collection years (2015 and 2016, respectively) (Nunes et al. 2022). The TPC of *C. hildmannianus* fruit extracts was 1 337 mg CAE per 100 g (CAE – chlorogenic acid equivalents) fresh sample (Pereira et al. 2013). There is a significant difference in the TPCs between the cladodes of *C. hildmannianus* and *C. peruvianus*. These variations may be attributed to several factors, including the timing of collection, whether the plants were flowering or fruiting, and potential pathogen attacks (Ksouri et al. 2008; Dutra et al. 2019).

Despite the presence of many flowers with significant nutritional value and high levels of nutraceutical compounds, their consumption remains minimal in Brazilian food culture. In contrast, cladodes and fruits are already more commonly consumed (Shetty et al. 2012).

Identification of compounds by UHPLC-ESI-QToF-MS/MS. Chemical investigation of the fruit and flower extracts from *C. hildmannianus* resulted in the putative identification of 24 compounds (20 phenolic and organic acids and their derivatives, as well as four flavonoids). The samples were analysed by HRMS, and the identification of compounds was proposed after a review of the genus *Cereus* and family Cactaceae, in addition to the mass error value (ESM, Tables S2 and S3).

Some compounds were found to be extract-specific. In the SE, the compound vanillin was identified. In the ME, hydroxybenzoic acid-hexose, dihydroxybenzoic acid-O-dipentoside, and lucuminic acid were identified; while in the EE, benzyl alcohol-dihexose and sinapic acid-hexoside were identified.

The compounds identified in the extracts (epicarp, mesocarp, sepals, and petals) were: succinic acid, benzoic acid, malic acid, salicylic acid, gallic acid, 2-isopropylmalic acid, azelaic acid, citric acid, ferulic acid, glucoheptonic acid, piscidic acid, tianshic acid, caffeoyl-glucose, ferulic acid-hexose, kaempferol 3-O-rutinoside, quercetin 3-O-rutinoside (rutin), narcissin, and isorhamnetin 3-O-sophoroside-7-O-rhamnoside (ESM, Tables S2 and Table S3).

These results demonstrate that the profile of phenolic compounds in flowers and fruits is the same as that previously reported for the cladodes and callus cultures of *C. hildmannianus*, which were likewise determined by UHPLC-ESI-QToF-MS/MS (Santos et al. 2022b).

Antioxidant activity and chelating capacity. The extracts from fruits and flowers of *C. hildmannianus* presented antioxidant activity by both methodologies (DPPH and ABTS; Table 2). The greatest antioxidant potential was obtained with the flower extracts as the PE presented results of 199.2 ± 1.6 µmol Trolox per mg DW and 59.8 ± 1.3 µmol Trolox per mg DW in the DPPH and ABTS assays, respectively ($P < 0.05$), followed by the SE with 176.1 ± 6.8 µmol Trolox per mg DW and 51.4 ± 2.3 µmol Trolox per mg DW, respectively. The EE showed activities of 81.2 ± 3.9 µmol Trolox per mg DW and 55.0 ± 1.2 µmol Trolox per mg DW in the DPPH and ABTS assays, respectively. The higher antioxidant activity observed for the flower extracts could be a reflection of the higher content of flavonoids, the compounds that have already shown an excellent antioxidant potential (Ouerghemmi et al. 2017).

Notably, the PE exhibited the highest significant antioxidant activity, despite having the second lowest total flavonoid content. This elevated activity may be attributed to the relatively higher concentrations of specific compounds, including kaempferol 3-O-rutinoside, isorhamnetin-3-O-rutinoside, and quercetin 3-O-rutinoside, as previously identified by Ouerghemmi et al. (2017).

The extracts from fruits of *C. hildmannianus* demonstrated antioxidant activity by the DPPH (3 249 g fresh sample per g DPPH) and ABTS methods (19 M

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Table 2. Antioxidant activity of extracts from the fruits and flowers of *Cereus hildmannianus* (1 mg·mL^{-1}) assessed by the DPPH and ABTS assays, chelating capacity of the extracts for Fe^{2+} and Fe^{3+}

Extracts	Antioxidant activity (µmol Trolox per mg DW)		Chelating capacity (%) for Fe^{2+} and Fe^{3+}	
	DPPH	ABTS	Fe^{2+}	Fe^{3+}
EE	81.2 ± 3.9^{c}	55.5 ± 1.2^{b}	1.9 ± 0.2^{d}	13.3 ± 0.2^{b}
ME	42.9 ± 2.1^{d}	22.4 ± 0.3^{c}	n.d.	2.2 ± 0.3^{e}
SE	176.0 ± 6.8^{b}	51.3 ± 2.3^{b}	3.1 ± 0.1^{c}	3.6 ± 0.8^{d}
PE	199.2 ± 1.6^{a}	59.8 ± 1.3^{a}	5.7 ± 0.5^{b}	5.7 ± 0.2^{c}
EDTA	n.t.	n.t.	99.9 ± 0.1^{a}	99.8 ± 0.1^{a}

$^{a-e}$Different letters indicate statistically significant differences by Tukey's test ($P < 0.05$); EE – epicarp extract; ME – mesocarp extract; SE – sepal extract; PE – petal extract; EDTA – ethylenediaminetetraacetic acid; DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); n.t. – not tested; n.d. – not detected

Trolox equivalents per g) (Pereira et al. 2013). Extracts obtained from *C. jamacaru* during the fruiting stage, meanwhile, exhibited antioxidant activity through a 43% inhibition of the ABTS radical and a 45.2% inhibition of the DPPH radical at concentrations of 62.5 µg·mL^{-1}. The peels of these fruits, however, had a 2.4% and 2.1% inhibition of the ABTS and DPPH radicals, respectively, at a concentration of 62.5 µg·mL^{-1}, while the fruit pulp did not demonstrate any antioxidant activity (Dutra et al. 2019).

The extracts with the highest iron ion chelating capacity were the EE with Fe^{3+} at 13.4 ± 0.2% ($P < 0.05$) and Fe^{2+} (1.9 ± 0.2%), and the PE with Fe^{2+} at 5.8 ± 0.5% ($P < 0.05$), at 1 mg·mL^{-1}, Table 2. The extracts from the pulp of the *C. jamacaru* fruit were able to chelate iron ions (2.7% for Fe^{2+} at 62.5 µg·mL^{-1}), whereas the extract from the bark did not show any activity in this test (Dutra et al. 2019). However, our study is the first to report on the iron ion sequestration activity of flower and fruit extracts from *C. hildmannianus*, demonstrating their potential as an alternative bioremediatory product for industrial water treatment.

Assessment of prebiotic activity. In the present study, extracts from *C. hildmannianus* flowers and fruits were assayed and their fermentability by bifidobacteria and lactobacilli was tested.

Bacterial multiplication, based on bacterial density, showed that the fruit and flower extracts were fermented and metabolised by all tested bacterial species/strains, as significantly higher growth ($P < 0.05$) was observed when compared to the growth in the BM alone (negative control), as well as in the BM containing Orafti® GR chicory inulin and in the WCH broth containing dextrose as the carbon source (positive controls). This may be related to the phenolic compounds present in the extracts. Therefore, the prebiotic effect of phenolic-rich extracts is reported for the first time for the *C. hildmannianus* species. Among the tested bacterial species, *Lactobacillus rhamnosus*, *Bifidobacterium dentium*, *B. bifidum*, and *B. animalis* ssp. *lactis* displayed the greatest fermentation capacity of the extracts from fruits and flowers of *C. hildmannianus*.

The greatest prebiotic effect, as assessed by bacterial density, was obtained with the extracts from fruits (ME and EE). The absorbances (A_{620}) ranged from 0.78 (*L. delbrueckii* ssp. *bulgaricus*) to 1.02 (*B. dentium*) with ME ($P < 0.05$), and 0.41 (*B. longum* ssp. *longum*) to 0.78 (*B. dentium*) with EE. For the flower extracts, absorbances of 0.48 (*L. paracasei* ssp. *paracasei*) to 0.79 (*B. dentium*) were observed with SE, and 0.47 (*B. longum* ssp. *longum*) to 0.71 (*B. dentium*) with PE (ESM, Table S4). The ME had the most prominent prebiotic potential against the bacterial species tested.

Complementary results to the bacterial density are the measurements of the pH of the culture medium, in which the acidity of the culture medium reflects the fermentation carried out by the bacteria using the offered carbon source, and consequently greater production of short-chain acids (SCFAs). The most significant results of this assay were in accordance with those found by the bacterial density assay, with the ME, followed by the EE being the best (ESM, Table S5).

Phenolic compounds are considered significant secondary metabolites of plants, fruits, vegetables, and seeds, comprising various classes based on their chemical structure, origin, or biological activity. This diverse group includes flavonoids, stilbenes, lignans, phenolic acids, and polyphenolic amides (Abbas et al. 2017). Each class exhibits distinct characteristics and contributes to the wide array of biological activities attributed to polyphenols. Their structural diversity allows them to exert various health benefits and play important roles in plant defence mechanisms. Plant polyphenols have the potential to meet the criteria for

https://doi.org/10.17221/128/2024-CJFS

prebiotics although further studies in the target host are necessary to confirm their prebiotic properties. It is estimated that approximately 90–95% of dietary polyphenols are not absorbed in the small intestine and, as a result, they reach the colon (Plamada and Vodnar 2021). Once in the colon, these polyphenols serve as substrate for the gut microbiota.

Numerous studies have been conducted to elucidate the role of phenolic compounds as prebiotic substrates. These studies have revealed that phenolic compounds could have the ability to promote the growth of beneficial bacteria, including members of the families *Bifidobacteriaceae* and *Lactobacillaceae*, as well as specific species such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*. In addition, phenolic compounds have demonstrated their potential in reducing the population of pathogenic bacteria including *Escherichia coli*, *Clostridium perfringens*, and *Helicobacter pylori* (Lavefve et al. 2020; Dias et al. 2021).

The results revealed that the ME exhibited significant utilisation by the tested bacterial species, particularly *L. rhamnosus*, *B. dentium*, *B. bifidum*, and *B. animalis* ssp. *lactis*, Table 3. This utilisation may be attributed to the higher TSC present in the ME compared to the other tested extracts. Furthermore, the results of our study also demonstrated a positive prebiotic effect associated with the EE, which contained a higher concentration of phenolic compounds in contrast to the ME.

In a previous study by Diaz-Vela et al. (2013), species belonging to the genera *Lacticaseibacillus* and *Pediococcus* have shown their ability to metabolise carbohydrates derived from *Opuntia ficus-indica* skins, which not only served as a carbon source but also exhibited prebiotic properties. In another study by Guevara-Arauza et al. (2012), the prebiotic effect of mucilage oligosaccharides (MO) and pectic oligosaccharides (PO) isolated from *O. ficus-indica* on the human colon microbiota was investigated. The results showed

Table 3. Production of lactic acid and acetic acid by bacterial strains cultivated with extracts from *Cereus hildmannianus*, as measured by isotachophoresis

Bacterial species/strains	GR	Fruit		Flower	
		EE	ME	SE	PE
	lactic acid [mg·(100 mL)$^{-1}$]				
Lacticaseibacillus paracasei ssp. *paracasei* CCDM 213	1 343 ± 51^{d}	807 ± 51^{b}	1 117 ± 104^{c}	530 ± 20^{a}	583 ± 15^{a}
Lacticaseibacillus rhamnosus CCDM 146	663 ± 15^{a}	903 ± 6^{c}	1 317 ± 29^{d}	630 ± 26^{a}	713 ± 6^{b}
Lacticaseibacillus helveticus CCDM 466	877 ± 21^{c}	763 ± 12^{b}	1 117 ± 29^{d}	592 ± 8^{a}	587 ± 42^{a}
Lacticaseibacillus delbrueckii ssp. *bulgaricus* CCDM 364	897 ± 21^{c}	703 ± 25^{b}	1 117 ± 29^{d}	543 ± 40^{a}	557 ± 25^{a}
Bifidobacterium dentium CCDM 318	923 ± 35^{a}	1 067 ± 58^{c}	1 377 ± 25^{d}	1 067 ± 29^{c}	990 ± 10^{b}
Bifidobacterium longum ssp. *longum* CCDM 775	720 ± 17^{b}	680 ± 20^{b}	1 167 ± 29^{c}	523 ± 31^{a}	490 ± 10^{a}
Bifidobacterium bifidum CCDM 559	713 ± 6^{a}	833 ± 15^{b}	1 167 ± 76^{c}	690 ± 10^{a}	683 ± 21^{a}
Bifidobacterium animalis ssp. *lactis* BB-12®	822 ± 8^{b}	983 ± 29^{c}	1 383 ± 76^{d}	833 ± 29^{b}	707 ± 15^{a}
Σ all the strains	870 ± 205^{B}	843 ± 132^{B}	1 220 ± 123^{C}	676 ± 182^{A}	664 ± 148^{A}
	acetic acid [mg·(100 mL)$^{-1}$]				
Lacticaseibacillus paracasei ssp. *paracasei* CCDM 213	653 ± 21^{d}	593 ± 21^{c}	643 ± 6^{d}	430 ± 26^{a}	500 ± 35^{b}
Lacticaseibacillus rhamnosus CCDM 146	577 ± 6^{b}	763 ± 15^{d}	813 ± 15^{e}	520 ± 26^{a}	607 ± 6^{c}
Lacticaseibacillus helveticus CCDM 466	663 ± 15^{b}	687 ± 15^{b}	702 ± 3^{c}	500 ± 17^{a}	500 ± 0^{a}
Lacticaseibacillus delbrueckii ssp. *bulgaricus* CCDM 364	477 ± 6^{a}	627 ± 6^{b}	723 ± 15^{c}	477 ± 25^{a}	457 ± 21^{a}
Bifidobacterium dentium CCDM 318	873 ± 25^{a}	910 ± 10^{a}	1 217 ± 104^{b}	997 ± 105^{a}	883 ± 21^{a}
Bifidobacterium longum ssp. *longum* CCDM 775	607 ± 25^{b}	690 ± 10^{c}	897 ± 6^{d}	480 ± 26^{a}	453 ± 15^{a}
Bifidobacterium bifidum CCDM 559	587 ± 15^{a}	810 ± 17^{b}	832 ± 16^{b}	607 ± 42^{a}	583 ± 29^{a}
Bifidobacterium animalis ssp. *lactis* BB-12®	770 ± 26^{b}	887 ± 12^{c}	1 107 ± 12^{d}	747 ± 45^{b}	573 ± 15^{a}
Σ all the strains	651 ± 119^{A}	746 ± 112^{B}	867 ± 195^{C}	595 ± 186^{A}	570 ± 134^{A}

$^{a-e}$Different lowercase letters indicate significant differences (P < 0.05) for each bacterial strain (within each row); $^{A-C}$different capital letters indicate significant differences (P < 0.05) for each medium (within each column); values are mean ± standard deviation (SD) from triplicate measurements; CCDM – Culture Collection of Dairy Microorganisms; GR – basal medium with Orafti® GR chicory inulin (positive control); EE – epicarp extract; ME – mesocarp extract; SE – sepal extract; PE – petal extract

https://doi.org/10.17221/128/2024-CJFS

that the addition of MO increased the growth of lactobacilli by 23.8%, while PO positively influenced the counts of bifidobacteria, resulting in a 25% increase. Numerous studies have consistently reported the positive impact of phenolic compounds from fruits, pulses, tea, and vegetables on the growth of species belonging to the genera *Lacticaseibacillus* (*L. casei*, *L. rhamnosus*), *Lactobacillus* (*L. acidophilus*, *L. delbrueckii*), and *Bifidobacterium*. Polyphenols may influence the gut microbiota by promoting the growth of beneficial bacteria due to their antioxidant properties, acting as signalling molecules, modulating gene expression in gut microbes or also creating a favourable environment for beneficial bacteria by reducing oxidative stress. Thus, their prebiotic effect could be related to mechanisms beyond just providing nutrients and energy source.

Overall, the findings of our study emphasise the potential of extracts obtained from the fruits and flowers of *C. hildmannianus* as valuable sources of prebiotic compounds. The specific compositions of the ME and EE, rich in sugars and phenolic compounds, respectively, contribute to their prebiotic effects and highlight their potential for application in promoting the gut health and overall well-being.

CONCLUSION

Extensive efforts are being made to explore cost-effective and environmentally friendly strategies to produce prebiotics. There is a growing interest in utilising cheap and abundant plant tissues as an alternative to conventional substrates. By tapping into these readily available plant tissues from *C. hildmannianus* (fruits and flowers), the production of prebiotics could be optimised in terms of efficiency, cost, and environmental impact. The fruits and flowers from *C. hildmannianus* proved to be efficient producers of phenolic compounds with antioxidant activity, iron chelation abilities, and prebiotic effects; more studies will be carried out in order to further clarify the prebiotic mechanisms and antioxidant effects. The perspective is the development of pharmaceutical and food products from fruits and flowers of *C. hildmannianus*.

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Received: June 26, 2024
Accepted: October 7, 2024
Published online: November 15, 2024