

Improvement of phenolic compounds production in callus cultures of *Cereus hildmannianus* (K.) Schum. by elicitation

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Abstract

Cereus hildmannianus (Cactaceae) is a medicinal plant with various pharmacological and industrial applications. Plant biotechnology is an attractive approach for specialized metabolites production under controlled conditions, with the elicitation process being considered a highly effective strategy for increasing the production of bioactive compounds. In this regard, the present study investigated the effects of elicitors, sucrose (S, 1–8 g L⁻¹), UV-C light (UV-C, 10–80 min), salicylic acid (SA, 50–200 µmol), and jasmonic acid (JA, 50–200 µmol), on the profile of esterase isozymes, total phenolic and total flavonoid contents, antioxidant activity (FRAP, DPPH, and ABTS), and the dereplication method by Ultra-High-Performance Liquid Chromatography coupled with Mass Spectrometry (UHPLC-ESI-Q-TOF-MS/MS). The isozyme profile was not significantly changed. The greatest accumulations of total phenolics were observed in callus tissues induced with 100 µmol JA (390.8), 200 µmol SA (355.5), 20 min UV-C (182.9), and 6 g L⁻¹ S (122.1 µg mg⁻¹ DW); the greatest concentrations of total flavonoids were observed following culture with 200 µmol SA (89.8) and 2 g L⁻¹ S (28.4 µg mg⁻¹ DW). Furthermore, the dereplication study allowed putative identification of thirty-six compounds (thirty phenolic and organic acids, and six flavonoids). The greatest antioxidant activities were obtained with callus tissues induced with 100 µmol JA, 200 µmol SA, 80 min UV-C, and 1 g L⁻¹ S. The elicitors were able to alter the production of phenolic compounds in callus cultures of *C. hildmannianus*. These results are promising for the clean and sustainable bioproduction of bioactive molecules for pharmaceutical and cosmeceutical purposes, prioritizing the conservation of the species.

Introduction

Cereus hildmannianus (K.) Schum. [syn. *C. peruvianus*] is a succulent plant from the Cactaceae family. Brazil, the country that possesses the greatest biodiversity in the world, is home to numerous different species of the Cactaceae family. The plant (Alvarez et al. 1995; Alvarez et al. 1992; Barros and Nozaki 2002; Nozaki et al. 1993; Nunes et al. 2022), as well as callus culture of the species (Jacomini et al. 2015; Jayme et al. 2015; Santos et al. 2022), have industrial, economic, and medicinal importance (Santos et al. 2021).

Plants have always offered humanity basic nutrients, proteins, fatty acids, and carbohydrates, in addition to being the richest and most biodiverse source of natural products (Santos et al. 2021; Sulaiman et al. 2022). Despite advancements in synthetic chemistry, biological sources are still incredibly important and, currently, more than 350,000 plant species are being investigated in the production of pharmaceuticals (Isah 2019; Naik and Al-Khayri 2016). However, with indiscriminate harvesting, several species are being threatened with extinction (Reyes-Martínez et al. 2019). Further to this, the production of these metabolites *in natura* is often low, occurring in specific seasons or plant organs (Dewick 2009; Smetanska 2008).

Plant biotechnology is a useful tool for the production of these metabolites of interest year-round (without seasonal periods) (Barbulova et al. 2014; Bourgaud et al. 2001), as the conditions of light,

humidity, and pressure can be controlled, and contaminants like microorganisms and heavy metals, as well as herbivory by insects, can be avoided (Aoyagi et al. 2002; Chattopadhyay et al. 2002; López Arnaldos et al. 2001; Pérez-Molphe-Balch et al. 2015; Sato et al. 2001). Furthermore, growth cycles are of weeks rather than years as occurs in the plant *in natura* (Ramirez-Estrada et al. 2016).

Phenolic compounds, which include flavonoids, are naturally-occurring plant specialized metabolites. Phytochemical studies have been widely conducted across plant species due to their high and diverse phenolic contents. In the genus *Cereus*, compounds obtained from cladodes of *C. jamacaru* (Araújo et al. 2008; Dutra et al. 2019; Dutra et al. 2018), fruits of *C. fernambucensis* (Souza et al. 2015), and cladodes of *C. peruvianus* (syn. *C. hildmannianus*) (Nunes et al. 2022) have already been characterized for their anti-tumor, anti-inflammatory, and/or antioxidant activity. Within the family Cactaceae, other compounds have also been characterized with potential in the treatment of cardiovascular diseases and antidiabetic activity from the cladodes of *Opuntia* spp. and *Opuntia littoralis* (Abd El-Moaty et al. 2020; Negre-Salvayre et al. 2018), and as having gastroprotective action such as those from the fruits of *Corryocactus brevistylus* (Areche et al. 2020). These studies show the importance of studying phenolic compounds and in implementing the most efficient strategies for better bioproduction, particularly for industrial purposes.

Plants *in natura* are continually exposed to stresses, which produce specialized metabolites that exert crucial functions in their defenses. This phenomenon can be mimicked in *in vitro* tissue cultures by approaches such as elicitation. Particular facets of plant metabolism can be stimulated via the use of elicitors, which can result in increases in the bioproduction of compounds (Bandhakavi and Kamarapu 2016; Ramirez-Estrada et al. 2016). Elicitors are chemicals or biofactors that can induce physiological and morphological changes in plants (Castro et al. 2021). Abiotic elicitors are non-biological in nature, and are grouped into physical and chemical factors that include light, osmotic, water, thermal, salinity, heavy metals, mineral salts and gaseous toxins (Naik and Al-Khayri 2016; Zhao et al. 2005), while biotic elicitors are biological substances/agents, characterized as compounds of microorganisms or plants, such as cell wall, chitin, polysaccharides, yeast extracts, and chemicals that are released in response to attack (Naik and Al-Khayri 2016; Thakur et al. 2019).

The impact of using elicitors in callus culture from *C. hildmannianus* has not yet been addressed. Our hypothesis in the current study is that the use of elicitors in callus cultures of *C. hildmannianus* may increase the production of phenolic compounds and antioxidant capacity. Therefore, the present study aimed to investigate the effects of elicitors (sucrose, UV-C light, salicylic acid, and jasmonic acid), on the profile of esterase isozymes, total phenolic and total flavonoid contents, antioxidant activity (FRAP, DPPH, and ABTS), and dereplication method by Ultra-High-Performance Liquid Chromatography coupled with Mass Spectrometry (UHPLC-ESI-Q-TOF-MS/MS).

Material And Methods

Callus culture of *C. hildmannianus*

Callus cultures were induced *in vitro* from hypocotyls of the *C. hildmannianus* [syn. *C. peruvianus*] plant (Oliveira et al. 1995). The species was identified by Dr. Daniela Cristina Zappi and a voucher was deposited at the Herbarium of the State University of Maringá, Paraná, Brazil (HUEM 36127). The species was registered (n° A05B398) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen).

The callus tissue culture was maintained in solid Murashige and Skoog (1962) (MS) medium supplemented with B5 vitamins (Gamborg et al. 1968), 0.8 g L⁻¹ agar (Sigma®), 30 g L⁻¹ sucrose (Sigma®) (except for callus tissues treated with different sucrose concentrations: 10 g L⁻¹, 20 g L⁻¹, 60 g L⁻¹, and 80 g L⁻¹), 4.0 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) (Sigma®), and 4.0 mg L⁻¹ *N*-(2-furanylmethyl)-1*H*-purine-6-amine (kinetin) (Sigma®), with a pH level of 5.8 (adjusted with KOH, prior to autoclaving at 121°C for 20 min). The cultures were incubated at 32°C with a photoperiod of 16 h (15 µmol·m⁻² s⁻¹ photosynthetic photon flux density, PPFD) (Oliveira et al. 1995) [Patent n° BR 102012007410-9].

The stock callus cultures were maintained under the same physical conditions as described above and subcultured at the 45-day intervals in 100 x 20 mm diameter glass Petri dishes containing 20 mL of solid medium. After this, callus tissue pieces of approximately equal weight (0.5 g) and size (0.5×0.5 cm) were excised and subcultured in fresh MS media supplemented with the different elicitors as described below.

Elicitor Preparation And Treatments Of Callus Tissue

The elicitors, sucrose (≥ 99.5%; Sigma-Aldrich®), salicylic acid (≥ 99.0%; Sigma-Aldrich®), (±)-jasmonic acid (≥ 97.0%; Sigma-Aldrich®), and UV-C lamp (12W, 253.7 nm), were used for the elicitation of callus cultures from *C. hildmannianus*. A stock solution for each elicitor was prepared and the pH of each stock was adjusted to 5.8 before implementation. Salicylic acid was dissolved in 0.01 M NaOH prepared with ultrapure water, while jasmonic acid and sucrose were dissolved in ultrapure water only. All the solutions were filter sterilized through a membrane filter of 0.22 µm pore size (Spritzen-TPP) into the MS medium at the desired concentrations. For the UV-C light elicitation, callus tissues were exposed to a UV-C lamp at a distance of 90 cm.

All the elicitors were added individually to the culture medium at final concentrations: A) Sucrose (S): 10 g L⁻¹ (S_{1%}), 20 g L⁻¹ (S_{2%}), 60 g L⁻¹ (S_{6%}), and 80 g L⁻¹ (S_{8%}); B) Salicylic acid (SA): 50 µmol (SA_{50µmol}), 100 µmol (SA_{100µmol}), 150 µmol (SA_{150µmol}), and 200 µmol (SA_{200µmol}); C) Jasmonic acid (JA): 50 µmol (JA_{50µmol}), 100 µmol (JA_{100µmol}), 150 µmol (JA_{150µmol}), and 200 µmol (JA_{200µmol}); D) UV-C light (UV-C): exposure for 10 min (UV-C_{10m}), 20 min (UV-C_{20m}), 40 min (UV-C_{40m}), and 80 min (UV-C_{80m}).

All procedures were performed under a laminar hood to avoid microbiological contamination.

Electrophoresis Of Callus Tissues For Esterase Isozyme Identification

Electrophoresis of callus tissues for esterase isozyme identification

Fresh callus tissues (250.0 ± 0.01 mg) were subjected to extractions with 20 μ L of solution (1 M phosphate buffer, pH 7.0; 5% PVP-40; 1 mM EDTA; 0.5% β -mercaptoethanol; and 10% glycerol). The samples were then centrifuged at 25,000 rpm for 25 min at -4°C , the supernatants were collected. A polyacrylamide running gel (12%) was made using 0.375 M *Tris*-HCl buffer (pH 8.8). This buffer was prepared as follows: 6.2 mL of acrylamide/*bis*-acrylamide solution (30 g of acrylamide and 0.8 g of *bis*-acrylamide dissolved in 100 mL of deionized H_2O), 4 mL of 1.5 M *Tris*-HCl buffer (pH 8), 6.2 mL of deionized H_2O , 320 μ L of 2% ammonium persulfate, and 16 μ L of *N,N,N',N'*-tetramethylethylenediamine (TEMED). The packaging gel was prepared with 3 mL of acrylamide/*bis*-acrylamide (5 g of acrylamide and 0.25 g of *bis*-acrylamide dissolved in 50 mL of deionized H_2O), 3 mL of 0.24 M *Tris*-HCl buffer (pH 6.8), 30 μ L of deionized H_2O , 250 μ L of 2% ammonium persulfate, and 3 μ L of TEMED. Electrophoresis was performed at 200 V for 4 h at 4°C and the running buffer used was 0.125 M *Tris*/0.0959 M glycine (pH 8.3).

The gel was incubated in 0.1 M sodium phosphate buffer (pH 6.2) for 30 min, then transferred to the same buffer plus 40 mg of β -naphthyl acetate, 40 mg of α -naphthyl acetate, 60 mg of Fast Blue RR salt, and 5 mL of *N*-propanol for 60 min at 37°C . After, the gel was fixed in 7.5% acetic acid/10% glycerol (Sala et al. 2011).

Preparation of methanolic extracts

The control and elicited callus tissues were lyophilized (5.0 ± 0.01 g) and exhaustively extracted by maceration with methanol (Santos et al. 2022), then subjected to a vacuum evaporator at a temperature of $35 \pm 1^{\circ}\text{C}$ until the organic solvent was completely evaporated. The extracts were stored at -20°C until further use.

Total Phenolic Content Assay

The total phenolic content (TPC) was determined as described previously by Singleton and Rossi (1965) with modifications by Sousa et al. (2007). In tubes, 40 μ L of the methanolic extracts were mixed with 3.1 mL of deionized H_2O and 600 μ L of Na_2CO_3 . After this, 200 μ L of Folin-Ciocalteu reagent was added. This mixture was incubated for 30 min, and the absorbance was measured in a spectrophotometer UV-Vis Varian (Cary-1E) at 765 nm. The calculation of the TPC was done by interpolating the measured sample absorbance from an analytical curve defined by standard solutions of gallic acid (Sigma®). The results are expressed as the equivalents of gallic acid per dry weight of extracts ($\mu\text{g GAE mg}^{-1}$ dry weight (DW)).

Total Flavonoid Content Assay

Total flavonoid content (TFC) was measured using an adapted method by Pothitirat et al. (2009). Briefly, in tubes, 1.5 mL of the methanolic extracts were mixed with 3.4 mL of acetic acid solution (5.0%; MeOH)

and 100 μL of aluminum chloride solution (5.0%; MeOH). This mixture was incubated for 30 min, and the absorbance was measured in a spectrophotometer at 425 nm. The values of TFC were calculated by interpolating the measured sample absorbance from an analytical curve defined by standard solutions of quercetin (Sigma®). The results are expressed as the equivalents of quercetin per dry weight of extracts ($\mu\text{g QE mg}^{-1} \text{ DW}$).

Ultra-high Performance Liquid Chromatography (Uhp lc-esi-q-tof-ms/ms)

Methanolic extracts ($5.0 \pm 0.01 \text{ mg}$) were analyzed by UHPLC (Shimadzu, Nexera X2) coupled with high-resolution mass spectrometry (HRMS-QTOF Impact II, Bruker Daltonics Corporation, USA) equipped with an electrospray ionization source. The capillary voltage was operated in negative and positive ionization mode, set at 4500 V, and with an endplate offset potential of -500 V. The dry gas parameters were set to 8 L min^{-1} at 200°C with a nebulization gas pressure of 4 bar. Data was collected from m/z 50-1300 with an acquisition rate of 5 spectra per second, and the ions of interest were selected by auto MS/MS scan fragmentation. Chromatographic separation was performed using a C18 column (75×2.0 mm i.d.; 1.6 μm Shim-pack XR-ODS III). The gradient mixture of solvents A (H_2O) and B (acetonitrile with 0.1% formic acid; v:v) was as follows: 5% B 0–1 min, 30% B 1–3 min, 95% B 3–12 min, maintained at 95% B 12–15 min, and 5% B 15–17 min, at 40°C. Ion chromatograms, MS and MS/MS spectra were compared to the literature, identified using a mass spectrometry database (HMDB and NIST), in addition to error calculation using Eq. 1 (Brenton and Godfrey 2010).

$$\text{Error (ppm)} = \frac{T_M - E_M}{T_M} \times 1.000.000$$

1

Where, T_M is the theoretical exact mass, and E_M is the experimental mass.

Evaluation Of Antioxidant Potential

Assay for the ferric-reducing antioxidant power (FRAP)

The FRAP method was performed according to Rufino et al. (2006). In tubes, 100 μL of the methanolic extracts, 300 μL of deionized H_2O , and 3 mL of FRAP (0.3 M acetate buffer; 10 mM TPTZ (2,4,6-*Tris*(2-pyridyl)-s-triazine) solution; 20 nM ferric chloride solution) (10:1:1, v/v/v) were mixed and maintained for 30 min at 37°C. The absorbance was measured in a spectrophotometer at 593 nm, and an analytical curve with Trolox solution was constructed. The results are expressed as $\mu\text{mol Trolox mg}^{-1} \text{ DW}$ (Shi et al. 2019).

Assay For The Dpph Radical-scavenging Activity

The antioxidant activity was obtained by the DPPH (2,2-Diphenyl-1-picrylhydrazyl, Sigma®) method performed according to Ma et al. (2011). In tubes, 25 µL of the methanolic extracts and 2 mL of the 6.25×10^{-5} mol L⁻¹ of DPPH[•] solution were mixed and maintained for 30 min at room temperature. The absorbance was measured in a spectrophotometer at 517 nm, and an analytical curve with Trolox solution ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma®) was constructed. The results are expressed as µmol Trolox mg⁻¹ DW (Rufino et al. 2007b).

Assay For The Abts Radical-scavenging Activity

The ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt, ~98%; Sigma®) method was performed according to Rufino et al. (2007a). In tubes, 30 µL of the methanolic extracts and 3 mL of the ABTS⁺ solution (5 mL of ABTS – 7 mmol L⁻¹ solution, and 88 µL of potassium persulfate – 140 mmol L⁻¹; reaction for 16 h) were mixed and maintained for 6 min at room temperature. The absorbance was measured in a spectrophotometer at 734 nm, and an analytical curve with Trolox solution was constructed. The results are expressed as µmol Trolox mg⁻¹ DW (Dutra et al. 2019).

Statistical analysis

The analyzes were performed in triplicate (independent experiments), and the results are presented as mean ± standard deviation (SD). The data were analyzed in the Statistica 10 (StatSoft Inc.®, Tulsa, OK) software, using the ANOVA and *post-hoc* Tukey's test. The results were considered statistically significant for values of $p < 0.05$. The figures were generated in the Origin® software version 9.5.1.

Results And Discussion

Elicitation method

Callus tissues are defined as a cluster of undifferentiated cells obtained by specific physiological conditions, which are cultured and result in a homogeneous cell mass growth (Guilford-Blake and Strickland 2008). Consequently, elicitation in the callus occurs homogeneously throughout the tissue as opposed to that which occurs in the plant. The growth curve of the control callus of *C. hildmannianus* has already been well established by Santos et al. (2022).

Macroscopic changes were observed in the callus tissues submitted to the different elicitation treatments. Characteristics of the control callus, such as greenish color and friable texture, were altered in calluses submitted to elicitation (Fig. 1), while the growth was also altered.

Cultures elicited with higher concentrations of sucrose (6 and 8%) displayed changes in their texture, as well as a color change (to yellowish) (Fig. 1b). Greater growth, as well as a change in color to reddish,

was observed in the calli maintained at increased concentrations of salicylic acid (concentration-dependent) (Fig. 1d). With regards to jasmonic acid concentration, greater growth and a color change to yellowish, was only seen in the calluses maintained in medium containing 150 μmol (Fig. 1e). The irradiation of UV-C light for 80 min negatively influenced the growth of callus tissues (Fig. 1c).

Figure 1

Electrophoresis Of Callus Tissues

The expression of esterase isozymes has an additive effect on the stress response in plants by constitutively priming the defense pathways with signaling agents to bioproduce secondary metabolites (Gershater and Edwards 2007; Swaminathan et al. 2021).

The α/β -esterase isozymes of the callus tissues were numbered sequentially, according to the decrease in the negative charge following separation by electrophoresis (starting from the anode), and based on previous studies with plants and callus tissues of *C. peruvianus* (syn. *C. hildmannianus*).

The Est-7, Est-8, Est-12, and Est-13 isozymes are specific for α -naphthyl acetate (α -esterases). The Est-3 isozyme preferentially hydrolyzes α -naphthyl acetate (α -esterase), while the Est-4 isozyme hydrolyzes β -naphthyl acetate (β -esterase). Est-3 and Est-4 isozymes are classified as arylersterases/carboxylesterases. Isoesterases produced from *Est-7* and *Est-12* loci are also classified as carboxylesterases; while those encoded by the *Est-1*, *Est-2*, *Est-9*, *Est-10*, *Est-11*, and *Est-14* loci are classified as acetylersterases (Fig. 2) (Sala et al. 2011).

Figure 2

The electrophoresis pattern for esterase isozymes in polyacrylamide gel showed increased expression of the Est-1, Est-2, and Est-3 isozymes in UV-C-elicited callus tissues. However, the profile for callus tissues maintained in culture media containing sucrose, salicylic acid, and jasmonic acid was not markedly altered.

Extractive yield

The extractive yield of methanolic extracts of callus tissues after elicitation with sucrose showed a greater mass production for those maintained in $S_{8\%}$ (53.4%), followed by $S_{1\%}$ and $S_{2\%}$ (43.1 and 41.3%), respectively. The highest extractive yield of methanolic extracts (59.2%) was observed for the callus tissue cultures that had been exposed to UVC_{80m} , followed by UVC_{40m} (53.5%), UVC_{20m} (50.5%), and UVC_{10m} (45.7%). The extractive yield of methanolic extracts from callus tissues maintained in culture medium with salicylic acid varied from 30.3 to 33.3%, and with jasmonic acid ($JA_{200\mu\text{mol}}$) the highest yield obtained 38.9%, which was still higher than the methanolic extracts obtained from control callus tissues (25.2%).

Phenolic compounds

The elicitation with the lower concentrations of sucrose did not result in significant differences in the TPC: S_{1%} (106.2 µg GAE mg⁻¹ DW) and S_{2%} (107.9 µg GAE mg⁻¹ DW) compared to the control (107.8 µg GAE mg⁻¹ DW). A significant decrease in the production of phenolic compounds was, however, observed in the callus tissues maintained under S_{8%} (68.4 µg GAE mg⁻¹ DW). The elicitation of callus tissues with 6% sucrose showed the highest production of TPC (122.1 µg GAE mg⁻¹ DW) (Fig. 3a), which was significantly higher than the control. On the other hand, the TFC was increased in the callus tissues of S_{2%} (28.4 µg QE mg⁻¹ DW) compared to the control (11.8 µg QE mg⁻¹ DW) (Fig. 3a). The production of TFC was not observed in callus tissues maintained in medium containing S_{6%} or S_{8%}.

Sucrose plays an essential role in the regulation of metabolic processes, including carbon and nitrogen assimilation and transport, and responses to oxidative damage (Horacio and Martinez-Noel 2013). Sucrose acts as an osmoprotectant in plants under abiotic stress, when the levels of specific sugars are severely affected (Gupta and Singh 2014). According to Kumar et al. (2018), sucrose concentrations can modulate the production of secondary metabolites in callus cultures. Carbonic stress is a type of physical elicitation and several studies have already shown that the elicitation with sucrose promotes the greatest production of phenolic compounds in the callus of *Fagonia indica* (Khan et al. 2018).

The callus cultures of *C. hildmannianus* had an improvement in the TPC under the different exposure times to UV-C light. The highest TPC was obtained in callus tissues exposed to UVC_{20m} (182.9 µg GAE mg⁻¹ DW), followed by UVC_{80m} (176.8 µg GAE mg⁻¹ DW). The TFC, however, decreased in the calli after exposure to UV-C light (Fig. 3b). These results suggest that the time of exposure to UV-C light in the callus cultures directly influences the production of these compounds.

According to Raúl et al. (2017), UV photoreceptors are present in plants and regulate the growth, development, and expression of genes involved in metabolite production processes, along with altering the production of reactive oxygen species and antioxidant enzymes. According to Cetin (2014) and Zhan and Huang (2015), the composition of phenolic compounds in the callus culture of *Vitis vinifera* was significantly altered with the elicitation with UV-C light. Similar results were reported by Camarena-Rangel et al. (2017), who evaluated UV-C light elicitation on the callus cultures of *Opuntia ficus-indica*, *O. megacantha*, and *O. streptacantha* and observed that phenolic compounds increased 2 to 3 times after exposure.

Figure 3

UV light functions as an elicitor by degrading the plant cell wall, while a variety of chemical and physical agents act by damaging the cells (Fosket 1994). UV-C light covers wavelengths from 200 to 280 nm, and this energy is a small part of solar radiation reaching the Earth's surface, exerting significant biological impacts on living organisms (Katerova et al. 2012). This highly energetic radiation can strongly affect cell function and DNA structure (Costa et al. 2013). In the literature, the impact of UV radiation on the

elicitation of secondary metabolites in vegetable cultures *in vitro* is not extensively studied, despite being considered a simple, inexpensive technique that can already be found in most laboratories.

In the elicitation of callus tissues with salicylic acid, there was a greater TPC and TFC compared to the control culture (Santos et al. 2022) (Fig. 3c). The highest TPC and TFC in callus tissues was obtained from those elicited with SA_{200µmol} (TPC: 355.5 µg GAE mg⁻¹ DW; and TFC: 89.8 µg QE mg⁻¹ DW). The TPC was lower with the addition of SA_{150µmol} (298.5 µg GAE mg⁻¹ DW), SA_{100µmol} (236.6 µg GAE mg⁻¹ DW) and SA_{50µmol} (240.9 µg GAE mg⁻¹ DW), as well as lower TFC for SA_{150µmol} (29.3 µg QE mg⁻¹ DW), SA_{100µmol} (28.0 µg QE mg⁻¹ DW) and SA_{50µmol} (11.4 µg QE mg⁻¹ DW), in comparison to the values obtained following elicitation with SA_{200µmol}.

Salicylic acid is a signaling molecule of aggression in plants, which generally increases the accumulation of many secondary metabolites with biological activities, including the induction of gene expression (Taguchi et al. 2001). Salicylic acid already has industrial application for eliciting specialized metabolites in cell cultures *in vitro* (Mahalakshmi et al. 2013). Several studies have shown that elicitation with salicylic acid promotes the greatest production of phenolic compounds in the callus cultures of *Phyllanthus pulcher* (Danaee et al. 2015), *Fagonia indica* (Khan et al. 2019), *Digitalis trojana* (Cingoz and Gurel 2016), and *Ruta graveolens* (Sharifi et al. 2019).

The jasmonic acid also induced an increase in the phenolic compounds. The highest TPC of calluses was obtained following the addition of JA_{100µmol} (390.8 µg GAE mg⁻¹ DW). The TPC was, however, lower than this value with the addition of JA_{50µmol} (265.1 µg GAE mg⁻¹ DW), JA_{150µmol} (277.3 µg GAE mg⁻¹ DW), and JA_{200µmol} (272.3 µg GAE mg⁻¹ DW), although still higher than the control. The TFC was also lower with the addition of JA_{50µmol} (0.15 µg QE mg⁻¹ DW), JA_{100µmol} (2.2 µg QE mg⁻¹ DW), JA_{150µmol} (4.7 µg QE mg⁻¹ DW), and JA_{200µmol} (9.5 µg QE mg⁻¹ DW) in comparison to the control (11.8 µg QE mg⁻¹ DW) (Fig. 3d).

The biosynthesis of phenolic compounds was also significantly induced in the callus cultures of *Scrophularia kakudensis* with the elicitors of salicylic acid and jasmonic acid (Manivannan et al. 2016). According to Camarena-Rangel et al. (2017), elicitation with 50 µmol of jasmonic acid in callus cultures of *O. ficus-indica* and *O. streptacantha* increased the phenolic compound production. The TPC was also two times higher in the callus culture of *O. robusta* elicited with jasmonic acid (Astello-García et al. 2013). The increased production of specialized metabolites by elicitation with jasmonic acid has been generally associated with a rapid and transient increase in enzyme activity in the phenylpropanoid pathway, such as ammonia from phenylalanine lyase and chalcone isomerase (Anusha et al. 2016).

Jasmonic acid is a natural growth regulator found in higher plants, with several physiological roles during development and in response to biotic and abiotic stresses (Anusha et al. 2016). Jasmonic acid is able to stimulate the production of various substances and is considered a signal molecule for secondary metabolism in plants (Simões et al. 2016).

According to Vasconsuelo and Boland (2007), it is essential to empirically determine the optimal elicitation time and concentration conditions for each particular system. Specificity, concentration, elicitor exposure duration, nutrient composition, and age of culture are some factors that can influence elicitation. According to Fosket (1994), elicitors do not seem to be highly specific, although they are extremely potent, where nanomolar concentrations can cause hypersensitivity reactions.

Dereplication Method By Uhp lc-esi-q-tof-ms/ms

It has been possible to identify and characterize a broad-spectrum of phenolic compounds with the development of highly sensitive analytical methods (Nielsen et al. 2019), and modulate bioproduction by means of stressors, elicitors, and mutagens (Akula and Ravishankar 2011; Coste et al. 2011; Zárte et al. 2013).

The chemical investigation of methanolic extracts from callus tissues of *C. hildmannianus* by UHPLC-ESI-Q-TOF-MS/MS resulted in the putative identification of thirty-six compounds (thirty phenolic acids, organic acids, and derivatives), and six flavonoids (five glycosylated) (Table 1). The samples were analyzed by high-resolution mass spectrometry and the identification of these compounds was proposed after a review of the genus *Cereus* and the Cactaceae family (Cabañas-García et al. 2019; Hernández-García et al. 2019; Kumar et al. 2015; Slimen et al. 2017), in addition to the value mass error (error ppm).

In the callus tissues elicited with S_{1%}, twenty-eight compounds (twenty-two phenolic acids, organic acids and derivatives, and five flavonoids) were identified while twenty-three compounds (nineteen phenolic acids, organic acids, and derivatives, and four flavonoids) were identified in the calluses elicited with S_{2%} (Table 1). The lowest sucrose concentration induced greater production of these metabolites.

Twenty-nine compounds (twenty-four phenolic acids, organic acids, and derivatives, and five flavonoids) were obtained after elicitation with SA_{100μmol}, and twenty-six compounds (twenty-two phenolic acids, organic acids, and derivatives, and four flavonoids) were characterized after elicitation with JA_{150μmol} (Table 1).

Table 1

Antioxidant Activity

The plant tissue culture was used as a platform to investigate the antioxidant activity in a controlled environment with elicitors, particularly as the UHPLC-ESI-Q-TOF-MS/MS data indicated the presence of phenolic compounds. Antioxidants are defined as any substance that, in low concentration compared to the oxidizable substrate, regenerates or significantly prevents its oxidation (Halliwell and Gutteridge 2015). Phenolic compounds are the most abundant antioxidants in the human diet, being the most widespread natural products of plant phytochemical classes (Es-Safi 2012).

The greatest antioxidant activity was observed in the callus tissues after elicitation with sucrose (S₁%) by the methods of FRAP (81.5 $\mu\text{mol Trolox mg}^{-1}$ DW) and DPPH (56.4 $\mu\text{mol Trolox mg}^{-1}$ DW). The antioxidant activity evaluated in the callus tissues after elicitation with S₂% by the methods of ABTS was 39.2 $\mu\text{mol Trolox mg}^{-1}$ DW (Fig. 4a).

Changes in sucrose concentration levels in *in vitro* cultures can alter the production of phenolic compounds and antioxidant activity (Khan et al. 2018; Modarres et al. 2018).

In the callus tissues exposed to UV-C light, the highest antioxidant activity was obtained with the elicitation of UV-C_{80m} in the FRAP (80.4 $\mu\text{mol Trolox mg}^{-1}$ DW), DPPH (56.0 $\mu\text{mol Trolox mg}^{-1}$ DW), and ABTS (36.2 $\mu\text{mol Trolox mg}^{-1}$ DW) methods (Fig. 4b).

Figure 4

The top-level antioxidant activities were obtained from callus tissues elicited with the SA and JA hormones. The highest antioxidant activity observed in the callus tissues after elicitation with salicylic acid was with the addition of 200 μmol in the culture medium by the methods of FRAP (124.1 $\mu\text{mol Trolox mg}^{-1}$ DW), DPPH (67.7 $\mu\text{mol Trolox mg}^{-1}$ DW), and ABST (57.0 $\mu\text{mol Trolox mg}^{-1}$ DW) (Fig. 4c). The highest antioxidant activity observed in the callus tissues after elicitation with jasmonic acid was with the addition of 100 μmol in culture medium by the methods of FRAP (115.2 $\mu\text{mol Trolox mg}^{-1}$ DW), DPPH (83.7 $\mu\text{mol Trolox mg}^{-1}$ DW), and ABST (57.1 $\mu\text{mol Trolox mg}^{-1}$ DW) (Fig. 4d).

These results of the antioxidant activity of the callus elicited from *C. hildmannianus*, which is known to be associated with promising antiaging activity, indicates that it could potentially be used as a natural product for this purpose. The perspective of this study will be to analyze the antioxidant activity in cellular models, and to quantify by chromatographic methods, the major phenolic compounds of these elicited callus tissues from *C. hildmannianus*.

Conclusion

The elicitors were able to qualitatively and quantitatively alter the phenolic compounds in callus tissues of *C. hildmannianus*, although not significantly changing the esterase isozyme profile. The current study showed that the use of the elicitors (salicylic acid and jasmonic acid) may increase the production of phenolic compounds and antioxidant activity in callus tissues of *C. hildmannianus*. These results are very significant and promising for the clean and sustainable bioproduction of bioactive molecules for therapeutic or food purposes, prioritizing the conservation of the species, combined with the industrial potential that callus cultures.

Abbreviations

DW Dry weight

JA	Jasmonic acid
MS	Murashige and Skoog medium
PPFD	Photosynthetic photon flux density
S	Sucrose
SA	Salicylic acid
TFC	Total flavonoid content
TPC	Total phenolic content
UHPLC-ESI-Q-TOF-MS/MS Ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry	
UV-C	Ultraviolet light C
UV-Vis	Ultraviolet-visible spectroscopy

Declarations

Supplementary Information The online version contains supplementary material available at.

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Author contributions All authors approve of the version to be submitted. ÉSS, MRPC, TFOS, AJBO, MFPSM, and CAM performed the study and examined the experimental data. ÉSS prepared the manuscript. RACG participated in the interpretation of data and revision for important intellectual content, and completed the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

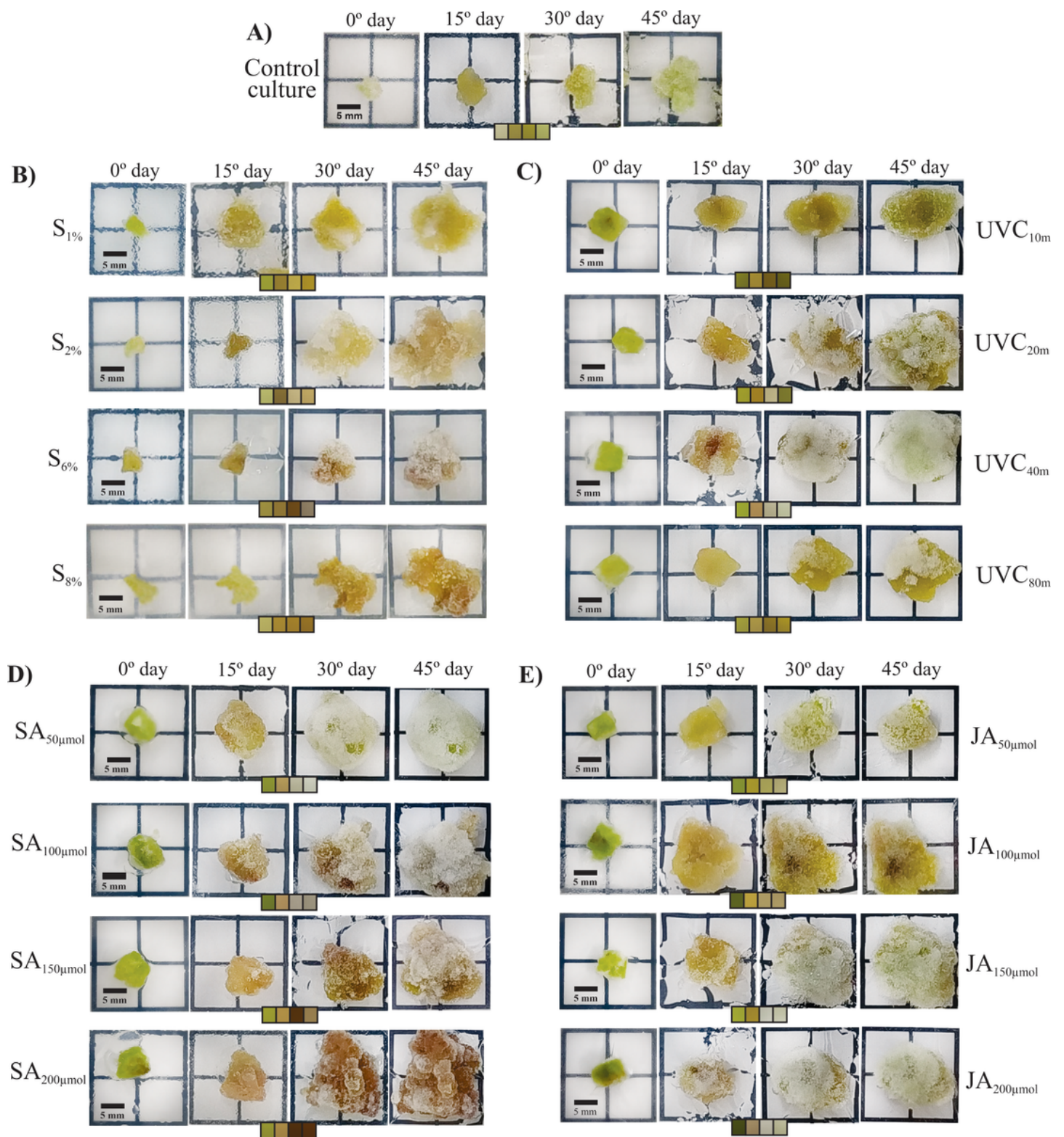


Figure 1

Growth of callus cultures of *C. hildmannianus*, **A)** Control culture; **B)** Sucrose (S); **C)** UV-C light (UV-C); **D)** Salicylic acid (SA); and **E)** Jasmonic acid (JA).

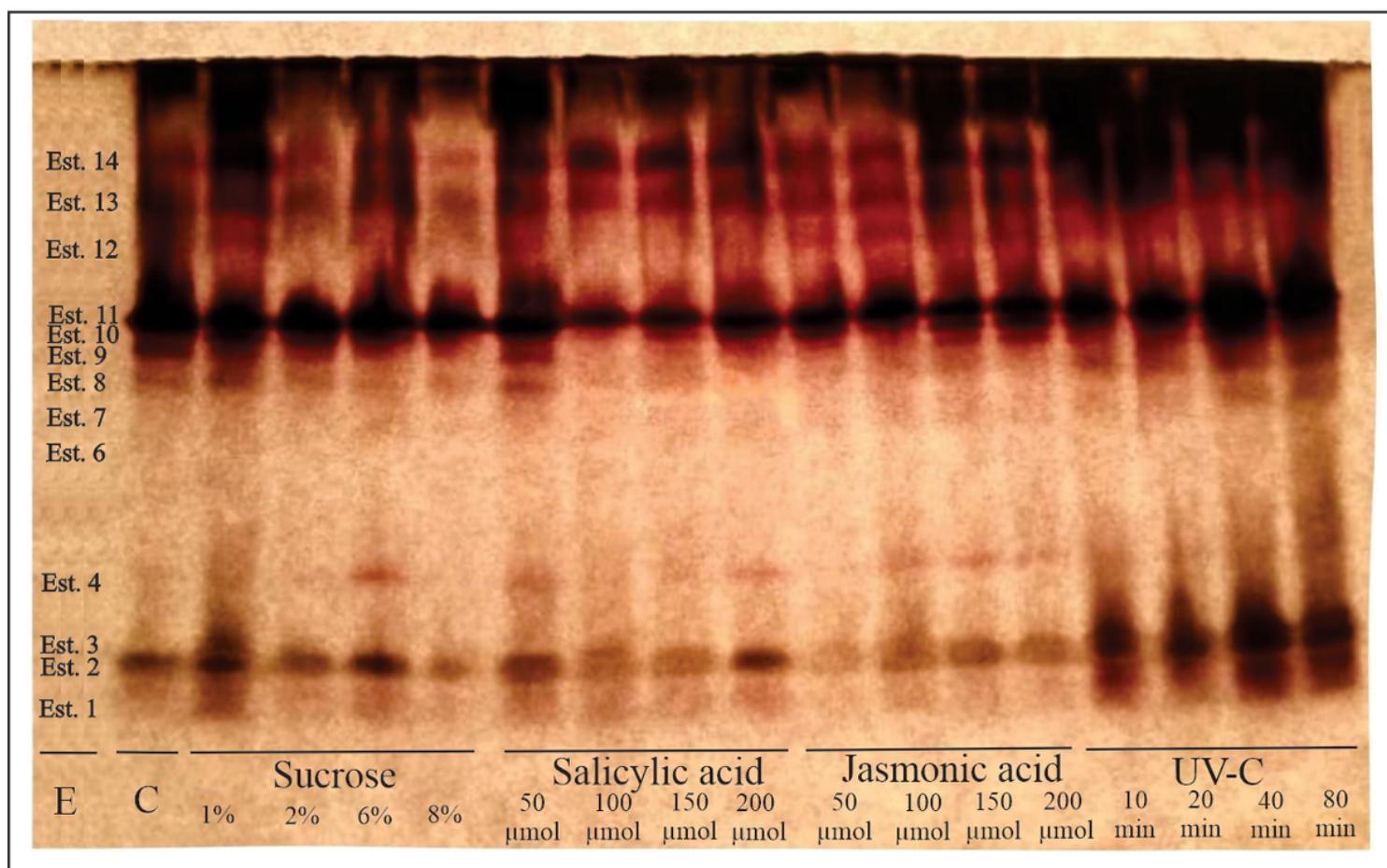


Figure 2

Esterase isozymes in polyacrylamide gel from extracts of callus cultures of *C. hildmannianus* elicited with sucrose, salicylic acid, jasmonic acid, and UV-C light. E and Est: Esterases; C: Control culture; Black: α -esterases; Red: β -esterases.

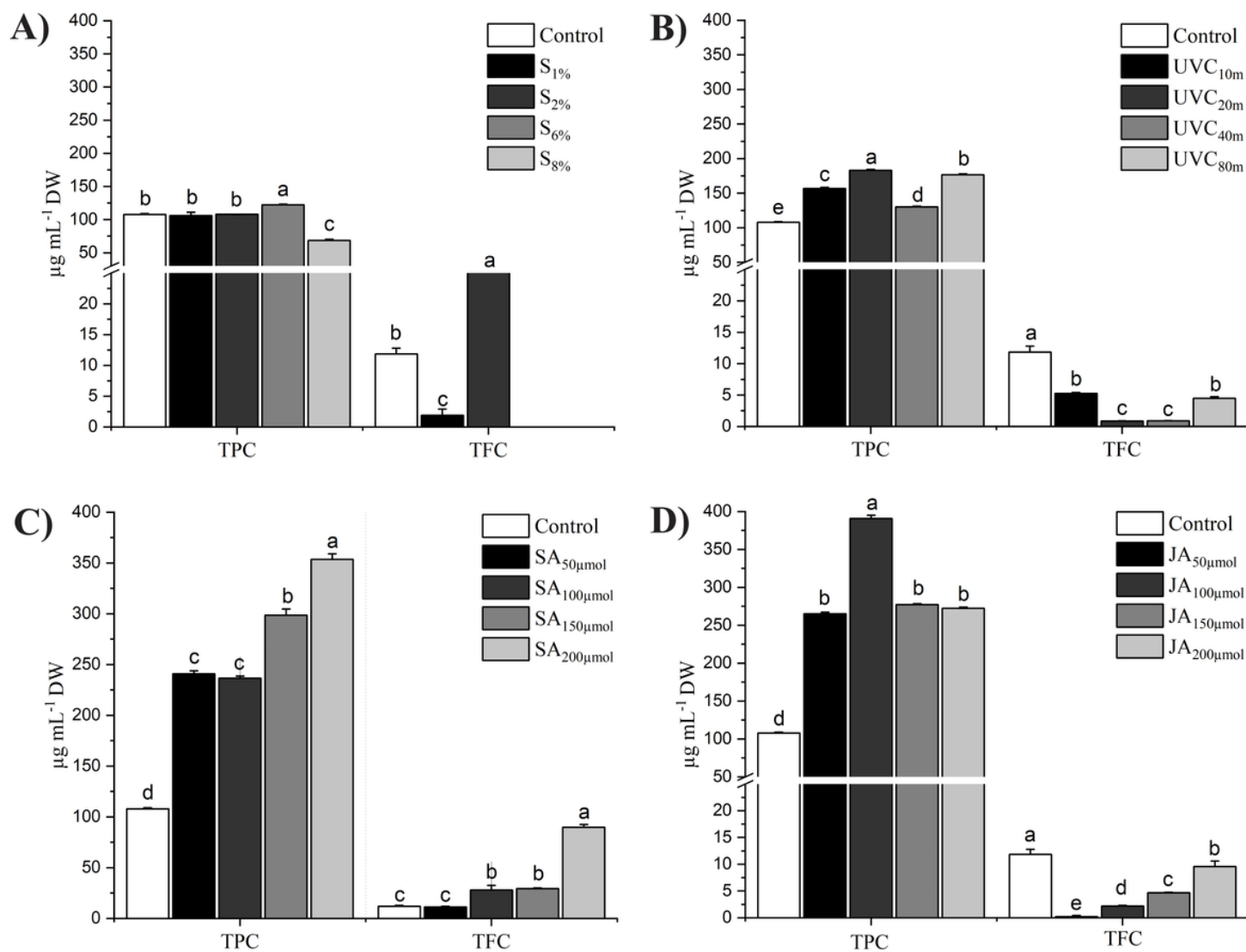


Figure 3

Total phenolic ($\mu\text{g GAE mg}^{-1}$ DW) and flavonoid ($\mu\text{g QE mg}^{-1}$ DW) contents of methanolic extracts of callus cultures of *C. hildmannianus* elicited with **A)** sucrose; **B)** UV-C light; **C)** salicylic acid; and **D)** jasmonic acid. Data of callus control (Santos et al. 2022). Lowercase letters indicate statistically significant differences by one-way ANOVA post hoc Tukey's test at $p < 0.05$.

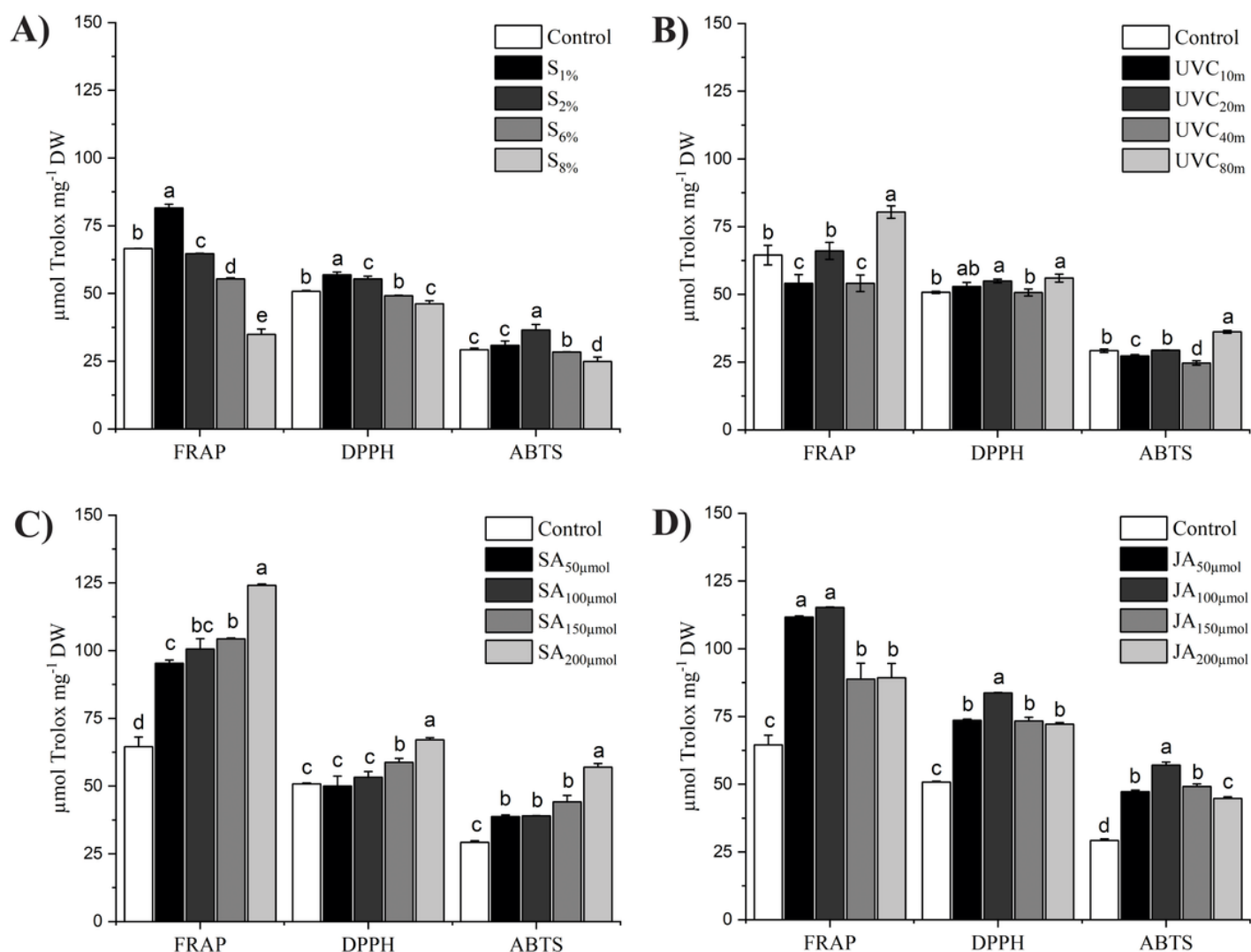


Figure 4

Antioxidant activity by FRAP, DPPH, and ABTS methods (equivalent $\mu\text{mol Trolox mg}^{-1} \text{DW}$) of callus from *C. hildmannianus* elicited with **A)** sucrose; **B)** UV-C light, **C)** salicylic acid, and **D)** jasmonic acid. Data of callus control (Santos et al. 2022). Lowercase letters indicate statistically significant differences by one-way ANOVA post hoc Tukey's test at $p < 0.05$.

Supplementary Files

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