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Subsequent treatment of leafy vegetables with low doses of UVB-radiation does not provoke cytotoxicity, genotoxicity, or oxidative stress in a human liver cell model

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ABSTRACT

Ultraviolet B (UVB) radiation in low but ecological-relevant doses acts as a regulator in the plant's secondary metabolism. This study investigates the effect of UVB radiation from light-emitting diodes (LEDs) [peak wavelength of (290 ± 2) nm] on the biosynthesis of health-promoting secondary plant metabolites (carotenoids, phenolic compounds, and glucosinolates) of green and red leafy vegetables of *Lactuca sativa*, *Brassica campestris*, and *Brassica juncea* followed by evaluation of potential adverse effects in a human liver cell model.

UVB radiation led to a significant increase in individual secondary plant metabolites, especially of phenolic compounds and glucosinolates, e.g. alkenyl glucosinolate content. Kaempferol und quercetin glycoside concentrations were also significantly increased compared to untreated plants.

The plant extracts from *Lactuca sativa, Brassica campestris*, and *Brassica juncea* were used to assess cytotoxicity (WST-1 assay and trypan blue staining), genotoxicity (Comet assay), and production of reactive oxygen species (EPR) using metabolically competent human-derived HepG2 liver cells. No adverse effects in terms of cytotoxicity, genotoxicity, or oxidative stress were detected in an extract concentration ranging from 3.125 to 100 μg ml^{-1}. Notably, only at very high concentrations were marginal cytostatic effects observed in extracts from UVB-treated as well as untreated plants.

In conclusion, the application of UVB radiation from LEDs changes structure-specific health-promoting secondary plant metabolites without damaging the plants. The treatment did not result in adverse effects at the human cell level. Based on these findings, UVB LEDs are a future alternative, promising light source to replace currently commonly used high-pressure sodium lamps in greenhouses.

1 Introduction

Feeding the rising world population adequately while avoiding malnutrition and hidden hunger is one of the major challenges facing the world today (https://sustainabledevelopment.un.org/sdg2). One solution could be the exploitation of new resources and the development of tools to produce fresh vegetables in novel local production facilities. Another possible approach could be to enhance the content of health-promoting secondary plant metabolites of vegetables in existing greenhouses or nurseries.

UV radiation (wavelengths between 100 and 400 nm) is one environmental factor that affects plants and their secondary plant

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metabolites. Due to passing through the atmosphere, the ozone layer, water vapour, oxygen, and carbon dioxide, all UVC and 90% of UVB radiation is absorbed. Of note is that UVB radiation (280–315 nm) leads to opposing effects in plants depending on the dose. Low, but ecologically relevant UVB radiation exposure activates the UVB photoreceptor UV-B RESISTANT LOCUS 8 (UVR8) and triggers photomorphogenic UVB responses such as the inhibition of stem elongation, differentiation of chloroplasts and accumulation of chlorophyll, and the expansion of leaves (Huché-Thélier et al., 2016; Jenkins, 2009). Low UVB doses also change the secondary plant metabolite composition (Jansen et al., 2008; Schreiner et al., 2012). On the other hand, high UVB doses stress the plants by, e.g. damaging the DNA, oxidation of relevant compounds, and inhibition of photosynthetic activities (Hideg et al., 2013; Jordan, 2002). Finally, the impact of UVB radiation depends also on the wavelength. For example, shorter wavelengths have a higher efficacy than longer wavelengths [general plant action spectrum (Caldwell, 1971)].

Leafy vegetables are a major research focus with the aim of increasing secondary plant metabolite content via light treatment, especially in species grown for fresh consumption or "to go" salads. These vegetables are consumed mainly raw and, therefore, the enhanced content of plant secondary metabolites can contribute to human health. The health-promoting effects of freshly consumed vegetables are described in many meta-analyses and the daily intake is suggested to play a crucial role in reducing the risk of numerous diseases (Albuquerque et al., 2020; Cena & Calder, 2020; Liu, 2013). However, the recommended daily intake is mostly not reached. Carotenoids, for example, are known to reduce the risk of chronic diseases, including cardiovascular diseases (Gammone et al., 2017) and type-2 diabetes mellitus (Akbaraly et al., 2008). Glucosinolates and their breakdown products confer a wide range of health-promoting properties and as signature secondary metabolites in *Brassica* vegetables are of interest for, amongst others, anti-diabetic (Guzman-Perez et al., 2016; Waterman et al., 2015) and anti-inflammatory effects (Bentley-Hewitt et al., 2014; Herz et al., 2016). The polyphenols are also of special importance due to both their antioxidant activity (Zietz et al., 2010) and anti-inflammatory effects (Pan et al., 2010). Importantly, all these secondary metabolites have been reported to lower the risk for several types of cancer (Bolhassani, 2015; Chen & Chen, 2013; Wu et al., 2013).

In most greenhouses for horticultural production, light-emitting diodes (LEDs) are not in routine use due to the associated high investment costs involved (Mitchell et al., 2015; Singh et al., 2015). However, as the cost of LEDs decreases, it is predicted that the number of greenhouses equipped with LEDs will steadily increase. One big advantage of using LEDs in greenhouses is the precise control of both the light spectrum and intensity (Singh et al., 2015). For example, it is possible to use LEDs of different wavelengths like 450 nm (blue light), 530 nm (green light), and 650 nm (red light) apart from white light to create an optimized lighting system in respect to growth, biomass, and plant development. Currently, additional light applications in horticultural production and scientific research are mainly limited to the addition of red and blue light (Agarwal & Gupta, 2016; Kaiser, Ouzounis, et al., 2019; Kaiser, Weerheim, et al., 2019; Piovene et al., 2015; Thoma et al., 2020). Since natural UVB radiation in greenhouses is low because of the UV-impermeable glass, artificial broad-band UVB radiation is usually applied with fluorescent light sources, e.g. TL 40W 12 RS lamp produced by Philips (Hamburg, Germany). To date, most experiments with additional UV radiation in greenhouses have mostly focused on the effects on photomorphogenesis (Barnes et al., 2005, p. 66; Koti et al., 2004; Li et al., 2013) and pathogen resistance (Mewis et al., 2012; Rechner et al., 2016). Indeed, only a few studies have investigated the effect of additionally applied (Goto et al., 2016; Neugart et al., 2012, 2014; Rodriguez et al., 2014) or reduced (Heinze et al., 2018) UV radiation on plant secondary metabolites.

Recent advances in the performance of visible and UV LEDs now make it possible to develop and manufacture special lighting systems for greenhouses and climate chambers (Kneissl et al., 2019). The implementation of such LEDs in greenhouses is one possibility to cover the required light demands of vegetables, especially within the off-season or low light season. However, an economic survey by The Netherlands' Ministry of Economic Affairs, Agriculture and Innovation discovered that 25–35% of costs for tomato production in Germany is for heating and lighting (Voss, 2011). Therefore, the horticultural production industry requires more energy efficient approaches, and LEDs in greenhouses could be part of the solution. Currently, German food law does not allow the treatment of vegetables with UV radiation except for disinfection of dried herbs and spices. However, there are no regulations regarding the doses of UV radiation or the differentiation between the different UV radiation wavelengths of UVA, UVB, and UVC.

We hypothesise that treatment with low doses of UVB radiation does not have the same effect as treatment with harmful, DNA-damaging UVC radiation, but only acts as a trigger for the biosynthesis of secondary metabolites in the plant. To test this hypothesis, we used an UVB radiation in our experiment lower than the sunlight's UVB. Nevertheless, this is a relatively high dose of UVB radiation emitted by LEDs at the current technical level. However, not much is known about any potential harmful effects caused by UVB radiation in food production. Therefore, we investigated the effects of UVB radiation on secondary plant metabolites, including carotenoids, phenolic compounds and glucosinolates, in the model plants *Lactuca sativa*, *Brassica campestris*, and *B. juncea*.This work aims to clarify whether UVB radiation could influence the plant in a way that may have harmful effects for the human consumer. For this purpose, we evaluated the cytotoxic and the genotoxic potential of UVB-treated plants compared with untreated ones using the human, metabolically competent liver cell line HepG2.

2. Materials and methods

Krebs HEPES buffer (KHB), deferoxamine methanesulfonate (DFO), diethyldithiocarbamic acid sodium (DETC), and 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrolidine hydrochloride (CMH) were purchased from Noxygen Science Transfer & Diagnostics GmbH (Elzach, Germany). Triton-X 100 was from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Dimethyl sulfoxide (DMSO; purity >99%) was purchased from Applichem GmbH (Darmstadt, Germany). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), trypsin 10x (25 mg mL^{-1}), trypsin-EDTA 10x (5 and 2 mg mL^{-1}), sodium phosphate buffered saline (PBS, without Ca and Mg), L-glutamine, and penicillin/streptomycin (P/S) 50:50 v/v solution were purchased from Gibco™ Life Technologies GmbH (Darmstadt, Germany). Low melting point agarose (LMPA) and normal melting point agarose (NMPA) were purchased from Serva GmbH (Heidelberg, Germany). Absolute ethanol, hydrochloric acid (37%), trypan blue, and ethidium bromide were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Ethylmethanesulfonate (EMS) was from Alfa Aesar (Haverhill, MA, USA). For the chemical analyses of the secondary metabolites, the following chemical products were used: acetonitrile (LC grade, Th. Geyer GmbH, Berlin, Germany), methanol (LC grade; Th. Geyer GmbH, Berlin, Germany), tetrahydrofuran (HiPerSolv Chromanorm, VWR ProLabo Chemicals, Germany), tert-butylmethylether (Rotisolv HPLC, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), dichloromethane (≥99.8%, SupraSolv® for GC-MS, VWR International GmbH), ammonium acetate (≥97% p.a. ACS, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), 2-propanol (Rotisolv HPLC, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), DEAE Sephadex A-25 chloride form (Sigma Aldrich, Germany), acetic acid supra-quality (ROTIPURAN®Supra 100%; Roth GmbH, Karlsruhe, Germany), imidazole (≥99%, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), formic acid (≥98% p.a. ACS, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), and β-glucuronidase/arylsulfatase (from *Helix pomatia*, Roche Diagnostics GmbH, Mannheim, Germany).

2.1. Plant material

Seeds of *Lactuca sativa* 'Navarro' (red leafy; Albert Treppens & Co Samen GmbH, Berlin, Germany), *Brassica campestris* 'Mizuna' (green leafy, Albert Treppens & Co Samen GmbH, Berlin, Germany), and *B. juncea* 'Red Giant' (red leafy, Albert Treppens & Co Samen GmbH, Berlin, Germany) were grown under controlled conditions (day/night temperature: 20 °C/18 °C; relative humidity 70%; light: 590 μmol m^{-2} s^{-1} for 12 h) in 12 cm pots on soil (Einheitserde type P, Fitz Kausek GmbH & Co. KG, Mittenwalde, Germany) for 3 weeks. Plants at the 4–5 leaf stage (baby leaf salads) were treated with a dose of 4.51 kJ m^{-2} d^{-1} of biologically active UVB radiation (UVB_{BE}) for four consecutive days at a distance of 30 cm and an intensity of 60 mW m^{-2} with a peak wavelength of (292 ± 2) nm for 6 h from 9 a.m. to 3 p.m. This corresponds to a high dose for cultivation using UVB LEDs. On the fifth day, 24 h after treatment, the plants were harvested. Control plants were cultivated at the same time without UVB radiation treatment. The plant material of each pot was individually harvested and directly frozen in liquid nitrogen, lyophilized, and grinded to a fine powder by a Retsch mill, and analyzed regarding their content of secondary plant metabolites. Afterwards, the plant material was pooled, homogenized, and analyzed again to confirm the homogeneity of the plant material. The plant material was stored at −60 °C until chemical analyses.

2.2. UVB radiation treatment

An UV radiation module was constructed from 20 UVB LEDs provided by SETi [Sensor Electronic Technology, Inc. (part of Seoul Semiconductor and Seoul Viosys), Court Columbia, South Carolina, USA] each with a peak wavelength of (292 ± 2) nm (Fig. 1a). The LEDs were arranged over an area of 50 cm × 50 cm, as shown in Fig. 1b. The UV LEDs are hermetically packaged to protect them from environmental impact, particularly humidity. The arrangement of the LEDs as well as the shape of the surrounding aluminum reflector was optimized with respect to a maximum uniformity of the UV irradiance distribution by ray tracing simulations using the ZEMAX-EE commercial software package from ZEMAX Development Corporation. Separately adjusting the current of each LED through individual current drivers resulted in a uniformity factor of about 85% at a distance of 30 cm (Fig. 1b) and more than 90% at a distance of 50 cm to the LEDs. To ensure maximum efficiency and reliability of the UV LEDs, self-heating of the devices was minimized using ceramic AlN packages, aluminum-core printed circuit boards with a high heat conductivity, and metal heat sinks actively cooled by fans. As a result, the temperature rise at the LEDs could be limited to 3 K above ambient temperature. The operation of the irradiation modules was managed by a microcontroller, which monitors the temperature and controls the current of all LEDs as well as the sequence of irradiation experiments. The mean value of the UV irradiance across the target area of 50 cm × 50 cm at a distance of 30 cm was adjusted to 60 mW m^{-2} as determined by a calibrated spectrometer.

Before plant irradiation began, the spectral irradiance of the UVB radiation was measured over the entire irradiated area using a calibrated spectrometer (USB 2000+ Fiber Optic Spectrometer, Ocean Optics Inc., Ostfildern, Germany). The spatially averaged spectral irradiances were weighted according to the relative photon effectiveness given by Caldwell (1971) and Green et al. (1974), setting it to 1 at 300 nm. By up-integration over the spectral range 280–313 nm, a biologically effective UVB irradiance was calculated, which is the basis of the UVB dose values given in this paper. It should be noted that by normalizing the photon effectiveness to 300 nm, the dose values given here correspond to a fictitious UVB irradiation at 300 nm, from which the same biological effect is expected as the irradiation actually applied at 290 nm.

2.3. Chemical analyses of secondary plant metabolites

2.3.1. Carotenoids and chlorophylls

The pigments of 5–7 mg lyophilized, powdered, and exactly weighted material were extracted completely using methanol:tetrahydrofuran (1:1, v:v). Qualitative and quantitative analyses were performed by means of UHPLC-DAD coupled to a ToF-MS (1290 Infinity UHPLC, 6230 TOF LC/MS, Agilent Technologies, Waldbronn, Germany) using external calibration standard curves (Frede et al., 2019).

2.3.2. Phenolic compounds

For analyzing the phenolic compounds, 10 mg lyophilized plant sample was extracted according to Neugart et al. (2015). For the quantitative analysis of flavonoid glycosides and hydroxycinnamic acid derivatives, a HPLC series 1100 (Agilent Technologies, Waldbronn, Germany) was used (Heinze et al., 2018). Identification was performed by a mass spectrometer (Bruker Ion Trap, Bremen, Germany) according to Neugart et al. (2015). For semi-quantification of phenolic compounds, external calibration curves were used for chlorogenic acid, quercetin-3-glycoside, kaempferol-3-glycoside, isorhamnetin-3-glucoside, and cyanidin-3-glucoside.

2.3.3. Glucosinolates

Glucosinolate concentration was determined as desulfo-glucosinolates of 10 mg of powdered plant samples according to Hanschen (2020). The concentration of desulfo-glucosinolates was calculated by the peak area relative to the area of the internal standard of desulfated 1 mM 2-propenyl glucosinolate.

2.4. Plant extract preparation

The plant extract was prepared as described before with

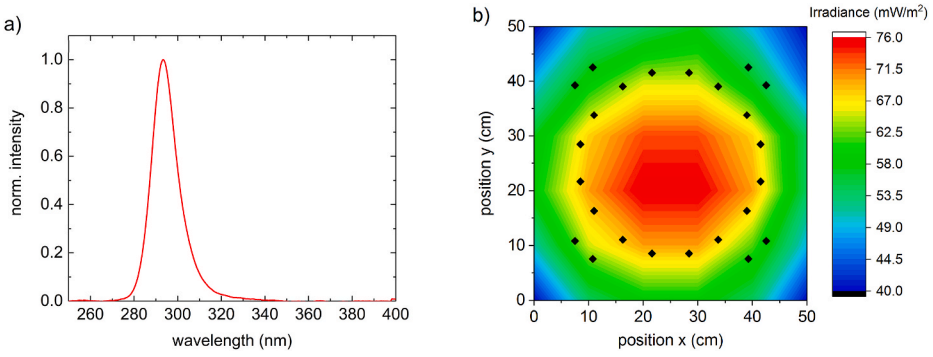

Fig. 1. a) Exemplary emission spectra of 292 nm LEDs as used for the module. The LEDs were operated at a current of 20 mA and passively cooled at room temperature. b) Irradiance distribution of the 292 nm LED module measured at a distance of 30 cm over a 50 cm × 50 cm target area. The black dots mark the positions of the LEDs on the module.

modifications (Tran et al., 2016). Briefly, 250 mg plant powder was added to 5 ml PBS (w/o Mg and Ca) and incubated for 30 min at room temperature. The aqueous extract was strained through gauze and sterile filtered (Millex-GP 0.22 μm filters, Merck-Millipore, Darmstadt, Germany). For each experiment, plant extracts were freshly extracted.

2.5. Cell line and cell culture

The HepG2 cell line (ACC-180) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cells were cultured in DMEM supplemented with 15% FCS and 1% penicillin/streptomycin solution and incubated in a 95% humidified incubator at 37 °C and 5 % CO_2 as described before (Odongo et al., 2017). After freezing, cells in passage number four to ten were used.

2.6. Assessment of cell viability using WST-1 assay

As a parameter for cell viability, the WST-1 assay was used for quantification of mitochondrial dehydrogenase activity, which cleaves the tetrazolium salt WST-1 to formazan. The assay was performed according the manufacturer's instructions (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) and as described before (Kupke et al., 2016). In brief, HepG2 cells were seeded in 96-well plates ($5*10^3$ cells/well), incubated for 48 h, and then exposed to serial dilutions of extracts or 1% PBS (solvent control) for 72 h. As positive control, 0.1% triton X-100 was used. Subsequently, medium was removed, 100 μl WST-1 working solution per well was added and incubated for another 1 h in a 95% humidified incubator at 37 °C and 5% CO_2. Absorbance was measured at 435 nm (reference 620 nm) using a multiplate reader (infinite M200, Tecan Deutschland GmbH, Crailsheim, Germany).

2.7. Assessment of cytotoxicity and cytostatic activity using trypan blue dye exclusion test

The cytotoxicity and cytostatic activity of extracts were assessed using the trypan blue dye exclusion test as described before (Odongo et al., 2017). Briefly, HepG2 cells were cultured in 12-well plates ($1*10^5$ cells/well) for 48 h and then exposed to extracts or 1% PBS (solvent control) for 72 h. Subsequently, cells were trypsinized, mixed 1:1 with a trypan blue solution (0.4 % w/v in PBS), and the cell number was counted using a Neubauer chamber and light microscopy. Cytotoxicity was determined as:

$$\%\ viability = \frac{number\ of\ viable\ cells}{total\ cell\ number} \times 100\ \%$$

Cytostatic activity was determined by comparing the total number of extract-treated cells with the respective solvent control. As positive control, 0.1% triton X-100 was used.

2.8. Assessment of genotoxic activity using the Comet assay

The alkaline Comet assay was used to assess genotoxic activity as described before (Lamy et al., 2004) with modifications. In brief, HepG2 cells were cultured in 12-well plates for 48 h, and then treated with the plant extracts or 1% PBS (solvent control) for 24 h. Subsequently, cells were washed twice with prewarmed PBS, harvested, and 4×10^4 cells were mixed with 90 μl of 0.7% LMPA in PBS. The cells were transferred to a glass slide coated with a layer of 0.7% LMPA and 1% NMPA. Afterwards, the slides were covered with a coverslip and placed on a cold plate to allow the agarose to solidify. The coverslips were then removed and the slides were incubated in a lyzing buffer (pH 10; 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris and 1% triton-X 100) for at least 90 min at 4 °C. After alkali unwinding (pH 13.5, 1.3 mM Na_2EDTA and 300 mM NaOH) for 30 min, electrophoresis was run at 25 V and 300 mA for 30 min at 4 °C. Afterwards, the slides were neutralized in a 400 mM Tris buffer (pH 7.5) for 5 min and rinsed in distilled water for another 5 min. The samples were fixed in absolute ethanol for 5 min and stored at 4 °C until analysis. The slides were stained with 75 μl ethidium bromide solution (10 μg/ml in distilled water) and analyzed using a Leica fluorescence microscope (Leica DMLS, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) with an excitation filter (BP 546/10 nm, barrier filter: 590 nm) equipped with an image analysis system (Comet 5.5, Optilas GmbH, München, Germany), and 100 cells per slide were systematically screened. The indicator of DNA damage was percentage of tail DNA. As positive control, 1.25 mM ethylmethanesulfonate (EMS) was used (Odongo et al., 2018).

2.9. Assessment of oxidative stress induction using electron paramagnetic resonance spectroscopy (EPR)

Oxidative stress induction by the plant extracts was assessed by electron paramagnetic resonance spectroscopy (EPR) equipped with a temperature controller Bio III (Noxygen, Elzach, Germany) that is capable of detecting reactive oxygen species (ROS) in the cells as described before (Lamy et al., 2013; Odongo et al., 2017). To analyze ROS production, HepG2 cells were seeded in 12-well plates ($1*10^5$ cells/well) and cultured for 48 h. Subsequently, cells were treated with serial dilutions of extracts or 1% PBS (solvent control) for 1 or 24 h. Cells were washed once with pre-warmed KHB and incubated with 100 μM spin probe CMH in KHB, supplemented with 25 μM DFO and 5 μM DETC for 30 min in a 95 % humidified incubator at 37 °C and 5 % CO_2. Supernatants were transferred to a reaction tube and kept on ice until EPR analysis. A 10 scan screening was performed for each sample using 50 μL glass capillaries. As positive control, cells were treated with 200 μM menandione for 30 min.

2.10. Data analyses

Statistical analysis was conducted with GraphPad PRISM (version 5 and 6.07, GraphPad Software Inc., San Diego, CA, USA). Significant differences between secondary plant metabolites of untreated and UVB-treated plant material were determined by Mann Whitney test ($p \leq 0.05$) of plant replicates ($n = 8$).

Results of the cell culture experiments are presented as means and standard deviation (SD) of three independent experiments (if not stated otherwise). Due to the sample size of three independent experiments, the test for normal distribution was not performed. Statistical significance between solvent control and cells exposed to plant extracts was analyzed using two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Statistical significance between untreated and UVB-treated plant extracts was assessed using two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. Differences were assumed as significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

3. Results and discussion

3.1. UVB radiation

The treatment with (292 ± 2) nm UVB radiation at a dose of UVB_{BE} = 4.51 kJ m^{-2} per day for four days (in total 18.04 kJ m^{-2}) did not damage the plants and no growth or morphological differences between the untreated and UVB-treated plants were found (data not shown).

3.2. Chemical analyses of secondary plant metabolites

The plant material (see 2.1) was analyzed regarding the content of secondary plant metabolites [carotenoids, phenolic compounds, and glucosinolates (Table S1)]. For extract preparation, the plant material was pooled, homogenized, and analyzed again. The results presented in Table S2 confirm a homogeneous plant material for the bioactivity tests.

The groups of secondary plant metabolites are discussed separately in the following sections.

3.2.1. Carotenoids and chlorophylls

Carotenoids and chlorophylls are secondary plant metabolites that are important for human health, and which are affected by visible light and UVB radiation. In all three vegetables tested here, the same carotenoids and xanthophyll compounds were detected with slightly different levels: chlorophyll *a* and *b*, lutein, β-carotene, zeaxanthin and two derivatives thereof, as well as violaxanthin (Table S1). Since UVB radiation just slightly affected the carotenoid and chlorophyll contents, changes were not significant. Comparing the absolute amounts of individual carotenoids and chlorophylls in *Lactuca sativa* and *Brassica campestris*, the content of chlorophyll *b* was significantly increased in UVB-treated compared to untreated plants (Table S1).

Carotenoids and chlorophylls are pigments that play an important role in light harvesting and photoprotection (Frede et al., 2018, 2019; Klopsch et al., 2019). Since UV radiation has been reported to reduce chlorophyll *a* contents (Marwood & Greenberg, 1996), our results of increased levels are therefore surprising. Interestingly, Salama et al. (2011) found reduction or increase of carotenoids depending on the wavelengths of the UV radiation (Salama et al., 2011). Additionally, low doses of UVB radiation led to accumulation of chlorophyll (Caldwell & Britz, 2006; Huché-Thélier et al., 2016; Jenkins, 2009) and increased the carotenoid concentration compared to control plants in bell pepper (León-Chan et al., 2017) and tobacco (Shen et al., 2017). In the present study, a species-specific response was observed. In detail, although no significant change was found when looking at total carotenoid and chlorophyll contents, the results do reveal that individual compound concentrations, namely chlorophyll *b* and lutein, increased due to UVB irradiation (Table S1). Previous studies also found species-specific plant's responses. For example, in broccoli sprouts, UVA radiation led to an increase of carotenoids, but UVB radiation did not (Moreira-Rodriguez et al., 2017). Moreover, Caldwell and Britz (2006) analyzed different cultivars of green and red leafy lettuce grown in a greenhouse with additional UV radiation. They found a reduction in carotenoid concentration and an increase in chlorophyll concentrations. Besides different wavelengths and light sources, the reduction in carotenoid concentration could be due to carotenoid degradation by ROS as part of the plant's response against stress induced by UVB radiation (Götz et al., 1999; Sandmann, 2019).

3.2.2. Phenolic compounds

Phenolic compounds act as shielding components and antioxidants against UV radiation (Jansen et al., 2008; Sharma et al., 2019). It is known that the plant's response to exposure to increasing UV radiation results in increasing content of phenolic compounds with anti-oxidative properties (Neugart et al., 2012), which protect the plant cells against UV damage (Agati & Tattini, 2010; Edreva, 2005).

The phenolic compound profiles, including flavonoid glycosides, hydroxycinnamic acids, and anthocyanins in the red leafy *L. sativa* and *B. juncea,* were fundamentally different between the plants (Table S1). *L. sativa* is characterized by various caffeoyl derivatives and quercetin monoglycosides of which most are acylated (Becker et al., 2013). In contrast, *B. juncea* had a high number of sinapoyl and feruloyl derivatives as well as complex kaempferol triglycosides. The same is true for *B. campestris*, a green leafy species. These compounds had been previously identified in many *Brassica* species, and thus, are signature phenolic compounds (Cartea et al., 2011; Neugart et al., 2018).

Daily treatment with UVB radiation at a moderate dose of 4.51 kJ m^{-2} had the strongest effect in *L. sativa* with increased concentrations of kaempferol, quercetin, and cyanidin glycosides (Fig. 2) – a finding that is consistent with a previous study (Assumpção et al., 2019). Moreover, malonylated quercetin, and cyanidin glycosides as well as dicaffeoyl tartaric acid and dicaffeoyl quinic acid were identified as the compounds with the highest antioxidant activity in *L. sativa* (Caldwell, 2003). Although quercetin and kaempferol glycosides increased and the concentration of quercetin glycosides was high, the quercetin to kaempferol ratio of the corresponding glucuronides decreased from 5.5 to 2.1, suggesting that kaempferol glycosides can also play a significant role in the UV response. Majer et al. (2014) showed that kaempferol glycosides have similar absorption spectra to quercetin glycosides and that the

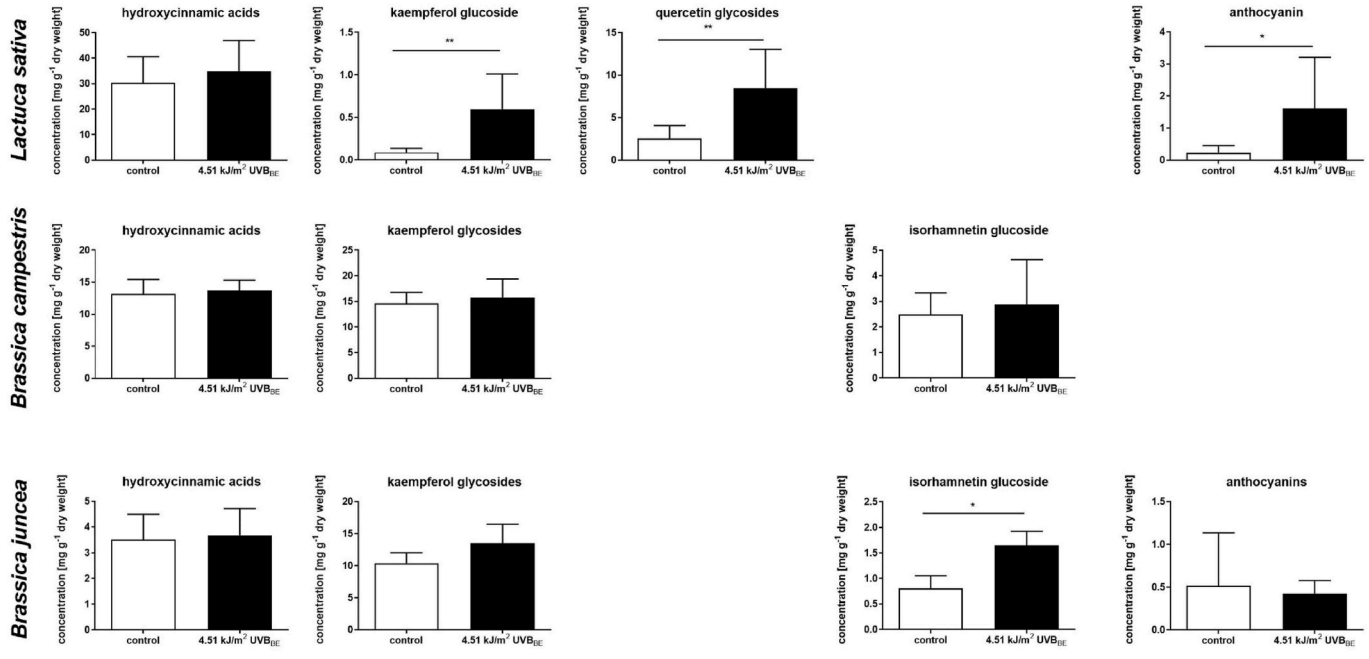

Fig. 2. Phenolic compound concentrations of *Lactuca sativa, B. campestris*, and *B. juncea* in mg g^{-1} dry weight of the plant material used for the extract preparation. The figure shows the total hydroxycinnamic acid, kaempferol, quercetin, and isorhamnetin glycosides, and total anthocyanin concentrations. Data are means and standard deviations of eight plants. Significant differences between UVB-treated and untreated plant material are marked (Mann Whitney test, *, $p < 0.05$; **, $p < 0.01$).

difference in antioxidant activity is less than that of aglycones, which do not occur in the plant *per se*. It can therefore be assumed that kaempferol-3-glucuronide in *L. sativa* exerts both functions, namely as an UV absorber and antioxidant compound. In *L. sativa*, the expression of *chalcone synthase* (CHS), *flavanone 3-hydroxylase* (F3H), and *dihydroflavonol* 4-*reductase* (DFR) genes showed a positive correlation with anthocyanin accumulation after UVB treatment, and hence, explains the red coloration of these plants (Park et al., 2007), which was also seen in our experiment.

The analyses of the red leafy species *B. juncea* revealed no significant effects on the total kaempferol glycosides. However, we did find increases of the non-acylated kaempferol-3-sophoroside and kaempferol-3-sophoroside-7-glucoside as well as the coumaroyl and sinapoyl monoacylated kaempferol triglycosides and even a doubling in concentration of the isorhamnetin glucoside (Fig. 2, Table S1). In contrast, the total flavonoid glycosides of *B. campestris* were not affected by UVB treatment and only the kaempferol-3-coumaroyl-sophoroside-7-glucoside concentration increased compared to untreated plants (Table S1). Several studies have shown that *Brassica* kaempferol glycosides play a role in UV defense (Harbaum et al., 2007; Neugart et al., 2012, 2014; Olsson et al., 1998; Rechner et al., 2016). Kaempferol glycosides are not known for their antioxidant activity. However, the glycosylation and acylation patterns are expected to change their functionality as has already been shown for kale (Zietz et al., 2010).

In our study, the hydroxycinnamic acids were unaffected by the UVB radiation. However, in a previous study in *L. sativa*, the hydroxycinnamic acids were slightly increased with lower daily UVB doses of 0.5 kJ m^{-2} which clearly underlines the dose-dependent outcomes of UVB radiation (Assumpção et al., 2019). Moreover, there are several studies on *Brassica* species showing the same effect (Harbaum-Piayda et al., 2010; Neugart et al., 2014; Reifenrath & Muller, 2007), whereas others even found a decrease with UVB radiation treatments (Moreira-Rodríguez et al., 2017; Neugart et al., 2012; Rechner et al., 2016).

3.2.3. Glucosinolates

To date, there is no information on the relevance of glucosinolates in protecting plants against excessive light and UV radiation, although glucosinolates content changes after treatment with UVB radiation probably due to a joint or UVB-induced regulation via the jasmonic acid signaling pathway (A.-H.-Mackerness et al., 1999).

Plant species of the order Brassicales contain characteristic secondary plant metabolites namely glucosinolates. Unlike *Brassica campestris* and *B. juncea*, *Lactuca sativa* does not belong to this plant order. Therefore, changes in glucosinolate content could only be investigated in two of the leafy vegetables (*B. campestris*, *B. juncea*) used in this study. Both *Brassica* species show different glucosinolate profiles. In *B. campestris*, the aliphatic glucosinolates 5-methylsulfinylalkyl glucosinolate, 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, 2-hydroxy-4-pentenyl glucosinolate, the arylaliphatic glucosinolate 2-phenylethyl glucosinolate, and the indolic glucosinolates indol-3-ylmethyl glucosinolate, 4-hydroxyindol-3-ylmethyl glucosinolate, 4-methoxyindol-3-ylmethyl glucosinolate, and 1-methoxyindol-3-ylmethyl glucosinolate were detected. The results from *B. juncea* differed from those of *B. campestris* in terms of the presence of diverse aliphatic glucosinolates, namely 2-propenyl glucosinolate, 3-butenyl glucosinolate, and 4-pentenyl glucosinolate.

The treatment with UVB radiation for four days did not change the total glucosinolate content in *B. campestris* but it did in *B. juncea* (Fig. 3). A closer look at the glucosinolate groups and the individual glucosinolate reveals that in *B. campestris*, just one aliphatic glucosinolate (2-hydroxy-3-butenyl glucosinolate) changed significantly after treatment with UVB radiation, and that this individual glucosinolate does not belong to the major glucosinolates (Table S1). Furthermore, the content of the total indolic glucosinolates significantly increased after UVB treatment due to increased contents of indol-3-ylmethyl and 4- and 1-methoxyindol-3-ylmethyl glucosinolates (Table S1). Conversely, in *B. juncea*, the overall total glucosinolate content increased significantly (Fig. 3) due to an elevation of the alkenyl glucosinolates, including the main aliphatic glucosinolate 2-propenyl (Table S1). In contrast, the arylaliphatic and indolic glucosinolate contents in *B. juncea* were not affected by UVB treatment. Of note is that these results further confirm other studies which reports that UVB radiation in a moderate dose can be used as an elicitor to enhance glucosinolate contents. Mewis et al. (2012) investigated the effect of a single or double dose of low to moderate UVB radiation on *B. oleracea* sprouts and found a significant increase in total aliphatic and indole glucosinolates as well as individual glucosinolates compared to untreated sprouts (Mewis et al., 2012). In our study, individual aliphatic glucosinolates were increased in both *Brassica* species and individual indole glucosinolates were also increased in *B. campestris*.

3.3. Assessment of cytotoxic and genotoxic effects as well as ROS-producing potential of the plant extracts using the human liver cancer cell line HepG2

High levels of UV radiation of plants is mostly linked to its harmful properties as a stressor, which can lead to the plant's death (Hideg et al., 2013; Jordan, 2002; Nawkar et al., 2013). In contrast, low to moderate doses of UV radiation could act more as a trigger for the elevated biosynthesis of secondary plant metabolites (Mosadegh et al., 2019). However, whether this plant treatment could have adverse effects on human health still remains to be elucidated. To the best of our knowledge, this is the first *in vitro* study to describe the effect of UVB-treated plant material on its bioactivity in a human cell line.

3.3.1. Cell viability in terms of mitochondrial dehydrogenase activity

First, to assess the effects on cell viability of the UVB-treated plant extracts *in vitro,* HepG2 cells were exposed to different concentrations (1.56–500 mg ml^{-1}) of aqueous extracts from UVB-treated and untreated *Lactuca sativa*, *Brasscica campestris*, and *B. juncea*. For this purpose, the cell viability was measured by using the WST-1 assay, which quantifies the mitochrondrial dehydrogenase activity by cleavage of the tetrazolium salt to formazan. The amount of the formazan dye generated by activity of dehydrogenase is directly proportional to the number of living cells. Cells exposed to extracts from *Lactuca sativa* either treated with UVB or untreated (Fig. 4A) showed decreased cell viability starting at 250 μg ml^{-1}, whereas extracts from *Brassica campestris* (Fig. 4B) and *B. juncea* (Fig. 4C) did not impact the cell viability at all tested concentrations. The observed results provide the first evidence that UVB radiation does not affect the bioactivity of plants beyond that observed for the untreated ones.

3.3.2. Cytotoxicity and cytostatic activity using trypan blue staining

Cytotoxicity is defined as toxicity due to not clearly defined mechanisms of compounds on living cells that cause in consequence cell death. HepG2 cells were treated with the extracts from untreated and UVB-treated plant material for 72 h, and cytotoxicity was determined using the trypan blue dye exclusion test, depicted as percentage of viable cells of total cell number (Fig. 5). None of the tested aqueous plant extracts showed any cytotoxic effects, neither the aqueous control plant extracts nor the extracts from UVB-treated plants (Fig. 5A–C).

The cytostatic activity was analyzed by comparing the total cell number of the extract-treated cells to the solvent control cells (Fig. 5D–F). At concentrations starting from 250 μg ml^{-1}, the extract from *Brassica juncea* showed cytostatic effects (Fig. 5D), the extracts from *Lactuca sativa* and *B. campestris* showed only in the highest concentration of 500 μg ml^{-1} a cytostatic effect of 45% and 44%, respectively (Fig. 5E and F). Again, there were no differences between the extracts of UVB-treated and the untreated plant material in their cytotoxic or cytostatic activity. In another study conducted earlier on HepG2 cells, the aqueous extract of the Brassicaceae *Eruca sativa* (rocket plant) (Lamy et al., 2008) also did not impact cell viability at all concentrations

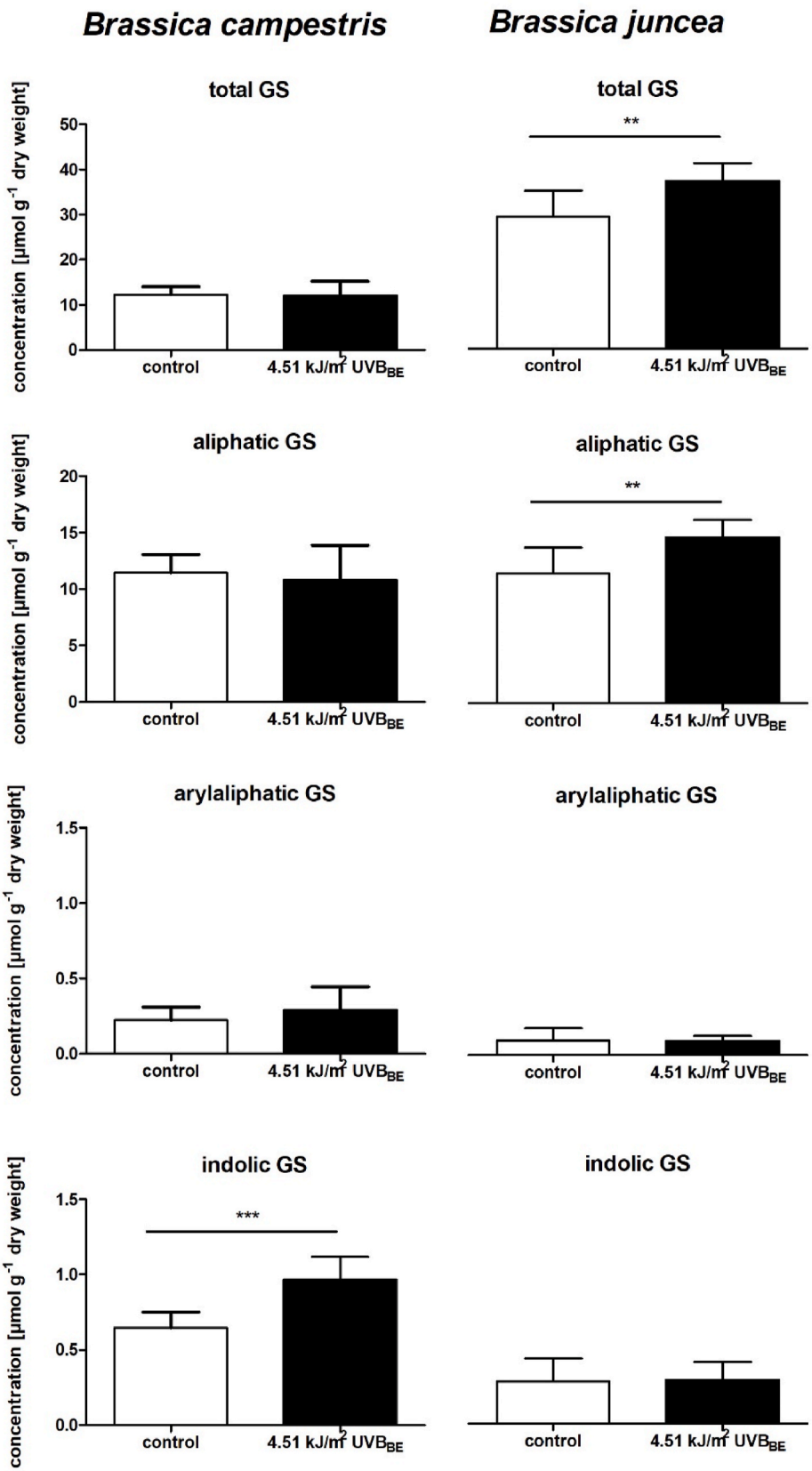

Fig. 3. Glucosinolate (GS) contents of *B. campestris* and *B. juncea* in µmol g^{-1} dry weight of the plant material used for the extract preparation. The figure shows the total GS content and total GS content of the different GS groups (aliphatic, arylaliphatic, and indolic GS). Data are means and standard deviation of eight plants. Significant differences between UVB-treated and untreated plant material are marked (Mann Whitney test; **, $p < 0.01$; ***, $p < 0.001$).

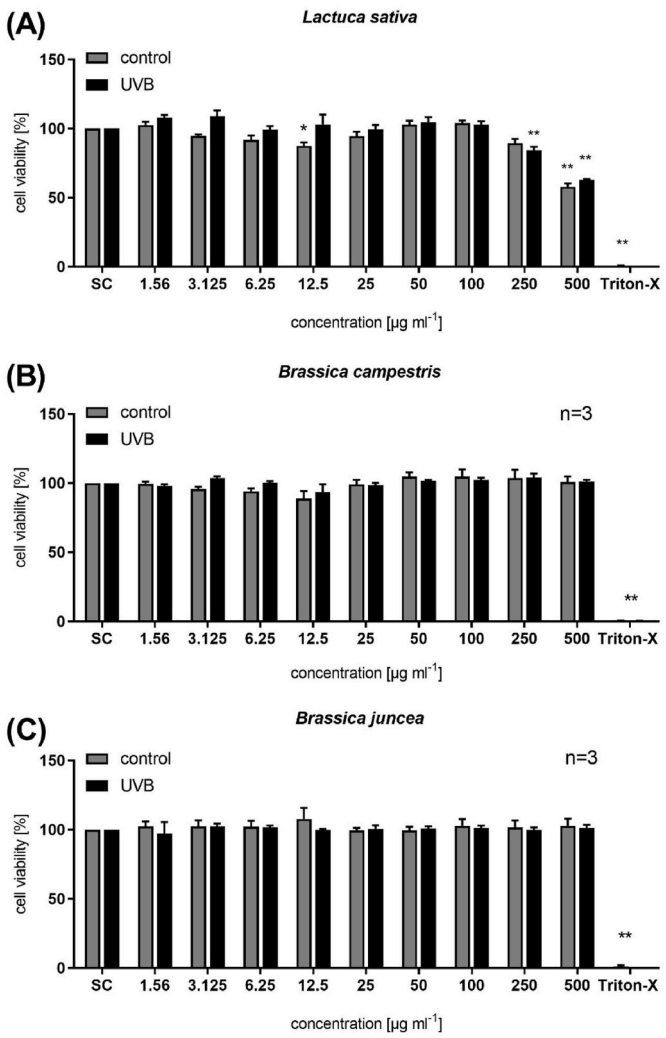

Fig. 4. Cell viability assessed using the WST-1 assay. HepG2 cells were exposed to aqueous extracts prepared from untreated and UVB-treated plant material from (A) *Lactuca sativa*, (B) *Brassica campestris*, and (C), *B. juncea* for 72 h. (A) measured in quadruplicate, (B) and (C) measured in three independent experiments. Data are given as means and standard deviations. Significant differences between the solvent control (SC, 0.1% PBS) and the plant extracts or triton-X (0.1%) are marked (two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test; **, $p < 0.01$).

tested, as determined by the trypan blue method. Similarly, for extracts of *B. carinata*, only at the highest concentration tested (333 µg ml^{-1}) were cytostatic effects of 30–40% observed in HepG2 cells (Odongo et al., 2017). Compared to the present study, the total content in the *B. carinata* plant extracts was then comparably lower (*B. campestris*) or the same (*B. juncea*) for the GS and much higher for the polyphenols. At the highest extract concentration tested, the content of total phenolic compounds was 11.9 mM (10.805 µg ml^{-1}) which is about 24-fold higher compared to highest total phenolic content of the extracts used in the present study (0.5 mM, approx. 227 µg ml^{-1}).

For isolated phenolic compounds, e.g. cytotoxic effects were only reported at high concentrations in the micromolar range (Habtemariam & Dagne, 2010). The flavonoids kaempferol and quercetin showed cytotoxicity at concentrations from 221 µM to 303 µM in human umbilical vein endothelial (HUVE) cells and 167 µM and 61 µM in human diploid fibroblast-like cells (TIG-1), respectively (Matsuo et al., 2005). Another study demonstrated the cytotoxic effect of quercetin in HepG2 cells in concentrations higher than 33 µM (Barcelos et al., 2011). Besides the concentration, the cytotoxic effect of phenolic compounds seems to depend on the catechol group in the B-ring of their chemical structure as well as on the conjugation between the A- and B-ring (Dickancaité et al., 1998).

3.3.3. Assessment of genotoxic activity

The comet assay was used to assess the genotoxic activity of the plant material after UVB radiation treatment. As seen in Fig. 6, no relevant genotoxicity of the extracts from untreated but also not the UVB-treated plants was observed, not even in the highest extract concentrations tested. This finding is in accordance with other studies reporting on genotoxicity assessment. In the study on *Eruca sativa* plant extracts, no genotoxicity could be seen after HepG2 cell treatment for 24 h with 50 µg ml^{-1} (Lamy et al., 2008). In another study analyzing the genotoxic activity of several raw and processed *Brassica* vegetables using human HT29 colon carcinoma cells, the extracts also did not show any genotoxic activity (Ferrarini et al., 2012) up to 100 µg ml^{-1} raw vegetable extract. Moreover, aqueous extracts of *Brassica* vegetables of up to 2500 µg ml^{-1} did not lead to persistent strands breaks or DNA disintegration (Kołodziejski et al., 2019), which was also seen in mouse cells (GonçalvesÁ et al., 2012).

Again, for single phenolic compounds, e.g. quercetin, it could be found that the investigated concentrations of up to 16 µM did not provoke any genotoxic effects (Barcelos et al., 2011). Another researcher group found that hydroxycinnamic acids with concentrations up to 400 µM alone did not cause detectable DNA damage, but in the presence of copper, they did (Zheng et al., 2008). This effect was dependent on the chemical structure of the hydroxycinnamic acid. The genotoxic effects were detected depending on the cell lines used, plant secondary metabolites, and concentration ranges. This was also shown by Duthie et al. (1997) who investigated different flavonoids at concentrations ranging from 0 to 1000 µM in human Caco-2, HepG2, and HeLa cells, as well as human lymphocytes.

3.3.4. Assessment of ROS production using electron paramagnetic resonance spectroscopy (EPR)

ROS serve as cell signaling molecules for normal biologic processes and play an important role as regards the cell's redox status (Patel et al., 2018). An imbalance between ROS production and the cellular antioxidant defense mechanism can disrupt the physiological cell status and might be a cause for the development of several diseases such as cancer, cardiovascular diseases, pulmonary hypertension, and retinopathy (Liu et al., 2018). To determine whether the UVB radiation of the plant could lead to oxidative stress in the cells, ROS production was determined after exposure to the cells for 1 or 24 h (Fig. 7). None of the used extracts of the UVB-treated plants were able to induce ROS production compared to the solvent control.

Investigations with other vegetables have shown both pro-oxidant and antioxidant effects. For example, Quassinti et al. (2016) found a concentration-dependent pro-oxidant activity of aqueous Savoy cabbage (*B. oleracea)* sprout extracts using human dermal fibroblasts (HuDe). The pro-oxidant activity was shown for a tested concentration of 0.25 and 0.5 mg ml^{-1} on HuDe cells after 30 min incubation. Moreover, Girard-Lalancette et al. (2009) showed pro- and antioxidant activities of different vegetable juices depending on their processing. In this context, broccoli and carrot juices showed a concentration-dependent pro-oxidant activity starting from 2 µg ml^{-1} up to 500 µg ml^{-1} after 60 min incubation on murine fibrosarcoma (L-929) cells and subsequent exposure to 200 µM *tert*-butylhydroperoxide (*tert*-BuOOH). In contrast, a concentration-dependent antioxidant activity was observed when tested as boiled juices.

For isolated phenolic compounds, both anti- and pro-oxidant actions have also been described (Sak, 2014). Zheng et al. (2008) investigated the pro-oxidant effect of 0.4 mM hydroxycinnamic acids on DNA-strand breakage and found that the DNA-damaging activity and the oxidative potential of the substances are dependent on the presence of Cu(II) ions and the chemical structure of the hydroxycinnamic acids, here the

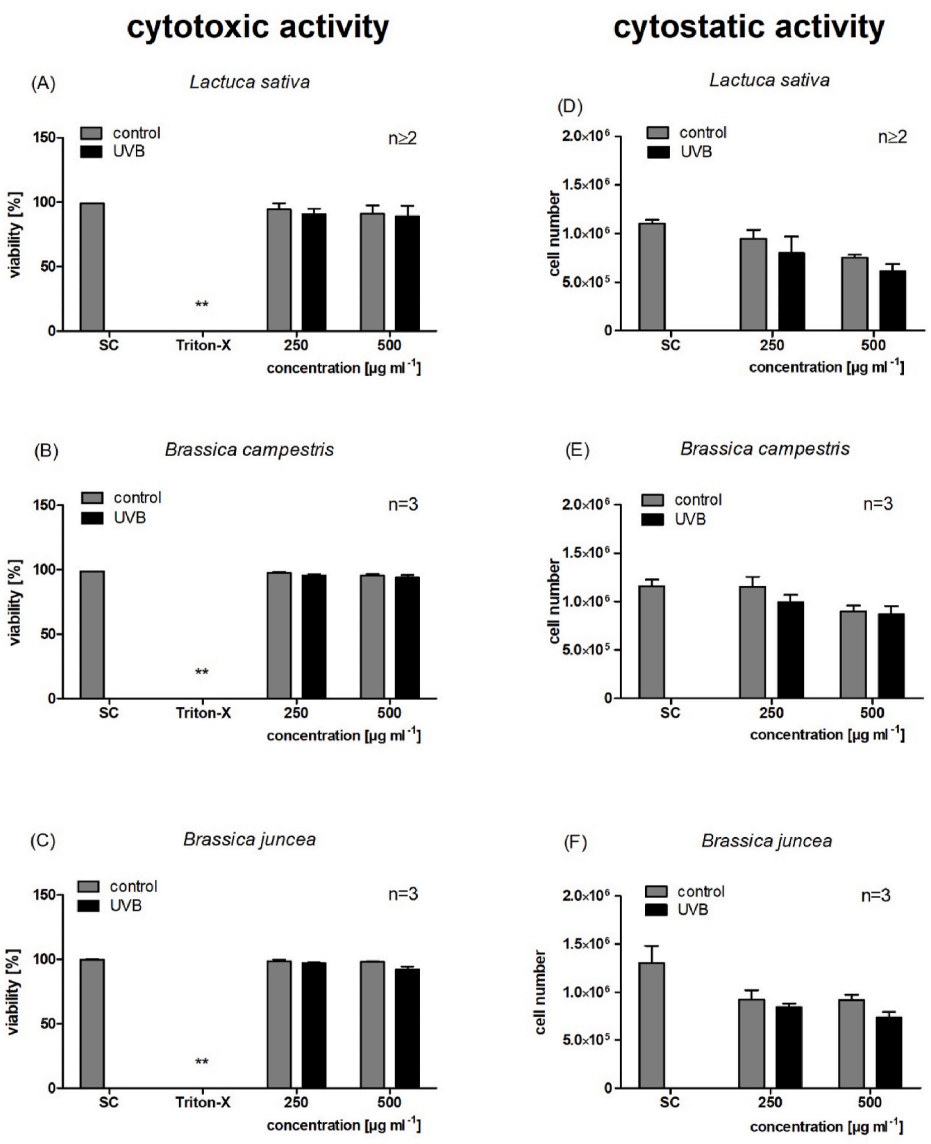

Fig. 5. Cytotoxic and cytostatic activity of aqueous extracts from untreated and UVB-treated plant material using the trypan blue dye exclusion test. HepG2 cells were exposed to extracts prepared from untreated and UVB-treated plant material from (A, D) *Lactuca sativa*, (B, D) *Brassica campestris*, and (C, F), *B. juncea* for 72 h. Means and standard deviations of independent experiments are given. Significant differences between the solvent control (SC, 0.1% PBS) and the plant extracts or positive control triton-X are marked (two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test, **, $p < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

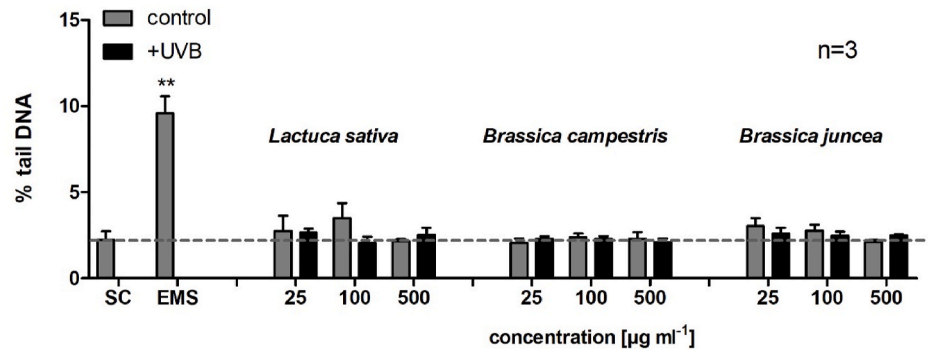

Fig. 6. Genotoxic activity was determined using the C omet assay. Cells were exposed to aqueous extracts prepared from untreated and UVB-treated plant material from *Lactuca sativa*, *Brassica campestris*, and *B. juncea.* Data are means and standard deviations of three independent experiments. Significant differences between the solvent control (SC; 0.1% PBS) and the plant extracts or the positive control ethylmethanesulfonate (EMS; 1.25 mM) are marked (two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test; **, $p < 0.01$).

ortho-dihydroxyl group of the acids. At lower concentrations (0.004–4 μM), the same hydroxycinnamic acid (caffeic acid) did not show pro-oxidant activity and was found to inhibit concentration-dependent ROS production induced by *tert*-BuOOH in a cell-based assay (Girard-Lalancette et al., 2009). Further, Kim et al. (2016) analyzed the role of quercetin-induced ROS production and found a pro-oxidant activity starting at 25 μM on human embryonic stem cells, but no effects on human dermal fibroblasts with a quercetin treatment of up to 200 μM. Moreover, for kaempferol, pro-oxidant effects were reported in different glioblastoma cell lines (LN229, U87MG, and T98G). Here, with increasing exposure time to 50 μM kaempferol, an increase in ROS production was observed compared to control cells (Sharma et al., 2007). In the present study, the highest concentration of total quercetin glycosides was 78.7 μM in the extract of *Lactuca sativa* and of total

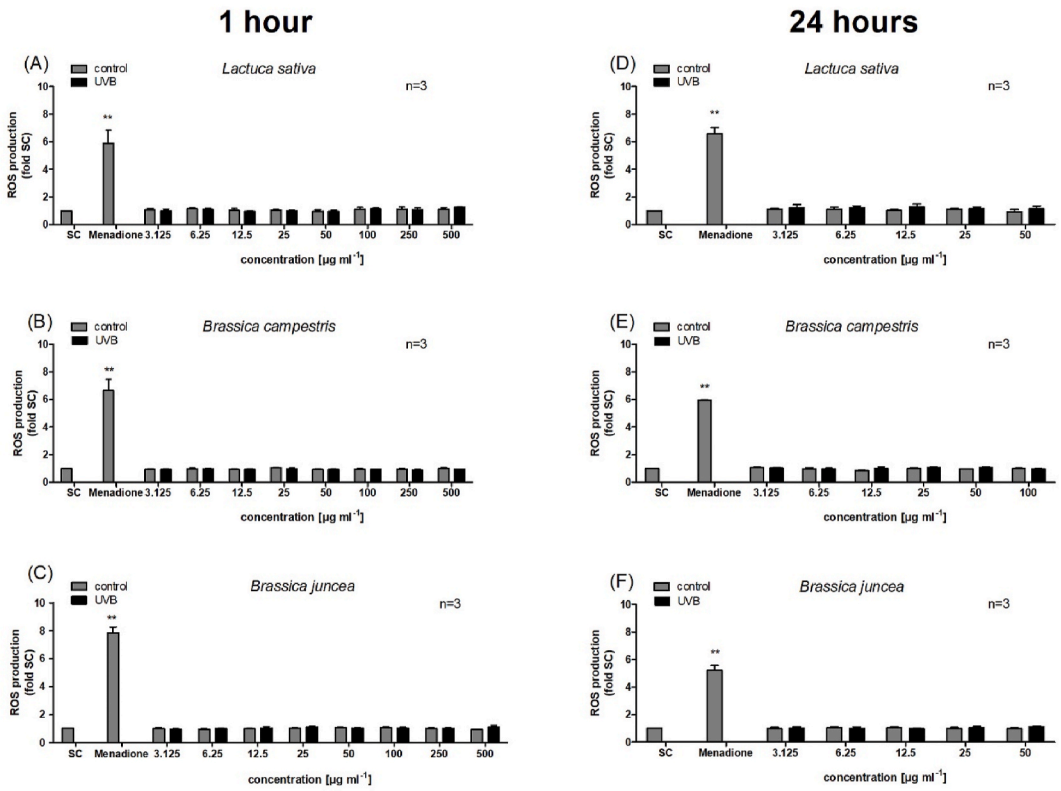

Fig. 7. ROS production was measured using electron paramagnetic resonance spectroscopy (EPR). HepG2 cells were exposed to aqueous extracts prepared from untreated and UVB-treated plant material from (A, D) *Lactuca sativa*, (B, E) *Brassica campestris*, and (C, F) *B. juncea* for 1 h (A, B, C) or 24 h (D, E, F). Data are means and standard deviations of three independent experiments. Significant differences between the solvent control (SC, 0.1% PBS) and the plant extracts are marked (two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test; **, $p < 0.01$).

kaempferol glycosides was 83.9 μM in *B. campestris* plant extracts treated with UVB without showing any pro-oxidant activities.

4. Conclusion

It is a long-held opinion among horticultural producers that UVB radiation is predominantly harmful for plants. This study has shown that treatment of plants with UVB radiation from LEDs at low doses changes individual plant secondary metabolites, like flavonoids or glucosinolates, mainly by increasing their content. The UVB radiation effect was both plant- and structure-specific. Carotenoids, anthocyanins, and quercetin glycosides acting as shielding compounds accumulated inside the vegetables. As described for the first time in this *in vitro* study, UVB-treated plants did not elicit any adverse effects on the human cell level regarding increased cytotoxicity, genotoxicity, or oxidative stress in a concentration range that is relevant for human vegetable consumption.

Thus, as soon as it is legally permissible, LEDs in greenhouses and climate chambers which emit UVB radiation can be safely used in the horticultural industry. The advantage of LED usage would be to allow more efficient vegetable production by providing a light environment that is adjusted to the needs of the plants. Finally, it is predicted that as with high-pressure sodium lamps, LED will be produced at lower costs in the future, thereby enabling energy savings and providing a more environmentally friendly lighting option.

Author statement/contributions of authors

MWR conducted the experiments, analyzed the glucosinolates, and wrote the manuscript with the support of the co-authors. SN set up the UVB experiments, analyzed the phenolic compounds, and supported writing the relevant sections. SB analyzed the carotenoids and chlorophylls and supported writing the relevant sections. MS and EL revised the manuscript. EL, JVDG, CH, and HTTT conceived, designed, performed, and analyzed the *in vitro* experiments on bioactivity and supported writing the relevant sections. SE, JG and TF planned and installed the UVB radiation modules, including programming the lighting sequence.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2021.101327.

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