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Antioxidant responses of damiana *(Turnera diffusa* Willd) to exposure to artificial ultraviolet (UV) radiation in an *in vitro* model; part I; UV-C radiation

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Abstract

Introduction: **Ultraviolet type C (UV-C) radiation has higher energy than the UV-B radiation and has been less studied because it is completely absorbed by the ozone layer. However, artificial UV-C radiation can generate diverse modifications in the plants. Given that exposure to UV-C for short periods of time increases the antioxidant content, improving the appearance and shelf-life of products, its potential application in postharvest treatments to modify the antioxidant content of medicinal plants, such as damiana *(Turnera diffusa),* is novel and relevant.**

Objective: **To determine the effects of UV-C radiation on enzymatic and non-enzymatic antioxidant defenses, as well as oxidative damage levels, in damiana *(Turnera diffusa)* plants *in vitro.***

Results: **UV-C radiation decreased superoxide dismutase (SOD, EC 1.15.1.1) and total peroxidases (POX, EC 1.11.1) activities, the concentration of chlorophylls *(a* and *b),* carotenes, vitamin C, and total antioxidant capacity. UV-C radiation increased the phenolic compound levels in damiana. Loss of antioxidant defenses was higher in damiana plants exposed to higher UV-C doses and/or for longer periods. This study suggests that UV-C radiation induces oxidative stress, evidenced as increased protein carbonyls and phenolic compound content, in damiana *(T. diffusa).***

Conclusion: **Low dose, short exposure to UV-C stimulates phenolic compound content in damiana. Thus, controlled UV-C treatments could be used as postharvest treatment to increase phenolic compound content in damiana plants.**

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Key words: *Antioxidants. Antioxidant capacity. Phenolic compounds.* Turnera diffusa. *UV-C radiation. Vitamin C.*

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RESPUESTA ANTIOXIDANTE EN DAMIANA *(TURNERA DIFFUSA* WILLD) EXPUESTA A RADIACIÓN ULTRAVIOLETA (UV) ARTIFICIAL EN UN MODELO *IN VITRO;* PARTE I; RADIACIÓN UV-C

Resumen

Introducción: **La radiación ultravioleta tipo C (UV-C) presenta mayor energía y es menos estudiada que la radiación UV-B, debido a que se considera que es totalmente absorbida por la capa de ozono. Sin embargo, la radiación UV-C artificial es capaz de generar diversas modificaciones en las plantas. Dado que la exposición a UV-C por intervalos de tiempo cortos incrementa la concentración de compuestos antioxidantes, mejorando la apariencia y vida de anaquel de los productos, su potencial aplicación en tratamientos poscosecha para modificar el contenido antioxidante de plantas medicinales, como la damiana *(Turnera diffusa),* es novedoso y relevante.**

Objetivo: **Determinar el efecto de la radiación UV-C sobre las defensas antioxidantes enzimáticas y no enzimáticas, así como en los niveles de daño oxidativo de damiana *(Turnera diffusa) in vitro.***

Resultados: **La radiación UV-C disminuyó la actividad de las enzimas superóxido dismutasa (SOD, EC 1.15.1.1) y peroxidasas totales (POX, CE 1.11.1), la concentración de clorofila *(a y b),* carotenos, vitamina C y la capacidad antioxidante total, e incrementó el contenido de compuestos fenólicos en damiana. La disminución de las defensas antioxidantes fue mayor en plantas de damiana expuestas a dosis más altas de UV-C o por períodos más largos. Estos resultados sugieren que la radiación UV-C induce estrés oxidativo, evidenciado por el incremento del contenido de carbonilos proteicos y el contenido de compuestos fenólicos en damiana *(T. diffusa).***

Conclusión: **Dosis bajas y menor exposición a UV-C estimulan la síntesis de compuestos fenólicos en damiana. Por ello, tratamientos controlados con UV-C podrían emplearse como tratamientos poscosecha para incrementar el contenido de compuestos fenólicos en plantas de damiana.**

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Palabras clave: *Antioxidantes. Capacidad antioxidante. Compuestos fenólicos. Radiación UV-C.* Turnera diffusa.

Abbreviations

DPPH: 2,2-diphenyl-2-picrylhidrazyl.
GAE: Gallic acid equivalents.
GAEAC: Gallic acid equivalent antioxidant capacity.
HPLC: High-performance liquid chromatography.
POX: Total peroxidases.
SOD: Superoxide dismutase.
TBARS: Thiobarbituric acid-reactive substances.
UV: Ultraviolet radiation.
UV-C: Ultraviolet radiation type C.

Introduction

Ultraviolet type C (UV-C) radiation produces harmful effects because it is highly energetic and can induce damage to DNA[1-2]; thus, is widely used artificially as germicide and for the conservation of food, mainly in postharvest handling and in minimally processed products, requiring only short-duration treatments[3-4]. UV-C radiation, as compared to UV-A and UV-B radiation, is considered to be totally absorbed by ozone[2,5]. The weakening of the ozone layer over the past years has generated an increase in the ultraviolet (UV) radiation levels that reach the troposphere[6]. Plants respond to increased UV radiation by modifying the antioxidant defense system to minimize oxidative damage[7]. UV-C radiation can induce modification of the antioxidant content in plants[8], mainly due to stimulation of the activity of phenylalanine ammonia-lyase (PAL), which is a precursor in the synthesis of phenolic compounds[9]; essential nutrients and the major contributors of the antioxidant potential in the human diet[10]. Therefore, UV-C radiation could be used to stimulate the production of molecules of biological and/or commercial interest, avoiding the use of genetically modified organisms[11]. The importance of including spices and/or plants products with elevated concentration of phenolic compounds in the diet and their contribution to human health have been recently reported[12].

Damiana (*Turnera diffusa*, Willd) is used in traditional medicine, as treatment of genito-urinary diseases, and as an aphrodisiac[13,14]. Despite the traditional and economic importance of this plant, knowledge of the composition and properties of bioactive molecules in damiana is scarce[15]. The potential application of exposure to UV-C as a postharvest treatment in order to increase the antioxidant content, with the possibility of the additional benefits of improving the appearance and shelf-life of medicinal plants, such as damiana, is novel and relevant. The objective of this study was to determine if exposure to UV-C radiation can be an alternative for increasing phytochemical compounds of interest in damiana (*T. diffusa*) plants in an *in vitro* model. The effect of UV-C radiation was assessed by quantifying the enzymatic and non-enzymatic antioxidant compound content, total antioxidant capacity, and oxidative damage (TBARS and protein carbonyls) levels in damiana leaves.

Materials and methods

Plant material and experimental setup

Damiana plants were introduced in tissue culture following the methodology of Alcaraz-Meléndez *et al.*[16], where Murashigee-Skoog (MS) medium culture was used. Five plantlets were used in triplicate as the experimental unit per treatment and day of irradiance. Bottles were covered with plastic and plantlets were grown for 6 weeks in 24 h photoperiod with white light at 25°C. UV-C radiation treatments were performed within a wooden box fully lined on the inside with aluminum foil. Germicidal lamps (G15T8 of 253.7 nm, The Medical Technology, Neuhäusler. A. G.) with an output of 0.38 mW cm^{-2} were used as a source of artificial UV-C radiation. The UV-C lamp was positioned and fixed at 45 cm above the cup of the plants. Treatments were: 1) control, plants grown with white light for 24 hours each day; 2) 5 min of exposure to UV-C daily; 3) 10 min of exposure to UV-C daily; 4) 20 min of exposure to UV-C daily. The rest of the time, the plantlets were under white light (24 hours). At the onset of the experiment (day zero), recently irradiated (day one), and every third day, samples were collected for up to 10 days of treatment. Samples were fixed by immersion in liquid N_2 and stored at -80°C prior to being analyzed.

Enzymatic and non-enzymatic antioxidants

Analytical methods used to obtain plant extracts and homogenates, and to quantify the antioxidant defenses in damiana plants have been described previously[15,17]. All the leaves from 5 plantlets were pooled and used for analysis; all analyses were run in triplicate. Briefly, chlorophyll *a*, chlorophyll *b*, and total carotenoid content were analyzed by the method of Lichtenthaler and Wellburn[18] and are expressed in μg g^{-1} of fresh weight. The coefficients of variability for chlorophyll *a*, chlorophyll *b*, and total carotenoid content were 5.5, 7.3, and 24.3, respectively. Superoxide dismutase (SOD, EC 1.15.1.1) activity was spectrophotometrically determined at 560 nm (Beckman Coulter DU-800, CA, USA) using the method of Suzuki[19]. One unit of SOD activity is defined as the quantity of enzyme necessary to inhibit the maximal rate of NBT reduction by 50%; the coefficient of variability for this method was 8.8. Total peroxidases (POX, EC 1.11.1) activity was detected spectrophotometrically at 420 nm[20]. One unit of POX activity is considered as the amount of enzyme required to increase absorbance by 0.1. The coefficient of variability for POX activity was 9.0. Antioxidant enzyme activities are expressed in units mg^{-1} protein.

Vitamin C (L-ascorbic acid) and vitamin E (α-tocopherol) content was quantified using high-performance liquid chromatography (HPLC, Waters, Milford, MA, USA). Chromatographic conditions and methods were

as described by Gratzfeld-Hüsgen and Schuster[21] and Soriano-Melgar *et al.*[15,17] Results were obtained by using standard curves of L-ascorbic acid (5-200 ng μL^{-1}) and α-tocopherol (5-200 ng μL^{-1}), respectively. Results are expressed as mg vitamin C g^{-1} fresh weight and mg of vitamin E g^{-1} fresh weight. The coefficients of variability for vitamin C and E were 0.26 and 4.0, respectively.

Total phenolic compound content was quantified by using the method of Singleton and Rossi[22], modified to use on a microplate reader (BioRad™, CA, USA), and using a standard curve of gallic acid (0-350 µg mL^{-1}). Results are expressed as mg gallic acid equivalents (GAE) g^{-1} fresh weight. The coefficient of variability was 3.2 for this method.

Total antioxidant capacity was determined following the method described by Brand-Williams *et al.*,[23] using the commercial free radical 2.2-diphenyl-2-picryl-hidrazyl (DPPH) and a standard curve of gallic acid (50-300 mg mL^{-1}). Total antioxidant capacity is reported as µM gallic acid equivalent antioxidant capacity (GAEAC) g^{-1} fresh weight; the coefficient of variability was 7.0.

Oxidative damage

The concentrations of thiobarbituric acid reactive substances (TBARS), as indicator of lipid peroxidation, and of protein carbonyls were analyzed as proxy for oxidative damage as previously described[15,17]. Briefly, the method of Persky *et al.*[24] was employed to quantify TBARS levels by using a standard curve of 1-bis tetraetoxipropane (TEP, 0-20 nmoles mL^{-1}); results are expressed as nmol of TBARS mg^{-1} protein. The coefficient of variability for TBARS analyses was 8.3. The content of protein carbonyls was analyzed following the method of Levine *et al.*[25], and results are expressed in µM protein carbonyls g^{-1} fresh weight. The coefficient of variability for protein carbonyls was 10.0.

Total protein content

Total protein content was used to standardize results of enzyme activities and TBARS levels, and were analyzed by the method of Bradford[26] modified for use in a microplate reader (BioRad™) as previously described[15,17]; a curve of bovine serum albumin (BSA) was included as standard. Total protein content was expressed in mg g^{-1} fresh weight. The coefficient of variability was 17.9 between days and treatments in this methodology.

Statistical analyses

Data were tested for normality and homoscedasticity of variance. Analysis of variance (ANOVA) and Tukey post-hoc tests were used to determine significant differences between days (0, 1, 4, 7 and 10 days) and treatments (control, 5, 10 and 20 min UV-C treatments). Statistical significance was considered when $p < 0.05$. All statistical analyses were run using the software Statistica 6 (StatSoft, Inc., 2001). Data are shown as mean ± standard error.

Results and Discussion

UV (UV-A, -B and -C) radiation, mainly UV-C, has been used to increase the content of bioactive molecules (flavonoids and phenolic compounds) in medicinal plants[27]. Therefore, in this study, the effect of UV-C on antioxidant compounds in damiana was determined in an *in vitro* model. Figure 1 shows the morphological conditions of plants treated with different UV-C doses for up to 10 days. Damiana plants showed immediate responses to the UV-C radiation; loss of leaves was observed after the initial exposure (day one), and on the following (4, 7 and 10) days of treatment. By the 10th day of treatments, damiana plants showed damage (chlorosis) in leaves; the most extensive damage was observed in the plants exposed to UV-C radiation for 20 min day^{-1} (fig. 1). Exposure to UV-C can generate damage to chloroplasts, produce premature senescence, and growth reduction[1,28-29]. In damiana plants irradiated with UV-C no growth of new leaves and signs of senescence in mature leaves were observed. Chlorophyll *a* content significantly decreased in damiana plants exposed to UV-C radiation for 5 and 20 min day^{-1} relative to control plants ($p = 0.001$, table I). Chlorophyll *b* and carotenoid content significantly decreased in damiana treated with UV-C radiation for 20 min day^{-1} when compared to control plants ($p = 0.001$ and $p = 0.035$, respectively; table I). Similar reduction in chlorophyll *a* and *b* were observed by Najeeb *et al*.[1] in shoots of mat rush (*Juncus effusus* L .) at different exposure times (15, 30 and 45 min) of UV-C radiation. Maharaj *et al.*[4] observed a significant increase in carotenoid content in tomato fruits in postharvest treat-

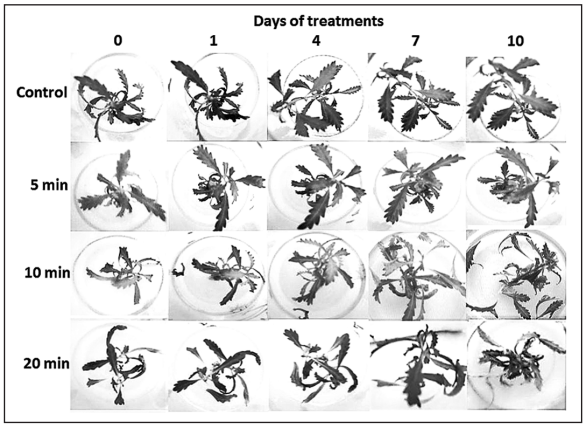

Fig. 1.—Appearance of damiana (Turnera diffusa) *plants irradiated* in vitro *with ultraviolet type-C (UV-C; 5, 10 and 20 min day^{-1}) and control (white light) for 10 days.*

Table I

Content of photosynthetic pigments, chlorophyll a (μg g^{-1} fresh weight), chlorophyll b (μg^{-1} fresh weight), and carotenoids (μg g^{-1} fresh weight) in leaves of damiana (Turnera diffusa) *plants irradiated* in vitro *with ultraviolet type-C (UV-C; 5, 10 and 20 min day^{-1}) for 10 days*

Treatment	*Chlorophyll a (μg g^{-1} FW)*					*Chlorophyll b (μg g^{-1} FW)*					*Carotenoids (μg g^{-1} FW)*				
Day	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*
Control	277.09±7.44a*	277.09±7.44a*	323.42±17.68a*	347.76±7.62a*	331.42±8.22a*	276.16±6.55a*	276.16±6.55a*	332.65±20.60a*	318.54±2.97a*	327.28±10.86a*	36.59±3.02a*	36.59±3.02a*	44.60±8.69a*	51.16±3.06a*	47.04±4.44a*
5 min UV-C	277.09±7.44a*,**	248.14±4.83a*,**	325.30±10.15a*,**	212.47±0.89b*,**	290.31±1.82b*,**	276.16±6.55a*,**	255.28±3.40a*,**	340.970±43.00a*,**	233.52±5.41b*,**	297.15±5.70b*,**	36.59±3.02a*,**	33.71±2.08a*,**	57.80±17.06a*,**	19.88±1.88b*,**	36.20±2.51a*,**
10 min UV-C	277.09±7.44a*,**	239.74±6.70a*,**	318.15±17.83a*,**	254.90±3.97c*,**	366.63±15.28a*,**	276.16±6.55a*	252.84±5.99a*	314.32±20.16a*	314.62±29.10a*	357.91±21.41ab*	36.59±3.02a*,**	30.66±3.64a*,**	45.02±9.49a*,**	22.70±8.55b*,**	48.63±8.36a*,**
20 min UV-C	277.09±7.44a*,**	253.07±12.62a*,**	269.11±7.91a*,**	228.11±12.58bc*,**	103.71±2.61c*,**	276.16±6.55a*,**	264.23±12.01a*,**	277.97±11.65a*,**	262.42±15.49ab*,**	102.84±2.25c*,**	36.59±3.02a*,**	33.09±7.15a*,**	35.91±3.12a*,**	24.17±6.51b*,**	13.23±1.40b*,**
• p value (day)	—	0.06	0.22	<0.001	<0.001	—	0.20	0.39	0.018	<0.001	—	0.82	0.57	0.01	0.003
• p value (treatment)			<0.001					0.001					0.035		

Day one is considered as newly irradiated.
Data are shown as mean ± standard error of a sample of n = 5; all analyses were run in triplicate.
• ANOVA and Tukey's post-hoc tests were applied to probe for differences between treatments.
Different letters indicate significant differences between days within each treatment; * denotes significant differences between treatments; p < 0.05.

ments with UV-C radiation in comparison with non-irradiated fruits. It is possible that different plant tissues have a specific capacity to react to UV-C radiation, and the pigment concentration in each tissue may reflect its potential for protection against UV radiation damage. A decrease or loss of photosynthetic compounds might be due to UV-C radiation being too severe[6].

Protein content in plants decreases in response to UV-C radiation[1]. Thus, the specific activity of antioxidant enzymes was determined in damiana plants exposed *in vitro* to UV-C radiation. No significant differences were found in SOD activity of damiana leaves between UV-C irradiated and control plants ($p = 0.078$, table II). Total POX activity significantly decreased in damiana plants irradiated with UV-C compared to control plants ($p = 0.003$, table II). In contrast, in shoots of mat rush (*Juncus effusus* L.) activity of SOD, POX and ascorbate peroxidase (APX) increased in response to UV-C radiation for 15 and 30 min, and antioxidant enzyme activities decreased at 45 min, at the same time when the protein content decreased[1]. In pepper (*Capsicum annuum* L.) leaves and roots activities of antioxidant enzymes, such as SOD, increased in response to UV-C radiation (5.7 W m^{-2} for 27 min per day during 14 days)[30]. In strawberry fruits irradiated with UV-C (0.25, 0.5 and 0.75 kJ m^{-2}) in postharvest treatments, SOD activity decreased[31]. Tang *et al.*[32] suggest that changes in antioxidant enzyme activities may be involved in the oxidative stress induced by UV-C exposure.

UV-C radiation did not have an effect on vitamin C (ascorbic acid equivalent) content in damiana plants throughout the experimental period ($p = 0.911$, table II). However, vitamin C content had a tendency to decrease (up to 40%) in both irradiated and control plants (table II), but no significant differences ($p = 0.911$) were observed between treatments. Artés *et al*.[33] reported similar results in minimally processed watermelon cubes irradiated with UV-C at different doses (1.6, 2.8, 4.8 and 7.2 kJ m^{-2}), as well as a decline of vitamin C content throughout the storage period[33].

In this study, higher vitamin E (α-tocopherol equivalent) content was observed in leaves of damiana irradiated with UV-C for 20 min day^{-1} than in controls ($p = 0.048$, table II). Given its solubility in lipids and its antioxidant properties, vitamin E contributes to the protection of membranes[34]. The increased vitamin E content in damiana plants exposed to UV-C radiation may provide protection against the oxidative damage potentially induced by UV-C radiation.

The phenolic compounds have beneficial effects in the organism and are present in spices and vegetables[10,12]. Wang *et al.*[8] reported in blueberry (*Vaccinium corymbosum* L.) fruits that UV-C doses significantly increased phenolic content compared to control (non-irradiated) fruits. Phenolic compound content in damiana increased immediately upon exposure to UV-C radiation for 5 min day^{-1} ($p < 0.001$), and in all UV-C treatments by day 7 ($p < 0.001$, table III). However, on

Table II

Enzymatic and non-enzymatic oxidant defenses, activity of superoxide dismutase (SOD, U mg^{-1} protein) and total peroxidase (POX, U mg^{-1} protein); vitamin C content (mg ascorbic acid g^{-1} fresh weight), and vitamin E concentration (mg α-tocopherol g^{-1} fresh weight) in leaves of damiana (Turnera diffusa) *plants irradiated in vitro with ultraviolet type-C (UV-C, 5, 10 and 20 min day^{-1}) for 10 days*

Treatment	*SOD (U mg^{-1} protein)*					*POX (U mg^{-1} protein)*					*Vitamin C (mg ascorbic acid g^{-1} FW)*					*Vitamin E (mg α-tocopherol g^{-1} FW)*				
Day	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*
Control	9.49 ±0.26a	9.49 ±0.26b	4.26 ±0.36a	14.81 ±0.35a	7.99 ±0.54a	0.32 ±0.02a*	0.32 ±0.02a*	0.134±0.01b*	0.44 ±0.01a*	0.20 ±0.01b*	1.11 ±0.03a	1.11 ±0.03a	0.76 ±0.02b	0.76 ±0.03a	0.64 ±0.03b	0.83 ±0.09a**	0.83 ±0.09a**	0.08 ±0.02a**	0.83 ±0.21a**	0.04 ±0.01c**
5 min UV-C	9.49 ±0.26a	12.94 ±0.25a	5.234 ±0.14a	8.50 ±0.46b	7.72 ±0.10ab	0.32 ±0.02a*,**	0.26 ±0.07b*,**	0.185±0.07a*,**	0.25 ±0.01b*,**	0.22 ±0.02a*,**	1.11 ±0.04a	1.27 ±0.21a	0.79 ±0.01b	0. 70 ±0.02a	0.78 ±0.03a	0.83 ±0.09a*,**	0.90 ±0.36a*,**	0.92 ±0.43a*,**	1.01 ±0.15a*,**	0.16 ±0.02c*,**
10 min UV-C	9.49 ±0.26a	9.39 ±0.24b	4.689 ±0.28a	8.49 ±0.15b	6.34 ±0.25b	0.32 ±0.02a*,**	0.15 ±0.01c*,**	0.17 ±0.02a*,**	0.24 ±0.01c*,**	0.24 ±0.01a*,**	1.11 ±0.03a	1.21 ±0.01a	0. 77 ±0.01b	0.69 ±0.05a	0.66 ±0.01b	0.83 ±0.09a*,**	0.25 ±0.01b*,**	1.20 ±0.09a*,**	0.87 ±0.34a*,**	1.16 ±0.16a*,**
20 min UV-C	9.49 ±0.26a	6.46 ±0.76c	4.72 ±0.26a	7.78 ±0.35b	6.83 ±0.29ab	0.32 ±0.02a**	0.16 ±0.01c**	0.13 ±0.01b**	0.24 ±0.02c**	0.134 ±0.02c**	1.11 ±0.03a	0.92 ±0.03a	0.99 ±0.02b	0.67 ±0.05a	0.72 ±0.01ab	0.83 ±0.09a*	1.16 ±0.04a*	1.19 ±0.43a*	0.93 ±0.09a*	0.67 ±0.25b*
• p value (day)	—	<0.001	0.17	<0.001	0.02	—	<0.001	<0.001	<0.001	<0.001	—	0.21	<0.001	0.35	<0.001	—	0.04	0.09	0.93	0.001
• p value (treatment)			0.078					0.003					0.911					0.048		

Day one is considered as newly irradiated.
Data are shown as mean ± standard error of a sample of n=5; all samples were analyzed in triplicate.
• ANOVA and Tukey's post-hoc tests were applied to probe for differences between treatments.
Different letters indicate significant differences between days of treatments; *denotes significant differences between treatments; $p < 0.05$.

Table III

Phenollic compound content (mg gallic acid g^{-1} fresch weight), total antioxidant capacity (mM gallic acid g^{-1} fresch weight) and oxidative damage (lipid peroxidation (nM TBARS g^{-1} protein) and protein carbonyls (μM protein g^{-1} fresch weight)) in leaves of damiana (Turnera diffusa) *plants irradited* in vitro *with ultraviolet type-C (UV-C; 5, 10 and 20 min day^{-1}) for 10 days*

Treatment	*Phenolic compounds (mg gallic acid g^{-1} FW)*					*Antioxidant Capacity (mM fg allic acid g^{-1} FW)*					*Lipid Peroxidation (nM TBARS g^{-1} protein)*					*Protein Carbonyls (μM protein g^{-1} FW)*				
Days	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*	*0*	*1*	*4*	*7*	*10*
Control	9.49 ±0.26a	9.49 ±0.26b	4.26 ±0.36a	14.81 ±0.35a	7.99 ±0.54a	0.32 ±0.02a*	0.32 ±0.02a*	0.134±0.01b*	0.44 ±0.01a*	0.20 ±0.01b*	1.11 ±0.03a	1.11 ±0.03a	0.76 ±0.02b	0.76 ±0.03a	0.64 ±0.03b	0.83 ±0.09a**	0.83 ±0.09a**	0.08 ±0.02a**	0.83 ±0.21a**	0.04 ±0.01c**
Control	12.04 ±0.13a**	12.04 ±0.13b**	12.45 ±0.36b**	9.19 ±0.40c**	10.85 ±1.27b**	330.43 ±5.37a*	330.43 ±5.37a*	366.82 ±9.70ab*	448.38 ±7.04a*	440.63 ±5.50b*	213.13 ±10.89a	213.13 ±10.89ab	155.02 ±2.07b	181.95 ±10.12a	169.56 ±8.58a	494.40 ±31.05a	494.40 ±31.05a	281.96 ±2.55b	170.00 ±16.61b	194.57 ±26.24c
5 min UV-C	12.04 ±0.13a*	29.59 ±1.08a*	13.17 ±0.39b*	30.29 ±2.23b*	37.69 ±4.50a*	330.43 ±5.37a**	265.39 ±2.60b**	332.57 ±11.43b**	295.80 ±10.52c**	349.70 ±12.91c**	213.13 ±10.89a	245.77 ±3.71a	74.75 ±5.27c	198.33 ±10.65a	174.90 ±13.29a	494.40 ±31.05a	241.33 ±17.88b	315.57 ±20.09b	236.31 ±13.98a	337.99 ±16.84ab
10 min UV-C	12.04 ±0.13a*	14.82 ±1.47b*	10.83 ±1.35b*	33.11 ±1.12b*	41.07 ±1.76a*	330.43±5.37a*,**	227.81 ±1.76c*,**	352.20 ±6.45ab*,**	393.01 ±9.79b*,**	432.09 ±5.27b*,**	213.13 ±10.89a	145.36 ±8.61c	218.08 ±11.53a	165.56 ±7.93a	172.42 ±8.11a	494.40 ±31.05a	206.19 ±31.06b	374.63 ±10.53ab	275.17 ±8.01a	271.03 ±9.47bc
20 min UV-C	12.04 ±0.13a*	12.70 ±1.38b*	36.01 ±1.96a*	41.24 ±0.42a*	17.04 ±1.01b*	330.43 ±5.37a*	345.12±5.85a*	384.42 ±9.67a*	383.54 ±13.25b*	501.72 ±21.14a*	213.13 ±10.89a	194.20 ±8.71b	206.33 ±3.30a	183.09 ±1.83a	173.82 ±3.90a	494.40 ±31.05a	266.92 ±26.38b	425.58 ±40.77a	222.30 ±5.84ab	355.66 ±10.11a
• p value (day)	—	<0.001	<0.001	<0.001	<0.001	—	<0.001	0.014	<0.001	<0.001	—	<0.001	<0.001	0.129	0.977	—	<0.001	0.0107	0.0017	<0.001
• p value (treatment)			<0.001					0.0001					0.763					0.743		

Day one is considered as newly irradiated.
Data are shown as mean ± standard error of a sample of n=5; all samples were analyzed in triplicate.
•ANOVA and Tukey's post-hoc tests were applied to probe for differences between treatments.
Different letters indicate significant differences between days of treatments; * denotes significant differences between treatments; $p < 0,05$.

the 10th day of exposure to UV-C radiation for 20 min day^{-1} phenolic compound content decreased significantly ($p < 0.001$, table III). These results suggest that while low, short exposure doses stimulate, excessive UV-C radiation reduces phenolic compound content in damiana. Therefore, it is important to assess the appropriate UV-C dosage in order to maximize the content of phenolic compounds in plants; it has been suggested that obtaining compounds such as carotenoids, vitamin C and vitamin E in the human diet is preferred to intake of supplements[10].

The DPPH radical is usually employed to analyze the total antioxidant capacity of vegetables[12]. In damiana, total antioxidant capacity decreased significantly ($p < 0.001$) on day one in plants treated for 5 and 10 min day^{-1} with UV-C radiation (table III). By the 7th day of treatment, plants irradiated with UV-C (for 5, 10 and 20 min day^{-1}) had lower antioxidant capacity than control plants ($p < 0.001$, table III). This may be the product of the combined decreases in non-enzymatic (photosynthetic pigments and carotenoids, table I) and enzymatic antioxidants (SOD and POX activities, table II) observed in UV-C irradiated damiana.

No significant differences in TBARS levels were found in damiana between treatments and control ($p = 0.763$) throughout the exposure period (table III). This could be due to the simultaneous increase in vitamin E levels in UV-C irradiated plants (table II). However, higher protein carbonyl content was observed on the 7th and 10th days in UV-C treated damiana plants in comparison with control plants ($p = 0.0017$ and $p < 0.001$, respectively; table III). Protein carbonyl levels are an indicator of damage to proteins due to oxidative stress[35]. These results suggest that antioxidant defenses in damiana plants are sufficient to curtail oxidative damage to lipids but not to proteins.

UV-C radiation increased total phenolic compound and vitamin E content in damiana plants. The time course of the observed effects of UV-C radiation in damiana plants suggests that the strongest responses occur immediately upon exposure, and that the effects may lessen with time. Similar results were reported by Wang *et al.*[8] in blueberry fruits exposed to UV-C radiation, and Ribeiro *et al.*[35] mentioned that the UV-C effects depend on the dosage. Therefore, exposure to controlled, mild levels of UV-C radiation may be an alternative to increase the content of antioxidants and other phytochemicals in damiana plants, as suggested by Jacobo-Velázquez and Cisneros-Zevallos[11] and Ribeiro *et al.*[35].

Conclusion

UV-C radiation had differential effects on the antioxidant defenses of damiana (*T. diffusa*) plants *in vitro,* depending on the time of the exposure. Short-term exposure increased vitamin E and phenolic compound content in damiana. Results suggest that UV-C treatment, when appropriately controlled, can be used as an alternative mechanism to increase the content of antioxidants and other phytochemicals in damiana plants.

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