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# PHENOLIC COMPOUNDS INCREASE THEIR CONCENTRATION IN CARICA PAPAYA LEAVES UNDER DROUGHT STRESS

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**Abstract**

*Carica papaya* L. cv. Maradol is a tropical plant with high commercial value due to its consumption and high nutritional value. Recent studies have corroborated a great diversity of biological activities in extracts from different tissues of the plant that seem to be caused by the presence of phenolic compounds. In this study, the effect of drought stress on the contents of phenolic compounds and the antioxidant capacities of aqueous extracts of papaya leaves were studied. Results show drought stress in plants increased their antioxidant capacity and the content and diversity of phenolic compounds. Several phenolics were identified by high-performance liquid chromatography with photodiode array coupled to electrospray quadrupole time-of-flight mass spectrometry and some of them were exclusively detected in papaya leaves under drought stress. Since this is the first report of the drought stress influence on the accumulation of phenolic compounds in leaves from papaya plants, this research opens many perspectives for obtaining a greater quantity and diversity of phenolics from vegetal tissues under abiotic stress conditions that could be exploited in food, cosmetic and pharmaceutical industries.

**Keywords**: *Carica papaya*; chromatographic profile; drought stress; mass spectrometry; phenolic compounds.

## *Introduction*

*Carica papaya* is a fruiting plant distributed in several tropical countries. It is extensively cultivated since its fruit is known for its high nutritional value (Chan 2009). Different medicinal properties of different papaya tissues have been reported: unripe green fruits: wound healing and abortifacient activity (Anuar et al. 2008); leaves: treatment of asthma and as abortifacient (Krishna et al. 2008); fruits and seeds: anthelminthic and antiamebic activities (Okeniyi et al. 2007).

Phenolic compounds or polyphenols are a unique group of phytochemicals found in many plants and present in fruits, leaves, and other different parts of the plant. Their antioxidant capacity is based on their functional groups which are capable of accepting the negative charges of free radicals and are widely studied due to their benefit for human health and treatment of diseases (Manach et al. 2004). Extracts of different plants with high phenolic content have been in high demand in the food industry because they delay oxidative decomposition of lipids and thus enhance the nutritional value of food (Kähkönen et al. 1999). This has led to a particular interest in environmental factors that modify phenolic concentration at their origin source within the plant as well as in the methodologies to isolate, analyze and identify them (Watson 2014). Recent studies on the identification of phenolic compounds present in extracts from papaya leaf tissues have determined the presence of different phenolic compounds using separation techniques such as HPLC coupled to mass spectrometry (HPLC-MS) and gas chromatography mass spectrometry (GC-MS) (Canini et al. 2007; Rivera-Pastrana et al. 2010).

Fields of plants cultivated for commercial interest are exposed to many abiotic stresses, such as salinity and drought (Vinocur and Altman 2005). Drought stress is the status in which the quantity of available water at the root zone of the plant is lower than that which is needed to

keep its optimum development and productivity (Deikman et al. 2012). As drought stress increases the formation of reactive oxygen species, plants counteract this condition by increasing the production of antioxidant molecules such as phenolic compounds (Król et al. 2014). Stomata are specially designed cells which react to external and endogenous signals and modify their form to enable gas exchange. The movement occurs as a consequence of increasing osmotic potential and turgor pressure (Croxdale 2007). In water dehydration stress conditions, partial or total stomatal closure enables plants to maintain a suitable water balance while restricting the carbon intake (Agurla et al. 2018). Hence the concentration of $CO_2$ inside the leaves decreases, and much less NADPH + $H^+$ and ATP are utilized for $CO_2$ fixation in the Calvin cycle, causing a substantial excess of NADPH + $H^+$. Plants under drought stress conditions amass greater concentrations of highly reduced compounds, for example, terpenoids, phenolics, or alkaloids (Selmar and Kleinwächter 2013; Kleinwächter and Selmar 2015).

The papaya plant is considered to be relatively durable as it relates to drought stress and exhibits responses classified as dehydration postmortem (Marler 2000; Mahouachi et al. 2006). In spite of the existence of some studies carried out in different tissues of papaya plants, most of the research focuses on the identification of phenolic compounds and their biological activities. To date, there have been no reports about the effects of drought stress on the diversity and concentration of phenolic compounds in papaya leaves as a possible defense mechanism of the papaya plant. The aim of this study was to evaluate the effects that drought stress can have on the variety and total content of phenolic compounds present in extracts from papaya leaves.

***Materials and methods***

*Chemical and reagents*

All the chemicals were of analytical grade. Methanol and ethanol of HPLC grade were purchased from Scharlab Chemie (Barcelona, Spain). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), potassium persulfate, ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and proline were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol and formic acid of HPLC-MS grade were supplied by Fisher Scientific (Leicesthershire, UK). Ultrapure water (18.2 MΩ/cm) was obtained from a Milli-Q instrument (Millipore, Billerica, MA, USA). Sulphosalycilic acid, glacial acetic acid and phosphoric acid were purchased from Fermont (Monterrey, Mexico). Ninhidryn was obtained from Applichem (Darmstadt, Germany). Toluene was supplied by Reproquifin (Estado de Mexico, Mexico).

*Plant material*

*Carica papaya* L. cv. Maradol seeds (Clontech) were sown in pots containing solid medium of Sunshine Blend Seedling Mix, Peat Moss (2:1, w/w) until they reached the age of three months. Plants were grown under glasshouse conditions: temperature of 38 °C, humidity of 40-80% and a photoperiod of 12 h light/12 h dark (Figueroa-Yañez et al. 2016; Gamboa-Tuz et al. 2018).

Afterwards, plants were transferred to a room with controlled conditions: temperature of 25 °C, humidity of 40-80% and a photoperiod of 12 h light/12 h dark with a photon flux density of 200 μmol $m^{-2}$ $s^{-1}$ (Arroyo-Herrera et al. 2016). The drought stress experiment (which consisted on the lack of irrigation upon a time period) started after two weeks of acclimation. All plants were watered at the beginning of the drought experiment. Leaves samples were collected after a water deficit period of 0 and 14 days (0DC and 14DD, respectively). The

drought stress experiment was carried out in three different plants. Also, three watered plants were used as control samples and their leaves were collected at the 14$^{th}$ day of the experiment (14DC). The samples were immediately frozen in liquid nitrogen and stored at −80 °C until freeze-drying (FreeZone® 4.5 Liter Freeze Dry Systems, Kansas City, USA). Samples were grounded and stored at −80 °C until analysis.

*Relative water content and osmotic potential*

Relative water content (RWC) was carried out as described by Hameed et al. (2015). Osmotic potential (Ψs) was carried out as described by of Cach-Pérez et al. (2018). Osmotic pressure of papaya leaves was calculated using van't Hoff equation on osmolarity measured using a vapor pressure osmometer (Vapro-5600, Wescor, Logan, UT, USA).

*Photosynthesis and transpiration rate*

Net photosynthetic rate (A) and transpiration rate (T) were measured employing a portable infrared gas analyzer (Li-6400XT, Licor, Lincoln, NE, USA) with an adaptable leaf chamber of 6 $cm^2$. The third larger leaf of each plant was selected to carry out the measurements. The internal $CO_2$ flow was of 400 µmol $mol^{-1}$ and the light source was of 300 µmol $m^{-2}s^{-1}$.

*Proline quantification*

Proline quantification was carried out as described by Bates et al. (1973). Proline content was measured using a proline standard curve. Results were expressed as µg of proline per gram of D.W. leaf sample.

*Scanning electron microscopy*

The phenotypic analysis of the effect of drought stress on stomatal closure in samples from papaya leaves was carried out as follows: square (1 $cm^2$) leave samples were fixed using a 2.5% glutaraldehyde solution diluted in buffer solution (0.2 M sodium phosphate, pH 7.2) for two days. Samples were kept in the dark at 4 °C. They were then rinsed two times with

buffer solution and were subsequently dehydrated with consecutive washes of ethanol:water solutions at different concentrations (30:70, 50:50, 70:70, 85:15, 96:4 % (v/v) and absolute ethanol) stored in the dark at 4 °C. Each wash was realized twice. Immediately after dehydration process, a critical point drying process was performed at 1072 psi/31 °C (Samdri1-795 Tousimis Rockville, MD, USA). Samples were subsequently added to metallic stubs with carbon conductive adhesive tape (Electron Microscopy Science) and sputter coated with a 150 Å gold layer (Denton Vaccum Desk II, USA). Stomatal aperture percentage was calculated for the abaxial epidermis at a magnification of 200× (0.1213 $mm^2$). Length of the guard cells was also measured in five stomata per field. Counts and measurements were done in ten fields of each leaf. Sample analysis and image recording were made employing a Scanning Electron Microscope (Jeol, JSM-6360LV, Japan).

*Ultrasound-assisted extraction of phenolic compounds*

A previous standardization process was performed to determine the best extraction conditions to obtain the maximum concentration of phenolic compounds from papaya leaves. Control leaves samples 0DC were selected as test samples for standardization process. The extractions of phenolic compounds from the different freeze-dried leaves of papaya were performed on a high intensity focused ultrasound probe (model VCX130, Sonics Vibra-Cell, Hartford, CT, USA). Aliquots of freeze-dried leaves (20 mg) were transferred into microcentrifuge tubes containing 1 mL of water each. The samples were placed into an ice bath and sonicated for 10 or 20 min at three different amplitude waves of 30%, 50% and 70%. After sonication, samples were centrifuged twice (13,000 rpm at 4 °C for 15 min) and the supernatant was removed. The extracts were evaporated to dryness using a concentrator (Concentrator Plus, Eppendorf, Hamburg, Germany) for at least 4 h at 30 °C. The dried extracts were dissolved in water at a concentration of 10 mg/mL prior to their analysis by

HPLC-DAD. The number of signals, the size of the peak areas and the resolution of the different chromatograms were compared as selection criteria. Water was chosen as the best extraction solvent in comparison to several hydroalcoholic mixtures through a series of HPLC analysis of the corresponding profiles (data not shown). The extraction process of all papaya leaves samples was subsequently carried out.

*DPPH radical scavenging assay*

The antioxidant capacity of the different kinds of leaves extracts was measured with the DPPH radical scavenging assay according to Plaza et al. (2014). The absorbance was measured at 516 nm in a UV-vis spectrophotometer (Agilent Cary 8454, Agilent Technologies, Santa Clara, CA, USA). The DPPH-methanol solution was employed as a reference. The remaining DPPH concentration in the reaction medium was determined from a calibration curve. The percentage of remaining DPPH against the extract concentration (μg of dry weight (D.W.) extract $mL^{-1}$) was then plotted to calculate the amount of antioxidant necessary to reduce the initial DPPH concentration by 50% or $EC_{50}$. Therefore, the lower $EC_{50}$, the higher antioxidant capacity.

*Trolox equivalent antioxidant capacity (TEAC) assay*

The TEAC assay described by Plaza et al. (2014) was employed. The absorbance of each sample was measured using a UV-Vis spectrophotometer (Agilent Cary 8454) at 734 nm. The reference standard was Trolox and results were expressed as TEAC values (mmol Trolox per g D.W. extract). These values were acquired from four different concentrations (ranging from 0.5 to 2.5 mg $mL^{-1}$) of each extract in order to give a linear response between 20 and 80% of the initial absorbance.

*Analysis of phenolic compounds by HPLC-DAD*

The analysis of phenolic compounds was carried out employing a 1100 HPLC system from Agilent (Agilent Technologies, Santa Clara, CA, USA), by using a previously published method with some modifications (Plaza et al. 2016). The HPLC instrument was equipped with an online degasser, a quaternary solvent pump, an auto-sampler, a column heater compartment and a photodiode array detector (DAD) with scanning capabilities, all controlled by the ChemStation (Agilent) software. The detection wavelengths used were 250, 280, 350, and 520 nm. Separation was performed using a porous-shell fused-core Ascentis Express C18 analytical column (150 × 2.1 mm, 2.7 μm) with an Ascentis Express C18 guard column (0.5 cm × 2.1 mm, 2.7 μm), both from Supelco (Bellefonte, PA, USA). The flow rate was 250 μL $min^{-1}$ and the column temperature 50 ºC. Two microliters of extract were injected. The mobile phases were (A) water with 0.5 % formic acid and (B) methanol with 0.5% formic acid. The gradient analysis was as follows: 0 min, 5% (B); 0-5 min, 5% (B); 5-35 min, 45% (B); 35-45 min, 45 % (B); with 10 min of post time. Each extract was injected in duplicate.

*Identification of phenolic compounds by HPLC-ESI-QTOF-MS*

The structural elucidation of phenolic compounds was achieved by a 1100 HPLC system from Agilent (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole-time-of flight mass spectrometer (QTOF-MS) Agilent 6530 equipped with an orthogonal electrospray ionization (ESI) source (Agilent Jet Stream, AJS). Agilent Mass Hunter Workstation software B.07.00 from Agilent was used for HPLC and MS control, data acquisition, and data analysis. The chromatographic conditions were the same as stated in section “Analysis of phenolic compounds by HPLC-DAD”.

The mass spectrometer was operated in negative and positive ion modes and the mass range was from 100 to 1700 m/z. MS parameters were: capillary voltage, 3500 V; drying gas flow rate, 12 L $min^{-1}$; nebulizer pressure, 50 psig; and gas temperature, 350 ºC. The fragmentor voltage was set at 80 V. The skimmer voltage was 60 V while octapole voltage was 750 V. Source sheath gas temperature and flow were 400 ºC and 12 L $min^{-1}$, respectively. MS/MS was performed employing the auto mode and the following optimized conditions; 2 precursors per cycle, dynamic exclusion after two spectra (released after 1 min), and collision energy of 5 V for every 100 Da. Internal mass calibration of the instrument was performed using an AJS ESI source with an automated calibrant delivery system. The reference compound solution for internal mass calibration containing purine and HP-0921 (hexakis(1H,1H,3H-tetrafluoropropoxy) phosphazine) in acetonitrile-water (90:10, v/v) (4 μM and 2.5 μM, respectively, 15 μL $min^{-1}$) from Agilent was used, *m/z* 121.0509 and *m/z* 922.0098, respectively.

*Statistical analysis*

All statistical analyses were performed using the GraphPad Prism 7.0 software (GraphPad Software, La Jolla California USA, www.graphpad.com). The collections of tissue samples as well as the measurements of physiological parameters were repeated at least three times in different individuals in each of the different experiments. All analyses were performed in triplicate. The means of most experiments were compared using one-way analysis of variance (ANOVA) except for the data obtained from the HPLC-DAD analysis that was analyzed using a two-way ANOVA. Significant differences among the treatment groups were determined with Dunnett's test ($p \leq 0.05$).

***Results and discussion***

*Drought stress characterization: net photosynthetic rate, transpiration rate, and proline content*

Samples were collected from the third or fourth youngest leaf. The effects of drought stress on the phenotype of *C. papaya* plants are illustrated in Fig. 1. Watered plants had turgid young dark green leaves with rigid petioles at the beginning of the experiment and little turgid old yellow-green leaves with fallen petioles at the end of the trial. As the drought stress experiment advanced, the younger leaves became flaccid and turned yellowish while older leaves wrinkled, dried and turned brown as result of water deficit. The RWC, osmotic pressure, A values, T values, and the content of free proline in leaves were used as physiological related parameters to analyze the effects of drought stress (Ashraf and Foolad 2007; Mahouachi et al. 2012).

Relative water content (RWC) is an important parameter to assess plant water status under drought stress conditions because it represents the equilibrium between water supply to the tissue and transpiration rate (Lugojan and Ciulca 2011). The means of RWC of the samples 0DC (81.56 ± 3.11 %) and 14DC (82.31 ± 3.51 %) did not show significant differences ($p \geq 0.05$). Oppositely, the mean RWC values of 14DD samples (51.33 μmol ± 3.51 %) decreased significantly (Fig. 2a). Osmotic adjustment is an essential mechanism for improving plant resistance to drought stress which results in a lower osmotic potential through the increase of intracellular solutes such as amino acids, betaines and sugars (Bajji et al. 2001). The means of Ψs of the samples 0DC (−0.47 ± 0.02 MPa) and 14DC (−0.46 ± 0.02 MPa) did not show significant differences ($p \geq 0.05$). The mean Ψs values of 14DD samples (−0.69 μmol ± 0.03 MPa) decreased significantly (Fig. 2b).

During stress conditions, photosynthesis rate in plants decreases due to stomatal closure to avoid water loss and the entry of $CO_2$ is reduced (Li et al. 2017). The means of A of the

samples 0DC (3.07 ± 0.10 µmol $CO_2m^{-2}s^{-1}$) and 14DC (2.73 ± 0.21 µmol $CO_2m^{-2}s^{-1}$) did not show significant differences ($p \geq 0.05$). By contrast, the mean A values of 14DD samples (−1.81 µmol ± 0.31 $CO_2m^{-2}s^{-1}$) decreased significantly (Fig. 2c). The transpiration measurements of the leaves showed the same behavior: the T means of samples 0DC (1.13 ± 0.12 mmol $H_2Om^{-2}s^{-1}$) and 14DC (0.92 ± 0.04 µmol $H_2Om^{-2}s^{-1}$) did not show significant differences ($p \geq 0.05$) and the mean T values of 14DD samples (0.27 µmol ± 0.11 $H_2Om^{-2}s^{-1}$) decreased significantly (Fig. 2d). Proline is an amino acid that acts as osmolyte during osmotic stress and other kinds of abiotic stresses (Ashraf and Foolad 2007). Proline content levels in the samples 0DD (487± 77 µg $g^{-1}$ D.W.) and 14DC (478 ± 48 µg $g^{-1}$ D.W.) did not exhibit significant differences ($p \geq 0.05$) (Fig. 2e). Oppositely, proline content levels in the 14DD samples (1793 ± 304 µg $g^{-1}$ D.W.) increased significantly. The behavior of these physiological parameters during the drought stress treatment was the expected (Mahouachi et al. 2012; Gamboa-Tuz et al. 2018). A scanning electron microscopy analysis of the abaxial surface of leaves was performed in watered plants and plants under drought stress to know the effect of drought treatment on the structures and functionality of stomatal aperture (Fig. 3). The turgid physiology and partial opening of most of the stomata in leaves of watered control plants from day 0 and until day 14 of the experiment did not show differences among treatments. However, almost all stomata of leaves in drought stress were totally closed. This behavior has been previously described in plants under drought stress (Huang et al. 2009).

The absence of changes in the RWC, osmotic potential, photosynthetic activity measurements, transpiration measurements, proline content and stomata aperture of the 0DC and 14DC samples proved that the environmental conditions did not cause negative effects on the homeostasis of the plants. 14DD samples presented lower RWC values, lower osmotic potential values, negative A values, lower transpiration rates, an increase of about four times

the proline content and the closure of almost all stomata. These results indicated that a period of two weeks without watering was enough to cause significant physiological damage in papaya plants.

*Extraction of phenolic compounds and antioxidant capacity*

The effect of the conditions employed to perform the extraction of phenolic compounds from papaya leaves by ultrasound-assisted extraction (UAE) was studied considering three different solvents (water, 50:50 methanol:water (v/v) and 50:50 ethanol:water (v/v)) at different extraction times (10 and 20 min) and with different amplitude waves (30%, 50% and 70%). These solvents were employed because methanol, water, ethanol, and alcohol-water mixtures are most frequently employed for recovering phenolic compounds due to their different polarities (Lattanzio et al. 2006). The extraction temperature was kept as low as possible by using an ice bath. Based on our results (data not shown), water extraction solvent, an amplitude wave of 70% and 10 min of sonication were selected as the optimal extraction conditions to extract higher amounts of phenolic compounds from papaya leaves by UAE. Thus, these optimal extraction conditions were used to get the extracts from all the samples (0DC, 14DC and 14DD) in order to measure their antioxidant capacity and to carry out the characterization of their phenolic composition by HPLC-DAD and HPLC-MS.

The antioxidant capacity of the extracts was calculated with the use of TEAC and DPPH assays. The $DPPH^{\cdot}$ free radical scavenging capacity of extracts from 14DC samples (100.42 ± 5.80 μg D.W. $mL^{-1}$) decreased in comparison with the extracts of 0DC samples (71.00 ± 3.29 μg D.W. $mL^{-1}$). In contrast, the antioxidant capacity of extracts from 14DD samples (41.91 ± 2.21 μg D.W. $mL^{-1}$) increased significantly (Fig. 4a). The results of the TEAC assay displayed a similar behavior than that of the DPPH test: the antioxidant capacity of papaya leaves extracts of 14DC samples (0.37 ± 0.06 mmol Trolox g D.W. $^{-1}$) was lower than 0DC

samples (0.74 ± 0.09 mmol Trolox g D.W. $^{-1}$), while the antioxidant capacity of 14DD samples (0.99 ± 0.10 mmol Trolox g D.W. $^{-1}$) increased significantly (Fig. 4b). These results confirm that all different kinds of extracts presented antioxidant properties, especially those of plants under drought stress. Our results are in agreement with previous reports on the antioxidant capacities of papaya leaves extracts (Vuong et al. 2015). Interestingly, the antioxidant capacities of 14DC samples decreased compared to the extracts of 0DC samples. To date there are no reports about the effect of the age difference of papaya plants on the antioxidant capacity of leaves. The analysis of the antioxidant capacity variations between young and old leaves within papaya plants was analyzed in this study (Gogna et al. 2015). It was determined that the extracts of the young leaves showed higher antioxidant capacity in comparison with the old leaves. In addition, the extracts of 14DD samples presented the highest antioxidant capacity from the different kinds of extracts, probably due to the generation of additional phenolic compounds which act as antioxidants. These results indicated that the natural decrease of the antioxidant capacity of papaya leaves seems to revert in stress conditions.

*Characterization of phenolic compounds from papaya leaves extracts*

A HPLC-DAD analysis was performed so as to identify the different kinds of phenolic compounds present in the different extracts. Different wavelengths were chosen for HPLC analysis due to the diversity of absorption wavelengths of the phenolic compounds (Lattanzio et al. 2006). When analyzing DAD results of the different leaves extracts, the detected peaks of the chromatograms obtained at 250, 280 and 350 nm were very similar, of which the highest number of peaks was observed at 280 nm. Chromatograms obtained at 520 nm did not present any peak. Therefore, the chromatograms obtained at 280 and 350 nm were analyzed in depth.

A total of 53 well defined peaks were detected in the different chromatograms obtained from the HPLC-DAD analysis of the extracts at wavelengths of 280 and 350 nm: 25 peaks were detected only at 280 nm, one peak ($t_R$=29.9 min) only at 350 nm and 27 peaks were detected at both wavelengths (Table S1, Fig. S1 and Fig. S2). A total of 26 detected peaks showed significant differences among the different kinds of papaya leaves extracts, probably due to the growth of the plants and the drought stress treatment.

Although both chromatogram groups of samples 0DC and 14DC shared many peaks, the areas of many of these (13 peaks at 280 nm and 9 peaks at 350 nm) decreased significantly ($p \leq 0.05$) and some of these peaks were absent in the groups of chromatograms of 14DC samples. Regarding data obtained from the chromatograms group of 14DD samples, many of the areas of the previously detected peaks increased significantly ($p \leq 0.05$) (18 peaks at 280 nm and 10 peaks at 350 nm) and the areas of new peaks (12 peaks at 280 nm and 4 peaks at 350 nm) were detected (see Table S1, Fig. S3 and Fig. S4). Plants under drought stress conditions showed a decrease in their biomass, probably as a strategy of adaptation to stress conditions. This decrease in biomass caused an increase in the concentration of certain metabolites under drought stress (Paulsen and Selmar 2016). The results obtained in this investigation showed that several of the peaks detected in the chromatograms showed a significant increase in their respective areas in the extracts of 14 DD samples (Fig S3 and FigS4). However, it was also observed that some peaks (peaks 4, 5 and 6) of the extracts of 14 DC samples had the same signal intensity as the extracts of 14 DD samples (Fig S3). Additionally, some peaks (peaks 3 and 8) did not present significant differences between the different treatments (Fig S3). These results indicated that the increase of the peaks in stress conditions of the 14DD samples were due to an increase in the production of the metabolites and not only due to an increase in their concentrations. Interestingly, the significant changes

in the peak areas obtained at 280 and 350 nm as well as the total number of peaks from the different extracts presented the same behavior as their respective antioxidant capacities: the extracts of 14DC samples decreased their antioxidant capacities while the extracts of 14DD samples increased their antioxidant capacities (see Fig. 4). These results indicate that the increase of antioxidant capacity in extracts from leaves under drought stress could be mainly caused by the increase in phenolic composition.

*Identification of phenolic compounds by HPLC-ESI-QTOF-MS*

In order to characterize the phenolic compounds found in the extracts from papaya leaves, a HPLC-DAD-QTOF-MS/MS method was optimized on the base of previous study from our research group (Plaza et al. 2016). The in-depth study of the separated metabolites, with the data provided by the MS, along with information reported in literature and MS databases (FooDB and PhytoHub), allowed the preliminarily identification and classification of 23 phenolic compounds (Table 1). The metabolites were grouped according to one of the following behaviors: group 1: only peak areas of the extracts of 14DC samples decreased and those of 14DD samples increased in comparison with those of the extracts of 0DC samples; group 2: only peak areas of the extracts of 14DC samples decreased compared with those of the extracts of 0DC samples; group 3: only peak areas of the extracts of 14DD samples increased in comparison with those of the extracts of 0DC samples.

*Hydroxycinnamic acid derivatives*

The mean areas of peaks 35, 36, 32, 2, 38 and 39, compared with the samples of 0DC, decreased in the 14DC samples and increased in the 14DD samples. The mean area of peak 31 only increased in the 14DD samples. The highest peak (**peak 35**) was tentatively identified as D-malic acid-p-coumarate derivative 1 and presented a molecular ion with m/z 279.0533 $[M-H]^-$ and deprotonated analyte adduct with m/z 559.0983 $[2M-H]^-$. This ion showed

fragments at m/z 163 and 132 that could most probably be the fragments corresponding to its aglycone and malic acid residues, respectively; and m/z 119 corresponding to the aglycone loss of $CO_2$ (see Table 1). The **peak 36** was also tentatively identified as D-malic acid-p-coumarate derivative 2, because the mass spectra presented the same molecular ion (m/z 279.0533 $[M-H]^-$) and fragmentation pattern (m/z 163, 132 and 119) as **peak 35**. This MS/MS pattern has been previously proposed for D-malic acid-p-coumarate (Regos et al. 2009). Increase of D-malic acid-p-coumarate has been previously reported in plants under mechanical damage (Housti et al. 2002).

**Peaks 31** and **32** presented a $[M-H]^-$ ion at m/z 295.0449, a $[2M-H]^-$ ion at 591.1075 and the $[M-H]^-$ ion produces clear fragments corresponding to its aglycone (m/z 179) and malic acid residue (m/z 133). These peaks were preliminarily identified as caffeoylmalic acid derivatives 1 and 2, respectively in accordance with the fragmentation reported in literature (Lin and Harnly 2008).

The **peak 2** showed a molecular ion with m/z 353.0896 $[M-H]^-$, its main fragment ion was shown at m/z 191 (Table 1) and corresponded to quinic acid ion. This compound was preliminarily identified as caffeoylquinic acid according to the fragmentation explained in literature (Regos et al. 2009; Ncube et al. 2014). **Peaks 38** and **39** were tentatively identified as feruloylmalic acid derivatives 1 and 2, respectively. They displayed a molecular ion with m/z 309.0637 $[M-H]^-$ and an analyte dimerization with m/z 619.0437 $[2M-H]^-$. MS/MS fragmentation of this ion gave fragmentation ions at 193 m/z and 134 m/z that are equivalent to its aglycone and malic acid residues, respectively (Spínola et al. 2015). Caffeoylquinic derivatives due to their antioxidant capacities have exerted neuroprotective properties (Nakajima et al. 2007) and feruloylmalic acid has shown antioxidative and anti-apoptotic activities (Luo et al. 2018).

It was also observed that the areas of some of the peaks (9, 11, 12 and 15) increased in the samples of drought-stressed plants extracts when compared to the other types of extracts. **Peaks 9**, **11, 12** and **16** were preliminarily identified as 2-O-caffeoyl hexoside acid derivatives 1, 2, 3 and 4, respectively. They exhibited $[M-H]^-$ ion at m/z 371.0626, their dimerization at m/z 743.1312 $[2M-H]^-$ and their fragmentation pattern presented product ions at m/z 209 and 191. The fragment at m/z 209 could be interpreted as an aldaric acid moiety, particularly an hexoside acid like glucaric and galactaric that underwent dehydration, giving rise to the fragment at m/z 191 $[209-H_2O]^-$ (Spínola et al. 2015). The **peaks 15** and **18** were preliminarily identified as 2-O-feruloyl hexoside acid derivatives 1 and 2, respectively. They had a molecular ion at m/z 385.0779 $[M-H]^-$. Their fragmentation patterns were similar to 2-O-caffeoyl hexoside acid derivatives with product ions at m/z 209 and 191 that can be a hexoside acid like glucaric and galactaric (m/z 209) and its dehydration (m/z 191) (Spínola et al. 2015). Also, the **peaks 15** and **18** showed a fragment from ferulate ion at m/z 193. Moreover, peaks **13**, **17** and **21** which had molecular ions with m/z 355.0646 $[M-H]^-$, showed very similar fragmentation pattern to 2-O-caffeoyl hexoside acid derivatives and 2-O-feruloyl hexoside acid derivatives (see Table 1). Their main fragments at m/z 209 and 191 corresponded to product ions of a hexoside acid and the loss of the water molecule from the hexoside acid, and at m/z 163 which could be the coumarate ion, being tentatively identified as p-coumaroyl hexoside acid derivatives 1 (peak 13), 2 (peak 17) and 3 (peak 21). This fragmentation pattern was previously described in literature (Coutinho et al. 2016). These peaks did not show significant differences among the different extracts from papaya leaves. A decrease in the concentration of hydroxycinnamic glucosides has been reported in tomato fruits during a hydric stress (Sánchez-Rodríguez et al. 2011). Sinapic and ferulic acid glycoside esters have reported antioxidant capacities (Kylli et al. 2008).

*Flavonoids*

The **peak 44** was preliminarily identified as the flavonol quercetin glucoside rhamnoside because it showed a $[M-H]^-$ at m/z 609.1462 and MS/MS yielding ions at m/z 463 and 301. These fragments are the products of the loss of a deoxyhexose (rhamnose-like) sugar $[M-146-H]^-$, a deoxyhexose and a hexose (glucose-like) sugars $[M-146-162-H]^-$, respectively (Guimarães et al. 2013). The **peak 48** presented a molecular ion with m/z 593.1524 $[M-H]^-$, and was tentatively identified as kaempferol glucoside rhamnoside. MS/MS yielded the main ions at m/z 447 and 285 as products of the loss of a deoxyhexose sugar (rhamnose-like) $[M-146-H]^-$ and a deoxyhexose and a hexose (glucose-like) sugars $[M-146-162-H]^-$, respectively (Benayad et al. 2014). Quercetin glucoside rhamnoside was identified only in extracts of leaves under drought stress (14DD), while kaempferol glucoside rhamnoside was identified in all extracts but its peak area decreased in watered plants extracts at day 14 (14DC). Some flavonol glycosides with molecular structures similar to those previously mentioned present neuroprotective (Nakayama et al. 2011) and hepatoprotective (Wang et al. 2015) properties .

The peaks **37** and **42** did not show significant differences between the different extracts from papaya leaves. The **peak 37** could be preliminarily identified as quercetin 3-O-dirhamnosyl-glucoside because it showed a $[M-H]^-$ at m/z 755.1997 and MS/MS yielding a main ion at m/z 300 (aglycone ion) (Barros et al. 2013). The **peak 42** was preliminarily identified as kaempferol-3-robinoside-7-rhamnoside (robinin). Its molecular ion was shown at m/z 739.2082 $[M-H]^-$, and generated a fragment that is equivalent to its aglycone (m/z 285) (Mönchgesang et al. 2016). Quercetin 3-O-dirhamnosyl-glucoside has been identified in root exudates of *S. vulgaris* and seems to participate in the protection mechanism against

chromium toxicity (Pradas del Real et al. 2017). Robinin has been reported to have antiinflammatory properties (Ficarra et al. 1995).

*Other compounds*

The **peaks 50** and **51** both displayed maximum absorbance at 314 nm and molecular ions with m/z 677.2834 $[M-H]^-$. These compounds were tentatively identified as So-NCC-2 derivatives 1 and 2, respectively, which belong to the group of tetrapyrroles. Peaks 51 and 52 produced fragments with m/z 659 $[M-H_2O-H]^-$, 645 $[M-CH_3OH-H]^-$, 627 $[M-CH_3OH-H_2O-H]^-$, 617, 541, 520 [M−ring A−H]$^-$, 488, 448 and 402 (see Table 1). These produced fragments are in agreement with the predicted MS/MS spectra acquired in CFM-ID (http://cfmid.wishartlab.com) and in literature (Scherl et al. 2012). Nonfluorescent chlorophyll catabolites (NCCs) are common products of chlorophyll degradation in leaf senescence (Oberhuber et al. 2001). The presence of NCCs has been reported in plants under drought stress (Borrmann et al. 2009). NCCs isolated from pear peels have shown potential antioxidant capacity (Müller et al. 2007). Both of our compounds were only identified in extracts of leaves under drought stress (14DD), probably as part of the response mechanisms to oxidative stress.

The **peak 6** was tentatively identified as guanosine which is a purine nucleoside. Its molecular ion was shown at m/z 282.0822 $[M-H]^-$, and produced a clear fragment at m/z 150 (Table 1). This fragmentation pattern has been previously described in literature (Hartmann et al. 2006). Cyclic guanosine monophosphate (cGMP) acts as a secondary mediator and participates in diverse kinds of plant responses to abiotic stresses (Van Damme et al. 2014). The mean area of peak 6, compared with the extracts of 0DC samples, increased in extracts of samples 14DC and 14DD but no significant differences were observed between both treatments.

Different studies have corroborated that all the tissues of the papaya plant present different types of biological activities such as antioxidant capacity, owing to the presence of phenolic compounds. However, to date there are very few studies on the variations of chromatographic profiles of phenolic compounds in tissues from papaya plants under conditions of abiotic stress. The increase in the production and concentration of phenolic compounds during stress conditions may be due to a significant increase in the expression and activity of enzymes that participate in the synthesis of metabolites in response to stress conditions (Selmar et al. 2017). The results obtained in the bioinformatic analysis conducted by Gamboa-Tuz et al. (2018) show a significant increase in the expression levels of enzyme genes involved in the synthesis of different metabolites (such as abscisic acid and suberin) in papaya plants in response to drought stress conditions. The results in this study demonstrated that the content and diversity of phenolic compounds, as well as their antioxidant capacities, increased in papaya leaves under drought stress. Additionally, results showed that the content of phenolic compounds and the antioxidant capacity of leaves in watered papaya plants tend to decrease as the plants aged. Some of the identified compounds have been previously characterized in other plant tissues and we found that several of these compounds presented different kinds of biological activities and antioxidant capacities under stress conditions. Based on our results, we can conclude that the application of an abiotic stress on the different tissues of papaya plants is a good alternative for obtaining a higher quantity and diversity of phenolic compounds that can be commercially exploited in food, cosmetic and pharmaceutical industries. Furthermore, *Carica papaya* plants that have undergone drought stress due to environmental factors are a potential new source of phenolic compounds.

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**Figure captions**

**Figure 1** Effect of drought stress on *C. papaya* plants phenotypes: 0DC (a), 14DC (b) and 14DD (c). White arrows indicate the leaves collected for the analyzes

**Figure 2** Physiological effects of drought stress in *C. papaya* plants. Relative water content (a), osmotic potential (b), photosynthetic net rate (c), transpiration rate (d), and proline content (e) of leaves of watered plants (0DC and 14DC) and plants under drought stress treatment (14DD). Columns represent the mean obtained from three independent experiments for each treatment and vertical lines mark standard deviation. Data were analyzed by one-way ANOVA using the Dunnett's test at $p \leq 0.05$. Asterisks above the columns indicate values that are statistically different from the control values (**** $p \leq 0.0001$)

**Figure 3** Scanning electron micrographs of the abaxial surface of leaves: 0DC (a), 14DC (b) and 14DD (c)

**Figure 4** The $DPPH^{\cdot}$ free radical scavenging capacity (a) and trolox equivalent antioxidant capacity$^{\cdot}$ (c) of extracts from leaves of *C. papaya* plants under drought stress treatment. Columns represent the mean obtained from three independent experiments for each treatment and vertical lines mark standard deviation. Data were analyzed by one-way ANOVA using the Dunnett's test at $p \leq 0.05$. Asterisks above the columns indicate values that are statistically different from the control values (* $p \leq 0.0332$, ** $p \leq 0.0021$, *** $p \leq 0.0002$)

## TABLES

**Table 1.** List of signal peaks of chromatographic profiles at 280 and 350 nm of compounds detected in extracts of papaya leaves of watered plants at day 0 (0DC), watered plants at day 14 (14DC), and plants under drought stress at day 14 (14DD). Data represent the mean and relative standard deviation (RSD, %) obtained from three independent experiments for each treatment. ID: peak assignment number. N.D.: not determined. G: number of group assigned according to the behavior of the changes of the areas of peaks (each group description has been explained above). Asterisks signs indicate peaks signals that presented significant differences between treatments and plus signs indicate peaks that did not present significant ($p \leq 0.05$) differences between treatments but only appear in extracts of leaves of plants under drought stress.

| ID | $t_R$ (min) | UV-vis max (nm) | 280 nm | | | | | | | 350 nm | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 DC | | 14 DC | | 14 DD | | | 0 DC | | 14 DC | | 14 DD | | |
| | | | Area | RSD | Area | RSD | Area | RSD | G | Area | RSD | Area | RSD | Area | RSD | G |
| 1 | 1.4* | 264 | 116.7 | 6.0 | 84.0 | 4.3 | 110.5 | 1.2 | 2 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 2 | 1.5* | 276 | 364.6 | 7.9 | 268.2 | 1.2 | 441.3 | 7.4 | 1 | 15.3 | 8.7 | 20.3 | 5.4 | 15.6 | 4.8 | |
| 3 | 1.7 | 268 | 50.8 | 9.9 | 50.6 | 8.5 | 41.8 | 7.3 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 4 | 1.9 | 258 | 252.4 | 4.9 | 354.3 | 1.6 | 367.4 | 4.9 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 5 | 2.1 | 260 | 160.3 | 9.9 | 93.1 | 7.0 | 107.0 | 9.4 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 6 | 2.3 | 254 | 223.7 | 4.2 | 350.7 | 4.4 | 399.8 | 1.0 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 7 | 2.8 | 252 | 5.5 | 9.1 | 19.5 | 4.8 | - | - | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 8 | 2.9 | 326 | 20.6 | 8.0 | 33.8 | 3.2 | 36.7 | 7.5 | | 13.6 | 3.9 | 11.3 | 5.6 | 26.7 | 9.0 | |
| 9 | 3.4* | 326 | 16.5 | 9.6 | 11.1 | 4.3 | 45.8 | 9.8 | 3 | 18.8 | 8.4 | 14.9 | 6.1 | 39.8 | 7.8 | |
| 10 | 4.1 | 264 | 12.6 | 3.9 | - | - | 21.2 | 9.0 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 11 | 4.3* | 324 | 40.8 | 8.6 | 40.2 | 5.0 | 80.1 | 10.0 | 3 | 28.1 | 9.0 | 22.5 | 8.7 | 54.4 | 9.8 | |
| 12 | 5.4* | 322 | 78.2 | 9.8 | 60.4 | 6.1 | 129.2 | 8.9 | 3 | 66.4 | 9.4 | 58.4 | 7.0 | 124.4 | 10.0 | 3 |
| 13 | 7.1 | 312 | 18.7 | 8.2 | - | - | 25.2 | 9.5 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 14 | 7.8* | 228 | 46.3 | 9.2 | 14.6 | 4.3 | 314.5 | 5.0 | 1 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 15 | 8.2* | 326 | 21.0 | 9.6 | 12.9 | 7.7 | 66.5 | 6.3 | 3 | 30.5 | 7.0 | 18.5 | 7.2 | 74.8 | 9.8 | 3 |
| 16 | 9.2* | 314 | 66.0 | 7.0 | 17.2 | 7.8 | 55.9 | 8.2 | 2 | 14.9 | 9.3 | - | - | 24.6 | 9.3 | |
| 17 | 10.2 | 312 | 25.7 | 8.6 | - | - | 21.6 | 8.0 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 11.2 | 326 | 43.4 | 6.9 | 27.1 | 2.8 | 66.0 | 4.9 | | 36.6 | 6.9 | 24.9 | 9.6 | 68.3 | 4.0 | |
| 19 | 12.7+ | 324 | - | - | - | - | 14.2 | 6.3 | | - | - | - | - | 14.4 | 4.3 | |
| 20 | 12.9+ | 326 | - | - | - | - | 20.9 | 4.0 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 21 | 13.9 | 314 | 26.8 | 5.3 | - | - | 34.8 | 5.0 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 22 | 14.5 | 326 | 15.3 | 4.2 | - | - | 18.6 | 8.3 | | 9.9 | 8.6 | - | - | 21.7 | 9.0 | |
| 23 | 15.6+ | 276 | - | - | - | - | 9.5 | 1.4 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 24 | 16.2 | 314 | 35.1 | 5.6 | 20.2 | 8.6 | 62.0 | 9.9 | | 17.1 | 3.4 | - | - | 18.5 | 8.9 | |
| 25 | 16.8+ | 324 | - | - | - | - | 9.5 | 3.9 | | 9.9 | 4.3 | 9.1 | 5.0 | 17.0 | 3.5 | |
| 26 | 17.3 | 312 | 16.4 | 4.8 | - | - | 11.4 | 2.2 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 27 | 17.9+ | 326 | - | - | - | - | 16.3 | 5.2 | | - | - | - | - | 13.2 | 9.5 | |
| 28 | 18.2 | 308 | 11.2 | 6.6 | - | - | 15.4 | 7.6 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 29 | 19.4 | 326 | 15.0 | 1.3 | - | - | 21.8 | 0.9 | | 21.9 | 6.5 | 13.6 | 3.3 | 28.0 | 7.8 | |
| 30 | 19.6 | 312 | 28.3 | 6.1 | 8.8 | 3.0 | - | - | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 31 | 21.5* | 320 | 55.9 | 2.7 | 51.1 | 2.2 | 92.2 | 9.8 | 3 | 44.9 | 0.8 | 45.0 | 3.8 | 62.9 | 8.2 | |
| 32 | 21.8* | 328 | 885.5 | 1.2 | 690.8 | 3.2 | 1250.7 | 6.2 | 1 | 1069.5 | 1.6 | 816.3 | 3.9 | 1520.5 | 7.2 | 1 |
| 33 | 24.0 | 264 | 8.4 | 1.4 | - | - | 8.3 | 6.4 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 34 | 24.6+ | 250 | - | - | - | - | 13.5 | 3.4 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 35 | 25.3* | 312 | 1071.5 | 9.1 | 548.8 | 1.8 | 1316.0 | 5.5 | 1 | 279.7 | 9.1 | - | - | 350.1 | 7.3 | 1 |
| 36 | 25.5* | 310 | 636.8 | 9.3 | 434.0 | 9.0 | 773.5 | 6.1 | 1 | 153.1 | 3.5 | 154.8 | 2.1 | 208.3 | 9.9 | 1 |
| 37 | 26.8 | 255 | 10.6 | 4.8 | - | - | 12.4 | 7.9 | | 10.4 | 7.8 | 110.5 | 8.7 | 15.1 | 9.0 | |
| 38 | 27.3* | 256 | 107.5 | 1.1 | 26.9 | 8.3 | 142.0 | 6.0 | 1 | 244.0 | 5.1 | 59.0 | 6.8 | 323.5 | 1.8 | 1 |
| 39 | 27.5* | 326 | 380.8 | 1.0 | 176.8 | 7.2 | 504.4 | 9.7 | 1 | 495.0 | 2.4 | 226.0 | 6.2 | 648.2 | 9.8 | 1 |
| 40 | 27.9* | 256 | 103.1 | 9.3 | 60.1 | 8.7 | 108.5 | 4.5 | 2 | 114.5 | 5.4 | 63.8 | 1.9 | 142.1 | 6.7 | 2 |
| 41 | 28.1* | 252 | 66.9 | 6.2 | 88.5 | 5.5 | 283.2 | 8.4 | 3 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 42 | 29.0 | 266 | 9.3 | 1.6 | - | - | - | - | | 9.9 | 4.9 | - | - | 13.8 | 0.6 | |
| 43 | 29.6* | 264 | 309.1 | 6.3 | 65.1 | 1.8 | 354.3 | 10.0 | 1 | 535.0 | 7.4 | 108.6 | 2.2 | 603.0 | 9.8 | 1 |
| 44 | 29.9* | 260 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | | - | - | - | - | 30.6 | 7.1 | 3 |
| 45 | 30.3* | 256 | 93.8 | 2.6 | 28.2 | 8.4 | 124.8 | 9.3 | 1 | 196.7 | 2.5 | 58.1 | 9.0 | 278.6 | 10.0 | 1 |
| 46 | 31.7 | 278 | 9.1 | 5.4 | - | - | 12.9 | 4.6 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 47 | 32.3+ | 288 | - | - | - | - | 14.4 | 2.9 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 48 | 33.7* | 264 | 71.5 | 6.8 | 11.9 | 4.8 | 90.4 | 9.6 | 2 | 128.7 | 8.9 | 16.6 | 3.0 | 131.6 | 6.5 | 2 |
| 49 | 34.4* | 290 | - | - | - | - | 61.2 | 5.5 | 3 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 50 | 37.0+ | 314 | - | - | - | - | 17.5 | 5.1 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 51 | 37.8* | 314 | - | - | - | - | 72.2 | 8.9 | 3 | - | - | - | - | 16.1 | 4.7 | |
| 52 | 38.8+ | N.D. | - | - | - | - | 10.2 | 2.8 | | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |
| 53 | 43.2* | 240 | - | - | - | - | 102.8 | 2.5 | 3 | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | |

**Table 2**. List of tentatively identified compounds from papaya leaves extracts by HPLC-DAD-QTOF-MS and -MS/MS analysis (peak assignment number (ID), retention time ($t_R$), proposed assignment name, MS characteristics (experimental m/z (monoisotopic ion), molecular formula (MF) and mass precision (ppm)), main MS/MS fragments (relative abundance) and compound classification).

| ID | $t_R$ (min) | Compounds identified | $[M-H]^-$, MF, ppm | Main fragments detected (m/z, relative abundance) | Classification | References |
|---|---|---|---|---|---|---|
| 2 | 1.5 | Caffeoylquinic acid | 353.0896, $C_{16}H_{17}O_9$, 6 | 191.0365 (20), 173.0262 (3), 135.0284 (0.4) | Organic oxygen compounds, alcohols and polyols, quinic acid derivates, coumaric acid derivatives, cinnamic acid esters | (Regos et al., 2009) |
| 6 | 2.3 | Guanosine | 282.0822, $C_{10}H_{12}N_5O_5$, -6 | 150.0387 (100) | Purine nucleosides | (Hartmann et al., 2006) |
| 9 | 3.4 | 2-O-caffeoyl hexoside acid derivative 1 | 371.0638, $C_{15}H_{15}O_{11}$, 6 | 209.0294 (100), 191.0179 (16) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, glucaric acid derivatives | (Spínola et al., 2015) |
| 11 | 4.3 | 2-O-caffeoyl hexoside acid derivative 2 | 371.0611, $C_{15}H_{15}O_{11}$, -1 | 209.0289 (100), 191.0185 (16) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, glucaric acid derivatives | (Spínola et al., 2015) |
| 12 | 5.4 | 2-O-caffeoyl hexoside acid derivative 3 | 371.0626, $C_{15}H_{15}O_{11}$, 3 | 743.1312 (25), 209.0287 (100), 191.0184 (37), 147.0280 (5) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, glucaric acid derivates | (Spínola et al., 2015) |
| 13 | 7.1 | p-coumaroyl hexoside acid derivative 1 | 355.0636, $C_{15}H_{15}O_{10}$, -8 | 209.0289 (72), 191.0185 (100), 163.0373 (16), 147.0292 (48) | Phenylpropanoids and polyketides, cinnamic acid derivatives, coumaric acid esters, cinnamic acid esters | (Coutinho et al., 2016) |
| 15 | 8.2 | 2-feruloyl hexoside acid derivative 1 | 385.0780, $C_{16}H_{17}O_{11}$, 2 | 209.0290 (30), 193.0472(17), 191.0207 (100), 147.0315 (66) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, galactaric acid derivatives | (Spínola et al., 2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | 9.2 | 2-O-caffeoyl hexoside acid derivative 4 | 371.0630, $C_{15}H_{15}O_{11}$, 4 | 209.0181 (100), 191.0069 (42) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, glucaric acid derivatives | (Spínola et al., 2015) |
| 17 | 10.2 | p-coumaroyl hexoside acid derivative 2 | 355.0642, $C_{15}H_{15}O_{10}$, -6 | 209.0278 (73), 191.0190 (100), 163.0390 (14), 147.0299 (46) | Phenylpropanoids and polyketides, cinnamic acid derivatives, coumaric acid esters, cinnamic acid esters | (Coutinho et al., 2016) |
| 18 | 11.2 | 2-O-feruloyl hexoside acid derivative 2 | 385.0779, $C_{16}H_{17}O_{11}$, 2 | 209.0318 (46), 193.0469 (12), 191.0189 (100), 147.0268 (12) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives, cinnamic acid esters, galactaric acid derivates | (Spínola et al., 2015) |
| 21 | 13.9 | p-coumaroyl hexoside acid derivative 3 | 355.0646, $C_{15}H_{15}O_{10}$, -5 | 209.0272 (70), 191.0163 (100), 163.0354 (15), 147.0269 (45) | Phenylpropanoids and polyketides, cinnamic acid derivatives, coumaric acid esters, cinnamic acid esters | (Coutinho et al., 2016) |
| 31 | 21.5 | Caffeoyl malic acid derivative 1 | 295.0449 $C_{13}H_{11}O_8$, 4 | 179.0332 (28), 133.0127 (100) | Phenylpropanoids and polyketides, hydroxycinnamic acids derivates, coumaric acid derivatives, cinnamic acid esters | (Lin and Harnly, 2008) |
| 32 | 21.8 | Caffeoyl malic acid derivative 2 | 295.0471, $C_{13}H_{11}O_8$, -4 | 591.1075 (100), 179.0336 (27), 133.0127 (100) | | (Lin and Harnly, 2008) |
| 35 | 25.3 | D-malic acid p-coumarate derivative 1 | 279.0533, $C_{13}H_{11}O_7$, 8 | 559.0983 (6), 163.0244 (100), 132.9994 (99), 119.0358 (24) | Phenylpropanoids and polyketides, cinnamic acids and derivates, coumaric acid esters, cinnamic acid esters | (Regos et al., 2009) |
| 36 | 25.5 | D-malic acid p-coumarate derivative 2 | 279.0533, $C_{13}H_{11}O_7$, 8 | 559.0983 (6), 163.0244 (44), 132.9994 (100), 119.0358 (15) | | (Regos et al., 2009) |
| 37 | 26.8 | Quercetin 3-O-dirhamnosyl-glucoside | 755.1997, $C_{33}H_{39}O_{20}$, 6 | 300.10345 (100), 178.99966 (3) | Phenylpropanoids and polyketides, flavonoids, flavonoid-3-O -glycosides | (Barros et al., 2013) |
| 38 | 27.3 | Feruloylmalic acid derivative 1 | 309.0637, $C_{14}H_{13}O_8$, 7 | 619.0437 (8), 193.0437 (100), 134.0303 (19) | Phenylpropanoids and polyketides, cinnamic acid derivates, malic acid derivates, cinnamic acid esters | (Spínola et al., 2015) |
| 39 | 27.5 | Feruloylmalic acid derivative 2 | 309.0605, $C_{14}H_{13}O_8$, 4 | 193.0497 (100), 133.0133 (20) | Phenylpropanoids and polyketides, hydroxycinnamic acid derivatives, coumaric acid derivatives | (Spínola et al., 2015) |
| 42 | 29.0 | Kaempferol-3-robinoside -7-rhamnoside (robinin) | 739.2082, $C_{33}H_{39}O_{19}$, 1 | 575.1416 (2), 394.0624 (0.3), 284.0303 (100), 285.0350 (42) | Phenylpropanoids and polyketides, flavonoids, flavonoid-7-O-glycosides | (Mönchgesang et al., 2016) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 44 | 29.9 | Quercetin 3-rutinoside (rutin) | 609.1462, $C_{27}H_{29}O_{16}$, 0 | 463.0999 (0.1), 343.0532 (1), 301.0438 (58), 271.0336 (22), 179.0069 (5) | Phenylpropanoids and polyketides, flavonoids, flavonoid-3-O-glycosides | (Guimarães et al., 2013) |
| 48 | 33.7 | Kaempferol glucoside rhamnoside | 593.1524, $C_{27}H_{29}O_{15}$, -2 | 447.0995 (1), 327.0581 (2), 285.0484 (100) | Phenylpropanoids and polyketides, flavonoids, flavonoid-3-O-glycosides | (Benayad et al., 2014) |
| 50 | 37.0 | So-NCC-2 derivative 1 | 677.2839, $C_{35}H_{41}N_4O_{10}$, 2 | 659.2707 (70), 645.2536 (60), 627.2431 (43), 617.2601 (100), 541.2454 (30), 520.2077 (98), 488.1823 (57), 448.1870 (75), 402.1828 (62) | Organoheterocyclic compounds, tetrapyrroles and derivatives | (Scherl et al., 2012) |
| 51 | 37.8 | So-NCC-2 derivative 2 | 677.2834, $C_{35}H_{41}N_4O_{10}$, 1 | 645.2544 (100), 627.2445 (41), 585.2067 (36), 520.2067 (60), 489.1870 (39), 444.1914 (18), 402.1645 (9) | | (Scherl et al., 2012) |

**FIGURES**

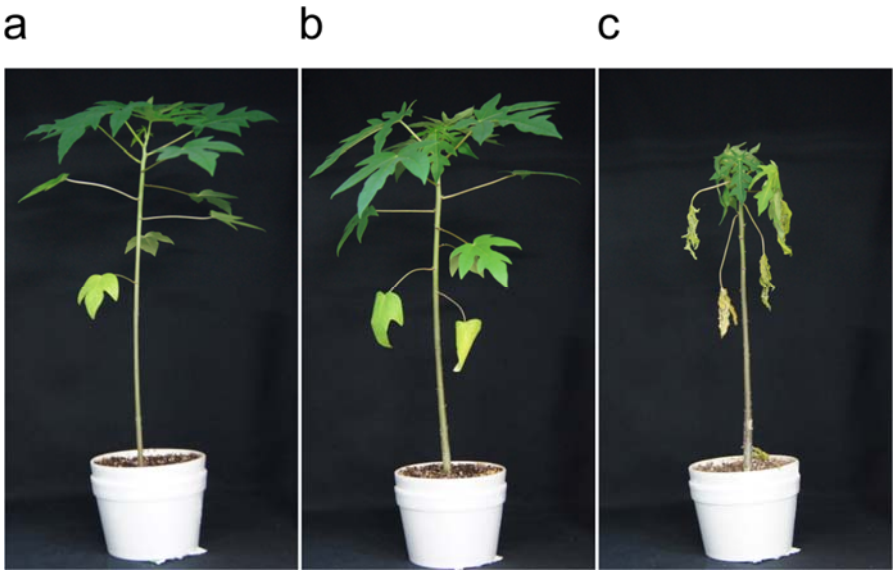


**Figure 1.**

**Figure 2.**

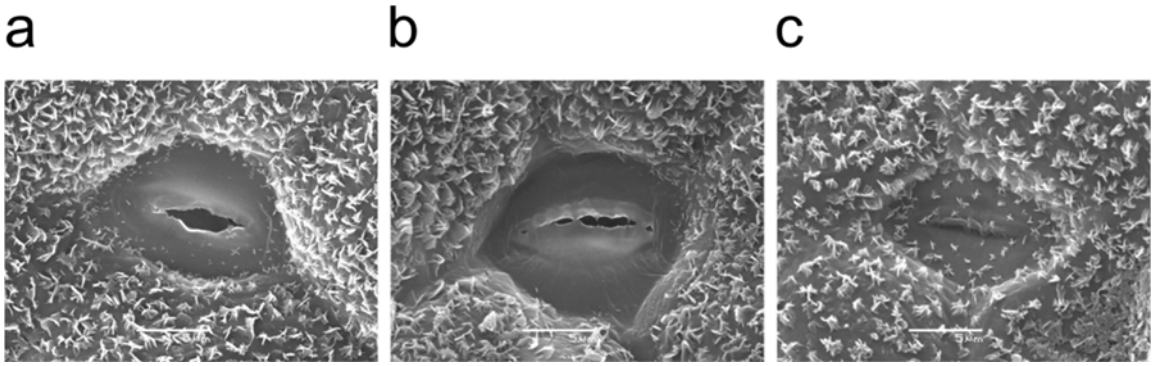


**Figure 3.**

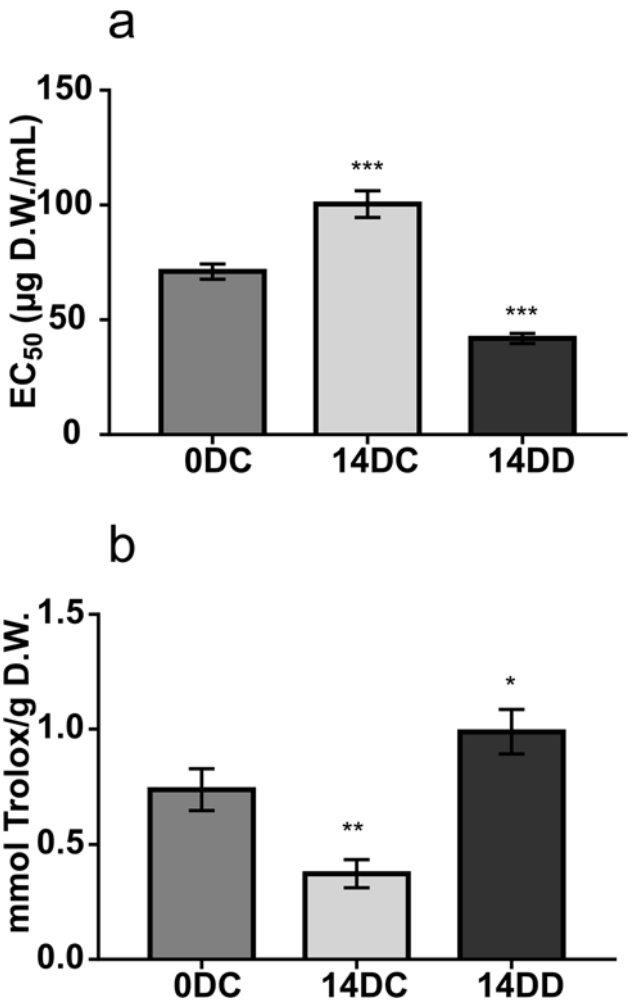
a
150
100
50
0
EC50 (µg D.W./mL)
***
***
0DC
14DC
14DD
b
1.5
1.0
0.5
0.0
mmol Trolox/g D.W.
**
*
0DC
14DC
14DD

**Figure 4.**