

## Bioactive compounds and antioxidant activities of selected types of chilli peppers

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**Abstract:** Chilli peppers (*Capsicum* spp.) are important plants usually consumed as food or used as a spice or medicine. They contain a wide range of phytochemicals such as capsaicinoids, polyphenols (PPH), carotenoids (CC) and vitamins. This study aimed to quantify the contents of capsaicin, dihydrocapsaicin, total PPH, phenolic acids (PHA) and total CC, and the antioxidant activities of the chilli peppers Trinidad Moruga Scorpion, Bhut Jolokia, Habanero Red, Habanero Maya Red, Habanero Red Savina, Jamaica Rosso, Serrano, and Jalapeño. High-performance liquid chromatography was used to quantify the contents of the bioactive compounds. The capsaicin contents in the peppers ranged from 2.1 to 124.2 mg·g<sup>-1</sup>, the dihydrocapsaicin content ranged from 5.1 to 151.3 mg·g<sup>-1</sup>, the total PPH content ranged from 3.53 to 25.9 mg GAE·g<sup>-1</sup>, and the total CC content ranged from 114.7 to 1 390.8 µg·g<sup>-1</sup>. The chlorogenic acid content was highest in Habanero Red (82.6 µg·g<sup>-1</sup>). The 1,1-diphenyl-2-picrylhydrazyl test of free-radical scavenging activity indicated that the ethanolic extract of the Trinidad Moruga Scorpion pepper had the highest antioxidant activity correlated with the contents of phenolic substances.

**Keywords:** capsaicin; dihydrocapsaicin; polyphenols; carotenoids; phenolic acids

Chilli peppers (*Capsicum* spp.) are among the most important vegetables worldwide (García-González and Silvar 2020). They originate from the tropical and humid regions of Central and South America, where they have spread to all inhabited continents.

The genus *Capsicum* belongs to the *Solanaceae* family. Currently, it contains more than 35 species, five of which (*Capsicum annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens*) are domesticated and are examples of the most important peppers for

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their nutritional value, economic importance, and cultivation (Bosland and Votava 2012). Chilli peppers contain many important bioactive compounds, such as anthocyanins, vitamins, polyphenols (PPH), phenolic acids (PHA), flavonoids, carotenoids (CC), and capsaicinoids. Among the most well-known properties of alkaloid capsaicinoids is their pungency (Sarpas et al. 2016). Capsaicinoids, capsaicin (CPS) and dihydrocapsaicin (DCPS), responsible for 90% of the intense organoleptic sensation of heat, are most abundant in peppers (Campos et al. 2013). Peppers' pungency degree is calculated based on the concentration of capsaicinoid compounds in the fruit. Flavonoids, PHA and their derivatives are the main phenolic compounds in chilli peppers (Carvalho et al. 2015). The diverse colouring of the fruits of different types of peppers at various stages of maturity is partly due to their CC profiles and chromogenic activity. Capsanthin is the primary CC in red fruits, accounting for 35–50% of the total CC content. Chlorophylls and CC are the dominant pigments in green peppers. Red or yellow CC, xanthophylls and anthocyanins gradually form during ripening. CC and chlorophylls in paprika are also involved in its antioxidant activity (Ornelas-Paz et al. 2010).

Many factors, such as climatic conditions, ripening time, genotype, fruit position on the plant and growing conditions influence the morphology and biochemical composition of pepper fruits. These factors affect the accumulation of capsaicinoids, PHA, CC, and other components in peppers. In connection with the importance of phytochemicals and their antioxidant activity, the aim of this study was to determine the contents of CPS, DCPS, total PPH, PHA and total CC, and the antioxidant activities of extracts from several types of chilli peppers.

## MATERIAL AND METHODS

**Samples.** Eight types of chilli peppers were selected for the study: Bhut Jolokia, Habanero Maya Red, Habanero Red, Habanero Red Savina, Jalapeño, Jamaica Rosso, Serrano, and Trinidad Moruga Scorpion. The peppers were grown and harvested at the Department of Food Hygiene, Technology and Safety of the University of Veterinary Medicine and Pharmacy in Košice. Experimental research on plants complies with relevant institutional, national, and international guidelines and legislation. The experiment started by buying seeds, sowing, germination, and gradually transplanting and caring for mature plants, includ-

ing adaptation to climatic conditions and fertilisation. Samples of mature chilli peppers (12 pieces of each variety were used in the experiment) were dried immediately after harvest in a laboratory oven with ventilation (MRC Lab, United Kingdom). Before drying, the fruits were cut into halves or quarters (depending on size) to accelerate drying and prevent undesired changes (moulds). The fruits were dried along with the placentae and seeds. Drying was carried out in two stages, at  $40 \pm 5$  °C for 24 h in the first stage and 12–24 h in the second stage, depending on the water content. After drying, the chilli peppers were stored in sealed glass containers in a dry, dark place until analysis (not more than one month).

**Preparation of extracts.** Fully dried chilli peppers were destemmed and ground in an electric stain-less-steel blender. From each sample of ground pepper, 0.2 g was weighed and placed into a 10 mL volumetric flask. Two mL of 96% (v/v) ethanol were added, and the mixture was mixed on a vortex homogeniser (Argolab, Italy) and placed in an ultrasonic bath (MRC Lab, United Kingdom) for 5 min. The flasks were then closed and stored in a dry, dark place. The peppers were macerated for 24 h under laboratory conditions and were then filtered through filter paper into 10 mL volumetric flasks. The filter paper was washed with a small amount of ethanol (96% v/v), and the extract in the flask was diluted with the ethanol solvent to the 10 mL mark. The diluted extract was filtered through a membrane syringe filter before analysis. The extracts were stored in sealed flasks at 5 °C.

**Quantification of CPS and DCPS.** Capsaicinoids in the prepared extracts were quantified using a Dionex UltiMate 3000 RS HPLC system (Thermo Fisher Scientific, USA) with a UV diode detector (UV-DAD; Thermo Fisher Scientific, USA) and a programmable Chromeleon Chromatography Data System, version 7.2 (Thermo Fisher Scientific, USA). Separation was achieved using a Polaris 5 C18-A reversed-phase column ( $250 \times 4.6$  mm, 5  $\mu$ m) (Agilent Technologies, Netherlands). Extracts were filtered through 25 mm diameter, 0.22  $\mu$ m pore size Q Max<sup>®</sup>RR PVDF membrane filters (Frisenette, Denmark) before measurement. The analysis conditions were based on the data presented by Al-Othman et al. (2011) and were modified in this study. An isocratic method was used for chromatographic separation with a mobile phase consisting of acetonitrile and water in a ratio of 70:30 (v/v) at a flow rate of 1 mL·min<sup>-1</sup> and a temperature of 40 °C. Absorbance was recorded at a wavelength of 282 nm using a DAD detector. The total analysis time was

10 min. A sample with a volume of 10  $\mu\text{L}$  was dosed using an autosampler. Stock solutions of the CPS and DCPS standards were prepared in ethanol with 10 and 5  $\text{mg}\cdot\text{mL}^{-1}$  concentration, respectively. The standard calibration curves were constructed in the concentration ranges of 10–500  $\mu\text{g}\cdot\text{mL}^{-1}$  for CPS and 5–500  $\mu\text{g}\cdot\text{mL}^{-1}$  for DCPS. The Scoville Heat Units (SHU) value expresses the pungency based on the Scoville organoleptic test (Scoville 1912). The pungency of the examined samples was calculated by multiplying the capsaicin content expressed in micrograms of capsaicin per gram of dry pepper by a coefficient of 16, which corresponds to the pungency of pure capsaicin.

**Quantification of total PPH.** To determine total PPH, 1.6 ml of distilled water and 20  $\mu\text{L}$  of extract solution or gallic acid standard solution and 100  $\mu\text{L}$  of Folin-Ciocalteu reagent were mixed in glass tubes. After 5 min, 300  $\mu\text{L}$  of a sodium carbonate solution (0.1  $\text{g}\cdot\text{mL}^{-1}$ ) was added to the mixture and thoroughly mixed. The mixture was incubated for 2 h at room temperature in the dark. The absorbance of the solution was then measured at a wavelength of 765 nm on a UV-1280 UV-VIS Spectrophotometer (Shimadzu, Japan). A standard stock solution of GA with a concentration of 5  $\text{g}\cdot\text{L}^{-1}$  was prepared for the calibration dependence of the absorbance of the solutions on the concentration of GA. Solutions with a concentration of 0.05 to 1.5  $\text{mg}\cdot\text{mL}^{-1}$  were prepared by diluting the stock solution with water. Total PPH content was expressed as mg of GA equivalent per gram dry weight ( $\text{mg GAE}\cdot\text{g}^{-1}\text{ DW}$ ).

**Quantification of PHA.** The HPLC quantification of PHA in the chilli pepper extracts was performed on the Ultimate 3000 system (Thermo Fisher Scientific, USA) and was controlled using Chromeleon 7.2 (Thermo Fisher Scientific, USA). PHA was chromatographically separated using an ACE Excel 2u C18-Amide (150  $\times$  4.6 mm) 100A column (Advanced Chromatography Technologies Ltd, Scotland). UV absorption was monitored at wavelengths between 194 and 500 nm. The samples were quantified at 260 and 300 nm. The data were evaluated using Chromeleon 7.2. Standard calibration curves were obtained in a 2–100  $\text{mg}\cdot\text{mL}^{-1}$  concentration range. The amounts of PHA were expressed as  $\mu\text{g}\cdot\text{g}^{-1}\text{ DW}$ .

**Quantification of total carotenoids.** The extracts for the quantification of total CC were prepared by mixing 0.25 g of dried and ground chilli peppers with 5 mL of acetone for 1 h. After filtering the mixture, the extract was diluted with acetone to the

25 mL mark of a volumetric flask. Total CC in the chilli pepper extracts was spectrophotometrically determined on a UV-1280 UV-VIS Spectrophotometer (Shimadzu, Japan) at wavelengths of 470, 647, and 663 nm in a 1 cm cuvette. Total CC concentration was calculated according to Ducsay (2011) and expressed as  $\mu\text{g}$  of CC per 1 g DW pepper.

**Quantification of antioxidant activity.** A diphenyl-2-picrylhydrazyl (DPPH) assay was used to determine free-radical scavenging activity. A mixture of 0.250 mL of sample solution, 0.750 mL of methanol and 2 mL of a 0.2  $\text{mmol}\cdot\text{L}^{-1}$  DPPH solution was mixed well and stored in closed glass tubes for 30 min in the dark. The absorbance of the solution was then measured in a 1-cm glass cuvette at a wavelength of 517 nm against methanol on a UV-1280 UV-VIS Spectrophotometer (Shimadzu, Japan). Ascorbic acid at a 1  $\text{mg}\cdot\text{mL}^{-1}$  concentration was used as a standard. Calibration solutions with concentrations from 5 to 300  $\text{mg}\cdot\text{L}^{-1}$  were prepared by diluting the ascorbic acid solution. DPPH free-radical scavenging was calculated according to Chen and Kang (2013).

**Statistical analysis.** Data were analysed using GraphPad Prism 9.2.0 (332) 2021 (GraphPad Software Inc., USA) using one-way analyses of variance with Tukey's multiple-comparison post hoc tests when the overall effect was significant ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

The CPS and DCPS contents were highest in the Trinidad Moruga Scorpion and Bhut Jolokia peppers (Table 1).

For Habanero peppers, the CPS content was highest in the Habanero Red pepper (25.5  $\text{mg}\cdot\text{g}^{-1}$ ), and the DCPS content was highest in the Habanero Red Savina pepper (40.2  $\text{mg}\cdot\text{g}^{-1}$ ). The concentrations of DCPS in the extracts did not differ among the Bhut Jolokia, Habanero Red and Habanero Red peppers. The concentrations of DCPS in the extracts were similar between Habanero Maya Red and Serrano peppers. The concentrations of DCPS in the extracts and fruits were lowest in Jalapeño peppers. The pungencies of the peppers were calculated based on the CPS contents. The hottest pepper was Trinidad Moruga Scorpion, followed by Bhut Jolokia. CPS and DCPS account for about 90% of the total capsaicinoid content (Bosland and Baral 2007). Bajer et al. (2015) reported that methanolic extracts of Trinidad Moruga Scorpion peppers contained 42.9  $\text{mg}\cdot\text{g}^{-1}$  of CPS and 18.1  $\text{mg}\cdot\text{g}^{-1}$  of DCPS, but Duelund and Mouritsen (2017) found higher contents,

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Table 1. Capsaicin (CPS) and dihydrocapsaicin (DCPS) in the peppers

Peppers	Capsaicin		Dihydrocapsaicin		SHU
	extract ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	fruit ( $\text{mg}\cdot\text{g}^{-1}$ )	extract ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	fruit ( $\text{mg}\cdot\text{g}^{-1}$ )	
Bhut Jolokia	1 085.5 $\pm$ 6.08 <sup>f</sup>	54.3 $\pm$ 0.30 <sup>e</sup>	847.1 $\pm$ 43.15 <sup>d</sup>	42.4 $\pm$ 2.16 <sup>e</sup>	868 416
Habanero Red	509.0 $\pm$ 1.41 <sup>e</sup>	25.5 $\pm$ 0.70 <sup>d</sup>	776.6 $\pm$ 4.07 <sup>d</sup>	38.8 $\pm$ 0.22 <sup>d</sup>	407 200
Habanero Maya Red	212.4 $\pm$ 1.66 <sup>c</sup>	10.6 $\pm$ 0.08 <sup>b</sup>	188.3 $\pm$ 0.86 <sup>b</sup>	9.5 $\pm$ 0.01 <sup>b</sup>	169 952
Habanero Red Savina	362.6 $\pm$ 0.76 <sup>d</sup>	18.1 $\pm$ 0.04 <sup>c</sup>	804.6 $\pm$ 1.64 <sup>d</sup>	40.2 $\pm$ 0.08 <sup>d,e</sup>	290 056
Jalapeño	41.1 $\pm$ 0.04 <sup>a</sup>	2.1 $\pm$ 0.01 <sup>a</sup>	102.5 $\pm$ 1.46 <sup>a</sup>	5.1 $\pm$ 0.06 <sup>a</sup>	32 840
Jamaica Rosso	343.4 $\pm$ 1.49 <sup>d</sup>	17.2 $\pm$ 0.07 <sup>c</sup>	597.6 $\pm$ 6.11 <sup>c</sup>	29.7 $\pm$ 0.30 <sup>c</sup>	274 720
Serrano	85.6 $\pm$ 0.88 <sup>b</sup>	4.3 $\pm$ 0.04 <sup>a</sup>	228.7 $\pm$ 0.63 <sup>b</sup>	11.4 $\pm$ 0.04 <sup>b</sup>	68 496
Trinidad Moruga Scorpion	2 483.5 $\pm$ 6.93 <sup>g</sup>	124.2 $\pm$ 0.35 <sup>f</sup>	3 026.1 $\pm$ 22.80 <sup>e</sup>	151.3 $\pm$ 1.14 <sup>f</sup>	1 986 880
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	–

<sup>a–g</sup> significant differences at  $P < 0.05$ ; values – means  $\pm$  standard deviations (SD); SHU – Scoville Heat Units

98.1 and 34.4  $\text{mg}\cdot\text{g}^{-1}$  for CPS and DCPS, respectively. Sarpras et al. (2016) analysed 63 samples of Bhut Jolokia peppers and reported that the content of CPS in dried samples ranged from 11.5 to 53.9  $\text{mg}\cdot\text{g}^{-1}$  and that the content of DCPS ranged from 2.6 to 26.5  $\text{mg}\cdot\text{g}^{-1}$  DW. Schmidt et al. (2017) found 21.9  $\text{mg}\cdot\text{g}^{-1}$  of CPS and 1.91  $\text{mg}\cdot\text{g}^{-1}$  of DCPS in Bhut Jolokia peppers grown in Austria. Our results for Bhut Jolokia peppers were comparable to those from Sarpras et al. (2016), but our CPS content for Trinidad Moruga Scorpion peppers was higher than those reported previously (Bajer et al. 2015). Giuffrida et al. (2013) analysed the capsaicinoid contents of Habanero Golden, Habanero Orange, Habanero Red (dark orange), Habanero Red (dark red), Habanero Chocolate and Habanero White peppers. The highest capsaicinoid content was in the acetone extract of dried Habanero Red (dark red) peppers, with 38.9  $\text{mg}\cdot\text{g}^{-1}$  of CPS and 14.1  $\text{mg}\cdot\text{g}^{-1}$  of DCPS. Duelund and Mouritsen (2017) reported 36.8  $\text{mg}\cdot\text{g}^{-1}$  of CPS and 11.4  $\text{mg}\cdot\text{g}^{-1}$  of DCPS in Habanero Red Savina chilli peppers grown in the mild climate of Denmark. CPS content was higher, and DCPS content was lower in both of these studies than ours. Generally, the CPS content is lowest in Jalapeño and Serrano peppers. Duelund and Mouritsen (2017) reported 3.6  $\text{mg}\cdot\text{g}^{-1}$  of CPS and 2.3  $\text{mg}\cdot\text{g}^{-1}$  of DCPS in Serrano peppers. The contents of these capsaicinoids in our Jalapeño and Serrano samples were higher than those previously reported (Schmidt et al. 2017). The CPS content in our Jalapeño sample was comparable with the results from Giuffrida et al. (2013), where the CPS content was 1.1  $\text{mg}\cdot\text{g}^{-1}$ . The SHU value and the CPS and DCPS contents of the Trinidad Moruga Scorpion pepper in our samples were higher than previously reported (Bajer

et al. 2015; Duelund and Mouritsen 2017). The Bhut Jolokia pepper has the highest reported pungency (Popelka et al. 2017). High SHU values (927 199 and 879 953) for Bhut Jolokia peppers were determined by independent tests in two commercial laboratories (Bosland and Baral 2007), comparable to our results. SHU values for Bhut Jolokia peppers, however, can range from 347 301 to 845 296 SHU (Hazari et al. 2014), with values of 537 000 SHU and 802 406 SHU (Popelka et al. 2017) also reported. The pungency of our Bhut Jolokia peppers was consistent with previously reported data (Bosland and Baral 2007) and was within the range of pungency for this pepper (600 000 to 1 041 427 SHU). Habanero red peppers are generally considered the hottest, with the highest reported pungency of 867 189 SHU (Hazari et al. 2014). Generally, the spiciness of our Habanero peppers was consistent with the spiciness scale of 150 000 to 500 000 SHU. The pungency of Habanero Red Savina peppers in our study was similar to previously reported values (Duelund and Mouritsen 2017; Tobolka et al. 2021) but lower than that reported by Giuffrida et al. (2013). The Jalapeño and Serrano peppers were the least hot in our study. The pungency of our Jalapeño peppers was 32 640 SHU, and Serrano was 68 496 SHU. Giuffrida et al. (2013) found that the pungency of Jalapeño pepper was 33 321 SHU, comparable to the value in our study, and the pungency of Serrano pepper was 21 034 SHU. Many factors can influence capsaicinoid contents in peppers, e.g. genetic factors, geographic location, the position of the fruits on the plant and the fruit ripening stage (Ananthan et al. 2018). Total PPH in the chilli peppers ranged from 3.53 to 25.9  $\text{mg GAE}\cdot\text{g}^{-1}$  DW (Table 2).



Table 2. Total polyphenols (PPH) in the extracts and peppers ( $P < 0.001$ )

Peppers	Total PPH	
	extract (mg GAE·cm <sup>-3</sup> )	fruit (mg GAE·g <sup>-1</sup> DW)
Bhut Jolokia	0.307 ± 0.0127 <sup>d</sup>	15.3 ± 0.629 <sup>d</sup>
Habanero Red	0.071 ± 0.0091 <sup>a</sup>	3.53 ± 0.451 <sup>a</sup>
Habanero Maya Red	0.121 ± 0.0016 <sup>b</sup>	6.06 ± 0.081 <sup>b</sup>
Habanero Red Savina	0.179 ± 0.0059 <sup>c</sup>	8.94 ± 0.299 <sup>c</sup>
Jalapeño	0.098 ± 0.0031 <sup>a,b</sup>	4.89 ± 0.156 <sup>a,b</sup>
Jamaica Rosso	0.114 ± 0.0062 <sup>b</sup>	5.72 ± 0.306 <sup>b</sup>
Serrano	0.075 ± 0.0049 <sup>a</sup>	3.76 ± 0.247 <sup>a</sup>
Trinidad Moruga Scorpion	0.518 ± 0.0158 <sup>e</sup>	25.9 ± 0.79 <sup>e</sup>

<sup>a–e</sup> significant differences at  $P < 0.05$ ; values – means ± standard deviations (SD); GAE – gallic acid equivalent; DW – dry weight

PPH content was highest in the Trinidad Moruga Scorpion pepper, followed by Bhut Jolokia. PPH content was lowest in the Habanero Red and Serrano peppers. Ornelas-Paz et al. (2010) reported total PPH contents in raw Mexican Jalapeño peppers (1 782.4 µg·g<sup>-1</sup>) and Serrano peppers (1 746 µg·g<sup>-1</sup>). The content of vanillic acid was lowest in the Jalapeño pepper and highest in the Trinidad Moruga Scorpion pepper (Table 3). GA content was highest in the Habanero Red and Habanero Red Savina peppers, which differed greatly from the other peppers. GA content was lowest in the Trinidad Moruga Scorpion, Serrano, and Jalapeño peppers. Protocatechuic acid was detected only in the Bhut Jolokia, Habanero Red and Serrano peppers. Chlorogenic acid content was highest in the Habanero Red pepper. The content of sinapic acid in the analysed samples ranged from 2.0 to 18.0 µg·g<sup>-1</sup> DW and ferulic acid from 4.0 to 26.0 µg·g<sup>-1</sup> DW. Oney-Montalvo et al. (2020) showed that subsequent harvesting cycles of pepper reduced the content of total polyphenols, chlorogenic acid and ellagic acid but increased the

content of gallic and protocatechuic acid. The authors report that the degree of ripeness, the harvest time and the interaction between these factors significantly affect almost all polyphenols evaluated. The type of pepper, agronomic conditions, and maturity influences the contents of PPH and PHA in fruits.

Total CC concentrations differed significantly among the extracts and fruits and were highest in the Trinidad Moruga Scorpion pepper ( $P < 0.001$ , Table 4).

Total CC concentrations in the extracts were lowest in the Jamaica Rosso and Habanero Maya Red peppers. The total CC contents in the fruits were lowest in the Jamaica Rosso, Habanero Maya Red and Jalapeño peppers. Pepper fruits contain more than 50 different CC. The diverse colouring of the fruits of different types of peppers at different stages of maturity is partly due to the CC profile in the fruits and their chromogenic activities. Capsanthin is the main CC in red fruits, accounting for 35–50% of the total CC content. Other CC include lutein, β-carotene, β-cryptoxanthin, zeaxanthin, violaxanthin and capsorubin (Antonio et al. 2018). Total

Table 3. The concentration of phenolic acids (PHA) (µg·g<sup>-1</sup> DW) ( $P < 0.001$ )

Peppers	Gallic	Protocatechuic	Chlorogenic	Vanillic	Sinapic	Ferulic
Bhut Jolokia	9.83 ± 0.501 <sup>c</sup>	0.08 ± 0.001 <sup>a</sup>	19.7 ± 1.21 <sup>d</sup>	90.1 ± 1.12 <sup>c</sup>	17.6 ± 0.95 <sup>d</sup>	22.7 ± 1.23 <sup>c</sup>
Habanero Red	12.8 ± 0.99 <sup>d</sup>	3.56 ± 0.458 <sup>c</sup>	82.6 ± 6.99 <sup>e</sup>	97.1 ± 2.51 <sup>c</sup>	6.93 ± 0.567 <sup>b</sup>	24.1 ± 0.74 <sup>c</sup>
Habanero Maya Red	9.08 ± 0.419 <sup>b</sup>	ND	7.45 ± 0.131 <sup>b</sup>	27.0 ± 0.37 <sup>b</sup>	3.15 ± 0.223 <sup>a</sup>	11.0 ± 0.30 <sup>b</sup>
Habanero Red Savina	11.3 ± 0.19 <sup>c,d</sup>	ND	15.9 ± 0.76 <sup>c</sup>	80.4 ± 1.34 <sup>c</sup>	2.04 ± 0.229 <sup>a</sup>	9.04 ± 1.051 <sup>b</sup>
Jalapeño	3.94 ± 0.344 <sup>a</sup>	ND	16.3 ± 1.64 <sup>d,c</sup>	4.96 ± 0.702 <sup>a</sup>	16.3 ± 1.644 <sup>c,d</sup>	4.64 ± 0.420 <sup>a</sup>
Jamaica Rosso	7.42 ± 0.763 <sup>b</sup>	ND	19.4 ± 2.25 <sup>d</sup>	8.78 ± 1.227 <sup>a</sup>	17.4 ± 1.06 <sup>d</sup>	9.33 ± 0.569 <sup>b</sup>
Serrano	4.76 ± 0.391 <sup>a</sup>	0.23 ± 0.001 <sup>b</sup>	5.48 ± 1.054 <sup>d</sup>	176 ± 7.8 <sup>e</sup>	14.5 ± 0.87 <sup>c</sup>	25.9 ± 1.68 <sup>c</sup>
Trinidad Moruga Scorpion	4.49 ± 0.937 <sup>a</sup>	ND	6.05 ± 1.045 <sup>a</sup>	120 ± 4.8 <sup>d</sup>	4.41 ± 0.605 <sup>a</sup>	9.49 ± 0.386 <sup>b</sup>

<sup>a–e</sup> significant differences at  $P < 0.05$ ; values – means ± standard deviations (SD); DW – dry weight; ND – not detected

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Table 4. Total carotenoids (CC) in the extracts and fruits of pepper ( $P < 0.001$ )

Peppers	Total carotenoids	
	extract ( $\mu\text{g}\cdot\text{cm}^{-3}$ )	fruit ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)
Bhut Jolokia	$3.80 \pm 0.134^c$	$380.2 \pm 13.37^b$
Habanero Red	$4.19 \pm 0.050^c$	$418.8 \pm 5.05^b$
Habanero Maya Red	$1.57 \pm 0.037^{a,b}$	$156.7 \pm 3.66^a$
Habanero Red Savina	$5.34 \pm 0.093^d$	$534.5 \pm 9.26^c$
Jalapeño	$2.29 \pm 0.124^b$	$114.7 \pm 1.24^a$
Jamaica Rosso	$1.43 \pm 0.523^a$	$142.8 \pm 5.23^a$
Serrano	$5.27 \pm 0.127^d$	$527.4 \pm 12.72^c$
Trinidad Moruga Scorpion	$13.9 \pm 0.62^e$	$1\,390.8 \pm 62.34^d$

<sup>a–e</sup> significant differences at  $P < 0.05$ ; values – means  $\pm$  standard deviations (SD); DW – dry weight

CC contents of  $234 \mu\text{g}\cdot\text{g}^{-1}$  for fresh red Jalapeño peppers,  $446 \mu\text{g}\cdot\text{g}^{-1}$  for Habanero peppers,  $266 \mu\text{g}\cdot\text{g}^{-1}$  for Serrano peppers (Cervantes-Paz et al. 2014) and  $362 \text{ mg CC per } 100 \text{ g fresh weight (FW)}$  for ripe Habanero peppers (Menichini et al. 2009) have been reported. Giuffrida et al. (2013) identified 52 CC in the fruits of Habanero Golden, Habanero Orange, Habanero Red (dark orange), Habanero Red (dark red), Habanero Chocolate, Habanero White, Serrano (red) and Jalapeño (red) peppers. Some Habanero cultivars have high  $\beta$ -carotene contents, and Serrano and Jalapeño peppers have high capsanthin contents but no  $\beta$ -carotene. Total CC contents in the fruits of Habanero peppers grown in Yucatan, Mexico, range from  $1.00$  to  $1.26 \text{ mg}\cdot 100 \text{ g}^{-1}$ , and CC content differ between genotypes (Campos et al. 2013). Cervantes-Paz et al. (2014) reported a CC content in red fruits of Jalapeño peppers of  $1\,178.4 \mu\text{g}\cdot\text{g}^{-1}$  DW. Total CC contents in the peppers in our study vary greatly due to many factors such as variety, type, and growing conditions (Wall et al. 2001).

The Trinidad Moruga Scorpion pepper had the highest inhibition and ascorbic acid equivalent (AAE)

(Table 5). The Habanero Maya Red, Habanero Red Savina, Jalapeño and Jamaica Rosso peppers had comparable antioxidant activities. The Habanero Red pepper had the lowest inhibition and AAE. Six of the eight extracts had antioxidant activities exceeding 50%. Antioxidants can reduce the amount of DPPH, manifested by a change in the colour of the solution from purple to yellow after receiving an electron or hydrogen radical. The antioxidant activity of 63 samples of Bhut Jolokia peppers grown in north-eastern Indian states ranged from 40.69 to 81.78% (Sarpras et al. 2016). Medina-Juárez et al. (2012) reported that antioxidant activity was 44.66% in Serrano pepper extracts and 8.45% in Jalapeño fruits, and Cervantes-Paz et al. (2014) found that the antioxidant activity of Jalapeño pepper extracts varied little during ripening. An extract of Bhut Jolokia peppers grown in north-eastern India had an antioxidant activity of 46.24% scavenging DPPH free radicals. The antioxidant activity of extracts of raw fruits may be due to the synergistic or additive activity of CC and chlorophylls but also of other compounds such as capsaicinoids and especially PHA. A recent study has also

Table 5. Inhibition of diphenyl-2-picrylhydrazyl (DPPH) radicals ( $P < 0.001$ )

Peppers	Inhibition (%)	Ascorbic acid equivalent ( $\text{mg}\cdot\text{dm}^{-3}$ )
Bhut Jolokia	$79.53 \pm 0.420^e$	$105.20 \pm 0.561^e$
Habanero Red	$28.30 \pm 1.016^a$	$37.07 \pm 1.352^a$
Habanero Maya Red	$58.32 \pm 0.795^c$	$76.98 \pm 1.056^c$
Habanero Red Savina	$62.45 \pm 2.460^c$	$82.48 \pm 3.270^{c,d}$
Jalapeño	$64.42 \pm 1.687^c$	$85.09 \pm 2.242^d$
Jamaica Rosso	$74.05 \pm 0.495^d$	$97.94 \pm 0.658^e$
Serrano	$40.61 \pm 2.339^b$	$53.43 \pm 3.111^b$
Trinidad Moruga Scorpion	$88.71 \pm 1.845^f$	$117.40 \pm 2.457^f$

<sup>a–f</sup> significant differences at  $P < 0.05$ ; values – means  $\pm$  standard deviations (SD)

shown a high correlation between vitamin C content, capsaicinoid and the antioxidant activity of pepper fruits (Lidíková et al. 2021). The capsaicinoid contents in the peppers we tested varied greatly, and the extracts of the eight peppers had high antioxidant activities.

## CONCLUSION

The capsaicinoid contents were high, especially in the Trinidad Moruga Scorpion and Bhut Jolokia peppers, corresponding to their high pungencies. These chilli peppers are good sources of CPS and DCPS. Total PPH and CC contents were highest in the Trinidad Moruga Scorpion pepper but were also high in the Habanero Red Savina and Bhut Jolokia peppers. The high antioxidant activities of the extracts from the Bhut Jolokia and Trinidad Moruga Scorpion peppers were correlated with the contents of PHA. The peppers had levels of phenolic constituents that contributed to high antioxidant activity and may be considered good sources of natural antioxidants.

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