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Antimicrobial and antioxidant activities of *Pimenta malagueta* (*Capsicum frutescens*)

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***Pimenta malagueta* (*Capsicum frutescens*), as it is known in Brazil, is one of the most commonly used pepper species in cooking and in Brazilian folk medicine. In this work, the total phenolic compounds and the capsaicin, dihydrocapsaicin, and chrysoeriol contents of *C. frutescens* were analysed. Additionally, the antioxidant and antimicrobial activities were determined. The contents of capsaicin, dihydrocapsaicin, and chrysoeriol found were 9.2, 4.0, and 2.1 mg/g extract, respectively. The minimal inhibitory concentration was determined against six strains of Gram positive and Gram negative bacteria species and one yeast strain (*Candida albicans* UFPEDA 1007), but for all of these microorganisms, the necessary concentrations were higher than 1000 µg/ml. The total phenolic content was 9.1 mg of gallic acid equivalent/g extract. The ethanolic extract of *C. frutescens* had effective 2,2-diphenyl-1-picrylhydrazyl (DPPH) ABTS• and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) ABTS•+ scavenging activities (EC_{50} values of 302.3 and 82.6 g/ml, respectively), and the percentage of antioxidant activity determined using the β-carotene/linoleic acid assay ranged from 15 to 47%. The primary groups of compounds extracted from plants with biological properties are essential oils, alkaloids, glycosides, phenolic compounds, terpenoids, and flavonoids. Our results suggest that *C. frutescens* had a potential antimicrobial and antioxidant effect due to its content in phenolic, capsaicinoid, and flavonoid substances.**

Key words: DPPH, β-carotene/linoleic acid system, capsaicin, *Capsicum frutescens*.

INTRODUCTION

The genus *Capsicum* comprises more than 200 varieties, and the fruits vary widely in size, shape, flavour and sensory heat. Five main species are cited in literature: *Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens* and *Capsicum pubescens*. Peppers from *Capsicum* species are native to the tropical and humid zones of Central and South America and belong to the *Solanaceae* family, which includes peppers of important economic value (Zimmer et al., 2012).

The greatest number of species is concentrated in Brazil (Barboza et al., 2011). *Capsicum frutescens* is a popular plant found in many parts of the world and is widely used as a food flavouring agent, a colouring agent, and an additive in livestock feed and in the food and pharmaceutical industries (Boonkird et al., 2008; Li et al., 2009; Zhuang et al., 2012). Although this pepper is widely consumed in Brazil and is known as “pimenta malagueta”, there have been few studies on its chemical composition and biological properties.

Fruit pungency is characteristic of the genus *Capsicum* due to some substances specific to peppers known as “capsaicinoids,” a group of compounds that includes

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more than 20 alkaloids (vanillylamines). Capsaicin, the principal compound, is located in the placenta section of the fruits. It is an odourless, fat-soluble compound that is rapidly absorbed through the skin and causes a burning sensation in mammalian tissue with which it comes in contact (Hayman and Kam, 2008). The genus *Capsicum* also is a rich source of phenolics, particularly flavonoids such as quercetin and luteolin (Howard et al., 2000). Dietary polyphenols like phenolic acids and flavonoids, are a primary source of antioxidants for humans and are derived from plants including fruits, vegetables, spices, and herbs (Martin and Appel, 2010). Some studies have demonstrated protective roles of flavonoids and carotenoids against coronary heart disease, stroke, and some forms of cancer. These protective effects of phenolic compounds are attributed to their antiradical and signalling activities (Martysiak-Żurowska and Wenta, 2012).

Natural antioxidants are considered multifunctional, and their activity depends on various parameters such as the multiplicity and heterogeneity of the matrix, the experimental conditions and, predominantly, the reaction mechanism (Gioti et al., 2009). Antioxidant compounds in food play important roles as health-protecting factors and are widely used as additives in fats and oils and in food processing to prevent or delay their spoilage (Ghasemnezhad et al., 2011).

The antimicrobial activities of polyphenols present in vegetable food and medicinal plants have been extensively investigated against a wide range of microorganisms (Daglia, 2012). Infectious diseases caused by bacteria, fungi, viruses, and parasites are still a major threat to public health, despite the tremendous progress in human medicine. The impact of infectious diseases is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance (Cos et al., 2006). Ideally, drug resistance should be overcome by the development of new classes of antimicrobial agents whose structures are different from those of the antibiotics to which pathogens have already developed resistance. The inhibition of resistance mechanisms through the development of novel antimicrobial drugs, without any selective activity, as adjuvants to antibiotics also represents an important antiinfectious strategy (Cushnie and Lamb, 2011).

In this study, the antioxidant and antimicrobial activities and the total phenolic content of the ethanolic extract of *C. frutescens* were determined, and the quantities of capsaicin, dihydrocapsaicin, and chrysoeriol were measured.

MATERIALS AND METHODS

General procedure

Infrared absorption spectra were recorded in KBr pellets using a Varian 640 FT-IR spectrophotometer with a PIKE ATR accessory operating in the 4000-400 cm^{-1} range. Silica gel 60 F_{254} (E. Merck) plates were used for thin layer chromatography (TLC). ^{1}H and ^{13}C NMR spectra were obtained using a Bruker DPX300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) in dimethyl sulfoxide-d_6. An Ultra Cleaner 1400 ultrasonicator (Unique, Indaiatuba, Brazil) was used to obtain the extracts. The extracts were analysed using a high-performance liquid chromatography (HPLC) system (Shimadzu Corp. Kyoto, Japan) equipped with preparative C18 Luna column (250 x 21.20 mm x 5µm) and a Luna C18 guard column (21 mm; Phenomenex, California, USA). The detector was an SPD-M20A Prominence diode array detector (DAD) (Shimadzu Corp. Kyoto, Japan), and the oven temperature was 40°C. The system consisted of two Model LC-6ADsolvent pumps and an SPD-M20A diode array detector (DAD) (Shimadzu Corp. Kyoto, Japan). The samples were injected into a rheodyne 7125i injector with a loop of 20 µl. The chromatographic separation was performed with a Luna 5µ C-18 80A column (150 x 4.6 mm x 5µm, Phenomenex, USA). All solvents used were of commercial HPLC grade. Capsaicin, β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, and the Folin–Ciocalteu reagent were purchased from Sigma–Aldrich (St. Louis, USA).

Pimenta malagueta samples

The ripe fruits of *C. frutescens* used in this study were purchased from a local market. The calyces and pedicels were removed manually.

Extraction, isolation, and quantification of compounds from Pimenta malagueta

The fresh ripe fruit (800 g) was extracted successively with ethanol, and an amount of 157.69 g of dry extract was obtained. The ethanolic extract was subjected to antioxidant and antimicrobial analyses.

To isolate and quantify the three compounds present in the ethanolic extract using HPLC-DAD (Figure 1), the fruits (2.78 kg) were dried in a circulating air oven (50°C) for 24 h and then triturated. The dried sample (1.9 kg) was initially extracted in hexane and after in acetonitrile with ultrasonication for 30 min. The extracts were filtered and evaporated to dryness in a rotary evaporator at 40°C. The acetonitrile extract was dissolved in methanol and injected into the HPLC-DAD system. The mobile phase was methanol/water (70:30), the flow rate was 15 ml/min, and the injection volume was 500 µl. The methanol was evaporated, and the compounds were extracted from the aqueous phase with dichloromethane and ethyl acetate. The organic solvent was evaporated, and 1184 mg of capsaicin, 778 mg of dihydrocapsaicin, and 36 mg of a flavonoid were obtained. Capsaicin and dihydrocapsaicin were identified using thin layer chromatography and melting point analysis by comparison with authentic samples. The ^{1}H and ^{13}C NMR spectra were used to identify the flavonoid as chrysoeriol.

The chrysoeriol, capsaicin, and dihydrocapsaicin contents were quantified using the external standard method based on peak area. The analyses were performed by plotting a calibration curve. To construct the calibration curve for each compound, working solutions with concentrations between 31.2 and 500 µg/ml were prepared from each stock solution by dilution with the appropriate volumes of methanol. The concentrations of these working solutions were then correlated with the measured area. The areas of these peaks were plotted, and the corresponding concentrations of the compounds were calculated from the calibration curve. For each sample, the quantitative analyses were performed in triplicate at 290 nm.

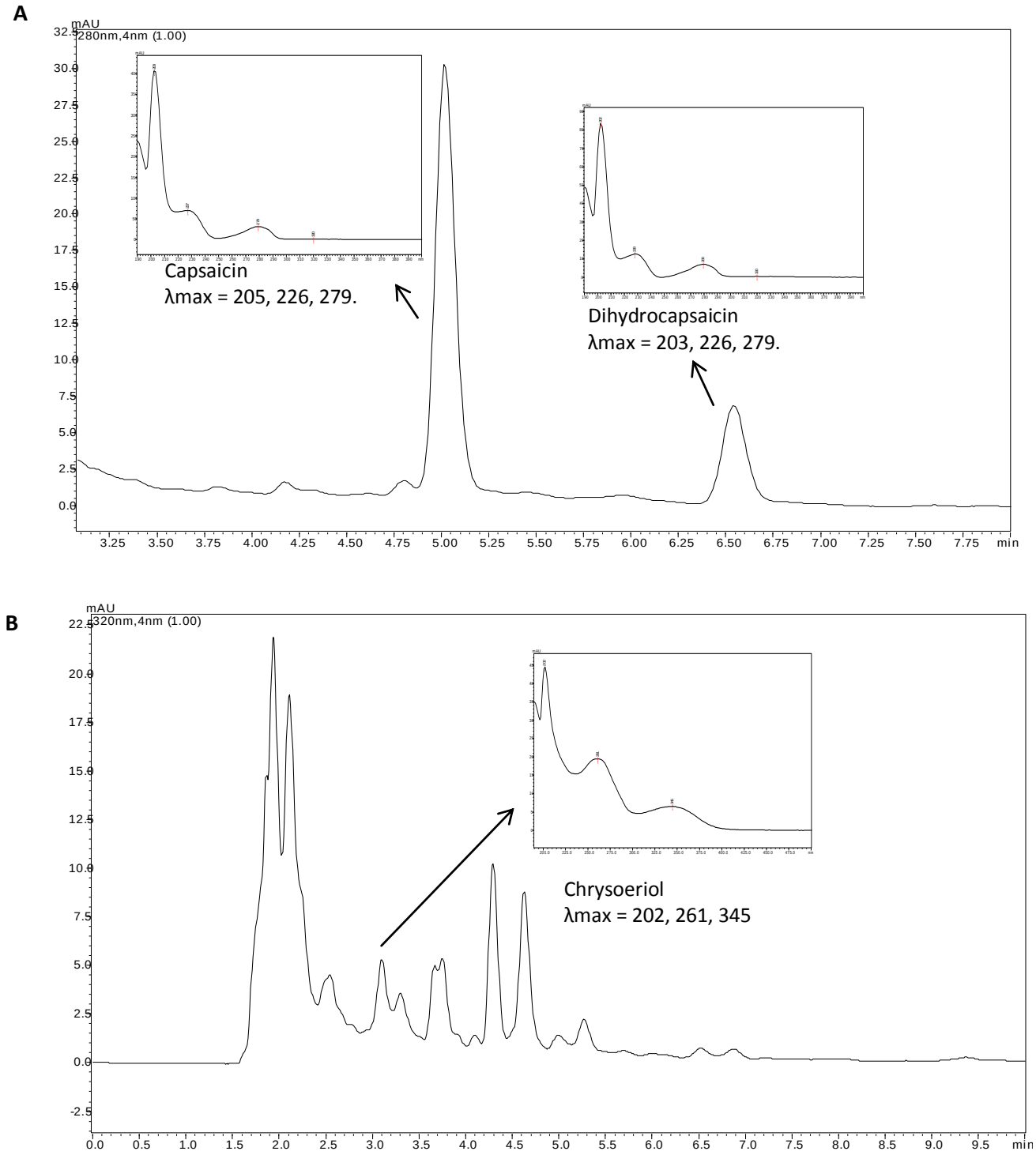

Figure 1. Chromatogram and UV spectra (HPLC-DAD) for the ethanolic extract of *C. frutescens*. (A) capsaicin and dihydrocapsaicin (280 nm), (B) chrysoeriol (320 nm).

Determination of the total phenolic content

The determination of the total phenolic content present in the ethanolic extract of *C. frutescens* was performed by the spectrophotometric method of Folin-Ciocalteu (Slinkard and Singleton, 1977) with modifications. Gallic acid was used as a standard. Briefly, 300 µl of a solution of the ethanolic extract (1 mg/ml) was added to 60 µl of Folin-Ciocalteu reagent and 2460 µl of distilled water, and this mixture was stirred for 1 min. Then, 180 µl of sodium carbonate (2%) was added to the mixture, which was then shaken for 30 s, resulting in a final concentration of 100 µg/ml. After two hours of incubation, the absorbance of each sample was determined spectrophotometrically at 760 nm. The results were expressed as mg of gallic acid equivalents (GAE)/g extract.

DPPH free radical scavenging assay

The free radical scavenging activities of the samples were determined using the 2,2,-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric method according to the study of Silva et al. (2006). This method is often used to determine the ability of plant species to capture free radicals (Ghasemnezhad et al., 2011).

A stock solution of the ethanolic extract of *C. frutescens* was prepared at 5.0 mg/ml. The appropriate quantities of an ethanol solution of $DPPH^{\bullet}$ (23.6 µg/ml) were added to samples to obtain final concentrations ranging from 100 to 500 µg/ml. The absorbance was measured at 517 nm after an incubation interval of 30 min with ultrasonication in the dark. The EC_{50} value is the sample concentration necessary to decrease by 50% the absorbance of DPPH; ascorbic acid was used as a standard.

ABTS radical cation assay

This test involves the generation of the chromophore $ABTS^{\bullet+}$ by oxidation of ABTS [2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] with potassium persulfate. The test was performed according to the method of Re et al. (1999) with modifications. ABTS was dissolved in water at a concentration of 7 mM. The ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for 12–16 h before use. Then, the $ABTS^{\bullet+}$ solution was diluted with ethanol (approximately 1:100 v/v) until an absorbance of 0.7±0.05 nm was reached. A stock solution of the ethanolic extract was prepared with a concentration of 1.0 mg/ml. An appropriate amount of $ABTS^{\bullet+}$ (2700 µl) was added to each sample to give final concentrations ranging from 20 to 120 µg/ml, and the samples were ultrasonicated in the dark for after 10 min. Then, the absorbance of the samples was measured at 734 nm. Trolox (0.1 mg/ml) was used as a positive control.

β-Carotene bleaching test

The level of antioxidant activity was determined using the β-carotene bleaching test according to the method of Bamoniri et al. (2010) with modifications. A solution of linoleic β-carotene/linoleic acid was prepared by adding an aliquot of 150 µl of β-carotene solution (20 mg/ml in chloroform) to 160 µl of linoleic acid and 660 µl of Tween 20 (in a 250 ml Erlenmeyer). Next, 140 ml of oxygenated distilled water was added to the system. The absorbance of this emulsion at 470 nm was adjusted to 0.6-0.7 nm. Aliquots of the crude ethanolic extract of *C. frutescens* (50 µg/ml) were compared to the control (no antioxidant) and to Trolox (16 µg/ml), which was used as a standard antioxidant. An initial reading of the absorbance was performed immediately after the addition of the samples and the standard to the system to determine the baseline. Subsequently, the absorbance was monitored every 20 min for 120 min. The samples were kept in a water bath at 40°C between the readings. The antioxidant capacity was expressed as the percentage inhibition of oxidation.

Antimicrobial assay

The microbial strains used belong to Gram positive (*Staphylococcus aureus* UFPEDA02*,* *Enterococcus faecalis* ATCC6057, *Bacillus subtilis* UFPEDA 86*),* Gram negative (*Escherichia coli* ATCC25922, *Klebsiella pneumonia* ATCC29665, *Pseudomonas aeruginosa* UFPEDA416*)* bacteria, and to yeasts (*Candida albicans* UFPEDA1007), which were acquired from the Antibiotics Department of the University Federal of Pernambuco, Brazil.

The broth microdilution assay was performed according to CLSI reference methods M7-A6, for bacteria (Clinical Laboratory Standards Institute, 2003), and M27-A3, for yeasts (Clinical Laboratory Standards Institute, 2008). Ninety-six-well microplates were used to determine the MIC of the crude ethanolic extract of *C. frutescens*. The final concentration of extract ranged from 5 mg/ml to 25 mg/ml, and dilutions were prepared in DMSO. The MIC was determined by measuring the absorbance of each well with microplate reader (ASYS UVM 340, Cambridge, UK). The MIC was defined as the lowest sample concentration that inhibited bacterial growth relative to the growth of the controls. Chloramphenicol (50 µg/ml) was used as a positive control for all bacterial strains, and itraconazole (25 µg/ml) was used for the yeast.

Statistical analysis

All samples were analysed in triplicate, and the results were pooled and expressed as the means ± standard error. Statistical analysis was performed with GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego CA, USA). The anti-free radical activity was determined using linear regression analysis with a confidence interval of 95% ($p<0.05$). The results were expressed as the EC_{50} ± SEM, which represents the concentration of sample necessary to reduce the absorbance of DPPH or $ABTS^{\bullet+}$ by 50% compared with the negative control.

RESULTS AND DISCUSSION

Total phenolic content

The Folin-Ciocalteu method is a convenient, simple, and quick procedure for the estimation of the total phenolic contents of various samples (Singleton et al., 1999). Several authors have evaluated the phenolic contents of different pepper species of the genus *Capsicum* using various techniques and solvents for the extraction of the chemical constituents of peppers.

Peppers contain moderate to high levels of neutral phenolics or flavonoids, phytochemicals that are important antioxidant components of a plant-based diet and that, in addition to traditional nutrients, may reduce the risk of degenerative diseases (Ghasemnezhad et al., 2011). The ethanolic extract of *C. frutescens* prepared in this study had a higher phenolic content (Table 1) than the most of the extracts prepared in previous studies (Table 2). This difference could be explained by variations in the sample preparation, extraction, and quantification methods; the chemical forms of the compounds analysed; the diversity of the varieties and genotypes of peppers (as sweet or hot peppers); the maturity stage and the use of fresh or dehydrated fruits.

Quantification of the principal compounds of *C. frutescens*

The group of pungent components unique to the fruits of these plants are called capsaicinoids (Alvarez-Parrilla et al., 2011). Flavonoids and capsaicinoids are the predominant phenolics found in *Capsicum* species (Alvarez-Parrilla et al., 2011). The amounts of two members of the capsaicinoid family and a flavonoid in the ethanolic

Table 1. Free radical scavenging activity ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) and total phenolic content of the ethanolic extract of *C. frutescens*.

Total Phenolic (mg GAE/g extract ±SD)	EC_{50} ABTS (µg/ml ± SD)		EC_{50} DPPH (µg/ml ± SD)	
Ethanolic extract	Ethanolic extract	Trolox	Ethanolic extract	Ascorbic acid
9.1±0.07	82.6±0.19	2.9±0.05	302.3±3.97	3.3±0.02

Table 2. Phenolic content by various species from *Capsicum*.

Specie	Extraction solvent	Phenolic Content	Reference
C. annuum	Methanol	1.0mg GAE/g extract	Alvarez-Parrilla et al., 2011
C. annuum	Methanol	5.7mg CAE/g extract	Howard et al., 2000
C. frutescens		5.1mg CAE/g extract	
C. chinense		4.3mg CAE/g extract	
C. annuum	Solution of acetone, water and acetic acid (70:29.5:0.5)	2.7mg GAE/g extract	Isabelle et al., 2010
C. chinense	Ethanol	7.5 mg GAE/g extract	Menichini et al., 2009
C. chinense	Water	17mg GAE/g extract	
C. annuum			
C. baccatum		10-12mg GAE/g extract	Ranilla et al., 2010
C. pubescens	Ethanol	4.9mg GAE/g extract	Zhuang et al., 2012
C. frutescens			
C. annuum		3.8 mg GAE/g extract	

GAE/g extract - Gallic acid equivalent/g extract; CAE - chlorogenic acid equivalent/g extract.

Table 3. Concentrations of capsaicin, dihydrocapsaicin and chrysoeriol in the ethanolic extract of *C. frutescens* (mg/g extract ± SD).

Capsaicin	Dihydrocapsaicin	Chrysoeriol
9.2 ± 0.03	4.0 ± 0.02	2.1 ± 1.24

extract of *C. frutescens* were determined (Table 3). Capsaicin and dihydrocapsaicin are the most pungent capsaicinoids and are responsible for up to 90% of the total pungency of peppers (Menichini et al., 2009). Some authors have evaluated the contents of capsaicin and dihydrocapsaicin in *C. frutescens* and found values ranging from 0.72 to 3.7 and from 0.75 to 2.4 mg/g dry weight, respectively (Garcés-Claver et al., 2006; Ozguven and Yaldiz, 2011). Interestingly, we found high contents of capsaicin and dihydrocapsaicin (9.2 and 4.0 mg/g extract). Regarding the flavonoid content, the concentration of the flavonoid chrysoeriol (3'-methoxy-luteolin) found in this study was 2.1 mg/g. This finding is remarkable compared with the concentration of 0.03 mg/g for *C. frutescens* found by Howard et al. (2000). The capsaicinoid and flavonoid contents can be affected by different factors such as the development stage of the fruit, the extraction technique, the species, and the environmental growth conditions (Menichini et al., 2009; Ornelas-Paz et al., 2010).

DPPH free radical scavenging and ABTS radical cation assays

Studies based on the use of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals are among the most popular spectrophotometric methods for the determination of the antioxidant capacities of foods, beverages and plant extracts. These methods have been frequently used to evaluate the antioxidant activity of compounds because these procedures are simple, rapid, sensitive, and easily reproducible (Martysiak-Żurowska and Wenta, 2012).

In the $DPPH^{\bullet}$ assay, antioxidants reduce the DPPH radical, which is purple, yielding a stable yellow compound upon receiving an electron or a hydrogen radical. The antiradical activity is assessed by the decrease in absorbance (Martysiak-Żurowska and Wenta, 2012). This assay has frequently been used to evaluate the antioxidant capacities of fruits and vegetables (Ghasemnezhad et al., 2011).

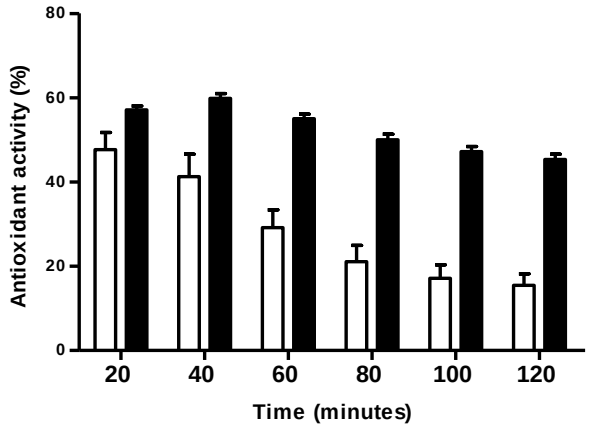

Figure 2. Antioxidant activity of the ethanolic extract of *C. frutescens* determined using the β-carotene bleaching test. Black columns – Trolox (16 µg/ml); white columns – *C. frutescens* ethanolic extract (50 µg/ml).

As shown in Table 1, the ethanolic extract of *C. frutescens* had a lower antioxidant capacity than the positive control, ascorbic acid. These results are corroborated by those of Zhuang et al. (2012). However, other species of *Capsicum* have been evaluated and exhibited EC_{50} values higher than the control (Locatelli et al., 2009; Zimmer et al., 2012).

The basis of the ABTS radical cation assay is to monitor the decay of the radical $ABTS^{\bullet+}$ produced by the oxidation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) due to the addition of a sample containing phenolics. $ABTS^{\bullet+}$ has a strong absorption, and its concentration can be easily determined spectrophotometrically by measuring the absorbance at a wavelength of 600-750 nm. In the absence of phenolics, $ABTS^{\bullet+}$ is very stable, but it reacts with H^+ donors and is converted into a colourless compound (Martysiak-Żurowska and Wenta, 2012). The EC_{50} value for ABTS obtained in this study (Table 1) was lower than that found by Hervert-Hernandez et al. (2010), who researched other pepper species. The lower antioxidant activity compared with that of the standard, despite the high content of phenolic compounds, may be explained by the fact that the raw extracts also contain non-phenolic substances such as sugars, organic acids, proteins, and pigments, which can interfere in the antioxidant evaluation assay (Kähkönen et al., 2001).

β-Carotene bleaching test

The β-carotene bleaching assay is based on the loss of the yellow colour of β-carotene due its reaction with radicals formed by linoleic acid oxidation in an emulsion (Silvestre et al., 2012). This test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O_2). The measurement of the amount of remaining β-carotene provides an estimate of the antioxidant potential of the sample (Bamoniri et al., 2010).

Table 4. Minimal inhibitory concentrations (M.I.C.) for the ethanolic extract of *C. frutescens* tested on different microorganisms.

Microorganisms	M.I.C. (mg/ml)
Gram positive bacteria	
Enterococcus faecalis	5
Bacillus subtillis	5
Staphylococcus aureus	5
Gram negative bacteria	
Escherichia coli	10
Klebsiella pneumoniae	10
Pseudomonas aeruginosa	25
Yeast	
Candida albicans	5

The rate of β-carotene bleaching can be slowed in the presence of antioxidants (Oke et al., 2009). The ethanolic extract of *C. frutescens* exhibited an antioxidant activity at all times tested. The percentage of antioxidant activity ranged from 15 to 47% (Figure 2). At 60 minutes (half of the total time), the antioxidant activity was 29%.

Pepper fruits have a wide array of phytochemicals with well-known antioxidant properties such as carotenoids, capsaicinoids and phenolic compounds particularly flavonoids, quercetin and luteolin (Zimmer et al., 2012). The antioxidant activity of pepper fruits can also be attributed to ascorbic acid. Therefore, the observation of a significant correlation between the antioxidant activity values and the concentrations of pepper constituents is noteworthy because other soluble compounds, besides polyphenols, present in extracts can affect the total antioxidant capacity (Hervert-Hernández et al., 2010).

Antimicrobial assay

The minimum inhibitory concentrations (MICs) in mg/ml for the ethanolic extract of *C. frutescens* are shown in Table 4. Our results show that all tested strains of Gram-positive and Gram-negative bacteria and of the yeast strain too were inhibited by the ethanolic extract of *C. frutescens*, but better inhibitory effects and lower MIC values were observed for the Gram-positive bacteria. Generally, due to their extra protective outer membrane and other particularities, Gram-negative bacteria are usually considerably more resistant to antibacterial agents than Gram-positive bacteria (Bamoniri et al., 2010). Nevertheless, the MIC values found in this study do not meet the stringent endpoint criteria used by some authors (Cos et al., 2006; Mbosso et al., 2010), which consider concentrations of up to 1 mg/ml for extracts or 0.1 mg/ml for isolated compounds to display antimicrobial activities. Chloramphenicol inhibited the growth of all the bacteria

tested and itraconazole inhibited the growth of the yeast. DMSO showed no antimicrobial activity against the microorganisms tested.

CONCLUSION

The ethanolic extract of pimenta malagueta showed antimicrobial and antioxidant activities, with high concentrations necessary for the extract to be effective. The antioxidant activity may be related to the presence of phenollics, such as the flavonoids found in some species of *Capsicum*. The subsequent isolation of compounds having antimicrobial activity could reduce the concentration needed to inhibit the microbial growth and the data can be used for further research to establish the relationship between specific bioactive compounds and antimicrobial activity.

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REFERENCES

Alvarez-Parrilla E, La Rosa LA, Amarowicz R, Shahidi F (2011). Antioxidant activity of fresh and processed Jalapeño and Serrano peppers. J. Agric. Food Chem. 59(1): 163-173.

Bamoniri A, Ebrahimabadi AH, Mazoochi A, Behpour M, Kashi FJ, Batooli H (2010). Antioxidant and antimicrobial activity evaluation and essential oil analysis of *Semenoviatragioides* Boiss. from Iran. Food Chem. 122: 553-558.

Barboza GE, Agra MF, Romero MV, Scaldaferro MA, Moscone EA (2011). New endemic species of *Capsicum* (Solanaceae) from the Brazilian Caatinga: comparison with the re-circumscribed *C. parvifolium*. Syst. Bot. 36(3): 768-781.

Boonkird S, Phisalaphong C, Phisalaphong M (2008). Ultrasound-assisted extraction of capsaicinoids from *Capsicum frutescens* on a lab- and pilot-plant scale. Ultrason. Sonochem. 15: 1075-1079.

Clinical and Laboratory Standarts Institute. CLSI. (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Wayne, PA: National Committee for Clinical Laboratory Standards. M7-A6.

Clinical and Laboratory Standarts Institute. CLSI. (2008). Method for broth dilution antifungal susceptibility testing of yeasts: proposed standard. 3rd. ed. Wayne, PA: CLSI. M27-A3.

Cos P, Vlietinck AJ, Berghe DV, Maes L (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol. 106: 290-302.

Cushnie TPT, Lamb AJ (2011). Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrob. Agents. 38: 99-107.

Daglia M (2012). Polyphenols as antimicrobial agents. Curr. Opin. Biotechnol. 23: 174–181.

Garcés-Claver A, Arnedo-Andrés MS, Abadia J, Gil-Ortega R, Álvarez-Fernández A (2006). Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruits by liquid chromatography–electrospray/time-of-flight mass spectrometry. J. Agric. Food Chem. 54(25): 9303-9311.

Ghasemnezhad M, Sherafati M, Payvast GA (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. J. Funct. Foods. 3: 44–49.

Gioti ME, Fiamegos YC, Skalkos DC, Stalikas CD (2009). Antioxidant activity and bioactive components of the aerial parts of *Hypericum perforatum* L. from Epirus, Greece. Food Chem. 117: 398-404.

Hayman M, Kam PCA (2008). Capsaicin: a review of its pharmacology and clinical applications. Curr. Anaesth. Crit. Care. 19: 338-343.

Hervert-Hernandez D, Sayago-Ayerdi SG, Goñi I (2010). Bioactive compounds of four hot pepper varieties (*Capsicum annuum* L.), antioxidant capacity and intestinal bioaccessibility. J. Agric. Food Chem. 58(6): 3399-3406.

Howard LR, Talcott ST, Brenes CH, Villalon B (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J. Agric. Food Chem. 48(5): 1713-1720.

Isabelle M, Lee BL, Lim MT, Koh WP, Huang D, Ong CN (2010). Antioxidant activity and profiles of common vegetables in Singapore. Food Chem. 120: 993-1003.

Kähkönen MP, Hopia AI, Heinonen M. (2001). Beery phenolics and their antioxidant activity. J. Agric. Food Chem. 49(8): 4076–4082.

Li F, Lin Y, Wang X, Geng Y, Wang D (2009). Preparative isolation and purification of capsaicinoids from *Capsicum frutescens* using high-speed counter-current chromatography. Sep. Purific. Technol. 64: 304–308.

Locatelli M, Gindro R, Travaglia F, Coïsson J, Rinaldi, M, Arlorio M (2009). Study of the $DPPH^{\bullet}$ - scavenging activity: development of a free software for the correct interpretation of data. Food Chem. 114: 889-897.

Martin KR, Appel CL (2010). Polyphenols as dietary supplements: a double-edged sword. Nutri. Diet. Sup. 2: 1–12.

Martysiak-Żurowska D, Wenta W (2012). A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci. Pol. Technol. Aliment. 11(1): 83-89.

Mbosso EJT, Ngouela S, Nguedia JCA, Beng VP, Rohmer M, Tsamo E (2010). *In vitro* antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. J. Ethnopharmacol.128: 476-481.

Menichini F, Tundis R, Bonesi M, Loizzo MR, Conforti F, Statti G, De Cindio B, Houghton PJ, Menichini F (2009). The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinese* Jacq. cv Habanero. Food Chem. 114: 553-560.

Oke F, Aslim B, Ozturk S, Altundag S (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem. 112: 874-879.

Ornelas-Paz JJ, Martínez-Burrola JM, Ruiz-Cruz S, Santana-Rodríguez V, Ibarra-Junquera V, Olivas GI, Pérez-Martínez JD (2010). Effect of cooking on the capsaicinoids and phenolics contents of Mexican peppers. Food Chem. 119: 1619-1625.

Ozguven M, Yaldiz G (2011). Capsaicin contents of different *Capsicum* (Red Peppers) populations and varieties. Adv. Environ. Biol. 5, 1991-1995.

Ranilla LG, Kwon YI, Apostolidis E, Shetty K (2010). Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour. Technol. 101(12): 4676-4689.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9/10): 11231-1237.

Silva TMS, Câmara CA, Lins ACS, Barbosa JM, Silva EMS, Freitas BM, Santos FAR (2006). Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke. J. Food Compos. Anal. 19: 507-511.

Silvestre RG, Moraes MM, Lins ACS, Ralph MT, Lima-Filho JV, Camara CA, Silva TMS (2012). Chemical composition, antibacterial and antioxidant activities of the essential oil from *Vismia guianensis* fruits. Afr. J. Biotechnol. 11(41): 9888-9893.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 299: 152-178.

Slinkard K, Singleton VL (1977). Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Viticult. 28(1): 49-55.
Zhuang Y, Chen L, Sun L, Cao J (2012). Bioactive characteristics and antioxidant activities of nine peppers. J. Funct. Foods. 4: 331-338.
Zimmer AR, Leonardi B, Miron D, Schapoval E, Oliveira JR, Gosmann G (2012). Antioxidant and anti-inflammatory properties of *Capsicum baccatum*: from traditional use to scientific approach. J. Ethnopharmacol. 139: 228-233.