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Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.)

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Abstract

An aqueous coriander extract obtained through a sequential extraction process, was analysed using chromatography and mass spectrometry in order to identify the phenolic compounds responsible for its antioxidant activity. Four fractions were identified from the crude extract using chromatography in a silica gel column. Their antioxidant activity, according to the β-carotene/linoleic acid model, was similar to one another but inferior to that of the crude extract and of butylated hydroxytoluene. Of the phenols identified through gas chromatography and mass spectrometry, it was noted that caffeic acid was present in high concentration (4.34 μg/ml in fraction I and 2.64 μg/ml in fraction III), whereas protocatechinic acid and glycitin were present in high concentration in fraction II (6.43 μg/ml) and fraction IV (3.27 μg/ml), respectively. These results, when considered with the recognized antioxidant ability of phenolic acids, suggest that they are principal components responsible for the antioxidant activity of the aqueous coriander extract.

Keywords: Spice; Coriander; Antioxidant; Aqueous extract; Phenolic compounds

1. Introduction

The phenolic compounds or polyphenols, secondary vegetal metabolites, constitute a wide and complex array of phytochemicals that exhibit antioxidant action and consequently a beneficial physiological effect (Bravo, 1998; Martinez-Valverde, Periago, & Ros, 2000). Their ability to delay lipid oxidation in foodstuffs and biological membranes, in addition to their propensity to act as a prophylactic agent has motivated research into food science and biomedicine (Farombi, Britton, & Emerole, 2000). Considering their bioactivity and their presence in a wide range of vegetables, these substances are considered natural antioxidants and the vegetable source that it contains as functional food (McDonald, Prenzler, Antolovich, & Robards, 2001).

Phenolic substances with an antioxidant activity, including phenolic acids and flavonoids, have been isolated from a variety of sources: rosemary and sage (Wu, Lee, Ho, & Chang, 1982; Houlihan, Ho, & Chang, 1985; Cuvelier, Berset, & Richard, 1994; Okamura, Haraguchi, Hashimoto, & Yagi, 1994; Lu & Foo, 2001) oregano, thyme and pepper (Nakatani, 1992; Vekiari, Oreopoulou, Tzia, & Thomopoulos, 1993). Melo (2002) noted in the aqueous coriander extract (*Coriandrum sativum*) 2.734 mg of total phenolics (catechin equivalents) per 100 g of dry sample, exhibiting considerable antioxidant activity. This finding formed the main support for the present study, the aim of which was to identify the principle components responsible for this activity.

2. Material and methods

The coriander (*Coriandrum sativum* L.) donated by the Agronomic Department—UFRPE, Brazil, was transported immediately after harvest to the Food

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Analysis and Experimentation Laboratory of the Nutrition Department of UFPE, where the study was performed. After copious washing in running water, the roots were removed and the leaves and shoots were laid out on a nylon mesh and dried with forced circulating air at 45°C for 48 h. The dried product was ground into a fine power, passed through an 80-mesh sieve and kept frozen at −18°C in polyethylene bags during the study.

2.1. Extract obtainment

The aqueous extract was obtained through sequential extraction where the coriander powdered (10 g) was submitted to extraction with ethyl ether (100 ml), for 60 min, under agitation at room temperature (25°C ± 2°C), and then the mixture was centrifuged at 3000*g* for 10 min. After transferring the supernatant into flask, the residue resuspended in ethyl ether (100 ml) and again submitted to the same extraction process. The supernatants were combined and the residue reused for ethanol extraction and, subsequently, with distilled water and submitted to extraction process as described above. The ether and ethanol extracts were stored for later studies and the aqueous extracts were transferred to amber flasks, flushed with nitrogen and stored in a freezer at −18°C until used for analysis.

2.2. Aqueous extract fractionating

The coriander water extract was fractionated by silica gel column (1.5 × 21 cm, 60–200 mesh—Merck). The following solvents were used for elution:ethyl ether:petroleum ether:methanol:water (20:45:35:10, v/v); chloroform:methanol (65:35, v/v); methanol:water (25:75 and 60:30, v/v) and water, with a flow-rate of 2.5 ml/min, were collected in tubes 10 ml/fractions. Each fractions obtained was investigated with regards the presence of phenolic compounds using Folin-Ciocalteau reagent (Merck), according to the procedure described by Wettasinghe and Shahidi (1999). The fractions were pooled according to the absorbance peaks recorded at 725 nm.

The crude extract and the fractions obtained from the column chromatographic were submitted to thin-layer chromatography (TLC) in silica gel plates (60-F_{254}, 20 × 20 cm with thickness 0.25 mm-Merck), using BAW (butanol:acetic acid:water—4:1:5, v/v/v), as mobile phase. Detection of spots on TLC plates was conducted under UV light (366 nm), ammonia vapour and spraying with ferric chloride/potassium ferrocyanide reagent (aqueous solution of $FeCl_3$ 1% and aqueous solution of $K_3Fe(CN)_6$−1:1). The Rf values were calculated for each visible spots.

Fractions aliquots from chromatographic column (0.5 ml) were submitted to nitrogen to evaporate all solvent, before and after the addition of 0.25 ml of the internal standard (heptadecanoic acid methyl ester) at the concentration of 0.256 mg/ml. Subsequently, dried fractions were derivatized by addition of 0.5 ml of BSA [N, O, bis acrilamida] and heated in a water-bath at 50°C for 30 min. There after, the samples were injected into the HP 6890 gas chromatograph (version A 03.03) that was coupled to the HP 5973 mass spectrometer. A fused-silica capillary column (25 cm × 0.2 mm), covered with DB5 (J & W) was used. The flame ionization detector was connected to a computer, the initial temperature of 112°C held for 3 min and programmed up to 290°C, the injector temperature was 250°C and the detector was 300°C. The identification of the phenolic compounds present in the fractions was based on samples relative retention times, in comparison to the standard phenolics chromatograms (Sigma) and their mass spectra.

2.3. Total phenolic compounds

The concentration of total phenolic compounds in fractions obtained through column chromatography was measured with the Folin-Ciocalteau reagent (Merck), according to procedure described by Wettasinghe and Shahidi (1999) using catechin as a standard. Suitable aliquots of the fractions were taken in a test tube. Then, 0.5 ml of Folin-Ciocalteau reagent and 1 ml of saturated sodium carbonate solution were added sequentially in each tube. The total volume of the system was adjusted to 10 ml with distilled water. The tubes were vortexes, placed in the dark for 60 min and the absorbance was recorded at 725 nm. The results were expressed as microgram of total phenolic in catechin equivalent per milliliter of the fraction.

2.4. Antioxidant activity

The antioxidant activity of the fractions, containing approximately 200 μg of total phenolic compounds, was determined by the coupled oxidation of β-carotene and linoleic acid, according to the methodology described by Marco (1968) and modified by Hammerschmidt and Pratt (1978). A solution of β-carotene was prepared by dissolving 1.0 mg of β-carotene in 10 ml of chloroform. 1.0 ml of this solution was pipetted into a round-bottom flask, which contained 20 mg linoleic acid and 200 mg Tween 40 emulsifier. After removal of chloroform on a rotary evaporator at 50°C, 50 ml of aerated distilled water were added to the flask with vigorous shaking. Aliquots (5 ml) of this emulsion were transferred into a series of tube that contained 0.2 ml of the antioxidant solution being tested. Tubes were placed in a water bath at 50°C. Reading at 470 nm were taken at 15 min intervals for 105 min. Antioxidant activity was subsequently expressed as an oxidation inhibition percentage, calculated in relation to the 100% oxidation that occurs

in the control (no antioxidant). The BHT antioxidant activity was determined under the same conditions as a means of comparison.

2.5. Statistical analysis

Results were processed by analysis of variance (ANOVA) using the general linear models procedure to compare due the inhibition of oxidation that was used as independent variable. The Tukey test was used for comparison the antioxidant activity of individual fractions of aqueous coriander extract. The analysis was performed using the statistical programme "Minitab for Windows" (Minitab Inc., State College, PA, USA). Evaluations were based on a significant level of $P<0.05$.

3. Results and discussion

Seven fractions were identified from the crude aqueous extract that was submitted to thin-layer chromatography. The fractions fluoresced under UV light (366 nm), became shiny and well-defined when exposed to the ammonium vapour, and became blue after spraying with $FeCl_3/K_3Fe(CN)_6$ reagent, confirming the presence of phenolic compounds. However, four fractions were obtained by column chromatography according to peaks clearly defined at 725 nm, which resulting from positive reaction to phenolic compounds (Fig. 1).

The total phenolic compounds level in the fractions I, II, III and IV, eluted with ethyl ether:petroleum ether:methanol:water (20:45:35:10, v/v), chloroform:-methanol (65:35, v/v), methanol:water (25:75 and 60:30, v/v) and water, respectively (Table 1) was determined by Folin-Ciocalteau reagent. The fraction II presented the greatest concentration of these compounds.

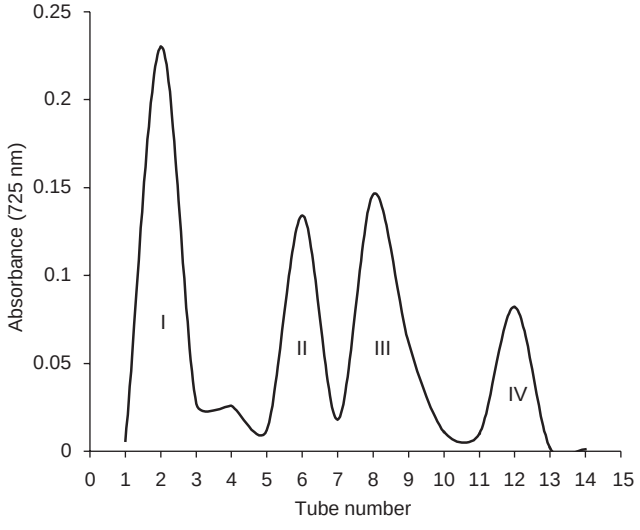

Fig. 1. Column chromatographic fractionation elution profile for aqueous extract from coriander.

The four fractions demonstrated by thin-layer chromatography that each one was composed of various phenolic compounds, whose chromatograph characteristic can be found in Table 2. Amarowicz, Wanasundara, Karamac, & Shahidi (1996); Amarowicz, Karamac, Wanasundara, and Shahidi (1997) reported similar findings by observing four and five fractions using the mustard ethanol extract, and the flaxseed methanol extract, respectively. Mancini Filho, Van-Voiij, Mancini, Cozzolino, and Torres (1998) obtained five fractions using the cinnamon aqueous extract. All the fractions were composed of various phenolic compounds.

The antioxidant activity was similar in all four the fractions (I, II, III and IV), less than, however, the BHT and the crude aqueous extract (69.83%) (Fig. 2). These data were compared by Tukey test and the evaluations were based on a significant level of $P<0.05$. Gu and Weng (2001) reported that the crude extract of *Salvia plebeia* R.Br, a Chinese herbal medicine, had greater antioxidant activity than individual fractions, probably due to a synergy between the phenolic compounds and/or between these and other constituents. According to Shahidi (2000), the mechanisms by which the phenolic

Table 1
The concentration of total phenolic compounds in aqueous coriander extract fractions

Fractions	Catechin equivalents (μg/ml)
I	5.88±0.13
II	17.08±0.90
III	4.42±0.22
IV	5.62±0.40

Each value is mean±standard deviation of three replicate analyses.

Table 2
Chromatographic characteristic of fractions of aqueous extract of coriander

Fractions	UV	UV + NH_3	$FeCl_3/K_3Fe(CN)_3$	Rf[a]
	Bright yellow	Bright yellow	Blue	0.86
I	Bright green	Bright green	Blue	0.67
	Bright green	Bright green	Blue	0.45
	Blue	Blue	Blue	0.84
	Bright yellow	Bright yellow	Blue	0.80
II	Bright yellow	Bright yellow	Blue	0.73
	Blue	Blue	Blue	0.65
	Bright green	Bright green	Blue	0.63
	Bright green	Bright green	Blue	0.54
	Bright green	Bright green	Blue	0.41
	Bright yellow	Bright yellow	Blue	0.82
III	Bright yellow	Bright yellow	Blue	0.72
	Yellow	Yellow	Blue	0.88
IV	Bright green	Bright green	Blue	0.53
	Bright green	Bright green	Blue	0.38

[a] Rf value using BAW (butanol:acetic acid:water—4:1:5).

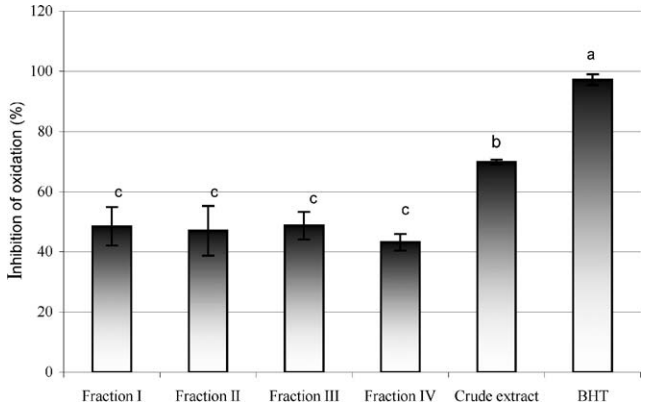

Fig. 2. Antioxidant activity of individual fractions of aqueous coriander extract. Reported values are mean ± standard deviations of three replications. Bars sharing the same letter are not significantly ($P > 0.05$-Tukey test) different from one another.

Table 3
Phenolic compounds in aqueous coriander extract fractions identified by gas chromatography and mass spectrometry

Phenolic compounds	Fractions			
	I	II	III	IV
Catechol (μg/ml)	0.0	0.0	0.0	0.25
Salicylic acid (μg/ml)	0.69	0.0	0.0	0.0
Glycitin (μg/ml)	0.0	0.0	1.77	3.27
Pyrogallol (μg/ml)	0.85	1.85	0.0	0.0
Gentisic acid (μg/ml)	0.0	1.16	0.0	0.0
Protocatechinic acid (μg/ml)	0.0	6.43	0.0	0.0
Quinic acid (μg/ml)	0.0	0.88	0.0	0.0
Caffeic acid (μg/ml)	4.34	2.44	2.64	2.07
No identified	0.0	4.44	0.0	0.0
Total	5.88	17.08	4.42	5.62

compounds exert their antioxidant effect are varied and may involve several mechanisms. In this context, identifying the compounds should help to elucidate the mode of action of each one.

Table 3 shows the fractions and their components as determined by gas chromatography and mass spectrometry. The results demonstrate that all the fractions contain caffeic acid, a phenolic that is correlated to the derivatives of hydroxycinnamic acids group, the principal component that process antioxidant activity (Godow, Joubert, & Hansmann, 1997; McDonald et al., 2001; Meyer, Heinonen, & Frankel, 1998; Pratt & Birac, 1979). This action is due to its orthodihydroxybenzene structure that facilitates the donation of hydrogen and the existence of an unsaturated aliphatic chain located on the aromatic ring, which increases its stability of the phenoxy radical by resonance (Lu & Foo, 2001; Moran, Klucas, Grayer, Abian, & Bacana, 1997).

It was observed that fraction II, only contain protocatechinic acid, in conjunction with the smallest concentration of caffeic acid results in an antioxidant activity comparable to that of the fractions I and III. Protocatechinic acid, found widely distributed in nature, has demonstrated effectiveness at inhibiting the human LDL catalysed by copper (Masella et al., 1999; Zhang, et al., 2001). This effect, according to Zhang et al. (2001), was less than that of chlorogenic acid, quercetin and rutin, however comparable to caffeic acid (Masella et al., 1999). Moreira, Torres, and Mancini Filho (2001) reported that this component showed 50% lipid oxidation inhibition in the β-carotene/linoleic acid model system. These findings support the results obtained for fraction II in this study.

For fractions III and IV, apart from caffeic acid, a considerable proportion of glycitin was noted. This is an isoflavone found predominantly in vegetables from the *Leguminosae* family especially soybeans which, in addition, contains genistin, daidzin and their respective aglycones (glycitein, genistein and daidzein) (Naim, Gestetner, Zilkah, Birk, & Bondi, 1974). It is worthwhile reiterating that glycitin differs from genistin and daidzin by possessing a hydroxy group at the C4 and a methoxy group at the C6 positions, respectively (Arora, Nair, & Strasburg, 1998; Merken & Beecher, 2000; Naim et al., 1974), a characteristic that seems to be responsible for its reduced antioxidant action, and therefore explaining the result obtained with fraction IV.

Although present in their free form in some vegetables (Harborne, 1973), the presence of quinic acid, catechol and pyrogallol in come fractions could have been as a result of the extraction process, despite all efforts to minimize this possibility. Quinic acid is considered a phenolic for being a precursor of many aromatic compounds in vegetable metabolism (Harborne, 1973; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). Moreira (1999) noted its antioxidant effect (69.15%), after extracting it from mustard-seed flour.

According to the literature, the antioxidant action of the compounds identified in these fractions could be due to the main phenolic compounds, their combined effect or even a common substance to all the fractions. This last hypothesis becomes a distinct possibility when considering that the fractions III and IV contain only two components (glycitin and caffeic acid), in inverse proportions with no consequent difference in their antioxidant ability. Fraction IV, with a similar caffeic acid concentration that of fraction II, demonstrated an antioxidant activity slightly lower, despite the presence of glycitin. This associative effect was also repeated in fraction II whose antioxidant activity was similar to that of fractions I and III.

4. Conclusions

The reported data indicated that the four coriander aqueous extract fractions possess similar antioxidant

activity that can be measured by the β-carotene/linoleic acid system. The four fractions contained several phenolic acids, and caffeic acid was presented in all four fractions. The phenolic acids are related to the antioxidant activity of coriander aqueous extract.

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