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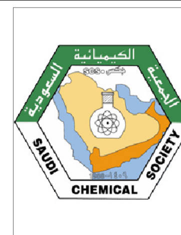

ORIGINAL ARTICLE

Antioxidant activity of methanolic extracts from three coriander (*Coriandrum sativum* L.) fruit varieties

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Abstract In this study, fruit methanolic extract of three coriander (*Coriandrum sativum* L.) varieties (Tunisian, Syrian and Egyptian) was assayed for their antioxidant activities. Obtained results showed that there are significant ($P < 0.05$) variations in total polyphenols (0.94 ± 0.05–1.09 ± 0.02 mg GAE/g DW), total flavonoids (2.03 ± 0.04–2.51 ± 0.08 mg EC/g DW) and total condensed tannin (0.09 ± 0.01–0.17 ± 0.01 mg EC/g DW) contents. The RP-HPLC analysis revealed the identification of phenolics in coriander fruits with chlorogenic and gallic acids as main compounds in Tunisian, Syrian and Egyptian varieties, respectively. Moreover, fruit methanolic extracts exhibited remarkable DPPH radical scavenging activity with IC_{50} values ranged from 27.00 ± 6.57 to 36.00 ± 3.22 μg/mL. EC_{50} values of reducing power activity varied significantly ($P < 0.05$) from 54.20 ± 6.22 to 122.01 ± 13.25 μg/mL. The IC_{50} values of β-carotene bleaching assay were between 160.00 ± 18.63 and 240.00 ± 26.35 μg/mL. Our results indicated that coriander fruit might constitute a rich and novel source of natural antioxidants and may be suggested as a new potential source of natural antioxidant and could be used as food additive.
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1. Introduction

Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules, and thus prevent or repair the damage of body cells caused by oxygen (Shahidi and Naczk, 2004; Tachakittirungrod et al., 2007). Various studies have focused on natural antioxidants in plant kingdom and their applications in food systems to prevent oxidation. The most widely used synthetic antioxi-

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dants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability or their suspected action as promoters of carcinogenesis (Namiki, 1990). For this reason, there is a growing interest in the studies of natural healthy (nontoxic) additives as potential antioxidants (Tomaino et al., 2005). In addition, Phenolic compounds are secondary plant metabolites that possess in common an aromatic ring bearing one or more hydroxyl substituent's. They are water soluble and may occur combined with a sugar molecule, as glycoside (Harbone, 1998). They are divided into sub-groups and these include phenols, phenolic acids, phenylpropanoids, flavonoids, flavones, glycoflavonones and biflavonyls, minor flavonoids, aurones, flavonones, dihydrochalcones, isoflavones, xanthones and stilbenes, hydrolysables and condensed (proanthocyanidines) tannins and quinines (Strack, 1997; Harbone, 1998). They are reported to have diverse biological activities ranging from toxicity to hormonal mimicry and act as cell wall material, colorful attractants for birds and insects helping seed dispersal and pollinization. These compounds also act as defense mechanisms of plants under different environmental stress conditions such as wounding, infection, excessive light or UV irradiation (Harbone, 1998) and constitute a wide and complex array of phytochemicals that exhibit antioxidant action and consequently a beneficial physiological effect (Bravo, 1998; Martinez-Valverde et al., 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 in food science and biomedicine (Farombi et al., 2000; Jaffel et al., 2011). Considering their bioactivity and their presence in a wide range of vegetables, these substances are considered as natural antioxidants and the vegetable source that it contains as functional food (McDonald et al., 2001). Phenolic substances with an antioxidant activity, including phenolic acids and flavonoids, have been isolated from a variety of sources such as rosemary and sage (Wu et al., 1982; Houlihan et al., 1985; Cuvelier et al., 1994; Okamura et al., 1994; Lu and Foo, 2001) oregano, thyme and pepper (Nakatani, 1992; Vekiari et al., 1993). Melo (2002) noted in the aqueous coriander extract 2.73 mg of total phenolics (catechin equivalents) per 100 g of dry sample, exhibiting considerable antioxidant activity.

On the other hand, our previous investigations on the compositional analysis of *Coriandrum sativum* L. fruits have described essential oil changes during maturation (Msaada, 2007; Msaada et al., 2006, 2007a, 2009a), essential oil composition of different coriander parts (Msaada et al., 2003, 2007b), fatty acid composition of fruits (Msaada et al., 2009b), effects of stage of maturity and growing region on fatty acid composition (Msaada et al., 2009c, 2010), regional and maturational effects on essential oils yields and composition (Msaada et al., 2009d), effects of crop season and maturity stage on the yield and composition of coriander fruit essential oil (Msaada et al., 2012) and variation in glycerolpipids and their fatty acids composition during maturation of coriander fruits (Msaada et al., 2011).

The composition of phenolic fraction present in coriander is still incompletely studied and some data are contradictory. To the best of our knowledge, there are no reports on the antioxidant capacities of coriander fruits cultivated in Tunisia, Syria and Egypt. In this work, we evaluated for the first time the total polyphenols, flavonoids and condensed tannin contents and the antioxidant potential of the methanolic extracts of coriander fruit by DPPH, reducing power and β-carotene/linoleic acid assays. The results could provide information about the potential utility of *C. sativum* L. as a raw material source for industrial utilization of phenolic components.

2. Materials and methods

2.1. Chemicals

All reagents and solvents used in these experiments (diethyl ether, chloroform, acetonitrile and methanol) were purchased from Merck (Darmstadt, Germany). Sulfuric acid (H_2SO_4), acetic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), β-carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), polyvinyl polypyrolidone Folin–Ciocalteu reagent and aluminum chloride ($AlCl_3$) were purchased from Sigma–Aldrich (Steinheim, Germany). Vanillin, catechin, gallic acid and tween 40 were purchased from Fluka (Biochemika, Switzerland). Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminum foil and stored at 4 °C. All other used chemicals were of analytical grade.

2.2. Plant material

Three coriander fruits varieties (Tunisian, Syrian and Egyptian) were purchased from the local supermarket in Menzel Temime (North Eastern of Tunisia). Fruits were at full ripeness and were completely dried at air temperature. The obtained fruits were analyzed before the expiry date. They were manufactured for food flavoring purposes. Fruits were kept at room temperature before extraction.

2.3. Polyphenol extraction

The air-dried fruits were finely ground with a blade-carbide grinding (IKA-WERK Type: A: 10). Triplicate sub-samples of 3 g of each ground variety were separately extracted by stirring with 10 mL of pure methanol for 30 min. The extracts were then kept for 24 h at 4 °C, filtered through a Whatman No. 4 filter paper, evaporated under vacuum to dryness and stored at 4 °C until their analysis (Mau et al., 2001).

2.4. Total polyphenol content

The amount of the total polyphenol was assayed colorimetrically by using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). An aliquot (0.125 mL) of a suitable diluted methanolic fruit extract was added to 0.5 mL of deionized water and 0.125 mL of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23 °C, the absorbance versus prepared blank was read at 760 nm. Total

polyphenol contents of coriander fruit (three replicates per treatment) were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL (R^2 = 0.99). All samples were performed in triplicates.

2.5. Total condensed tannin content

Total condensed tannin content was determined by the Folin–Ciocalteu procedure, as described above, after their adsorption onto hide powder of the polyvinyl polypyrrolidone (PVPP) (Hagerman and Butler, 1978). In brief, samples of plant extract were homogenized with adsorbent material and the mixture was stirred for 30–60 min, the obtained preparation was stored for 1–2 h at +4 °C to develop tannin–PVPP complex. Then the pH was acidified (pH 3) in case of using PVPP. After centrifugation at 4000 rpm/15 min, no adsorbed phenolics in the supernatant were determined by the Folin–Ciocalteu procedure as described above. Calculated values were subtracted from total polyphenol contents and the amount of total condensed tannins expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. The calibration curve range was 50–400 mg/mL (R^2 = 0.99).

2.6. Total flavonoid content

Total flavonoid content was measured according to the method described by Dewanto et al. (2002). Two hundred and fifty microliter of methanolic extract of coriander fruit was appropriately diluted and mixed with 75 μL $NaNO_2$ (5%). After 6 min, 150 μL of 10% $AlCl_3$ and 500 μL of NaOH (1 M) were added to the mixture. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid contents of coriander fruit (three replicates per treatment) were expressed as mg of catechin equivalents per gram of dry weight (mg CE/g DW) through the calibration curve with catechin. The calibration curve range was 50–500 mg/mL.

2.7. Hydrolysis and identification of phenolic compounds using RP-HPLC

Dried samples from coriander fruit were hydrolyzed according to the method of Proestos et al. (2006) which was slightly modified. The acidic hydrolysis was used to release the aglycones in order to simplify the identification process since the free forms of phenolic compounds are rarely present in plants and they occur as esters, glycosides or bound to the cell wall (Nuutila et al., 2002). Twenty milliliter of methanol containing BHT (1 g/L) was added to 0.5 g of a dried sample. Then 10 mL of 1 M HCl was added. The mixture was stirred carefully and sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was injected to HPLC. The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatography (RP-HPLC) coupled with an UV–vis multiwavelength detector. The separation was carried out on a 250 × 4.6-mm, 4 μm Hypersil ODS C_{18} reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulfuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15%A/85%B 0–12 min, 40%A/60%B 12–14 min, 60%A/40%B 14–18 min, 80%A/20%B 18–20 min, 90%A/10%B 20–24 min, 100%A 24–28 min (Bourgou et al., 2008). The injected volume was 20 μL, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 μm membrane filter before injection. Phenolic compounds were identified according to their retention times and spectral characteristics of their peaks against those of standards, as well as by comparing the sample with standards. Analyses were performed in triplicate.

2.8. DPPH assay

The electron donation ability of the obtained extracts was measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Hanato et al. (1988). One milliliter of different concentrations of extracts (1, 10, 100 and 200 μg/mL) prepared in methanol was added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was vigorously shaken and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (μg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample after 30 min. BHT was used as a positive control. Tests were carried out in triplicate.

2.9. β-Carotene bleaching test

The β-carotene bleaching method is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). A modification of the method described by Koleva et al. (2002) was employed. β-Carotene (2 mg) was dissolved in 20 mL of chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Reference compound (BHT), sample extracts were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of different concentrations of extract (1, 10, 100 and 200 μg/mL). The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50 °C for 120 min, when the absorbance was measured again. BHT was used as the positive control. In the negative control, the extract was substituted by an equal volume of methanol. The antioxidant activity (%) of the coriander fruit extracts was evaluated in terms of the bleaching of the β-carotene using the following formula:

$$\% \text{ Inhibition} = \frac{A_t - C_t}{C_0 - C_t} \times 100$$

where A_t and C_t are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and C_0 is the absorbance values for the control measured at zero time during the incubation. The results are expressed as IC_{50} values (μg/mL), the concentration required to cause a 50% β-carotene bleaching inhibition. Tests were carried out in triplicate.

2.10. Reducing power

In this assay, the yellow color of the test solution changes to green depending on the reducing power of the test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by the measurement of the absorbance at 700 nm (Zou et al., 2004). The method of Oyaizu (1986) was used to assess the reducing power of different coriander fruit extracts. One milliliter of different concentrations of fruit extracts (1, 10, 100 and 200 μg/mL) in methanol was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6, prepared from 62.5 mL of a 0.2 M Na_2HPO_4 and 37.5 mL of 0.2 M $NaH_2PO_4H_2O$) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ and incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged at 650*g* for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the blue–green color was measured at 700 nm. The EC_{50} value (mg/mL) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as the positive control. Tests were carried out in triplicate.

2.11. Statistical analyses

Results are presented as means ± standard deviation (SD) from three replicates of each experiment. A P value < 0.05 was used to denote significant differences among mean values determined by the analysis of variance with the assistance of "Statistica v 5.1" software (Statsoft, 1998). The means were compared by using the one-way and multivariate analysis of variance (ANOVA) followed by Duncan's multiple range tests. A principal component analysis (PCA) was performed in order to discriminate between the three studied coriander varieties on the basis of their phenolic percentage composition.

3. Results and discussion

3.1. Total polyphenol, total flavonoid and total condensed tannin contents

Results from the quantitative determination of total polyphenols, total condensed tannins and total flavonoids of the methanolic extracts of the three coriander fruit varieties are summarized in Table 1. Total flavonoids and total condensed tannin contents were determined as catechin equivalents in milligrams per gram of dry weight (mg CE/g DW), while total polyphenol contents were calculated as gallic acid equivalents in milligrams per gram of dry weight (mg GAE/g DW) The total polyphenol contents varied significantly ($P < 0.05$) between the three studied varieties, Syrian coriander extract had higher total polyphenol content (1.09 ± 0.02 mg GAE/g) than Tunisian (1.00 ± 0.06 mg GAE/g) and Egyptian (0.94 ± 0.05 mg GAE/g) ones. The previous study showed that Tunisian coriander methanolic extract had a polyphenol content of 1.04 mg GAE/g DW (Neffati et al., 2010). Sriti et al. (2011) reported that total polyphenol contents found in coriander fruit methanolic extracts were 15.16 mg GAE/g DW for Canadian variety and 12.10 mg G GAE/g DW for Tunisian one. Wangensteen et al. (2004) reported that the extraction of total polyphenols with ethyl acetate from coriander fruit samples from Norway contained 1.9 g GAE/100 g DW. On the other hand, Ali Al-Mamary, 2002 found that polyphenol contents of coriander cultivated in Yemen were 701.21 ± 6.07 mg/100 g DW. The difference in the extraction yield could be the result of using different extraction solvents in other works and methanol in ours. The importance of the solvent type used in the extraction has already been mentioned (Liu et al., 2007; Salem et al., 2011). These authors showed significant variability on phenolic contents in the same extract when using solvents with different polarities.

The highest total flavonoid content was obtained in Syrian variety (2.51 ± 0.08 mg CE/g DW) followed by Egyptian (2.07 ± 0.05 mg CE/g DW) and Tunisian (2.03 ± 0.04 mg CE/g DW) ones and varied significantly ($P < 0.05$) between them (Table 1). It is well known that an important function of flavonoids and phenolic acids was their action in plant defense mechanisms (Dixon and Paiva, 1995). Indeed, flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release, potent nitric oxide radical scavenging activity and exhibiting antibacterial, antivi-

Table 1 Total polyphenol, flavonoid and tannin contents, DPPH·scavenging activity, reducing power and β-carotene bleaching of three varieties of coriander fruit methanolic extracts.

	Coriander varieties				BHT	Ascorbic acid
	P value	Tunisia	Syria	Egypt		
Phenolic contents (mg GAE/g DW)	0.000***	1.00 ± 0.06[b]	1.09 ± 0.02[a]	0.94 ± 0.05[c]		
Flavonoid contents (mg CE/g DW)	0.000***	2.03 ± 0.04[c]	2.51 ± 0.08[a]	2.07 ± 0.05[b]		
Tannin contents (mg CE/g DW)	0.000***	0.09 ± 0.01[c]	0.17 ± 0.01[a]	0.16 ± 0.01[ab]		
DPPH·scavenging activity (IC_{50} μg/mL)	0.000***	27.00 ± 6.57[c]	36.00 ± 3.22[a]	32.00 ± 2.87[b]	18.00 ± 1.61	
Reducing power (EC_{50} μg/mL)	0.000***	122.01 ± 13.25[a]	54.20 ± 6.22[c]	56.11 ± 7.45[b]		40.00 ± 5.23
β-Carotene bleaching (IC_{50} μg/mL)	0.000***	160.00 ± 18.63[b]	240.00 ± 26.35[a]	240.00 ± 25.84[a]	85.00 ± 9.68	

Values are means of triplicates ± SD. Values in the same row with different superscripts (a–c) are significantly different at $P < 0.05$.
*** $P < 0.001$.

Table 2 Contents (µg/g) and percentages (%) of phenolic compounds of Tunisian, Syrian and Egyptian coriander fruit varieties.

Compounds	Tunisia		Syria		Egypt		*P* value
	µg/g	%	µg/g	%	µg/g	%	
Phenolic acids	797.88 ± 82.45^{A}	81.47 ± 9.68^{a}	413.43 ± 52.79^{C}	38.66 ± 4.22^{c}	459.43 ± 51.74^{B}	49.17 ± 6.85^{b}	0.000***
Gallic acid	84.37 ± 9.68^{B}	8.61 ± 0.98^{b}	147.65 ± 16.14^{A}	13.81 ± 1.58^{a}	74.12 ± 9.55^{C}	7.93 ± 6.52^{c}	0.000***
Chlorogenic acid	147.8 ± 16.39^{A}	15.09 ± 2.33^{a}	76.61 ± 8.46^{B}	7.16 ± 8.22^{b}	14.07 ± 1.91^{C}	1.51 ± 1.66^{c}	0.000***
Cafeic acid	87.6 ± 10.21^{A}	8.94 ± 9.55^{a}	6.73 ± 0.88^{C}	0.63 ± 0.08^{c}	34.95 ± 4.11^{B}	3.74 ± 0.40^{b}	0.000***
Vanillic acid	73.54 ± 8.46^{B}	7.51 ± 8.59^{a}	76.24 ± 7.81^{A}	7.13 ± 0.86^{ab}	37.37 ± 4.97^{C}	4.00 ± 0.34^{b}	0.000***
p-Coumaric acid	43.02 ± 5.26^{A}	4.39 ± 0.54^{a}	24.9 ± 3.24^{C}	2.33 ± 0.31^{c}	29.05 ± 3.21^{B}	3.11 ± 0.40^{b}	0.000***
Ferulic acid	108.4 ± 11.23^{A}	11.07 ± 2.01^{a}	64.69 ± 7.29^{B}	6.05 ± 0.81^{b}	43.7 ± 5.11^{C}	4.68 ± 0.50^{c}	0.000***
Rosmarinic acid	47.3 ± 5.26^{B}	4.83 ± 0.56^{b}	5.06 ± 0.61^{C}	0.47 ± 0.07^{c}	83.56 ± 9..21^{A}	8.94 ± 0.97^{a}	0.000***
o-Coumaric acid	89.87 ± 10.53^{A}	9.18 ± 0.99^{a}	2.61 ± 0.31^{C}	0.24 ± 0.03^{c}	14.11 ± 1.65^{B}	1.51 ± 0.17^{b}	0.000***
trans-Hydroxycinnamic acid	41.91 ± 5.28^{B}	4.28 ± 0.53^{b}	2.48 ± 0.26^{C}	0.23 ± 0.03^{c}	64.96 ± 7.52^{A}	6.95 ± 0.77^{a}	0.000***
Salycilic acid	20.72 ± 3.24^{A}	2.12 ± 0.31^{a}	4.03 ± 0.51^{C}	0.38 ± 0.05^{c}	14.46 ± 1.66^{B}	1.55 ± 0.17^{b}	0.000***
trans-Cinnamic acid	53.35 ± 6.35^{A}	5.45 ± 0.66^{a}	2.43 ± 0.26^{C}	0.23 ± 0.03^{b}	49.08 ± 5.03^{B}	5.25 ± 0.62a^{b}	0.000***
Flavonoids	181.53 ± 20.15^{C}	18.53 ± 1.91^{c}	655.89 ± 71.43^{A}	61.34 ± 7.26^{a}	474.99 ± 52.33^{B}	50.83 ± 6.25^{b}	0.000***
Quercetin-3-rhamnoside	55.98 ± 5.36^{C}	5.72 ± 0.66^{b}	98.2 ± 10.23^{A}	9.18 ± 0.95^{a}	85.9 ± 8.99^{B}	9.19 ± 0.95^{a}	0.000***
Rutin trihydrate	10.99 ± 2.23^{C}	1.12 ± 0.13^{c}	139.6 ± 14.26^{A}	13.06 ± 1.51^{a}	47.04 ± 5.26^{B}	5.03 ± 0.69^{b}	0.000***
Luteolin	1.67 ± 0.18^{C}	0.17 ± 0.01^{c}	193.86 ± 20.50^{A}	18.13 ± 1.95^{a}	36.55 ± 4.26^{B}	3.91 ± 0.44^{b}	0.000***
Quercetin dihydrate	2.15 ± 0.23^{C}	0.22 ± 0.03^{c}	5.84 ± 0.64^{B}	0.55 ± 0.07^{b}	52.53 ± 6.28^{A}	5.62 ± 0.61^{a}	0.000***
Resorcinol	–	–	58.27 ± 6.29^{B}	5.45 ± 0.67^{b}	60.43 ± 7.55^{A}	6.47 ± 0.77^{a}	0.000***
Kaempferol	10.28 ± 1.56^{B}	1.05 ± 0.12^{b}	4.91 ± 0.52^{C}	0.46 ± 0.05^{c}	32.59 ± 4.02^{A}	3.49 ± 0.47^{a}	0.000***
Naringin	8.84 ± 0.95^{C}	0.90 ± 0.08^{c}	14.79 ± 1.63^{B}	1.38 ± 0.15^{b}	18.25 ± 2.01^{A}	1.95 ± 0.20^{a}	0.000***
Apigenin	30.11 ± 4.11^{B}	3.07 ± 0.40^{b}	3.66 ± 0.38^{C}	0.34 ± 0.04^{c}	121.34 ± 13.11^{A}	12.99 ± 1.45^{a}	0.000***
Flavone	44.78 ± 5.22^{A}	4.57 ± 0.55^{a}	14.54 ± 1.53^{B}	1.36 ± 0.14^{b}	10.12 ± 1.12^{C}	1.08 ± 0.11^{c}	0.000***
Coumarine	16.73 ± 1.77B	1.71 ± 0.18^{b}	122.22 ± 14.53^{A}	11.43 ± 1.42^{a}	10.24 ± 1.06^{C}	1.10 ± 0.12^{c}	0.000***
Total	979.41 ± 90.45^{B}	100.00 ± 8.23^{a}	1069.32 ± 115.23^{A}	100.00 ± 11.27^{a}	934.42 ± 91.70^{C}	100.00 ± 10.29^{a}	0.000***

Values in the same row with different superscripts [small letters a–c (between percentages) and capital letters A–C (between contents)] are significantly different at $P < 0.05$, (Duncan test).
– Not detected.
*** $P < 0.001$.

ral, anti-inflammatory and antiallergenic effects (Cook and Samman, 1996).

Significant differences ($P < 0.05$) were also found in total condensed tannin contents among different coriander varieties, representing 0.17 ± 0.01 mg GAE/g, 0.16 ± 0.01 mg GAE/g and 0.09 ± 0.01 mg GAE/g in Syrian, Egyptian and Tunisian varieties, respectively (Table 1). From data presented in Table 1, statistical analysis showed that there are strong ($P < 0.001$) effects of coriander varieties on total polyphenol, total flavonoids and total condensed tannins contents. These results could be due to environmental conditions (light, nutrient availability, and day length) (Circella et al., 1995; Skoula et al., 2000). In addition, variation in phenol, flavonoid and tannin contents could be attributed to the genetic potential of individual variety and to some other factors like conditions of cultivation especially the extent of the use of fertilizers and irrigation (Arganosa et al., 1998). Syrian variety was the rich variety in total polyphenol, total flavonoid and total condensed tannin contents.

3.2. Identification and quantification of phenolic compounds by RP-HPLC

In order to quantify and separate phenolic compounds of the three studied coriander fruit varieties, RP-HPLC coupled with a UV–vis multiwavelength detector was used and the obtained results are summarized in Table 2. Twenty-one compounds were identified in different coriander varieties including eleven phenolic acids (gallic, chlorogenic, cafeic, vanillic, *p*-coumaric, ferulic, rosmarinic, *o*-coumaric, *trans*-hydroxycinnamic, salycilic and *trans*-cinnamic acids) and ten flavonoids (quercetin-3-rhamnoside, rutin trihydrate, luteolin, quercetin dihydrate, resorcinol, kaempferol, naringin, apigenin, flavone and coumarine). In Tunisian coriander variety, phenolic acids were predominant with 81.47 ± 9.68% and represented mainly by chlorogenic (15.09 ± 2.33%), ferulic (11.07 ± 2.01%) *o*-coumaric (9.18 ± 0.99%) cafeic (8.94 ± 9.55%) and vanillic acids (7.51 ± 8.59%). The remaining percentage (18.53 ± 1.91%) was attributed to flavonoids class which was dominated by quercetin-3-rhamnoside (5.72 ± 0.66%), apigenin (3.07 ± 0.40%) and flavones (4.57 ± 0.55%). On the other hand, Syrian variety was characterized by the predominance of flavonoid class (61.34 ± 7.26%) due to the high presence of luteolin (18.13 ± 1.95%) and rutin trihydrate (13.06 ± 1.51%). For Egyptian coriander variety, phenolic acids (49.17 ± 6.85%) and flavonoids (50.83 ± 6.25%) were approximately distributed equally in the methanolic extract. In the later variety, apigenin was the major compound (12.99 ± 1.45%) followed by quercetin-3-rhamnoside (9.19 ± 0.95%), rosmarinic acid (8.94 ± 0.97%), gallic acid (7.93 ± 6.52%) and *trans*-hydroxycinnamic acid (6.95 ± 0.77%). From Table 2, it appears that phenolic composition was origin and/or variety dependant.

To the best of our knowledge, there are no previous reports dealing with the distribution of phenolic compounds in coriander methalonic extracts. The amounts of total phenolic compounds detected by the Folin–Ciocalteu method were 979.41 ± 90.45, 1069.32 ± 115.23 and 934.42 ± 91.70 µg/

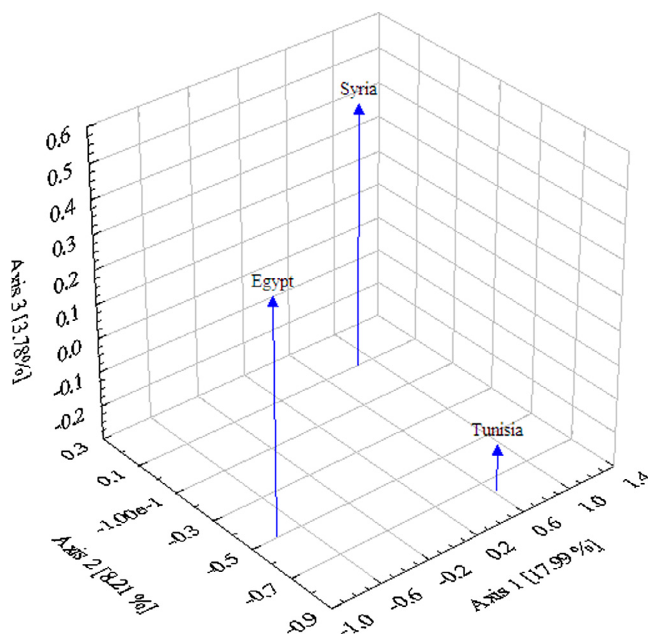

Figure 1 A three-dimensional visualization of PCA results of the three *Coriandrum sativum* L. varieties based on their percentage of phenolic composition (Table 2).

g DW in Tunisian, Syrian and Egyptian varieties, respectively. On the other hand, the amounts of total phenolic compounds assessed by HPLC were significantly lesser than those obtained by the Folin–Ciocalteu method. This latter method was predictable due to the weak selectivity of the Folin–Ciocalteu reagent, as it reacts positively with different antioxidant compounds (phenolic and nonphenolic substances) (Que et al., 2006).

Principal component analysis (PCA) was performed in order to determine the relationship between the three coriander varieties on the basis of their percentages of phenolic composition. A better discrimination was revealed on the three-dimensional visualization of the plotted scores, where the three PC accounted for 29.98% of total variance. As can be seen in Fig. 1, Egyptian and Syrian varieties were grouped together. Tunisian variety was clearly distinguished from this group. So, this fact could be due to the near environmental conditions in Syria and Egypt, Tunisia ones where so far.

3.3. Antioxidant assays

3.3.1. DPPH radical-scavenging

The antioxidant activity, determined by three different methods namely DPPH, reducing power and β-carotene-linoleic acid, is presented in Table 1. The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for the determination of free radical scavenging activity, usually expressed as IC_{50}, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. Lower IC_{50} value indicates a higher antioxidant activity (Molyneux, 2004). The Tunisian coriander variety extract showed the highest capacity to neutralize this radical. Moreover, the obtained IC_{50} values were 27.00 ± 6.57 μg/mL, 36.00 ± 3.22 μg/mL and 32.00 ± 2.87 μg/mL in Tunisian, Syrian and Egyptian varieties, respectively. The IC_{50} of BHT was 18.00 ± 1.61 μg/mL. In this study, DPPH radical scavenging activity of the tested samples was in the order BHT > Tunisian variety > Egyptian variety > Syrian variety. On the other hand, the ethanolic extracts from both leaves and seeds showed a concentration-dependent DPPH scavenging activity with IC_{50} values of 389 ± 5 and 510 ± 12 μg/mL, respectively (Wangensteen et al., 2004). Moreover, the DPPH radical scavenging activity was strongly ($P < 0.001$) affected by the coriander varieties (Table 1). Sriti et al. (2011) have showed a lower free radical scavenging activity (IC_{50} of 32 ± 0.78 μg/mL) in comparison with our result (27.00 ± 6.57 μg/mL). In addition, the ethyl acetate extract from coriander leaves showed the most potent activity (IC_{50} value of 147 ± 3 μg/mL) and indicate that compounds with strongest radical-scavenging capacity are of medium polarity (Wangensteen et al., 2004). This antiradical activity could be due to the phenolic compounds. In fact, it has been found that antioxidant molecules such as polyphenols, flavonoids, and tannins reduce and discolor DPPH due to their hydrogen donating ability (Kumaran and Karunakaran, 2007).

3.3.2. Reducing power

The reducing power of a bioactive compound may also serve as a significant indicator of its potential antioxidant activity (Roginsky and Lissi, 2005). Table 1 showed that the Fe^{3+} reducing power of fruit extracts differs greatly depending on varieties. Syrian variety showed the higher reducing capacity (EC_{50} = 54.20 ± 6.22 μg/mL) followed by Egyptian (EC_{50} = 56.11 ± 7.45 μg/mL) and Tunisian (EC_{50} = 122.01 ± 13.25 μg/mL) ones. Furthermore, in comparison to the positive control: asorbic acid (EC_{50} = 40.00 ± 5.23 μg/mL), the Syrian, Egyptian and Tunisian methanolic extracts exhibit 1.35, 1.40 and 3.5-fold lower activities. These results indicate that the different methanolic extracts are able to act as electron donor and, therefore, react with free radicals, converting them to more stable products and, thereby, terminating radical chain reactions. The presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Ferreira Isabel et al., 2007). On the other hand, statistical analysis revealed a higher ($P < 0.001$) effect of varieties on reducing power (Table 1).

3.3.3. β-Carotene bleaching inhibition

The antioxidant activity of coriander fruit methanolic extract was also evaluated by the β-carotene-linoleate bleaching method (Table 1) because β-carotene shows strong biological activity and constitutes physiologically important compound (Sarkar et al., 1995; Kumazawa et al., 2002; Sakanaka et al., 2005). This method was based on the loss of the yellow color of β-carotene due to its reaction with radicals formed after linoleic acid oxidation in emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). Syrian and Egyptian varieties have the same ability to prevent the bleaching of β-carotene (IC_{50} = 240.00 ± 26.35 and IC_{50} = 240.00 ± 25.84 μg/mL, respectively) whereas Tunisian variety had the strongest activity (IC_{50} = 160.00 ± 18.63 μg/mL). On the other hand, all the methanolic extracts had lower antioxidant activities than BHT with IC_{50} of 85.00 ± 9.68 μg/mL (Table 1). In addition, the β-carotene-linoleate bleaching values were highly ($P < 0.001$) affected by the provenance of varieties.

4. Conclusion

The results presented here constitute the first information on the antioxidant activities of coriander fruit methanolic extract of three different varieties (Tunisian, Syrian and Egyptian). Syrian variety showed the highest amount on total polyphenol, total flavonoid and total condensed tannin contents but the Tunisian one showed the strongest DPPH scavenging activity and the β-carotene bleaching test than the Syrian and Egyptian ones. Syrian variety showed the highest reducing power activity. In addition, antioxidant capacities varied significantly according to coriander varieties. In the light of these results, we can conclude that antioxidant activity depends on phenolic composition and not on polyphenol contents (Cheung et al., 2003). These preliminary results open the way for deep studies of methanolic extracts of the other different organs of coriander (leaves, stems, roots, flowers) which are few to be investigated but could be more interesting than coriander fruit methanolic extract. Coriander antioxidant activity was high enough for the plant to be a new and natural source of antioxidant substances for its use as natural additives in food. To understand their mechanism of action as bioactive components, further fractionation of methanolic extracts, isolation of phenolic compounds and determination of their biological activities in vitro and in vivo are needed.

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