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Effects of Extraction Solvents and Provenances on Phenolic Contents and Antioxidant Activities of Cumin (*Cuminum cyminum* L.) Seeds

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Abstract Polyphenol contents and 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) scavenging activity of cumin (*Cuminum cyminum* L.) seed extracts were compared depending on their geographical origin (Tunisia and India: TCS and ICS, respectively) and the extraction solvent polarity. The β-carotene bleaching assay, the chelating ability and the reducing power of the most promising solvent extracts were also assessed. In addition, TCS and ICS extracts were acid-hydrolyzed and the phenolics identified by reversed-phase high-performance liquid chromatography (HPLC). Seed phenolic contents and antioxidant activity appeared to be accession and solvent dependent. Extraction with 80% acetone led to the highest polyphenol (18.60 and 16.50 mg gallic acid equivalents (GAE)/g dry weight (DW)), flavonoid (5.91 and 4.99 mg catechin equivalents (CE)/g DW) and tannin (83.23 and 80.23 mg CE/g DW) contents, respectively for TCS and ICS. DPPH scavenging activity, β-carotene bleaching assay, chelating ability and reducing power were maximal in 80% acetone for both TCS and ICS. HPLC analysis revealed several phenolic compounds in *C. cyminum* seeds, with *p*-coumaric (4.83 and 2.33 mg/g DW), *trans*-2-dihydrocinnamic (1.09 and 1.20 mg/g DW) and rosmarinic (0.70 and 1.04 mg/g DW) acids as major phenolics in TCS and ICS, respectively. Thus, phenolic composition of cumin seeds is also origin dependent. Taken together, our findings indicate that cumin might constitute a rich and novel source of natural antioxidants as food additives in food industry and that acetone 80% would be the most appropriate solvent for seed extraction.

Keywords *Cuminum cyminum* L. · Seeds · Solvent · Geographic origin · Antioxidant activity · Phenolics

Introduction

Plants constitute a valuable source of natural antioxidants including vitamins, phenolic compounds and flavonoids (El-Ghorab et al. 2007). Because of their potential carcinogenicity, the utilization of synthetic antioxidants is progressively restricted. This trend is concomitant with an increasing interest for the identification and valorization of natural antioxidants of plant origin. Consumers are also more attentive about healthy and balanced diets, which greatly rely on the utilization of natural preservatives for a healthier lifestyle and natural ways of preserving nutrients (Raghavan 2007). Spices are a potent source of antioxidants, some of them being more efficient and safer than the synthetic antioxidants. According to Pietta (2000), the antioxidant effect of plant products could be mainly ascribed to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes. Phenolics play an important role in human health owing to their anti-inflammatory, anti-allergic, antimicrobial, anticarcinogenic and antiviral activities (Medina et al. 2007). In plants, these compounds prevent lipid peroxidation and oxidative modification of low-density lipoproteins due to their antioxidant activities (Servili et al. 2004).

Extraction process aims at providing a maximum yield of biosubstances of the highest quality (concentration of

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target compounds and antioxidant power of the extracts). Yet, literature about the most effective methods and the solvents to extract these compounds is abundant but to some extent contradictory (Contini et al. 2008). Considering the structure of these compounds and their physicochemical properties, it would be impossible to propose a universal extraction protocol. Different solvent systems have been used for the extraction of polyphenols from plant material (Pinelo et al. 2004), but the extraction yield is influenced by the solvent nature and the extraction method (Goli et al. 2004). Phenolic content during the plant growth cycle is also affected by genetic, cultural practices and climatic factors. According to Akowuah et al. (2005), depending on the solvent used for extracting phenolic compounds, extracts obtained from the same plant may vary widely with respect to their antioxidant concentration and activities. In tea, aqueous ethanol seems to be more efficient than methanol and acetone for extracting flavonoids (Wang and Helliwell 2001), whereas water was found to be a better solvent for extracting catechins, as compared to 80% methanol or 70% ethanol (Khokhar and Magnusdotti 2002).

Cumin (*Cuminum cyminum* L.) of the Apiaceae produces highly nutritional oleaginous seeds which consist in a stripped paired or separate carpel showing nine ridges and oil canals (Pottier-Alapetite 1979). Cumin originates from Egypt, Turkistan and East Mediterranean, but it is cultivated in Iran, China, India, Morocco, south Russia, Japan, Indonesia, Algeria and Turkey (Tuncturk and Tuncturk 2006). In Tunisia, this plant, commonly known as "kammoun", is a widely used spice in local kitchen. Cumin is an ingredient of most curry powders and many savory spice mixtures and is mainly used to prepare fish dishes in mixture with salt and olive oil. In the city of Sfax (south of Tunisia), it is largely used to prepare many dishes like "Kammounia" and fish plates. It gives bite to plain rice, beans and cakes.

C. cyminum seeds are used for treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice (Eikani et al. 1999; Nostro et al. 2005) whereas essential oil of *C. cyminum* seed exhibits diuretic, carminative, emmenagogic antibacterial, antioxidant, cytotoxic, antidiabetic and antispasmodic properties (Allahghadri et al. 2010). Seeds contain fixed oil (approximately 10%), protein, cellulose, sugar, mineral elements and volatile oil (Li and Jiang 2004). They also contain volatile oil (1–5%) that account for the typical aroma of cumin seeds. Data on *C. cyminum* phenolics are still scarce and even lacking for Tunisian accessions. In order to assess the effect of solvent system and the geographic origin on phenolic content and antioxidant activities of cumin seeds, we compared the phenolic composition and antioxidant activities of Tunisian and Indian accessions under different extracting solvents. The expected results will be useful for the valorization of this species as a new local source of bioactive compounds for economic and health utilization.

Materials and Methods

Plant Material and Growth Conditions

Two provenances of mature green cumin (*C. cyminum* L.) seeds were used. The first one was purchased from an herbal market in Menzel Temime, a little town situated in the northeast of Tunisia and called TCS whereas the second one was imported from India (ICS). Seeds of both provenances were cultivated from January 2009 to July 2009 in a greenhouse under controlled conditions (14 h photoperiod; photosynthetic photon flux density 400 mol m^{-2} s^{-1}; mean temperature and relative humidity 30±5 °C, 55±5% day and 16±2 °C, 90±5% night, respectively). Seeds were sown in 10-L pots filled with a clayey-loamy agricultural soil and were irrigated with tap water. After their harvest, mature seeds were air-dried and stored at 4 °C until used for further analysis.

Preparation of Extracts

Harvested seeds were finely grounded with a type A10 blade-carbide grinding (Ika-Werk, Staufen, Germany), and 2.5 g of the samples was then stirred with 25 mL of solvent for 30 min. Extracts were kept for 24 h at 4 °C, filtered through a Whatman N° 4 filter paper, dried under vacuum and stored at 4 °C until analysis was achieved. Seed extracts were obtained using different pure solvents with increasing polarity: hexane, acetone, acetone 80%, ethanol, methanol, ethanol 80%, methanol 80% and water.

Total Phenolic Content Determination

Total phenolic content was assayed using the Folin–Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. (2002). This method was employed to evaluate the phenolic content of the samples. A calibration curve of gallic acid (ranging from 50 to 400 mg/mL) was prepared and the results, determined by the regression equation of the calibration curve (y=62.94–0.67, R^2=0.99), were expressed as milligram gallic acid equivalents (GAE) per gram dry weight of raw material (DW). In this method, an aliquot (0.125 mL) of a suitable acetone sample was added to 0.5 mL of deionized water and 0.125 mL of Folin–Ciocalteu reagent. The solutions were mixed and incubated at room temperature. After 1 min, 1.5 mL of 7% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken thoroughly and then incubated for 90 min in the dark at room temperature.

The absorbance of all samples was measured at 760 nm using a HACH UV–Vis spectrophotometer. Triplicate measurements were made for each sample.

Flavonoid Content Determination

Total flavonoid contents (TFC) were measured according to Dewanto et al. (2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to a 75 μL of $NaNO_2$ solution and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as milligram catechin equivalents (CE) per gram DW, through the calibration curve of (+)-catechin. The calibration curve range was 50–400 μg/mL ($R^2 = 0.99$). All samples were analysed in three replications.

Assessment of Total Condensed Tannins

Total tannin contents were measured using the modified vanillin assay described by Sun et al. (2002). To 50 μL of properly diluted sample, 3 mL of methanol vanillin solution (4%) and 1.5 mL of H_2SO_4 were added. The absorption was measured at 500 nm against extract solvent as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW. The calibration curve range was 50–400 μg/mL ($R^2 = 0.99$). All samples were analysed in three replications.

Antioxidant Activities

DPPH Assay

The donation capacity of the obtained extracts and essential oils was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. (1998). One millilitre of the extract at different concentrations was added to 0.5 mL of a DPPH·methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC50 (micrograms per millilitre), the antiradical dose required to cause a 50% inhibition. A lower IC50 value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect(\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. BHT was used as a positive control. Samples were analysed in triplicate.

β-Carotene Bleaching Test

A modified method described by Koleva et al. (2002) was used. β-Carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween 40 (polyoxyethylene sorbitan monopalmitate) were mixed with 0.5 mL of chloroform. Chloroform was removed at 45 °C, under vacuum, using a rotary evaporator (Heidolph, Germany). The resulting mixture was immediately diluted with 10 mL of triple-distilled water and was mixed well for 1 to 2 min. The emulsion was further made up to 50 mL with oxygenated distilled water. Aliquots (4 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of test samples. BHA was used for comparative purposes. A control, containing 0.2 mL of corresponding solvent and 4 mL of the above emulsion, was prepared. The tubes were placed, at 50 °C, in a water bath. Absorbances of all the samples at 470 nm were taken at zero time ($t=0$), and measurement of absorbance was continued, until the colour of the β-carotene disappeared in the control reaction ($t=180$ min), at 15 min intervals. A mixture prepared as above, without β-carotene, served as blank. All determinations were performed in triplicate. The antioxidant activity of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula:

$$\%\ Inhibition = [(A_t - C_t)/(C_0 - C_t)] \times 100$$

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 value for the control measured at zero time during the incubation. The results are expressed as IC_{50} (micrograms per millilitre), the concentration required to cause a 50% β-carotene bleaching inhibition.

Chelating Effect on Ferrous Ions

The ferrous ion chelating activity of different organ extracts was assessed as described by Zhao et al. (2006). Different sample concentrations of the sample were added to 0.05 mL of $FeCl_2$–$4H_2O$ solution (2 mM) and incubated at room temperature for 5 min. Then, the reaction was initiated by adding 0.1 mL of ferrozine (5 mM), and the mixture was adjusted to 3 mL with deionized water, shaken vigorously and left standing at room temperature for 10 min. The solution absorbance was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–

Fe^{2+} complex formation was calculated using the formula given below:

$$Metal\ chelating\ effect(\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the ferrozine–Fe^{2+} complex and A_1 is the absorbance of the test compound. Results were expressed as IC_{50}, efficient concentration corresponding to 50% ferrous iron chelating. EDTA was used as a positive control. Samples were analysed in triplicate.

Reducing Power

The method of Oyaizu (1986) was used to assess the reducing power of different seed extracts. Each extract was mixed with 2.5 mL of sodium phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (10 g/L), and the mixture was incubated at 50 °C for 20 min; 2.5 mL of trichloroacetic acid (100 g/L) was then added, and the mixture was centrifuged at 650×g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of ferric chloride (0.01 g/L) and thoroughly mixed. The absorbance was measured at 700 nm against a blank in a spectrophotometer. A higher absorbance indicates a higher reducing power. EC50 value (milligrams per millilitre) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from linear regression analysis. Ascorbic acid was used as control.

RP-HPLC Evaluation of Phenolic Compounds

Dried samples from TCS and ICS were hydrolysed according to the method of Proestos et al. (2006) and slightly modified. Twenty millilitres of acetone 80% 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 high-performance liquid chromatography (HPLC). The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatography (reversed-phase (RP)-HPLC) coupled with an UV–vis multi-wavelength detector. The separation was carried out on a 250×4.6-mm, 4-μm Hypersil ODS C18 reversed-phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient programme 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 and 100% A 24–28 min. The injection volume was 20 μl, and peaks were monitored at 280 nm.

Samples were filtered through a 0.45-μm membrane filter before injection. Peaks were identified by congruent retention times compared with those of pure standards. Analyses were performed in triplicate.

Statistical Analysis

All extractions and determinations were conducted in triplicates. Data are expressed as mean±SD. The means were compared by using the one-way and multivariate analysis of variance followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $p<0.05$. All analyses were performed by using the "Statistica v 5.1" software.

Results and Discussion

Effect of Provenance and Solvent on Extraction of Phenolics

Total Phenolic Contents

Usually, the solvent used for extraction is chosen according to the purpose of extraction (preparation or analysis), the nature of the assayed components, the physicochemical properties of the matrix, the availability of reagents and equipments, the cost and safety concerns (Yu et al. 2002). Depending on the solvent used, the amount of total phenolic contents (TPC) extracted from cumin seeds ranged from 1.09 to 18.60 mg GAE/g DW, respectively, for water and acetone 80% (Table 1). TPC extracted from TCS were 1.3-fold higher compared to ICS. In both accessions, the highest TPC was obtained with acetone 80%. With respect to TPC extraction yield, solvents used in the present study could be classified in the following decreasing order: acetone 80%, methanol 80%, ethanol 80%, acetone, methanol, ethanol and water. Some exceptions were observed, particularly when water and ethanol were used as extracting solvents. Consistent with previous studies (Turkmen et al. 2006), our findings point out the influence of the extracting solvent and accession on TPC. Zielinski and Kozlowska (2000) showed that 80% acetone is the most efficient solvent mixture for phenol extraction from cereal seeds. Similarly, Zhou and Yu (2004) reported that 50% acetone wheat extracts contained the highest TPC whereas ethanol was the least effective solvent. Eighty percent ethanol and 80% methanol were more efficient than water for extracting total phenolics from peanut skin (Yu et al. 2005).

Recently, El-Ghorab et al. (2010) reported that the highest total phenolic content was found in the cumin methanolic extract (35.5 mg GAE/g DW), whereas the

Table 1 Phenolic contents of different solvent extracts of *C. cyminum* seeds

		Ethanol	Acetone	Methanol	Ethanol (80%)	Acetone (80%)	Methanol (80%)	Water
Polyphenol (mg GAE g^{-1} DW)	TCS	2.24±0.33aD	5.97±0.02aC	4.31±0.03aCD	14.20±0.34aB	18.60±0.03aA	17.42±0.02aAB	1.13±0.01aE
	ICS	2.03±0.02aD	4.23±0.01abC	3.33±0.02bCD	11.73±0.77bB	14.15±0.01bA	15.24±0.06bAB	1.09±0.01aE
Flavonoid (mg CE g^{-1} DW)	TCS	1.72±0.01abB	2.23±0.08aB	0.56±0.01aC	2.03±0.02aB	5.91±0.03aA	1.44±0.01aB	0.77±0.01aC
	ICS	2.43±0.03aB	1.93±0.02aB	0.88±0.02aC	1.92±0.11aB	4.19±0.31abA	1.78±0.07aB	0.52±0.02aC
Tannin (mg CE g^{-1} DW)	TCS	36.70±0.34Fa	52.71±0.51Da	46.50±0.26Ea	76.58±1.32Ba	83.23±1.83Aa	65.71±1.03Ca	32.11±0.08Fa
	ICS	32.98±0.26Eb	41.93±0.33Db	40.83±0.51Db	72.44±2.93Bab	74.03±1.93Ab	61.37±1.11Cab	30.39±0.93Ea

Values are means of three replications ($N=3$±SD). The data marked with the different capital letter, for the solvents, and small letter, for the provenance, in the table, value share significant differences at $P<0.05$ (Duncan test)

GAE gallic acid equivalents, *CE* catechin equivalents

hexane extract showed the lowest one (10.6 mg GAE/g DW). Thippeswamy and Naidu (2005) reported that TPC evaluated in cumin methanolic extract was 9 mg/g DW. In another study, Shan et al. (2005) found a TPC of 2.3 mg/g DW for 80% methanolic extracts of cumin. These changes could be ascribed to the variations in pedoclimatic conditions.

Moreover, the 80% acetone extract obtained from Tunisian provenance showed higher polyphenols content than the Indian one, suggesting that phenolics biosynthesis in *C. cyminum* is greatly influenced by genetic factors. Previous reports showed that the production of secondary metabolites (such as essential oil) in this species is affected by several factors including genetic ones (Bettaieb et al. 2011).

Total Flavonoids

TFC of cumin seeds varied from 0.52 to 5.91 mg CE/g DW, respectively, for water and 80% acetone. Regarding flavonoid solubility, the solvent classification with respect to their extraction efficiency was similar to that made for polyphenols. Zielinski and Kozlowska (2000) also showed that 80% acetone is an efficient solvent mixture for extracting flavonoids from cereal seeds. Similarly, it was demonstrated that water addition to organic solvents (especially acetone, ethanol or methanol) improved flavonoid extraction yield (Robards and Antolovich 1997). Variance analysis showed that there were significant differences in total flavonoid concentration among the two accessions. TFC extracted from TCS were 11.05% higher than those from ICS.

Condensed Tannins

Cumin is known to contain significant amount of tannins (Uma Pradeep et al. 1993). Our findings confirm this since values of condensed tannin contents (CTC) ranged from 30.92 to 83.23 mg CE/g DW for water and 80% acetone, respectively. As found for phenolics and flavonoids, CTC were found to vary depending on the solvent used. In both accessions, the highest CTC values were recorded when extraction was achieved using 80% acetone (ca. 81 mg EC/g DW in the average), followed by 80% ethanol (ca. 74 mg EC/g DW in the average). Generally, CTC extracted in TCS were 1.3-fold higher than in ICS. Chavan et al. (2001) reported that 70% acetone with or without acid was more efficient than absolute acetone to extract condensed tannins from different peas. Aqueous acetone was chosen as the extraction solvent because it has been reported to be the most effective medium for samples presumably rich in tannin (Naczk and Shahidi 2004). Moreover, the concentrations of total acetone-extractable polyphenols were statistically ($P<0.05$) affected by geographical origin.

Several studies have highlighted that genetic factors modulated phenolic concentration in different crops; this has been shown for various pecan cultivars grown in Texas State (Villarreal-Lozoya et al. 2007).

Effect of Provenance and Solvent on Cumin Antioxidant Activities

The solvent nature is the most controversial parameter that influences antioxidant capacities (Naczk and Shahidi 2004; Turkmen et al. 2006). The solubility of phenolic compounds is strongly governed by the type of solvent used, the degree of polymerization of phenolics, as well as by the interaction of phenolics with other food constituents and the formation of insoluble complex (Djeridane et al. 2006).

In order to identify the most suitable solvent for assessing antioxidant capacity, the antiradical activity (DPPH test) was evaluated either using the pure solvents or varied mixtures (Fig. 1). Solvent type had significant effect on the DPPH$^{\bullet}$ scavenging activity of *C. cyminum* extracts. IC$_{50}$ values ranged from 6.24 μg/mL (80% acetone) to 236 μg/mL (water). Seed extracts with 80% acetone showed the highest ability to reduce DPPH, with IC$_{50}$ values reaching 6.24 and 15.14 μg/mL for TCS and ICS, respectively. Eighty percent methanol was found also to be the second suitable system (IC$_{50}$ value of 19.70 and 21.14 μg/mL), followed by ethanol 80% (IC$_{50}$ value of 22 and 41 μg/mL), respectively, for TCS and ICS. IC$_{50}$ values using acetone, methanol and ethanol were higher than 100 μg/mL. The lowest antiradical capacity was found in water extracts of *C. cyminum* seeds (IC$_{50}$ values were 222 and 236 μg/mL, respectively, for TCS and ICS) (Fig. 1). In addition, ICS exhibited significantly the lowest antiradical activity compared to the TCS irrespective of the solvent used in our study. These results indicate that aqueous solvents are more suitable for extracting DPPH$^{\bullet}$ scavenging
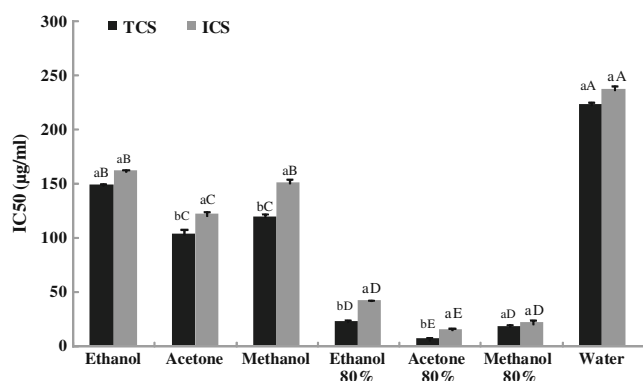
Fig. 1 DPPH scavenging activity (IC$_{50}$) of different seed extracts (TCS and ICS). Values are means of three replications (*N*=3±SD). The data marked with the different *capital letter*, for the solvents, and *small letter*, for the provenance, in the histograms of each IC$_{50}$ value share significant differences at *P*<0.05 (Duncan test)

agents from *C. cyminum* seeds. Moreover, the significant variations in antioxidant potential according to the solvents used is essentially due to the difference in polarity, and thus different extractability, of the antioxidative compounds (Djeridane et al. 2006; Maisuthisakul et al. 2007). Several studies showed that solvent polarity leads to significantly different extraction capacities for phenolic compounds in plants (Parida et al. 2004; Galvez et al. 2005).

In accordance with several studies, adding water at low percentages to the solvent (especially acetone) improved the extracting power and antioxidant activity (Zhao et al. 2006; Zhou and Yu 2004). Zhao et al. (2006) suggested that acetone/water (8:2) is the most recommended solvent system to obtain the highest antioxidant activity in barley. The same correlation is established by Maisuthisakul et al. (2007) by comparing phenolic and DPPH scavenging activities of 26 Thai indigenous plants.

Our findings indicate that aqueous solvents were more effective in terms of extraction power of seed polyphenols and the quenching ability of DPPH than the other solvents. Eighty percent acetone, 80% methanol and 80% ethanol extracts were assessed for complementary antioxidant activities (β-carotene bleaching assay, chelating ability and reducing power). Concerning the β-carotene bleaching assay, the oxidation of linoleic acid generates peroxyl-free radicals due to the abstraction of hydrogen atom from diallylic methylene groups of linoleic acid (Kumaran and Karunakaran 2006). The free radical then will oxidize the highly unsaturated β-carotene. The presence of antioxidants in the extract will minimize the β-carotene oxidation by hydroperoxides. These will be later neutralized by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. Eighty percent acetone, 80% methanol and 80% ethanol extracts differed in their capacity to inhibit linoleic acid oxidation (Table 2), indicating that the solvent system may significantly influence the antioxidant activity. As observed in the DPPH radical-scavenging capacity measurements, 80% acetone was the most effective means for extracting β-carotene bleaching activities compounds from *C. cyminum* (followed by 80% methanol and 80% ethanol). Moreover, Tunisian variety exhibited significant higher activity (65.86 μg/mL) than the Indian one (77.05 μg/mL) with acetone 80%. The antioxidative components in the *C. cyminum* extracts can probably reduce the extent of β-carotene destruction by neutralizing the linoleate-free radical and other free radicals formed in the system.

The effect of solvent and provenance on the antioxidant ability of *C. cyminum* seeds was also assessed by the estimation of chelating and reducing powers estimation (Table 2). The different extracts showed high antioxidant activities, but 80% acetone led to the highest high chelating power with IC$_{50}$ values of 23.65 and 19.73 mg/mL,

Table 2 Antioxidant activities of TCS and ICS extracts using 80% ethanol, 80% acetone and 80% methanol

	β-carotene bleaching (IC$_{50}$, μg/mL)		Chelating ability (IC$_{50}$, mg/mL)		Reducing power (EC$_{50}$, μg/mL)	
	TCS	ICS	TCS	ICS	TCS	ICS
Ethanol (80%)	112.65±1.34Ba	123.75±1.09Aa	35.46±0.36Ba	29.35±0.25Aa	233.45±2.43Ba	154.63±2.94Aa
Acetone (80%)	65.86±0.23Bb	77.05±0.05Ab	23.65±0.87Ab	19.73±0.83ABb	110.34±3.74Ac	93.87±2.11ABc
Methanol (80%)	71.34±0.66Bbc	80.46±0.55Ab	31.34±0.24Aab	32.45±0.59Aa	210.34±3.64Abc	124.64±2.77Bb
EDTA	–		0.03±0.01		–	
Ascorbic acid	–		–		40±0.84	
BHA	43±0.56		–		–	

Each value in the table was obtained by calculating the average of three experiments. The data marked with the different capital letter, for the provenance, and small letter, for the solvents, in the table of each IC$_{50}$ or EC$_{50}$ value share significant differences at $P<0.05$ (Duncan test)

respectively, for TCS and ICS as well as a strong reducing capacity, with IC$_{50}$ values of 110.34 mg/mL and 93.87 μg/mL, respectively. Comparing TCS and ICS antioxidant capacities showed that unlike to the DPPH and β-carotene bleaching assays, the Indian accession exhibited lower IC$_{50}$ values and thus higher chelating and reducing powers than the Tunisian one. The reducing capability may serve as a significant indicator of the potential of antioxidant activity (Tanaka et al. 1998). This may be due to a slightly different phenolic composition between the 80% acetone extracts. It has been reported that the solvent polarity and the type of antioxidant substance influence the antioxidant capacity of the extract (Naczk and Shahidi 2004). The reducing property is generally associated with the presence of reductones (Duh 1998), such as ascorbic acid, which have been shown to exert antioxidant action by breaking the free radical chain (Gordon 1990). Reductones are also reported to react directly with peroxides and also with certain precursors of peroxides, thus preventing peroxide formation (Shimada et al. 1992; Xing et al. 2005). On the other hand, ferrous ions are commonly found in foods and are the most effective pro-oxidants (Yamaguchi et al. 1988). Hence, the ferrous ion chelating abilities of the various extracts from *C. cyminum* seeds would be beneficial.

Identification of Phenolic Compounds by RP-HPLC Assay

Phenolic compounds are naturally present in fruits and vegetables. They are part of everyday diet and are also used as medicines or supplements. Acetone 80% was the most efficient solvent to extract phenolics. For this reason, phenolic composition of TCS and ICS was only evaluated for this extract. No data are available concerning the phenolic composition of *C. cyminum* seeds. Here, for the first time, polyphenol qualitative and quantitative determination in cumin seeds were performed by RP-HPLC coupled with an UV–visible multi-wavelength detector. Phenolics occur in plants mainly as aglycones, glycosides

and esters or are bounded to the cell wall (Soong and Barlow 2006). Therefore, acidic hydrolysis was used to release aglycones. Qualitative and quantitative differences were found between the two accessions (Table 3). TCS and ICS contained comparable phenolic acids (74.53%, 9.49 mg/g DW and 66.24%, 6.41 mg/g DW, respectively). However, TCS contained more flavonoids than ICS (11.43%, 2.88 mg/g DW and 9.68%, 1.77 mg/g DW, respectively).

A total of 19 phenolic compounds were successfully identified in TCS and ICS. The phenolic composition of *C. cyminum* seeds revealed that *p*-coumaric acid was more present in TCS (30.49%, 4.83 mg/g DW) compared to ICS (22.91%, 2.33 mg/g DW). Seeds were also characterized by the presence of other phenolic compounds in interesting proportions. TCS showed higher proportions of luteolin (4.65%, 1.29 mg/g DW), syringic acid (9.72%, 0.64 mg/g DW) and even cinnamic acid (6.35%, 0.94 mg/g DW), compared to ICS. However, Indian seeds were richer in *trans*-2-dihydrocinnamic acid (14.52%, 1.20 mg/g DW) and flavone (5.53%, 0.61 mg/g DW) than TCS.

Variations in phenolic composition could be explained by several factors, whether genetic or related to the geographic origin, plant organ, harvest time, extraction method, type of cultivar and storage conditions (Lawrence 2002). The season and even sunlight duration are also known to influence the plant metabolism since some compounds may be accumulated at a particular period to respond to environmental changes (Koenen 2001).

Ani et al. (2006) studied the phenolic composition of *Cuminum nigrum* seeds and phenolics including gallic, protocatechuic, caffeic, ellagic, ferulic acids and also flavonols such as quercetin and kaempferol. These authors also found that caffeic and ferulic acids were the predominant compounds. Epidemiological studies have suggested the association between the consumption of phenolic acid rich foods or beverages and the prevention of many

Table 3 Phenolic compounds of *C. cyminum* extract (TCS and ICS)

	TCS		ICS	
	%	mg/g	%	mg/g
Phenolic acids	**74.53**	**9.49**	**66.24**	**6.41**
Gallic acid	0.71±0.02[b]	0.09±0.02[a]	1.58±0.03[a]	0.09±0.01[a]
Cafeic acid	0.69±0.01[a]	0.07±0.00[b]	0.30±0.01[b]	0.22±0.05[a]
Dihydroxyphenolic acid	0.45±0.01[a]	0.02±0.00[b]	0.27±0.02[ab]	0.20±0.03[a]
Dihydroxybenzoic acid	1.06±0.01[a]	0.39±0.02[a]	1.11±0.01[a]	0.08±0.01[b]
Chlorogenic acid	0.97±0.01[a]	0.22±0.01[a]	1.20±0.02[a]	0.06±0.01[b]
Syringic acid	9.72±0.41[a]	0.64±0.02[bc]	5.43±0.33[b]	0.43±0.04[a]
Vanillic acid	0.57±0.01[a]	0.03±0.01[b]	0.60±0.02[a]	0.35±0.02[a]
p-Coumaric acid	30.49±0.33[a]	4.83±0.11[a]	22.91±0.26[b]	2.33±0.07[b]
Ferrulic acid	1.30±0.01[a]	0.47±0.03[a]	2.40±0.02[b]	0.14±0.04[ab]
Rosmarinic acid	9.99±0.89[a]	0.70±0.04[ab]	10.20±1.65[a]	1.04±0.03[a]
trans-2-dihydrocinnamic acid	12.23±1.99[ab]	1.09±0.41[a]	14.52±1.87[a]	1.20±0.03[a]
Cinnamic acid	6.35±1.17[a]	0.94±0.02[a]	1.54±0.05[b]	0.27±0.00[b]
Flavonoids	**11.43**	**2.88**	**9.68**	**1.77**
Luteolin	4.65±0.82[a]	1.29±0.24[a]	1.92±0.43[b]	0.39±0.11[b]
Catechin	4.28±0.01[a]	0.23±0.02[b]	4.18±0.01[a]	0.52±0.03[a]
Coumarin	0.52±0.05[a]	0.04±0.01[a]	0.28±0.02[ab]	0.11±0.05[a]
Quercetin	0.13±0.01[a]	0.02±0.01[a]	0.16±0.01[a]	0.08±0.01[a]
Apigenin	0.30±0.01[a]	0.03±0.00[a]	0.13±0.03[a]	0.03±0.01[a]
Amentoflavone	0.09±0.01[a]	0.01±0.01[a]	0.12±0.01[a]	0.03±0.00[a]
Flavone	1.46±0.07[b]	0.12±0.02[b]	5.53±0.99[a]	0.61±0.07[a]
Unknown	14.04±0.88[b]	1.14±0.32[b]	24.08±1.22[a]	3.58±0.21[a]
Total	100	13.51	100	11.76

Values are represented as mean ± standard deviation of triplicates; Values followed by the same small letter did not share significant differences at $p < 0.05$ (Duncan test)

diseases (Morton et al. 2000). Recent interest in food phenolics has increased owing to their roles as antioxidants and scavengers of free radicals and their implication in the prevention of many pathologies such as cardiovascular diseases (Ursini et al. 1999) and certain types of cancer (Hudson et al. 2000). Quantitative analysis of total phenolic compounds using HPLC indicates that TCS contained more TPC than ICS (13.51 and 11.76 mg/g, respectively). However, contents obtained by HPLC were significantly lower than those obtained by the spectrophotometrical method. This was predictable due to the low selectivity of Folin–Ciocalteu reagent, as it reacts positively with different phenolic and non-phenolic substances (Que et al. 2006).

Conclusion

The extracting solvent as well as the provenance significantly affected total polyphenol, flavonoids, tannins and antioxidant activities in *C. cyminum* seeds. In our conditions, the optimal extraction solvent yielding the highest content of phenolic compounds (TPC, TFC and CTC) and their antioxidant capacities (DPPH radical-scavenging capacities, β-carotene bleaching assay, chelating ability and reducing power) was acetone 80%. The major phenolic compound in seeds of the two accessions was *p*-coumaric acid, which contributes greatly to the high antioxidant activities of this species. The qualitative variability observed in both accessions suggests that phenolic biosynthesis in *C. cyminum* seeds is greatly influenced by genetic factors. Seeds of both accessions contained high amounts of phenolic compounds and strong antioxidant activities, which is of high economical significance in the perspective of breeding strategies, selecting provenances and solvents with high polyphenols, flavonoids and tannins contents and high antioxidant potential for producing specific health-promoting antioxidants in the food industry.

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