DOI:10.1021/jf102248j

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Essential Oils, Phenolics, and Antioxidant Activities of Different Parts of Cumin (*Cuminum cyminum* L.)

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Cuminum cyminum L. roots, stems and leaves, and flowers were investigated for their essential oils, total phenolics, flavonoids, and tannins contents, individual phenolic compounds, and antioxidant activities. The essential oil was investigated by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS), whereas identification and quantification of individual target polyphenolic compounds was performed by reversed-phase high-performance liquid chromatography (RP-HPLC). Essential oil yields were 0.03% in roots, 0.1% in stem and leaves, and 1.7% in flowers. Major components of the oils were bornyl acetate (23%), α-terpinene (34%), and γ-terpinene (51%) in roots, stems and leaves, and flowers, respectively. In all *C. cyminum* organs, total phenolics content ranged from 11.8 to 19.2 mg of gallic acid equivalents per gram of dry weight (mg of GAE/g of DW). Among the polyphenols studied, 13 were identified in roots, 17 in stem and leaves, and 15 in flowers. The major phenolic compound in the roots was quercetin (26%), whereas in the stems and leaves, *p*-coumaric, rosmarinic, *trans*-2-dihydrocinnamic acids and resorcinol were predominant. In the flowers, vanillic acid was the main compound (51%). The antioxidant activities of *C. cyminum* essential oils and acetone extracts obtained from the three organs were assessed using four tests [1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene/linoleic acid, reducing power, and chelating power assays]. The acetone extract of flowers was strongly effective as a DPPH radical scavenger, lipid peroxidation inhibitor, and reducing agent, with IC_{50} values of 4, 32, and 8 μg/mL, respectively. Moreover, the acetone extract of stems and leaves showed the highest chelating power. However, the essential oils exhibited moderate activities in the different tests.

KEYWORDS: ***Cuminum cyminum* L.; organs; essential oil; phenolics; antioxidant activities**

INTRODUCTION

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, color, flavor, texture, and safety of foods (*1*). Recently, there is a growing interest in oxygen-containing free radicals in biological systems. Several studies investigated the biological activity of different secondary metabolites, including polyphenols and essential oils (*2*−*4*). Polyphenols, are considered as powerful active compounds expressing strong antioxidant activities (*5*). The presence of phenolic compounds (phenolic acids, polyphenols, and flavonoids) in many species of fruits, vegetables, herbs, cereals, sprouts, and seeds, along with the essential oil, has been investigated in terms of antioxidant activity (*2*−*4*). This activity is mainly due to their redox potential, which can play an important role in adsorbing and neutralizing free radicals, quenching reactive oxygen species, and chelating metal, especially iron and copper cations (*6*). Essential oils constitute a heterogeneous group of complex mixtures of organic substances. In addition to their classical roles as natural food additives and/or fragrances, many essential oils have also been confirmed to possess antioxidant activities (*7*−*10*). Plants that are rich in antioxidant compounds are attractive to the food industry, prompting their use as replacements for synthetic ones and also as nutraceuticals, playing a role in preventing many diseases. Therefore, there is an increasing demand to evaluate the antioxidant properties of plant extracts (*11*).

Cumin (*Cuminum cyminum* L.), a member of the Apiaceae family, is an annual herbaceous plant, growing to a height of about 25 cm. Its flowers are small, with white or pink color in compound umbels form. The seeds come as paired or separate carpel, about 3−6 mm long. They have a striped pattern, having nine ridges and oil canals (*12*). Cumin originated from Egypt, Turkistan, and east Mediterranean, but it is widely cultivated in Iran, China, India, Morocco, South Russia, Japan, Indonesia, Algeria, and Turkey (*13*). Its seeds are used mainly as a spice, constituting a major ingredient of most curry powders and many savory spice mixtures, used in stews and on the grill, especially for lamb and chicken dishes. Traditionally, farmers have been adding cumin straw to their animal nutrition, and the result, particularly in lactating animals, has been beneficial. However, information on the food value of cumin organs is scarce. Many phytochemical

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 Published on Web 09/01/2010

studies have been conducted thus far to investigate the chemical composition of the essential oil of cumin seeds. They state that the major components of cumin are aldhehydes, where the most prominent one is cuminaldehyde (*14*−*18*). Other major components of cumin include cuminic alcohol, γ-terpinene, *p*-cymene, and β-pinene, which were previously found in the essential oil obtained from Turkey (*19*), Pakistinian (*20*), and Iranien cumin seeds (*14*). Cumin oil is a pale yellow to brownish yellow liquid; it occasionally displays a greenish tint (*19*). However, the study of this plant as a source of bioactive compounds requires analysis of not only its seeds but also other different parts of the plant. It has been shown that the phenology of some species also affects the composition of their secondary metabolites (*21*). Besides, it was shown that the different parts of the same plant may have different oil, phenolic composition, and thereby, different antioxidant potentialities (*22*−*24*). Qualitative and quantitative data indicated that oil production from cumin herb is possible (*25*). Nevertheless, no work has been undertaken concerning the biochemical characterization of cumin flowers, stems and leaves, and roots. The aim of the present study was to determine essential oil and polyphenol composition and contents of different *C. cyminum* organs (flowers, stem and leaves, and roots) and to evaluate their antioxidant properties.

MATERIALS AND METHODS

Chemicals. All solvents used in the experiments (diethyl ether, acetonitrile, ethanol, acetone, and methanol) were purchased from Merck (Darmstadt, Germany). Sulfuric acid, acetic acid, tricloroacetic acid, and anhydrous sodium sulfate (Na_2SO_4) were purchased from Sigma−Aldrich (Steinheim, Germany). Butylated hydroxytoluene (BHT), β-carotene, linoleic acid, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), iron(II) chloride ($FeCl_2$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and polyvinyl polypyrolidone were purchased from Sigma. Folin−Ciocalteu reagent, aluminum chloride, sodium nitrite, and sodium carbonate were purchased from Aldrich. Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in high-performance liquid chromatography (HPLC)-grade methanol. These solutions were wrapped in aluminum foil and stored at 4 °C. All other chemicals used were of analytical grade.

Plant Material. Samples of stems and leaves, flowers, and roots of cumin (*C. cyminum* L.) were collected at the flowering stage from Menzel Temime (northeastern Tunisia; latitude, 36° 46′ 17.80″ N; longitude, 10° 46′ 03.38″ E; altitude, 141.43 m) on May 2009. This site was characterized by a low annual rainfall of 700 mm and a mean annual temperature of 16.8 °C. After harvest, samples were transported from the field to the laboratory and were air-dried before being treated.

Botanical identification of this species was carried out by Prof. A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia), and a voucher specimen has been kept in our laboratory for future reference.

Essential Oil Extraction. A total of 50 g of air-dried material was subjected to hydrodistillation for 90 min after a kinetic survey for 30, 60, 90, and 120 min. The obtained distillate was extracted using diethyl ether as the solvent and dried over anhydrous sodium sulfate. The obtained essential oil was stored at −20 °C prior to analysis.

Gas Chromatography−Flame Ionization Detector (GC−FID). Analytical GC was carried out on a Hewlett-Packard 6890 gas chromatograph series II (Agilent Technologies, Palo Alto, CA) equipped with HPInnowax and HP-5 (60 m × 0.25 mm, 0.25 μm film thickness) capillary column. Samples (1 μL) were injected with a split ratio of 1:60, and a continuous flow rate of 1.6 mL/min of chromatographic-grade nitrogen was used. The oven temperature was initially held for 10 min at 35 °C, ramped at 3 °C/min up to 205 °C, and held isothermal for 10 min. Injector and detector FID temperatures were held at 250 and 300 °C, respectively.

Gas Chromatography−Mass Spectrometry (GC−MS). The GC−MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm, 0.25 μm film thickness) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was helium, with a flow rate of 1.2 mL/min. The scan time and mass range were 1 s and m/z 50−550, respectively. The injected volume was 1 μL, and the total run time was approximately 63 min.

Compound Identification. The identity of the oil components was assigned by a comparison of their retention indices relative to C8−C22 *n*-alkanes to those of the literature or to those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC−MS data system and other published mass spectra (*26*). Determination of the percentage composition was based on peak area normalization without using correction factors.

Polyphenol Extraction. The air-dried stem and leaf, root, and flower were finely ground with type A10 blade-carbide gringing (Ika-Werk, Staufen, Germany). A total of 1 g of this ground material was extracted by stirring with 10 mL of 80% acetone 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 analyzed.

Total Phenolic Content. The total phenolic content was assayed using the Folin−Ciocalteu reagent, following Singleton's method, slightly modified by Dewanto et al. (*27*). An aliquot (0.125 mL) of a suitable diluted acetone sample 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% sodium carbonate (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 phenolic contents of stem and leaf, root, and flower (three replicates per treatment) were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg of GAE/g of DW) through the calibration curve with gallic acid. The calibration curve range was 50−400 mg/mL (R^2 = 0.99). Triplicate measurements were taken for all samples.

Assessment of Total Condensed Tannins. The total tannin content was measured using the modified vanillin assay described by Sun et al. (*28*). A total of 3 mL of 4% methanol vanillin solution and 1.5 mL of concentrated H_2SO_4 were added to 50 μL of suitably diluted sample. The mixture was kept for 15 min, and the absorbance was measured at 500 nm against methanol as a blank. The amount of total condensed tannins was expressed as milligrams of (+)-catechin equivalent per gram of dry weight (mg of CE/g of DW) through the calibration curve with catechin. Triplicate measurements were taken for all samples.

Total Flavonoid Content. The total flavonoid content was measured according to Dewanto et al. (*27*). A total of 250 μL of the sample appropriately diluted was mixed with 75 μL of 5% $NaNO_2$ (sodium nitrite). After 6 min, 150 μL of 10% aluminum chloride ($AlCl_3$) and 500 μL of 1 M NaOH were added to the mixture. Finally, the mixture was adjusted to 2.5 mL with distilled 120 water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid contents of leaf and stem and flower (three replicates per treatment) were expressed as milligrams of catechin equivalents per gram of dry weight (mg of CE/g of DW) through the calibration curve with catechin. The calibration curve range was 50−500 mg/mL. Triplicate measurements were taken for all samples.

Reversed-Phase (RP)-HPLC Evaluation of Major Antioxidant Compounds. Dried samples from different cumin parts were hydrolyzed according to the method of Proestos et al. (*29*), which was slightly modified. A total of 20 mL of 80% acetone 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, 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 chromatograph (RP-HPLC) coupled with an UV−vis multiwavelength 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% sulfuric acid (solvent B). The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 15% A/85% B at 0−12 min, 40% A/60% B at 12−14 min, 60% A/40% B at 14−18 min, 80% A/20% B at 18−20 min, 90% A/10% B at 20−24 min, and 100% A at 24−28 min (*30*). 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 to standards. Analyses were performed in triplicate.

DPPH Assay. The donation capacity of the obtained extracts and essential oils was measured by bleaching of the purple-colored solution of the DPPH radical according to the method of Hanato et al. (*31*). A total of 1 mL of different concentrations of extracts and essential oils prepared in 80% acetone was added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity was expressed as IC_{50} (μg/mL), the concentration required to cause 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

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

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 analyzed in triplicate.

β-Carotene Bleaching Test. A modified method described by Koleva et al. (*32*) was employed. β-Carotene (2 mg) was dissolved in 20 mL of chloroform. Then, 4 mL of this solution was added to linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was evaporated under vacuum at 40 °C, and 100 mL of oxygenated ultrapure water was added. Then, the emulsion was vigorously shaken. Reference compounds [BHT and butylated hydroxyanisole (BHA)], sample extracts, and essential oils were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of different concentrations of essential oils and extract. 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 and BHA were used as a positive control. In the negative control, the essential oil or the extract were substituted with an equal volume of methanol. The antioxidant activity (%) of the acetone extracts and essential oils was evaluated in terms of the bleaching of the β-carotene using the following formula:

$$\text{percent inhibition (\%)} = [(A_t - C_t)/(C_0 - C_t)] \times 100$$

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 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 50% β-carotene bleaching inhibition.

Chelating Effect on Ferrous Ions. The ferrous ion chelating activity of different organ extracts and essential oils was assessed as described by Zhao et al. (*33*). Different concentrations of the sample were added to 0.05 mL of $FeCl_2 \cdot 4H_2O$ solution (2 mM) and left for incubation 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. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine$-Fe^{2+}$ complex formation was calculated using the following formula:

$$\text{metal chelating effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

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 analyzed in triplicate.

Reducing Power. The method of Oyaizu (*34*) was used to assess the reducing power of different organ extracts and essential oils. A total of 1 mL of different concentrations of organ extracts and essential oils in 80% acetone was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6) 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 of 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 a positive control.

Statistical Analysis. All extractions and determinations were conducted in triplicate. Data are expressed as mean ± standard deviation (SD). The means were compared using the one-way and multivariate analysis of variance (ANOVA) 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 using the "Statistica, version 5.1" software (*35*).

RESULTS AND DISCUSSION

Essential Oil Yield. Hydrodistillation of different plant parts did not yield the same production and gave rise to different amounts of essential oil, with average yields of 0.03% in roots, 0.1% in stems and leaves, and 1.7% in flower. Essential oil obtained differed by their colors: pale yellow in flowers, marked for stems and leaves, and translucent in roots. The essential oil odors of all of these parts were agreeable and characteristic. To the best of our knowledge, there are no data concerning the essential oil yield from organs of *C. cyminum*, except by El Sawi et al. (*25*), who found that cumin herbal oil gave 0.3%. This difference could be related to abiotic factors and the phenological stage. The latter affects the physiological behavior of the plant between different development steps (vegetative, flowering, and fruiting stages). On the other hand, it is known that essential oil accumulation is organ-dependent. The highest yield found in flower in our experiment is in agreement with the results obtained for the Apiaceae family; in fact, that flower has been found to exhibit generally the highest essential oil yield as compared to the other organs, such as *Coriandrum sativum* (*36*). Similar results were reported in species of other families, such as *Hyptis suaveolens* (*37*), *Origanum vulgare* (*38*), and *Hypericum perforatum* (*39*). In most cases, flowers produce a wide variety of compounds, mainly terpenoids and benzenoids, that facilitate the pollinization of this species (*40*), whereas leaves produce mainly lower molecular-weight compounds that can attract the natural enemies of the herbivores (*41*); i.e., these compounds have important roles in insect−plant interactions.

In contrast, Spanish *Eryngium glaciale* (Apiaceae) showed a higher essential oil content in roots, followed by the stems and leaves and finally the inflorescences. These differences could be explained by the accumulation of the essential oil in *Eryngium* roots during the winter, as with other Apiaceae species, and later distributed throughout the plants during the vegetative season (*22*).

Essential Oil Composition. The identified oil components representing 95.6, 88.2, and 99.6% for roots, stems and leaves, and flowers, respectively, are summarized in **Table 1**. A total of 57 compounds was identified, where 8 characterized the three organs studied. A total of 47 compounds were identified in the essential oils of stems and leaves. In contrast, the number of identified compounds in roots was significantly ($p < 0.05$) lower (21 compounds).

The isolated essential oils were a complex mixture of terpenic hydrocarbons, alcohols, aldehydes, ketones, epoxides, phenols, acids, and esters. Essential oils obtained from roots, stems and leaves, and flowers were characterized by the predominance of terpenic hydrocarbons. Also, we noted that the aerial parts (stems and leaves and flowers) were significantly richer in terpenic hydrocarbons (61.3−47.4%) and alcohols (20.1−17.6%) than roots. The latter contained more esters (24.5%), acids (10.4%), and ketones (6.4%).

The essential oil of roots showed as main constituents bornyl acetate (23.8%), diepi-α-cedrene (18.5%), β-pinene (13.1%), hexadecanoic acid (10.4%), and camphor (6.4%). The main

Table 1. Essential Oil Composition of Different Parts of *C. cyminum* L. (Roots, Stem and Leaves, and Flowers)[a]

compounds	RI[b]	RI[c]	identification[d]	root	leaf and stem	flower
terpenic hydrocarbons				39.98 ± 1.94 c	47.49 ± 2.88 b	61.37 ± 2.97 a
α-thujene	928	1035	MS	1.00 ± 0.24 a	–	0.03 ± 0.01 b
camphene	954	1076	RI, MS	–	–	0.12 ± 0.03 a
β-pinene	980	1118	RI, MS, co-GC	13.16 ± 0.71 a	0.51 ± 0.05 c	7.03 ± 0.93 b
sabinene	975	1132	RI, MS	–	–	0.28 ± 0.07 a
α-phellandrene	1006	1176	RI, MS	–	–	8.04 ± 1.52 a
α-terpinene	1018	1188	MS, co-GC	–	34.40 ± 2.53 a	0.28 ± 0.09 b
limonene	1030	1203	RI, MS, co-GC	–	1.52 ± 0.05 a	0.42 ± 0.08 b
1.8-cineol	1033	1233	RI, MS, co-GC	–	3.60 ± 0.74 a	0.98 ± 0.06 b
(*E*)-β-ocimene	1040	1266	RI, MS	–	0.93 ± 0.04 a	–
γ-terpinene	1062	1255	RI, MS	–	1.62 ± 0.03 b	51.75 ± 2.63 a
p-cymene	1026	1280	RI, MS, co-GC	–	3.03 ± 0.50 a	0.76 ± 0.01 b
terpinolene	1092	1290	RI, MS, co-GC	3.53 ± 0.08 a	0.15 ± 0.02 b	–
α-humulene	1452	1687		tr	tr	tr
(*E*)-β-farnesene	1461	1770	RI, MS, co-GC	2.02 ± 0.33 a	0.35 ± 0.03 b	–
diepi-α-cedrene	1450	1762	MS	18.51 ± 0.09 a	–	–
α-curcumene	1474	1786	MS	–	0.78 ± 0.04 a	tr
γ-cadinene	1525	1773	RI, MS, co-GC	0.29 ± 0.03 a	–	tr
germacrene-D	1480	1715	RI, MS	1.47 ± 0.02 a	0.60 ± 0.01 b	–
alcohols				2.98 ± 0.77 c	17.61 ± 1.45 b	20.15 ± 2.93 a
2-ethyl-1-hexanol	1101	1553	MS, co-GC	2.74 ± 0.07 a	0.52 ± 0.02 b	–
Z-3-hexenol	855	1370	RI, MS, co-GC	–	0.30 ± 0.01 a	–
linalool	1098	1553	RI, MS, co-GC	–	0.09 ± 0.01 a	–
p-menth-2-en-1-ol	1130	1638	MS	–	0.09 ± 0.02 b	0.38 ± 0.03 a
1.4-*p*-menthadien-7-ol	1315	1948	MS	–	3.21 ± 0.07 a	0.03 ± 0.002 b
p-cymene-8-ol	1183	1864	RI, MS, co-GC	–	0.24 ± 0.02 a	–
terpinene-4-ol	1178	1611	RI, MS, co-GC	–	0.67 ± 0.01 a	0.14 ± 0.03 b
1-phenyl-1-butanol	1355	1970	MS	–	2.28 ± 0.08 b	5.31 ± 0.20 a
1-phenyl-1.2-ethanediol	1350	1973	MS	–	4.41 ± 0.32 b	13.04 ± 0.63 a
cis-carveol	1247	1861	RI, MS	–	–	0.14 ± 0.05 a
carotol	1300	1897	RI, MS	0.24 ± 0.09 b	4.23 ± 0.43 a	0.47 ± 0.06 b
cis-sabinene hydrate	1464	1078	RI, MS, co-GC	–	0.40 ± 0.02 a	0.13 ± 0.03 b
geraniol	1255	1857	RI, MS, co-GC	–	0.10 ± 0.01 a	tr
farnesol			RI, MS	–	1.07 ± 0.03 a	tr
α-terpineol	1198	1709	RI, MS, co-GC	–	–	0.50 ± 0.09 a
aldehydes				8.24 ± 0.05 a	7.75 ± 1.01 ab	9.25 ± 0.03 a
citronellal	1201	1464	RI, MS, co-GC	5.78 ± 1.03 a	1.52 ± 0.04 b	–
myrtenal	1237	1472	RI, MS, co-GC	–	0.07 ± 0.02 a	tr
safranal	1211	1460	RI, MS, co-GC	–	0.19 ± 0.04 a	tr
nonanal	1157	1528	RI, MS, co-GC	2.02 ± 0.07 a	–	–
cuminaldehyde	1283	1785	MS	–	5.41 ± 0.32 b	8.85 ± 0.65 a
heptanal	902	1194	MS, co-GC	0.44 ± 0.04 a	0.18 ± 0.03 b	0.14 ± 0.01 b
cinnamaldheyde	1283	1785	RI, MS, co-GC	–	0.38 ± 0.04 a	0.25 ± 0.01 a
ketones				6.48 ± 0.96 a	1.35 ± 0.07 b	–
camphor	1143	1532	RI, MS, co-GC	6.48 ± 0.77 a	1.07 ± 0.14 b	–
pulegone	1220	1511	RI, MS, co-GC	–	0.28 ± 0.02 a	–
epoxides				–	0.52 ± 0.03 a	0.04 ± 0.01 b
cis- linalool oxide	1074	1478	RI, MS	–	0.08 ± 0.02 a	–
trans-linalool oxide	1088	1475	RI, MS	–	0.20 ± 0.05 a	0.04 ± 0.01 b
caryophyllene oxide	1596	2008	RI, MS	–	0.24 ± 0.011 a	–
phenols				2.34 ± 0.17 a	2.82 ± 0.33 a	0.79 ± 0.03 b
eugenol	1356	2192	RI, MS, co-GC	0.39 ± 0.05 b	0.17 ± 0.02 b	0.70 ± 0.09 a
thymol	1290	2198	RI, MS	0.43 ± 0.02 a	0.05 ± 0.02 b	tr
carvacrol	1296	2215	MS	–	0.87 ± 0.03 a	–
apiole			MS	–	0.51 ± 0.04 a	–
methyl eugenol	1408	2004	RI, MS, co-GC	1.52 ± 0.07 a	1.22 ± 0.02 a	0.08 ± 0.01 b
acids				10.43 ± 1.66 a	2.68 ± 0.10 b	–
hexadecanoic acid	1598	1600	MS	10.43 ± 1.66 a	2.68 ± 0.10 b	–
ester				24.54 ± 1.56 a	5.86 ± 0.87 b	0.13 ± 0.77 c
bornyl acetate	1295	1597	RI, MS	23.83 ± 1.03 a	5.27 ± 0.33 b	0.13 ± 0.02 c
α-terpenyl acetate	1343	1389	RI, MS	–	0.21 ± 0.03 a	–
geranyl acetate	1383	1765	MS	0.71 ± 0.0.05 a	0.38 ± 0.05 a	–
others				0.56 ± 0.02 a	0.32 ± 0.03 a	–
3-buthylphtalide	–	2023	MS	0.56 ± 0.02 a	0.25 ± 0.01 a	–
n-eicosane	1996	2000	MS	–	0.07 ± 0.02 a	–
NI				0.56 ± 0.03 b	1.07 ± 0.02 a	0.04 ± 0.02 c

[a] – = not detected. Values followed by the same letter did not share significant differences at 5% (Duncan test). Means of three replicates ± SD. Values with different letters are significantly different at $p < 0.05$. [b] RI = retention indices calculated using an apolar column (HP-5). [c] RI = retention indices calculated using a polar column (HP-Innowax). [d] RI, retention indices relative to C8−C22 *n*-alkanes on the (HP-Innowax); MS, mass spectrum; co-GC, co-injection with authentic compound.

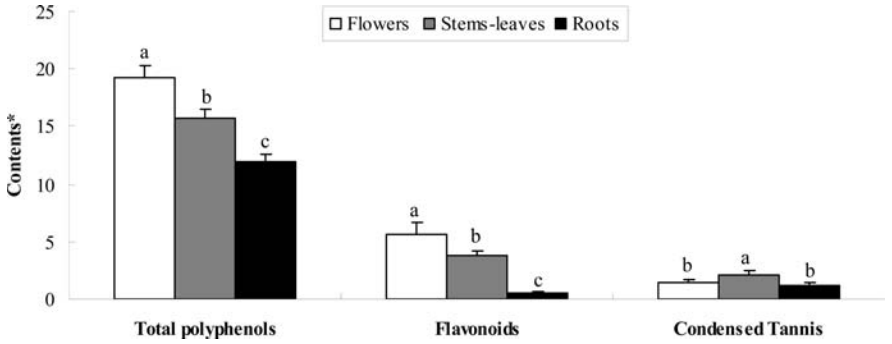

Figure 1. Total phenolic, flavonoid, and tannin contents of different parts of *C. cyminum* L. (∗) Total phenolics were expressed by mg of GAE/g of DW. Total flavonoid and condensed tannin contents were expressed by mg of CE/g of DW. Values are represented as mean ± SD of triplicates. The data marked with the different letters in the histograms of each phenolic category share significant differences at $p < 0.05$ (Duncan test). GAE, gallic acid equivalents; CE, catechin equivalents.

component found in the oil obtained from stems and leaves was α-terpinene (34.4%). This compound is responsible for the oil herbal note (*42*). Hydrodistilled oil of flowers contained mainly γ-terpinene at a high percentage (51.7%), followed by 1-phenyl-1,2-ethanediol (13%), cuminaldehyde (8.8%), α-phellandrene (8%), and β-pinene (7%). Fresh and spicy notes of this oil were related to γ-terpinene and cuminaldehyde, respectively (*42*). It is to be noticed that diepi-α-cedrene and nonanal were found only in the essential oil of roots.

When our results obtained from different organs were compared to cumin herb oil composition gathered in Egypt (*25*), we noted a significant difference in composition. They reported that cuminaldehyde was considered as the principal constituent (40.5%), followed by acordiene (7.6%) and 4-(1-methylethyl)-benzoic acid (5.3%).

Moreover, ours results revealed that the essential oil of roots showed great differences in the percentages of its constituents compared to that of stems and leaves. It was rich in β-pinene (13.1%), terpinolene (3.5%), camphor (6.4%), hexadecanoic acid (10.4%), and bornyl acetate (23.8%) but exhibited a lower percentage of carotol (0.24%). Our results also illustrated differences in the proportions of some compounds in flowers and stems and leaves. The most important differences are attributed to γ-terpinene, 1-phenyl-1,2-ethanediol, and cuminaldehyde. These components were found in larger proportions in oil from flowers than in those of aerial parts (stems and leaves), in which bornyl acetate was more aboundant (5.2%). Also, we noticed that some compounds, such as sabinene, α-phellandrene, and α-terpineol, were present only in flowers, whereas nonanal was found only in roots. α-Terpinene, limonene, 1,8-cineol, 1-phenyl-1-butanol, 1-phenyl-1,2-ethanediol, and cuminaldehyde were found in both flowers and stems and leaves, but they were absent in roots. It seems that different metabolic pathways closely related to genetic information are elicited in *C. cyminum* L. secondary metabolism during plant development, generating a great variability in essential oil composition (*39*).

These findings suggest that the irregular allocation of essential oil compounds was organ-dependent. Different from our results, in several Apiaceae species, the essential oil extracted from different plant parts was characterized by the same main compound, such as for fennel (*Foeniculum vulgare*), where *trans*-anethole was the main component in all organs (stems and leaves and buds and inflorescences of flowers), as reported by Stefanini et al. (*43*). Besides, (*E*)-2-dodecanal was found as the main compound in all *Coriandrum sativum* organs (upper leaves, basal leaves, flowers, stems, and roots) (*36*).

Contents of Total Phenols, Tannins, and Flavonoids. Quantitative evaluation of total phenolic in acetone extracts revealed that cumin organs exhibited high and variable contents (**Figure 1**). Thus, the flower extract has the highest total phenol content (19.2 mg of GAE/g of DW), followed by stems and leaves (15.6 mg of GAE/g of DW) and roots (11.8 mg of GAE/g of DW). Data concerning the phenolic content of cumin are very limited. Thippeswamy and Naidu (*44*) studying cumin seed methanolic extracts, reporting a weaker content of total phenolics (8.6 mg of GAE/g of DW). This result confirms that the phenolic content is organ-dependent; however, the influence of the solvent must not be excluded because the solvent polarity could lead to variation in extract polyphenolic content (*45*, *46*). Cumin parts also significantly differed on their flavonoid and condensed tannin contents. As for polyphenols, flowers had the highest total flavonoid content (5.6 mg of catechin equivalent per gram of dry weight (mg of CE/g of DW) followed by stems and leaves, whereas the root extract content was very weak (0.4 mg of CE/g of DW). However, concerning condensed tannin contents, the extract of stems and leaves displayed the highest content (2.1 mg of CE/g of DW), which was about 1.5-fold higher than that of the extracts of flowers and roots (1.4 and 1.2 mg of CE/g of DW, respectively). Phenolic content variability between plant organs has been previously reported (*21*,*47*). Phenolics occur ubiquitously in plants and are not uniformly distributed in plants at the tissue level (*48*). Within a same species, diversity may occur because of genetic factors, environment conditions, and growth stage (*49*).

Identification and Quantification of Phenolic Compounds. No data are available concerning the phenolic composition of *C. cyminum*. Here, for the first time, polyphenol qualitative and quantitative determination of the different plant parts was performed by RP-HPLC analysis. Phenolics occur in plants mainly as aglycones, glycosides, or esters or are bond to the cell wall (*50*). For this reason, acidic hydrolysis was used to release aglycones, and the results are presented in **Table 1**. Great qualitative and quantitative differences were noticed between the organs. In fact, roots were dominated by the presence of flavonoids (59.1%), whereas phenolic acids appeared to be the main phenolics present in the extracts of leaves and stems and flowers, representing 72.6 and 75.7% of total phenolic compounds, respectively.

A total of 12 phenolic compounds were successfully identified in the roots, where quercetin was the major one (26.1%). This organ was also characterized by the presence of cinnamic acid (14.4%), amentoflavone (8.9%), and ferulic acid (7.2%) at interesting proportions.

In stems and leaves, 17 compounds were identified. The most abundant compounds were *p*-coumaric, rosmarinic, and *trans*-2-dihydrocinnamic acids as well as resorcinol, with percentages of

Table 2. Contents (mg/g) and Percentages (%) of Phenolic Compounds of Different Parts of *C. cyminum* L.[a]

	root		leaf and stem		flower	
	mg/g	%	mg/g	%	mg/g	%
phenolic acids	1.57 ± 0.01 c	37.18	5.21 ± 0.08 b	72.61	9.34 ± 0.04 a	75.79
gallic acid	0.08 ± 0.01 b	1.86	0.41 ± 0.03 a	5.69	0.04 ± 0.03 b	0.34
cafeic acid	0.11 ± 0.01 a	2.61	0.14 ± 0.01 a	1.99	0.11 ± 0.03 a	0.89
dihydroxyphenolic acid	–	–	0.04 ± 0.01 a	0.61	0.05 ± 0.01 a	0.36
dihydroxybenzoic acid	–	–	0.08 ± 0.01 a	1.14	–	–
chlorogenic acid	–	–	0.11 ± 0.02 a	1.54	0.10 ± 0.01 a	0.82
syringic acid	0.19 ± 0.02 b	4.56	0.32 ± 0.04 a	4.49	–	–
vanillic acid	–	–	0.60 ± 0.02 b	8.40	6.36 ± 0.33 a	51.61
p-coumaric acid	0.11 ± 0.16 b	2.58	1.23 ± 0.22 a	17.18	–	–
ferulic acid	0.31 ± 0.01 b	7.24	0.29 ± 0.04 b	4.02	0.73 ± 0.04 a	5.89
rosmarinic acid	–	–	1.05 ± 0.01 a	14.69	0.04 ± 0.01 b	0.33
trans-2-dihydrocinnamic acid	0.16 ± 0.01 b	3.89	0.81 ± 0.04 a	11.28	0.64 ± 0.01 a	5.17
cinnamic acid	0.61 ± 0.07 b	14.40	0.11 ± 0.01 c	1.52	1.27 ± 0.02 a	10.33
flavonoids	2.50 ± 0.03 a	59.17	1.53 ± 0.04 b	21.27	2.35 ± 0.01 a	19.06
resorcinol	0.25 ± 0.01 b	5.95	0.77 ± 0.13 a	10.66	–	–
catechin	0.15 ± 0.01 b	3.61	0.24 ± 0.04 b	3.39	0.53 ± 0.01 a	4.31
coumarin	–	–	0.10 ± 0.01 b	1.46	0.42 ± 0.02 a	3.36
quercetin	1.10 ± 0.01 a	26.13	–	–	0.35 ± 0.91 b	2.86
apigenin	0.20 ± 0.01 b	4.72	–	–	0.41 ± 0.04 ab	3.33
amentoflavone	0.38 ± 0.02 a	8.91	0.01 ± 0.04 b	0.20	0.31 ± 0.13 a	2.55
flavone	0.28 ± 0.01 a	6.55	0.02 ± 0.05 c	0.28	0.18 ± 0.07 b	1.42
unknown	0.36 ± 0.03 b	3.65	0.82 ± 0.11 a	6.12	0.58 ± 0.05 a	5.15
total	4.22 ± 0.55 c	100	7.18 ± 0.03 b	100	12.32 ± 1.22 a	100

[a] Values are represented as mean ± SD of triplicates. Values followed by the same letter did not share significant differences at $p < 0.05$ (Duncan test). – = not detected.

17.1, 14.6, 11.2, and 10.6%, respectively. Additionally, vanillic and gallic acids were also found at appreciable levels (8.4 and 5.6%). Although dihydroxybenzoic acid was detected in a small percentage (1.1%) in the stems and leaves, this phenolic acid remained absent in the other organs (roots and flowers). Different from the last organs, the phenolic composition of the extract of flowers was mainly dominated by vanillic acid (51.6%). The other phenolic compounds were present at relatively low percentages (< 5.9%), except cinnamic acid (10.3%).

This is the first report showing the presence of an array of phenolic compounds in *C. cyminum* organs. Ani et al. (*51*) studied the phenolic composition of *C. nigrum* seeds and phenolics, including gallic, protocatechuic, caffeic, ellagic, and ferulic acids and also flavonols, such as quercetin and kaempferol. These authors also found that caffeic and ferulic acids were the predominant compounds.

Quantitation of total phenolic compounds by HPLC indicates that the flower was the richest organ followed by the stems and leaves and the root, with respective quantities of 12.3, 7.1, and 4.2 mg/g. Such order is in concordance with that estimated by the Folin−Ciocalteu assay. However, contents obtained by HPLC were significantly lower than those obtained by the Folin−Ciocalteu method. This is predictable because of the weak selectivity of the Folin-Ciocalteu reagent, because it reacts positively with different phenolic and non-phenolic substances (*52*).

Antioxidant Activity. The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition-metal ion catalysts (*53*). Thus, to evaluate antioxidant effectiveness, several analytical methods and different substrates are used.

The free-radical-scavenging activity of essential oils and acetone extracts was assessed using DPPH (**Table 2**). We noticed that the different *C. cyminum* acetone organ extracts were more effective to scavenge the DPPH radical than the organ essential oils. Indeed, IC_{50} values of acetone extracts ranged from 4 to 65 μg/mL, whereas the essential oils presented high IC_{50} values of 548, 753, and 1487 μg/mL, respectively, for flower, stem and leaf, and root. Moreover, the DPPH-scavenging activity of the three different acetonic extracts was greater than that reported by Thippeswamy and Naidu (*44*) for the methanolic seed extract of *C. cyminum* (IC_{50} = 520 μg/mL). Our results indicated also that the acetone extracts of flowers and roots were particularly active, exhibiting IC_{50} values of 4 and 8 μg/mL, respectively, which were lower than the positive control BHT (IC_{50} = 25 μg/mL). It is well-known that antioxidants react with DPPH free radical and convert it to the stable form. Therefore, *C. cyminum* acetone extracts, especially those of flowers and roots, seem to contain powerful inhibitor compounds, which may act as primary antioxidants that react with free radicals as efficient hydrogen donors.

As for the DPPH assay, the assessment of the reducing power showed that *C. cyminum* acetone extracts were more effective than the essential oils as reducing agents, exhibiting IC_{50} values ranging from 8 to 136 μg/mL. The acetone extracts of flowers and leaves and stems demonstrated particularly a strong activity with IC_{50} values of 8 and 32 μg/mL, respectively. Furthermore, in comparison to the positive control asorbic acid (IC_{50} = 40 μg/mL), the two acetone extracts exhibit 5- and 1.3-fold higher activities. These results indicate that the different acetone extracts are able to act as a 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 (*54*). Furthermore, the activity of the essential oils was relatively weak in comparison to acetone extracts, with the essential oil of flowers being the most active, with an IC_{50} value of 696 μg/mL.

The potential of *C. cyminum* to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test. This test measures the potential of the plant to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation (*55*).

The different acetone extracts were effective in the β-carotene/linoleic acid bleaching test (**Table 3**). The acetone extracts of flowers and stems and leaves exhibited high activity, with IC_{50} values of 32 and 55 μg/mL, respectively. This activity was stronger than that of BHT (IC_{50} = 70 μg/mL) and close to that of BHA (IC_{50} = 43 μg/mL). Among the *C. cyminum* essential oils, that obtained from the flower was the most active, exhibiting an interesting activity, with an IC_{50} value of 115 μg/mL, followed by the essential oils of roots and stems and leaves, showing moderate (IC_{50} = 228 μg/mL) and weak (IC_{50} = 1120 μg/mL) activity, respectively.

The study of the metal chelating power revealed that the extracts differed on their chelating power; essential oils of stems and leaves and roots were completely inactive, whereas those from flowers as well as the different acetone extracts were capable of interfering with Fe^{2+}−ferrozine complex formation, suggesting that they have the ability to capture ferrous ions before ferrozine (**Table 3**). These results also indicate that the acetone extract of stems and leaves exhibited the highest power, with an IC_{50} value of 4 mg/mL, followed by the essential oil and acetone extract of flowers, which showed comparable activities (IC_{50} values of 12 and 18 mg/mL, respectively). However, in comparison to the positive control, these activities remained weak. The chelating power is important because transition-metal ions can stimulate lipid peroxidation by two mechanisms, namely, by participating in the generation of initiating species and by accelerating hydroperoxide production from lipid oxidation.

Generally, a positive correlation between the phenolic content and antioxidant capacity is reported. Thus, this high performance of the acetone extract of flowers in the different systems, except for the chelating test, is related to their phenolic composition in comparison to that of stems and leaves and roots. Recently, it has been shown that the antioxidant activity of extracts is roughly connected to their phenolic composition and strongly depends upon their phenolic structures (*56*). Our RP-HPLC analysis showed that the *C. cyminum* flower exhibits a high vanillic acid content. This phenolic acid has been reported as an efficient antioxidant compound, scavenging reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical (*46*). Moreover, Andjelkovic et al. (*57*) studied the capacity of several phenolic acids for complex formation with iron and found that vanillic acid was not able to chelate iron because of the absence of the catechol or galloyl moiety. This may explain the low chelating power of the extracts of flowers in comparison to their efficiency in other systems. In the chelating test, the acetone extract of stems and leaves was the most effective; this could be mediated at least in part by its high quercetin content. In fact, this flavonol is a powerful chelating agent, which is derived from three structural features: 3′,4′-*o*-dihydroxycatechol on the B ring, 3-hydroxyl in conjugation with a 4-oxo function on the C ring, and 5-hydroxyl on the A ring in conjugation with a 4-oxo function on the C ring (*58*). In addition, quercetin is known to exhibit strong radical scavenging and reducing activities (*59*).

On the other hand, the moderate activity of the essential oils found in our study may be connected to their composition, dominated by terpenic hydrocarbons. Antioxidant property is usually attributed to the phenolic volatiles, mainly carvacrol and thymol (*60*, *61*). These compounds were weakly represented in our experiment (< 0.9%). However, some monoterpene hydrocarbons have been found active as radical scavengers and antioxidant compounds. This is the case of β-pinene and γ-terpinene (*61*). The essential oil of flowers was found to be the most effective in the different antioxidant assays. Its moderate activities could be attributed at least in part to its richness in γ-terpinene (51.7%), whose activity has been reported to be due

Table 3. Antioxidant Activities of the Essential Oils and the Acetone Extracts from Different Parts of *C. cyminum* L.[a]

	essential oil			acetone extract			synthetic antioxidant			
	root	leaf and stem	flower	root	leaf and stem	flower	BHT	EDTA	ascorbic acid	BHA
DPPH (IC_{50}, μg/mL)	1478 ± 2.12 a	753 ± 1.87 b	548 ± 3.24 c	8 ± 0.44 f	65 ± 0.55 d	4 ± 0.05 f	25 ± 0.54 e	–	–	–
β-carotene bleaching (IC_{50}, μg/mL)	228 ± 0.66 b	1120 ± 2.84 a	115 ± 0.78 c	224 ± 0.59 b	55 ± 0.47 e	32 ± 0.07 g	70 ± 0.98 d	–	–	43 ± 0.56 f
chelating ability (IC_{50}, mg/mL)	–	–	12 ± 0.44 c	26 ± 1.11 a	4 ± 0.22 d	18 ± 1.02 b	–	0.03 ± 0.01 e	–	–
reducing power (EC_{50}, μg/mL)	1200 ± 0.87 a	920 ± 1.33 b	696 ± 2.78 c	136 ± 0.69 d	32 ± 1.75 f	8 ± 0.3 g	–	–	40 ± 0.84 e	–

[a] Each value in the table was obtained by calculating the average of three experiments. Means followed by the same letters are not significantly different at $p = 0.05$ based on Duncan's multiple range test. – = not detected.

to the presence of activated methylene groups. Tepe et al. (*61*) reported that this monoterpene was more active in the carotene/linoleic acid test than in the DPPH assay. This is in agreement with our results concerning essential oil activities of flowers.

Our study is the first report on the activities of *C. cyminum* essential oil and acetone extracts from different plant parts, its chemical composition, and its antioxidant activity. Essential oils, which were rich in γ-terpinene (flowers), thymol and α-terpinene (stems and leaves), and bornyl acetate (roots), exhibited a moderate antioxidant activity. The major phenolic compounds were quercetin (roots), *p*-coumaric (stems and leaves), and vanillic acid (flowers). Acetone extracts have been proven to be alternative sources of natural antioxidants and more efficacious than various synthetic antioxidants, BHA, EDTA, ascorbic acid, and BHT. This activity was high enough for the plant to be a new and natural source of strong antioxidant substances for use as natural additives in food. To understand their mechanism of action as bioactive components, further fractionation of acetonic extracts, isolation of phenolic compounds, and determination of their biological activities *in vitro* and *in vivo* are needed.

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Received for review June 15, 2010. Revised manuscript received August 23, 2010. Accepted August 25, 2010.