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Quantitative and Qualitative Analysis and Evaluation of Antioxidant Activity of Phenolic Compounds Extracted from Apiaceae Family Spices

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ABSTRACT

The aim of the present study was to analyze the phenolic compounds extracted from seeds of four spices belonging to Apiaceae family, namely caraway (*Carum carvi*), coriander (*Coriandrum sativum* L.), mystical cumin (*Ammodaucus leucotrichus*), and cumin (*Cuminum cyminum* L.). The extraction was carried out using solvents of different polarities (water, ethanol, methanol, and hexane). The antioxidant activity of each studied spice was performed by using different methods, including total antioxidant capacity (TAC), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2 picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and metal ion chelation power (CP). On the basis of the obtained results, a significant difference in the contents of phenolic compounds of the analyzed seeds was observed. The lowest phenolic compounds level was obtained by the hexane extract (HE) for the four studied spices. Besides, the aqueous extract (AE) of conventional cumin showed the highest level of phenolic compounds (16.49 mg Gallic acid equivalents (GAE)/g dry weight (DW)), followed by the AE of caraway (15.9 mg GAE/g DW), then the AE of mystical cumin (15.01 mg GAE/DW), and, at the end, by the AE of coriander (12.89 mg GAE/g DW). This work revealed a good correlation between the antioxidant activities of the studied seed and the type of solvents used for their extraction. The studied spices present a much-diversified molecular weights distribution of the phenolic compounds evaluated by exclusion chromatography on the Sephadex G50 gel. The climatological analysis also showed that the production of phenolic compounds was strongly influenced by some environmental factors, such as the mean annual rainfall and temperature values registered in the planting zones of the four studied spices in Morocco.

Keywords: spices, coriander, phenolic compounds, exclusion chromatography, mystical cumin, caraway, antioxidant activities.

INTRODUCTION

Spices are widely used in meat products and as additives in their processing (Trifan *et al.*, 2021). Thus, these spices and their components are used in the food industry as flavoring to replace artificial antioxidants (Chen *et al.*, 2014, Li and Jiang, 2004, Westh *et al.*, 2004). Several Apiaceae seeds are used as spices, such as caraway, coriander, and cumin, which are essential in many kitchens for seasoning and flavoring foods. Additionally, they are also used in traditional medicine, pharmaceuticals, food technology, cosmetics and as biopesticides (Mandal *et al.*, 2022). Apiaceae spices are known for their antifungal (Westh *et al.*, 2004), antibacterial (Demir and Korukluoglu, 2020, Hamdani and Antony, 2022), anticancer and antidiabetic effects (Abouri *et al.*, 2012). The spices from the Apiaceae family, as indicated by (Mandal *et al.*, 2022), are abundant as antioxidants grouping together a large group of bioactive compounds consisting of phenolic compounds, flavonoids, tannins, and vitamins. The diverse chemical compositions of these spices primarily contribute to their antioxidant properties.

Caraway, coriander, mystical cumin, and cumin, as plants of the Apiaceae family planted in different climatic zones in Morocco, are used as food additives seasoning. In more developed countries, they are also used in the pharmaceutical industries or for medicinal purposes, such as antifungal (Gherraf *et al.*, 2017, Singh *et al.*, 2002), anti-inflammatory (Mohammedi *et al.*, 2018, Sandra *et al.*, 2012), antimicrobial, antidiabetic, anti-hyperglycemic activities (Eddouks *et al.*, 2004), anticancer (Eddouks *et al.*, 2004) and anti-hypercholesteroloid (Laribi *et al.*, 2010) and as antioxidants compounds (Abouri *et al.*, 2012, Bravo, 1998, Gazdik *et al.*, 2008, Khalfaoui *et al.*, 2021, Nostro *et al.*, 2005, Oganesyan *et al.*, 2007, Trifan *et al.*, 2020).

Thus, the main purposes of this study were to highlight the importance of mystical cumin with studied spices as preservative additives in meat products in Morocco as potential sources of bioactive molecules. There has been a growing awareness among both food producers and consumers regarding the substitution of synthetic preservatives with natural alternatives.

Industries, such as meat production, are particularly concerned about the quality and safety of their products. There is a pressing need to develop and implement effective processes for preserving these products in a more natural manner, with the aim of reducing the adverse effects currently associated with the use of nitrates and nitrites in industrial meat processing (Petcu *et al.*, 2023). In this regard, spices offer a promising alternative as natural food additives for meat preservation, contributing to prolonged shelf life, inhibition of bacterial growth, reduction or elimination of foodborne pathogens (Qiu *et al.*, 2022), and improvement in flavor.

The study aimed at the comparison of mystical cumin with the studied spices. Since they already have been used as meat products additives (Trifan *et al.*, 2021), phenolic compounds (extracted by different solvents: hexane, methanol, ethanol, and water) and their antioxidant activities were targeted to determine the culinary and medical classification of these spices which constitute an important financial source to fight against social insecurity in less developed areas in Morocco. The fraction of flavonoids relative to phenolic compounds (F/PC) was used in this study as an indicator to select the effectiveness of the candidate spice for more generalized applications.

EXPERIMENTAL PROCEDURE

Biological material

Samples of caraway, coriander, mystical cumin, and cumin were obtained from their plantation areas in Morocco (Figure 1). *Carum carv*i was harvested from the middle and high mountains of Morocco (Rif and Atlas). *Coriandrum sativum* was recovered from Central and Northern Morocco due to its adaptability to humid and subhumid climates. In Morocco, *Ammodaucus leucotrichus* is more found in semi-arid coastal regions. Cumin (*Cuminum cyminum*) is generally grown in the regions with hot, arid climates. The collected samples of caraway, coriander, mystical cumin, and cumin were dried in shade in a dry place, then ground into a fine powder (0.01 mm) as well as stored in bags protected from light and moisture before complete analysis.

Climatological study

Climatic study was measured to inquire about the climatic effect on the studied spices plantations at the four areas (stations). The Emberger bioclimatic quotient was then calculated

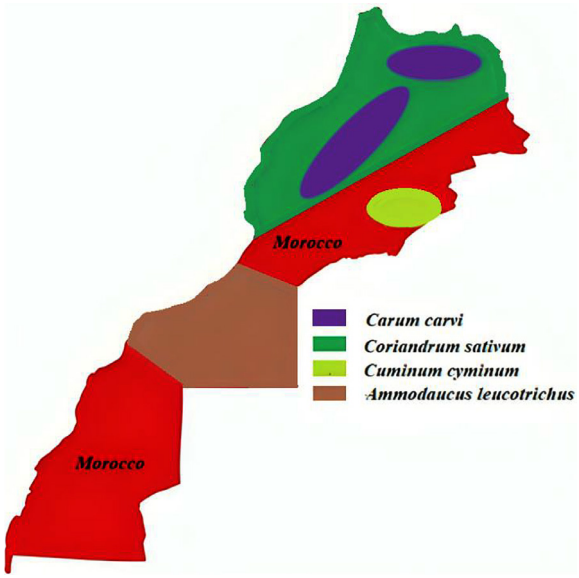

Figure 1. Geographical localization of the production sites of studied spices

according to the following formula modified by (Mokhtari *et al.*, 2014):

$$Q2 = \frac{2000\,p}{\left((M+m+546.4)\times(M-m)\right)} \quad (1)$$

where: p – annual rainfall in mm/m^2/year; M – maximum temperature of the hottest month in °C, m – minimum temperature of the coldest month in °C.

Phenolic compounds extraction

The solubility of the phenolic contents was strongly governed by the type and polarity of the used solvent (Djeridane *et al.*, 2006). Thus, the extracts were prepared in triplicate by adding 100 mL of polar solvent to 10 g of spice powder at room temperature. The extract was then recovered by filtration through Whatman paper and stored in the dark at 4°C before use. Hexane, as a nonpolar solvent, was used as a control.

Phenolic compounds and flavonoid contents analysis

Phenolic compound contents were determined by absorption spectrophotometry, using the colorimetric method with the Folin-Ciocalteu reagent established by (Dranca and Oroian, 2016) taking into account the modifications made by (Cheok *et al.*, 2013). The calibration curve was read from the equation (y = 1.2259 x + 0.174, R^2 = 0.9989) ranging from 0 to 1 mg mL^{-1} in ethanolic Gallic acid solution. The results were expressed in mg of Gallic acid equivalent (GAE) g^{-1} of dry seed. The flavonoid content was determined according to (Shraim *et al.*, 2021) using the colorimetric test of aluminum chloride with $NaNO_2$ at an absorption wavelength of 480 nm. The calibration curve (y = 3.8733 x + 0.0455; R^2 = 0.9994) varied from 0 to 1 mg mL^{-1} in ethanolic quercetin solution.

An enrichment factor was introduced, in this study, entitled flavonoids percentage in phenolic composition (*% Fl/PC*) determined according to the following Equation 2:

$$\%\frac{Fl}{PC} = \frac{Flavonoids\ concentration}{Phenolic\ compound\ concentration}\times 100 \quad (2)$$

Antioxidant activities

Antioxidant activities were carried out by applying different methods: total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2 picrylhydrazyl (DPPH), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and metal ion chelation power (CP).

Total antioxidant capacity – TAC

The total antioxidant activity of the spices (caraway, coriander, cumin, and mystic cumin) was assessed by the formation of the Phosphorus-Molybdenum complex according (Prieto *et al.*, 1999). The aqueous ascorbic acid solution used as a calibration curve (y = 1.7355 x + 0.235; R^2 = 0.9999) had a concentration ranging from 0 to 1 mg mL^{-1}. The experiment was carried out on three separate runs, and the results indicated (antioxidant activity in ascorbic acid equivalent) were average values expressed in g-ascorbic acid equivalent per gram of dry plant.

Ferric reducing-antioxidant power – FRAP

The reducing power was carried out according to (El-Maati *et al.*, 2016). Sample or standard was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $K_3Fe(CN)_6$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The top layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and then the absorbance was measured at 700 nm.

Free radical scavenging activity – DPPH

DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared according to the method described by (Miraliakbari and Shahidi, 2008). For this purpose, 25 µL of different concentrations of samples or standards were added to 60 µM ethanolic solution of DPPH (1 mL). The absorbance measurements were read at 517 nm, after incubation (60 min) at room temperature. A blank sample containing the same amount of methanol and DPPH solution served as a negative control. The experiment was carried out three times and the percentage inhibition of free radical scavenging activity of each extract was calculated, as shown below:

$$\%\,Inhibition = \left(1 - \frac{Abs\ sample}{Abs\ control}\right) \times 100 \qquad (3)$$

Inhibition was plotted as a function of the sample or standard content (% DPPH free radical inhibition).

ABTS radical scanning activity

For ABTS test, the procedure followed was carried out according to the method reported by (Re *et al.*, 1999). Stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal amounts and allowing them to react for 14 h in the dark. The solution was then diluted by mixing 1 ml of ABTS solution with methanol (60 mL) to obtain an absorbance of 0.832 ± 0.01 units at 734 nm. A fresh ABTS solution was prepared for each test. The plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution and absorbance was taken at 734 nm after 7 min. Extract ABTS scavenging capacity was compared to that of BHT and ascorbic acid, whereas the percentage inhibition calculated as radical scavenging activity was determined according to Eq. 3, where Abs control is ABTS radical absorbance in methanol. Abs Sample is the absorbance of ABTS free radical solution mixed with the sample extract/standard. All determinations were carried out in three separate runs.

Chelation power of metal ions: CP

The chelation degree of ferrous ions is the previously described method (Miguel, 2010). Briefly, the samples were incubated with 0.05 mL of $FeCl_2,4H_2O$ (2 mM). The addition of 0.2 mL of 5 mM ferrozine triggered the reaction, and after 10 min, absorbance was measured at 562 nm. An untreated sample served as a control. The percentage of chelating power was calculated according to Eq. 3.

Gel filtration chromatography

Fractionation of the crude aqueous extract (AE) was carried out on a Sephadex G50 open column. This method is based on size exclusion chromatography, which separates molecules according to their molecular size. Sephadex gel is made up of highly porous microbeads, where high molecular weight molecules only diffuse outside the bead pores and are evacuated from the column first. Smaller molecules, on the other hand, diffuse into all the microbeads and are then eluted from the column.

A column with a diameter of 2.5 cm and a length of 50 cm was used with a flow rate set at 1 mL/min, based on the method of (El Massoudi *et al.*, 2023) with some modifications. An amount of 20 grams of Sephadex G50 was mixed with 150 mL of lithium chloride buffer solution (5 mM NaOH, 2.5 mM LiCl). Half mg/mL of each extract was fractionated on Sephadex gel, and analyzed with a spectrophotometer at 380 nm to separate phenolic compounds (El Massoudi *et al.*, 2023).

Statistical analysis

All experiments selecting the solvent proportion, as well as the total phenolic compound contents, trapping activity of a free radical, and total antioxidant capacity, were performed in triplicate and the results were reported as a medium ± standard error. Statistical comparisons were made with one-way ANOVA followed by the Tukey HSD test done by using SPSS software. The significance level was set at $p \leq 0.05$. Principal component analysis (PCA) was generated by past software package.

RESULTS AND DISCUSSION

Climatology of the studied spices localities

The average values of the Emberger bioclimatic quotient, reported in Table 1, and evaluated by the method developed in this work, as reported by Mokhtari and his collaborators (Mokhtari *et al.*, 2014) clearly confirm that the caraway,

Table 1. Emberger bioclimatic quotient (Q2) values in the studied localities

Spice	Cuminum cyminum	Coriandrum sativum	Carum carvi	Ammodaucus leucotrichus
Q2	1–2	60–180	200–300	20–30

coriander, mystical cumin and cumin in Morocco planting areas present well-differentiated bioclimatic positions. Cumin belongs to a desert bioclimatic environment, coriander to a semi-arid and humid environment, mystic cumin to a desert and coastal climate, while caraway belongs to a high mountain environment. This result shows that bioclimatic conditions are very different and can therefore affect the metabolism as well as the chemical composition of the plants studied.

Solvent screening for phenolic compounds and flavonoids extraction

The levels of polyphenolic compounds and flavonoids of the studied spices (caraway, coriander, mystical cumin, and cumin), depending on the extraction solvent, are presented in Table 2. The highest values of phenolic and flavonoids are found in the aqueous extract, while the lowest values are obtained in the hexane extract (HE).

This difference reveals the considerable impact of the solvent on the yield of extraction. Thus, water, methanol and ethanol are significantly ($p > 0.05$) the most effective solvents to extract a good yield of polyphenols and flavonoids. Table 2 clearly shows that cumin contains the highest flavonoid fortification values *Fl*/*PC*. Therefore, these results suggest that FC could be the main contributor to the antioxidant potential and inhibitory actions of oxidative reactions.

Results are expressed in dry weight (DW) mean ± standard deviation (n = 3). Total phenolics expressed in mg GAE/100 g, Flavonoids expressed in mg QE/100 g expressed in values that do not share the same letter are significantly different ($p < 0.05$). Upper case represents the difference between samples and the lower case represents the difference between solvents, determined by ANOVA followed by the Tukey HSD test. Caraway – *Carum Carvi* seeds, Coriander – *Coriandrum sativum* L. seeds, Mystical cumin – *Ammodaucus leucotrichus* seeds and Cumin – *Cuminum cyminum* seeds.

Fl/PC – fortification factor

The dimensional analysis carried out by PCA (Figure 1) shows that the solvents (water and ethanol) are strongly correlated with the phenolic compounds and the Fl/PC fortification factors of cumin and coriander of the spices studied. Methanol is strongly correlated with flavonoids, as that of cumin and caraway fortification indicators.

According to the literature data (Abderrezag *et al.*, 2021) reported that the mystical cumin seeds extracted using green solvents (ethanol and water) give a yield of phenolic compounds which

Table 2. Total phenolic and flavonoid compounds and the percentage of flavonoids from total phenolic content

Compound	Spice	Extraction solvent			
		Methanol	Ethanol	Water	Hexane
Phenolic compounds (mg/100g DW)	Caraway	13.66 ± 0.03 $^{b, B}$	11.28 ± 0.64 $^{c, C}$	15.9 ± 0.21 $^{a, B}$	0.96 ± 0.42 $^{d, C}$
	Coriander	5.43 ± 0.82 $^{c, D}$	8.4 ± 0.88 $^{b, D}$	12.89 ± 0.27 $^{a, D}$	1.59 ± 0.37 $^{d, C}$
	Mystical cumin	12.59 ± 0.17 $^{c, C}$	13.64 ± 0.13 $^{b, B}$	15.01 ± 0.16 $^{a, C}$	1.03 ± 0.35 $^{d, C}$
	Cumin	14.33 ± 0.21 $^{c, A}$	16.53 ± 0.06 $^{a, A}$	16.49 ± 0.74 $^{b, A}$	1.31 ± 0.65 $^{d, C}$
Flavonoids (mg /100g DW)	Caraway	11.45 ± 0.60 $^{a, B}$	6.86 ± 0.81 $^{b, B}$	4.80 ± 0.12 $^{c, B}$	0.52 ± 0.93 $^{d, C}$
	Coriander	4.31 ± 0.57 $^{b, D}$	4.91 ± 0.27 $^{a, D}$	3.85 ± 0.47 $^{c, D}$	0.64 ± 0.07 $^{d, B}$
	Mystical cumin	8.00 ± 0.27 $^{a, C}$	6.74 ± 0.41 $^{b, C}$	3.06 ± 0.53 $^{c, C}$	0.50 ± 0.18 $^{d, C}$
	Cumin	12.3 ± 0.27 $^{b, A}$	14.23 ± 0.40 $^{a, A}$	6.50 ± 0.50 $^{c, A}$	0.82 ± 0.10 $^{d, A}$
Fl/PC (%)	Caraway	83.82 ± 0.001 $^{a, B}$	60.82 ± 0.011 $^{b, B}$	30.19 ± 0.02 $^{d, B}$	54.17 ± 0.53 $^{c, B}$
	Coriander	79.37 ± 0.008 $^{a, C}$	58.45 ± 0.10 $^{b, C}$	29.87± 0.001 $^{d, C}$	40.25 ± 0.81 $^{c, D}$
	Mystical cumin	63.54 ± 0.009 $^{a, D}$	49.41 ± 0.25 $^{b, D}$	20.39± 0.110 $^{d, D}$	48.54 ± 0.04 $^{c, C}$
	Cumin	85.83 ± 0.002 $^{b, A}$	86.09 ± 0.04 $^{a, A}$	39.42± 0.310 $^{d, A}$	62.60 ± 0.07 $^{c, A}$

was around 22.3-24 mg GAE/g DW. In addition (Zhang *et al.*, 2014) reported phenolic compound contents of 8.33 mg GAE/g DW in the methanolic extract of cumin. (El-Ghorab *et al.*, 2010) also reported a higher value of 35.5 mg/g in the methanolic extract of cumin while the hexanic extract contained values like that obtained in the presented work. In another study, (Bukhari *et al.*, 2009) found that the content of phenolic compounds in cumin was 5.38, 5.97 and 1.90 mg/g for methanolic, ethanolic and hexanic.

Regarding these results, coriander flavonoids are much higher than those reported by (Msaada *et al.*, 2017) who observed that the flavonoid contents in Syria and Egypt were 2.51 and 2.07 mg QE/gDw, respectively. In Iraq, (Sadoun *et al.*, 2021) analyzed the flavonoid contents of the ethanolic extract of coriander which was ranged from 3.422 to 8.112 mg QE/g DW. This value is close to the present obtained results, while the methanolic extract was about (2.811-3.793) mg QE/g Dw (Sadoun *et al.*, 2021). Other studies carried out in Morocco by (Derouich *et al.*, 2020) demonstrated that the total flavonoids reached 10.24 mg QE/g DW in the methanolic extract of coriander. On the other hand, a study carried out in Algeria reported by (Djeridane *et al.*, 2006) revealed that extraction solvents significantly affected the flavonoid contents of the extract.

Flavonoids stand out as the primary antioxidant compounds found in spices (Trifan *et al.*, 2021). Beyond their role as natural food preservatives and flavor enhancers, the consumption of spices can offer additional health benefits associated with oxidative stress-related disorders. This includes conditions such as gastrointestinal disorders, cardiovascular injuries. In essence, the utilization of spices extends beyond culinary applications, contributing to potential health advantages by combating oxidative stress-related issues (Trifan *et al.*, 2021).

Molecular weights distribution of phenolic compounds extracts from studied spices

Figure 3 illustrates the evolution of the molecular weights of the phenolic compounds extracted from the studied spices. A large difference in molecular weights of monophenols and polyphenols can be seen in Figure 3. This difference confirms the great diversity of profiles, for example mystical cumin is richer in polyphenols, while cumin is rich in monophenols. Coriander is less rich in polyphenols and monophenols. This study is very limited in regard to aqueous extracts, but it gives a very clear idea of the culinary fractions linked to the studied solvent (water).

Antioxidant activity (AA)

The antioxidant activity of caraway, coriander, mystical cumin, and cumin was investigated in the present study by in vitro tests; potential reduction tests to evaluate the free radical scavenging activity and antioxidant capacity of each solvent. The variability of the free radical scavenging activity and total antioxidant activity of spices depending on the different solvents used is presented in Table 3. Results revealed that the anti-radical activity of (caraway, coriander, mystical cumin, and cumin) is significantly influenced by the nature of the solvent. Thus, it should be noted that for TAC, the best activity is observed with the solvents water and ethanol, followed by methanol and hexane.

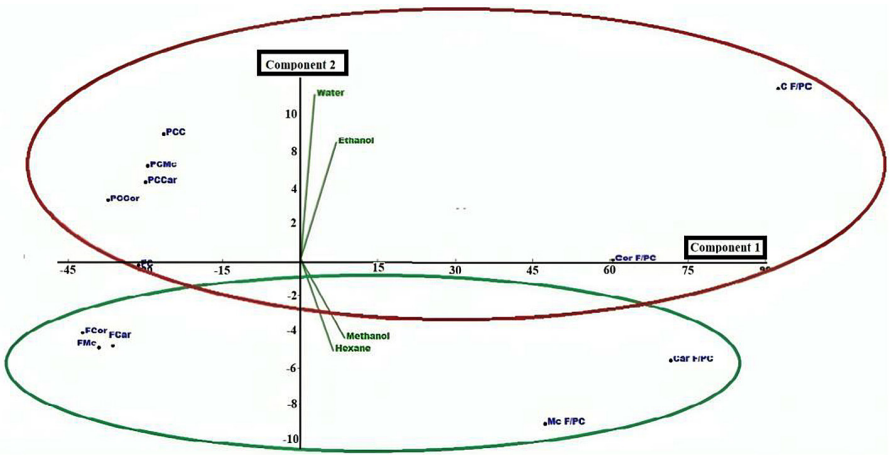

Figure 2. Principal component analysis of the phenolic and flavonoid compound contents of the studied spices

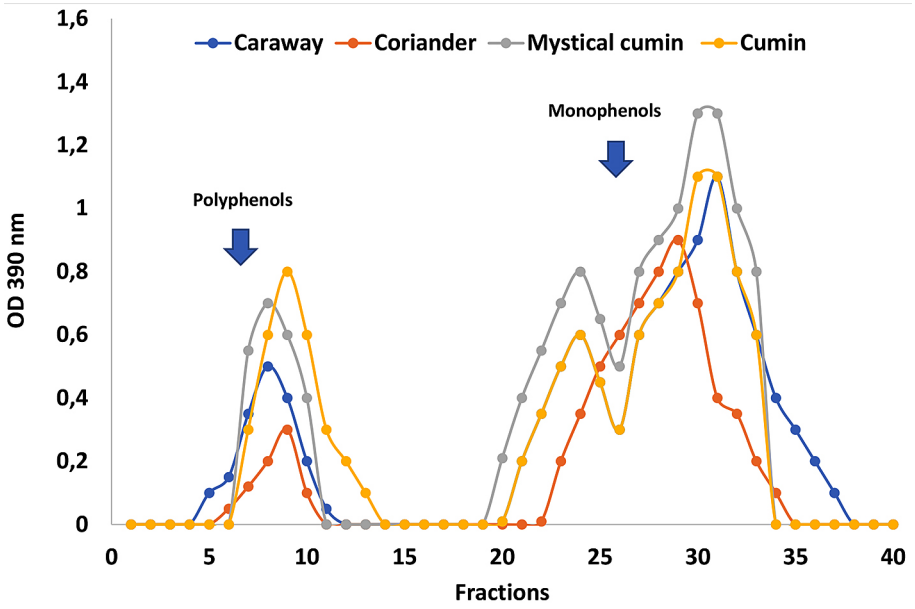

Figure 3. Distribution of molecular weight of phenolic compounds extracted from the studied spices

Total antioxidant capacity

The results reported in Table 3 show that cumin is the most active among the spices tested where values of 33.20, 20.20, 33.90 and 27.13 (mg EAA/g DW) were recorded for water, hexane, ethanol, and methanol, respectively. These values fall within a range of values reported by (El-Maati *et al.*, 2016) and (Mouderas *et al.*, 2020), who carried out the extraction of mystical cumin using different solvents.

Table 3. Antioxidant activities of different seed extracts (Cw, Cd, Cs, Cm) using water, methanol, ethanol, and hexane. Values are means of three replications (n = 3 ± SD) by five methods (DPPH, TAC, FRAP, ABTS, CP)

Method	Spice	Extraction solvent			
		Methanol	Ethanol	Hexane	Water
TAC	Caraway	18.29 ± 0.001 $^{d, C}$	21.16 ± 0.08 $^{c, C}$	23.49 ± 0.04 $^{b, B}$	31.36 ± 0.01 $^{a, C}$
	Coriander	13.85 ± 0.42 $^{a, D}$	13.37 ± 0.06 $^{b, D}$	9.95 ± 0.01 $^{d, D}$	12.80 ± 0.02 $^{c, D}$
	Mystical cumin	28.23 ± 0.14 $^{c, A}$	31.20 ± 0.18 $^{b, B}$	31.11 ± 0.13 $^{b, A}$	31.64 ± 0.77 $^{a, B}$
	Cumin	27.13 ± 0.06 $^{c, B}$	33.90 ± 0.16 $^{a, A}$	20.20 ± 0.09 $^{d, C}$	33.20 ± 0.08 $^{b, A}$
DPPH	Caraway	76.18 ± 0.60 $^{a, A}$	63.24 ± 0.81 $^{b, C}$	12.00 ± 0.93 $^{d, C}$	48.09 ± 0.12 $^{c, C}$
	Coriander	46.17 ± 0.5 $^{a, D}$	36.03 ± 0.27 $^{b, D}$	11.24 ± 0.07 $^{d, D}$	35.65 ± 0.47 $^{c, D}$
	Mystical cumin	75.92 ± 0.2 $^{a, B}$	74.22 ± 0.20 $^{b, A}$	15.11 ± 0.18 $^{d, A}$	48.40 ± 0.53 $^{c, B}$
	Cumin	64.35 ± 0.2 $^{b, C}$	71.91 ± 0.40 $^{a, B}$	13.53 ± 0.40 $^{d, B}$	55.95 ± 0.04 $^{c, A}$
FRAP	Caraway	1.53 ± 0.08 $^{b, C}$	1.02 ± 0.02 $^{c, A}$	1.00 ± 0.07 $^{c, A}$	2.00 ± 0.07 $^{a, A}$
	Coriander	1.35 ± 0.04 $^{b, B}$	1.00 ± 0.04 $^{c, A}$	0.49 ± 0.02 $^{d, C}$	2.05 ± 0.03 $^{a, A}$
	Mystical cumin	1.84 ± 0.04 $^{b, A}$	1.50 ± 0.04 $^{c, A}$	0.81 ± 0.01 $^{d, B}$	1.941 ± 0.01 $^{a, A}$
	Cumin	1.17 ± 0.04 $^{c, D}$	1.37 ± 0.06 $^{b, A}$	0.47 ± 0.03 $^{d, C}$	2.00 ± 0.02 $^{a, A}$
ABTS	Caraway	98.117 ± 0.25 $^{a, A}$	93.87 ± 0.75 $^{b, B}$	79.49 ± 0.80 $^{c, C}$	46.43 ± 0.18 $^{d, D}$
	Coriander	95.352 ± 0.30 $^{a, C}$	82.61 ± 0.50 $^{b, D}$	79.05 ± 0.56 $^{c, C}$	76.04 ± 0.87 $^{d, C}$
	Mystical cumin	96.554 ± 0.97 $^{a, B}$	93.573 ± 0.11 $^{b, C}$	92.15 ± 0.82 $^{c, B}$	87.34 ± 0.25 $^{d, A}$
	Cumin	84.01 ± 0.97 $^{c, D}$	94.11 ± 0.67 $^{a, A}$	93.63 ± 0.12 $^{b, A}$	80.93 ± 0.76 $^{d, B}$
CP	Caraway	73.81 ± 0.17 $^{c, C}$	93.56 ± 0.74 $^{a, A}$	58.44 ± 0.34 $^{d, D}$	91.00 ± 0.23 $^{b, D}$
	Coriander	63.69 ± 0.34 $^{c, D}$	48.03 ± 0.28 $^{d, B}$	65.29 ± 0.68 $^{b, C}$	97.84 ± 0.23 $^{a, C}$
	Mystical cumin	79.43 ± 0.57 $^{b, B}$	43.08 ± 0.12 $^{d, D}$	74.52 ± 0.67 $^{c, B}$	99.44 ± 0.30 $^{a, B}$
	Cumin	98.88 ± 0.04 $^{b, A}$	46.99 ± 0.40 $^{d, C}$	97.51 ± 0.23 $^{c, A}$	99.89 ± 0.01 $^{a, A}$

All extracts had a reducing capacity on molybdate ions ranging from 20.52 ± 2.2 to 134.79 ± 1.8 mg AAE/g DW.(Tangkanakul *et al.*, 2009) noted that the total antioxidant capacity ranged from 92.18 to 302.26 (mg ECV1/100 g) for coriander, cumin, respectively ,as well as phenolic compounds at the highest level. Several studies, reported elsewhere, like (Sharififar *et al.*, 2009) have shown that phenolic compounds, particularly flavonoids, contribute significantly to the phosphomolybdate scavenging activity of medicinal plants.

Results are expressed in dry weight mean ± standard deviation (n = 3). CAT expressed in mg AAE/g, DPPH expressed in %, ABTS expressed in %, CP expressed in %, FRAP expressed in DO, expressed in values that do not share the same letter are significantly different ($p < 0.05$) Upper case represents the difference between samples and the lower-case represents the difference between solvents, determined by ANOVA followed by the Tukey HSD test. Caraway – *Carum Carvi* seeds, Coriander – *Coriandrum sativum* L. seeds, Mystical cumin – *Ammodaucus leucotrichus* seeds and Cumin – *Cuminum cyminum* seeds.

Reducing-antioxidant power (FRAP)

According to the results illustrated in Table 3, the reducing power of the extracts increases along with concentration. A strong reducing power is observed for aqueous and ethanolic values comparable to those of the control antioxidant ascorbic acid. A much lower reducing power is observed for the hexane extraction. The results show a significant correlation between phenolic compounds and the reducing power of all spices (caraway, coriander, cumin, and mystical cumin).

Samples are reduced in the following order: water > methanol > ethanol > hexane. A similar relationship between phenolic constituents and potential-reducing activity has been reported for several plant extracts (Amarowicz *et al.*, 2004). (Muzolf-Panek and Stuper-Szablewska, 2021) performed the extraction of caraway with water, and showed that 50% aqueous ethanol or pure ethanol, had an antioxidant activity that did not exceed 12.8 ± 1.01 µmol TE/g. (Trifan *et al.*, 2021) using the FRAP method (87.71 mg TE/g). Cumin EO possessed higher antioxidant activity than theee extract obtained by maceration, which is known for its food preservation – particularly meat products – mainly due to cuminaldehyde (Homayonpour *et al.*, 2021, Salanţă and Cropotova, 2022).

Free radical scavenging activity: DPPH

According to the results reported in Table 3, all extracts exhibit dose-dependent antioxidant activity. In this study, the mystical cumin extracts showed the highest antioxidant activity (75.92, 74.22, 48.40, and 15.11) in methanol, ethanol, water and hexane, respectively. Also, the caraway extracts (76.18, 63.24, 48.09, and 12.00%), respectively is followed by cumin. These results revealed that the methanolic extract is more active (76.18 ± 0.11%) while the hexanoic extract is the least active (13.53 ± 0.17%).

In comparison, (Usha Rani and Meena, 2014) reported that the DPPH activity in methanolic extract of cumin did not exceed 82.8 µg ml^{-1}. (Demir and Korukluoglu, 2020) also reported the DPPH activity in cumin and coriander of 1.48 ± 0.03 and 2.2 ± 0.2, respectively, for the methanolic extract; and 3.25 ± 0.2 and 5.6 ± 0.1 for the ethanolic extract, respectively. For caraway, (Synowiec *et al.*, 2019) reported that the aqueous extracts of caraway had an activity of 31.65, as well as (Demir and (Demir and Korukluoglu, 2020) reported an antioxidant activity value of 57.75 measured with the DPPH test expressed as percentage inhibition for coriander extracts; in turn, (Manssouri *et al.*, 2020) reported that the mystical cumin extracts with different solvents did not exceed 83.09 ± 1.20%. (Muzolf-Panek and Stuper-Szablewska, 2021) reported the results of caraway related to DPPH ranged (12.23-20.17µmol TE/g).

ABTS radical scanning activity

The antioxidant activity evaluated with the ABTS test allowed obtaining the results reported in Table 3. Cumin and coriander in water, methanol and ethanolic extract have strong antioxidant activity when compared to hexane extract. The results show that the highest antioxidant activity is obtained by the aqueous extraction of caraway, coriander, cumin, and mystical cumin are 46.43 ± 0.18, 76.04 ± 0.18, 87.34 ± 0.18, 80.93 ± 0.18, respectively, followed by methanol with 98.117 ± 0.14, 95.35 ± 0.18, 96.55 ± 0.18 and 84.01 ± 0.18, respectively. However, hexane showed the lowest antioxidant activity for all the studied spices due to limited hexane extraction of phenolic composition as previously reported by (Ndaba *et al.*, 2023).

(Thiviya *et al.*, 2021) reported that species of the Apiaceae family, caraway, coriander, and cumin, are rich in antioxidant activity, mainly phenolic and flavonoid components. In particular, coriander

contains 103.0 µmol TE/g. (Muzolf-Panek and Stuper-Szablewska, 2021) showed that caraway extracted with different solvents (water, 50% aqueous ethanol or pure ethanol) had an antioxidant activity ranging from 19.71 ± 0.09 to 35.37 ± 0.39 µmoL TE/g. (Ksouda *et al.*, 2018) reported that the antioxidant activity of species of the Apiaceae family did not exceed 52 ± 14 (mg TEAC/100 g DW), especially for the coriander. (Shan *et al.*, 2005) also reported that the radical scavenging activity of caraway, coriander, cumin was in the range of 4 and 11 mmol/100 g Dw (Gallo *et al.*, 2010).

Chelation capacity

The chelating effect of caraway, coriander, cumin, and mystical cumin on the formation of Fe^{2+} and ferrozine complexes was also investigated in this study. As it is shown in Table 3, water exhibits the best chelating effect with 91.00 ± 0.12, 97.84 ± 0.12, 99.44 ± 0.12, 99, 89 ± 0.12% for caraway, coriander, mystical cumin, and cumin, respectively. It is followed by methanol with 73.81 ± 0.16, 63.69 ± 0.16, 79.43 ± 0.16, 98.88 ± 0.16% for caraway, coriander, mystical cumin, and cumin, respectively, followed by ethanol and hexane. In this study, the metal chelating capacity of various extracts is significantly affected by the nature of the solvent. Indeed, it has been reported that the ferric chelating power of plant extracts is attributable to phenolic compounds. (Hajlaoui *et al.*, 2021) noted that the extraction of essential oils from caraway and coriander carried out with hydrodistillation gave a chelation activity of 36.33 and 70.00 EC50 µg mL^{-1}, respectively. (Hinneburg *et al.*, 2006) reported that the iron chelation of caraway did not exceed 100 mg/g of extract.

PCA of antioxidants activities and PC

The PCA is used in this study to better understand the effective extraction of high content of phenolic and flavonoids, as (Thiviya *et al.*, 2021) reported that a strong correlation exist between antioxidant activities and phenolic compounds. The dimensional analysis of Figure 4 shows that hexane and water are grouped together and contribute negatively to component 1 (PC1), whereas ethanol and methanol contribute to the positive part of components 1 and 2, and they are strongly correlated with the DPPH and ABTS carried out on the phenolic compounds of mystical cumin. The CAT and Pc technique contributes massively to the negative part of component 2 and1. The first principal component axis (PC1) explains 59.98% of the total variation and the second (PC2) explains 18.85% of the variance. These distributions show that the extraction solvents carried out make it possible to group the spices and characterize them according to their content of phenolic compounds and their AA antioxidant activity.

In summary, the PCA study confirms the strong correlation, demonstrated by a good separation between antioxidant activity and spice extraction types. These results are similar to those already reported by (Dahmani *et al.*, 2018), who

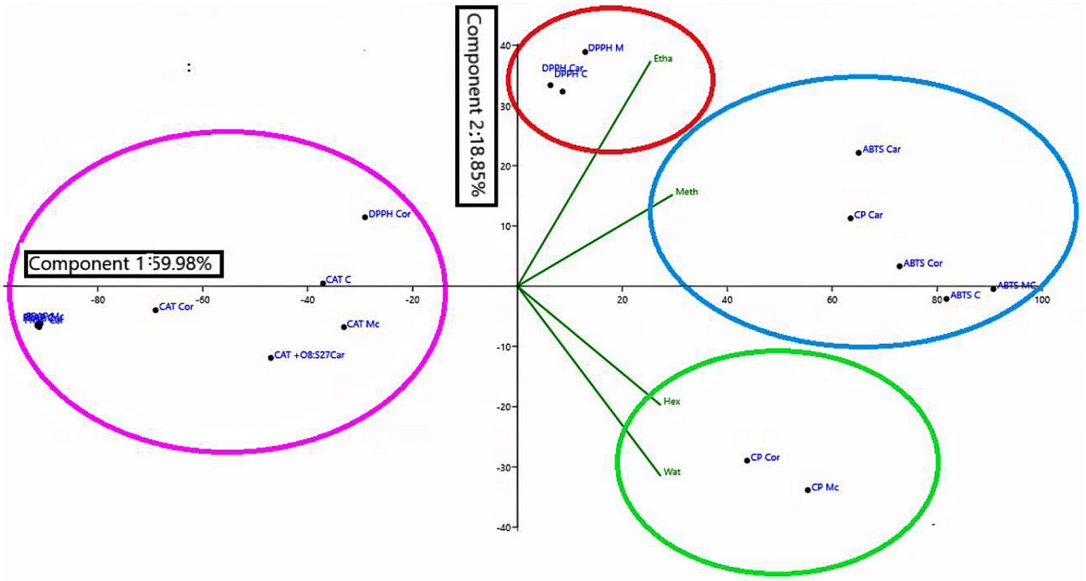

Figure 4. Principal component analysis of antioxidant activity and total phenolic compounds of extracts from selected culinary spices and herbs (Mc: mystical cumin, car: caraway, c: cumin, cor: coriander)

used another matrix (principal component analysis of the sensory attributes of artisanal table olives). Some reports attributed the antioxidant activity to compounds other than phenolic and flavonoids (Hamdouch *et al.*, 2022), and this is the case for chelating capacity. Therefore, phenolic compounds, especially flavonoids, are likely the main contributors to the antioxidant capacity.

CONCLUSIONS

The total phenolic contents and the antioxidant capacities of caraway, coriander, cumin, and mystical cumin were assessed. The composition of these spices was found to exhibit high antioxidant capacities, which is a valuable source of natural antioxidant. The antioxidant properties of the aqueous extract and its derivative fractions correlated well with their total phenolic content. These results may be useful for a further application of caraway, coriander, cumin, and mystical cumin or their constituents in the natural conservation of meat products after performing formulation of meat products and testing their preservation. This type of research could significantly contribute to the treatment and prevention of human damage related to active chemical and synthetically antioxidants in industry.

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