



Antioxidant Activity of Some Spices and Their Two Traditional Mixture Named Ras el Hanout Used in Northwestern of Algeria

**Meryem Seladji^{1,2*}, Fatima Zohra Khedim²,
Hanane Dib² and Nassima Bendimerad²**

¹*Ahmed Ben Bella University-Oran 1, Faculty of Science of Nature and Life, Department of Biology, BP 1524, 31000, Oran, Algeria.*

²*Laboratory of Natural Products, Department of Biology, Abou Bekr Belkaïd University, Department of Biology, Tlemcen, BP 119, 13000, Tlemcen, Algeria.*

Authors' contributions

This work was carried out in collaboration among all the authors. Authors MS and FZK performed the analyses. The authors MS and HD wrote the manuscript. All authors have read and approved the final manuscript.

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ABSTRACT

Aims: Our study aimed to evaluate the antioxidant activity of ten spices in the composition of two spice blends named Ras el Hanout I and Ras el Hanout II. The spices studied are *Alpinia officinarum*, *Cinnamomum cassia*, *Carum carvi*, *Coriandrum sativum*, *Cuminum cyminum*, *Curcuma domestica*, *Foeniculum vulgare*, *Piper cubeba*, *Piper nigrum*, *Zingiber officinale*, mixtures were prepared well defined measures.

Place and of Study: Laboratory of Natural Products, Department of Biology, Abou Bekr Belkaïd University, Department of Biology, Tlemcen.

Methodology: The contents of polyphenols were determined by spectrophotometric techniques. The antioxidant activities were determined in vitro through trapping evaluation tests of the free radical DPPH and iron reduction (FRAP). The dried crude extracts of spices were prepared in a water-methanol mixture.

Results: Yields vary considerably from one spice to another with values ranging between 4.01% for *Coriandrum sativum* and 12.26% for *Cuminum cyminum*. The evaluation of the antioxidant activity by the scavenging of free radical DPPH and the iron reduction method (FRAP) showed that our extracts have an antioxidant potential. The extract of *Cinnamomum cassia* has better reducing capability of iron in comparison to all other spices and even compared with standard antioxidant ascorbic acid. Also, a stronger free radical scavenging activity was observed compared to that of ascorbic acid and BHA. On the other hand, the concentration which inhibits the 50% effect free radical DPPH is equal to 0.15 ± 0.035 mg / mL for Ras el Hanout I and 0.51 ± 0.07 mg / mL for Ras el Hanout II but which remains higher than that of 0.09 ± 0.05 mg / mL ascorbic acid.

Conclusion: This study demonstrated that crude extracts of spices exhibit (individually and as a mix) significant antioxidant activity.

Keywords: Spices; Ras el hanout; phenolics content; antioxidant activity; FRAP; DPPH.

1. INTRODUCTION

Herbs and spices have been used in many different ways. Since the ancient times, spices and aromatic herbs have been added to food to enhance flavor and improve their organoleptic properties. Spices and herbs have also been widely used as preservatives and medicine [1]. Spices plants are a great source of antioxidants. They are used widely in food manufacturing and processing and are an alternative to unhealthy, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which contribute to liver damage and carcinogenesis [2,3].

Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins [1]. In recent years, antioxidant properties of spices are well explored by scientific community due to its natural origin [4]. Spices studies have revealed a wide range of biological activities, including antioxidant, anti-inflammatory, antitumor, immunomodulatory, and antiradical activities [5,6]. Their antioxidant constituents are identified as responsible for the health-promoting properties of spices, such as antiathero-sclerotic, anticancer, and anti-inflammatory activities [7].

In north Mediterranean countries, many blends of spices are used to make traditional delicacies, and « Ras el Hanout » is one of the most popular. The name is Arabic for "head of the shop" and implies a mixture of the best spices [8]. The mixture is used in the preparation of Soup, some tagine and couscous. There is no definitive composition of spices that makes up Ras el Hanout. The mixture usually consists of over a dozen spices, in different proportions [9].

According to the herbalists, this mixture is usually composed of coriander, cumin, cinnamon, ginger, black pepper, turmeric, caraway, fennel, red pepper, nutmeg, laurel and cloves [8]. To our knowledge there is only one study on effect of thermal treatment on phenolic compounds, antioxidant and antimicrobial activities of some spices contained in the mixture of Ras el Hanout [8]. Our objective is devoted to the comparison of the phytochemical study and the evaluation of the antioxidant activity of two Ras el Hanout mixtures and the spices that compose them.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant material consisting of a mixture of spices was obtained from an herbalist in the province of Tlemcen (Algeria). Each spice was purchased raw. Those plants materials are crushed using a mortar, then finely ground and stored at room temperature in tightly closed glass jars to preserve its initial quality. The extract proportions of spices constituting the mixture « Ras el Hanout » were determined in the laboratory, by evaluating the amount of each spice in the mixture. These values are reported in the Table 1.

2.2 Preparation of Extracts

The extraction of crude hydro-methanolic extracts from the two Ras el Hanout mixture and their component spices, was carried out according to Harborne [10]. Briefly, 2 g of powder of spice material was macerated in 100 ml of methanol / distilled water (80/20, V / V), for 24h with stirring at room temperature. The solution is filtered and then evaporated to dryness at 60 °C using a Büchi R-200 type Rotavapor. The residues were suspended in 3 mL of methanol and stored at -4 °C for further use.

Table 1. Composition in g of the two Ras el Hanout mixtures

Spices	Botanical Name	Ras el hanout I (g)	Ras el hanout II (g)
Cinnamon	<i>Cinnamomum cassia</i> (Lauraceae)	17	2
Caraway	<i>Carum carvi</i> (Apiaceae)	17	10
Coriander	<i>Coriandrum sativum</i> (Apiaceae)	16	10
Ginger	<i>Zingiber officinale</i> (Zingiberaceae)	17	10
Black pepper	<i>Piper nigrum</i> (Piperaceae)	8	5
Turmeric	<i>Curcuma domestica</i> (Zingiberaceae)	18	10
Fennel	<i>Foeniculum vulgare</i> (Apiaceae)	-	5
Galangal	<i>Alpinia officinarum</i> (Zingiberaceae)	-	2
Cumin	<i>Cuminum cyminum</i> (Apiaceae)	-	5
Cubeb	<i>Piper cubeba</i> (Piperaceae)	17	-

2.3 Determination of Phenolics Content (TPC)

TPC was determined using Folin-Ciocalteu reagent. A volume of 200 μ L of the extract was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times with water and 800 μ L of a 7.5% sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve ($Y=0.3037x+0.2761$, $R^2=0.9977$). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) [11].

2.4 Antioxidant Activity

2.4.1 Scavenging of the free radical DPPH

The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple-coloured methanol solution of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [12]. 50 μ L of various concentrations of the extracts in methanol were added to 1950 μ L of a 0.025 g/L methanol solution DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

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

Where A_0 and A_1 are the absorbance at 30 min of the positive control and the extract, respectively. The antiradical activity was expressed as IC_{50} (mg/ml), this is the extract concentration required to cause a reduction of

50% to absorbance at 517 nm. A lower IC_{50} value corresponds to the extract effectiveness [13].

2.4.2 Ferric reducing antioxidant potential (FRAP) assay

1 mL of each extract at different concentrations was mixed with 2.5 mL of 0.2 M phosphate buffer at pH = 6.6 and 2.5 mL of a 1 % potassium ferricyanide solution. The mixture obtained is incubated for 20 minutes at 50 °C, and then 2.5 mL of 10 % trichloroacetic acid are added to stop the reaction. The mixture was centrifuged at 650 g for ten minutes at room temperature and 2.5 mL of the supernatant added to 2.5 mL of distilled water and 0.5 mL of 0.1 % iron chloride. The absorbance is read at 700 nm against a blank. The results made it possible to calculate the effective concentration (EC_{50}), concentration of the extract corresponding to an absorbance equal to 0.5, the linear regression curve (density of the optics as a function of the different concentrations). The activity of the extract is finally compared with that of the positive control (BHA) [14].

2.5 Statistical Analysis

All experiments were performed at least in triplicate and the results are presented as mean \pm standard deviation (SD).

3. RESULTS AND DISCUSSION

Yield of crude hydro-methanolic extracts present in vegetables have received considerable attention because of their potential biological activities. Table 2 summarizes the yield (%) of crude hydro-methanolic extracts from the two Ras el Hanout studied and the spices that compose them.

Table 2. Yields of the extracts from the spices studied

Spices	Yield (%)
Cinnamon (<i>Cinnamomum cassia</i>)	7.38±0.021
Caraway (<i>Carum carvi</i>)	8.06±0.014
Coriander (<i>Coriandrum sativum</i>)	4.01±0.007
Ginger (<i>Zingiber officinale</i>)	9.1±0.141
Black pepper (<i>Piper nigrum</i>)	7.18±0.014
Turmeric (<i>Curcuma domestica</i>)	9.16±0.042
Fennel (<i>Foeniculum vulgare</i>)	11.965±0.01
Galangal (<i>Alpinia officinarum</i>)	10.26±0.028
Cumin (<i>Cuminum cyminum</i>)	12.26±0.021
Cubeb (<i>Piper cubeba</i>)	8.94±0.031
Ras El Hanout I	7.08±0.014
Ras El Hanout II	6.9±0.318

The data are displayed with mean ± standard deviation of triplicate replications.

3.1 Determination of Phenolics Content (TPC)

In general, antioxidant and radical scavenging properties of plants extracts is associated with the presence of phenolic compounds possessing the ability to donate hydrogen to the radical [15]. The concentration of phenolics compounds in crudes hydro-methanolic extracts of the twelve spices studied was determined by Folin-Ciocalteu method. This were expressed in mg of gallic acid (Table 3). As shown in Table 3, the amount of phenolics varied widely, it's ranging between 1.62±0.190 and 62.4±0.848 mg GAE/g DS. The extract of Cinnamon showed the highest level of phenolics while coriander showed the lowest (Cinnamon > Galangal > Ras El Hanout I > Ginger > Ras El Hanout II > Turmeric > Black pepper > Cumin > Cubeb > Fennel > Caraway > coriander).

Results of phenolic content of Cinnamon, black pepper and caraway corroborate those of Kim et al. [16], Annou et al. [8] and Słowianek & Leszczyńska [17]. On the other side, our results for coriander, turmeric and cumin do not agree those of Denre, [18] who found that cumin is more rich polyphenols than turmeric, ginger and cinnamon.

3.2 Antioxidant Activity

In the present study, two commonly used antioxidant evaluation methods such as DPPH radical scavenging activity and reducing power assay (FRAP) were chosen to determine the antioxidant potential of the hydro-methanolic extracts of the two Ras el Hanout mixture and different spices that compose them.

3.2.1 Scavenging of the free radical DPPH

The antioxidant activities of spice extracts have been widely demonstrated. IC₅₀ value was determined from plotted graph of scavenging activity against the different concentrations of spices extracts, ascorbic acid, and BHA. The scavenging activity was expressed by the percentage of DPPH reduction after 30 min of reaction.

As shown at the Table 3, IC₅₀ values reveal that *Cinnamomum cassia* is a high antioxidant spice because the concentration which makes it possible to inhibit the effect of 50% of free radicals is equal is equal to 0.025±0.0003 mg / mL. This activity is clearly greater than that of the ascorbic acid evaluation, which is 0.09±0.05 mg/ml. *Alpinia officinarum*, Ras Hanout I, *Zingiber officinale* have similar antioxidant activities. This resulted in the value of their respective IC₅₀ 0.11±0.042, 0.15±0.035 and 0.18±0.06 mg / mL. A similarity is also observed for *Piper cubeba*, *Piper nigrum*, *Curcuma domestica*, in which Ras el hanout II had respective IC₅₀ of 0.28±0.147, 0.29±0.08, 0.30±0.141, 0.51±0.07 mg / mL. *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare* and *Carum carvi* inhibited the DPPH radical at respective effective concentrations of 0.94±0.03; 0.98±0.014; 1.19±0.57 and 1.21±0.009 mg / mL.

The efficiency of scavenging free radicals is inversely proportional to the IC₅₀ value and is of increasing order according to the following classification: *Cinnamomum cassia*> BHA> Ascorbic acid > *Alpinia officinarum* > Ras el Hanout I > *Zingiber officinale* > *Piper cubeba* > *Piper nigrum* > *Curcuma domestica* > Ras el

Hanout II > *Coriandrum sativum* > *Cuminum cyminum* > *Foeniculum vulgare* > *Carum carvi*.

IC₅₀ values for free radical scavenging DPPH founds for cinnamom (0.0025±0.0003mg/mL), ginger (0.18±0.06 mg/mL), turmeric (0.30±0.141 mg/mL), Coriander (0.94±0.03 mg/mL) and cumin (0.98±0.014 mg/ mL), are lower than those found by Annou et al. [8] who determined for the same hydro-methanolic extracts IC₅₀ values of the order: 0.05±0.0 mg/mL, 0.78±0.58mg/mL, 0.43±0.31mg/mL, 3.87±0.26 mg/mL and 1.54±0.31 respectively. Others studies have also found that cinnamon is more efficient than ginger, cumin and turmeric on the DPPH radical scavenging [18,19]. The high antioxidant activity of the extracts of Cinnamon (*Cinnamomum cassia*) in the trapping of the free radical DPPH could be attributed to their high content of phenolic compounds. These are bioactive compounds naturally found in plants and which are very important for human health [20]. In the other hand, the scavenging activities of the mixtures must be related to the scavenging activities of the antioxidants used. Therefore, an antioxidant having high scavenging activity but in low concentration could contribute to the overall activity of the mixture in less grade than other antioxidants having lower activity, but present in greater concentration [21].

3.2.2 Ferric reducing antioxidant potential (FRAP) assay

The results show (Table3) that all the spice extracts have variable capacities to reduce iron,

the most important of which are *Cinnamomum cassia*, *Zingiber officinale*, including their EC₅₀ which varies between (0.03±0.007 and 0.04±0.014 mg/mL). These have better iron-reducing activity than ascorbic acid and BHA. That of *Piper nigrum* is similar to the ascorbic acid standard, however, both Ras el hanout I and II mixtures have iron-reducing activity that was approximately to that of ascorbic acid. For *Cuminum cyminum*, and *Foeniculum vulgare* the EC₅₀ values vary between 0.79 and 1.59 mg / mL respectively. *Piper cubeba*, *Alpinia officinarum* and *Curcuma domestica* also have higher iron-reducing activity than ascorbic acid but lower than BHA. The efficiency of the reduction of iron is inversely proportional to the EC₅₀ value, it is of the increasing order according to the following classification: *Cinnamomum cassia* > *Zingiber officinale* > BHA > *Piper cubeba* > *Alpinia officinarum* > *Curcuma domestica* > Ras el hanout I > Ras el hanout II > *Piper nigrum* > Ascorbic acid > *Cuminum cyminum* > *Carum carvi* > *Coriandrum sativum* > *Foeniculum vulgare*.

The antioxidant activity of spices can be influenced by many factors. It is related to the botanical origin and antioxidant content of spices. The literature suggests that the amount of antioxidants in the sample also depends on the type of solvent used for their extraction [22]. Many experimental investigations have demonstrated that a number of secondary metabolites, such as polyphenol compounds, extracted from medicinal and aromatic plants possess a high antioxidant potential, due to their hydroxyl groups and protect more efficiently

Table 3. Total phenolics content and IC₅₀ and EC₅₀ values of antioxidants activities of hydro-methanolic extracts

Spices	Total phenolics content* (mgGAE/g DW)	FRAP EC ₅₀ (mg/mL)	DPPH IC ₅₀ (mg/mL)
Cinnamon (<i>Cinnamomum cassia</i>)	62.4±0.848	0.03±0.007	0.0025±0.0003
Caraway (<i>Carum carvi</i>)	2.32±0.247	1.22±0.311	1.21±0.009
Coriander (<i>Coriandrum sativum</i>)	1.62±0.190	1.45±0.056	0.94±0.03
Ginger (<i>Zingiber officinale</i>)	7.5±0.707	0.04±0.014	0.18±0.06
Black pepper (<i>Piper nigrum</i>)	4.09±0.127	0.30±0.155	0.29±0.08
Turmeric (<i>Curcuma domestica</i>)	6.6±0.07	0.19±0.127	0.30±0.141
Fennel (<i>Foeniculum vulgare</i>)	2.47±0.098	1.59±0.014	1.19±0.57
Galangal (<i>Alpinia officinarum</i>)	13.05±0.141	0.17±0.027	0.11±0.042
Cumin (<i>Cuminum cyminum</i>)	3.06±0.071	0.79±0.035	0.98±0.014
Cubeb (<i>Piper cubeba</i>)	2.7±0.141	0.12±0.014	0.28±0.147
Ras El Hanout I	10.57±0.042	0.22±0.035	0.15±0.035
Ras El Hanout II	7.2±0.283	0.28±0.028	0.51±0.07
Ascorbic acid	-	0.296±0.063	0.09±0.05
BHA	-	0.09±0.007	0.08±0.03

*Expressed as mg GAE/g of dry Weight plant material; the data are displayed with mean ± standard deviation of triplicate replications

against some free radical-related diseases [23]. Some studies have shown a high correlation between the content of polyphenols and antioxidant activity of spices [17,16, 24].

4. CONCLUSION

This study demonstrated that crudes extract of spices exhibit (individually and as a mix) significant antioxidant activity. *Cinnamomum cassia* extract represents the most active extract for scavenging the free radical DPPH and reducing iron, which is comparable to standard antioxidants ascorbic acid and BHA, which allows us to say that it is an extract that has powerful antioxidant activities. The mixtures of the studied spices of Ras el Hanout II compared to Ras el Hanout I have showed low radical scavenging activity as high concentration is required to inhibit the activity of DPPH by 50%. This suggests that there are interrelations molecular, which can be antagonistic, between the different metabolites of the constituent spices of Ras el Hanout which still remains to be clarified. In addition to enhancing the taste of dishes, and flavouring, spices can be considered as useful sources of materials for human health. They represent a good source of natural antioxidants, and are much safer than the synthetic antioxidants.

CONSENT

It is not applicable.

ETHICALAPPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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