



PHENOLIC COMPOUNDS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SPICES MIXTURES USED BY POPULATION OF OUARGLA (ALGERIA)

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ABSTRACT

Two spice mixtures "Ras el Hanout" composed of coriander, Caraway, cumin, fennel, green anise, black pepper, ginger, turmeric, cinnamon, and nutmeg at different proportions were studied. Phenolic profiles, as well as antioxidant and antibacterial activities of spice extracts were determined. The colorimetric assays revealed the richness of turmeric in polyphenols and flavonoids, of which the respective contents were 98.8 ± 5.29 mgGAE/gDS and 48.62 ± 1.07 mg QE/gDS. While the contents of coriander in these metabolites did not exceed 5.72 ± 0.51 mgGAE/gDS and 0.77 ± 0.19 mg QE/gDS, respectively. Both DPPH and FRAP tests revealed the strong antioxidant activity of the extracts of cinnamon and nutmeg. Coriander had the lowest antioxidant activity based on the DPPH test. Ginger was also found to be weakly active but by the FRAP test. The inhibitory effects of the extracts of black pepper (40 ± 4.25 mm) and cumin (37 ± 1.05 mm) against *E. coli*, ginger (30 ± 1.25 mm) against *P. aeruginosa*, curvi (40 ± 3.65 mm), and cumin (37 ± 2.44 mm) against *S. aureus*, ginger (40 ± 1.25 mm) and turmeric (38 ± 1.29 mm) against *C. albicans* were determined. The study revealed the impact of the relative proportions of constituent spices of the selected mixtures, on their secondary metabolite content, and also their antimicrobial and antioxidant activities.

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Introduction

The medicinal usage of herbs and spices has been well documented [1]. Even though they have become forgotten because of the coming of the modern westernized diet, they could be called the first "functional food" [2]. Spices are natural herbal products utilized as food preservatives and folk medicines and also, as coloring and flavoring agents worldwide since several years ago. Also, various spices have anti-carcinogenic potential, digestive stimulant, anti-mutagenic, antioxidant, anti-inflammatory, antimicrobial, carminative action, etc. [3]. Spices should be included as an integral part of healthy, nutritious, and as functional ingredients [4]. The active constituents like phenolics, flavonoids, tannins, and terpenes present in the spices are mainly responsible for spices' activities [5]. These secondary metabolites have an immense potential to produce new drugs, which benefit humankind. Systemic screening may lead to exploitation and exploration of novel effective products [6]. Several spice mixes are used in gastronomy, to combine taste properties or produce an aesthetic effect. We know the "curry" and "grama-massala" characteristic of Indian cuisine [7] and "Ras el Hanout" from North Africa. Etymologically, «Ras el Hanout» means, "head of the shop" indicating that it is a noble product and highly in demand. This mixture, known for its ancestral popularity, has the distinction of being different in composition from a spice merchant to another. Fifty different spices can be intervened in the constitution of "Ras el hanout". Among these spices, around twenty are selected to prepare the mixture; this gives a constitutional diversity to the "Ras el hanout" varying from one region to another. Citizens of Ouargla (City of the southwest of Algeria) are well known for extensive use of "Ras el hanout", especially in local traditional dishes. According to the herbalists of the region, this mixture is usually composed of coriander, cumin, cinnamon, ginger, black pepper, turmeric, caraway, fennel, red pepper, nutmeg, laurel, and cloves. Several investigations reported the phenolic constituents and antibacterial and antioxidant activities of spices. However, the

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information about the phenolic compounds of mixtures spices as "Ras el hanout" is limited. The objective of this research was to comparatively determine the total flavonoids and phenolic content, as well as antioxidant and antibacterial activities of spices commonly used in the preparations of "Ras el hanout" mixtures.

Materials and Methods

Spices Selection by Herbalist Investigation

The determination of the spices constituting "Ras el hanout" was made possible by the investigation carried out at the level of six herbalists in the Ouargla region (Algeria), from whom samples of no ground "Ras el hanout" were purchased and examined. Then, the proportions of each spice in each preparation were calculated and recorded in **Table 1**. The choice was made on the common spices between the different mixtures, which were coriander (*Coriandrum sativum* L.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* L.), black pepper (*Piper nigrum* L.), green anise (*Pimpinella anisum* L.), cinnamon (*Cinnamomum cassia* L.), caraway (*Carum carvi* L.), nutmeg (*Myristica fragans* Houtt.), fennel (*Foeniculum officinale* Mill.), and cumin (*Cuminum cyminum* L.). Among the examined mixtures, two showed a qualitative but not quantitative similarity, were also retained for the study to highlight the effect of quantitative change of spices on their mixtures.

Table 1. Principal constituents of different "Ras el hanout" mixtures according to herbalists.

spices	The proportion of different spices in different mixtures (%)					
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6
Nutmeg	1.15	4.35	1.99	2.37	0.12	0.8
Black pepper	4.97	8.28	9.79	3.80	4.56	3.66
Cinnamon	3.94	4.02	6.94	5.29	5.68	3.85
Turmeric	5.21	5.45	5.56	4.01	4.23	5.89
Ginger	5.17	7.63	6.93	4.30	7.01	5.98
Fennel	4.2	12.60	5.74	3.79	3.66	1.00
Cumin	3.89	13.10	8.30	4.77	5.24	3.58
Green anise	3.81	8.43	3.68	5.61	/	3.55
Coriandre	62.06	22.79	45.56	60.80	56.85	53.11
Caraway	5.49	13.30	5.46	4.69	3.95	4.35
Long pepper	/	/	/	/	2.81	/
Rose	/	/	/	/	/	1.36
Absinthe	/	/	/	/	/	6.54
Cardamom	/	/	/	/	2.5	2.31
Cloves	/	/	/	/	1.02	/
Chili pepper	/	/	/	1.23	/	2.22
Cubeb	/	/	/	/	3.74	/
Laurel	/	/	0.78	/	0.19	2.19

Extraction of Plant Material

Net powders of selected spices and their two mixtures were soaked in a mixture of methanol-water (80:20V/V) for 24 hours at ambient temperature [8]. The extraction and filtration were repeated three times with the renewal of the solvent. Using a rotary evaporator the extracts were concentrated and dried at 40°C. Dry extracts were then dissolved in methanol for the quantification of secondary metabolites and evaluation of the antioxidant activity and in dimethyl sulfoxide (DMSO) for the antimicrobial activity.

Determination of Total Phenolic Content

Using wong *et al.*'s (2006) method, the total phenolic content of plant extract was evaluated [9]. To 1 mL of Folin-Ciocalteu's reagent (10 %), 200 µL of the sample was added. After 4 minutes, 800 µL sodium carbonate (75g /L) was added, then vortexed and incubated at room temperature in the dark for 30 minutes. The absorbance was measured at 765 nm.

Using various concentrations of gallic acid, a standard curve was obtained. The results were expressed as mg gallic acid equivalent/g dry weight of the sample (mgGAE/gDS).

Determination of Total Flavonoid Content

Total flavonoids were determined by AlCl₃ colorimetric assay [10]. To 1 mL of extract, 1 mL sodium nitrate (2% in methanol) and after 5 min, 150 µL AlCl₃ solution (10%) was added and vortexed, then incubated for 10 minutes. The absorbance was measured at 430 nm. The appearance of yellow colour indicated the presence of flavonoids. Quercetin was used as a standard. The results were expressed as mg quercetin equivalent/g dry weight of the samples (mg QE/gDS).

DPPH Radical Scavenging Assay

DPPH• test was performed according to the procedure described by Sanchez-Moreno (1998) [11]. A volume of 50 µL of each extract at different concentrations was added to 1950 µL of freshly prepared methanolic solution of DPPH (0.024 g/L). Negative control was also prepared, by the addition of 50 µL of methanol to 1950 µL of DPPH. The positive control was ascorbic acid. After incubation at ambient temperature for 30 min in the dark, absorbance was measured at 517 nm. DPPH• scavenging activity (inhibition %) was calculated using the equation: $I\% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$. Where, A control: absorbance of the DPPH solution without extract, A sample: absorbance of the solution containing the sample. Inhibitory concentration (IC₅₀) values were calculated from the graph plotting inhibition percentage against different extracts concentrations.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP was determined according to Oyaizu (1986) [12]. 2.5 mL of different concentrations of each extract diluted in methanol was mixed with 2.5 mL PBS (0.2 M; pH 7.4) and 2.5 mL of potassium ferricyanide at 1%. The mixtures were incubated at 50°C for 30 minutes. Then, 0.5 mL of 0.1% FeCl₃, 2.5 mL trichloroacetic acid (10%), and 2.5 mL distilled water were added to the reaction medium. Absorbance was measured at 700 nm. Ascorbic acid was utilized as a positive control. To explore the results obtained, the absorbance curves obtained as a function of different concentrations were plotted. The increased absorbance corresponded to an increase in the reducing power of the extracts.

In vitro Antibacterial Activity

The crude extracts of plants were screened for their antimicrobial activity against food-borne pathogens and spoilage bacteria. The standard strains of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 11303, and *C. albicans* were obtained from the internal laboratory of Mohamed BODIAF hospital and laboratory of Quality Control and repression of fraud (Ouargla-Algeria). The Agar disc diffusion method was used to study this activity [13]. Standard inoculums standardized to 0.5 McFarland (10⁶ CFU.mL⁻¹) were introduced into the surface of sterile agar plates. The sterile discs prepared from Whatman N°3 filter paper (6 mm in diameter) previously soaked in a known concentration of the test compound (50 g/L) were placed in Mueller Hinton medium for bacteria and in Sabouraud medium for yeast and incubated at 37°C for 24 hours. The results were recorded by measuring the zone of inhibition around the discs. The successive dilution method was used to determine the minimum inhibitory concentration (MIC). The concentrations tested were 25, 12.5, 6.12, 3.12 and, 1.56 mg/ml.

Statistical Analysis

The results of the chemical analyses were illustrated as the means standard deviation of three independent measurements. Data were analyzed using Excel (Microsoft Inc.) and IBM SPSS v.21. Significant differences between samples were analyzed using ANOVA. A P-value of <0.05 was considered statistically significant.

Results and Discussion

Total Polyphenol Content

Our study showed that the phenolics concentrations varied widely, ranging between 98.97±5.29 and 5.72±2.51 mg GAE/g DS (**Figure 1**).

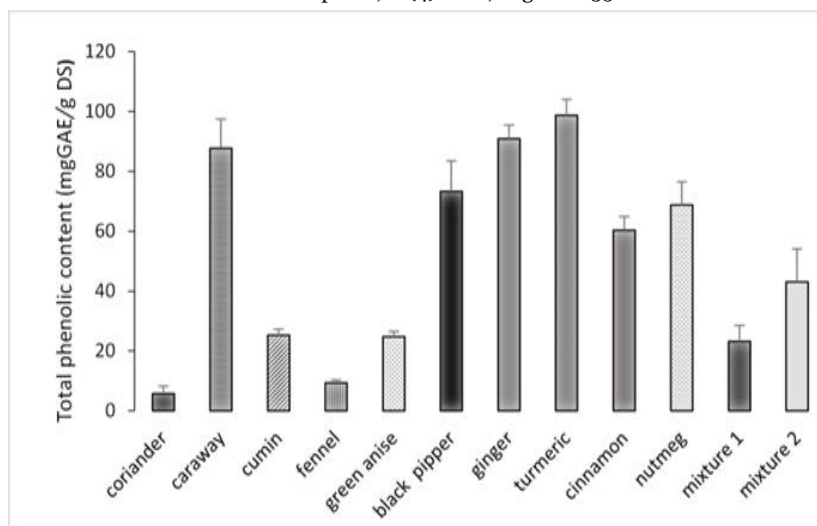


Figure 1. Total phenolic contents of the spices extracts and their mixtures. Values are means \pm SD of 3 replications. mgGAE/g DS= mg Gallic Acid Equivalent/g Dry Sample.

Turmeric followed by ginger represents the spices richest in polyphenols with contents of 98.8 ± 5.29 and 90.93 ± 4.48 mgEAG/gDS, respectively. Caraway, pepper, nutmeg, and cinnamon are also rich giving, in descending order, contents between 87.77 ± 9.67 and 64.71 ± 4.55 mgEAG/gDS. Cumin and green anise show a quantitative similarity in these active ingredients ($P > 0.05$) (Figure 1). The spice least endowed with polyphenols is coriander. The determination of the polyphenols in the mixtures prepared highlights significant differences between the two mixtures ($p < 0.05$). The classification of our spices according to their richness in polyphenols is in agreement with the results of Denre (2014) [14] but is in contrast with data of Maizura (2011) [15] reported that ginger is richer in polyphenols than turmeric. Kabera (2014) reported that the differences are probably due to the analytical methods, time of sample collection, environmental factors (moisture, salinity, temperature, soil, etc.) genotype, etc. [16]. According to the literature, polyphenols are antioxidant, antimicrobial, antimutagenic, anticarcinogenic, anti-inflammatory, analgesic, antiallergic, antispasmodic, hepatoprotective, neuroprotective, anti-atherosclerosis, cardioprotective, antidiabetic, protective effect on immune cell functions, modulator of hormonal effects regulator of cell cycle progression, estrogenic agents, etc. [17, 18].

Flavonoids Content

Flavonoids' contents of spices and their mixtures are illustrated in Figure 2.

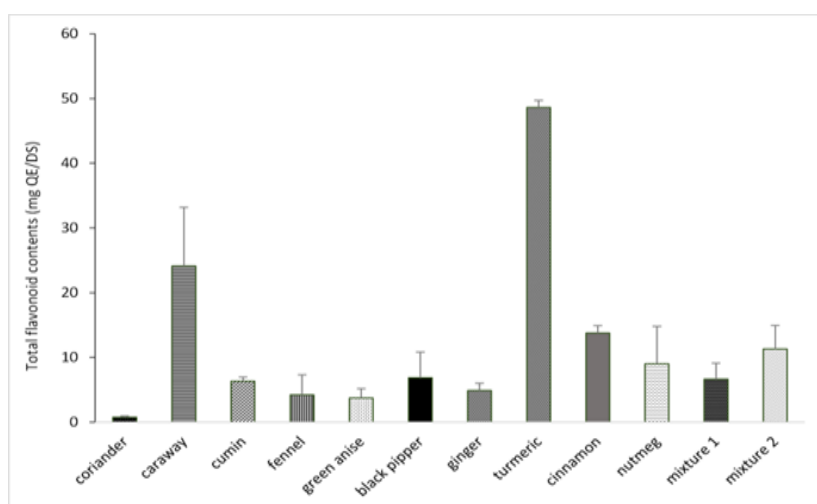


Figure 2. Total flavonoids contents of the spices extracts and their mixtures. Values are means \pm SD of 3 replications. mg QE/g DS=mg quercetin equivalent/g Dry sample.

It is apparent from Figure 2 that turmeric had the highest flavonoids content (48.62 ± 1.07 mg QE/g DS) followed by caraway (24.13 ± 9.03 mg QE/gDS). Whilst coriander had the lowest value (0.77 ± 0.19 mg QE/g DS). The difference in the flavonoids contents of the spices studied was significant ($p < 0.05$), except between cumin and black pepper and between ginger and fennel. The selected mixtures had significantly different flavonoid contents ($p < 0.05$) (26.65 ± 2.44 and 11.32 ± 3.63 mg QE/gDS, respectively for the first and second mixtures). The majority of polyphenol's benefits are achieved by the flavonoid subclass. It constitutes the largest group of plant phenolics and has extensively been studied. Regarding the flavonoid

content of our spices, the high flavonoid content of turmeric corroborates the results of Kim *et al.* (2011) [19]. Curcuminoids make up 2-4% of dry turmeric root powder including curcumin, which represents the major bioactive compound of turmeric [20, 21]. Various adjuvants can be recommended to increase the bioavailability of curcumin, the highest one is the piperine contained in the black pepper [22, 23]. Our data was revealed also the high content of caraway in flavonoids; this corroborates the results of Agrahari and Singh (2014) [24]. These flavonoids giving the spice a multitude of biological activities, such as antimicrobial, antifungal, anti-hyperglycemic, anti-hyperlipidemic, anti-tumor, and others [25, 26]. The existence of flavonoids in other spices, even in small quantities, gives them medicinal properties specific to the nature of the flavonoids they contain [27].

DPPH Free Radical-Scavenging Activity

Cinnamon and nutmeg had a strong capacity to trap the DPPH radical than the positive control, their IC_{50} recorded were 0.05 ± 0.0 and 0.16 ± 0.02 mg/mL, respectively (**Figure 3**). Fennel, cumin, caraway, and coriander had the lowest antioxidant activity; their IC_{50} were 1.30 ± 0.12 , 1.54 ± 0.15 , 1.95 ± 0.18 , and 3.87 ± 0.13 mg/mL, respectively. The second mixture ($IC_{50} = 0.23 \pm 0.04$ mg/mL) exhibited better antioxidant activity than the first mixture whose IC_{50} value was 0.85 ± 0.12 mg/mL ($p < 0.05$) (**Figure 3**).

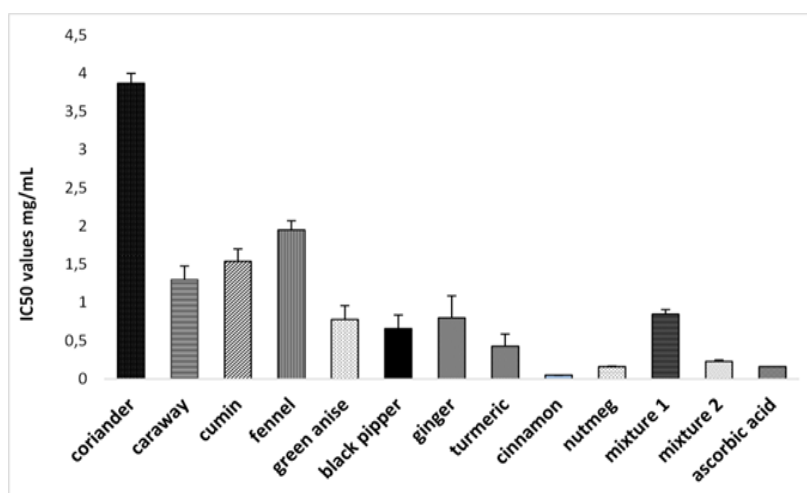


Figure 3. Antioxidant activity by DPPH method of the spice extracts and their mixtures. Values are means \pm SD of 3 replications.

Ferric Reducing Antioxidant Potential (FRAP) Assay

The absorbance values measured made it possible to draw logarithmic trend curves, which thus demonstrated a proportional relationship between the increase in the concentration of the extract and the reduction of iron in the samples studied (**Figure 4**).

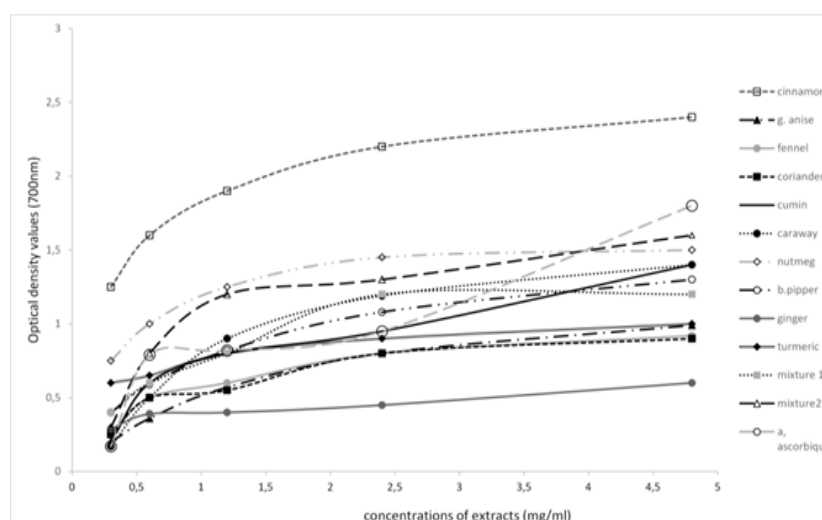


Figure 4. Antioxidant activity by FRAP methods of the spice extracts and their mixtures.

Cinnamon, nutmeg, caraway, and black pepper had a better ability to reduce iron than the positive control, at concentrations of 0.3 to 2.4 mg/mL. Beyond that, only cinnamon, which is by far the spice with the highest capacity to reduce iron, reaching at the concentration of 4.8 mg/mL an optical density of 2.4 against 0.95 of the control. Furthermore, cumin,

turmeric, green anise, fennel, coriander, and ginger had lower antioxidant activity than that of the positive control. From low concentrations up to 2.4 mg/mL, mixtures were more iron-reducing than ascorbic acid. **Figure 4** reveals also that, from the concentration of 0.6 mg/mL, mixture 2 is more effective in the reduction of Fe³⁺ to Fe²⁺. The antioxidant effectiveness of cinnamon has been reported by several authors. Asimi *et al.* (2013) [28] found that cinnamon is more significantly effective than cumin and ginger in reducing the DPPH radical. This effectiveness, compared to that of turmeric and ginger, has also been reported by Denre (2014) [14]. Murcia (2004) mentioned that the ethanolic extract of cinnamon has a significant inhibitory effect (96.3%) than α -tocopherol as a natural antioxidant [29]. The antioxidant potential of cinnamon can be explained by its richness in metabolites including cinnamaldehyde and cinnamic acid, which have antioxidant properties [30, 31]. The antioxidant activity of nutmeg has also been confirmed in the literature. Gupta and Rajpurohit (2011) and Nokilic *et al.* (2021) attributed this activity to the richness of nutmeg in antioxidant compounds, mainly myristicin, caffeic acid, lignan, eugenol, and others [32, 33]. Nonetheless, an overdose of nutmeg is toxic. Ingestion of 20 grams can be fatal. The molecules responsible for this toxicity are myristicin and safrole [34, 35]. The notion of toxicity is undoubtedly taken into consideration by the ancients in the preparation of spice mixes such as "Ras el hanout" where we note that the strong spices are only present in small amounts. Therefore, before using and combining spices, we need to know how they are listed and what categories they belong to maximize their benefits and minimize their harm.

Evaluation of Antibacterial Activity of Extracts

The antibacterial activities of the spices extracts at concentrations of 50mg/mL and their MIC are summarized in **Table 2**.

Table 2. Antibacterial activities of crude extracts of the spices

Spices	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		<i>C. albicans</i>	
	Inhibition diameter (mm) at 50mg/mL	MIC (C) (mg/mL)	Inhibition diameter (mm) at 50mg/mL	MIC (C) (mg/mL)	Inhibition diameter (mm) at 50mg/mL	MIC (C) (mg/mL)	Inhibition diameter (mm) at 50mg/ML	MIC (C) (mg/mL)
Coriander	16±0.05	25<C<50	10±1.02	25<C<50	7±0.01	25<C<50	6	-
Caraway	22±0.0	6.25<C<12.5	10±0.0	25<C<50	40±3.65	1.56<C<3.12	25±1.89	12.5<C<25
Cumin	32±0.6	C<1.56	37±1.05	3.12<C<6.25	37±2.44	1.56<C<3.12	36±0.56	C<1.56
Fennel	16±0.4	25<C<50	6	-	16±0.58	25<C<50	6	-
green anise	11±0.0	25<CI<50	6	-	11±0.0	25<C<50	12±0.0	25<C<50
B. pepper	40±4.25	1.56<C<3.12	20±0.0	6.25<C<12.5	35±5.23	1.56<C<3.12	25±1.25	6.25<C<12.5
Ginger	35±1.5	C<1.56	30±1.25	6.25<C<12.5	24±0.54	12.5<C<25	40±1.25	C<1.56
Curcuma	25±1.99	3.12<C<6.25	20±1.2	3.12<C<6.25	33±2.56	1.56<C<3.12	38±1.29	C<1.56
Cinnamon	29±1.55	3.12<C<6.25	23±1.33	6.25<C<12.5	22±0.21	6.25<C<12.5	33±2.60	C<1.56
Nutmeg	19±0.0	25<C<50	6	-	14±0.45	25<C<50	20±1.05	25<C<50
Mixture 1	20±0.25	6.25<C<12.5	12±0.25	25 <C< 50	14± 2.4	12.5 <C< 50	14±2.3	12.55 <C<50
Mélange 2	23±3.14	6.25<C<12.5	16±1.2	25 <C< 50	23±0.07	6.25<C<12.5	20±1.56	6.25<C<12.5

Note. MIC=C= Minimum inhibitory concentration.

It appears from the results recorded in **Table 2** that the crude extract of black pepper followed by that of ginger was extremely active against *E. coli*, forming zones of inhibition of 40±4.25 and 35±1.5 mm, respectively. Ginger and cumin extracts were the best inhibitors of *P. aeruginosa* with inhibition zones of 30±1.25 and 37±1.05 mm, respectively. In contact with *S. aureus*, caraway and cumin extracts were more inhibitory. The yeast *C. albicans* was particularly sensitive to the extracts of ginger and turmeric with which, zones of inhibition of 40±1.25 and 38±1.29 were formed with a MIC was less than 1.56 mg/ml. Regarding the spice mixtures studied, it emerged that the extract of the second mixture is more inhibitory than that of the first in contact with all the strains tested. This difference was significant (p >0.05) with *S. aureus* and *C. albicans*. The effectiveness of cumin, ginger, cinnamon, pepper, and turmeric against the germs tested corroborates with the literature. Indeed, Islam *et al.* (2014) reported a potent antimicrobial effect of ginger against a range of pathogenic bacteria including *Escherichia coli*, *Pseudomonas aruginosa*, *Staphylococcus aureus* [36]. An antimicrobial screening evaluated by Mahfuzul-Hoquea *et al.* (2008) showed an extreme activity of the cinnamon extract against several germs contaminating food [37]. Turmeric extract is an excellent inhibitor of *S. aureus*, *E. coli*, and *C. albicans* [38]. The antibacterial mode of action is still not completely understood. Spices contain many different bioactive compounds divided into volatile (essential oils) and non-volatile (phenolics), which could impact the swarming, motility, and biofilm production of bacteria. Overall,

the action of one compound cannot confirm the antimicrobial activity of spices. The final activity is a synergistic effect of more components [39, 40].

Conclusion

This analysis revealed the richness of turmeric in polyphenols and flavonoids. The spices least endowed with these metabolites being coriander. We suggest that the second mixture acquired its richness in phenolics as well as their activities due to its higher content of spices endowed with rich in these compounds and good antioxidant and antimicrobial activities. The coriander strongly presents in the first mixture caused the poverty of this mixture in metabolites and the decrease of their biological activities. The spices are complex by their nature, representing a potential for the development of synergistic interactions, additives, or antagonists. We, therefore, favor the second mixture "Ras el hanout" and to improve its quality, we suggest increasing its content on turmeric "friend of health" and minimizing its content on nutmeg known by its toxicity.

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