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CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTI-FOOD-BORNE BACTERIAL ACTIVITIES OF ESSENTIAL OILS FROM SOME SPICES CONSUMED IN TUNISIA

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ABSTRACT

The essential oils of five cooking spices: Clove, nutmeg, cinnamon, carvi and coriander were obtained by hydro distillation with a 14.40 ± 1.20 %, 13.00 ± 1.20 %, 0.95 ± 0.10 %, 3.50 ± 0.50 % and 0.53 ± 0.01 % (v/w) yield, respectively. Their chemical compositions were analyzed by gas chromatography-mass spectrometry. The quantitative analysis showed that the major compounds of clove, Cinnamon, coriander and carvi essential oils were eugenol (78.60%), Z-cinnamaldehyde (86.00%), Linalool (80.00%) and carvone (88.00%), respectively, and those of nutmeg are Sabinene (22.30%), α -terpineol (17.30%), Myristicin (19.50%) and Alpha-pinene (14.6%). The antioxidant activity was assessed by 2, 2-Diphenyl-1-picrylhydrazyl free radical assay. All essential oils exhibited antioxidant activity, except for cinnamon. The highest antioxidant activity was detected in clove with minimum inhibitory concentration value of $4.27 \mu\text{g/ml}$. The results of disk diffusion assay indicate that Cinnamon was the most effective at inhibiting food-borne bacteria. This study suggests that Spicy foods are not only appealing but also healthy.

KEYWORDS

Spice, Essential oil, Chemical composition, Antioxidant activity and Antibacterial activity.

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INTRODUCTION

Spices have been an integral part of culinary cultures around the world and have a long history of use for flavoring, coloring, and preserving food, as well as for medicinal purposes. The increasing use of spices as flavorings in foods is a major trend worldwide. Their bioactive ingredients have long been documented in experimental¹⁻² and have recently attracted scientific interest as sources of natural medicines possessing many beneficial effects on

health, such as antioxidant, antiviral, antibacterial, antifungal, anti-inflammatory and acetyl cholinesterase inhibitory activities³⁻⁸. Using essential oils from flowering plants (angiosperms) as food preservatives can be traced back to the ancient Egyptians⁹. In modern society, synthetic drugs are developed for use as food preservatives and antibacterial regents. The practical functions of essential oils have been completely substituted by industrial products. However, in recent years, many concerns have been raised regarding the overuse of synthetic drugs as preservatives and additives in food products. It has been showed that the excessive use of these synthetic drugs leads to potential health hazards and carcinogenicity¹⁰. The urge to search for healthy chemical substitutes has drawn massive attention to essential oils¹¹. Additional of essential oils into food, drugs, and cosmetics is now desired in various products. Many performance results have been made possible by the advent of essential oil (EO) products. It is therefore important to scientifically evaluate the possible applications of essential oils. One of the most intriguing properties of essential oils is their antioxidant and antibacterial property. Depending on the spice's growth condition, such as location and climate, the chemical composition of their same essential oils may vary. In this paper, we report the extraction, the chemical composition, the antioxidant and antibacterial activities of Five spices from India used in cooking as flavorings in Tunisia: Clove (*Syzygium aromaticum*, Myrtaceae), Coriander (*Coriandrum sativum*, Apiaceae), Nutmeg (*Myristica fragrans*, Myristicaceae), Cinnamon (*Cinnamomum zeylanicum*, Lauraceae) and Carvi (*Carum Carvi*, Apiaceae). Most of the data published on the antioxidant and antimicrobial properties of Clove, coriander, nutmeg, Cinnamon and carvi essential oils are fragmented and employ only basic screening techniques. However, this paper takes the work a stage further to establish precise antioxidant, bacteriostatic and bactericidal concentrations against four food-borne bacteria and nine other pathogenic bacteria.

MATERIAL AND METHODS

Plant material and extraction

Five spices: Clove (*Syzygium aromaticum*, Myrtaceae), Coriander (*Coriandrum sativum*, Apiaceae), Nutmeg (*Myristica fragrans*, Myristicaceae), Cinnamon (*Cinnamomum zeylanicum*, Lauraceae) and Carvi (*Carum Carvi*, Apiaceae) were purchased from a local market in Sfax, Tunisia (from India). Plant materials consisted of flower buds (clove), fruits (coriander, nutmeg, Carvi) and stem bark (cinnamon). The samples of spice plant materials were deposited in the Laboratory of Chemistry of Natural Substances, Faculty of Sciences of Sfax, Tunisia. One hundred grams of spices were powdered then soaked in 1l of distilled water. Hydro distillation was performed simultaneously for 3h by means of the Clevenger-type apparatus¹². The oil obtained was dried with anhydrous sodium sulphate and kept at 4°C until use. The chemicals and all applied solvents were purchased from Flukan Chemie, Buchs, Switzerland.

GC-mass spectrometry analysis

The analysis of the essential oils was performed on a GC-MS HP model 5975B inert MSD (Agilent Technologies, J and W Scientific Products, Palo Alto, CA, USA), equipped with an Agilent Technologies capillary DB-5MS cross bond (5% diphenyl-polysiloxane and 95% dimethyl-polysiloxane; 30 m × 0.25 mm, 0.25 µm) and coupled to a mass selective detector (MSD5975B, ionization voltage 70 eV; all Agilent, Santa Clara, CA). The carrier gas was He and was used at (1 mL/min) flow rate. The oven temperature program was as follows: 1 min at 100 °C ramped from 100 to 260 °C at 4°C.min⁻¹ and 10 min at 260 °C. The injection volume was 1µl, and the injection mode was split with a1:100 ratio. Identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components. For further confirmation, Kovats retention index (RI) was calculated relatively to standard n-alkanes of n-paraffin mix C7, C8, C9, C10, C11, C12, C13, C14, C15, C16 (Aldrich, St. Louis; USA). The calculated RI was compared with those reported by Olivera¹³ and Po-Chenl¹⁴. The

relative percentage amounts were calculated on the basis of peak areas for GC-MS analysis.

Free radical scavenging activity

DPPH is one of the compounds that possess a proton free radical, when DPPH encounters proton radical scavengers its purple color fades rapidly. This assay determines the scavenging of stable radical species according to the method of Marinova and Batchvarov¹⁵. Briefly, the assay was carried out by mixing 1.5 mL ethanolic solution of each of the essential oils with 1.5 mL of a 0.06 mM methanolic DPPH solution at four final concentrations (0.12, 0.25, 0.5 and 1 mg essential oil/mL). The mixture was then incubated in the dark for 30 minutes at 25°C and the absorbance at 517 nm was recorded as (A_{sample}), using a SHIMADZU UV-1280 Multipurpose UV-Visible Spectrophotometer. A blank experiment was also carried out applying the same procedure to a solution without the EO and the absorbance was recorded as (A_{blank}). The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance against a blank.

The percentage of antiradical activity (%ArA) was calculated as follows:

$$(\%ArA = ((A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}) \times 100)$$

The results are expressed as IC_{50} , the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. The lower IC_{50} values indicate a higher antioxidant activity. The synthetic antioxidants butylated hydroxytoluene (BHT) and vitamin E were used as reference compounds. The assays were performed twice with three replicates.

Microorganisms and growth conditions

The antibacterial potentials of the EOs against 13 microorganisms obtained from international culture collections (ATCC) was investigated in vitro. The pathogenic bacteria isolated strains were as follows: *Staphylococcus xylosus*, *Staphylococcus epidermidis*, *Escherichia coli* (ATCC 25922) and *Salmonella*. *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC), *Brevibacterium flavum*, *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecium* (ATCC 29212), *Micrococcus luteus* (ATCC 4698), *Klebsiella pneumoniae* (ATCC 13883),

Pseudomonas aeruginosa (ATCC 27853) and *Enterobacter cloacae* and Using the standard disk, the susceptibility of the micro-organisms to EOs was determined as previously described by Fattouch¹⁶. All microorganisms were cultivated at 37°C. The cultures were started by adjusting the bacterial suspension in broth to 0.5 McFarland turbidity. Using a serial 10-fold dilution method, the bacterial suspension was spread plated on count Muller-Hinton (MH) 1.5% agar in order to give a population of 10^8 colony-forming units (cfu) per plate. For the disk diffusion method, 20 µg (1 mg/mL) of the EO dissolved in 10% (v/v) Dimethylsulfoxide (DMSO) were placed on sterile paper disk (6 mm diameter, Sanofi) and placed onto the inoculated agar surface. The petri dishes were incubated for 24 h and the diameters of the resulting inhibition zones were measured. Ampicillin (10 µg) was used as positive control and 10% (v/v) DMSO was as negative control. The assays were performed twice with three replicates.

Determination of minimum inhibitory and bactericidal concentration

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)¹⁷. All tests were performed in Mueller Hinton broth. A serial doubling dilution of the EO was prepared in a 96-well microtiter plate over the range 1.00-0.02 mg/mL. Overnight, broth cultures of each strain were prepared and the final concentration in each well was adjusted to 5×10^5 cfu/mL. Plates were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The MICs were determined as the first well in ascending order that did not produce a pellet. The MIC was confirmed and MBC was estimated when removing an aliquot (25 µL) of the broth from each well and spread onto MH-agar plates, then inoculated for 2-16 h at 37°C for bacteria. The enumeration of surviving organisms allowed the determination of the MBC at 99% of bacterial death¹⁶. Each test was performed in duplicate and repeated twice. Ampicillin was served as positive control.

RESULTS AND DISCUSSION

The hydro distillation of 100 g of Clove, nutmeg, cinnamon, carvi and coriander gave essential oils with yields of $14.4 \pm 1.40\%$, $13 \pm 1.20\%$, $0.95 \pm 0.10\%$, $3.5 \pm 0.50\%$ and $0.53 \pm 0.01\%$ based on dry weight (v/w), respectively. The chemical composition of EOs obtained from the studied spices was identified by GC-MS chromatography with Wiley and NIST library data. The chemical components of five EOs, ranked according to their Kovats retention indices, and their relative abundance (above 0.1%) are listed in Table No.1. The quantitative analysis revealed that the major compounds of clove, Cinnamon, coriander and carvi essential oils were eugenol (78.60%), Z-cinnamaldehyde (86.00%), Linalool (80.00%) and carvone (88.00%), respectively, and those of nutmeg are Sabinene (22.30%), α -terpineol (17.30%), Myristicin (19.50%) and Alpha-pinene (14.6%). The main components mentioned above constitute more than 73% of the essential oils. This high percentage contents indicate potential for special applications. It is of interest to compare the composition of Indian spices used in Tunisian Cooking and other spices grown elsewhere. By comparing with the literature data, we found that the clove, coriander and carvi had the same major compound everywhere¹⁸⁻²² but those of nutmeg and cinnamon was different. Cinnamon grown in India contains primarily Z-Cinnamaldehyde along with other minor components, and while that grown in Sri Lanka contains a relative abundance of eugenol in addition to Z-Cinnamaldehyde²³. The contents of cinnamon from Malaysia are very different from those of their counterparts in other parts of the world and the major constituents were linalool (36.0%), methyl eugenol (12.8%), limonene (8.3%), α -terpineol (7.8%) and terpinen-4-ol (6.4%)²⁴. These results demonstrate that essential oils have a large diversity in their composition in line with their different origins.

The reduction ability of DPPH radicals by the spices EOs was evaluated and the obtained results were summarized in Table No.3 where BHT and Vitamine E were used as positive controls ($IC_{50} = 11.40 \pm 0.20$ $\mu\text{g/ml}$ and $IC_{50} = 9.80 \pm 0.20$ $\mu\text{g/ml}$, respectively). Results showed that the antioxidant activity of EOs

of clove and coriander were endowed with a powerful antioxidant activity ($IC_{50} = 4.27 \pm 0.20$ and $IC_{50} = 50.00 \pm 0.20$ $\mu\text{g/ml}$, respectively) higher than that of BHT and vitamine E. These observations are in full agreement with some previous results which evaluate the efficiency of clove and coriander as antioxidant agents against Lipid oxidation after adding to cake's ingredients during preparation, which demonstrated that the antioxidant activity of their essential oils exhibited high antioxidant activity in cake when compared with the synthetic antioxidant during storage²⁵⁻²⁶. The EOs of Carvi and nutmeg displayed a moderate activity with IC_{50} values of 430.00 ± 0.30 and 740.00 ± 0.30 $\mu\text{g/ml}$, respectively. Those essential oils could be interesting antioxidants only if applied at the highest concentration tested. However, there was no evaluation of the antioxidant activity of cinnamon essential oil. In order to provide additional data to support their utilization and development as bactericides for food disease control, we also reported, at this stage, the antibacterial activity of the five spices against nine clinically isolated strain and four food-borne bacteria. Subsequently, the antibacterial activities of the EOs were qualitatively and quantitatively assessed by the determination of inhibition zone (DD), MIC and MBC values where ampicillin was used as a positive control. The result of disk diffusion assay given in Table No.4 indicates that the five EOs have a stronger inhibitor activity against food-borne bacteria than the other pathogenic bacteria. From food-borne bacteria, *Staphylococcus xylosus* was the most sensitive microorganism tested with a DD ranging from 4.00 ± 0.31 to 22.00 ± 0.50 mm. On the other hand, *Klebsiella pneumoniae* was the most bacteria affected with DD ranging from 2.00 ± 0.50 mm to 14.00 ± 0.50 mm. The potent bacteria zone inhibitor was obtained with Cinnamon and clove reaching those of the positive control Ampicillin. This important antibacterial activity may be due to the presence of the major compounds Z-Cinnamaldehyde (86.00%) and (Eugenol 78.60%)²⁷⁻²⁹. Carvi and coriander EOs were more active against clinical bacteria *Enterococcus faecium*, and *Enterococcus faecalis* than the other strain. However, nutmeg was active against *Enterobacter*

cloacae 8.00±0.50 mm. These results were confirmed by the determination of MBC/MIC ratios. In fact, MIC is the lowest concentration of an antibiotic that will inhibit the visible growth of a microorganism after overnight incubation³⁰. Although the MICs and MBCs results varied between organisms tested, in most cases the MIC was equivalent to the MBC indicating a bactericidal activity of the oil³¹. The MIC and MBC/MIC ratios of the five essential oils were presented in Table No.5 and Table No.6, respectively. Results indicated that *Enterococcus faecium* was the most sensitive microorganism tested with MIC (62.50±6.10 µg/ml) from Coriander and carvi and (125.00±10.50 µg/ml) from Cinnamon, clove and nutmeg. Only EO of Cinnamon have a bactericidal effect against *Echerchia coli* with MIC = 500 µg/ml. The data obtained from MBC/MIC ratios demonstrated that the five EOs have a stronger inhibitory growth of food-borne than clinical bacteria. The highest bactericidal concentration of the five EOs against *Staphylococcusxylosus* and *Salmonella* was 250µg/ml and against *Staphylococcus epidermidis* was 500µg/ml. The low bactericidal concentration of the five essential oils studied against some bacterial food poisoning and some clinical bacteria provide an exciting move away from artificial preservative and a move towards more natural alternatives.

Some previous results, which evaluated the efficiency of coriander and Clove antimicrobial agent against mold growth after adding to bakery's ingredients during preparation, demonstrated that their essential oils could be used as antimicrobial in foodstuffs and could increase shelf-life of those foods²⁵⁻²⁶. Other studies indicated that the EO of Cinnamon has a strong inhibitory growth of food-borne pathogenic and spoilage microorganisms²⁴ and that the application of EOs for microbial control are more effective when applied to ready to use foods containing a high protein level at acidic pH, as well as lower levels of fats or carbohydrates³².

Table No.1: General characteristics and preliminary data of antibacterial and antioxidant potential of essential oils from five spices tested

S.No	Family	Plant species	Common name	Part used
1	Myrtaceae	<i>Syzygiumaromaticum</i> ,	Clove	Flower buds
2	Myristicaceae	<i>Myristicafragrans</i> ,	Nutmeg	Fruits
3	Apiaceae	<i>Carum Carvi</i> ,	Carvi	Fruits
		<i>Coriandrum sativum</i> ,	Coriander	Fruits
4	Lauraceae	<i>Cinnamomumzeylanicum</i>	Cinnamon	Stem bark

Table No.2: Volatile components of the five essential oils and their relative abundance

S.No	Compound ^a	KI ^b	KI ^c	KI ^d	Coriander	Carvi	Clove	Cinnamon	Nutmeg
					% of Total ^e				
1	Alpha-pinene	930	936	925	1.10	0.70	1.30	1.10	14.60
2	Sabinene	976	975	963	-	-	-	-	22.30
3	Beta-pinene	980	972	969	-	1.20	-	-	7.20
4	α -myrcena	991	1009	991	-	-	-	-	2.20
5	α -terpinene	1018	994	1016	-	-	-	-	4.00
6	Limonene	1029	1023	1031	8.80	4.00	0.10	-	0.30
7	1-8 Cineol	1031	1027	1033	-	-	-	0.30	-
8	γ -terpinene	1060	1049	1061	-	-	-	-	7.20
9	Linalool	1088	1085	1102	80.00	3.60	-	-	-
10	α -terpineol	1189	1295	1175	0.70	-	-	0.30	17.30
11	Carvone	1243	-	1234	-	88.00	-	0.40	-
12	Geraniol	1252	1252	1244	0.40	-	-	-	1.40
13	Z-cinnamaldehyde	1272	1280	-	-	-	-	86.00	-
14	Terpinylacetate	1350	1333	-	-	0.10	-	-	-
15	Eugenol	1359	1377	1334	5.50	0.30	78.60	2.20	-
16	Beta-caryophyllene	1419	-	1404	-	-	8.00	3.00	-
17	Z-cinnamylacetate	1445	-	-	-	-	-	5.60	-
18	Eugenylacetate	1493	-	-	-	-	0.30	-	-
19	Myristicin	1523	1496	-	-	-	11.00	-	19.50
20	Delta-Cadinene	1529	1527	-	-	-	0.20	-	-
21	Total e%				96.50	97.90	99.50	98.90	95.80

^aThe chemical compositions were identified by Database Wiley and NIST.

^bCalculated Kovats retention index on the DB-5MS column.

^ckovats retention index (RI) from Olivera *et al*²¹.

^dkovats retention index (RI) from Po-Chen *et al*²².

^eIntegral of peak area in the GC-MS chromatograms is used.

Table No.3: Antioxidant activity using DPPH-free radical method of the five spices essential oils and positive controls

S.No	Sample	IC ₅₀ ^a
1	Clove	4.27±0.20
2	Nutmeg	740.00±0.30
3	Cinnamon	-
4	Coriander	50.00±0.20
5	Carvi	430.00±0.30
6	BHT	11.40±0.20
7	Vitamine E	9.80±0.20

IC₅₀, the concentration of antioxidant which reduces the free radical DPPH[•] about 50% ^aValues given as µg/ml/.

Table No.4: Antibacterial activity of the five essential oils expressed as diameters of inhibition zones against four bacteria food-born and nine clinically bacteria after 24h

S.No	Miroorganismes	DD ^a					
	Clinically bacteria	Coriander	Carvi	Clove	Cinnamon	Nutmeg	Amp ^b
1	<i>Enterococcusfaecium</i>	8.00±0.56	8.00±0.56	4.00±0.31	12.00±0.50	3.00±0.10	30.00±0.50
2	<i>Enterococcusfaecalis</i>	8.00±0.56	8.00±0.56	4.00±0.31	NA	3.00±0.10	32.00±0.50
3	<i>Bacillus subtilis</i>	2.00±0.50	2.00±0.50	NA	10.00±0.50	2.00±0.10	20.00±0.50
4	<i>Bacillus cereus</i>	NA	NA	4.00±0.31	12.00±0.50	NA	32.00±0.50
5	<i>Brevibacteriumflavum</i>	2.00±0.50	2.00±0.50	4.00±0.31	10.00±0.50	2.00±0.10	15.00±0.50
6	<i>Micrococcusluteus</i>	NA	NA	6.00±0.31	12.00±0.50	2.00±0.10	30.00±0.50
7	<i>Pseudo aeruginosa</i>	2.00±0.50	2.00±0.50	3.00±0.10	10.00±0.50	2.00±0.10	20.00±0.50
8	<i>Klebsiella pneumoniae</i>	2.00±0.50	2.00±0.50	6.00±0.31	14.00±0.50	NA	14.00±0.50
9	<i>Enterobactercloacae</i>	2.00±0.50	2.00±0.50	4.00±0.31	8.00±0.50	8.00±0.50	28.00±0.50
10	Food -borne bacteria	-	-	-	-	-	-
11	<i>Staphylococcus xylosus</i>	10.00±0.56	10.00±0.56	14.00±0.50	22.00±0.50	4.00±0.31	40.00±0.50
12	<i>Staphylococcusepidermidis</i>	2.00±0.50	NA	4.00±0.31	14.00±0.50	2.00±0.10	28.00±0.50
13	<i>Escherchia coli</i>	NA	NA	4.00±0.31	12.00±0.50	NA	24.00±0.50
14	<i>Salmonella</i>	2.00±0.50	4.00±0.31	6.00±0.31	20.00±0.50	2.00±0.10	20.00±0.50

DD, diameter of Zone of inhibition (mm) including disc diameter of 6 mm. NA, not active.

^aTested at a concentration of 1mg/disc

^bTested at a concentration of 10µg /disc

Table No.5: Antibacterial activity of the five essential oils expressed as minimum inhibitory concentration against four bacteria food-born and nine clinically bacteria after 2 16 h

Microorganisms	MIC ^a					
	Coriander	Carvi	Clove	Cinnamon	Nutmeg	Amp
<i>Enterococcusfaecium</i>	62.50±6.10	62.50±6.10	125.00±10.50	125.00±10.50	125.00±10.50	10.00±0.50
<i>Enterococcusfaecalis</i>	125.00±10.50	125.00±10.50	250.00±10.50	-	250.00±10.50	10.00±0.50
<i>Bacillus subtilis</i>	125.00±10.50	125.00±10.50	-	125.00±10.50	250.00±10.50	6.00±0.50
<i>Bacillus cereus</i>	-	-	500.00±12.50	500.00±12.50	-	12.00±0.50
<i>Brevibacteriumflavum</i>	250.00±10.50	250.00±10.50	250.00±10.50	250.00±10.50	250.00±10.50	14.00±0.50
<i>Micrococcusluteus</i>	-	-	500.00±12.50	500.00±20.50	500.00±12.50	12.00±0.50
<i>Pseudo aeruginosa</i>	500.00±12.50	-	250.00±10.50	500±12.50	500.00±12.50	18.50±0.5
<i>Klebsiella pneumoniae</i>	250.00±10.50	250.00±10.50	500.00±12.50	500±12.50	-	12.00±0.50
<i>Enterobacter cloacae</i>	500.00±12.50	500.00±12.50	-	500±12.50	500.00±12.50	20.00±0.50
Food -borne bacteria						
<i>Staphylococcus xylosus</i>	250.00±10.50	250.00±10.50	250.00±10.50	250.00±10.50	250.00±10.50	6.00±0.50
<i>Staphylococcusepidermidis</i>	500.00±12.50	-	500.00±12.50	500.00±12.50	500.00±10.50	12.00±0.50
<i>Escherchia coli</i>	-	-	-	500.00±12.50	-	14.50±0.50
<i>Salmonella</i>	250.00±10.50	500±12.50	-	500.00±12.50	500.00±12.50	14.50±0.50

MIC, minimal inhibition concentration, NA, not active.

^aValues given as µg/ml

Table No.6: Antibactericidal activity of five essential oils expressed as minimal bactericidal concentration/minimal inhibition concentration (MBC/MIC) ratios

S.No	Microorganisms	MBC/MIC ratios					
	Clinically bacteria	Coriander	Carvi	Clove	Cinnamon	Nutmeg	Amp
1	<i>Enterococcusfaecium</i>	3 ^a	2	2	2	2	2
2	<i>Enterococcusfaecalis</i>	2	2	1	NA	1	1
3	<i>Bacillus subtilis</i>	2	2	NA	2	1	2
4	<i>Bacillus cereus</i>	NA	NA	1	2	NA	1
5	<i>Brevibacteriumflavum</i>	1 ^b	1	1	1	1	1
6	<i>Micrococcusluteus</i>	NA	NA	1	1	1	1
7	<i>Pseudo aeruginosa</i>	1	NA	2	1	1	1
8	<i>Klebsielle pneumoniae</i>	1	2	1	1	NA	1
9	<i>Enterobactercloacae</i>	1	1	NA	1	1	1
10	Food -borne bacteria	-					
11	<i>Staphylococcus xylosus</i>	1	1	1	1	1	1
12	<i>Staphylococcusepidermidis</i>	1	NA	1	1	1	1
13	<i>Escherchia coli</i>	NA	NA	NA	1	NA	1
14	<i>Salmonella</i>	2	1	NA	1	1	1

MIC, Minimal Inhibition Concentration, NA, not active.

^aMIC < MBC indicating a bacteriostatic activity of the EO.

^bMIC was equivalent to the MBC indicating a bactericidal activity of the EO.

CONCLUSION

The extraction, the chemical composition, the antioxidant and the antibacterial activities of the essential oils of five spices used in cooking as flavorings were evaluated. Most of essential oils exhibited antioxidant and antimicrobial activities against microorganism tested and could be used as healthy chemical substitute preservatives and antimicrobial agents. This study suggests that Spicy foods are not only appealing but also healthy.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Srinivasan K. Role of spices beyond food flavoring: Nutraceuticals with multiple health effects, *Food. Rev. Int*, 21(2), 2005, 167-188.
2. Lampe J W. Spicing up a vegetarian diet: Chemo preventive effects of phytochemicals, *Am. J. Clin. Nutr*, 78(3), 2003, 579S-583S.
3. Graca Miguel M Da, Doughmi O, Aazza S, Antunes D, Lyoussi B. Antioxidant, anti-inflammatory and acetylcholinesterase inhibitory activities of propolis from different regions of Morocco, *Food Sci. Biotechnol*, 23(1), 2014, 313-322.
4. Wang H F, Yih K H, Huang K F. Comparative Study of the Antioxidant Activity of Forty-five Commonly Used Essential Oils and their Potential Active Components, *J Food Drug Anal*, 18(1), 2010, 24-33.
5. Aruoma O I, Spencer J P, Rossi R, Aeschbach R, Khan A, Mahmood N, Munoz A, Murcia A, Butler J, Halliwell B. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal

- herbs, *Food Chem. Toxicol*, 34(5), 1996, 449-456.
6. Friedman M, Henika P R, Mandrell R E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *listeria monocytogenes* and *Salmonella enteric*, *J. Food Protect*, 65(10), 2002, 1545-1560.
7. Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients, *International Journal of Food Microbiology*, 124(1), 2008, 91-97.
8. Sokovic M, Tzakou O, Pitarokili D, Couladis M. Antifungal activities of selected aromatic plants growing wild in Greece, *Nahrung*, 46(5), 2002, 317-320.
9. Abdel-Maksoud G, El Amin A R. A review on the materials used during the mummification process in ancient Egypt, *Mediterr Archaeol Archaeol*, 11(2), 2011, 129-150.
10. Moch R W. Pathology of BHA and BHT induced lesions, *Food Chem. Toxicol*, 24(10-11), 1986, 1167-1169.
11. Raut J S, Karuppayil S M. A status review on the medicinal properties of essential oils, *Ind crop Prod*, 62, 2014, 250-264.
12. Clevenger J F. Apparatus for determination of volatile oil, *J Am Pharm Assoc*, 17(4), 1928, 341-346.
13. Olivera P, Mila J and Mladen M. Chemical Composition and Antioxidant Activity of Essential Oils of Twelve Spice Plants, *Croatia Chemica Acta*, 79(4), 2006, 545-552.
14. Po-Chen L, Jason J L, I-Jy C. Essential oils from Taiwan: Chemical composition and antibacterial activity against *Escherichia coli*, *Journal Food Drug Anal*, 24(3), 2016, 464-470.
15. Marinova G and Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH, *Bulg. J. Agric. sci*, 17(1), 2011, 11-24.
16. Fattouch S, Caboni P, Coroneo V, Tuberoso C I, Angioni A, Dessi S, Marzouki N, Cabras P. Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts, *J Agric Food Chem*, 55(3), 2007, 963-969.
17. Fattouch S, Caboni P, Coroneo V, Tuberoso C, Angioni A, Dessi S et al. Comparative analysis of polyphenolic profiles and antioxidant and antimicrobial activities of Tunisian pome fruit pulp and peel aqueous acetone extracts, *Journal of Agricultural and Food Chemistry*, 56(3), 2008, 1084-1090.
18. Yin H W, Yield and composition variation of essential oil from leaves of different *cinnamomum osmophloeum* kanehira clones in Taiwan, *Q J Chin For*, 24, 1991, 83-104.
19. Wang C L, Yin H W. The location and seasonal variations of leaf essential oil from cultivated *Cinnamomum osmophloeum* Kaneh, *Taiwan J for Cci*, 6, 1991, 313-328.
20. Razafimamonjison G, Jahiel M, Duclos T, Ramanoelina P, Fawbush F, Danthu P. Bud, leaf and stem essential oil composition of clove (*Syzygium aromaticum* L.) from Indonesia, Madagascar and Zanzibar, *Int. J. Basic App. Sci*, 3(3), 2014, 224-233.
21. Misharina T A. Influence of the Duration and Conditions of Storage on the Composition of the Essential Oil from Coriander Seeds, *Appl. Biochem. Microbiol*, 37(6), 2001, 622-628.
22. Razzaghi-Abyaneh M, Ghahfarokhi M S, Rezaee M B, Jaimand K, Alinezhad S, Saberi R, Yoshinari T. Chemical composition and anti-aflatoxinigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils, *Food Contr*, 20(11), 2009, 1018-1024.
23. Paranagama P A, Wimalasenava S, Jayatilake G S, Jayawardena A L, Senanayake U M and Mubarak A M. A Comparison of essential oil constituents of bark, leaf root and fruit of cinnamon (*Cinnamomum zeylanicum* Blum) grown in Sri Lanka, *J. Natn. sci. Foundation Sri Lanka*, 29(3,4), 2001, 147-153.

24. Boniface Y, Philippe S, Rose de Lima H, Jean Pierre N, Alain A G, Fatiou T and Dominique S. Chemical composition and Antimicrobial activities of *Cinnamomumzeylanicum* Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms, *Int. res. J. Biological. Sci*, 1(6), 2012, 18-25.
25. Ibrahim M I, Abd El-Ghany M E and Ammar M S. Effect of Clove Essential Oil as Antioxidant and Antimicrobial Agent on Cake Shelf Life, *World J. Dairy Food Sci*, 8(2), 2013, 140-146.
26. Darughe F, Barzegar M and Sahari M A. Antioxidant and antifungal activity of Coriander (*Coriandrum sativum* L.) essential oil in cake, *Int Food Res. J*, 19(3), 2012, 1253-1260.
27. Didry N, Dubreuil L, Pinkas M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria, *Pharm. Acta Helv*, 69(1), 1994, 25-28.
28. Shan B, Cai Y Z, Brooks J D, Corke H. Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): Activity against food borne pathogenic bacteria, *J. Agric. Food Chem*, 55(14), 2007, 5484-5490.
29. Nunez L, Aquino M D. Microbicide activity of clove essential oil (*Eugenia caryophyllata*), *Braz. J. Microbiol*, 43(4), 2012, 1255-1260.
30. CLSI. Performance standards for antimicrobial susceptibility testing, Twenty-fourth Informational Supplement. CLSI document M100-24, Wayne, PA: *Clinical and Laboratory Standards Institute*, 35(3), 2014, 1-236.
31. CLSI. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guidelin, *Document M26-A. USA*, 1st Edition, 19(18), 1999, 1-32.
32. Aliakbarlu J, Sadaghiani S K, Mohammadi S. Comparative evaluation of antioxidant and anti-Food-borne bacterial activities of essential oils from some spices commonly consumed in Iran, *Food sci, Biotechnol*, 22(6), 2013, 1487-1493.

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