

Extraction, Antioxidant Activity and Characterization of Essential Oils of Different Spices

A. Hassan¹, A. Ali Redha^{2*}, Z. Adel³

^{1,2,3}Dept. of Chemistry, College of Science, University of Bahrain, Sakhir, Kingdom of Bahrain

*Corresponding Author: ali96chem@gmail.com

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Abstract — Essential oils of five different common spices in cinnamon, cumin, cardamom, wisteria and coriander were extracted by hydrodistillation and steam distillation. The yield percentages of oils extracted by each method were calculated and compared. The yield percentages of cinnamon, cumin, cardamom, wisteria, and coriander oils obtained by hydrodistillation are 1.9, 2.0, 2.8, 1.7 and 1.0%, respectively. The yield percentages of cinnamon, cumin, cardamom, wisteria, and coriander oils obtained by steam distillation are 0.7, 0.8, 1.2, 0.5 and 0.5%, respectively. Hydrodistillation provided higher yield compared to steam distillation. The antioxidant activity of each spice was determined in terms of antioxidant activities due to volatile oil and non-volatile components of spice by DPPH radical scavenging activity method. The highest percentage antioxidant activity due to volatile oil was of cinnamon with 69.27%. The highest percentage of antioxidant activity due to non-volatile component of spice was of coriander with 90.79%. The composition of fresh spice extracts and essential oil of each spice was determined by gas chromatography-mass spectrometry (GC-MS). The most common components found in spice samples were D-Limonene, α -Terpinene, γ -Terpinene, β -Thujene, Ocimene, Camphene, α -Phellandrene, 3-Carene, α -Fenchene, Cuminaldehyde, α -Terpineol and 4-Terpinyl acetate.

Keywords — essential oil, distillation, spice, antioxidant activity, gas chromatography-mass spectrometry

I. INTRODUCTION

Essential oils are secondary metabolites, that have been used widely for centuries for different purposes in different cultures. Historically, essential oils were used for their odour and strong taste. Later on, during the 18th and 19th centuries, scientists started exploring the composition of essential oils analytically and their biological effects of some compounds such as caffeine, morphine, quinine and atropine were noticed [1]. Essential oils having a wide range of pharmacological actions showed positive effects on anxiety, agitation, stress, fatigue, insomnia and pain management [2]. In addition, different essential oils have exhibited antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anti-lice and anti-dandruff potential [2]. The bioactivity of essential oils is highly dependent on the content and composition of the oil, which is influenced by several factors including the variety of plant, part of plant, the area in which the plant was grown, harvesting time of the plant, climate changes of the growth area, chemotype of the plant and storage conditions [1].

Essential oils are primarily classified into two main groups: oxygenated compounds and hydrocarbon compounds. The class of oxygenated compounds involves alcohols, phenols, aldehydes, ketones, esters, oxides and aromatics [3]. On the other hand, hydrocarbons mainly

include terpenes of different isoprene units (C₅), including monoterpenes (C₁₀) and sesquiterpenes (C₁₅) [3].

Several techniques have been used to extract essential oils from their natural sources. These techniques include solvent extraction, water or steam distillation, microwave assistance extraction, ultrasound assisted extraction, supercritical fluid extraction, and subcritical water extraction [4], [5].

In the present study, the essential oil of cinnamon, cumin, cardamom, wisteria and coriander, which are widely used in the Middle East, were extracted by hydrodistillation and steam distillation to compare the effect of extraction method yield percentages and determine the antioxidant activity of each oil. The components of each oil were then qualitatively determined by gas chromatography-mass spectrometry analysis (GC-MS).

The rest of the paper is organized as follows: Section II includes detailed description of materials and methods involved in this research (preparation of samples, extraction of essential oils, antioxidant analysis, and GC-MS analysis of spice extracts and essential oils), Section III includes the results and discussion of extraction methods of essential oils, their antioxidant potential and identification of their components by GC-MS, finally

Section IV includes the conclusions of this research and future scope.

II. METHODOLOGY

Materials

Cinnamon (*Cinnamomum verum*), cumin (*Cuminum cyminum*) seeds, cardamom (*Elettaria cardamomum*) seeds, wisteria (*Wisteria sinensis*) seeds and coriander (*Coriandrum sativum*) seeds were purchased from the local market. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ethanol, methanol, sodium chloride and anhydrous sodium sulphate were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Hyphenated gas chromatograph (Perkin Elmer, Clarus 600, USA) and mass spectrometer (Perkin Elmer, 600C, USA) were used in the analysis of samples.

Methods

Preparation of Samples

Five commonly used as spices which are cinnamon, cumin (seeds), cardamom (seeds), wisteria (seeds) and coriander (seeds) were bought from the local market, washed with distilled water and dried at room temperature. The dried samples were then crushed with a commercial grinder to have a coarse powder. The samples were then stored in dark glass bottles at room temperature until use.

Extraction of Essential Oils

About 100 g of each type of spice sample was mixed with 500 ml distilled water and subjected to hydrodistillation (water immersion) or steam distillation for 8 h. The distillate was collected and cooled down to room temperature. The distillate consisted of an organic phase (containing the essential oil) and an aqueous phase (known as aromatic water). To the distillate, 15 mL of saturated sodium chloride solution was added to each distillate. Then, the distillates were subjected to liquid-liquid extraction with hexane-ethyl acetate (4:1) three times (with three portions of 35 mL of extracting solvent). The organic phase was collected and dried using anhydrous sodium sulphate, and then it was concentrated under reduced pressure using rotary evaporator. The essential oil was collected in a flask with known weight. The yield percentage of oil extracted and isolated was calculated (using the following equation) for each method. The essential oils were kept in dark glass vials and stored at 4 °C.

$$\text{Yield percentage} = (\text{mass of extracted oil} \div \text{mass of plant material}) \times 100\%$$

Antioxidant Analysis

An amount of 2 g of each powdered spice (to prepare total spice extract [TSE]) and 2 g of the remaining residue of each spice after steam distillation (to prepare distilled residue extract [DRE]) were extracted separately in 50 mL of ethanol by continuous stirring for 8 hours at room temperature. The extracts were filtered, and the filtrate (liquid extract) was collected. A volume of 50 µL of each extract was added to 2.95 mL DPPH solution (freshly

prepared, 4.5 mg DPPH dissolved in 100 mL of methanol). The solutions were vortexed and incubated at room temperature for 30 minutes. After completion of 30 minutes, the absorbance of each solution was measured at 517 using ORION AQUAMATE 8000 UV/VIS spectrophotometer [6]. Freshly prepared DPPH solution was used as blank. Triplicate sample tests were done. The percentage radical scavenging activity towards DPPH radical, percentage antioxidant activity due to volatile oil and the percentage antioxidant activity due to non-volatile component of spices were calculated using the equations below.

$$\text{Percentage Radical Scavenging Activity (PRSA)} = \left(\frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \right) \times 100\%$$

$$\text{Percentage Antioxidant Activity due to Volatile Oil} = \left(\frac{\text{PRSA of TSE} - \text{PRSA of DRE}}{\text{PRSA of TSE}} \right) \times 100\%$$

$$\begin{aligned} \text{Percentage Antioxidant Activity due to Non-volatile component of spice} \\ = 100\% - \text{Percentage Antioxidant Activity due to Volatile Oil} \end{aligned}$$

Identification of Spice Extract & Essential Oil Components by GC-MS

A volume of 1.0 µL of total spice extract (prepared by extracting 2 g of spice with 25 mL ethanol), distilled residue extract (prepared by extracting 2 g of spice residue of steam distillation with 25 mL ethanol) and essential oil (obtained in 2.2.2 by steam distillation) of each spice were injected into the gas chromatography-mass spectrometry (GC-MS) instrument. The mode of the mass spectrometer was electron impact with a mass range of m/z 50-600. The capillary column used was 30 m long with a volume of 250 µL and film thickness of 25 µm. Gas chromatography was performed in splitless mode with 1 min splitless time. The temperature of the GC oven was programmed to start at 70°C with a 10 min holding time, then, from 70 to 135°C with a rate of increase of 2°C min⁻¹ and a holding time for 10 min, then from 135 to 220°C with a increasing rate of 4°C min⁻¹ and holding time of 10 min, finally, from 220 to 270°C with 3.5°C min⁻¹ increasing rate and holding of 20 min [6]. The flow rate of mobile phase (helium gas), was maintained at 1.0 mL min⁻¹ [7]. Identification of the components of each sample was achieved with the aid of the instrument's database.

III. RESULTS AND DISCUSSION

Comparison of Essential Oil Extracted by Distillation Methods

The yield percentages of all the oils extracted by hydrodistillation were higher than that extracted using steam distillation. The highest yield percentages of oil extracted was of cardamom (2.8%) by hydrodistillation and the lowest yield was of wisteria and coriander (0.5%) by steam distillation.

Table 1. Comparison between yield percentage (%) of oil extracted by hydrodistillation and steam distillation

Spice	Yield Percentage of Oil Extracted by Hydrodistillation (%)	Percentage Yield of Oil Extracted by Steam Distillation (%)
Cinnamon	1.9	0.7
Cumin	2.0	0.8
Cardamom	2.8	1.2
Wisteria	1.7	0.5
Coriander	1.0	0.5

Antioxidant Activity of Spices

The antioxidant activities of the spices were determined by the radical scavenging method of DPPH. Due to the insolubility of the extracted essential oils in methanol (solvent of DPPH solution), the antioxidant activities of the essential oils were determined indirectly. The antioxidant activity of each TSE determined resembles the total antioxidant activity of the spice, and the antioxidant activity of each DRE determined resembles the total antioxidant activity of spice content excluding the essential oil content which was extracted by steam distillation.

Table 2. Comparison between percentage radical scavenging activity and antioxidant activity due to volatile oil and non-volatile oil of different spices

Spice	Extract	Radical Scavenging Activity towards DPPH Radical (%)	Percentage Antioxidant Activity due to Volatile Oil of Spice (%)	Percentage Antioxidant Activity due to Non-volatile Component of Spice (%)
Cinnamon	TSE	31.04 ± 1.39	69.27	30.72
	DRE	9.54 ± 0.51		
Cumin	TSE	10.33 ± 1.72	19.34	80.66
	DRE	8.33 ± 0.78		
Cardamom	TSE	7.17 ± 1.79	37.41	62.58
	DRE	4.49 ± 0.63		
Wisteria	TSE	6.81 ± 1.05	15.91	84.08
	DRE	5.72 ± 0.48		
Coriander	TSE	7.75 ± 1.27	9.30	90.69
	DRE	7.03 ± 1.79		

As expected, the antioxidant activity of TSE was higher than that of DRE (Table 2). The TSE contained the volatile and non-volatile compounds. On the other hand, the volatile compounds did not exist in the distillate extracts. Thus, the percentage of antioxidant activities due to volatile and non-volatile oil were calculated and shown in Table 2 and Figure 1. The highest antioxidant activity was observed in the TSE of cinnamon as 31.04 ± 1.39%, the antioxidant activity of the remaining extracts changed in the range 5-10% ca. The volatile oil component of cinnamon exhibited the highest antioxidant activity (69.27%) which was followed by the volatile oil of cardamom (37.41%), and the activities of the components

changed in the range 10-20% ca. On the other hand, the antioxidant activity due to non-volatile component of spices was comparably high in cumin (80.66%), wisteria (84.08%) and coriander (90.69%). The antioxidant activity of the non-volatile component of cinnamon and cardamom, which had a relatively high amount volatile oil, were found as 30.72% and 62.58%, respectively. The composition of the extracts varies from spice to spice, thus resulting in the differences in the antioxidant activity of each of them. It has been reported previously that cinnamon had an effective potential in decreasing the lipid oxidation of palm oil and was suggested to replace the synthetic antioxidants used in the food products [8].

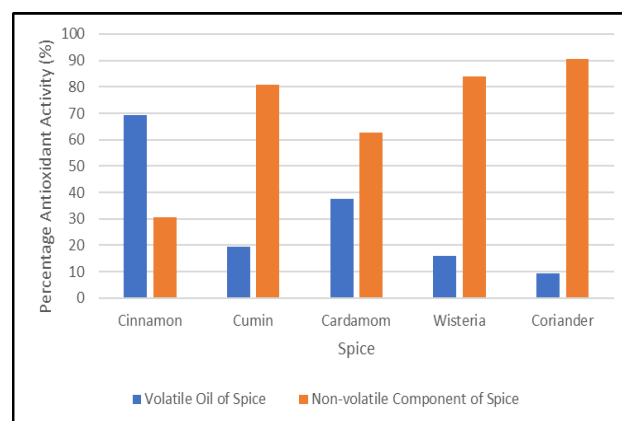


Figure 1. Percentage antioxidant activity due to volatile & non-volatile component of different spices

Identification of Spice Extract & Essential Oil Components

GC-MS was used to analyse the constituents of the spice extract and essential oil. Natural essential oils contain a mixture of terpenoids (primarily monoterpenoids and sesquiterpenoids), aromatic compounds and aliphatic compounds (Table 3). The mass spectra of these compounds are usually very similar, and therefore the identification of these compounds could be difficult. To determine the composition of the complex samples by GC-MS qualitatively and to increase the reliability of the analytical results, it is necessary to utilize retention indices identities. The total spice extract, distilled spice residue extract and the spice essential oil were analysed by GC-MS. The data were collected in full scan (m/z 50-600) and the compounds were identified based on their retention time and mass spectra referring to the database of the instrument as presented in Table 3. Some of the monoterpenes and sesquiterpenes were present in the spice oil only, while absent in whole extract which can be explained by their non-detectability because of their presence in low concentrations in the whole extract.

No compounds were detected in the GC-MS analysis of distilled spice residue extracts, suggesting that the distillation process was efficient in extracting the volatile components of the spices and isolating them into their essential oil.

Cinnamon total spice extract and cinnamon oil constituents were Cinnamaldehyde, 1H-Indenol, α -Phellandrene and Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-dione.

In addition, cinnamon oil also contained α -Copaene, α -Cubebene, β -Cubebene, α -Ylangene, β -Ylangene, trans-Cadina-1,4-diene, Isoledene and α -Cedrene, which were absent in the total spice extract.

The constituents of cumin total spice extract were γ -Terpinene, β -Thujene, α -Phellandrene, 3-Carene and Cuminaldehyde. The essential oil of cumin contained p-Cymene, α -Terpinene, γ -Terpinene, β -Thujene, 3-Carene, α -Fenchene, Cuminaldehyde, Safranal and Cuminy alcohol.

The total spice extract of cardamom contained D-Limonene, α -Terpinene, γ -Terpinene, α -Pinene, Ocimene, Camphene, α -Terpineol and Germacrene. Cardamom oil also contained D-Limonene, α -Terpinene, γ -Terpinene, α -Pinene, Ocimene, Camphene, α -Phellandrene, β -Phellandrene, Terpinolene, cis-Sabinene, α -Fenchene, 4-Carvomenthenol, α -Terpineol, Sabinene hydrate, 4-

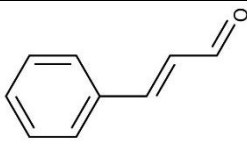
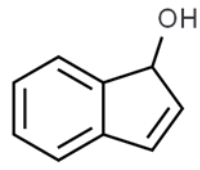
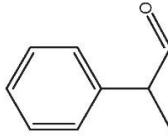
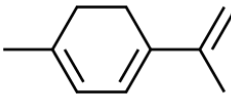
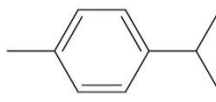
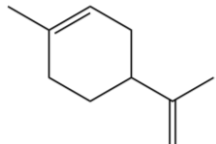
Terpinyl acetate, β -Copaene, Arteannuic acid and Linalyl phenyl acetate.

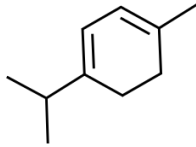
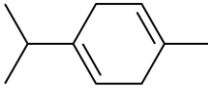
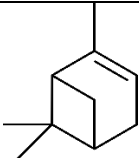
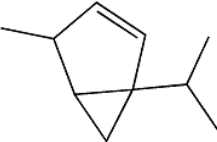
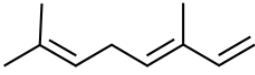
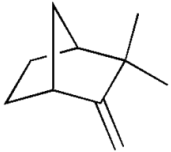
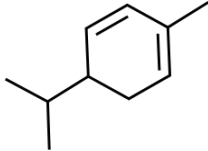
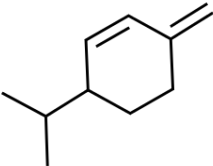
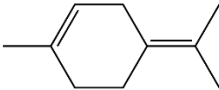
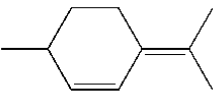
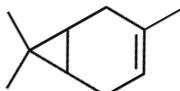
Wisteria total spice extract and wisteria oil constituents were D-Limonene, α -Terpinene, Ocimene, Camphene, Isoterpinolene, α -Fenchene, cis-Carveol, α -Terpineol, Isopulegol, Terpin (hydrate) and 4-Terpinyl acetate. On the other hand, wisteria oil also contained α -Copaene, Isoledene and α -Cedrene, which were absent in the total spice extract.

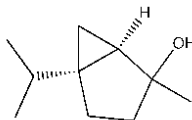
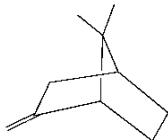
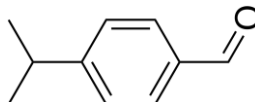
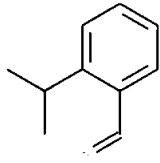
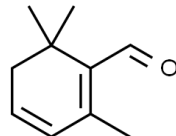
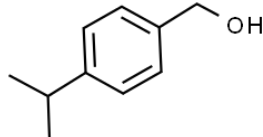
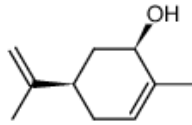
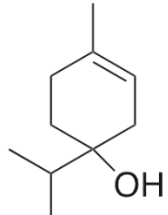
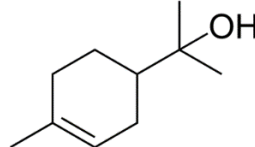
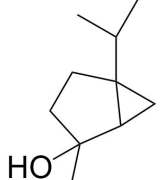
The total spice extract and essential oil of coriander contained 2-Phenylpropionaldehyde, γ -Terpinene, β -Thujene, α -Phellandrene, Terpinolene, 3-Carene, Cuminaldehyde, p-Isopropenylanisole and 2-Isopropylbenzaldehyde. The total spice extract also contained p-Menthatriene and α -Terpinene.

The antioxidant potential of D-Limonene, α -Pinene and α -Terpineol was recently reported [9]. In fact, these compounds were also found in interest for their antibacterial potential against *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus* [8].

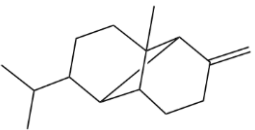
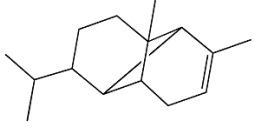
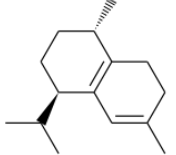
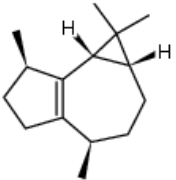
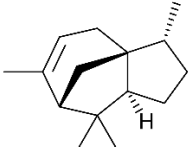
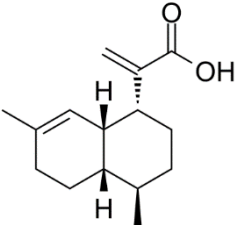
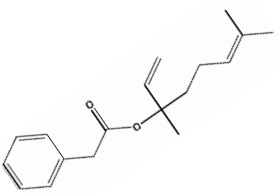
Table 3. Identified compounds of spice extracts and essential oil by GC-MS

Compound	Molecular Formula	Molecular Weight (g/mol)	Structure	Presence	Retention Time (min)
Cinnamaldehyde	C ₉ H ₈ O	132.162		Cinnamon Extract Cinnamon Oil	15.804 15.737
1H-Indenol	C ₉ H ₈ O	132.162		Cinnamon Extract Cinnamon Oil	15.804 15.367
2-Phenylpropionaldehyde	C ₉ H ₁₀ O	134.175		Coridaner Extract Coridaner Oil	11.023 11.251
p-Menthatriene	C ₁₀ H ₁₄	134.222		Coridaner Extract	4.234
p-Cymene	C ₁₀ H ₁₄	134.222		Cumin Oil	4.567
D-Limonene	C ₁₀ H ₁₆	136.238		Cardamon Extract Wisteria Extract Cardamon Oil Wisteria Oil	4.561 4.584 4.452 4.294

α -Terpinene	$C_{10}H_{16}$	136.238		Cardamon Extract Wisteria Extract Coriander Extract Cardamon Oil Cumin Oil Wisteria Oil	5.591 5.584 4.994 5.355 5.696 5.260
γ -Terpinene	$C_{10}H_{16}$	136.234		Cardamon Extract Cumin Extract Coriander Extract Cardamon Oil Cumin Oil Coriander Oil	4.448 4.348 4.984 4.355 4.361 4.552
α -Pinene	$C_{10}H_{16}$	136.238		Cardamon Extract Cardamon Oil	6.561 6.709
β -Thujene	$C_{10}H_{16}$	136.238		Cumin Extract Coriander Extract Cumin Oil Coriander Oil	6.348 5.938 6.361 5.684
Ocimene	$C_{10}H_{16}$	136.238		Cardamon Extract Wisteria Extract Cardamon Oil Wisteria Oil	17.622 16.684 17.472 16.234
Camphene	$C_{10}H_{16}$	136.238		Cardamon Extract Wisteria Extract Cardamon Oil Wisteria Oil	16.622 16.584 16.352 16.286
α -Phellandrene	$C_{10}H_{16}$	136.238		Cumin Extract Cinnamon Extract Coriander Extract Cardamon Oil Cinnamon Oil Coriander Oil	6.348 5.996 5.935 6.361 6.127 6.897
β -Phellandrene	$C_{10}H_{16}$	136.238		Cardamon Oil	7.451
Terpinolene	$C_{10}H_{16}$	136.238		Coriander Extract Cardamon Oil Coriander Oil	4.938 5.989 5.221
Isoterpinolene	$C_{10}H_{16}$	136.238		Wisteria Extract Wisteria Oil	6.584 6.236
3-Carene	$C_{10}H_{16}$	136.238		Cumin Extract Coriander Extract Cumin Oil Coriander Oil	6.348 5.938 6.361 5.684

cis-Sabinene	C ₁₀ H ₁₆	136.238		Cardamon Oil	10.989
α-Fenchene	C ₁₀ H ₁₆	136.238		Wisteria Extract Cardamon Oil Cumin Oil Wisteria Oil	6.584 7.006 7.325 7.206
Cuminaldehyde	C ₁₀ H ₁₂ O	148.205		Cumin Extract Coriander Extract Cumin Oil Coriander Oil	14.110 13.623 13.970 13.895
2-Isopropylbenzaldehyde	C ₁₀ H ₁₂ O	148.205		Coriander Extract Coriander Oil	11.623 11.623
Safranal	C ₁₀ H ₁₄ O	150.221		Cumin Oil	16.521
Cuminy alcohol	C ₁₀ H ₁₄ O	150.221		Cumin Oil	13.651
cis-Carveol	C ₁₀ H ₁₆ O	152.237		Wisteria Extract Wisteria Oil	4.057 4.601
4-Carvomenthenol	C ₁₀ H ₁₈ O	154.253		Cardamon Oil	11.641
α-Terpineol	C ₁₀ H ₁₈ O	154.250		Cardamon Extract Wisteria Extract Cardamon Oil Wisteria Oil	5.701 5.584 6.709 6.286
Sabinene hydrate	C ₁₀ H ₁₈ O	154.250		Cardamon Oil	10.989

Isopulegol	$C_{10}H_{18}O$	154.253		Wisteria Extract Wisteria Oil	4.407 4.601
Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-dione	$C_{10}H_8O_2$	160.174		Cinnamon Extract Cinnamon Oil	15.804 15.804
Terpin (hydrate)	$C_{10}H_{22}O_2$	172.27		Wisteria Extract Wisteria Oil	6.467 6.639
4-Terpinyl acetate	$C_{12}H_{20}O_2$	196.62		Wisteria Extract Cardamon Oil Wisteria Oil	5.584 6.582 6.639
Germacrene	$C_{15}H_{24}$	204.350		Cardamon Extract	5.561
α -Copaene	$C_{15}H_{24}$	204.350		Cinnamon Oil Wisteria Oil	18.559 18.929
β -Copaene	$C_{15}H_{24}$	204.357		Cardamon Oil	17.559
α -Cubebene	$C_{15}H_{24}$	204.350		Cinnamon Oil	20.559
β -Cubebene	$C_{15}H_{24}$	204.350		Cinnamon Oil	20.599

α -Ylangene	$C_{15}H_{24}$	204.350		Cinnamon Oil	20.599
β -Ylangene	$C_{15}H_{24}$	204.350		Cinnamon Oil	20.599
trans-Cadina-1,4-diene	$C_{15}H_{24}$	204.350		Cinnamon Oil	20.599
Isoledene	$C_{15}H_{24}$	204.350		Cinnamon Oil Wisteria Oil	20.599 19.929
α -Cedrene	$C_{15}H_{24}$	204.350		Cinnamon Oil Wisteria Oil	20.559 19.929
Arteannuic acid	$C_{15}H_{22}O_2$	234.334		Cardamon Oil	17.909
Linalyl phenyl acetate	$C_{18}H_{24}O_2$	272.388		Cardamon Oil	17.661

IV. CONCLUSION AND FUTURE SCOPE

Essential oils of five different common spices in cinnamon, cumin, cardamom, wisteria and coriander were extracted by hydrodistillation and steam distillation. The yield percentages of oils extracted by hydrodistillation were higher than that of steam distillation. The antioxidant activity of each spice was determined in terms of antioxidant activities due to volatile oil and non-volatile components of spice by DPPH radical scavenging activity method. The composition of fresh spice extracts and essential oil of each spice was determined by GC-MS. The most common components found in the samples were D-Limonene, α -Terpinene, γ -Terpinene, β -Thujene, Ocimene, Camphene, α -Phellandrene, 3-Carene, α -Fenchene,

Cuminaldehyde, α -Terpineol and 4-Terpinyl acetate. In terms of future research scope, quantitative GC-MS analysis could be conducted to determine the amount of each component. In fact, other antioxidant assays could also be evaluated.

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AUTHORS PROFILE

Dr. A. Hassan received her B.Sc. in Biochemistry from Concordia University (Canada) in 1985, M.Sc. in Organic Chemistry from Oregon State University (U.S.A.) in 1994, and Ph.D. in Organic Chemistry from University of Bristol (U.K.) in 1997. Her areas of research expertise include synthesis of organic compounds, isolation, purification and structure determination of natural products from microorganisms and plants, using stable and radiolabels in biosynthetic studies. She is an assistant professor of organic chemistry at University of Bahrain since 1997.



Mr. A. Ali Redha received his Bachelor of Science in Chemistry from University of Bahrain, Kingdom of Bahrain in 2018. As an undergraduate, he focused on phytochemical investigations and medicinal studies of a wide range of



plants. Ali then pursued his postgraduate Masters of Science degree in Analytical Chemistry and graduated with a distinction from Loughborough University, United Kingdom in 2019. Since September 2019, Ali is a chemistry instructor at Department of Chemistry, University of Bahrain, Kingdom of Bahrain.

Mrs. Z. Adel received her Bachelor of Science in Chemistry from University of Bahrain, Kingdom of Bahrain in 2019. As an undergraduate, she focused on extraction of essential oils, and analysis of their antioxidant activity.