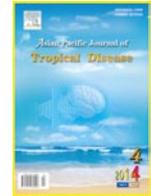




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## Comparison of chemical constituents and antimicrobial activities of three essential oils from three different brands' clove samples collected from Gulf region

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## PEER REVIEW

## ABSTRACT

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**Comments**

This is an interesting study in which the authors analyzed three essential isolated oils from three different brands' clove samples collected from Gulf region and tested their antimicrobial activities. The data are helpful in the further research.

Details on Page 267

**Objective:** To analyze three essential oils isolated from three different brands' clove samples collected from Gulf region and tested their antimicrobial activities.

**Methods:** The essential oils were isolated from powder clove samples by water distillation method and analyzed and identified by gas chromatography–mass spectrometry. Antimicrobial activities of the three isolated essential oils were calculated on the diameter inhibition zone by disc diffusion method against three bacterial strains with amoxicillin standard.

**Results:** Twenty-eight, twenty-two and twenty-six chemical ingredients with high percentage were characterized based on gas chromatography retention time from clove essential oils collected from Saudi Arabia, United Arab Emirate and Jordan. The highest antimicrobial activity was obtained from Saudi Arab against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the lowest was from Jordan and the order of antimicrobial activity was Saudi Arabia>United Arab Emirate>Jordan.

**Conclusions:** All three essential oils from Gulf region are containing very portent chemical ingredients and it could be used as medicine or antibiotics for different aliments.

## KEYWORDS

*Syzygium aromaticum*, Myrtaceae, Hydrodistillation, Essential oil, Gulf region, Antimicrobial activity, Gas chromatography–mass spectrometry analysis

### 1. Introduction

Cloves are aromatic plants belong to the family Myrtaceae. Its scientific name is *Syzygium aromaticum* and Arabic name is Kronfol. Firstly, the cloves are originated from Indonesia. Now it is commercially cultivated in tropical and sub-tropical countries like Indonesia, India, Madagascar, Zanzibar, Pakistan,

Bangladesh and Sri Lanka<sup>[1]</sup>. The maximum height is about 8–12 m having large leaves and cheerful flowers in numerous groups of terminal clusters. Secondly, the buds are pale green, then gradually they become deep green. So, during the harvesting time, the colour of buds should be bright red and the size is about 1.5 to 2 cm long. The morphological arrangement of buds consists of long calyx, terminating in four spreading sepals, and four unopened

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petals which form a small ball in the center.

It is an very important herb due to its medicinal values<sup>[1–3]</sup>. Different parts of this tree were used by the people in different way from the ancient time for different ailments. People use its oils, dried flower buds, leaves, and stems to prepare herbal medicine. The most important traditional use of clove is to cure upset stomach and expectorant<sup>[2,3]</sup>. Expectorants medicine makes it easier to cough up phlegm<sup>[2,4,5]</sup>. The essential oil of clove is used for diarrhea, hernia, intestinal gas, nausea, vomiting and bad breath<sup>[2,3,6,7]</sup>. Nowadays, commercially, it is used directly to the gums for toothache, for pain control during dental work, and for a complication of tooth extraction<sup>[2,3]</sup>. It is also used to the skin as a counterirritant for pain and for mouth and throat inflammation. In combination with other ingredients, clove is also applied to the skin as part of a multi-ingredient product used to keep men from reaching orgasm too early<sup>[8,9]</sup>. It is commonly and widely used as a flavoring agent in foods and beverages industries<sup>[10–12]</sup>. The powder of cloves is often used in the whole Asian countries; African and Middle Eastern for cooking meats, curries and meat marinades. It is also used to create sweet dishes, such as apples, pears, or rhubarb<sup>[13]</sup>.

Traditionally, it is widely used in Indian Ayurvedic medicine, Chinese medicine, and Western herbalism and dentistry medicine system for treatment of pain of dental emergencies<sup>[14]</sup>. They are used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis<sup>[6–8,15]</sup>. Cloves are also considered to be a natural anthelmintic<sup>[8]</sup>. The essential oil of clove is used in aromatherapy when stimulation and warming are needed, especially for digestive problems<sup>[2]</sup>. It is applied to a cavity in a decayed tooth and relieve toothache <sup>[5,8,9,16]</sup>.

In Chinese medicine, cloves or ding xiang are considered acrid, warm and aromatic, entering the kidney, spleen and stomach meridians, and are notable in their ability to warm the middle, direct stomach qi downward, to treat hiccough and to fortify the kidney yang. Because the herb is so warming that it is contraindicated in any persons with fire symptoms and according to classical sources, it should not be used for anything except cold from yang deficiency<sup>[3,5,7,17,18]</sup>. As such it is used in formulas for impotence or clearing vaginal discharge from yang deficiency, for morning sickness together with ginseng and patchouli, or for vomiting and diarrhea due to spleen and stomach coldness<sup>[2,3]</sup>. Clove oil has many uses in daily life especially for ailments that typically strike. Not only it is beneficial, but clove oil is also readily available at local grocery stores that specialize in all-natural products in the vitamin section<sup>[10,11,19,20]</sup>. Therefore, the first objective of the present study is to investigate and compare the chemical ingredients in three brands' clove essential oils by gas chromatography–mass spectrometry (GC–MS). The second objective of the present study is to evaluate the antimicrobial activity of three essential oils against two Gram-positive bacteria *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and one Gram-negative bacteria *Staphylococcus aureus* (*S. aureus*).

## 2. Materials and methods

### 2.1. Chemicals

All the chemicals and solvents used in this present study were analytical grade from BDH, UK. Clevenger apparatus was used for the isolation of essential oils from Borosil, India. Agar gel Petri dishes for antimicrobial activity were obtained from the Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa. Filter papers (Whatman, GE Healthcare companies, China, catalogue number: 1001090) were used as a disc.

### 2.2. Microorganisms

The bacterial strains such as Gram-negative *E. coli* (ATCC 9637) and *P. aeruginosa* (ATCC 9027) and Gram-positive bacterial strain *S. aureus* (ATCC 29213) were obtained from Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa, Sultanate of Oman.

### 2.3. Clove samples

The three sealed packet clove samples were collected from Saudi Arabia (SAS1), United Arab Emirate (UAES2) and Jordan (JS3). All the sealed packet clove samples were purchased from their local supermarkets. After collection, the cloves samples were again packed in a seal white polyethylene bags.

### 2.4. Sample preparation and extraction

Each clove sample (100 g) was weighed and ground separately by using blender machine for 2 min until its powder forms. The powder sample was packed in a sealed bottle until the extraction. Each powder sample (50 g) was taken in a round bottom flask (250 mL) and added water (200 mL) mixed together by shaking. After shaking, the Clevenger type apparatus was fitted and sealed. Then it was heated by heating mantle until the water boiled. The isolated crude essential oil was collected in a sealed bottle. The crude essential oil was extracted with dichloromethane solvent and dried over anhydrous sodium sulphate<sup>[13]</sup>. The same process was applied for other brand's clove samples. The water free essential oil was stored in sealed vials and kept in the freeze at 4 °C until further analysis.

### 2.5. Antimicrobial activity by agar disc method

The three essential oils from different clove samples were tested for their antimicrobial activity using three bacterial strains such as *E. coli*, *P. aeruginosa* and *S. aureus* grown on nutrient agar plates using disc diffusion technique<sup>[11]</sup>. The following four concentrations such as 2 mg/mL, 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL were prepared for each essential oil by using dimethyl sulphoxide (DMSO) solvent. The Petri

dishes were prepared by using agar gel layer. Filter paper discs' diameter (6 mm) were saturated with each prepared concentration and placed on the agar plate. The amoxicillin (0.5 mg/mL) was used as positive control and DMSO used as negative control. All the plates were incubated at 37 °C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition against the tested bacteria.

### 2.6. GC–MS analysis

Essential oils were analyzed by GC (PerkinElmer Clarus 600 GC) coupled with MS (PerkinElmer Clarus 600 MS) with fused silica capillary column (Rtx®–5MS). The specification of fused silica capillary column (30 m×0.25 mm i.d., film thickness 0.25 µm) was used to separate and identify the chemical ingredients in the oils. Electron ionization mode system with ionization energy of 70 eV was used for the ionization and separation of the compounds. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. The temperatures of mass transfer line and injector were set at 260 and 290 °C, respectively. The oven temperature was programmed from 40 °C (hold 2 min) to 270 °C at 4 °C/min, then held isothermal for 20 min and finally raised to 290 °C at 10 °C/min. Diluted samples (1/50, v/v, in dichloromethane) of 1 µL were injected in the split mode with a split ratio of 1:120. The relative percentage of the essential oil constituents was expressed as percentage by peak area normalization.

### 2.7. Identification of the compounds

The chemical ingredients of the essential oils were characterized on the basis of GC retention time on fused silica capillary column. The mass spectrum was matched with the GC–MS exiting library (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC–MS systems)[11,12,15].

### 2.8. Statistical analysis

The three essential oils from clove samples collected from different countries were evaluated for antimicrobial activity. Each experimental method was run in triplicate, and mean values were calculated. A *t*-test was computed for the statistical significance of the results.

## 3. Results

The essential oils were isolated from different varieties of clove samples by hydro distillation method and characterized their chemical ingredient by GC–MS method, which were collected from different countries of Gulf region. The recovery percentage yield of essential oil is about 0.7%–0.92%. The highest recovery of essential oil was from TS3 samples and the lowest recovery from SAS1.

### 3.1. Chemical composition of essential oil from SAS1

The characterization of chemical ingredients of the essential oil isolated from the clove samples by GC–MS which was collected from SAS1. Twenty–eight different chemical ingredients were identified, which represent about 92.65% of the total composition. The identified chemical ingredients in the essential oil are listed in Figure 1 and Table 1. The most important aroma ingredients with high percentage found in the essential oil were eugenol (57.17%), β–E–caryophyllene (29.94%), α–humulene (3.71%), acetyl eugenol (5.92%), α–cubebene (0.55%), α–amorphene (0.14%) and α–farnesene (0.51%).

**Table 1**

Chemical composition of essential oil from Saudi Arab (SAS1).

KI <sub>calc</sub>	Compound name	Retention time	MW	% SAS1	KI <sub>ref</sub>
840	Furfural	3.49	96	0.026	828
880	2–Heptanone	4.42	114	0.003	889
986.87	6–Methyl–5–hepten–2–one	6.82	126	0.004	981
1040.78	2–Heptanol, acetate	8.57	158	0.016	1035
1043.95	Phenylacetaldehyde	8.68	120	0.003	1098
1047.42	α–Pinene	8.80	136	0.004	932
1066.93	Acetophenone	9.47	120	0.008	1059
1092.65	2–Nonanone	10.36	142	0.033	1087
1096.87	Benzoic acid, methyl ester	10.51	136	0.012	1084
1164.52	Acetic acid phenyl methyl ester	13.18	150	0.079	1160
1171.43	Benzoic acid, ethyl ester	13.46	150	0.014	1160
1191.70	9–Terpineol	14.27	154	0.002	1172
1195.55	Methyl salicylate	14.42	152	0.154	1190
1255.07	Chavicol	16.88	134	0.083	1247
1293.47	2–Undecanone	18.47	170	0.015	1293
1349.51	α–Cubebene	20.77	204	0.020	1345
1362.58	Eugenol	21.30	164	57.172	1356
1377.34	α–Cubebene	21.91	204	0.550	1374
1392.58	β–Elemene	22.53	204	0.016	1389
1421.13	β–E–Caryophyllene	23.67	204	29.942	1424
1453.45	α–Humulene	24.95	204	3.711	1456
1476.01	α–Amorphene	25.85	204	0.140	1483
1493.93	Eremophilene	26.56	204	0.097	1486
1507.38	α–Farnesene	27.08	204	0.511	
1522.40	α–cadinene	27.64	204	0.324	1522
1522.69	δ–Cadinene	27.66	204	0.324	
1528.86	Acetyl eugenol	27.89	206	5.927	1552
1581.10	(–)–Caryophyllene oxide	29.87	220	0.183	1576

Retention index (KI<sub>calc</sub> and KI<sub>ref</sub>) relative to *n*-alkanes on fused silica capillary column (Rtx®–5MS).

### 3.2. Chemical composition of essential oil from UAES2

The identification and characterization of chemical bioactive ingredients in the essential oil which was collected from UAES2 and analysed by GC–MS. Total 22 different chemical ingredients of different molecular weight represent about 97.43% of the total composition. The identified chemical ingredients in this essential oil are listed in Figure 2 and Table 2. The most active aroma ingredients found in this essential oil were eugenol (63.64%), β–E–caryophyllene (23.04%), α–humulene (2.97%), acetyl eugenol (4.60%), α–amorphene (0.13%), α–farnesene (0.54%), 2–heptanone (0.75%), 2–heptanol (0.14%), 2–nonanone (0.33%), and chavicol (1.12%).

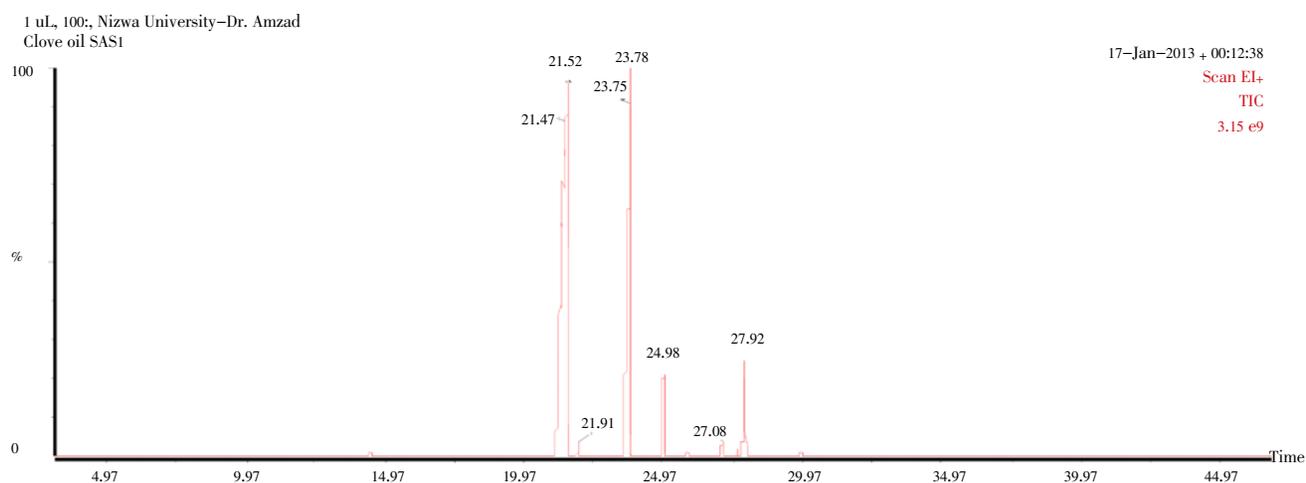


Figure 1. A typical chromatogram of essential oil isolated from Saudi Arabia clove samples.

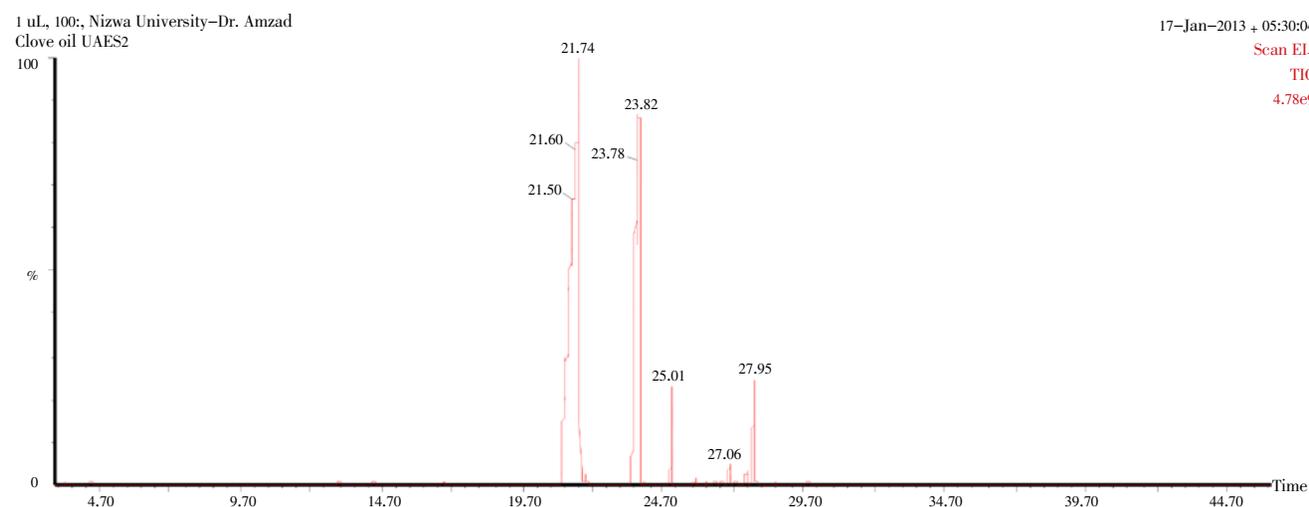


Figure 2. A typical chromatogram of essential oil isolated from United Arab Emirate clove samples.

Table 2

Chemical composition of essential oil from United Arab Emirate (UAES2).

KI <sub>calc</sub>	Compound name	Retention time	MW	% UAES2 essential oil	KI <sub>ref</sub>
880	2-Heptanone	4.43	128	0.758	871
900.54	2-Heptanol (s)	4.58		0.144	879
962.16	5-Methyl-2-furaldehyde	6.18	110	0.069	957
976.83	Eremophilene	6.56	204	0.101	1486
986.29	6-methyl-5-hepten-2-one	6.80	126	0.098	981
1000.57	Hexanoic acid, ethyl ester	7.18	144	0.082	976
1032.10	1,8-Cineole	8.27	154	0.062	1024
1039.91	2-Heptanol acetate	8.54	158	0.016	1035
1047.05	β-Trans-ocimene	8.78	136	0.131	1044
1092.36	2-Nonanone	10.35	142	0.338	1074
1096.87	Benzoic acid, methyl ester	10.51	136	0.166	1084
1164.52	Acetic acid, phenylmethyl ester	13.18	150	0.041	1160
1170.80	Benzoic acid, ethyl ester	13.43	150	0.010	1175
1191.20	9-Terpineol	14.25	154	0.001	1172
1195.17	Methyl salicylate	14.40		0.084	1190
1256.37	Chavicol	16.93		1.122	1247
1372.46	Eugenol	21.74	164	63.64	1356
1422.52	β-E-Caryophyllene	23.73	204	23.204	1417
1454.46	α-Humulene	24.99	204	2.974	1456
1450.50	α-Amorphene	25.85	204	0.138	1483
1506.74	α-Farnesene	27.08	204	0.545	1496
1520.48	β-cadinene	27.65	204	0.343	1514
1527.67	Acetyl eugenol	27.84	206	4.609	1521
1561.97	Nerolidol	29.14	222	0.046	1561
1580.44	(-)-Caryophyllene oxide	29.84		0.014	1576
1607.32	Humulene oxide	30.85	220	0.179	1601

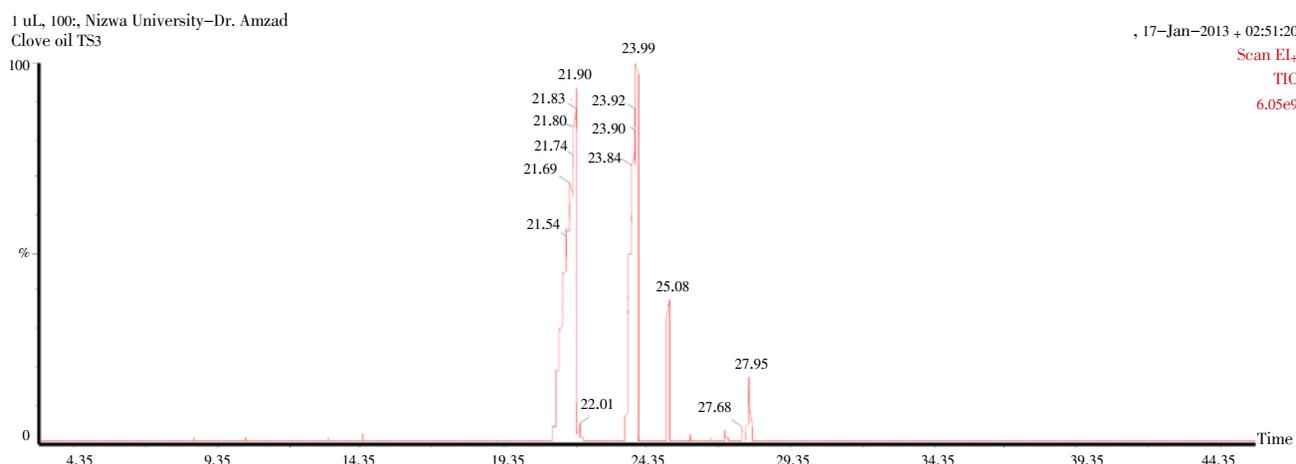
Retention index (KI<sub>calc</sub> and KI<sub>ref</sub>) relative to *n*-alkanes on fused silica capillary column (Rtx®-5MS).

Table 3

Chemical composition of essential oil from Jordan (TS3).

KI <sub>calc</sub>	Compound name	Retention time	MW	% TS3 essential oil	KI <sub>ref</sub>
960.23	Benzaldehyde	6.13	106	0.183	952
986.87	6-methyl-5-hepten-2-one	6.82	126	0.243	981
1000.14	Hexanoic acid, ethyl ester	7.16	144	0.180	997
1040.92	2-Heptanol acetate	8.57	158	0.075	1035
1047.42	β-ocimene	8.80	136	0.463	1044
1060.86	Benzenemethanol	9.26	122	0.099	1055
1066.35	Acetophenone	9.45	120	0.217	1059
1092.48	2-Nonanone	10.36	142	0.070	
1097.13	Benzoic acid, methyl ester	10.52	136	0.011	1084
1164.64	Acetic acid, phenylmethyl ester	13.19	150	0.088	1160
1171.05	Benzoic acid, ethyl ester	13.44	150	0.021	1160
1191.53	9-Terpineol	14.26	154	0.003	1186
1195.55	Methyl salicylate	14.42	152	0.149	1190
1255.41	Chavicol	16.89	134	0.913	1247
1293.71	2-Undecanone	18.48	170	0.233	1293
1376.00	Eugenol	21.85	164	54.974	1356
1429.04	β-E-caryophyllene	23.99	204	31.457	1417
1456.56	α-Humulene	25.08	204	4.250	
1462.07	Aromadrene	25.29	204	0.356	1439
1476.76	α-Amorphene	25.88	204	0.149	1483
1485.80	β-Selinene	26.23	204	0.942	1489
1494.44	Eremophilene	26.58	204	0.112	1486
1507.38	α-Farnesene	27.08	204	0.264	1499
1523.21	δ-cadinene	27.68	204	0.331	1514
1530.34	Acetyl eugenol	27.95	206	2.447	1521
1583.87	(-)-caryophyllene oxide	29.97	220	0.092	1576

Retention index (KI<sub>calc</sub> and KI<sub>ref</sub>) relative to *n*-alkanes on fused silica capillary column (Rtx®-5MS).



**Figure 3.** A typical chromatogram of the essential oil isolated from Jordan clove samples.

### 3.3. Chemical composition of essential oil from TS3

The identification and characterization of chemical bioactive ingredients in the essential oil which was collected from TS3 and analysed by GC–MS. Total 26 different chemical ingredients of different molecular weight, which represent about 94.89% of the total composition, were identified in this essential oil listed in Figure 3 and Table 3. The most active aroma ingredients found in this essential oil were eugenol (54.97%),  $\beta$ -E-caryophyllene (31.45%),  $\alpha$ -humulene (4.25%), acetyl eugenol (2.44%),  $\alpha$ -amorphene (0.15%),  $\alpha$ -farnesene (0.26%), 2-nonanone (0.07%), chavicol (0.93%), benzaldehyde (0.18%), acetophenone (0.21%), aromadrene (0.35%) and  $\beta$ -selinene (0.94%).

### 3.4. Antimicrobial activity

*In vitro* antibacterial activity of essential oils isolated from three different brands' clove samples collected from SAS1, UAES2 and TS3 against the above mentioned bacterial strains was qualitatively determined by the presence or absence of inhibition zones. The different essential oils of different concentration of clove samples displayed antibacterial activity against one Gram-positive *S. aureus* and two Gram-negative *E. coli* and *P. aeruginosa* bacteria strain at different concentrations such as 2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL and 0.25 mg/mL dilution with DMSO (Table 4).

**Table 4**

Antimicrobial activity of different essential oils of clove samples against *E. coli*, *P. aeruginosa* and *S. aureus*.

Essential oil	Concentration	<i>E. coli</i> <sup>a</sup> (mm)	<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)
Saudi Arab	2 mg/mL	10.00±0.19	9.00±0.12	nd
	1 mg/mL	9.00±0.78	6.00±0.45	nd
	0.5 mg/mL	7.00±0.12	nd	nd
	0.25 mg/mL	6.00±0.09	28.00±0.23	nd
	Standard	24.00±0.10	29.00±0.21	nd
UAE	2 mg/mL	8.00±0.11	nd	nd
	1 mg/mL	7.00±0.32	nd	nd
	0.5 mg/mL	7.00±0.43	24.00±0.32	nd
	0.25 mg/mL	8.00±0.10	29.00±0.25	nd
	Standard	24.00±0.89	35.00±0.54	nd
Jordan	2 mg/mL	5.00±0.33	17.00±0.14	nd
	1 mg/mL	nd	7.00±0.54	nd
	0.5 mg/mL	12.00±0.98	nd	nd
	0.25 mg/mL	nd	nd	nd
	Standard	18.00±0.19	31.00±0.09	nd

nd=not detected; <sup>a</sup> Values are represented as the mean±SD of three experiments.

The highest antimicrobial activity was in SAS1 and the lowest activity was in TS3 and in the order of activity was SAS1>UAES2>TS3.

## 4. Discussion

Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants, seeds, buds *etc.* Scientist and researcher have been looking for various ways to search medicinally important ingredients which are to cure or relieve disease. Several medicinal plant products and their formulated products have been used as medicines since the ancient time[4,16,21]. Recently, the scientist and researcher are involving to isolate and identify bioactive ingredients in the essential oils and crude extracts from plants which are used medicinally all over the world. The plant crude extracts and essential oils were isolated from medicinal plants which have been used as antimicrobial, anticancer, antifungal and antioxidant agents. More recently, those plants widely used in traditional medicines to cure diseases have been scientifically screening as potential sources of novel anticancer, antifungal and antioxidant agents[17,18,22,23]. Several reports published by the scientist have the use as anti-fungal and antibacterial activity of essential oils and plant extracts such as rosemary, peppermint, bay, basil, tea tree, celery seed and fennel[16–18,24].

The objective of this report was the isolate and chromatographic (GC–MS) analysis of the essential oils from different brands of clove samples collected from SAS1, UAES2 and TS3. All three essential oils were analyzed by using sensitive GC–MS. This kind of study has been done by the authors for the first time in the Sultanate of Oman. The differences of bioactive ingredients composition in the essential oil from cloves samples are depending on geographical distribution as well as the environmental conditions such as temperature, rainfall, altitude, hours of sunshine, *etc.* The different bioactive ingredients can affect the biological and chemical activities. In our present study, only few volatile ingredients were identified in all essential oils. Most of the identified chemical ingredients have low molecular weight and they are easily volatile at room temperature. So, during the samples' processing time, most of the low volatile chemical constituents evaporated due to hot weather. Eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene and

acetyl eugenol are predominant bioactive ingredients used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anesthetic[7]. In this study, the four predominant ingredients have different composition in three essential oils. The active ingredients identified in SAS1 were eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene and acetyl eugenol. The active ingredients found in UAES2 were eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene and acetyl eugenol. The chemical ingredients in TS3 essential oil were identified as eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene, acetyl eugenol. So, the chemical composition of predominant ingredients was not constant. The composition was varied due to the extraction process, samples process and environmental conditions. In previous studies, different clove samples done by several authors[4,20,23] were eugenol and eugenol, eugenol acetate and eugenol, eugenol acetate and  $\beta$ -E-caryophyllene, respectively. However, comparatively low percentage of major chemical ingredients was obtained from three brands' cloves samples.

The hydro distillation method was used to isolate the essential oils from different brands' power cloves samples. The colour of essential oils was deep yellow. The major chemical ingredients of the oils were eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene and acetyl eugenol and several minor chemical ingredients were also obtained from the same clove oils. Most of the chemical ingredients isolated and identified in the essential oil were mono and sesquiterpene, normal and cyclic hydrocarbons, phenolic derivatives as well as their oxygenated derivatives reported by several authors[9,16,18,25]. The major clove ingredients have potential to strongly inhibit microbial pathogens[9,16]. The antimicrobial activity of the essential oils and plant crude extracts depends on their chemical ingredients[9,16,18].

The three essential oils isolated from SAS1, UAES2 and TS3 showed potential antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* bacterial strains at the concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.25 mg/mL within the range 5–29 mm. SAS1 showed moderate activity against *E. coli* at all the prepared concentrations. The highest activity was obtained at the concentration 2 mg/mL. UAES2 also showed moderate activity against *E. coli* at all the prepared concentrations. The highest activity was obtained at the concentration 2 and 0.25 mg/mL. However, the essential oil from UAES2 did not show any activity against *E. coli* at the concentration of 1 and 0.5 mg/mL. Similarly, the essential oil from TS3 also did not show any activity against *E. coli* at the concentration of 1 and 0.25 mg/mL. However, 2 and 0.5 mg/mL showed moderate activity against the same bacterial strain. On the other hand, SAS1 essential oil showed predominant activity against at the concentration of 0.25 mg/mL and the other concentration showed moderate activity except 0.5 mg/mL. Similarly, UAES2 also showed high activity against *S. aureus* at the concentration of 0.5 and 0.25 mg/mL but the concentration of 2 and 1 mg/mL did not show any activity. TS3 essential oil showed high activity against *S. aureus* at the concentration of 2 mg/mL and moderate

activity at 1 mg/mL. But the essential oil also did not show any activity against *S. aureus* at the concentration of 0.5 and 0.25 mg/mL. However, no activity was found against *P. aeruginosa* in three essential oils at all concentrations. The negative control did not inhibit the growth of the bacteria tested and the positive control showed strong inhibition against *S. aureus* and *E. coli* except *P. aeruginosa*. Almost similar antimicrobial results were obtained from the essential oils of clove samples by other reported results[19].

The GC-MS is the most suitable technique for the identification and characterization of aroma ingredients in the essential oils and crude extracts. The results from the above experiment indicated that the essential oils contain mainly four compounds such as eugenol,  $\beta$ -E-caryophyllene,  $\alpha$ -humulene and acetyl eugenol. Further studies in progress in our laboratory are being designed to isolate the bioactive components from the essential oil of clove samples.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

Cloves are aromatic plants belong to the family Myrtaceae. Its scientific name is *Syzygium aromaticum*. Cloves are mainly originated from Indonesia. Now it is commercially cultivated in tropical and sub-tropical countries like Indonesia, India, Madagascar, Zanzibar, Pakistan, Bangladesh and Sri Lanka. Cloves are the most important herb due to its medicinal values.

#### Research frontiers

The present study is to analyze three essential isolated oils from three different brands' clove samples collected from Gulf region and test their antimicrobial activities.

### Related reports

Some reports already published in different journals but they were individual studies. This paper is completely different from others. The authors tried to compare data isolated from different brands' clove samples.

### Innovations & breakthroughs

The scientific work was done by the authors. It gives the new information and data to the scientific community.

### Applications

According to the paper, there are so many bioactive compounds that can be used to prepare herbal medicine.

### Peer review

This is an interesting study in which the authors analyzed three essential isolated oils from three different brands' clove samples collected from Gulf region and tested their antimicrobial activities. The data are helpful in the further research.

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