



**IN THE NAME OF ALLAH,
MOST GRACIOUS, MOST MERCIFUL**

Chemical Constituents and Antimicrobial Activity of Essential Oils Extract from Spices/Herbs Used in Saudi Arabia

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Abstract. Antimicrobial activity of various essential oils obtained from *Rosemarinus officinalis* (rosemary), *Mentha longifolia* (basil), *Mentha piperita* L. (mint), *Allium sativum* L. (garlic), *Zingiber officinale* (ginger), *Salvia officinalis* (sage), *Chamaemelum nobile* (chamomile), *Cuminum cyminum* (cumin), *Nigella sativa* (black seed), *Syzygium aromaticum* L. (clove) and *Piper nigrum* (black pepper) have been evaluated against six microorganisms including *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *B. cereus* and *Candida albicans*. The disc diffusion method and cup plate method were used to screen the antimicrobial activity of the essential oils and the test was performed for each in triplicates. The inoculated plates of MHA and SDA were incubated at 37°C and 25°C for 24 h for bacteria and yeast respectively, and then the inhibitory zones were measured (mm) for the respective oils and strains. The essential oils of basil and clove showed excellent antibacterial activity against all the test organisms. The essential oils from rosemary, sage and mint showed good antimicrobial activity against *Staphylococcus aureus*, *E. coli* and *Salmonella* spp., while garlic, ginger and black seed inhibited the growth of *Staph. aureus* only. The chamomile essential oil inhibited the growth of *Candida albicans*, however those from cumin and black pepper did not show any antimicrobial activity against the test organisms. In comparison, most of the essential oils showed high antimicrobial activity than four commercial antibiotics (nystatin, nalidixic acid, streptomycin and tetracycline).

Introduction

Spices and herbs have been used for thousands of years by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Zaika, 1988; Shelef, 1983). Essential oils as antimicrobial agents present two main characteristics: the first is their natural origin which means more safety for consumers, and the other is that they are considered to be low risk for resistance development by pathogenic microorganisms.

Food borne pathogens such as diarrheagenic

serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *B. cereus* are widely distributed in nature, causing considerable mortality and morbidity in the population. It has been reported that, worldwide, there are more than 1.3 billion cases of human salmonellosis annually, with three million deaths (Pang *et al.*, 1995). Among the various diarrheagenic serotypes of *E. coli*, enterohemorrhagic *E. coli* O157:H7 is implicated in large number of food borne outbreaks in many parts of the world including developed nations (Mead, 1999). It is also reported that *E. coli* O157:H7 has low infective dose (Doyle *et al.*, 1997; Elnima, *et al.*, 1983). *Listeria monocytogenes* has been isolated from various environments and is reported to cause 25% of all the deaths resulting from food-borne outbreaks in the

United States annually (CDC, 1995). Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens (Cohen, 1992; Gold and Moellering, 1996). This shift in susceptibility greatly affects our ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments (Hora and Nair, 1944). They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, the use of spices/herbs has been gradually increasing in developed countries also.

In the present study, we have evaluated the antimicrobial effect of the essential oil of 11 spices widely used in Saudi Arabia such as *Rosmarinus officinalis* (rosemary), *Mentha longifolia* (basil), *Mentha piperita* (mint), *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Salvia officinalis* (sage), *Chamaemelum nobile* (chamomile), *Cuminum cyminum* (cumin), *Nigella sativa* (black seed), *Syzygium aromaticum* L. (clove) and *Piper nigrum* (black pepper) against six different types of microorganisms including *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *B. cereus* and *Candida albicans*. The aims of the present study were to assess the antimicrobial activity of these essential oils and to compare them with the effect of four commercial antifungal and antibiotics (nystatin, nalidixic acid, streptomycin and tetracycline).

Material and Methods

Preparation of herbal/spices essential oil

Table 1. Plant classification which used in the experiment

Plant Name	Scientific Name
Rosemary	<i>Rosmarinus officinalis</i>
Basil	<i>Mentha longifolia</i>
Mint	<i>Mentha piperita</i> L.
Garlic	<i>Allium sativum</i>
Ginger	<i>Zingiber officinale</i>
Sage	<i>Salvia officinalis</i>
Chamomile	<i>Chamaemelum nobile</i>
Cumin	<i>Cuminum Cyminum</i>
Black seed	<i>Nigella sativa</i>
Clove	<i>Syzygium aromaticum</i>
Black Pepper	<i>Piper nigrum</i>

Essential oil extraction

The herbal/spices were obtained from the local market. The herbal/spices were cleaned, and the fresh herbal washed in sterile distilled water, all the herbal was drayed and about 100 g of each herbal/spices were crushed with mortar and pestle. The herbal/spices powder were sieved and subjected to steam distillation and the essential oil for each sample collected in closed vile. This essential oil was considered as the 100% concentration of the extract (Vukovic *et al.*, 2007).

Gas chromatography-mass spectrometry (GC-MS)

Analyses were carried out in a Shimadzu 2001 (QP5050A) mass spectrometry (GC-MS) fitted with a Rtx. 225 column (Crossbond 50% cyanopropylmethyl- 50% phenyl methyl polysiloxane 15 m_0.25 mm i.d., film thickness 0.25 µm), operated by LabSolutions software, oven temperature program: 75–250°C, at 107 min analysis time, injector temperature: 250°C; carrier gas: helium, adjusted to a column velocity of flow 0.8 ml/min; split ratio was 50:1, whereas split flow was 41.9 ml/min, interface temperature: 230°C; MS source temperature: 230°C; MS acquisition mode, temperature: 230°C. One microliter of sample (dissolved in hexane 100% v/v) was injected into the system (AOAC, 2000).

Microorganisms

Antimicrobial activity tests were carried out against the bacteria *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *B. cereus* and against the yeast *Candida albicans*. The microorganisms were obtained from Food & Drugs Lab, Ministry of Health, Riyadh, Saudi Arabia. The stock cultures of bacteria were maintained at 4°C on slopes of Nutrient Agar and *Candida albicans* on slants of Sabouraud Dextrose Agar.

Preparation of inoculums

Fresh cultures for experiments were prepared by transferring a loopfull of cell from the stock cultures to Brain Heart Infusion liquid for bacteria and Sabouraud Dextrose Broth for *Candida albicans* that were incubated for 24 h at 37°C and 25°C respectively with two consecutive transfers. The cell suspensions were diluted finally with fresh Brain Heart Infusion and Sabouraud Dextrose Broth to give inoculums concentrations of 10⁶-10⁷ cfu/ml for being used in the activity assays (Nanasombat and Lohasupthawee, 2005).

Antimicrobial susceptibility test

The disc diffusion method was used to screen the

antimicrobial activity (Jorgensen *et al.*, 1999). The Mueller Hinton Agar (MHA) plates and Sabouraud Dextrose Agar (SDA) were prepared by pouring 15 ml of molten media in sterile Petri dishes. The selected bacteria and yeast were inoculated into sterile BHI and SDB at 37°C and 25°C for 24 h respectively. Using a sterile cotton swab, the inoculum suspension of each bacteria and yeast were swabbed on the entire surface of Mueller Hinton Agar and Sabouraud Dextrose Agar, then the inoculum was allowed to dry for 5 min. Fifty microlitres of the various spices' extract (100%) were aseptically loaded on 6 mm sterile filter paper disc. The loaded disc was placed on the surface of medium and the plates were kept for incubation at 37°C and 25°C for 24 h. At the end of the incubation period, the diameter of inhibition zones were measured in mm and the results were recorded. Diameters between 12 and 16 mm were considered moderately active, and these with >16 mm were considered highly active. In addition to the disc diffusion procedure, the solid medium diffusion (cup plate method) was used to determine the antimicrobial activity. For this, 1 ml of the bacteria and yeast suspension was uniformly spread on sterile MHA and SDA Petri dishes. After inoculum absorption by agar, wells were made using sterile glass tubes (diameter 6 mm) which were filled with 50 µl of different essential oil of herbal/spices. The plates were incubated in inverted position at 37°C and 25°C for 24 h for bacteria and yeast respectively. The diameter of inhibition zones was measured in mm and the results were recorded. Controls included in this assay were essential oil replaced by sterile water. All the media used in the present study were obtained from Oxoid Company, Hampshire, England (Sagfildic *et al.*, 2002).

Antibiotic sensitivity testing

The microorganisms were tested also for their sensitivity against the antibiotics nalidixic acid (30 mcg), nystatin (100 unit), streptomycin (10 mcg) and tetracycline (30 mcg) by the disk diffusion method (Jorgensen *et al.*, 1999). The cultures were enriched in sterile Muller Hinton Broth for 24 h at 37°C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile MHA and SDA plates. Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed on the surface of MHA and SDA plates sufficiently separated from each other to avoid the overlapping of the inhibition zones. The plates were incubated for 24 h at 37°C for bacteria and at 25°C for yeast. At the end of the incubation period, the diameter of the inhibition zones were measured (mm). All the experiments were performed in triplicate.

Statistical analysis

Data were analyzed by using GLM procedure of SAS (1995). The differences were considered significant at level of ≤ 0.05 .

Results and Discussion

The chemical composition of the essential oils was analyzed using GC-MS technique, which allowed the identification of oil constituent. The chemical constituent of the entire essential oil have some component which showed highly antimicrobial activity (Doyle *et al.*, 1997; Thongson *et al.*, 2004). Table 2 represents the antimicrobial activity of the spices extracts (essential oils) and selected commercial antibiotics against *B. cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli*, *Salmonella* spp. and *Candida albicans*. The essential oils from rosemary, mint, garlic, ginger, chamomile, black seed, clove and sage showed antimicrobial activity to at least one of the tested microorganisms. Basil and clove essential oils presented antimicrobial activity against all the six microorganisms used in this study (the diameter of the inhibition zone, 11-20 mm) with high significant against *B. cereus* (20 mm $\pm$ 0.58) with basil essential oil, while this picture was less in case of *Listeria monocytogenes* (15 mm $\pm$ 1.04). However, in case of clove, the picture was opposite to basil. On the other hand, the extracts from cumin and black pepper did not show any antimicrobial activity against the above microbes.

The essential oil of garlic was effective against *E. coli* (20 mm $\pm$ 1.04); however, it did not show any inhibitory against other microorganisms, these results agreed with those reported by other researchers (Srinivasan *et al.*, 2001; Srinivasan and Lakshmanaperumalsamy, 1993). The volatile oil of rosemary has some antiseptic properties. It contains a high percentage of 1,8-cineole (providing the fresh eucalyptus-like fragrance), and other major terpenoid components including α -pinene, α -terpineol, and camphor, (Table 2). The British Herbal Pharmacopoeia reports that rosemary has antibacterial and antispasmodic action. An extract of rosemary can also produce an increase in bile secretion, thus aiding in fat digestion. The German Commission approves the internal use of rosemary leaf for dyspeptic complaints and the external use as supportive therapy for rheumatic conditions and peripheral circulatory disorders. Also, the volatile oil of basil and mint contain high level of 1,8-cineole, carvone, pulegone and D-limonene which having highly bacterial effect (Tables 3 and 4).

Table 2. The diameter of the inhibition zones (mm) of essential oils of herbal/spices extracts and four antibiotics against selected six microorganisms

Essential oil (50µl/desk) & Antibiotics	<i>Listeria</i> <i>monocytogenes</i>	<i>Staph.</i> <i>aureus</i>	<i>E. coli</i>	<i>Salmonella</i> <i>spp.</i>	<i>Bacillus cereus</i>	<i>Candida</i> <i>albicans</i>
Rosemary	R	20mm $\pm$ 0.76 ^b	16mm $\pm$ 0.76 ^b	17mm $\pm$ 0.29 ^{ab}	R	R
Basil	15mm $\pm$ 1.04 ^a	18mm $\pm$ 0.29 ^{bc}	16mm $\pm$ 0.76 ^b	16mm $\pm$ 1.32 ^b	20mm $\pm$ 0.58 ^a	17mm $\pm$ 0.76 ^c
Mint	R	19mm $\pm$ 1.53 ^{bc}	15mm $\pm$ 1.0 ^b	R	R	R
Garlic	R	R	20mm $\pm$ 1.04 ^a	R	R	R
Ginger	R	R	12mm $\pm$ 0.58 ^c	R	R	R
Sage	R	19mm $\pm$ 1.50 ^{bc}	17mm $\pm$ 0.76 ^b	19mm $\pm$ 1.53 ^a	R	R
Chamomile	R	R	R	R	R	20mm $\pm$ 0.76 ^a
Cumin	R	R	R	R	R	R
Black seed	R	18mm $\pm$ 1.04 ^{bc}	R	R	R	R
Clove	16mm $\pm$ 0.58 ^a	14mm $\pm$ 0.50 ^d	15mm $\pm$ 0.58 ^b	13mm $\pm$ 0.58 ^c	11mm $\pm$ 0.50 ^c	15mm $\pm$ 0.58 ^b
Black pepper	R	R	R	R	R	R
Streptomycin 10µg	R	16mm $\pm$ 0.58 ^{cd}	R	R	R	R
Nystatin 100 units	R	R	R	R	R	R
Tetracyclin 30µg	R	30mm $\pm$ 0.58 ^a	R	R	R	R
Nalidixic acid 30µg	R	20mm $\pm$ 0.58 ^b	17mm $\pm$ 1.53 ^b	R	17mm $\pm$ 0.58 ^b	R

Mean in the same column followed by different subscripted are significant different ($P \leq 0.05$)

R = resistant,

Table 3. Chemical composition of Rosemary essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA %*
ALPHA.-PINENE,	C ₁₀ H ₁₆	16.103	299718	0.32
.alpha.-pinene	C ₁₀ H ₁₆	16.695	9383464	10.08
Ethanone, 1-(3-ethyloxiranyl)	C ₆ H ₁₀ O ₂	17.103	240680	0.26
Camphene	C ₁₀ H ₁₆	17.703	7657464	8.22
.beta.-Pinene	C ₁₀ H ₁₆	19.462	3118285	3.35
MYRCENE	C ₁₀ H ₁₆	19.837	1247295	1.34
1-PHELLANDRENE	C ₁₀ H ₁₆	21.145	103513	0.11
.Alpha.-Terpinene	C ₁₀ H ₁₆	21.962	341979	0.37
D-Limonene	C ₁₀ H ₁₆	22.845	2707095	2.91
1,8-CINEOLE EUCALYPTOL	C ₁₀ H ₁₈ O	23.145	23260494	24.96
.gamma.-Terpinene	C ₁₀ H ₁₆	24.978	725929	0.78
1,8-Cineole	C ₁₀ H ₁₈ O	25.828	277843	0.3
.alpha.-terpinolene	C ₁₀ H ₁₆	27.245	489814	0.53
.beta.-Linalool	C ₁₀ H ₁₈ O	27.945	199168	0.21
TRANS-THUJAN-4-OL	C ₁₀ H ₁₈ O	28.262	186852	0.2
Camphor	C ₁₀ H ₁₆ O	32.045	7418878	7.97
l.-alpha.-Terpineol	C ₁₀ H ₁₈ O	33.662	204805	0.22
Borneol	C ₁₀ H ₁₈ O	33.82	1552671	1.67
4-TERPINEOL	C ₁₀ H ₁₈ O	34.437	518813	0.56
.alpha.-TERPINEOL	C ₁₀ H ₁₈ O	35.495	1717253	1.84
BORNYL ACETATE	C ₁₂ H ₂₀ O ₂	42.487	13956554	14.99
Copaene	C ₁₅ H ₂₄	49.953	471821	0.51
.beta.-CARYOPHYLLENE	C ₁₅ H ₂₄	53.245	11881936	12.76
globulol	C ₁₅ H ₂₆ O	54.462	204411	0.22
.ALPHA.-HUMULENE	C ₁₅ H ₂₄	55.328	1675435	1.8
germacrene d α -amorphene	C ₁₅ H ₂₄	56.395	588990	0.63
.ALPHA.-MUUROLENE	C ₁₅ H ₂₄	57.595	308931	0.33
germacrene d	C ₁₅ H ₂₄	58.453	647777	0.7
.DELTA.-CADINENE	C ₁₅ H ₂₄	58.753	1259697	1.35
CARYOPHYLLENE OXIDE	C ₁₅ H ₂₄	61.903	478468	0.51

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 4. Chemical composition of Mint essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA %*
ALPHA.-PINENE	C ₁₀ H ₁₆ ,136	16.687	568257	0.43
SABINENE	C ₁₀ H ₁₆ ,136	19.078	592311	0.44
2-.BETA.-PINENE	C ₁₀ H ₁₆ ,136	19.462	921357	0.69
MYRCENE	C ₁₀ H ₁₆ ,136	19.845	843890	0.63
D-Limonene	C ₁₀ H ₁₆ ,136	22.853	10722234	8.04
1,8-CINEOLE EUCALYPTOL	C ₁₀ H ₁₈ O ₁₅₄	23.128	5832325	4.37
cis-.beta.-Terpineol	C ₁₀ H ₁₈ O ₁₅₄	25.828	876354	0.66
.alpha.-TERPINEOL	C ₁₀ H ₁₈ O ₁₅₄	35.503	281282	0.21
CIS-DIHYDROCARVONE	C ₁₀ H ₁₆ O ₁₅₂	35.928	577042	0.43
CIS-CARVEOL	C ₁₀ H ₁₆ O ₁₅₂	38.837	496841	0.37
+) -PULEGONE	C ₁₀ H ₁₆ O ₁₅₂	39.237	1420240	1.06
CARVONE	C ₁₀ H ₁₄ O ₁₅₀	39.887	98570571	73.92
TRANS-CARVYL ACETATE	C ₁₂ H ₁₈ O _{2,194}	48.453	226014	0.17
BETA. BOURBONENE	C ₁₅ H _{24,204}	50.745	1577588	1.18
BETA. ELEMENE	C ₁₅ H _{24,204}	50.987	1020783	0.77
beta.-CARYOPHYLLENE	C ₁₅ H _{24,204}	53.22	2641214	1.98
GERMACRENE-D	C ₁₅ H _{24,204}	53.737	136861	0.1
ALPHA.-CUBEBENE	C ₁₅ H _{24,204}	54.728	640972	0.48
.beta.-Cubebene	C ₁₅ H _{24,204}	55.82	1082640	0.81
Germacrene D	C ₁₅ H _{24,204}	56.82	2047683	1.54
BICYCLOGERMACRENE	C ₁₅ H _{24,204}	57.628	938458	0.7
trans-calamenene	C ₁₅ H _{24,204}	58.903	310550	0.23
TAU.-CADINOL	C ₁₅ H ₂₆ O ₂₂₂	59.537	153931	0.12
Cubenol	C ₁₅ H ₂₆ O ₂₂₂	63.053	623436	0.47
alpha.-cadinol	C ₁₅ H ₂₆ O ₂₂₂	64.57	264466	0.2

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 5. Chemical composition of Garlic essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA %*
1,2-Dithiolane	C ₃ H ₆ S _{2,106}	12.387	483112	2.03
Oil garlic Allyl sulfide	C ₆ H ₁₀ S ₁₁₄	12.82	210345	0.88
Allyl disulfide	C ₆ H ₁₀ S _{2,146}	26.778	4383589	18.38
Allyl methyl ether	C ₄ H ₈ O ₇₂	28.345	152787	0.64
Trisulfide, methyl 2-propenyl	C ₄ H ₈ S ₃	31.578	843087	3.53
2-Vinyl-4H-1,3-dithiin	C ₆ H ₈ S _{2,144}	37.537	278827	1.17
Allyl trisulfide	C ₆ H ₁₀ S _{3,178}	44.045	14820834	62.12
Anthracene, 9-dodecyltetradecahydro-	C ₂₆ H _{48,360}	65.195	2682987	11.25

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 6. Chemical composition of Ginger essential oil

MS + t _{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
1,8-CINEOLE \$ EUCALYPTOL	C ₁₀ H ₁₈ O ₁₅₄	23.128	902941	0.1
LINALOOL L	C ₁₀ H ₁₈ O ₁₅₄	27.937	1654196	0.19
MENTHONE	C ₁₀ H ₁₈ O ₁₅₄	32.528	3946068	0.45
BORNEOL L	C ₁₀ H ₁₈ O ₁₅₄	33.803	6645395	0.76
alpha.-TERPINEOL	C ₁₀ H ₁₈ O ₁₅₄	35.503	3435876	0.39
CITRONELLOL	C ₁₀ H ₂₀ O ₁₅₆	37.878	1852549	0.21
(+)-PULEGONE	C ₁₀ H ₁₆ O ₁₅₂	39.228	4911994	0.56
2-UNDECANONE	C ₁₁ H ₂₂ O ₁₇₀	42.545	3175169	0.36
ALPHA.-COPAENE	C ₁₅ H _{24,204}	49.928	1787921	0.2
beta.-Farnesene	C ₁₅ H _{24,204}	54.662	4494430	0.51
UNKNOWN FROM LIME OIL	C ₁₅ H _{24,204}	54.97	5670477	0.65
gamma.-elemene	C ₁₅ H _{24,204}	55.587	34197723	3.91
beta.-Himachalene	C ₁₅ H _{24,204}	56.403	73910519	8.45
ALPHA.-CURCUMEN	C ₁₅ H _{22,202}	56.67	93520732	10.69
Zingiberene	C ₁₅ H _{24,204}	57.428	225941708	25.83
Farnesene	C ₁₅ H _{24,204}	57.728	68686625	7.85
beta. bisabolene BETA-BISABOLENE	C ₁₅ H _{24,204}	58.003	109843217	12.55
BETA-SESQUIPHELLANDRENE	C ₁₅ H _{24,204}	58.878	152136826	17.39
alpha.-Humulene	C ₁₅ H _{24,204}	59.412	2097910	0.24
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	C ₁₅ H _{24,204}	60.287	15150492	1.73
Guaiol	C ₁₅ H ₂₆ O ₂₂₂	63.545	20108378	2.3
Zingiberone	C ₁₁ H ₁₄ O _{3,194}	64.295	10718783	1.22
beta.-Eudesmol	C ₁₅ H ₂₆ O ₂₂₂	64.637	7354924	0.84
d-Nerolidol	C ₁₅ H ₂₆ O ₂₂₂	65.812	19044879	2.18

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 7. Chemical composition of Black Seed essential oil

MS + t _{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
alpha.-Thujene	C ₁₀ H _{16,136}	16.17	4169556	11.05
.alpha.-PINENE	C ₁₀ H _{16,136}	16.72	878841	2.33
SABINENE	C ₁₀ H _{16,136}	19.112	381607	1.01
2-BETA.-PINENE	C ₁₀ H _{16,136}	19.512	875619	2.32
PARA CYMENE	C ₁₀ H _{14,134}	22.637	10532671	27.92
-LIMONENE	C ₁₀ H _{16,136}	22.887	598105	1.59
.GAMMA. TERPINENE	C ₁₀ H _{16,136}	25.037	353622	0.94
DIHYDRO-CARVEOL	C ₁₀ H ₁₈ O ₁₅₄	29.712	1508539	4
CIS-LIMONENE OXIDE	C ₁₀ H ₁₆ O ₁₅₂	36.353	291210	0.77
p-Cymene-2,5-dione	C ₁₀ H ₁₂ O _{2,164}	40.162	12889555	34.17
Carvacrol	C ₁₀ H ₁₄ O ₁₅₀	44.312	773654	2.05
Junipene	C ₁₅ H _{24,204}	52.587	1445346	3.83
ACETOVANILLONE	C ₉ H ₁₀ O _{3,166}	60.82	3023953	8.02

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 8. Chemical composition of Sage essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
.alpha.-pinene	C ₁₀ H _{16,136}	16.72	22456949	5.08
Camphene	C ₁₀ H _{16,136}	17.72	10107143	2.29
beta.-Pinene	C ₁₀ H _{16,136}	19.487	6190861	1.4
.beta.-Myrcene	C ₁₀ H _{16,136}	19.87	7304123	1.65
o-Cymene	C ₁₀ H _{14,134}	22.637	4499402	1.02
Limonene	C ₁₀ H _{16,136}	22.953	2032224	0.46
1,8-CINEOLE EUCALYPTOL	C ₁₀ H ₁₈ O ₁₅₄	23.428	251234431	56.85
L-LINALOOL	C ₁₀ H ₁₈ O ₁₅₄	27.987	906937	0.21
.ALPHA. THUJONE	C ₁₀ H ₁₆ O ₁₅₂	28.845	3859797	0.87
beta.-thujone BETA-THUJONE	C ₁₀ H ₁₆ O ₁₅₂	29.67	1948543	0.44
Camphor	C ₁₀ H ₁₆ O ₁₅₂	32.187	32766291	7.41
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl	C ₁₀ H ₁₆ O ₁₅₂	33.27	650742	0.15
l.-alpha.-Terpineol	C ₁₀ H ₁₆ O ₁₅₂	33.778	11243116	2.54
4-TERPINEOL	C ₁₀ H ₁₈ O ₁₅₄	34.52	2023457	0.46
(+)-.alpha.-Terpineol (p-menth-1-en-8-ol)	C ₁₀ H ₁₈ O ₁₅₄	35.637	15869112	3.59
linalyl acetate	C ₁₂ H ₂₀ O _{2,196}	39.645	1741590	0.39
BORNYL ACETATE	C ₁₂ H ₂₀ O _{2,196}	42.512	802677	0.18
Phenol, 2-ethyl-4,5-dimethyl-	C ₁₀ H ₁₄ O ₁₅₀	43.47	676419	0.15
Phenol, 2,3,5,6-tetramethyl-	C ₁₀ H ₁₄ O ₁₅₀	44.278	768910	0.17
ISO-ISOPULEGYL ACETATE	C ₁₂ H ₂₀ O _{2,196}	44.795	472408	0.11
ALPHA-TERPINYL ACETATE	C ₁₂ H ₂₀ O _{2,196}	47.462	10104963	2.29
.beta.-CARYOPHYLLENE	C ₁₅ H _{24,204}	53.253	6340262	1.43
Alloaromadendrene	C ₁₅ H _{24,204}	54.478	4784160	1.08
VERIDIFLOROL	C ₁₅ H ₂₆ O ₂₂₂	62.395	12520922	2.83
CARYOPHYLLENE OXIDE	C ₁₅ H ₂₆ O ₂₂₂	64.828	9594314	2.17
CARYOPHYLLENE OXIDE	C ₁₅ H ₂₄ O ₂₂₀	65.32	5298081	1.2
Z-Citral	C ₁₀ H ₁₆ O ₁₅₂	65.712	15245336	3.45

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 9. Chemical composition of Clove essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
CHAVICOL	C ₉ H ₁₀ O ₁₃₄	41.078	10899348	0.34
TRANS-ANETHOLE	C ₉ H ₁₀ O ₁₄₈	42.82	1463674	0.05
ALPHA.-CUBEBENE	C ₁₅ H _{24,204}	47.67	14034228	0.43
EUGENOL	C ₁₀ H ₁₂ O _{2,164}	50.953	1697832074	52.47
beta.-CARYOPHYLLENE	C ₁₅ H _{24,204}	54.112	589678944	18.22
.ALPHA.-HUMULENE	C ₁₅ H _{24,204}	55.778	79507432	2.46
Aromadendrene	C ₁₅ H _{24,204}	56.078	2061848	0.06
CADINENE	C ₁₅ H _{24,204}	56.487	1211345	0.04
ALPHA.-COPAENE	C ₁₅ H _{24,204}	56.645	4195969	0.13
alpha.-Farnesene	C ₁₅ H _{24,204}	57.637	6125880	0.19
GERMACRENE-D	C ₁₅ H _{24,204}	57.07	2562002	0.08
Aromadendrene	C ₁₅ H _{24,204}	57.812	4412124	0.14
CADINENE	C ₁₅ H _{24,204}	58.978	58066725	1.79
EUGENYL ACETATE	C ₁₂ H ₁₄ O _{3,206}	59.803	701144080	21.67
CARYOPHYLLENE OXIDE	C ₁₅ H ₂₄ O ₂₂₀	62.262	62399813	1.93

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 10. Chemical composition of Chamomile essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
1,8-CINEOLE EUCALYPTOL	$C_{10}H_{18}O_{154}$	23.17	388625	0.43
Linalool	$C_{10}H_{18}O_{154}$	27.995	137498	0.15
MENTHONE	$C_{10}H_{18}O_{154}$	32.603	2239640	2.5
(+)-PULEGONE	$C_{10}H_{16}O_{152}$	39.303	2953214	3.3
CARVONE	$C_{10}H_{14}O_{150}$	39.662	990471	1.11
.beta.-Farnesene	$C_{15}H_{24,204}$	54.678	14063708	15.71
beta.-Chamigrene	$C_{15}H_{24,204}$	55.528	2267288	2.53
beta.-Himachalene	$C_{15}H_{24,204}$	56.287	3139230	3.51
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	$C_{15}H_{22,202}$	56.495	3860303	4.31
Zingiberene	$C_{15}H_{24,204}$	57.128	9652700	10.78
alpha.-Farnesene	$C_{15}H_{24,204}$	57.487	2516648	2.81
BETA-BISABOLENE	$C_{15}H_{24,204}$	57.803	5530405	6.18
beta.-sesquiphellandrene	$C_{15}H_{24,204}$	58.645	4588499	5.13
(+) spathulenol	$C_{15}H_{24}O_{220}$	61.737	2545451	2.84
.tau.-Cadinol	$C_{15}H_{26}O_{222}$	64.095	1341373	1.5
BISABOLOL OXIDE	$C_{15}H_{26}O_{2,238}$	64.52	18195040	20.33
alpha.-Bisabolol	$C_{15}H_{26}O_{222}$	65.345	4195929	4.69
Bisabolone oxide	$C_{15}H_{24}O_{2,236}$	65.603	10914635	12.19

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 11. Chemical composition of Cumin essential oil

MS + t_{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
alpha.-Thujene	$C_{10}H_{16,136}$	16.153	500673	0.12
.alpha.-PINENE	$C_{10}H_{16,136}$	16.712	1386948	0.34
SABINENE	$C_{10}H_{16,136}$	19.12	2072099	0.51
2-BETA.-PINENE	$C_{10}H_{16,136}$	19.545	38311875	9.34
MYRCENE	$C_{10}H_{16,136}$	19.887	2813331	0.69
ALPHA.-PHELLANDRENE	$C_{10}H_{16,136}$	21.187	789211	0.19
PARA CYMENE	$C_{10}H_{14,134}$	22.637	15123892	3.69
dl-Limonene	$C_{10}H_{16,136}$	22.887	950205	0.23
beta.-Phellandrene	$C_{10}H_{16,136}$	23.037	630233	0.15
Eucalyptol	$C_{10}H_{18}O_{154}$	23.203	335772	0.08
gamma.-Terpinene	$C_{10}H_{16,136}$	25.162	62515933	15.24
TRANS-SABINENE HYDRATE \$\$	$C_{10}H_{18}O_{154}$	28.378	686500	0.17
phellandral	$C_{10}H_{16}O_{152}$	35.703	9099966	2.22
CUMINAL	$C_{10}H_{12}O_{148}$	39.845	107562518	26.22
2-Caren-10-al	$C_{10}H_{14}O_{150}$	42.995	15314202	3.73
3-CAREN-10-AL	$C_{10}H_{14}O_{150}$	43.612	115742189	28.2
beta.-Farnesene	$C_{15}H_{24,204}$	54.67	2176431	0.53
Manool \$\$ 1-Naphthalenepropanol, 1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4- hexadiynylidene)	$C_{20}H_{34}O_{290}$	60.67	14295440	3.48
	$C_{13}H_{12}O_{2,200}$	60.928	18186295	4.43
carotol	$C_{15}H_{26}O_{222}$	62.545	1797649	0.44

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 12. Chemical composition of Black Pepper essential oil

MS + t _{ret} identification	FORMULA	R.T.*	AREA*	AREA%*
2,6-Dimethylheptane	¹²⁸ C ₉ H ₂₀	11.303	933287	0.14
Ethylcyclohexane	¹¹² C ₈ H ₁₆	11.878	790161	0.12
2,3-Dimethylheptane	¹²⁸ C ₉ H ₂₀	12.437	248742	0.04
1-Phenyl-3,3-dimethylbutane	¹⁶² C ₁₂ H ₁₈	13.062	1046029	0.16
p-Xylene	¹⁰⁶ C ₈ H ₁₀	13.437	3989117	0.62
n-Nonane Shellsol 140	¹²⁸ C ₉ H ₂₀	14.353	3325365	0.52
m-Xylene	¹⁰⁶ C ₈ H ₁₀	14.645	996390	0.15
ALPHA-THUJENE	¹³⁶ C ₁₀ H ₁₆	16.203	1801442	0.28
alpha.-PINENE	¹³⁶ C ₁₀ H ₁₆	16.762	8590476	1.33
SABINENE	¹³⁶ C ₁₀ H ₁₆	19.17	19665079	3.05
2-.BETA.-PINENE	¹³⁶ C ₁₀ H ₁₆	19.562	23914148	3.71
BETA.-MYRCENE	¹³⁶ C ₁₀ H ₁₆	19.945	6511659	1.01
DECANE	¹⁴² C ₁₀ H ₂₂	20.312	801524	0.12
ALPHA.-PHELLANDRENE	¹³⁶ C ₁₀ H ₁₆	21.253	7432271	1.15
(+)-3-CARENE	¹³⁶ C ₁₀ H ₁₆	21.703	52438890	8.13
PARA CYMENE	¹³⁴ C ₁₀ H ₁₄	22.695	3164934	0.49
D-Limonene	¹³⁶ C ₁₀ H ₁₆	23.012	61433263	9.52
beta.-Phellandrene	¹³⁶ C ₁₀ H ₁₆	23.128	4973042	0.77
.gamma.-TERPINENE	¹³⁶ C ₁₀ H ₁₆	25.103	684650	0.11
TERPINOLENE	¹³⁶ C ₁₀ H ₁₆	27.353	1561665	0.24
L-LINALOOL	¹⁵⁴ C ₁₀ H ₁₈ O	28.07	2751909	0.43
TERPINEOL-4	¹⁵⁴ C ₁₀ H ₁₈ O	34.595	879327	0.14
delta.-Elemene	²⁰⁴ C ₁₅ H ₂₄	46.645	9798970	1.52
CADINA-1,4-DIENE	²⁰⁴ C ₁₅ H ₂₄	47.678	630258	0.1
ALPHA.-COPAENE	²⁰⁴ C ₁₅ H ₂₄	50.078	1919773	0.3
BETA. ELEMENE	²⁰⁴ C ₁₅ H ₂₄	51.145	14206986	2.2
.beta.-CARYOPHYLLENE	²⁰⁴ C ₁₅ H ₂₄	53.67	326458992	50.61
.gamma.-elemene	²⁰⁴ C ₁₅ H ₂₄	53.862	1339718	0.21
ALPHA.-GUAIENE	²⁰⁴ C ₁₅ H ₂₄	54.228	7762263	1.2
.beta.-Farnesene	²⁰⁴ C ₁₅ H ₂₄	54.712	928749	0.14
ALPHA.-HUMULENE	²⁰⁴ C ₁₅ H ₂₄	55.487	22620223	3.51
GERMACRENE-D	²⁰⁴ C ₁₅ H ₂₄	56.937	2401047	0.37
beta.-Selinene	²⁰⁴ C ₁₅ H ₂₄	57.337	21183524	3.28
alpha.-selinene	²⁰⁴ C ₁₅ H ₂₄	57.737	9117033	1.41
beta.-Bisabolene	²⁰⁴ C ₁₅ H ₂₄	57.862	1104635	0.17
CARYOPHYLLENE OXIDE	²²⁰ C ₁₅ H ₂₄ O	61.995	7772691	1.2
Spathulenol	²²⁰ C ₁₅ H ₂₄ O	63.695	4641339	0.72

*RT is retention time, AREA is the peak volume, (Area %) is percent of area

Table 13. Chemical composition of Basil essential oil

MS + t _{ret} identification	FORMULA	R.T.*	AREA	AREA%
alpha.-PINENE	C ₁₀ H ₁₆	16.687	1131606	0.62
Camphene	C ₁₀ H ₁₆	17.703	627085	0.35
SABINENE	C ₁₀ H ₁₆	19.078	1106257	0.61
beta.-Pinene	C ₁₀ H ₁₆	19.462	1977227	1.09
beta.-Myrcene	C ₁₀ H ₁₆	19.837	1091842	0.6
D-Limonene	C ₁₀ H ₁₆	22.845	1633332	0.9
1,8-CINEOLE \$\$ EUCALYPTOL	C ₁₀ H ₁₈ O	23.137	17464681	9.62
MENTHONE	C ₁₀ H ₁₈ O	32.57	15323048	8.44
ISOMENTHONE	C ₁₀ H ₁₈ O	33.387	866900	0.48
1-.alpha.-Terpineol	C ₁₀ H ₁₈ O	33.687	705730	0.39
Borneol	C ₁₀ H ₁₈ O	33.812	1739641	0.96
Isopulegone	C ₁₀ H ₁₈ O	34.295	1866669	1.03
.alpha.-TERPINEOL	C ₁₀ H ₁₈ O	35.528	1944952	1.07
PULEGONE	C ₁₀ H ₁₈ O	39.52	119341441	65.76
CARVONE	C ₁₀ H ₁₄ O	39.67	652346	0.36
PIPERITONE	C ₁₀ H ₁₆ O	40.445	313191	0.17
tetrahydroedulan C	C ₁₃ H ₂₄ O	42.995	275424	0.15
PIPERITENONE	C ₁₀ H ₁₄ O	47.412	5761871	3.17
.beta.-CARYOPHYLLENE \$\$	C ₁₅ H ₂₄	53.228	4160356	2.29
LINALYL FORMATE	C ₁₁ H ₁₈ O ₂	55.328	265636	0.15
GERMACRENE-D	C ₁₅ H ₂₄	56.803	1024898	0.56
germacrene d	C ₁₅ H ₂₄	58.437	517374	0.29
.TAU.-CADINOL	C ₁₅ H ₂₆ O	64.02	1467173	0.81

*RT is retention time, AREA is peak volume, (Area %) is percent of area

The results agree with the observations of previous researchers. Antibacterial activity of garlic powder on *E. coli* O157:H7 was also reported (Sasaki *et al.*, 1999). However, garlic extract did not show any inhibitory effect on the growth of *L. monocytogenes*. The results were also comparable to those of other authors (Kumar and Berawal, 1998; Thongson *et al.*, 2004; Marques *et al.*, 2008; Irkin and Korukluoglu, 2009), who had reported reduced activity of garlic extract on *L. monocytogenes*, suggesting that Gram positive organisms may be better equipped naturally to prevent the action of garlic extract. The antibacterial activity of garlic is reported to be due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphide (Avato *et al.*, 2000). It is postulated that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group. Garlic can be used as a potent inhibitor of food pathogens. The use of garlic would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods. Table 2 represents the antibacterial activity of rosemary extract against *Staphylococcus*

aureus, *E. coli* and *Salmonella* spp. When tested against *L. monocytogenes*, *B. cereus* and *Candida albicans* were found to be sensitive. *Listeria monocytogenes* was found to be highly sensitive to all the extracts, except basil and clove extract which shown highly effect. Ginger extract was also found to have moderate antibacterial properties against *E. coli*. However, our results compare well with previous observations (Chen *et al.*, 1985). Ginger extract did not show any antibacterial activity against all other bacteria. These results are contradictory to the observations of others authors (Srinivasan and Lakshmanaperumalsamy, 1993; Suresh *et al.*, 2004), who had reported moderate activity of ginger extract on *E. coli*. The extracts of cumin and black pepper also did not show any antibacterial activity. The natural products of plant origin have played a significant role in the search for therapeutic drugs, such as cineole, pinene, carvone, eugenol and allyl disulfide from herbal under study. The search for new antimicrobials is very important in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria.

Since the majority of microbes were resistant to many antibiotics, only nalidixic acid-30 µg, nystatin 100 units, streptomycin 10 µg and tetracycline 30 µg were used as reference because the resistance of the three drugs of them was common in all the microorganisms tested. The inhibition zones obtained in this study for the essential oils of spices/herbals were compared with the zones obtained from the four commercial antifungal and antibiotics (Pang *et al.*, 1995). As can be seen from Table 2, *Listeria monocytogenes*, *Salmonella* spp. and *Candidia albicans* were resistant to nalidixic acid (30 µg), nystatin (100 units), streptomycin (10 µg) and tetracycline 30 µg, while *E. coli* and *B. cereus* were sensitive only to nalidixic acid (30 µg) (17 mm). On the other hand, *S. aureus* was resistant to nystatin (100 units) but highly sensitive to streptomycin (10 µg) (16 mm), tetracycline (30 µg) (30 mm) and nalidixic acid (30 µg) (20 mm). Therefore, our results revealed the importance of essential oils spices/herbals to control resistant microorganisms. Furthermore, essential oils of basil and clove can be used as antimicrobial because of their high effectiveness on all microorganisms tested above.

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³ مختبرات المعاينة والتشخيص والتحليل والاستشارات (IDAC)، قسم الأمراض، ص ب ٧١٣٣، الخرج ١١٩٤٢، المملكة العربية السعودية

(قدم للنشر في ١٤٣٠/٢/١ هـ؛ وقبل للنشر في ١٤٣٠/٦/٣٠ هـ)

: زيوت عطرية، نشاط مضاد للميكروبات، اشريشا قولون، سالمونيل، ستريا مونوسيتوجنيسس، عنقودية الذهبية، العصية
سيريومية، الخميرة البيضاء.

■ تم تقييم النشاط المضاد للبكتيري للعديد من الزيوت العطرية والمستخلصة من حصا ألبان (إكليل الجبل)، والحبق، والنعناع، والثوم، والزنجبيل، والمرامية، والبابونج، والكمون، والحبّة السوداء، والقرنفل، والفلفل الأسود ضد ستة أنواع من الميكروبات تشمل الاشريشا القولونية، والسالمونيلة، ولستريا مونوسيتوجنيسس، والمكورة العنقودية الذهبية، والعصية السيريومية، والخميرة البيضاء. استخدمت طريقة القرص المنتشر والإطباق ذات الأبيار في فحص نشاط الزيوت العطرية المضاد للميكروبات وأُجري الاختبار على أساس ثلاث مكررات. لقحت الأطباق التي تحتوي على منبت MHA و SDA وحضنت على درجة حرارة ٣٧°م و ٢٥°م لمدة ٢٤ ساعة للبكتريا والخميرة على التوالي والقطر المانع للنمو قيس للزيوت مع الميكروبات المختلفة. زيت الحبق والقرنفل أظهر تأثيراً جيداً على جميع الميكروبات المختبرة، أما الزيوت من حصا ألبان والمرامية والنعناع فقد أظهرت نتائج مضادة للبكتيريا العنقودية الذهبية والاشريشا القولونية والسالمونيلة بدرجة جيدة، أما الثوم والزنجبيل والحبّة السوداء فمنعت نمو البكتريا العنقودية الذهبية فقط، أما زيت البابونج فقد ثبت نمو الخميرة البيضاء، ولكن الزيت الكمون والفلفل الأسود لم يكن له أي تأثير مضاد على جميع الميكروبات المختبرة. أظهرت معظم الزيوت نشاطاً مضاداً للميكروبات أفضل من بعض المضادات التي استخدمت (النيستاتين، وحامض نلذكس، والستربتوميسين، والتتراسيكلين).

