IN THE NAME OF ALLAH,
MOST GRACIOUS, MOST MERCIFUL

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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 37ºC and 25ºC 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 oficinalis* (rosemary), *Mentha longifolia* (basil), *Mentha piperita* (mint), *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Salvia officnalis* (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	*Rosmarinus oficinalis*
Basil	*Mentha longifolia*
Mint	*Mentha piperita* L.
Garlic	*Allium sativum*
Ginger	*Zingiber officinale*
Sage	*Salvia officnalis*
Chamomile	*Chamaemelum nobile*
Cumin	*Cuminum Cyminum*
Black seed	*Nigella sativa*
Clove	*Syzygium aromaticum*
Black Pepper	*Piper nigium*

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: 75–250ºC, at 107 min analysis time, injector temperature: 250ºC; 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: 230ºC; MS source temperature: 230ºC; MS acquisition mode, temperature: 230ºC. 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 4ºC 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 37ºC and 25ºC 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 10^6-10^7 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 25ºC for 24 h respectively. Using a sterile cotton swab, the inoculums suspension of each bacteria and yeast were swabbed on the entire surface of Mueller Hinton Agar and Sabouraud Dextrose Agar, then the inoculums were 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 37ºC and 25ºC 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 inoculums 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 37ºC and 25ºC 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 (Sagfldıc *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 Mulluer Hinton Broth for 24 h at 37ºC. 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 37ºC 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 bepper 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 "-pinene, "-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	***Listeria monocytogenes***	***Staph. aureus***	***E. coli***	***Salmonella spp.***	***Bacillus cereus***	***Candida albicans***
Rosemary حصا لبان	R	20mm ± 0.76^{b}	16mm ± 0.76^{b}	17mm ± 0.29^{ab}	R	R
Basil حبق	15mm ± 1.04^{a}	18mm ± 0.29^{bc}	16mm ± 0.76^{b}	16mm ± 1.32^{b}	20mm ± 0.58^{a}	17mm ± 0.76^{c}
Mint نعناع	R	19mm ± 1.53^{bc}	15mm ± 1.0^{b}	R	R	R
Garlic ثوم	R	R	20mm ± 1.04^{a}	R	R	R
Ginger زنجبيل	R	R	12mm ± 0.58^{c}	R	R	R
Sage ميرامیه	R	19mm ± 1.50^{bc}	17mm ± 0.76^{b}	19mm ± 1.53^{a}	R	R
Chamomile بابونج	R	R	R	R	R	20mm ± 0.76^{a}
Cumin كمون	R	R	R	R	R	R
Black seed حبة سوداء	R	18mm ± 1.04^{bc}	R	R	R	R
Clove قرنفل	16mm ± 0.58^{a}	14mm ± 0.50^{d}	15mm ± 0.58^{b}	13mm ± 0.58^{c}	11mm ± 0.50^{c}	15mm ± 0.58^{b}
Black pepper فلفل أسود	R	R	R	R	R	R
Streptomycin 10µg	R	16mm ± 0.58^{cd}	R	R	R	R
Nystatin 100 units	R	R	R	R	R	R
Tetracyclin 30µg	R	30mm ± 0.58^{a}	R	R	R	R
Nalidixic acid 30µg	R	20mm ± 0.58^{b}	17mm ± 1.53^{b}	R	17mm ± 0.58^{b}	R

Mean in the same column followed by different subscripted are significant different (P≤ 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.-pipene	$C_{10}H_{16}$	16.695	9383464	10.08
Ethanone, 1-(3-ethyloxiranyl)	$C_6H_{10}O_2$	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	C10H16O	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 $$ alpha.-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	C10 H14 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_3H_6S $_{2,106}$	12.387	483112	2.03
Oil garlic Allyl sulfide	C_6 H_{10} $S_{,114}$	12.82	210345	0.88
Allyl disulfide	$C_6H_{10}S$ $_{2,146}$	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	$C_6H_{10}S$ $_{3,178}$	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} H18 $O_{,154}$	23.128	902941	0.1
LINALOOL L	C_{10} H18 $O_{,154}$	27.937	1654196	0.19
MENTHONE	C_{10} H18 $O_{,154}$	32.528	3946068	0.45
BORNEOL L	C_{10} H18 $O_{,154}$	33.803	6645395	0.76
alpha.-TERPINEOL	C_{10} H18 $O_{,154}$	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.-pipene	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	C10H14,134	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} $O_{,148}$	4282	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,	$C_{20}H_{34}O_{,290}$	60.67	14295440	3.48
1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)	$C_{13}H_{12}O_{2,200}$	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	$_{128,}C_9H_{20}$	11.303	933287	0.14
Ethylcyclohexane	$_{112,}C_8H_{16}$	11.878	790161	0.12
2,3-Dimethylheptane	$_{128,}C_9H_{20}$	**12.437**	**248742**	**0.04**
1-Phenyl-3,3-dimethylbutane	$_{162,}C_{12}H_{18}$	13.062	1046029	0.16
p-Xylene	$_{106,}C_8H_{10}$	13.437	3989117	0.62
n-Nonane Shellsol 140	$_{128,}C_9H_{20}$	14.353	3325365	0.52
m-Xylene	$_{106,}C_8H_{10}$	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, pipene, 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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المحتوى الكيميائي والنشاط المضاد البكتيري للزيوت العطرية المستخلصة من التوابل والأعشاب العطرية المستخدمة في المملكة العربية السعودية

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

ملخص البحث. تم تقييم النشاط المضاد البكتيري للعديد من الزيوت العطرية والمستخلصة من حصا ألبان (إكليل الجبل)، والحبق، والنعناع، والثوم، والزنجبيل، والمرامية، والبابونج، والكمون، والحبة 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 و٢٥°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 القولوني والسالمونيلة بدرجة جيدة، أما الثوم والزنجبيل والحبة السوداء فمنعت نمو البكتريا العنقودية الذهبية فقط، أما زيت البابونج فقد ثبط 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 نلدكس، والستربتوميسين، والتتراسيكلين).