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Chemical Composition, Antioxidant and Antimicrobial Properties of the Essential Oils and Extracts of Some Aromatic Plants.

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ABSTRACT

Essential oils and ethanolic extracts of lemongrass (*Cymbopgon citratus),* thyme *(Thymus vulgaris) and* marjoram (*Origanum marjorana*) were screened for their possible antioxidant and antimicrobial properties as well as their chemical compositions. According to gas chromatography (GC/ MS) were identified, 39, 16 and 54 compounds of essential oils lemongrass, thyme and marjoram, respectively. The major constituents were 9-cis-retinal , δ-2-carene and isomethyl-α-ionol for lemongrass; carvacrol, 2,5-dihydroxybenzoic acid and α –pinene for thyme and 9-cis-retinal, t-butylhydroquinone and p-mentha-3,8-diene for marjoram. The major phenolic and flavonoid compounds of ethanolic extracts identified using HPLC were benzoic , coumarin , hisperidin and hispertins for lemongrass; salicylic ,ellagic , hisperidin and rosmarinic for thyme and benzoic, pyrogalol, , hisperidin and narerigin for marjoram. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging. Results showed the superior of ethanolic extracts (low IC_{50}) at lower concentrations than essential oils for scavenged DPPH. The antimicrobial activity (by disc diffusion tests) of different plant essential oils and extracts against 11 strains of microorganisms was done. The essential oils of the tested plants have a stronger antimicrobial activity than those of ethanolic extracts which did not exhibit antimicrobial activity against some microbial strains.

Key words: lemongrass, thyme and marjoram essential oils, ethanolic extracts, chemical composition, antimicrobial activity, antioxidant activity.

Introduction

In the last decades, the essential oils and various plant extracts have been of great interest as the sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the foods preservation from the toxic effects of the oxidants. Particularly, the antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Kelen and Tepe, 2008).

The increasing interest in natural dietary components has focused attention on plants used as food or spices which are a rich source of bionutrients or bio-active phytochemicals. Phenolic phytochemicals are large group of substances and were found in significant quantities in vegetables, fruits, spices, and seeds. Since they have been regarded as possible antioxidants, their roles in food industry and in chemoprevention of diseases resulting from oxidative stress have become an area of active research in many fields (Undeger *et al*., 2009)

Preservation of food from degradation during production, storage and marketing is an important issue in the food industries. Chemical and/or microbial dilapidation of industrially produced foods can inversely affect their quality and even lead to the appearance of toxic materials within them. Herbs and spices have been employed since ancient times as flavouring and storing agents for food, but only in the last decade scientific research has focused its interest on their essential oils and extracts as natural sources of antimicrobial and antioxidant compounds. From this point of view, they have gained a special attention as safer alternative additives for food preservation. Governmental authorities and consumers are concerned about the safety of food and about the potential effects of synthetic additives on health. In fact, according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have already been documented. For example, these substances can show carcinogenic effects in living such as BHT induces liver tumours in long-term experiments and BHA induces in animals tumours of the forestomach, which are dose dependent (Kahl and Kappus,1993) . Hence, the presence of antioxidants in foods is essential for their quality, retention and safety. Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Tenore *et al*., 2011).

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As can be seen several studies have demonstrated the antibacterial and antioxidant properties of lemongrass, thyme and marjoram using in vitro assays. The objectives of this study were: To determine the chemical composition of different three plant (lemongrass, thyme and marjoram) essential oils and extracts by GC/MS and HPLC. To evaluate the effectiveness of the different plant essential oils and extracts as potential antimicrobial using the disc diffusion against 11 strains of spoilage and pathogen microorganisms, as well as to assess the antioxidant activity of different plant oils and extracts by (DPPH scavenging activity) and compare their activities with a synthetic antioxidant (BHT).

Materials and Methods

Materials:

Aromatic plants:

Dried aerial parts of lemongrass (*Cymbopgon citratus*), thyme (*Thymus vulgaris*) and marjoram (*Origanum marjorana*), were obtained from Royal company, El-Menia, Egypt, while essential oils of lemongrass, thyme and marjoram were obtained from Kato aromatic company ,Giza, Egypt.

Microbial strains:

The essential oils and ethanolic extracts of the aromatic plants were individually tested against a panel of microorganisms, including strains of Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 43495 , *Escherichia coli* ATCC 25922, *Listeria monocytogenes* NICPBP 54002 and *Salmonella typhimurium* ATCC14028) and strains of Gram-positive bacteria (*Bacillus cereus* DSM 351, *Klebseilla pneumonia* ATCC 1705 ,*Staphylococcus aureus* ATCC 12600 *, Lactobacilus plantarum ATCC14917 and Lactobacilus acidophilus ATCC20552) and two strains of yeasts (Candida albicans* DSM 11225 and *Saccharomyces cerevisiae* NRRL 1095) . These strains were obtained from the Microbiological Resources Center, (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Culture media:

Tryptone glucose extract agar was composed of : (Beef Extract 1.0 g, tryptone 5.0 g, glucose 2.0 g, agar No.(1) 15.0 g and 1000 ml distilled water) however yeast and malt extract agar (Y.M.Agar) was composed of : (Malt 3.0 g, yeast extract 3.0 g, peptone 5.0 g, dextrose 10.0 g, agar No.(1) 15.0 g and 1000 ml distilled water) according to APHA, (1992), while M.R.S.Agar was composed of : (Pepton 10.0 g, beef extract 10.0 g, yeast extract 5.0 g, glucose 20.0g, dipotassium hydrogen phosphate 2.0 g, sodium acetate 5.0 g, ammonium citrate 2.0 g, magnesium sulphate 0.2 g, manganous sulphate 0.05 g and tween 80 1.0 ml and 1000 ml distilled water) according to Oxoid, (1990) method.

Methods:

Preparation of plant ethanolic extracts:

Aromatic plants samples were extracted in 80% ethanol at ratio 1 /5 (w:v) of parts of plant : ethanol at 45–55°C in a water bath for 24h.The obtained ethanolic solution was filtered, concentrated in a water bath under vacuum using rotary evaporator, then dried under vacuum at 50°C for 8 h and kept in the dark at 4°C until used (Park *et al*., 2012).

GC-MS analysis of the essential oils:

The Gas Chromatography / Mass Spectrometry (GC/MS) technique was used for separation and identification the components of lemongrass, thyme and marjoram essential oils based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILLEY library. Analyses were performed using a G.C (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm I . d. and 0.25 µm film thickness) (Vasudeva and Sharma., 2012).

Fractionation and identification of phenolic compounds of ethanolic extracts by HPLC:

The phenolic compounds of ethanolic extracts were identified with Hewlett – Packard HPLC (Model 1100), using a hypersil C18 reversed-phase column (25 x 4.6 mm) with 5 µm particle size. The phenolic compounds were identified by comparing retention times and UV–VIS spectra with those of pure standards to indicate the preparations of standards and the range of calibration curves (Ozkan and Ozcan, 2014).

Evaluation of antimicrobial activity (disc diffusion method):

The agar disc diffusion method was employed for the determination of antimicrobial activities of the different plant essential oils and extracts. The extracts (200mg/ml) in suitable solvent were sterilized by filtration through 0.22µm nylon filter. Sterile filter paper discs (6 mm diameter), were impregnated with 50µl of essential oils and ethanolic extracts, then placed in the center of the inoculated Petri dishes. The control sample was prepared using the same solvent employed to dissolve the plant extracts. These plates after staying at 4 °C for 2 h were incubated at 37 °C for 24 h for bacteria, at 30°C for 48 h for yeast. The diameter of the zone of inhibition around each of the discs (disc diameter included) was taken as measured of the antimicrobial activity (Al Haiali *et al*., 2012 and Helal *et al*., 2006).

Evaluation of antioxidant activity (DPPH test) :

The free radical scavenging activity of different essential oils and extracts was measured by the 2, 2-diphenyl-1 picryl-hydrazil (DPPH) method in which the hydrogen atoms or electrons donation ability of the corresponding extracts was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. 2 ml of 0.002% methanolic solution of DPPH was added to 2ml of various concentrations of the extracts in methanol (5-200 µg/ml) and (2.5 – 25 µl/ml) for essential oils . After a 30 min incubation period at ambient temperature, the absorbance was measured against a blank at 517 nm. The scavenging activity was calculated using the formula: DPPH scavenging effect % = (A blank –A sample / A blank) x 100

Where A blank is the absorbance of the control reaction (containing all reagents except the test sample) and A sample is the absorbance in the presence of the tested sample (Rekha *et al*., 2012).

The concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration (Karabegovic *et al*., 2011).

Determination of total phenolic and total flavonoids contents:

Total phenolic and total flavonoids contents were determined according to Shiban *et al*.,(2012).

Results and Discussion

Chemical composition of essential oils of some aromatic plants:

The components of essential oils of lemongrass, thyme and marjoram were identified by GC/MS spectrometer analysis. Their percentage compositions, calculated as the ratio of peak area to the total chromatographic area, were listed in Table (1). About 39, 16 and 54 constituents were identified from lemongrass, thyme and marjoram essential oils, respectively. The most abundant components in lemongrass were 9-cis-retinal (22.82 %), δ-2-carene (10.68%), isomethyl-α-ionol (10.68%), 2,3-dimethylhydroquinone (10.33%) , p-mentha-3,8-diene (8.59%), β-terpinol (6.52%) and isogeraniol (2.57%).

The main components of the essential oil of thyme were carvacrol (81.15%), 2,5-dihydroxybenzoic acid (5.21%), α –pinene (2.55%) and cymene (2.45%), where results in Table (1) showed also that, 9-cis-retinal (11.12%), t-butylhydroquinone (7.75%), p-mentha-3,8-diene (6.70%), δ-2-carene (6.56%), trans-2,3-dimethoxycinnamic acid (5.94) and 8-Hydroxylinalool (5.51) were formed the majority of marjoram essential oil. This analysis is matching with those obtained by Snoussi *et al*., (2008) who found the main compounds of the essential oil of thyme were carvacrol (60.27%), α –pinene (2.83%) and cymene (7.58%) and those obtained by Boukhatem *et al*., (2014) who found some of main compounds of the essential oil of lemongrass are geraniol 2.42% and carene 0.99% and those reported by Tarjdn *et al*., (2002) who found the main compounds of the essential oil of marjoram were terpineol and linalool.

Fractionation and identification of phenolic and flavonoid components of three aromatic plants.

The phenolic and flavonoid components of lemongrass, thyme and marjoram ethanolic extracts were fractionated and identified using HPLC, results are shown in Table (2). It could be noticed that, the 28 compounds were fractionated, results showed that the lemongrass phenolic compounds were benzoic , coumarin , epicatechin , salicylic, ellagic and catechin were found in high concentrations (1185.28 ppm),(526.90 ppm) ,(417.75ppm), (305.73 ppm) , (262.72 ppm) and (140.19ppm), respectively.

While, the main flavonoid compounds of lemongrass were Hisperidin (3026.20 ppm) followed by Hispertins (2827.54 ppm), Quercitrin (1423.31 ppm) and Kaempferol (790.22 ppm). The main phenolic compounds in thyme extract were salicylic (2489.01 ppm), ellagic (1140.42ppm), pyrogalol (532.39 ppm) and benzoic (446.08 ppm), while the main flavonoid compounds were hisperidin (34798.0 ppm), rosmarinic (8672.01 ppm), quercitrin (4817.73ppm), narerigin (1817.42 ppm) and luteolin (87.99 ppm). On the other hand the main phenolic of marjoram were benzoic (851.26 ppm), pyrogalol (638.14 ppm), coumarin (549.64 ppm), P_hydroxy benzoic (442.27 ppm), salicylic (362.36 ppm)., while the main flavonoid compounds were hisperidin (15159.20

ppm), narerigin (6099.86 ppm), rutin (1485.32 ppm) and quercitrin (703.42 ppm). It could be noticed that the total phenolic content were 321.00, 384.50 and 375.50 (mg gallic acid/g dry extract), while total flavonoid

Table 1: Chemical composition of the essential oils of lemongrass, thyme and marjoram (%) identified by GC/MS.

No.	Name	Lemongrass %	Thyme %	Marjoram %
1	δ-2-Carene	10.68	-	6.56
2	p-Mentha-3,8-diene	8.59	-	6.70
3	β-Terpinol	6.52	0.20	0.34
4	9-cis-Retinal	22.82	-	11.12
5	α-Terpineol	0.50	-	-
6	Isogeraniol	2.57	2.16	6.23
7	cis-Carvol	0.19	-	0.09
8	cis-Verbenol	1.01	-	3.58
9	7,8-Dihydro- α-ionone	4.23	-	0.34
10	Isopergol	0.55	0.29	3.38
11	8-Hydroxylinalool	2.31	-	5.51
12	t-Butylhydroquinone	0.40	0.40	7.75
13	5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexane-1-ol	3.27	0.58	4.65
14	(±)-Solanone	3.38	-	0.62
15	α –pinene oxide	1.37	2.55	-
16	Isomethyl-α-ionol	10.68	0.26	3.05
17	(+)-(R)-pulegone	0.11	-	-
18	2,3-Dimethylhydroquinone	10.33	0.15	1.57
19	Isolongifolol	0.12	-	0.10
20	5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexene-1,4-diol	1.42	-	0.19
21	Pinane-2α,3α-diol	1.04	-	0.61
22	4-Pinanol	0.95	0.24	0.44
23	3-Methoxy-2,4,6-trimethylphemol	0.91	-	0.07
24	α –Selinene	0.24	-	1.96
25	Valencene	0.37	-	2.40
26	δ –Cadinene	0.11	-	0.50
27	Vitamin A aldehyde	0.18	-	0.31
28	Caryophylene oxide	0.12	-	1.33
29	Cis-11-Eicosenoic acid	0.11	-	0.18
30	Drimenol	0.47	-	0.30
31	Trypethelone	0.27	-	0.16
32	5,7,3',4'-Tetrahydroxyflavone	0.36	-	0.39
33	Trans-Geranylgeraniol	0.17	-	0.33
34	γ – Himachalene	1.11	-	-
35	Catechin	0.47	-	-
36	(-)-Campherenone	0.27	0.36	-
37	2-Bornanol, 2-methyl	1.10	-	-
38	Tetrahydrocannabinol(nor-delta-9-HOOC)	0.56	-	-
39	β-Cryptoxanthin	0.12	-	-
40	Cymene	-	2.45	-
41	D-(+)-Xylose	-	2.83	-
42	2,5-Dihydroxybenzoic acid	-	5.21	4.72
43	Carvacrol	-	81.15	-
44	Ethyl palmitate	-	0.24	-
45	Homovanillic acid	-	-	1.77
46	Gentisic acid	-	-	1.05
47	4-Hydroxy-2-methoxybenzaldehyde	-	-	0.36
48	δ –Tocophero	-	-	3.16
49	Cedrenol	-	-	0.53
50	1,4-dihydroxy-p-menth-2-ene	-	-	1.23
51	Nerol acetate	-	-	0.44
52	Geranyl-α-terpinene	-	-	0.12
53	A-Guaiene	-	-	0.25
54	Methyleugenol	-	-	0.10
55	α-Humulene	-	-	0.14
56	Globulol	-	-	0.37
57	Cis-Z-α-Bisabolene epoxide	-	-	2.63
58	Guaiol	-	-	0.86
59	β-Selinenol	-	-	0.72
60	2,6-Di-tert-butylbenzoquinone	-	-	2.34
61	Spathulenol	-	-	0.35
62	Xanthone,1-hydroxy-2,3,5-trimethoxy-	-	-	0.18
63	Cis-10-Heptadecenoic acid	-	-	1.10
64	Agatholic acid	-	-	0.95
65	Trypethelone	-	-	0.16
66	Trnas-2,3-Dimethoxycinnamic acid	-	0.94	5.94

content were 138.00, 285.02 and 266.92(mg quercetine /g dry extract) for lemongrass, thyme and marjoram ethanolic extracts ,respectively. These results are in agreement with those obtained by Bobis *et al*., (2015), they found the main phenolic and flavonoid compounds of thyme were chlorogenic acid, caffeic acid, rosmarinic and luteolin. Also, Ghasemzadeh *et al.,* (2012), found that, the main phenolic and flavonoid compounds of lemongrass extract were Catechin and Kaempferol, while Abo El-Maati *et al.,* (2012) recorded that the total phenolic content of thyme and marjoram ethanolic extracts were 233 and 204 (mg gallic acid/g dry extract),respectively.

Table 2: Fractionation and identification of phenolic and flavonoid components of three aromatic plants by HPLC.

Compound No.	Components	Lemongrass (ppm)	Thyme (ppm)	Marjoram (ppm)
	phenolic compounds			
1	Gallic	0.40	41.15	6.45
2	Catechin	140.19	61.49	36.36
3	Pyrogalol	10.87	532.39	638.14
4	Epicatechin	417.75	363.38	123.37
5	Chlorogenic	37.76	327.08	111.05
6	Aminobenzoic	8.02	22.95	4.72
7	Catechol	53.33	509.95	88.26
8	Caffeine	18.45	6.06	45.51
9	P_Hydroxy benzoic	122.88	215.31	442.27
10	Caffeic	56.79	135.58	22.29
11	Vanilic	84.25	24.34	176.76
12	Ellagic	262.72	1140.42	16.23
13	Benzoic	1185.28	446.08	851.26
14	Salicylic	305.73	2489.01	362.36
15	Coumarin	526.90	312.19	549.64
16	Cinnamic	12.65	19.33	4.18
17	Protocatchoic	---	90.01	34.14
Total phenolic content (mg gallic acid/g dry extract)		321.00	384.50	375.50
	Flavonoid compounds			
18	Rutin	360.64	230.44	1485.32
19	Hisperidin	3026.20	34798.00	15159.20
20	Rosmarinic	237.48	8672.01	121.81
21	Quercitrin	1423.31	4817.73	703.42
22	Querctin	276.71	485.92	151.27
23	Narenginin	14.57	194.32	87.32
24	Kaempferol	790.22	960.10	533.80
25	Hispertins	2827.54	230.37	94.07
26	Flavone	47.86	47.17	117.86
27	Narerigin	----	1817.42	6099.86
28	Luteolin	----	87.99	81.96
Total flavonoid content (mg quercetine /g dry extract)		138.00	285.02	266.92

Antioxidant activity of essential oils of some aromatic plant.

Data in table (3) showed the percentage scavenging activity of essential oils of lemongrass, thyme and marjoram of the DPPH free radical compared with (BHT) at 200 ppm. A high antioxidant activity was observed for marjoram essential oil (45.36%) at 2.5 µl/ml and (82.84%) at 25 µl/ml followed by lemongrass (42.09 %) at 2.5 µl/ml and (79.84%) at 25 µl/ml . The essential oils of thyme were also good radical scavenging with the inhibition (40.38%) and (70.47%) at 2.5 and 25 µl/ml, respectively.

Table 3: DPPH scavenging activity (%) of essential oils of some aromatic plants.

Concentrations (µl/ml)	DPPH scavenging activity (%)		
	Lemongrass	Thyme	Marjoram
2.5	42.09	40.38	45.36
5	61.58	60.9	63.95
10	73.00	62.19	74.53
15	76.04	65.69	78.74
20	77.94	69.85	80.47
25	79.84	70.47	82.84
BHT (200 ppm)	73.48 %		

Data in the same table indicated that, the concentration of 10 to 25 µl/ml of marjoram essential oil exhibited antioxidant activity from 74.53 to 82.84%, and antioxidant activity of lemongrass essential oil at 15 to 25µl/ml showed 76.04 and 79.84%, respectively. While, antioxidant activity of BHT was 73.48% at 200ppm, finally the antioxidant activity of thyme essential oil at (25 µl/ml) was 70.47%.

These results are in agreement with those of (Grigore *et al*., 2010) who found that the DPPH radical scavenging activities of *Thymus vulgaris* volatile oil obtained by two different methods at doses higher than 3mg/mL, both samples exhibit over 50% inhibition on DPPH free radical, while (Saleh *et al.,* 2010) recorded

that the antioxidant activity of the *Origanum marjoram* essential oil at 100 mg/mL causing more than 90% inhibition of DPPH.

Antioxidant activity of some aromatic plant extracts (DPPH radical scavenging activity):

Free radical–scavenging capacity of ethanolic extracts of lemongrass, thyme and marjoram measured by DPPH assay at different concentrations and compared with (BHT) at 200 ppm were given in Table (4). DPPH radical scavenging activities (%) were increased with increasing the concentration of tested ethanolic extracts from 5 to 200µg/ml. Data in the same table showed that, the extract of thyme recorded the highest inhibition percentage of radical DPPH from 45.47 % at 5 µg/ml to 74.34 and 75.07 % At 100 and 200 µg/ml, respectively. These last two concentrations were higher than the DPPH radical scavenging activity of BHT at 200 ppm which recorded 73.48 %, while the extract of marjoram and lemongrass showed the inhibition percentage of radical DPPH from 37.64 and 32.8 % at 5 µg/ml to 68.95 and 72.55 % at 200 µg/ml, respectively . This percentages were lower than the inhibition percentage of BHT (73.48 %) at 200 ppm.

Table 4: DPPH scavenging activity (%) of the ethanolic extracts of some aromatic plants.

Concentrations (µg/ml)	DPPH scavenging activity (%) of the ethanolic extracts		
	Lemongrass	Thyme	Marjoram
5	32.8	45.47	37.64
10	47.15	55.42	50.28
20	54.52	65.88	62.07
50	63.71	73.41	67.48
100	67.44	74.34	69.51
200	68.95	75.07	72.55
BHT (200 ppm)	73.48 %		

These results are in agreement with those obtained by (Amarowicz *et al*., 2009). They found that the radical-scavenging capacities of the ethanolic extracts of oregano, thyme and marjoram in DPPH test were in the order of oregano > thyme > marjoram.

Selim, (2011) stated that the methanol extract of lemongrass had shown significant DPPH radical scavenging activity (39.38 and 59.98 %) at 10 and 50 µg/ml, respectively.

Concentrations of essential oils and ethanolic extracts providing 50% inhibition of DPPH (IC50).

Concentrations of different plant extracts and essential oils providing 50% inhibition of DPPH (IC50) were used in evaluating the efficiency of different samples. Data in Table (5) showed that the antioxidant power of the ethanolic extracts, according to IC50 decreased in the order thyme> marjoram > lemongrass. The IC50 values of the ethanolic extracts studied were found to be 8.00, 10.00 and 12.00 µg/ml for thyme, marjoram and lemongrass, respectively.

Table 5: Concentrations of essential oils and ethanolic extracts providing 50% inhibition of DPPH (IC50).

Tested	Ethanolic extracts IC50 (µg/ml)	Essential oils IC50 (µl/ml)
Lemongrass	12.00	3.50
Thyme	8.00	3.75
Marjoram	10.00	3.25

Also, data in the same table showed the free radical scavenging capacities of the tested essential oils are as the following order: marjoram > lemongrass > thyme and the IC50 values of the essential oils recorded 3.25, 3.50 and 3.75 µl/ml, respectively. These results are in agreement with those obtained by Jumepaeng *et al.,* (2013) who reported that IC50 value of the antioxidant capacity of lemongrass oil was 4.73 µL/mL, while Benchikha *et al.,*(2013) found that the IC50 value of ethanolic extract of the *Origanum majorana* was 13.7 µg/ml.

Antimicrobial activity of essential oils and extracts of some aromatic plants.

Initial screening of the antimicrobial activity of the investigated essential oils and extracts were studied against tested microorganisms using the agar disc diffusion assay, which was assessed by the presence and absence of inhibition zones. The antimicrobial activity of essential oils can be classified into three levels: weak activity (inhibition zone ≤ 12mm), moderate activity (12mm<inhibition zone<20mm) and strong activity (inhibition zone ≥ 20), (Lv *et al.,* 2011).

The antimicrobial activity of the essential oils and ethanolic extracts of lemongrass, thyme and marjoram were studied against 9 strains of bacteria and 2 strains of yeasts using the filter paper disc agar diffusion technique and the results are given in Tables (6).

The data of the inhibition zones (mm) of various microorganisms indicated that the essential oil of thyme exhibited the highest antimicrobial activity against all the strains of microorganisms, especially for Gram-positive bacteria (*Bacillus cereus*, *Klebseilla pneumonia*, *Staphylococcus aureus* and *Salmonella typhimurium*)

and yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) , whose zones of inhibition ranged from 20 to 53mm. On the other hand the essential oil of lemongrass showed strong activity against (*Klebseilla pneumonia, Staphylococcus aureus, Pseudomonas fluorescens, Escherichia coli, Salmonella typhimurium and Saccharomyces cerevisiae*) with inhibition zones ranged from 20 to 33 mm ,while lemongrass oil showed moderate activity against (*Listeria monocytogenes and Lactobacilus plantarum*) with inhibition zones 14 mm and weak activity against (*Candida albicans*) with inhibition zone 12 mm. The essential oil of marjoram showed strong activity against (*Staphylococcus aureus, Pseudomonas fluorescens and Candida albicans*) with inhibition zones 22, 22 and 20 mm, respectively. On the other hand, marjoram oil showed moderate activity against (*Klebseilla pneumonia, Escherichia coli, Salmonella typhimurium and Saccharomyces cerevisiae*) with inhibition zones ranged from 13 to 17.5 mm, while the marjoram oil have weak activity against (*Bacillus cereus, Listeria monocytogenes, Lactobacilus plantarum and Lactobacilus* acidophilus) with inhibition zones 9.0, 12.0, 12.0 and 9.0 mm, respectively . Data in the same table showed the, ethanolic extract of thyme have strong activity only against (*Klebseilla pneumonia*) with inhibition zone 21 mm, while thyme extract showed moderate and weak activities against other strains with inhibition zone ranged from 15.0 to 3.0 mm. The ethanolic extract of lemongrass have strong activity against (*Pseudomonas fluorescens and Salmonella typhimurium*) with inhibition zone 21 and 22mm, while showed moderate activity against (*Staphylococcus aureus*, *Escherichia coli and Listeria monocytogenes)* with inhibition zone 15.0, 17.0 and 15.0 mm, respectively. Also lemongrass showed weak activity against (*Bacillus cereus and Klebseilla* pneumonia) with inhibition zone 8.5 mm. On the other hand the ethanolic extract of marjoram have moderate activity against (*Pseudomonas fluorescens, Escherichia coli ,Listeria monocytogenes and Salmonella typhimurium)* with inhibition zones 13, 13, 14 and 19 mm, respectively. Also results showed that the ethanolic extract of marjoram have a weak activity against (*Bacillus cereus, Klebseilla pneumoniaand, Staphylococcus aureus and Candida albicans*) with inhibition zone 7,8,9,and 8 mm, respectively. On the other hand, the extract of lemongrass did not exhibit antimicrobial activity against (*Lactobacilus plantarum, Lactobacilus acidophilus, Candida albicans and Saccharomyces cerevisiae*), while marjoram extract didn't exhibit antimicrobial activity against (*Lactobacilus plantarum, Lactobacilus acidophilus, and Saccharomyces cerevisia)*.

Similar results were obtained by Helal *et al*., (2006) who stated that the essential oils of lemongrass, thyme and marjoram showed significantly stronger antimicrobial properties against (*Pseudomonas sp., Escherichia coli, Staphylococcus aureus, Bacillus cereus, Saccharomyces cerevisia and Candida albicans*) ,while Kansoh *et al*., (2000) recorded antimicrobial activity of thyme ethanolic extract against *(Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisia and Candida albicans)*. Also Al-Haiali *et al*., (2011) reported that lemongrass ethanolic extract has antimicrobial activity against *(Klebseilla sp., Staphylococcus aureus, Pseudomonas sp., Escherichia coli and Salmonella sp*.). It can be concluded that the essential oils of all tested plants have stronger activity than those of alcoholic extracts and the antimicrobial activities of essential oils are predominantly related to their main components Oussalah *et al*., (2006).

Table 6: Antimicrobial activity of essential oils and extracts of some aromatic plants.

Microorganisms	Inhibition zone diameter (mm) caused by essential oils and extracts					
	Lemongrass		Thyme		Marjoram	
	Oil	Extract	Oil	Extract	Oil	Extract
Bacillus cereus	7.0	8.50	20.0	9.0	9.0	7.0
Klebseilla pneumonia	20.0	8.50	43.0	21.0	17.5	8.0
Staphylococcus aureus	33.0	15.0	53.0	7.0	22.0	9.0
Pseudomonas fluorescens	30.0	21.0	33.0	12.0	22.0	13.0
Escherichia coli	22.0	17.0	26.0	3.0	13.0	13.0
Listeria monocytogenes	14.0	15.5	24.0	15.0	12.0	14.0
Salmonella typhimurium	20.0	22.0	37.0	10.0	14.0	19.0
Lactobacilus plantarum	14.0	-	30.0	8.0	12.0	-
Lactobacilus acidophilus	9.0	-	20.0	9.0	9.0	-
Candida albicans	12.0	-	30.0	11.0	20.0	8.0
Saccharomyces cerevisiae	22.0	-	50.0	10.0	13.0	-

Conclusion

Major aroma components found in the essential oils such as 9-cis-retinal , δ-2-carene and isomethyl-α-ionol for lemongrass; carvacrol, 2,5-dihydroxybenzoic acid and α –pinene for thyme and 9-cis-retinal, t-butylhydroquinone and p-mentha-3,8-diene for marjoram, while the major phenolic and flavonoid compounds of ethanolic extracts were benzoic , coumarin , hisperidin and hispertins for lemongrass; salicylic ,ellagic , hisperidin and rosmarinic for thyme and benzoic, pyrogalol, hisperidin and narerigin for marjoram. The antioxidant activity of ethanolic extracts and essential oils examined by the (DPPH) radical scavenging method. Data showed that the superior of ethanolic extracts (low IC_{50}) than essential oils for scavenged DPPH. In addition, the antimicrobial activity of essential oils and ethanolic extracts were determined by using disc

diffusion method. Data ascertained that essential oils had an inhibitory effect against all tested microbial strains, while ethanolic extracts did not exhibit antimicrobial activity against some microbial strains.

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