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Effective Chemical Compounds and Antibacterial Activities of Marjoram Leaves, Teucrium Leaves and Fennel Fruits Essential oils

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ABSTRACT

Essential oils have been used for centuries ago in traditional medicine, for industrial applications and as food preservatives due to their antimicrobial activity. The antibacterial activity of essential oil of marjoram, teucrium and fennel were tested against Gram-negative bacteria (*Escherichia coli O157 :H7, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Serratia marcescens*) and Gram-positive bacteria (*Bacillus anthrakoids, Bacillus cereus*, *Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus Micrococcus roseus, Staphylococcus aureus* and *Streptomyces spp.*) in *vitro* by well agar diffusion method. Results showed that, the obtained data of GC-MS analysis revealed that, the essential oil of marjoram leaves contained on five groups, namely hydrocarbons (61.48%), alcohols (17.87%), esters (10.79%), ketones (4.02%) and phenols (2.66%). The major identified compounds were α-phyllandrene (8.87%), α-terpinene (7.37%), γ-terpinene (7.25%). Terpinene 4-ol (6.33%), trans-sabinene hydrate (4.00%) and carvacrol (2.25%). While, the essential oil of *teucrium polium* leaves contained 28 essential effective compounds, α-pineneα (12.52%), Linalool (10.63 %), Caryophyllene oxide (9.69%), β-pinene (7.09%) and β-caryophllene (6.98%). And the essential oil of fennel fruits contained 18 essential effective compounds, Estragole (57.94%), Limonene (20.64%), Fenchone (7.22%), *trans*-anethole (4.99%) and α-pinene (3.61%), The essential oil of marjoram and fennel had the best inhibitory effect against tested bacteria. Meanwhile, teucrium essential oil exhibited good antibacterial effect against all of these tested bacteria except *Klebsiella pneumonia, Bacillus anthrakoid, Streptomyces spp.* and *Proteus vulgaris,* also, essential oil of marjoram and fennel showed similar inhibitory effect against tested bacterial species. These essential oils may be used not only to enhance the product flavor but also to inhibit and delays the microbiological growth. Hence they may be considered as natural preservatives without any side effects on human health.

Key words: Marjoram, Teucrium and Fennel essential oils, GC-MS analyses, Antimicrobial activity.

Introduction

Medicinal plants and herbs are invaluable resources, useful in daily life as food additives, flavours, fragrances, pharmaceuticals, colours or directly in medicine. This use of plants has a long history all over the world, and over the centuries, humanity developed better methods for the extraction of essential oils from such materials (Greathead, 2003). People have used Medicinal plants and herbs for thousands of years for their flavours, but an added benefit is the antimicrobial properties of the essential oils, which can significantly reduce spoilage. First used of spices and herbs Egyptian, Greeks, Romans and Middle East. These plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin *et al*, 1998).

The use of natural antioxidants has the advantage of being more acceptable by the consumers as these are considered as non chemical. In addition, they do not require safety tested before being used (Loliger *et al,* 1996). Among the methods used to reduce lipid oxidation is the application of antioxidants. Recently, natural antioxidants have been gaining increasing popularity. These antioxidants include extracts obtained from plants belonging to the Labiatae family, especially from rosemary due to its well-documented antioxidative properties rosemary extracts are produced on a commercial scale and used in the production of foodstuffs (Solomakos *et al*, 2008). Recent researches are now directed towards finding naturally occurring antioxidants of plant origin. Interest in natural antioxidants has increased dramatically in recent times due to the following reasons:(1) the possible carcinogenic effects of synthetic antioxidants in foods (Shahidi, 2008), (2) the antioxidative efficacy of a variety of phytochemicals, (3) the consensus that foods rich in certain phytochemicals can affect the a etiology and pathology of chronic diseases and the aging process (Dorman and Hiltunen, 2004). Natural extracts from herbs and spices have been used long time ago in meats flavoring agents. Plant extract also exhibit antimicrobial

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activity by interfering and destabilizing the operation of the phospholipids bilayer of the cell membrane, enzyme systems and genetic material constituents of essential oils exhibit significant antimicrobial properties when tested separately (Kalemba and Kunicha 2003).

Spices, herbs and their derivatives such as essential oil and oleoresin are used in foods for their flavors and aroma. Recently, there has been considerable emphasis on studies involving essential oils and extracts of spices and their constituents for inhibiting the growth of microbes. It has also been known for some time that certain crude drugs and spices contain substances with antifungal activity in their derivatives Deans and Svoboda (1990).

Herbal spices, being a promising source of phenolics, flavonoids, anthocyanins and arytenoids', are usually used to impart flavour and enhance the shelf-life of dishes and processed food products (Cieślik *et al,*. 2006).

Marjoram is also added to mouth rinse and its cures inflamed skin wounds and infections (McCarroll *et al.,* 2008; Sundar *et al*., 2011). Fresh as well as dry marjoram is one of the most popular culinary herbs. It is used globally in salad, meat and vegetable dishes for flavor and as a natural antioxidant to avoid the use of synthetic antioxidants in food (Arts and Hollman, 2005; Shan *et al.,* 2005; Yanishlieva *et al*., 2006; Hossain *et al*., 2008).

Aromatic plants represent a renewable source of flavoring substances, which can be used in the food, perfumery and pharmaceutical industries. Medicinal, aromatic and herb spices from family Lamiaceae such as marjoram (*Origanum marjorana*) and spearmint (*Mentha spicata*), are widely distributed in Egypt. These plants are used as stomachic, spasmolytic, carminative and expectorant agents in folk medicine and in official medicine. Ethereal oils extracted from Lamiaceae family plants can contribute the quality of food with better odor and flavor what is consider as very important parameter in food manufacturing (Kovaaevi, 2001). The emulsified oil of marjoram was reported to possess strong antparasitic activity (Force *et al.,* 2000).

Fennel (*Foeniculum vulgare* Miller) is a major aromatic plant belonging to the Apiaceae family, which has long been considered as a medicinal and spice herb. Fennel oil is commonly used as a flavouring agent in food products and as a constituent of cosmetic and pharmaceutical products; furthermore, its antimicrobial properties against a wide range of microorganisms have been well established (Elgayyar *et al*., 2001; Aprotosoaie *et al.,* 2008).

Essential oils, derived from aromatic medicinal plants (fennel (*Foeniculum vulgare*), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*)), have been reported to be active against Gram-positive and Gram-negative bacteria as well as against yeasts, fungi and viruses. They are mixtures of different lipophilic and volatile substances, such as monoterpenes, sesquterpenes, and/or phenylpropanoids, and have a pleasant odor. Furthermore, they are considered to be part of the reformed defense system of higher plants (Reichling, 1999).

Teucrium polium L., commonly known as golden germander, is a steno-Mediterranean perennial shrub spices belonging to the Lamiaceae family. This medicinal plant and other species belonging to the genus Teucrium have been used for over two thousand years in traditional and herbal medicine. *T. polium* has been utilized in Mediterranean countries to treat manly diseases such as abdominal pain, indigestion and diabetes (Al-Khalil, 1995; Ljubuncic *et al*., 2006).

Some spices and herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and the attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications (Shan *et al*., 2007).

Therefore, this research was performed to determine the effective chemical compounds in the essential oils extracted from marjoram, teucrium and fennel essential oil by gas chromatography-mass spectrometry (GC/MS) and to throw the light on the antimicrobial activity.

Materials and Methods

1. *Collection of tested samples:*

The marjoram; *Origanum marjorana,* leaves, the fennel; *Foeniculum vulgare,* fruits were collected from local market at Egypt and teucrium; *Teucrium polium,* leaves was collected from North Sinai-Egypt. All other chemicals used in this study were obtained from EL-Gomhoria Company, Egypt.

2. *Extraction of the essential oil:*

Essential oils were obtained by steam distillation method according to Guenther, (1961). The plant material (about 300g), was cut into small pieces, and placed in a flask (4L) together with doubled distilled water (1.5L). The mixture was boiled for 3h, collected essential oil were dried with anhydrous sodium sulfate and kept at –18 C until use.

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3. Tested Microorganisms:

Gram-negative bacteria (*Escherichia coli O157:H7, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Serratia marcescens*). Gram-positive bacteria (*Bacillus anthrakoids, Bacillus cereu*s, *Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus Micrococcus roseus, Staphylococcus aureus* and *Streptomyces spp.*). All the previous strains were obtained from Botany Dept., Fac. of Sci. Al-Azhar Univ. Assuit, Egypt.

4. Determination of antibacterial activity:

Antibacterial activity was determined by the well agar diffusion method according to the NCCLS (1993). Petri plates containing 20 ml of nutrient agar medium were inoculated with a 24h culture of the bacterial strains wells (6 mm diameter) were cut into the agar using sterile cork borer. Using sterilized dropping pipettes and the essential oils were carefully added into the wells. Stock culture of all bacterial species were grown in nutrient broth at 35º C for 18-24 h.

Final concentration was 10^8 CFU /ml. 0.2 ml of this inoculum was added to each plate containing nutrient agar. Serial concentrations of all test essential oils were achieved (%w/v) in plates containing nutrient agar medium, as follow: 0, 2, 5, 10, 15, 20 and 25 mg/ml. concentrations in dimethylsulfoxide (DMSO) were filled to the holes using a pipette and DMSO without the essential oil was used as a control. Petri plates of nutrient agar were inoculated with bacterial species in triplicates was individually spread on the surface of the solid wed agar plates and added each essential oils test in the hole at plates in order to obtain a control of the solvent antimicrobial effect using triplicates. After inoculation procedures, essential oils and control plates were then incubated at 35ºC. Plates were evaluated for the presence or absence of visible growth of the each bacterial species on the agar plate after 24h of incubation. The absence of colonies on all plates tested was considered as an inhibitory effect and the zones of microbial inhibition (millimeter) were recorded.

5. Separation and identification of extract components:

Gas chromatographic Mass Spectrometric analysis (GC/MS):

GC/MS analysis of the essential oil was done according to Shahat *et al.,* (2008) GC/MS analysis of the essential oil was carried out using an HP5890 Series II Gas Chromatograph, HP 5972 Mass Selective Detector and Agilent 6890 Series Auto sampler (Agilent Technologies, USA). A Supelco MDN-5S 30mby 0.25mmcapillary column with a 0.5 μmfilm thickness was used with helium as the carrier gas at a flow rate of 1.0 ml/min. The GC oven temperature was programmed at an initial temperature of 40◦C for 5 minutes, then heated up to 140◦Cat 5◦C /min and held at 140◦C for 5 min, then heated to 280◦C at 9◦C/min and held for 5 additional minutes.

Results and Discussion

1. The Identified Effective Chemical Compounds in the Essential oils of Marjoram leaves, Teucrium leaves and Fennel fruits Analyzed by GC/MS Technique:

Marjoram essential oil was fractionated and identified by using gas chromatography coupled with mass spectrometry (GC/MS), results are shown in Table (1). It could be noticed that 38 compounds were identified representing 96.82% of total essential oil. The unknown compounds might be considered as trace compounds in such oil. The identified compounds were classified into 5 main groups; those were in the concentration as hydrocarbons (61.48%), alcohols (17.87%), esters (10.79%), ketones (4.02%) and phenols (2.66%). The major identified compounds were α-phyllandrene (8.87%), α-terpinene (7.37%), γ-terpinene (7.25%). Terpinene 4-ol (6.33%), trans-sabinene hydrate (4.00%) and carvacrol (2.25%).

In this concern, it should be mentioned that there are tow main chemotypes; one consists mostly of monoterpene alcohols and other of phenols. In the first chemotype terpinene-4-ol, either alone or together with other substances, such as cis- and/or trans-sabinene hydrate, was found to be the main volatile component; and second, marjoram oils rich in phenols (thyme and/or carvacrol) (Vera and Ming, 1999). The present study shows that our tested Egyptian marjoram volatile extracts represent characteristics of the first chemotype (El-Ghorab *et al.,* 2004; Rodrigues *et al.,* 2002 and Busatta *et al.,* 2008). Moreover, our results are in close agreement with those found by El-Ghorab *et al.* (2004) and Zawislak (2008) who found that there were some components dominated in marjoram essential oil such as trans-sabinene hydrate and terpinen-4-ol. They also added that sabinene, γ-terpinene, linalyl acetate, and cis-sabinene hydrate were identified in large concentrations. In addition, Busatta *et al.* (2008) found that terpinen-4-ol was the major component, followed by γ-terpinene, cis-

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sabinene hydrate, sabinene and α-terpineol. Lambert *et al*. (2001); Marino *et al*. (2001) and Deferera *et al*. (2003) performed gas-chromatographic analysis of marjoram essential oil and reported that, out of a large number of constituents, the carvacrol as major component.

Sweet marjoram (*Origanum majorana* L., syn.: *Majorana hortensis* Moench) has been used for centuries both as a culinary and medicinal herb. It has been evidenced in a number of studies that essential oil from sweet marjoram contains mainly terpinen-4-ol, α- and γ-terpinenes, linalool and carvacrol, which are the basis for their antimicrobial properties (Sarer *et al.* 1982, Daferera *et al.* 2000, Vagi *et al.* 2005, Nurzyńska-Wierdak and Dzida 2009).

Table 1: The effective chemical compounds in the essential oils of marjoram leaves analyzed by GC-MS technique.

Chemical constituents	Retention time (min)	Concentration (%)
Hydrocarbons		
1-Methyl-cyclohexene	9.80	3.27
α-Pinene	6.08	5.89
α-Myrcene	8.01	0.94
α-Terpinene	8.54	7.37
Champhene	8.60	4.64
d-Limonene	9.78	0.90
γ-terpinene	11.76	7.25
Methylbenzene	12.03	6.94
α-Thujene	13.28	0.31
α-Terpinolene	13.89	4.61
β-Pinene	28.38	1.13
β-Ocymene	28.41	0.11
δ- 3-Carene	28.61	0.71
α-Phyllandrene	30.10	8.87
P-Cymene	9.21	2.85
γ- Elemene	31.00	0.54
β- Caryophyllene	32.00	1.56
β- Myrcene	33.24	1.24
α-Humnlene	34.35	2.35
Total	61.48	
Esters		
Iso-bornyl acetate	12.21	0.71
Trans-sabinene hydrate	25.35	4.00
Cis-Sabinene hydrate	30.40	3.04
Linalyl formate	30.66	2.83
Linalyl acetate	33.67	0.21
Total	10.79	
Phenols		
Thymol	30.06	0.41
Carvacrol	63.21	2.25
Total	2.66	
Ketones		
Carvone	22.01	2.55
Champhor	34.01	1.46
Dehdrocarvone	43.11	0.31
Total	4.32	
Alcohols		
Champhor	21.32	3.35
Carvaeol	23.14	0.63
4-terpinol	32.01	2.83
Phytol	33.15	0.77
Cis-Piperitol	34.21	0.87
Cineole	35.17	0.85
Spathulenol	42.20	0.61
Terpinenol	42.41	6.33
Veterverol	43.10	1.63
Total	17.8	
Total identified compounds	97.02	
Total unidentified compounds	3.81	
Total phenolic content**	9.4	

***** Total phenolic content (%) were determined as Gallic acid mg/100mg.***

The identified combinations in teucrium essential oil and quantitative parentages of the compounds are presented (Table 2). It could be noticed that 28 compounds were identified compounds were classified those were in the concentration as α-pineneα (12. 52%), Linalool (10.63 %), Caryophyllene oxide (9.69%), β-pinene (7.09%) and β-caryophllene (6.98%).

Zare *et al.,* (2011) reported that the chemical analysis of *Teucrium polium* essential oil by Gas chromatography/ mass spectrophotometer (GC/MS) shows the presence of 58 substances (90.48%) mainly including Bicyclodec-1-ene and 1,3-Cyclooctadiene Respectively, and found that the Minimum inhibitory concentration of the essential oil determined using reassuring as bacterial cell growth indicator shows the highest antimicrobial activities against *Bacillus cereus* and *Pseudomonas aeruginosa*. The highest MIC values were observed in cases of *Escherichia coli* and *Salmonella typhimurium*.

In general, β-caryophyllene and caryophyllene oxide were reported as the main sesquiterpenes in many of the *Teucrium* species Mortezr *et al.,* (2007).

In Iranian folk medicine, *Teucrium polium* is used as anticonvulsant medicine (Zargari 1992). Of 28 compounds being identified in the essential oil of this plant with 99.75%, the combination of α-pinene (12.52%), linalool (10.63%), caryophyllen oxide (6.69%), β- pinene (7.09%), and caryophyllene (6.98%) with 46.91% percent constitute the highest percentage of essential oil. A study on oil obtained from *Teucrium polium* grown in Iran revealed the presence of sesquiterpenes as major components oil (Moghtader, 2009).

Table 2: The effective chemical compounds in the essential oil of Teucrium polium analyzed by GC-MS technique.

Compound No.	Chemical constituents	Restrictive index (RI)	Concentration (%)
1	(E)-2-hexenal	865	0.27
2	α-pinene	927	12.52
3	α-camphene	954	5.73
4	Sabinene	965	0.84
5	1-octen-3-ol	978	2.97
6	3-octanol	982	3.29
7	β-pinene	984	7.09
8	Myrcene	995	1.46
9	-cymeneρ	1025	0.45
10	1.8-cineole	1032	3.60
11	Limonene	1036	1.89
12	camphor	1092	5.21
13	Linalool	1127	10.63
14	α-terpineol	1139	0.33
15	Bornyl acetate	1142	5.34
16	Terpinen-4-ol	1198	0.19
17	Carvacrol	1272	5.23
18	β-myrcene	1296	0.45
19	Camphene	1385	0.27
20	β-caryophllene	1421	6.98
21	α-humulene	1437	2.75
22	γ-cadinene	1488	3.66
23	Germacrene D	1497	5.03
24	Elemol	1549	1.24
25	Spathulenol	1552	0.21
26	Caryophyllene oxide	1578	9.69
27	α-cadinol	1702	1.68
28	Exadecanoic acid	1896	0.75

The chemical constituents of fennel essential oil were fractionated and identified by GC and GC-MS techniques. From the results tabulated in Table (3). It could be noticed that 18 essential components were isolated from fennel essential oil; these identified compounds represented (85.35%) of the chemical components of fennel essential oil. Of the 18 compounds being identified in the essential oil of this plant with 85.35%, the combinations of Estragole (57.94%), Limonene (20.64%), Fenchone (7.22%), *trans*-anethole (4.99%) and α-pinene (3.61%)

GC and GC-MS analysis of *F. vulgare* essential oil led to identification and quantification of eighteen different components, representing 98.1% of the total oil composition which include monoterpene hydrocarbons (41.1%), oxygenated mono-terpenes (12.7%), sesquiterpenes hydrocarbons (0.3%) and benzene derivates (44.0%). Anethole (41.2%), limonene (24.8%), fenchone (11.9%), myrcene (5.2%) and a-pinene (4.1%) are the major constituents of this essential oil. These compounds were also found in the essential oil obtained from the green part of the plant *F. vulgare* as was reported in some studies (Piccaglia and Marotti, 2001; Muckenstrum *et al.,* 1997).

Estragole is nowadays recognized as a potential human carcinogen (Vincenzi *et al*., 2000). The over all result could be to change in the chemotype in terms of reduced quality of fennel and increased health risk. Additionally, estragole and fenchone are known to be carcinogenic and epileptogenic respectively (Burkhard *et al*., 1999). On the other hand, the insecticidal action of estragole and fenchone against Soryzae adults was very rapid, whereas only anethole exhibited rapid insecticidal action against L serricorne adults (Kim and Ahn, 2001). Ozcan and Chalchat, (2006) reported that estragole (61.08% and 40.49%), fenchone (23.46% and 16.90%) and limonene (8.68% and 17.66%), respectively, as the major constituents in the essential oil of bitter fennel (*F. vulgare* spp. *piperitum*) grown in Turkey. Such variations in the chemical composition of essential oil across countries might be attributed to the varied agro climatic (climatically, seasonal, geographical) conditions of the regions, stage of maturity and adaptive metabolism of plants.

Estragole and trans-anethole are the major constituents of the essential oils of bitter fennel chemotypes (*Foeniculum vulgare* Mill. var. *vulgare*) (Gross *et al.,* 2002). Cell free extracts from bitter fennel tissues display o-methyltransferase activities able to *in-vitro* methylate chavicol and transanol to produce estragole and trans-anethole, respectively. These results indicated that an apparent association between the levels of estragole and trans-anethole in the different plant parts. Thus the present results indicate that fruits had the most substantial content of estragole and the callus had the most substantial content of trans-anethole. *Foeniculum vulgare* var. *vulgare* presents great composition differences with varying populations with the aim of clarifying the status of var. *vulgare*, the proposed to subdivide it into three chemotypes according to their relative compositions (Muckensturm *et al.,* 1997).

The proportion of fenchone in the volatile fraction of both roots and hypocotyls extracts was a similar order and greater than that in fruits extract. These results are disagreement with previous reports of essential oils of bitter and sweet fennel (Akgül and Bayrak, 1988; Baldrich *et al*., 1986).

Table 3: The identified effective chemical Compounds of fennel fruits analyzed by GC-MS technique.

Compound No.	Chemical constituents	Retention time (min)	Concentration (%)
1	α-pinene	14.56	3.61
2	Camphene	15.25	0.19
3	Sabinene	16.13	0.56
4	β-pinene	16.35	0.21
5	Myrcene	16.72	0.32
6	α-Phellandrene	17.43	0.11
7	o-Cymene	18.13	0.71
8	Limonene	18.32	20.64
9	Eucalyptol	18.44	1.93
10	γ-Terpinene	19.35	0.38
11	Fenchone	20.54	7.22
12	Linalool	21.73	0.11
13	Camphor	22.60	0.29
14	Estragole	24.21	57.94
15	Fenchyl acetate	25.25	0.21
16	Cmic aldehyde	25.92	0.10
17	-Anisaldehydep	26.20	0.26
18	*trans*-Anethole	26.96	4.99

From another point of view, it can be observed that samples with a high Trans-anethole content contained small amounts of estragole and vice versa, these results are in agreement with Miraidi (1999). Anisaldehyhe, a degradation product of trans-anethole was also a high content in hypocotyls callus induced on MS media supplemented with 1.0 mg/l kinetin (Bilia *et al*., 2000). The results under discussion clearly show that the ratio between trans- anethole and estragole differs between chemotypes (Barazani *et al.,* 2002; Muckensturm *et al*., 1997).

2. Antibacterial activity of marjoram, teucrium and fennel essential oils extracts:

(a) Antibacterial activity of marjoram (Origanum majorana) essential oil:

The antibacterial activity of marjoram essential oil at different concentrations was determined by the Well agar diffusion method against six Gram-negative bacteria and eight Gram-positive bacteria. The diameters of inhibition zones were measured and taken as an indicator of the antimicrobial effect (inhibitory activity). Results are showed in Table (4) from this table it could be stated that no inhibition zone was observed at all concentrations of marjoram essential oil against *Listeria monocytogenes.* On the other side, marjoram essential oil inhibited Gram-negative bacteria in higher degree more than Gram-positive bacteria at concentration 15 to 25 mg/ml. These results are in the same line obtained by Pasqe *et al*., (2005) showed that concentrations of marjoram essential oil for Gram-negative bacteria was lower than Gram-positive bacteria. According to essential oil, the antimicrobial activity of marjoram essential oil is assigned to be a number of terpenoid (α-Terpinene, γ-Terpinene and Terpinolene) which also in pure from have been shown to exhibit antibacterial or antifungal activities (Conner, 1993).

Combination of essential oils of oregano and thyme, oregano with sweet marjoram and thyme with sage had the most effect against *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli* O157:H7 and *Listeria monocytogenes* (Tajkarimi *et al.* 2010).

Table 4: Diameters of inhibition zones (mm) of marjoram (Origanum majorana) essential oil against some selected microorganisms.

Microorganisms / Concentrations (mg/ml)	2	5	10	15	20	25
Bacillus anthrakoids	0.0	0.0	10	11	15	16
Bacillus cereus	10	11	11	12	13	15
Bacillus subtilis	0.0	0.0	0.0	10	11	13
Escherichia coli O157 :H7	0.0	0.0	0.0	10	11	12
Klebsiella pneumoniae	0.0	0.0	0.0	10	12	13
Listeria monocytogenes	0.0	0.0	0.0	0.0	0.0	0.0
Micrococcus luteus	9.0	9.0	10	11	12	13
Micrococcus roseus	8.0	8.0	9.0	10	11	12
Proteus vulgaris	0.0	0.0	0.0	10	14	16
Pseudomonas aeruginosa	0.0	0.0	0.0	10	12	13
Salmonella typhi	0.0	0.0	0.0	9	10	12
Serratia marcescens	14	15	16	17	18	20
Staphylococcus aureus	0.0	0.0	10	11	12	13
Streptomyces sp.	10	11	11	12	13	14

Whereas other isolates were used rarely in microbiological studies, it has been shown that aqueous and methanolic extracts from sweet marjoram contain multiple compounds with considerable antimicrobial action, e.g. phenolic derivatives (phenolic acids, flavonoids as apigenin, luteolin, quercitin and their glycosides as rutin or isovitexin) (Janicsak *et al*. 1999; Fecka and Turek 2008). Marjoram and thyme oils are rich in phenolic compounds being particularly active in both antioxidants and antimicrobials (Dorman and Deans, 2004). These results are in accordance with findings of (Kurata and Koike, 1983; Palmer *et al*., 1998). who pointed out that the antimicrobial effect of marjoram and thyme oils constituents were found to be phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons. Specific functional groups introduced into a compound can increase that antimicrobial activity, e.g., phenolic compounds can be bactericidal or bacteriostatic depending on their effective concentration. Antibacterial, antifungal, and antioxidant properties and chemical compositions of essential oils obtained from cinnamon, basil, lemon, lemon grass, frankincense, marjoram, and rosemary were reported by Baratta *et al*. (1998).

(b) Antibacterial activity of teucrium (teucrium polium) essential oil:

Antibacterial activity of *teucrium polium* leaves essential oil at 2, 5, 10, 15, 20 and 25 mg/ml, concentrations were examined against fourteen bacterial strains. Data in Table (5) clearly shown that *teucrium polium* leaves essential oil exhibited antibacterial activity against most tested microorganisms. It showed high activity in case of *Bacillus cereu*s, *Escherichia coli O157:H7*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Serratia marcescens*. Whereas, all concentrations of *teucrium polium* leaves essential oil recorded no inhibitory effects against *Bacillus anthrakoids, Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*. These results are in agreement with those obtained by Zare *et al.,* (2011) found that the Minimum inhibitory concentration of the essential oil of *Teucrium polium* determined using reassuring as bacterial cell growth indicator shows the highest antimicrobial activities against *Bacillus cereus* and *Pseudomonas aeruginosa*. The highest MIC values were observed in cases of *Escherichia coli* and *Salmonella typhimurium*.

The antibacterial activity of *T. polium* extracts can be attributed to its contents in flavonoids. In general the methanol extraction of *T. polium* plant material yielded to flavonoids (Sharififar *et al*. 2009).

Table 5: Diameters of inhibition zones (mm) of teucrium (teucrium polium) essential oil against some selected microorganisms

Microorganisms / Concentrations(mg/ml)	2	5	10	15	20	25
Bacillus anthrakoids	0.0	0.0	0.0	0.0	0.0	0.0
Bacillus cereus	8	9	10	10	11	12
Bacillus subtilis	0.0	0.0	0.0	0.0	9	10
Escherichia coli O157 :H7	9	10	11	11	12	13
Klebsiella pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0
Listeria monocytogenes	0.0	0.0	9	12	13	15
Micrococcus luteus	0.0	0.0	8	9	9	10
Micrococcus roseus	0.0	0.0	9	10	11	12
Proteus vulgaris	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas aeruginosa	9	10	11	12	14	15
Salmonella typhi	0.0	0.0	0.0	0.0	0.0	0.0
Serratia marcescens	0.0	0.0	9	10	11	13
Staphylococcus aureus	0.0	9	10	11	12	13
Streptomyces spp.	0.0	0.0	0.0	0.0	0.0	0.0

(c) Antibacterial activity of fennel (Foeniculum vulgare) essential oil:

Antibacterial activity of fennel essential oil at different concentrations (2, 5, 10, 15, 20 and 25 mg/ml) were determined and the inhibition zones were observed. Data presented in Table (6) showed that fennel essential oil exhibited antibacterial activity against most tested microorganisms. It showed high activity in case of *Bacillus anthrakoid*, *Escherichia coli O157:H7*, *Staphylococcus aureus* and *Serratia marcescens*. Whereas, all concentrations of fennel essential oil recorded no inhibitory effects against *Proteus vulgaris, Klebsiella pneumoniae* and *Salmonella typhi*

The antibacterial activity of fennel essential oil may be due to attributed the chemical compound which contained Estragole, Limonene, Fenchone and *trans*-Anethole. In conclusion, these essential oils may be used not only to enhance the product flavor but also to inhibit and delays of microbiological growth. Hence they may be considered as natural preservatives without any side effects on human health.

The antimicrobial activity of plant oils and extracts has been recognized for many years, and they have many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. The chloroform soluble fraction from the stems of fennel exhibited a potent antimicrobial activity against bacteria and fungi. Dillapional, scopoletin, dillapiol, bergapten, imperatorin, psolaren and dillapional were found to be antimicrobial principles against *Bacillus subtilis*, *Aspergillus niger* and *Cladosporium cladosporioides* (Kwon *et al.,* 2002). Essential oils from the fruits of fennel showed significant antibacterial activity to *Escherichia coli* and *Bacillus megaterium* (Araque *et al*., 2007; El-Adly *et al.,* 2007).

Table 6: Diameters of inhibition zones (mm) of fennel (Foeniculum vulgare) essential oil against some selected microorganisms.

Microorganisms \ Concentrations(mg/ml)	2	5	10	15	20	25
Bacillus anthrakoids	0.0	0.0	0.0	11	12	14
Bacillus cereus	0.0	0.0	8	9	9	11
Bacillus subtilis	0.0	0.0	0.0	0.0	9	11
Escherichia coli O157 :H7	10	11	12	12	13	14
Klebsiella pneumoniae	0.0	0.0	0.0	0.0	0.0	10
Listeria monocytogenes	0.0	0.0	0.0	10	12	14
Micrococcus luteus	0.0	0.0	9	10	11	11
Micrococcus roseus	0.0	8	10	11	11	12
Proteus vulgaris	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas aeruginosa	0.0	0.0	0.0	9	10	12
Salmonella typhi	0.0	0.0	0.0	0.0	10	11
Serratia marcescens	9	10	11	12	13	14
Staphylococcus aureus	0.0	0.0	9	10	10	10
Streptomyces sp.	0.0	0.0	0.0	8	9	10

In agreement with our results, Cantore *et al.* (2004) reported that the Gram-negative strains of bacteria, especially *E. coli*, have less sensitivity to fennel essential oils. Ozcan *et al.* (2006) found that fennel essential oils exhibit an inhibitory effect against a wide range of *Bacillius* species. In this connection (Dadalioglu and Evrendilek, 2004) reported that *Foeniculum vulgare* Mill exhibited a very strong antibacterial activity against *Escherichia coli* O157: H7 and *Staphylococcus aureus* and that *trans*-anethole (85.63%) were the predominant constituents in fennel essential oils.

Essential oils of *F. vulgare* and *M. spicata* showed significant antibacterial activity either against Gram-positive and Gram-negative bacteria. Alls essential showed antimicrobial activity against *S. aureus* that is a major human pathogen, which can colonize many different tissues and organs, thereby causing a wide variety of diseases. The resulting complexity of staphylococcal pathogenesis poses an urgent challenge, especially in light of increasing resistance to antibiotics and the emergence of severe invasive infections (Schwarz-Linek *et al.*, 2006).

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