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Antioxidant and Antimicrobial Activities of Marjoram (Origanum majorana L.) Essential Oil.

[1]A.Z.M. Badee, [2]R.K. Moawad, [1]M.M. ElNoketi, [2]M.M. Gouda

[1]Food Science Department, Faculty of Agriculture, Cairo University.
[2]Food Technology Department, National Research Centre, Dokki, Egypt.

ABSTRACT

Marjoram essential oil (MEO) was analyzed by gas chromatography coupled with mass spectrometry (GC/MS),the analysis revealed that, the major components were γ-terpinene (23.20%), α-terpinene (19.71%) and terpinen-4-ol (12.64%), followed by α-terpinolene (6.76%),sabinene hydrate (6.56%), p-cymene (5.81%), α-phellandrene (5.43%) and Limonene (5.40%),they constituted 85.51% of total essential oil, indicating that Egyptian marjoram oil belonged to terpinen-4-ol /sabinene-hydrate chemotype.GC/MS studies also indicated that,the oil was dominated by monoterpene-hydrocarbons (75.79%), followed by oxygenated- monoterpenes (21.50%) andSesquiterpene-hydrocarbons (2.34%). Results of total phenolic content (TPC) revealed that MEO is a rich source of polyphenols which are known natural antioxidants. MEO also exhibited strong antioxidant activities as shown by the consistently high values of DPPH free radical-scavenging inhibition(83.6 %) andβ-carotene / linoleic acid system inhibition (76.2 %), at 400µg / mL oil concentration. Antioxidant effectiveness was further confirmed on the essential oil using automatic determination of the oxidative stability for sunflower oil (Rancimat). MEO was found to be very effective in stabilizing sunflower oil in comparison with BHT, the stability of the oil was increased with the increasing in essential oil concentration.On the basis of the results obtained, marjoram essential oil could be used as a potential source of natural antioxidant with possible applications in food systems. In antimicrobial investigation with the agar disc diffusion assay, all the microorganisms tested were susceptible to the action of marjoram essential oil, with a range of inhibition zone diameter values from 13 to 34 mm/sample. *Candida albicans* was the most sensitive strain tested to the MEO with the strongest inhibition zone (34 mm). While, *Aspergillus flavus* was the most resistance strain tested, with the lowest inhibition zone (13 mm). Minimum inhibitory concentrations (MIC) for *Candida albicans* was determined as 32 µL/ ml MEO, in comparison to 98 µL/mL with commercial Amphotericin B (antifungal agent), According to the results, the studied essential oil potentially might be used as a natural preservative ingredient in food industry.

Key words: Marjoram, Natural Preservatives, DPPH, β-carotene, Phenolic Compound, GC/MS.

Introduction

Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity and formation of potentially toxic reaction products in food products, leading to their deterioration (Halliwell *et al*., 1995). In addition, many diseases are caused by cell oxidative stress, such as cardiovascular disease, cancer, and other chronic diseases (Benzie, 1996; Shahid *et al*., 2008). Synthetic antioxidants, such as butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT) and tert-butyl hydroquinone (TBHQ), are widely used in food industry to retard or minimize oxidative deterioration of foods (Goze *et al*., 2009). However, consumers have rejected synthetic antioxidants due to their suspected toxic and carcinogenic effects (Ito *et al*., 2005; Fasseas *et al*., 2007). Hence, research for new, safe and effective natural antioxidants is needed.

Recently, several natural plants have received much attention as sources of biological active substances including antioxidants. They are suggested as a superior alternative for the synthetic ones to improve lipid stability and enhance the sensory properties of food (Li *et al*., 2008). Natural antioxidants properties are mainly attributed to their phenolic contents, thus their antioxidants action is similar to synthetic phenolic antioxidants (Fecka and Turek, 2008). Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Elena *et al*., 2009). Therefore, natural antioxidants can protect the human body from free radicals and could retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (Robbins&Bean, 2004; Arts &Hollman, 2005).

Corresponding Author: A.Z.M. Badee, Food Science Department, Faculty of Agriculture, Cairo University.
E-mail: dr.badee47@Hotmail.com

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food-borne diseases (Bassole&Juliani, 2012). Currently there is a growing interest to use natural antimicrobial compounds, like extracts of herbs and spices for the preservation of foods, as these possess a characteristic flavor and sometimes show antioxidant activity as well as antimicrobial activity (Chan *et al*., 2012). Among several essential oils that may be useful as antimicrobial agents, marjoram oil possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi and therefore, it may have the greatest potential for use in industrial applications (Busatta *et al*., 2008; Mohamed & Mansour, 2012).

In recent years, the use of natural plant preservatives to increase the shelf-life of food products is promising technology since they derived substances have antioxidant and antimicrobial properties. Many herbs, spices, and their extracts have been added in a variety of foods to improve their sensory characteristics and extend shelf-life (Burt, 2004). Of the common herbs used for seasoning foods, sweet marjoram (Origanum majorana L.) is a popular herb, belongs to the Lamiaceae plant family, that widely distributed in Egypt and was known to the ancient Egypt for its healing properties (Tapsell *et al*., 2006). Traditionally, marjoram has been used as a folk remedy (Faleiro *et al*., 2005).For centuries, marjoram oil have been used for curing various diseases (Al-Harbi, 2011). Marjoram oil is also used in perfumes, soaps, and detergents for its spicy herbaceous notes (Hazzit *et al*., 2006).Marjoram is considered among the main crops for increasing Egypt income from foreign currency (El-Aeshmawiy *et al*., 2009). The fresh or dried marjoram leaves and their essential oil are widely used in the food industry as a food ingredient, a herbal tea, flavoring, coloring, nutritional and natural preservatives (Holley & Patel, 2005).

Marjoram essential oil (MEO) is obtained by steam distillation of dry marjoram leaves which contain 0.7–3.5% essential oil (Kumar *et al*., 2011). Considerable variations in the content and compositional pattern of MEO are observed depending on the species, growth stages, origin of herb, climatic and drying conditions (Sellami *et al*., 2009; Baatour *et al*., 2012). However, terpinene-4-ol alone or along with sabinene hydrate is responsible for the characteristic flavor and fragrance of marjoram oil (Vági *et al*., 2005). It was postulated that the oils exist in two forms, In the first chemotype, terpinene-4-ol either alone or together with sabinene hydrate, α- terpineol, α- and γ-terpinene were found to be main constituents of the essential oils (Vera and Chane-Ming, 1999; Banchio *et al*., 2008), and the other chemotype with thymol and/or carvacrol as predominant compounds (Daferea *et al*., 2003). Marjoram volatile oil is rich in terpinen-4-ol, sabinene hydrate, γ-terpinene, p-cymene, α-terpinene, and α-terpineol (Vera and Chane-Ming, 1999; Lis *et al*., 2007).

In order to reduce health hazards, food poisoning outbreaks, lipid oxidation and subsequent economic losses, the use of natural marjoram essential oils as green antioxidant and antimicrobial agents seems to be an attracting manner both in industrial applications and scientific research (Busatta *et al*., 2008; Chan *et al*., 2012). However, their use in foods requires detailed characterization of their compounds. Therefore, the current study was aimed to separate and identify the chemical constituents of marjoram leaves essential oil beside, detection of the antioxidant and antimicrobial activities of the volatile oil obtained.

Materials and Methods

2.1. Materials:

Shade-dried marjoram leaves (Origanum majorana L.),cultivated in Beni-Suef city, were purchased in June 2009 (during the flowering period),from Al-Dahlia Company, Nasr City, Egypt. These samples were manually crushed and kept at 4°C until use.

Refined sunflower oil, free of synthetic antioxidants, was kindly supplied from Cairo Oil and Soap Company, El Aiyat,Giza, Egypt. The chemicals and reagents used for analyzing the antioxidant compounds were of analytical grade.

2.2. Analytical Methods:

Essential Oil Isolation:

One hundred grams of shade- dried marjoram leaves were hydro-distilled in a Clevenger type apparatus for 3h. The essential oils were dried over anhydrous sodium sulphate, thenamount of oil obtained from plant material was calculated.MEO was stored in a dark- colored glass bottles, and kept at 4°C until analysis.

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Physico-Chemical Measurements:

Oil yield percentage, specific gravity at 20°C, refractive index at 20°C , optical rotation, solubility in ethyl alcohol and acid value of marjoram essential oil were determined according to the methods described by Guenther(1961).

GC/MS Analysis:

The essential oil was analyzed in a gas-chromatograph interfaced with a mass selective detector—GC/MSD, Model (Varian 240-MS), using a capillary column VF-5, MS (30mX0.25mm,ID,0.25 µm film thickness) and a flow rate of helium as the carrier gas at1ml/min. Marjoram essential oil were analyzed by direct injection (1µL) into split less injector. The injector and detector temperature were 250°C. The column oven was temperature-programmed from 45-240°C at a rate of 45°C/10min, and 6°C/ 15min up to 240°C. Mass spectra were acquired over a mass range of 20-425 amu, at 0.5 scan per second. The identification of marjoram essential oil compounds was accomplished by comparing their retention times with those of authentic standards, and by comparison of their mass spectra with those from the Wiley library. Compositions were then expressed as percentages of normalized peak areas.

Oxidative stability of sunflower oil:

Rancimat test was used to determine the antioxidant activity of marjoram essential oil added at levels of 0.02, 0.04, 0.06 and 0.08%(v/v) and mixed well with sunflower oil using magnetic stirrer, in comparison to BHT (0.02% w/v), and pure sunflower oil(without any addition, as control). Sunflower oil with and without addition of antioxidant was determined under accelerated conditions (100ºC, Oxygen flow at 20L/hr) using Rancimat743 (Metrohm, Switzerland), the induction period (IP) was conducted with Rancimat.The antioxidant activity index (AA) and increasing index %were calculated from the measured induction times, according to the following formulas by Holasova *et al*. (2006): AA = Ind. time of oil with antioxidant / Ind. time of control. Increasing Index % = {(IP with antioxidant–IP of the control)/(IPof the control)}x 100.

Antioxidant Activity:

Determination of total phenolic content (TPC):

The level of total phenols in the marjoram essential oil was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid. MEO,200µL and 4 mL of Folin–Ciocalteu reagent were added and the contents mixed thoroughly. After 4 min, 1 mL of 20% Na2CO3 was added, and then the mixture was allowed to stand for 1 h at normal temperature. The absorbance was measured at 760 nm using a spectrophotometer (Shimadzu,Kyoto,Japan). The concentration of the total phenolic was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve (Li *et al*.,2008).

β-Carotene-Linoleate Scavenging Assay:

The antioxidant activity of the marjoram essential oil was evaluated using β-carotene-linoleate model system. 0.1 mg of β-carotene in 0.2 mL of chloroform, 10 mg of linoleic acid and 100 mg of Tween-20 were mixed. The solvent was removed at 40°C under vacuum and the resulting mixture was diluted with 10 mL of water and was mixed well. To this mixture, 20 mL of oxygenated water was added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 200 µL of each marjoram essential oil concentrations (50, 100, 200 and 400 µg/mL "ppm") and BHT (50, 100,and200µg/mL) in ethanol. BHT was used for comparative purposes. A control containing 200 µL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50°C in a water bath and the absorbance at 470 nm was taken at zero time (t= 0). The absorbance was continued to be measured until the color of β-carotene disappeared in the control tubes (t =60 min) at an interval of 15 min (Gulcin *et al*., 2007). A mixture prepared as mentioned above without β-carotene served as blank. The antioxidant activity (AA) of the essential oil was evaluated in terms of bleaching of the β-carotene using the following formula:(% Inhibition = [(AB-AA)/AB] X100).Where: AB: absorption of blank sample (t=0 min). AA: absorption of sample solution (t=60 min).The results were expressed in % basis in preventing bleaching of β-carotene.

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Radical scavenging activity using DPPH assay:

Antioxidant activity was also determined by DPPH assay using spectrophotometer at 517 nm. Fifty microliters of various concentrations (50, 100, 200 and400 µg/mL: "ppm") of the essential oil in methanol as well as BHT, as standard antioxidant (50, 100, and 200 µg/mL) were put into appropriate tubes, and 4 mL of 0.004% methanolic solution of DPPH• was added to each tube and shaken vigorously (Gulcin, 2006). The tubes were allowed to stand at room temperature for 30 min.Control was prepared as the same time without any sample. The changes in the absorbance of the prepared samples were measured at 517 nm. Radical scavenging activity was estimated as the inhibition percentage and was calculated using the following formula: % Inhibition = [(AB-AA)/AB]X100. Where: AB: absorption of blank sample (t=0 min), AA: absorption of sample solution (t=30 min).

Antimicrobial investigations:

With the agar disc diffusion assay, six bacterial species *(Bacillus subtilis, Streptococcus faecalis, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli);* and two fungi *(Aspergillus flavus and Candida albicans),* were selected on the basis of the size of the halo formed and their possible occurrence in food products.Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method (Bauer *et al*., 1996). One hundred μL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5cells/mL for fungi (NCCLS, 2002). 100 μL of microbial suspension was spread onto agar plates (Muller – Hinton Agar), then Blank paper disks (Schleicher &Schuell, Spain), soaked with 10 μL of marjoram essential oil, were laid on the surface of previously agar plates inoculated with the different microorganisms tested in this study. Individual samples were examined in triplicate against each bacterium, together with a negative control (disks with $10\,\mu L\ of\ DMSO$), and positive control (discs with Tetracycline, 0.020 mg/mL DMSO, as antibacterial agent and Amphotericin B, 0.020 mg/mL DMSO, as antifungal agent). Bacteria were incubated at 35-37℃ for 24-48h; fungi at 25℃ for 48h; and yeast at 30℃ for 24-48h, then the diameters (mm) of the inhibition zones (size of the halo formed) were measured.

Minimum Inhibitory Concentration (MIC):

MIC value was measured by Agar Dilution Method. Stationary phase cultures of yeast were prepared at 32℃ and used to inoculate fresh 5.0 mL /culture to an OD_{600} of 0.05. the5.0 mL cultures were then incubated at 32 ℃ until an OD_{600} of 0.10 was achieved from which standardized yeast suspensions were prepared to a final cell density of 6×10^5 CFU/mL. Serial dilution from the treatment oil (0 –256 μL/mL) were prepared and mixedwith 5 mL of the standardized yeast suspension then added to the plates and incubated for 24 h at 32℃.The colony forming units (CFU) were counted for each dilution (NCCLS,2002).

Results and Discussion

3.1. Chemical Constituents of Marjoram Essential Oil:

MEO obtained by steam distillation were fractionated and identified by using GC/MS. The components and their relative proportions (Area %) are listed in Table 1, from which it is apparent that, the major components of MEO were γ-terpinene (23.20%),α-terpinene (19.71%) and terpinen-4-ol (12.64%), followed by α-terpinolene (6.76%), sabinene hydrate (6.56%), p-cymene (5.81%), α-phellandrene (5.43%) and Limonene (5.40%), they constituted 85.51% of total oil, indicating that Egyptian marjoram oil belonged to terpinen-4-ol/ sabinene hydrate chemotype.

GC/MS analysis also reveals that, some compounds are presented in marjoram essential oil in appreciable amounts such as myrcene (2.91%), α-thujene (2.41%), α-terpineol (1.94 %), and β-caryophyllene (1.58%). Same data indicated that, other constituents of the studied oil were traces (less than 1%), such as pinene, methyl benzene, ocimine, acetic acid, aromadendrene, carvone, linalool, sabinene, camphene, humulene, etc…(Table 1). These findings are consistent within the range reported for Origanum majorana L. (Vera and Chane-Ming, 1999; Lis *et al*.2007;Romeilah, 2009,and Soliman, 2009).

Display of data demonstrated in Table 1 it is obvious that GC/MS studies reveal the presence of 47 components classified mainly into the following groups: (1) Monoterpene-hydrocarbons (75.79%); (2) Oxygenated--monoterpenes (21.50%); (3) Sesquiterpene-hydrocarbons (2.34%); (4) esters (butyl acetate, 0.04%); (5) aldehyde (n-hexanal, 0.05%); (6) ketone (carvone, 0.18%), while total unknown (peaks 24, 25, 26, 27) represent 0.10%. Similar classification groups with little variations in component concentrations were observed for marjoram essential oil by other authors (Soliman, 2009; Kumar *et al* 2011; Darwish *et al*,

2012).However, the variations in the content and compositional pattern of marjoram essential oil could be due to many factors including species, growth stages, origin of herb, climatic and drying conditions, (Sellami *et al*., 2009, and Baatour *et al*., 2012).

Table1: Chemical Constituents of Marjoram *E*ssential Oil By GC/MS:

No.	RT.	Library ID	Area %	No.	RT.	Library ID	Area %
1	5.16	α-pinene (MH)	0.74	25	19.75	Unknown (UK)	0.03
2	5.31	α-thujene (MH)	2.41	26	21.02	Unknown (UK)	0.03
3	6.40	Camphene (MH)	0.10	27	26.87	Unknown (UK)	0.02
4	7.82	α-terpinolene (MH)	0.16	28	9.56	P-Menthen-1-ol (OM)	0.06
5	8.02	β-Pinene (MH)	0.27	29	13.29	Sabinene Hydrate (OM)	6.56
6	8.74	Sabinene (MH)	0.11	30	23.84	Linalool (OM)	0.12
7	11.28	α-phellandrene (MH)	5.43	31	24.51	P-menth-3-en-1-ol (OM	0.09
8	11.51	Myrcene (MH)	2.91	32	25.12	Terpinen-4-ol (OM)	12.64
9	12.13	α-terpinene (MH)	19.71	33	25.34	4-Vinyl-phenol (OM)	0.05
10	12.93	Limonene (MH)	5.40	34	25.77	Pyrimidinone (OM)	0.04
11	14.74	Cis-Ocimene (MH)	0.35	35	27.09	α–Terpineol (OM)	1.94
12	15.08	γ-terpinene (MH)	23.20	**	Total	Oxygenated –Monoterpenes	21.50
13	15.35	Trans-Ocimene (MH)	0.72	36	23.33	γ-selinene (Sesquiterp.)	0.03
14	15.69	1-methyl-Cyclohexene	0.10	37	23.41	Cadinene (Sesquiterp.)	0.03
15	15.82	P-Cymene (MH)	5.81	38	24.09	T-Caryophyllene (Ses)	0.04
16	16.34	α-terpinolene(MH)	6.76	39	24.30	Aristolene (Sesquiterp)	0.03
17	16.45	Isoterpinolene (MH)	0.56	40	24.45	Cis-caryophyllene (Ses)	0.09
18	19.87	β-Ocimene (MH)	0.06	41	24.68	Germacrene (Sesquiter)	0.02
19	20.92	Methylbenzene (MH)	0.73	42	24.85	Calarene (Sesquiterpen)	0.02
20	21.22	Acetic acid (MH)	0.26	43	24.96	β-Caryophyllene (Ses)	1.58
*	Total	Mono. Hydrocarbons	75.79	44	25.21	Aromadendrene (Sesq)	0.24
21	6.77	Butyl acetate (Ester)	0.04	45	25.95	Deh.Aromadendrene(S)	0.05
22	7.04	N-Hexanal (Aldehyde)	0.05	46	26.10	γ-Caryophyllene (Sesq)	0.11
23	27.81	L-Carvone (Ketone)	0.18	47	26.54	α–Humulene (Sesquit)	0.10
24	8.30	Unknown (UK)	0.02	#	Total	Sesquiterpene-Hydrocarbons	2.34

*MH: monoterpene-hydrocarbons, *OM: oxygenated-monoterpenes, SH: Sesquiterpene-hyrocarbons.

3.2. Physico-Chemical Properties ofMarjoram Essential Oil:

The yield and physico-chemical properties of MEO were determined and the obtained results are presented in Table 2.The results show that, the average yield of MEO was determined to be 1.90%(v/w). In this connection, different yield percentages (2.0, 2.1, 1.2, and 1.7 %) were reported by other authors in their frameworks on marjoram (Lis *et al*., 2007;Soliman, 2009; Busatta *et al*., 2008, and Romeilah 2009; respectively). These differences could be due to growth stages, origin, climatic and drying conditions (Kumar *et al*., 2011).

Table 2: Physico-Chemical Properties of Marjoram Essential Oil:

Parmeter	Value
Volatile oil Yield (on dry wt. basis)	1.9%
Specific Gravity (Density) at 20°C	0.9023gm/mL
Refractive index at 20°C	1.473
Optical Rotation at 20°C	+13
Acid Value	1.2 mg KOH/gm oil
Essential Oil Color	a pale or light yellow color
Solubility In Ethyl Alcohol	1 mL oildissolved in 1mL Ethanol 80%

Results in Table 2 also reveal that specific gravity of MEO was0.9023gm/cm3, which indicates high quality and purity of the volatile oil. However, the refractive index, solubility in alcohol, optical rotation, acid value, are also considered very important parameters determined the freshness, purity and quality of the volatile oil under investigation. Generally the values of these parameters presented in Table 2 are in agreement with those found byRiad, (2005) andSoliman (2009).Generally, the above mentioned values are in accordance with the critical limit values recommended by the Egyptian Standards (ES, 1361/2008).

3.3. Oxidative Stability 0f Sunflower Oil:

Rancimat test was used to determine the antioxidant activity of marjoram essential oil added to sunflower (S) oil at different levels, in comparison to BHT (0.02% w/v), and control refined sunflower oil (without antioxidant), the results are illustrated in Table 3.

The induction period for sunflower oil (control) was 9.42 h., which was increased to 12.10 h. in the same oil by addition of 200 ppm BHT, while, the levels of herb oil used (200 to 800 ppm) gave lower induction periods compared with synthetic one.

Table 3: Oxidative stability of sunflower oil as affected by MEO:

Sample	Induction period at 100°C (hours)	Antioxidant activity	Increasing index %
Control (no additives)	9.42	1.0	…
Sunflow.Oil +200 ppm BHT	12.10	1.28	28.45
Sunflow.Oil +200 ppm MEO	10.18	1.08	8.07
Sunflow.Oil +400 ppm MEO	10.74	1.14	14.01
Sunflow.Oil +600 ppm MEO	11.40	1.21	21.02
Sunflow.Oil +800 ppm MEO	11.91	1.26	26.43

Results demonstrated in Table 3 also indicated that, BHT exhibits the highest antioxidant activity (1.28) compared to all sunflower oil samples under investigation. Furthermore, all MEO concentrations presented a protection factor greater than 1(protection factor of the control) however, the MEO deserve special attention since it was more effective in delaying oxidation under all levels applied, the oxidative stability was directly proportional to the increase of MEO concentration (Table 3). However in the final level used (800 mg.kg–1), MEO presented an oxidation inhibition percentage of 26.43%, which is very nearly *from BHT sample (28.45%).* Unlike synthetic antioxidants, MEO can be added in larger quantities to get optimal effects (addition of synthetic antioxidants is limited under food laws and regulations). As a result the stability of refined oils was improved with the addition of the essential oil, which possesses antioxidant activity. Therefore, MEO could be used as a safe, effective and easily accessible source of natural preservative ingredient in food industry.

3.4.Antioxidant Activity of MEO:

Total Phenolic Contents:

Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Elena *et al*., 2009).Therefore, evaluation of the total antioxidant capacity of plant products cannot be performed accurately by any single method due to the complex nature of phytochemicals (Chan *et al*., 2012). Antioxidant capacity of herbal plants is closely correlated with the content of phenolic compounds. Total phenolic contents (TPC) of marjoram essential oil were determined by using Folin-Ciocalteu reagent method and the results (Not shown) are expressed as mg gallic acid equivalent /g dry weight. TPC was 5.6 mg GAE/g of marjoram essential oil. This finding confirmed the results obtained by Sellamia *et al*.,(2009) who reported that, TPC varied from 2.706 to 6.834 mg/g of dry weight of marjoram essential oils. Recently, Roby *et al*, (2013) found that, the total phenolic contents were 8.10, 5.95, and5.20 (mg gallic acid equivalent/g dry weight) for thyme, sage, and marjoram, respectively.

DPPH Scavenging Assay:

It is clear fromDPPH results illustrated in Table 4 that, the percentage DPPH scavenging activities of marjoram essential oil were concentration dependent. Considerable DPPH radical scavenging activity was evident at all the tested concentrations of MEO. Results in Table 4 also show that, at 400 ppm, marjoram essential oil exhibits high DPPH inhibition (83.6 %). However, none of the MEO samples evaluated here showed activity as strong as the synthetic antioxidant BHT at 200 ppm concentration (92.0%). These results confirmed the results obtained by Romeilah,(2009) who found that, radical scavenging activity of marjoram essential oil was 76.37% at 200 ppm concentration.

Table 4: Effect of Marjoram Essential Oil and BHT on DPPH and β-Carotene Inhibition %:

Treatment	DPPH Inhibition %	β-carotene Inhibition%
Essential oil 50 µg/mL	43.9	38.9
Essential oil 100 µg/mL	60.8	52.3
Essential oil 200 µg/mL	74.1	65.9
Essential oil 400 µg/mL	83.6	76.2
BHT 50 µg/mL	70.3	68.9
BHT 100 µg/mL	76.5	73.8
BHT 200 µg/mL	92.0	90.4

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Antioxidant assay using the β-carotene bleaching method:

In the case of β-carotene /linoleic acid test results (Table 4), the most active MEO concentration, as expected, was the 400 ppm sample (76.2%). The presence of marjoram essential oil can hinder the extent of β-carotene bleaching by acting on the linoleate-free radical and other free radicals formed in the system (Chan *et al*., 2012). Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of MEO, they retained their color and thus absorbance, for a longer time. Table 4 also reveals that BHTreached the percentages of β-carotene Inhibition of 68.9, 73.8 and 90.4% at 50, 100 and 200 ppm; respectively. However, current consumer trends are towards foods that contain natural preservatives rather than synthetic ones. From the results of Table 4 it could be concluded that, marjoram essential oil could be used as a potential source of natural antioxidant in food products.

Generally, it is worth mentioning that, available literature lacks uniform methodology of presenting research results concerning both antioxidant capacity and content of total phenolic contents. Authors use various methods of isolation (steam-distillation or extraction by solvent) while preparing solutions for research and express final results in various conversion units [Goze *et al*., 2009; Chan *et al*., 2012),which makes it difficult to compare results obtained in the research with research results by other authors.

3.5.Antimicrobial Activity and minimum inhibitory concentration (MIC)of MEO:

Results obtained in the antimicrobial and MIC of the essential oil are shown in Table 5.

Table 5: Antimicrobial Activity and minimum inhibitory concentration (MIC):

Microorganisms	Gram Reaction	Inhibition zone diameter (mm/sample)		
		Standard		Oil
		Tetracycline	AmphetericinB	
Bacillus subtilis	(G+)	32	-	17
Escherichia coli	(G-)	32	-	18
Neisseria gonorrhoeae	(G-)	31	-	18
Pseudomonas aeruginosa	(G+)	35	-	15
Staphylococcus aureus	(G+)	30	-	17
Streptococcus faecalis	(G+)	34	-	18
Aspergillus flavus	(Fungus)		18	13
Candida albicans	(Fungus)		19	34

Sample	MIC (μL/ mL)
	Candida albicans
Marjoram essential Oil	32
Standard: Amphetericin B Antifungal agent	98

Tetracycline: Anti -bacterial agent,Amphetericin B: Antifungal agent.

From Table 5 it could be observed that all tested microorganisms were susceptible to the action of marjoram essential oil, with a range of inhibition zone diameter values from 13 to 34 mm/sample (Table 5). These results confirmed the findings reported by Riad (2005).Results in Table 5 further reveal that *Candida albicans*was the most sensitive strain tested to the MEO with the strongest inhibition zone (34 mm), the modest activities of MEO were observed against *Pseudomonas aeruginosa*, with inhibition zones of 15 mm. While, *Aspergillus flavus* was the most resistance strain tested to MEO with the lowest inhibition zone (13 mm).In general, the antimicrobial activities have been mainly explained through terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bondswith active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essentialoils (Burt, 2004; Rota *et al*., 2008). However, these results confirmed the findings achieved by other authers (Riad, 2005; Busatta *et al*., 2008; Chan *et al*., 2012), who reported that marjoram essential oil possesses antimicrobial properties against food borne pathogens and spoilage microorganisms when tested in vitro, and therefore, it may have the greatest potential for use in industrial applications.

MIC was defined as the lowest concentration that inhibited the visible microbial growth. The minimal inhibitory concentration (MIC) was determined here for the microorganism that displayed the strongest inhibition zone in antimicrobial activity test, MIC was determined by agar dilution method (NCCLS,2002),serial dilution from the treatment essential oil (from0 to 256 µL / mL) was used. As shown in Table 5 it is clear that *Candida albicans*was the most sensitive strain tested to the MEO with the strongest inhibition zone (34 mm), the minimum inhibitory concentrations (MIC) was 32 µL/mL, in comparison to 98 µL/mL with commercial Amphotericin B (antifungal agent), indicating that the antifungal activity of MEO is even more than synthetic agent.These results are in agreement with the findings reported by Riad (2005) and Busatta *et al*., (2008).

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Conclusion:

Bassed on the above chemical constituents, physico-chemical properties, antioxidant and antimicrobial activities of marjoram essential oil, it is concluded that MEO could be used as natural preservative source in various food products to control microbial contamination; delay oxidative degradation of lipids; improve quality and nutritional value of foods; and replace synthetic preservatives, whose use is being restricted.

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