J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (1): 45 - 61, 2012

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SOME HERBS VOLATILE OILS AND THEIR ETHANOLIC EXTRACTS

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ABSTRACT

Antioxidative and antimicrobial properties of volatile oils and their ethanolic extracts from four herbs namely, thyme, rosemary, fennel and marjoram were determined. the volatile oils were analyzed by GC–MS, different compounds were identified, namely carvone, thymol, α- pinene, anisole , caryophyllene, eugenol ..... etc, and about 11 different phenolic compounds were identified namely . Catechien, Chlorogenic acid, Caffien, P-OH Benzoic, Ferrulic, Caffeic, Synergic , Salicylic, P-coumaric, Cinnamic and Vanillic acids Results indicated that all extracts were varied in their content of phenolic compounds,

Volatile oils and ethanolic extracts obtained from different herbs were evaluated for their antibacterial activities against five strains Gram-positive and Gram-negative pathogenic bacteria : *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella sp.*, *Shigella flaxnary* and *Enterobacter sakazakii* , respectively and two fungus strains namely , *Aspergillus niger and Aspergillus oryazae* . Thyme volatile oil proved the superiority over the other volatile oil against the tested microorganisms followed with marjoram volatile oil. Whereas fennel volatile oil had positive effect against tested microorganisms exception with *Enterobacter sakazaki* , also, inhibitory effect found by thyme , marjoram and rosemary extracts against all tested microorganisms .Results of minimum inhibitory concentration (MIC) indicated that both of thyme extract and volatile oil have a strong antimicrobial activity .

Obtained data suggested the possibility to use volatile oil and their ethanolic extracts in food processing to control pathogens for food preservation as well .

Keywords: Volatile oils, ethanolic extracts , DPPH, oxidative stability , rancimat test antimicrobial activity .

INTRODUCTION

Oxidative stability is one of the most important indicators for marinating the quality of edible oils and could be increased using natural and synthetic antioxidants, the effect of antioxidants on the behavior of edible oils is widely investigated , but very little known on the effective of antioxidants on the stabilization of oils *(*Sendzikiene *et al.*,2005*)*.

Natural antioxidants may be useful in retarding oxidative deterioration of food materials especially those with high lipid content Hence there is a tendency towards the use of natural antioxidants of herbs and to replace these synthetic ones. The ap ication of natural antioxidants to prevent edible oil rancidity had been studied (Xiong,*et al.* 2001).

Aromatic plants have been used for centuries as spices and condiments to confer aroma and flavor to food and beverages. Additionally, due to their constituents, medicinal and aromatic plants can act as stabilizer agents, playing an important role in the shelf-life of foods and beverages

(Salgueiro *et al.* 2010) but only in the last decade scientific research has focused its interest on their (Karaman *et al.* 2001).

Volatile oils and their extracts acts as natural sources of antimicrobial and antioxidant compounds (Sagdic etal ., 2003). Volatile oils are natural compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites (Bakkali *et al.* , 2008). They are usually obtained by hydro-distillation, steam distillation or dry distillation of a plant or of some parts of it. The main advantage of Eos is that they can be used in any food and are generally recognized as safe (GRAS) (USFDA, 2006)

Volatile oils are made up of many different volatile compounds and have shown to posses antimicrobial properties, volatile oil and plant extracts are arousing increasing interest because of their safe status, their wide acceptance by consumers and their exploitation for potential multi –purpose functions uses. So, volatile oil are the one of the most promising groups of natural compounds for the development of safer antifungal and antibacterial agents .

Use of volatile oil as antimicrobial agents in food systems may be considered as an additional intrinsic determinant to increase the safety and shelf life of foods (Nychas *et al.* 2003) . Food processors, food safety researchers and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks caused by some pathogens and/or their enterotoxins. *Escherichia coli, Staphylococcus aureus, Salmonella spp., Yersinia spp. and Clostridium spp.* are responsible for many cases of intestinal disorders, causing vomiting and diarrhoea. Moreover, some microorganisms are also associated with food spoilage, causing economic losses (Demirci, *et al.* 2008 and Friedman *et al.* 2002). Global interest in bio preservation of food systems has recently been increased because of great economic costs of deterioration and poisoning of food products by food pathogens. Volatile oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents Use of volatile oils as antimicrobial agents in food systems may be considered as an additional intrinsic determinant to increase the safety shelf life of foods (Nychas *et al.* 2003).

Many spices and herbs exert antimicrobial activity due to their volatile oil fractions. The most common herbs and spices in the miditerarrin region are thyme, fennel, ginger, mint, basil ..etc , these herbs and spices are widely used as a source of volatile oils for flavoring and recently have been used as a valuable source of the potent antioxidants in food industries

According , This study was carried out to compare between some volatile oils extracted from some herbs namely, thyme, rosemary, fennel and marjoram and their ethanolic extracts as natural antioxidants and antibacterial agents .

MATERIALS AND METHODS

Materials:

Palm oil, Tertiary Butyl Hydroquinone (TBHQ) were obtained from Misr Oil and Soap Company, Mansoura, Egypt.

Herbs : Dried herbs namely, **Thyme** (*Thymus. Vulgairs*), **Fennel** (*Foeniculum vulgare*) , **Rosemary** (*Rosmarinus officinalis*) and **Marjoram** (*Origanum majorana*)were purchased from local market in El-Azhar, Cairo, Egypt .

Microorganisms: Two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), three Gram negative bacteria (*Shigella flaxnary, Salmonella sp.* and *Enterobacter sakazakii*) and two fungal strains *(Aspergillus niger* and *Aspergillus flavus).* The bacteria strains were obtained from Dairy Dept., faculty of Agric., Mansoura University and the two fungal strains were obtained from Microbial. Dept., faculty of Agric., Mansoura University.

Chemicals :

DPPH (1.1 Diphenyl 1-2-picrylhydrazl) was obtained from sigma/ Aldrich company .USA , and Sodium sulphate anhydrous was obtained from El-Gomhoria company for El-Gomhoria Co. for trading in Medicines, Chemicals and Medical Supplies, El-Mansoura , Egypt.

Methods:

Separation of volatile oils:

The dried herbs were individually ground by domestic grinder type Moulinex and then pass through 80 mesh sieve, then each herb was extracted using hydro- distilled method for 5 hrs in a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate and stored in dark bottle for further analysis (Skandamis and Nychas 2001).

Ethanol Extracts: All herbs firstly were dried at 45-50°C for an hour using air drying oven (Officine Specializzate, GARBUIO, Essiccatioi, TREVISO, ITALY). Then, it was extracted using a method of maceration with ethanol (500 g dried herb / 500 ml solvent) for 12 hours. After maceration, the extracts were collected, filtered and evaporated with vacuum rotary evaporator (BÜCH, RE 111, SWIZERLAND). The evaporated extracts were collected in dark glass bottles and stored at 3-5°C until will be used (Wojdylo *et al* 2007).

Chemical analysis:

Identification of volatile oils compounds:

The volatile oils were identified and determined according to Adms, (2001) using Gas Chromatography –mass spectroscopy (GC-MASS) techniques at Food Technology Research Institute , Giza , Egypt .

The operation conditions: Mass selective xl inert detector 5975,obtained results were identified by HP 30908 N.

Extraction and identification of phenolic compounds :

Extractions of phenolic compounds of all herbs were carried out according to the method described by Wojdylo *et al.*, (2007) and were

determined according to the method described by *Waskmundzka et al.,(2007)*, Which calculated as mg Gallic acid /100g of dry weight material.

- Phenolic compounds of herbs ethanolic extract were identified using high performance liquid chromatography (HPLC) ,"HP1050" . Food Technology Research Institute , Giza, Egypt .

Radical scavenging assay DPPH:

DPPH radical was determined according to Mau *et al.*, (2004) with minor modifications . The extracts 100,200, 250,500, 1000 µg) in methanol (1 mL) was mixed with 4 mL of 0.004% methanolic solution of DPPH . The mixture was shaken vigorously and left to stand for 30 min in dark at 30°C , and the absorbance was then measured at 517 nm. using Spekol 11, Carl Zeiss Jena, German.

The percent of DPPH discoloration of the samples was calculated according to the equation:-

Antiradical Activity% =

Absorbance of control – absorbance of sample / absorbance of control .

Oxidative stability of palm oil : :

The influence of different methanolic extracts evaluated on oxidative stability of palm oil was evaluated by the rancimat method using Metrohm 679 as described by De Man and DeMan, (1984).Oil without any addition was used as control. The induction period (I.P.) was conducted at 100°C and calculated at 25° C at Food Technology Research Institute , Giza, Egypt according as described by Pardun and Kroll (1972)) .

Antioxidant Actively (S.F.) = I.P of sample / I.P of control

Increasing Index %= (I.P of sample - I.P of control / I.P of control) x 100 .

I.P. = Induction Period

Microbiological experiments:

Conditions for cultivation:

Bacteria were assayed on nutrient agar medium and fungi were assayed on potato dextrose agar media. Bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 $x10^6$ CFU/ml and spore suspensions were adjusted with sterile saline to a concentration of 1.0 x 10^4 spore/ml. 0.02% Tween 80 was added to the media to emulsify the oils (Hood *et al.*, 2003).

Assessment of antimicrobial activities :

Agar wells – diffusion method : was used and the antimicrobial test was performed according to the method of Wan *et al.*, (1998)_with some modification. Briefly, 0.5 ml of fresh overnight cultures of the tested bacteria (1.0 $x10^6$ CFU/ml), and fungi (10^4spore/ml) were spread on nutrient agar for bacteria and Potato dextrose agar for fungi in sterilized Petri dishes (90 mm diameter). Wells were created using a 6mm cork borer. Each well was filled with 10µl of the oils samples. The incubation was carried out at 37°C for the bacteria and 30°C for the fungi. After 24-48h of incubation, the antimicrobial activity was evaluated by measuring the width of the zone of inhibition. Experiments were performed in triplicate.

Minimum Inhibitory Concentrate (MIC):

The Broth dilution method was used to determine the minimum inhibitory concentration (MIC). 0.5 ml of fresh over night tested bacteria and

fungi were inoculated into tubes containing serial dilutions of volatile oils. All tubes were incubated at 37°C and 30°C for bacteria and fungi, respectively. Tubes were examined for visible signs of bacterial and fungal growth. The highest dilution without growth is the minimal inhibitory concentration (MIC) (Andrews, 2001).

Statistical analysis: Values represented are the means and standard error , significance was used at $p \leq 0.05$,(ANOVA) was done using SPSS 17 program for windows.

RESULTS AND DISCUSSIONS

Separation and identification of volatile compounds in herbs:

Volatile oils from different herbs were identified and determined_using(GC/MS) analysis. Data presented in Table (1) revealed that the volatile oils from thyme, fennel, rosemary and marjoram which contained a complex mixture consisting of mainly oxygenated mono-and sesquiterpenes mono hydrocarbons different compounds namely, linalool , caryophyllene, terpens and α- pinene

Thyme oil is considered as a source of natural antioxidants . Chemical composition of thyme oil showed a great chemical homogeneity characterized by high amount of thymol (44.5), carvone (11.20) Caryophyllene (14.30%),. There were some oxygenated monoterpens like Carvacol (15.5%) and cadinol 0.51% . Also, some of other volatile compounds were thymoquinone 5.81% , α- terpene (0.41%) and other Alcohols were defined Lanisole (0.21) (Zeyada *et al.*, 2007).

Fennel oil is not only use as natural source of food flavoring, but also have a medicinal properties for the treatment of various diseases .The chemical constitutes of commercial fennel oil and its main compounds was tested also, among them cavicol (11.31%) further more some interesting compounds were identified namely camphor, cryophellene. These compounds followed by some oxygenated mono terpenes Kampheriol (11.21) and phenol (1.32%) (Adms , 2001)

Marjoram oil is used also as natural flavoring agents nearly 15 compounds were identified, over 50% of the total volatile compounds from p- cymene (66.90%) the major volatile fraction responsible for the aromatic and sharp flavour, kurane and linalool (16.65 and 8.21) respectively terpinol+ terpinene were the predominant oxygenated mono terpenes compound with area *20.22%* other compounds were detected namely limone,. Major volatile detected in this study were in accordance with Yoshizaki *et al* ., (2010)

Rosemary oil : from abovementioned results in Table (1) , it could be noted that rosemary oil are characterized by the presence of large amount from monoterpenes hydrocarbons represented α- Pinene (22.3 %) and the predominant alcohol was cineole which presented (28.78 %)and α-terpineol was detected as a minor monoterpenes alcohols. While ester compounds were detected namely, camphor and camphene were(20.92 and 5.66 %) . these results were in accordance with (EL –Bastawesy *et al.* 2008 and Celiktas *et al.* 2007)

Table (1): Some identified compounds of volatile oils extracted from herbs.

Volatile oils	Identified constituents	Area %
Thyme oil (T_{oil})	thymol	44.5
	carvacol	15.5
	carvone	11.20
	thymoquinone	5.81
	linalool	0.81
	β- caryophyllene	0.8
	α- pinene	0.8
	anisole	0.62
	eugenol	0.5
	borneol	0.51
	cadinol	0.51
	Lanisole	0.21
	Cayrophyllene	0.21
	α- terpene	0.41
Fennel oil (F_{oil})	α- pinene	18.65
	cavicol	11.31
	Kampheriol	11.21
	phenol	1.32
	Bornyol	0.88
	camphor	0.74
	Cryophellene	0.55
	Cineole	0.32
Marjoram oil (Ma_{oil})	Apiol	15.62
	α-terpene	18.32
	p-cymene	66.90
	linalool	8.21
	kuarene	16.65
	Terpineol+ terpinene	20.22
Rosemary oil (Rm_{oil})	α- pinene	22.3
	borneol	5.6
	b-pinene	8.79
	Camphene	5.66
	cineole	28.78
	Limonene	2.33
	α-terpineol	5.66

A- Antioxidant Activity of herbs volatile oils and their ethanolic extracts

Radical scavenging activity of herbs and spices oils: DPPH is a free radical compound terminating the propagation of radicals and generating and has been widely used to test the free radical unreactive phenoxyl radicals as well as hydroperoxides, the DPPH assay, which measure the ability of compounds to transfer labile H-atoms to radicals, is the commonest method of antioxidants activity evaluation (Brand-Williams *et al.* 1995).

The radical scavenging activity of natural volatile oils from herbs depends greatly on their concentration as resulted in table (2). The highest DPPH was detected in rosemary , thyme and marjoram , were 80.21, 85.02 , 83.65 %, respectively. Thyme oil was found to effective in compare

with other volatile oils this could be attributed to it is composition of some main components namely carvacol,thymol and carvone . The lowest scavenging activity was 70.54 %detected for fennel oil at the concentrate of 1000 µg/ml .

Results in the same table indicated that the ethanolic extracts of herbs were also detected the same trend of scavenging effect were observed, the ethanolic extracts could be arranged designingly as follows: thyme > marjoram> rosemary > marjoram extracts.

These results indicated that natural oils from herbs under investigation have a noticeable effect on scavenging free radicals. These are also attribute to their molecular structure and their content of phenolics and aromatic compounds and α- tocopherol . The antiradical scavenging activity would be related to the substitution of hydroxyl groups in the aromatic rings of phenolics, thus contributing to their hydrogen donating ability. And their content of phenolics compounds and hydroxyl groups contribute markedly to the antioxidant activity. This results were nearly accordance with (Yen *et al.* 2005).

Table (2): Free radical of scavenging activity on of natural and synthetic antioxidants.

Antioxidants	Concentration				
	1000 µg/ml	500 µg/ml	250 µg/ml	200 µg/ml	100 µg/ml
TBHQ	93.1±2.08	89±7.36	74±2.92	70±2.56	48.3±3.19
A-Volatile oils :					
Rosemary oil (Ro)	80.21 ±2.7	70.38 ±2.92	50.68 ±3.70	40.58 ± 1.90	39.21 ±3.18
Thyme oil (To)	85.02 ±2.5	77.84 ±2.31	58.69 ±1.90	55.36 ±1.65	45.32 ±
Marjoram oil (Mo)	83.65 ±1.8	75.36 ±2.65	55.68 ±2.31	45.98 ±2.09	40.32 ±1.90
Fennel oil (Fo)	70.54 ±3.5	66.25 ±4.84	45.69 ±2.77	37.65 ±3.70	35.50 ±3.25
B- Ethanol extracts :	**Ethanol extract concentration**				
Rosemary Extract (R_m E)	60.32±2.88	55.98±2.56	50.65±22.56	35.69±3.74	33.61±1.90
Thyme Extract (TE)	70.35 ±2.31	68.24 ±3.71	55.48 ±3.19	39.65 ±2.33	35.58 ±3.25
Marjoram Extract (ME)	65.3 ±4.31	57.35 ±2.78	51.32 ±1.90	38.56±	31.54 ±1.95
Fennel Extract (FE)	55.31 ±1.87	55.00 ±4.79	48.60 ±2.77	34.87 ±2.44	24.32 ±3.74

All values are means of three replicates ± SD

Table (3): Total Phenolic Content (TPC) in Herbs Extracts as mg/g Gallic acid .

Phenolic Extracts	mg/g Gallic acid
Rosemary Extract (Rm)	114.23
Thyme Extract (TE)	265.96
Marjoram Extract (ME)	194.08
Fennel Extract (FE)	59.25

Separation and Identification of phenolic compounds extracts from some herbs :

Data in Table (3) showed the phenolic compounds content (TPC) in ethanolic extracts which is more frequently used for isolation of antioxidants.

Ethanolic extracts were always greater in total Polyphenolic compounds as gallic acid.

Data revealed that, the total phenolic compounds of thyme extract represented 265.96 mg/g. followed by marjoram extract exhibited 194.08, rosemary extract was 114.23 mg/g. gallic acid respectively, while Fennel extract exhibited the lowest amount of phenolic content was 59.25 mg/g. gallic acid . These data were in accordance with Moure *et al.*,(2001) who found that the phenolic compound have high total and active constitutes, these compounds resulted in the superiority, quenching and rerated oxidation of fatty products which herbs and spices where added .

Also, it could be noted that the variations in total phenolic content could be attributed to the specific nature of the plant type, (Kim *et al.*,1995) stated that the antioxidant activity of extracts produced from herbs and spices was dependent on the type of herbs than the solvent and these results were not doubtful because phenolic compounds in plant foods are largely influenced by genetic factors and environmental conditions. The difference in phenolic content could affect the antioxidant capacity of plants, because many phenolic compounds in plants are good sources of natural antioxidants. Therefore, these phenolic extracts should be used as commercial antioxidants substitution from synthetic ones according to their high amounts of phenolic compounds which protect oil from oxidation and rancidity and at as a synergistic agents (Juliano *et al.*, 2000).

Data in Table (4) showed the identification of different phenolic compounds of investigated herbs . The obtained results indicated that phenolic acids were the most abundant compounds in all examined extracts.

Thyme *(Thymus. vulgaris*) is usually rich in phenols ,these compounds known to have high antioxidants capacity. Results showed that Catechien was the most abundant phenolic compounds represented 94.75 mg/g followed with Ferrulic acid 49.19 mg/g . these results were in accordance with Zeyada *et al.*, (2007) and Zheng and Wang(2001) who mentioned that Catechien has been identified as a major components which represents an important contribution especially in the taste of thyme and could be attributed to its antioxidants property as a scavenger of the reactive oxygen species which potent antioxidants inhibiting the photo peroxidation of linolenic acid .

Other phenolic compounds were also detected namely P-coumaric and caffeic acid were 47. 51 and 14.12 mg/g, vanillic acid represented 9.22 mg/g . These obtained results are nearly with the same with those obtained by Moure *et al.*,(2001), who found same flavonoids and biphenols dimmers were found in thyme extract .

Rosemary (*Rosmarinus officinalis*) is a widely natural antioxidants in food flavoring. results of HPLC indicated eleven phenolic compounds were separated an eight of them were identified. Salysillic acid was the major phenolic compounds in rosemary (99.01 mg/g) followed by catechien (62.50 mg/g). Moreover , p-coumaric and p-oh benzoic were also detected (42.1 and 13.2 mg/g) these obtained results were in accordance with those given by (El-Bastawesy *et al.* 2008)

Fennel (*Foeniculum vulgare*) is not widely used as natural antioxidants source of food flavouring but also had antioxidants properties the HPLC

analysis of fennel extract showed a large number of flavonoids and phenolic acids were identified also . Data in Table (4) showed that Salicylic acid was 9.29 mg/g and Ferrullic acid 2.75 mg/g where the most abundant phenolic constitutes in the extract also catechien presented 3.36 mg/g these data were in accordance with those given by (Yansihtevia *et al.*, 2006 , Weinberg *et al.*, 1999 and Marinova and Yansihtevia, 1996).

Regarding to the same data , it could be noticed that caffeic , chlorogenic, and vanillic have the lowest level of phenolic compounds followed by cinnamic recorded the least concentrate in compare with the major extracted phenolic matters.

Marjoram (*Origanum majorana)* was natural antioxidants used for spicing and marenading in some areas.Data in the same table presented that Catechien was the predominant phenolic compounds with 27.14 mg/g followed by Salicylic acid 20.94 mg/g . Also the amount of chlorgenic and benzoic acid seemed to be equal. Only trace amounts of the phenolic acids were detected. Some phenolic compounds were disappeared namely Synergic, P-coumaric and Cinnamic, However there were lower amount of phenolic acids Chlorogenic, Caffeic and Vanillic acids were 4.97 , 2.21 and 1.77 mg/gm respectively . These obtained results were nearly the same with Lien *et al.*, (2008) who stated that catechien and Salicylic acid were presented in marjoram extracts these polyphenols constituent are capable of scavenging more free radicals .

Table (4): Identification of some Phenolic compounds in herbs ethanolic extracts (mg/gm).

Phenolic Extract / Phenolic Compounds	Thyme Extract (TE)	Rosemary extract (RM)	Marjoram Extract (ME)	Fennel Extract (FE)
Catechien	94.75	62.5	27.14	3.36
Chlorogenic acid	22.16	11.32	4.97	0.66
Caffien	---------	11.02	----	0.45
P-OH Benzoic	20.80	13.2	4.65	1.34
Ferrulic	49.19	12.33	----	2.75
Caffeic Acid	14.12	-	2.21	0.98
Vanillic Acid	9.22	-	1.77	0.36
Synergic Acid	11.18	-	----	0.88
Salicylic Acid	----	99.01	20.94	9.29
P- coumaric Acid	47.51	42.1	----	----
Cinnamic Acid	8.13	3.21	-----	0.22

B-Oxidative stability of herbs volatile oils and their ethanolic extracts:

Oxidation rancidity of oils and fats is one of a great concern , since it affects the quality of the food owing the development of various off flavours ,the efficiency of different volatile oils and their ethanolic extracts at the level of 0.2 ml compared with TBHQ (200ppm) were evaluated and the results are presented in table (5), also, the results for induction time which characterizes the resistance of oil and fats to oxidation and the stabilization factor (S.F), were expressed in Table (5) .

Results indicated that TBHQ exhibited the highest induction period (11.5) in compare with the others, addition of rosemary or thyme volatile oils at the concentration of 0.2 ml/l to palm oil resulted in (I.P) 11.2 hours and followed by fennel volatile oil was 10.3 hours at the same concentration .

Results in the same table indicated that The most effective antioxidants is obtained with the palm oil treated with thyme extract at the concentration of 0.2 ml /l represented 10.8 hours. On the other hand, there was a very little difference in induction period in all treated palm oil samples with different extracts at the concentration of 0.2 ml/l that may be due to the linked more of the volatile compounds to secondary degradation oxidation products. The effectiveness of the inhibitor represents the possibility of blocking the propagation phase through interaction with the peroxyl radical, which is responsible for the duration to reach the IP. For our study we focus on the effectiveness of the inhibitor which expressed as IP sample/ IP of control. (Guzman *et al.*, 2009;Tang *et al.*, 2008 ;and Marinova *et al.*, 2008)

In general it could be observed , there were appositive correlation between oxidative stability and the type of antioxidants , generally treated palm with thyme and rosemary volatile oil increased the induction period with the concentration of 0.2 ml in compare with their ethanolic extracts at the same concentration and delaying the oxidation process .

Table (5): Oxidative stability of Palm oil treated with different antioxidants .

Treated oil	I.P at 100° C (hrs)	Shelf Life (months)	Antioxidant activity	Increasing Index %
Control	10.00	16.02	--	---
Palm oil+TBHQ 200 ppm	11.50	16.93	1.15	15
Palm oil + RmO 0.2 ml	11.2	17.00	1.12	12
Palm oil + To 0.2 ml	11.2	16.1	1.11	11
Palm oil + Fo 0.2 ml	10.3	15.9	1.03	3
Palm oil + Mo o.2 ml	10.3	15.9	1.03	3
Palm oil + RmE 0.2 ml	10.3	15.7	1.03	3
Palm oil + TE 0.2 ml	10.8	15.9	1.08	8
Palm oil + FE0.2 ml	10.5	15.9	1.05	5
Palm oil + MaE 0.2 ml	10.5	15.0	1.05	5

RmO = Rosemary oil . To = thyme oil .. Fo = Fennel oil . Mo= Marjoram oil.
RmE = Rosemary extract . TE = thyme extract FE = Fennel extract
Ma E= Marjoram extract

C- Antimicrobial activity of herbs volatile oils and their ethanolic extracts :

The antimicrobial activity of some herbs volatile oils and their extracts namely , rosemary, marjoram, thyme and fennel and their ethanolic extracts against some food borne and pathogenic microorganisms by wells diffusion method were assessed and the results presented in Table (6) . Results indicated that, The volatile oils of thyme and rosemary were superior in compare with other volatile oils added, they have an inhibitory effect against *Fungi namely, A. niger* and *A. flavus* ,gram negative bacteria *Shigella*

flaxnary , salmonella sp. and enterobacter sakazakii , gram positive bacteria Staphylococcus aureus and *bacillus cereus*. From the microbial analysis it could be reported that rosemary volatile oils have a moderate effect against tested pathogenic strains G^+, and fungi. This could be attributed to gram positive bacteria are more sensitive to volatile oil than gram negative bacteria due to that marjoram volatile oil contained borneol, camphor , cineole and limonene which are good antimicrobial compounds., it could be also due to consisted mainly of carvacerol , linalyl acetate and thymol as major components exhibited a complete mycelia inhibition effect on the growth of fungi (Bourchra *et al.* 2003 and Juliano *et al.* 2000)

According to the results of wells diffusion method indicated marjoram volatile oil exhibited a considerable antimicrobial activity against the strains tested particularly gram positive bacteria namely, *Staphylococcus aureus* and *bacillus cereus* and gram negative bacteria *salmonella sp.* These results are in accordance with those given by Kuarta and Koike(1983) and Govaris *et al.* (2010) who mentioned that antimicrobial effect of marjoram and thyme oils constitutes were found to be phenols, alcohols, aldhydes, ketones, ethers and hydrocarbons can increase that antimicrobial activity and their bacterostatic effect depending on their effectiveness concentration . Data showed that, Thyme volatile had the most antifungal effect. Karaman *et al.*, (2001) reported that thyme oil which rich in carvacerol and thymol possessing high levels of antifungal and antibacterial activity.

Results also, indicated that Fennel and marjoram oil have also displayed high levels of antifungal activity, it has been demonstrated that the antimicrobial effects of volatile oils causing structural and functional damages to the bacterial cell membrane , it also indicated that the optimum range of hydrophobicity is involved the toxicity of volatile oils (Goni *et al.* 2009)

Several authors (Viuda-Martos *et al.*, 2008 , Tomaino *et al.*, 2005; and Gergis *et al.*, 1990).) stated that the major component of thyme is thymol, the antibacterial and antifungal activity of these compounds has been confirmed on bacteria.

Thymol has been shown to cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents (Oussalah *et al.*, 2006).

Most studies reporting the action of volatile oils against pathogenic bacteria agree that volatile oils are relatively more active against Gram+ve than Gram-ve bacteria (L Viuda-Martos *et al.*, 2008 and Ambert *et al.*, 2001). The cell wall structure of Gram - ve bacteria is constituted essentially with lipopolysaccharides. This constituent avoids the accumulation of oils on the cell membrane (Bezic` *et al.*, 2003).

Results in the same table showed that thyme and marjoram extracts were the most efficient ethanolic extracts against Gram positive bacteria, *S. aureus* ($Gram^+$ve) is more sensitive to thyme than *B. cereus* ($Gram^+$ve) and *E. sakazakii* ($Gram^-$ve). *Sh. flaxnary* and *salmonella sp* ($Gram^-$ ve bacteria) were resistant to the thyme extract while , all ethanolic extracts were effective against fungi activities . this could due to these extracts were rich in some phenolic compounds namely, carnosol , gallic acid chlorogenic acid. . This

results were in accordance with those given by (Oussalah *et al.*, 2006 and Kim *et al.* 1995).

(Table 6):. Antimicrobial activity of some herbs volatile oils and their ethanolic extracts (inhibition zone/mm).

Microbes / Oils	Gram negative bacteria(g+)			Gram positive bacteria		Fungi	
	Shigella flaxnary,	*Salmonella SP.*	*Enterobacter sakazakii*	*Staphylococcus aureus*	*bacillus Ceruis*	*Aspergillus niger*	*Aspergillus oryzae*
A-Volatile oils							
Rosemary oil (Ro)	12.1	8.2	10.7	11.1	11.3	14.0	13.1
Thyme oil	+	+	+	+	+	15.0	14.0
Marjoram oil	+	8.2	+	11.0	10.7	10.0	11.0
Fennel oil	1.9	1.7	NA	1.1	1.1	7.0	5.0
B-ethanol extracts :							
Rosemary Extract (R_m E)	5.6	5.2	5.0	3.5	2.1	5.4	5.5
Thyme Extract	8.1	8.2	7.6	7.5	7.2	8.5	8.4
Marjoram extract	7.2	7.1	7.5	7.3	3.0	6.1	6.1
Fennel extract	4.1	NA	NA	NA	NA	5.1	5.1

+ 100 % activity NA= non active

The Minimum Inhibitory Concentration (MIC) of the thyme volatile oil were tested and the results were illustrated in Fig. (1). Results indicated that MIC for thyme oil was varied between 12.5 – 50 µl/ml. The MIC values for *B. cereus* and *E. sakazakii* were higher than the MIC values of *S. aureus* and fungi. The lowest values were detected for the tested fungi namely *Aspergillus niger* and *Aspergillus oryaze* were the same (12.5 µl/ml).

Results in Fig. (1) showed that *Staphylococcus aureus* have the best susceptibility towards the ethanolic extract of *Thymus vulgaris* with a MIC value of 15.5_ µl /ml followed by *Bacillus cereus* and *salmonella sp.* MIC was 31.0_ µl /ml. On the other hand, the methanol extract of thyme demonstrated moderate activities against tested bacteria. The best activity was seen against *Staphylococcus aureus* and the lowest activity was against *Salmonella sp.*

Finally it could be concluded that volatile oils and their extracts could be a natural source of antioxidants and antimicrobial agents in reducing the total contamination level of foods beside their effect against food pathogen bacteria .

Our obtained results suggested that, thyme and marjoram volatile oil and their extracts have a strong antimicrobial activity.

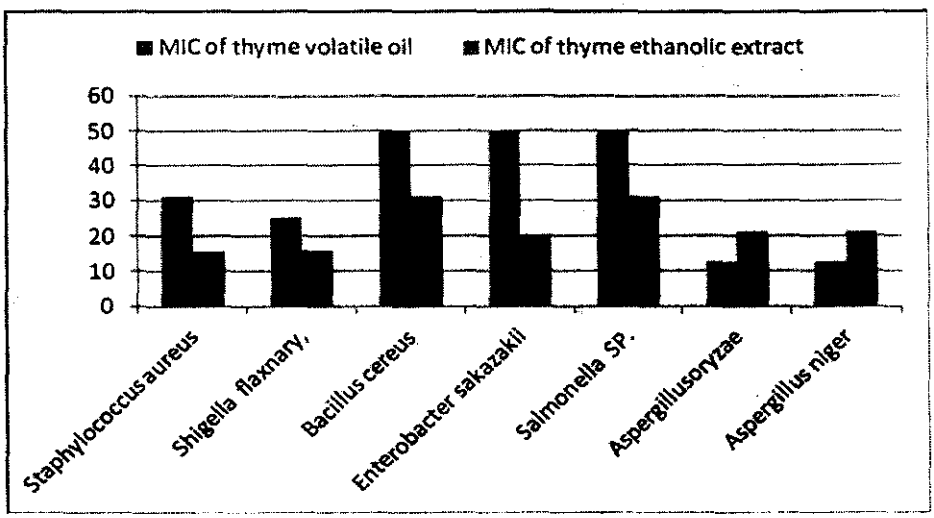

Fig. (1): Minimum Inhibitory Concentration (MIC) of thyme volatile oil and thyme extract (µl/ml) .

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فاعلية مضادات الاكسدة والنشاط الميكروبي للزيوت العطرية لبعض الاعشاب ومستخلصاتها الكحولية

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باستخدام جهاز الفصل الكروماتوجرافي GC-MS تم فصل وتقدير المواد الفعالة للزيوت العطرية المستخلصة من بعض الاعشاب وهي الزعتر وحصا اللبان والشمر والبردقوش وكذا مستخلصاتها الكحولية وكان اهمها carvone, thymol, α-pinene , anisole, cayrophyllene, , eugenol.

بالاضافة الي حوالي ١١ مركب فينولي مختلف . ١١ ساعة Catechien, Chlorogenic acid, Caffien, P-OH Benzoic, Ferrulic, Caffeic, Synergic , Salicylic, P- coumaric, Cinnamic and Vanillic acids.

كما اوضحت النتائج عند تقدير النشاط الميكروبي للزيوت العطرية للاعشاب ومستخلصاتها الكحولية علي خمسة سلالات من الميكروبات وهي *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella sp.*, *Shigella flaxnary* and *Aspergillus niger and enterobacter sakazakii respectively and Aspergillus oryazae* . اظهرت النتائج ان كلا من زيت الزعتر اعطي اعلي فاعلية تجاه جميع الميكروبات المختبرة تلاه الزيت العطري للبردقوش بينما الزيت العطري للشمر اثر بايجابية علي النشاط الميكروبي لجميع الميكروبات فيما عدا *Enterobacter sakazakii* وايضا اعطت المستخلصات الكحولية لكلامن الزعتر والبردقوش وحصا اللبان فاعلية تجاه جميع الميكروبات المختبرة . واوضحت نتائج اختبار MIC ان كلا من الزيت العطري والمستخلص الكحولي للزعتر اعطت اعلي فاعلية ضد النشاط الميكروبي . لذلك نقترح من النتائج امكانية استخدام الزيوت العطرية ومستخلصاتها الكحولية في مجال التصنيع الغذائي للحفظ والحد من نمو البكتريا الممرضة للانسان .

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