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Preliminary Study on Some Essential Oils Comprising Their Chemical Composition and Antibacterial Activity

***El-Hamahmy, A.F.; **Abul-Fadl, M. M;*El-Kotkat, M.B. and *Abd-Elraheim, A.A**

***Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt**
****Food science and Technology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt**

Abstract

Four essential oils (EOs) were extracted from leaves of Sage, Marjoram, Rosemary and seed of clove spice plants using hydrodistillation. They were fractionated to recognize and identify their ingredient components. In vitro by well agar diffusion and measuring the diameter of inhibition zone, the Eos were examined for their antibacterial activities against some bacterial isolates which caused spoilage of manufactured meats. The tested bacterial isolates were isolated from spoilage burger. They were identified and found to be belonged to three Gram negative bacteria (*Klebsiella pneumonia, pseudomonas aeruginosa and serratia marcescens*) and six Gram positive bacteria (*Bacillus anthrakoids, Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Lactobacillus sp.) The* obtained date showed great variation between the tested Eos in their chemical compounds especially that with high proportion. Eos were also varied in their antibacterial activity against the tested bacterial isolates. The gram negative bacterial isolates showed to be slightly less susceptible than gram positive bacterial isolates. The essential oils (Eos) are known as natural materials used to enhance the product flavor. In this respect the present study stated that Eos may be also used as natural preservatives to inhibit and delay the growth of bacteria causing meat spoilage.

INTRODUCTION

Essential oils (Eos) also called volatile or ethereal oils; (**Guenther,1948**) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) They can be obtained by expression, fermentation enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of Eos (**Van de Braak and Leijten, 1999**).An estimated 3000 Eos are known of which about 300 are commercially important destined chiefly for the flavours and fragrances market (**Van de Braak and Leijten, 1999**) the major components of the economically interesting Eos are summarized by (**Bauer *et al.*, 2001**). Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of the EO or its head spice (**Salzer, 1977; Scheffer and Baerheim Svendsen, 1981; Wilkins and Madsen, 1991; Deferera *et al.*, 2000; Juliano *et al.*, 2000; Jerkovic *et al.*, 2001; Delaquis *et al.*, 2002**). Eos can comprise more than sixty individual components (**Senatore, 1996; Russo *et al.*, 1998**). Major components can constitute up to 85% of the EO whereas other components are present only as a trace (**Senatore, 1996; Bauer *et al.*, 2001**). Isolation of spoilage bacteria has been received great attention and investigated by many researchers (**Fraizer, and Westhoff, 1977; Burt, 2004; Bektas *et al.*, 2005 and Ebrahim Abadi *et al.*, 2010**) Most studies investigating the action of whole Eos against food spoilage organisms and food borne pathogens. It is agreed that, generally, Eos are slightly more active against gram positive than gram negative bacteria (**Shelef; 1983; Shelef *et al.*, 1984, Farag *et al.*, 1989, Marino *et al.*, 1999, Marino *et al.*, 2001; Harpaz *et al.*, 2003; and Burt, 2004**).

The purpose of the present study was to perform a preliminary informative study on some economic plant essential oils (Eos) throughout their chemical composition as well as their antibacterial activity against some spoilage bacteria which isolated from local spoilage burger. This was made in attempt to contribute to use of Eos as alternative products for microbial control and food preservation.

Materials and Methods

Spice plants

Spice plants namely (Rosemary, marjoram, sage and clove) were purchased from local market at Cairo, Egypt. The part used of these plants were leaves from (rosemary, marjoram, sage and seeds for clove).

The tested bacteria

The tested bacteria were isolated from spoilage burger they were purified and identified according to Bergy's Manual of Determinative Bacteriology(1994) they were found to be three gram negative bacteria (*Klebsilla pneumonia, Pseudomonas aeruginosa and Serratia marcescens*) and six gram positive bacteria (*Bacillus anthrakoids, Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Lactobacillus sp.)*

Fractionation of the essential oils

The essential oil extracted from Rosemary, Marjoram, sage and clove by hydrodistillation were analyzed by using a gas chromatography and gas chromatography-mass spectrometry at National Research Center Giza, Egypt.

1. Gas chromatography analysis:

Gas chromatography was performed on a Hewlett-Packard-5890-series II plus.

2. Gas chromatography-Mass spectrometry analysis:

Analyses were carried out using a coupled gas chromatograph Hewlett-Packard (5890) mass spectrometry Hewlett-Packard-M (5970) Isolated peaks were identified by matching with data from the library of mass spectra (Wiely) and comparison with those of authentic compounds and published data (**Adams, 1995**). The quantitative determination was carried out based on peak area integration.

Determination of antibacterial activity

Antibacterial activity was determined by the well agar diffusion method according to the **NCCLS (1993).** Petri plates containing 20ml of nutrient agar medium were inoculated with a 24 hrs culture of tested isolates. Wells (6 mm diameter) were cut

into the agar using sterile cork borer and crude essential oil of 0.1ml was added into each well by sterilized drepping pipettes, control was carried out using the solvent dimethyl sulfoxide (DMSO) alone. All treatments were incubated at 35°C for 24 hrs. After the incubation period, inhibition zone diameter around the wells were recorded.

Media used

Nutrient broth medium

Nutrient broth medium was used for growing stock culture of bacteria according to (**Difco Manual, 1985**).

Ingredients per liter

Beef extract	3g
Peptone	5g

Final pH6.8at 25°C

The ingredients were mixed and boiled to dissolve, dispensed into flask and then outoclaved at 121°C for 15min

-Nutrient agar medium

Nutrient agar medium was used for determination of the antibacterial effect of plant extracts using well agar diffusion method (**Difco Manual, 1985**)

Ingredient per liter

Beff extract	3g
Pepton	5g
Agar	15g

Final pH6.8 at 25°C the ingredients were mixed and boiled to dissolve, dispensed into flasks and then autoclaved at 121°C for 15 min

Results and Discussion

- Essential Oils and Their Chemical Composition

Essential Oils (Eos) were extracted from four spice plants using steam distillation method. Extraction was from leaves of sage, marjoram, rosemary and seed of clove. The Eos were fractionated to their chemical composition (tables 1, 2, 3 and 4). Concerning the compounds with high proportion, it was observed that marjoram as in table (1). Contained two compounds i.e, carvacrol (45.23%) and borenol (12.66%). While clove as in table (2). Contained only one compound nemlay. Eugenol, Sage as indicated table (3). Contained two compounds i.e, cinol (34.47%) and Borenol (18.08%), Rosemary, table (4), contained three compounds i.e, cloven (12.33%), cinol (38.81%) and borenol (13.70%) Also, it was noticed that some essential oils of the tested spice plants contained some other compound with different proportions. For example cinol found in rosemary, sage and marjoram Eos with proportions 38.81%, 34.47% and 0.14% in descending order. Numerous publications have presented data on the composition of the various Eos. The major components of the economically interesting Eos are summarized by **Bauer *et al.*, (2001).** Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of the EO or its head space (**Salzar, 1977, Scheffer and Baerheim Svendsen, 1981, Wilkins and Madsen, 1991, Daferera *et al.*, 2000; Juliano *et al.*, 2000; Jerkovic *et al.*, 2001 and Delaquis *et al.*, 2002**) Eos can comprise more than sixty individual component (**Senatore, 1996; Russo *et al.*, 1998**). Major components can constitute up to 85% of the EO whereas other components are present only as a trace (**Senatore, 1996 and Bauer *et al.*, 2001**)

Table(1) Chemical Compound of Marjoram Essential Oil

Peak No.	TR*	Area%**	Component
1	14.14	.0.86	2-Butylfuran
2	14.46	0.35	Heptanol
3	16.16	3.02	1.2.5 tri methylpyrole
4	16.33	0.53	α-Thujene
5	16.92	0.65	Sabinene
6	17.56	1.24	α-pinene
7	18.10	6.67	Fluorovaline
8	18.55	2.69	β-cymene
9	18.73	5.85	Champhor
10	20.08	12.66	Borenal
11	21.40	2.97	2-choloro anisole
12	22.05	0.60	Geraniol
13	22.69	1.85	Menthol
14	23.92	0.20	Terpinen-4-ol
15	26.18	45.23	Carvacrol
16	27.38	8.38	3-Octanone
17	28.82	1.09	Citral
18	30.40	0.13	Anethole
19	30.96	1.17	a-terpinyl acetate
20	32.45	0.06	α-ionone
21	34.05	0.14	Cinol
22	36.17	3.41	Neral
23	36.93	0.09	Mevinphos
24	37.34	0.19	Clovene

*TR: Relation time

**Area%: Percentage of component

Table(2) Chemical Compound of Clove Essential Oil

Peak No.	TR*	Area%**	Component
1	34.59	93.35.	Eugenol
2	36.46	3.33	Caryophyllene
3	37.86	0.41	Benzaldehyde
4	41.39	0.80	n-Heptacosane
5	54.21	0.11	n-Wonacosane
6	54.51	0.16	n-Hentriacontane
7	56.06	0.20	n-Tri tria contane
8	58.42	0.17	n-Nonadecane
9	59.67	1.05	Eugeny acetate
10	61.06	0.42	n-Heneicosine

*TR: Relation time

**Area%: Percentage of component

Table (3) Chemical Compound of Sage Essential Oil

Peak No.	T R*	Area%**	Component
1	14.02	0.29	Ethyl2-methylbutenoate
2	14.19	0.25	1-Butoxy-2-propanal
3	14.56	7.76	3.4Diethylhexane
4	15.21	6.82	Heptanal
5	16.43	8.46	Octanal
6	16.91	3.93	3-Decene
7	18.39	0.34	Allyl trichlore acetate
8	18.88	34.47	Cinol*
9	20.09	0.81	1-Octanamine
10	21.54	0.22	1-Phenylathanone
11	22.43	1.50	Propyl hexanoate
12	22.98	1.14	Tetradecane
13	24.48	18.08	Borenal*
14	25.09	0.87	3-Chlorobenzylamie
15	25.78	0.18	Vinyl phenol
16	26.21	2.11	S-Methyl butanethioats
17	27.33	3.77	Dodecane
18	28.85	0.08	Ethyoctanate
19	30.40	1.77	Putyl butyrate
20	31.78	0.04	4-Chlorobenzoic acid
21	32.21	0.08	Alpha-cubebene
22	32.86	0.05	Glycolaldehyde
23	33.11	1.34	Benzene
24	35.66	0.11	3-Methyl-Ibutanol
25	36.24	4.37	2-3Dimethyl butanol
26	36.93	0.26	Mevinohos
27	37.56	0.82	Clovene
28	37.83	0.09	Thujone

*TR: Relation time

**Area%: Percentage of component

Table(4) Chemical Compound of Rosemarry Essential Oil

Peak No.	T R*	Area%**	Component
1	14.02	0.31	Tricycleme
2	14.53	12.33	Clovene
3	15.27	8.75	Camphene
4	15.43	0.27	Verbenene
5	16.41	4.47	β-pinene
6	17.00	1.71	Myrcene
7	17.70	0.63	α-phellandrene
8	18.43	1.22	3-Carene
9	18.90	38.81	Cinol
10	20.13	1.67	Para-cymene
11	21.56	0.43	Limonene
12	23.25	0.09	Terpinene
13	24.50	13.70	Borenol
14	25.10	0.29	Camphor
15	25.22	0.73	Furanone
16	25.76	0.31	Pinocarvone
17	26.11	2.98	Geraniol
18	27.02	5.74	Bornyl acetat
19	27.78	1.45	Methyl eugenal
20	30.41	0.84	2-Methyl jasmonate
21	33.59	0.85	Linaloal
22	33.99	0.09	E-caryephyllene
23	36.19	1.81	α-humulene
24	36.85	0.19	α-bisabalol
25	37.55	0.33	Terpin-4-ol

*TR: Relation time

**Area%: Percentage of component

-Isolation Of Some Bacteria From Spoilage Burger

Nine bacterial isolates were isolated from spoilage burger they were purified and identified according to **Bergey's Manual (1994).** They were found to be belonged to three gram negative bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Serratia marcescens*) and six gram positive bacteria (*Bacillus anthrakoids, Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Lactobacillus sp.*). In this respect, obtained results are in agreement with those mentioned for spoilage bacteria by many researchers **(Fraizer, and Westhoff, 1977; Ebrahimabadi *et al.*, 2010, Bektas *et al.*, 2005 and Burt, 2004)**

-The Antibacterial Activity Of Tested Essential Oils

The concentrated Eos were examined for their antibacterial activity Table (5). Against the obtained bacterial isolates of spoilage burger using the well agar diffusion method according to the **NCCL (1993)**. Petri plates containing 20 ml of nutrient agar medium were inoculated with a 24 hrs culture of tested isolates. Results showed that the Eos were greatly varied in their antibacterial activity according to the inhibition zones around wells. Clove essential oil showed to be the first one in the size of inhibition zone diameters which ranged from 12 to 38 mm against six bacterial isolates, followed by rosemary which recorded inhibition zone diameters ranged from 12 to 29 mm against four bacterial isolates. Whereas sage essential oil exhibited its inhibiting ability against five bacterial isolates with low diameters of inhibition zones ranged from 11-22 mm. While marjoram showed no inhibition zone against the tested bacterial isolates except *Pseudomonas aeruginosa* (32 mm inhibition zone) which appeared to be resisted to other tested essential oils. All tested Eos showed no inhibition zone against *Serratia marcescens* otherwise *Serratia marcescens* showed resistance to all tested Eos. Generally it was detected that gram negative bacterial isolates were slightly less susceptible than gram positive bacterial isolates. Similar results were obtained by **(Shelef *et al.*, 1984,**

Mendoza.Yepes *et al.*, 1997 Juliano *et al.*, 2000; pintore *et al.*, 2002; Harpaz *et al.*, 2003 and Burt, 2004). That gram negative organisms are less susceptible to the action of antibacterials is perhaps to be expected, since they possess an outer membrane surrounding the cell wall (**Ratledge and Wilkinson, 1988**), which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (**Vaara, 1992**). However, not all studies on Eos have concluded that gram positive are more susceptible (**Wilkinson *et al.*, 2003**). Concerning the antibacterial activity of EO compounds (**Burt, 2004**). Reported that a number of EO component has been identified as effective antibacterial e.g Carvacrol, Thymol, Eugenol, Perillaldehyde, Cinnamaldehyde and Cinamic acid, having minimum inhibitory concentration (MICs) of 0.05-5ULml in vitro. A higher concentration is needed to achieve the same effect in food (**Burt, 2004**).

Table (5) Antibacterial Activity of Concentrated Essential Oils Against Some Bacterial Isolates Of Spoilage Burger

Essential Oils of	Sage	Clove	Rosemary	Marjoram
Bacterial Isolates	Inhibition Zone diameter (mm)			
Klebsialla pneumonia	18	29	12	-
Pseudomonas aeruginosa	-	-	-	32
Micrococcus luteus	11	14	27	-
Serratia marcescens	-	-	-	-
Lactobacillus Sp	-	38	29	-
Bacillus anthrakoids	22	26	-	-
Bacillus cereus	12	12	-	-
Bacillus subtilis	22	31	-	-
Staph ylococcus aureus	-	38	28	-

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دراسة مبدئية على بعض الزيوت الطيارة شاملة تركيبها الكيميائي ونشاطها التضادي

*عبدالعزيز فتحي الحماحمي – **مصطفي أبو الفضل محمد – *محمد بدوي عمر القطقاط

*أحمد عبدالفتاح عبدالرحيم مصطفي

* قسم النبات الزراعي – كلية الزراعة – جامعة الأزهر – القاهرة

** قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة الأزهر – القاهرة

الملخص العربي

تم استخلاص اربعه زيوت نباتيه من أوراق نباتات المريميه و البردقوش وحصالبان ومن بذور نبات القرنفل بإستخلاصها بالغليان في الماء وتم فصل وتعريف المركبات الموجوده بتلك الزيوت فى المعمل. وبواسطه الانتشار خلال الاجار well agar diffusion تم قياس منطقه تثبيط النمو لعدد من عزلات البكتيريا المسببه لفساد اللحوم المصنعه والتى تم عزلها من البرجر المعرض للفساد فى جو المعمل، تلك العزلات قد تم عزلها فى حاله نقيه وتعريفها ووجد انها تنتمى الى ثلاث عزلات سالبه لجرام (*Klebsiella pneumonia-Pseudomonas aeruginosa-Serratia marcescens*). وستة عزالات موجبه لجرام (*Bacillus anthrakoids-Bacillus cereus-Bacillus subtilis-Micrococcus luteus-staphylococcus aureus-Lactobacillus* Sp).

اظهرت النتائج المتحصل عليها تباين واضح فى المكونات الكيميائيه للزيوت المختبره خاصه المركبات ذات النسب العاليه كما ان الزيوت المختبره تباينت فى قدرتها التضاديه للعزلات البكتيريه المختبره، ولقد تبين ان العزلات البكتيريه السالبه لصبغه جرام كانت اقل قابليه للتاثر عن العزلات الموجبه لصبغه جرام.

ومن المعروف عن الزيوت الطياره انها مواد طبيعيه تستخدم لاعطاء النكهه ولكن فى هذا البحث تبين قدرتها ايضا على تثبيط وتأخير نمو البكتيريا المسببه للفساد كمواد حافظه طبيعيه.