EVALUATING ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF VOLATILE OILS EXTRACTED FROM ANISE, FENNEL AND SPEARMINT PLANTS

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ABSTRACT

Essential oils of the three Aromatic plants growing in Egypt; anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and spearmint (*Mentha spicata*) as natural products were examined concerning their chemical constituents, antimicrobial and antioxidant activities. Essential oils were extracted by hydro-distillation method and were analyzed using Gas chromatography/mass spectrometry technique. GC/MS analysis of the essential oils revealed the percentage of major/main components in each volatile oil, which was greatly different among all examined oils. Antioxidant activities of the essential oils were evaluated using the DPPH radical scavenging assays. Essential oil of Spearmint was more effective antioxidant than those of fennel and anise. Antimicrobial activities of each oil were tested against some pathogenic bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium, Escherichia coli enterotoxigenic* and *Stenotrophomonas maltophilia*) and non-pathogenic bacteria (*Bacillus lichneformis*, *Escherichia coli* JM109*, E. coli* JM109 DE3, E. *coli* JM109 DE3 BL21 PLsS) as indicator strains. All tested were used at four different concentrations (50, 100, 200 and 500 µl/ml) using agar dilution method. The minimum inhibitory concentration (MIC) for each volatile oil was determined. The antibiotic susceptibility test was performed against the test organisms by disc diffusion method. All tested essential oils were used at concentrations of 70µl/well (diameter 6 mm) in Muller Hinton agar medium. Results showed that all tested essential oils exhibited markedly antibacterial effect against all tested organisms except for *P. aeruginosa* which was not affected by volatile oils from anise and fennel. Spearmint oil showing the highest inhibitory activity, it was observed for all tested concentrations, while anise oil was inhibitory against six bacterial members and fennel oil demonstrated the lowest inhibitory effect on bacterial growth. However, *Stenotrophomonas maltophilia* showed less sensitivity towards essential oil extracts. Present data underpin the great potential of anise, fennel and spearmint essential oils as biological weapons against various bacterial pathogens from Gram-negative and Gram-positive bacteria.

Keywords: Essential oils; Fennel; Anise; Spearmint; antioxidant activity; Antimicrobial activity; microorganisms; GC-MS; trans-Anethole; Fenchone

INTRODUCTION

Essential oils are made from a very complex mixture of volatile molecules that are produced by the secondary metabolism of aromatic and medicinal plants and can be obtained by different methods, including the use of low or high pressure distillation of different parts of plants or the employment of liquid carbon dioxide or microwaves. Several factors influence the quality and quantity of the extracted product, in particular the soil composition, plant organ, vegetative cycle phase and climate (Miguel *et al., 2005;* Angioni *et al.,* 2006 and Figueiredo *et al.,* 2008). Essential oils composition can be differentiated in two component group's .The main group has terpene and terpenoid origin and the second is constituted by aromatic and aliphatic components. Terpenes are the major group of plant natural products characterized by an extensive variety of structural types and the most valuable compounds (Degenhardt *et al., 2009*). Monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20) are the main terpenes, but hemiterpenes (C5), triterpenes (C30) and tetraterpenes (C40), also can be found. A terpene containing oxygen is designated terpenoid. The aromatic compounds result from phenylpropane and are less common than terpenes. Plants use different biosynthetic pathways to synthesize terpenes and the phenylpropane by-products, but may jointly occur in some, however one major pathway will prevail. See the example of fennel (*Foeniculum vulgare* Mill.) where trans-anethole (31-36%), α-pinene (14-20%) and limonene (11-13%) are produced (Miguel *et al,* 2010).

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal, and antioxidant properties (Baratta *et al.*, 1998; Cosentino *et al*., 1999 and Bounatirou *et al*., 2007). Biological activity of essential oils depends on their chemical composition, which is determined by the plant genotype and is greatly influenced by several factors such as geographicalorigin environmental and agronomic conditions (Rota *et al.*, 2004 and YesilCeliktas *et al.*, 2007). Plant volatile oils are variable mixtures of essential terpenoids, especially monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also be present, and of a variety a low-molecular-weight aliphatic hydrocarbons, acids, alcohol, aldehydes, phenolic compounds, acyclic esters, or lactones

(Rota *et al*., 2004). Many species and herbs exert antimicrobial activity due to their essential oil fractions. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity (Omidbeygi *et al*., 2007 and YesilCeliktas *et al.* 2007).

Several epidemiological studies have demonstrated that consumption of fruits and vegetables and those of selected natural antioxidants such as plant polyphenols, vitamin C and flavonoids are correlated with reduced incidence of cardiovascular and chronic diseases and certain cancers (Kaur and Kapoar, 2002; Sultana *et al*., 2007 and Majhenic *et al*., 2007). The use of synthetic antioxidants in foods for prevention of lipid oxidation is discouraged due to the perceived carcinogenic potential (Jeong *et al.,* 2004 and Ho and Jie, 2007). Much effort has been devoted to exploring viable, safer natural antioxidants (Ruberto *et al*., 2000; Anwar *et al*., 2007 and Majhenic *et al*., 2007). There is renewed interest in the use of plant-based antimicrobial and antioxidants compounds for preservation of foods and to control the diseases caused by microorganism (Singh *et al*., 2005; Tepe *et al*., 2005; Gulluce *et al*., 2007 and Majhenic *et al*., 2007). Many herbs and spices, customarily used to add flavor to dishes, are also valued as commendable sources of natural antioxidants (Mata *et al*., 2007). Spices and herbs have attracted a great deal of scientific interest for their potential uses as alternative remedies for the treatment of infectious diseases. The antioxidant and antimicrobial activities of spices and herbs are attributed to the presence of essential oils, bioactive constituents, and phenolic components (Ruberto *et al*., 2000; Singh *et al*., 2006;Yadegarinia *et al*., 2006 and Majhenic *et al*., 2007).

On the other hand, spreading of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.*, 2004). World Health Organization (WHO,2002 and 2002 b) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine and constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2004 and Kordali *et al*., 2005) Some oils have been used in cancer treatment (Sylvestre *et al.*, 2006) Some other oils have been used in food preservation (Faid *et al*., 1995) ,aromatherapy (Buttner *et al*., 1996) and fragrance industries (Van de Braak and Leijtein, 1999). Essential oils are a rich

source of biologically active compounds, there has been increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau *et al*., 1997) Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific, as well as, general antimicrobial activity and antibiotic potential (Darokar *et al*., 1998).

The aim of the present work is to screen volatile oils extracted from three aromatic plants (fennel, anise and spearmint) cultivated in different geographical areas of Egypt, for their chemical composition, antioxidant activity and antimicrobial activity of these essential oils against several bacterial strains was another goal for this work.

MATERIALS AND METHODS

Plant materials and extraction of essential oils:

Different plant organs mentioned in Table (1) used in this study were obtained from local market in Alexandria, Egypt at May 2014. All the tested herbal oils or extracts were sterilized by filtration using Millipore cellulose filter membrane (0.45 mm pore diameter).

Table (1): Aromatic plants and main part for the essential oils extract

Common name	Botanical name	Family	Plant organ	Properties
Spearmint	*Mentha spicata*	Lamiaceae	Whole Herb	Digestent, stimulant and tonic. flavoring agent
Anise	*Pimpinella anisum*	Apiaceae	Seeds	Carminative, stimulant, expectorant, condiment and flavouring agent.
Fennel	*Foeniculum vulgare*	Apiaceae	Seeds	diuretic, anti-inflammatory, analgesic, hepatoprotective and antispasmodic effects

Essential oil analysis:

The volatile oils were extracted separately by hydro-distillation method utilizing apparatus similar to European Pharmacopoeia (EP).The extracted essential oil was collected in glass tubes covered with aluminum foil to avoid the negative effects of light and stored at 4 ºC.The essential oils were analyzed with GC-MS (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 260 °C at a rate of 4 °C/min. The split injector was heated at 250 °C, the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of major

constituents were estimated by measuring the peak area of the different compounds of the chromatogram according to Gunther and Joseph (1978).

Indicator strains: sources and inoculum preparation

Microorganisms were obtained from the Department of Biotechnology, Institute of Graduate and Research, Alex University. Nine bacterial indicator strains; two strains of Gram-positiive bacteria [*Bacillus cereus* and *Bacillus licheformis*] and seven strains of Gram-negative bacteria [*Escherichia coli* enterotoxigenic strain, *Escherichia coli* JM109*, Escherichia coli* JM109 DE3, *Escherichia coli* JM109 DE3 BL21 PyLsS, *Pseudomonas aeruginosa*, *Salmonella Typhi* and *Stenotrophomonas maltophilia*] were used in the study. Stock cultures of all strains were maintained on different agar slants at 4°C. Microorganisms's inocula were prepared by innoculating ten milliliters of suitable broth medium in a 50 ml Erlenmeyer flask previously, sterilized by autoclaving at 121ºC for 20 minutes, by one loopful of indicator strains. Then the inoculated broth was incubated in a shaker incubator (New Brunswick Scientific-company, USA) at 37ºC for 24 h at 150 rpm.

Determination of antibacterial activity

The sensitivity of microorganisms was determined by the agar diffusion method. Essential oils concentration (50, 100, 200 and 500 µl/ml of each plant species) were prepared using DMSO; Controls were prepared using the same quantities of DMSO as blank. Muller Hinton agar media (Oxoid, England) and the bacterial inoculum were properly mixed then were poured into petri dishes and were allowed to cool down to room temperature. After media solidification, wells were punched out of agar medium using a sterile cork-porer (Ø6 mm). The base of each well was sealed with a drop of melted sterile water agar (20g agar per liter of H_2O). Each concentrations of the test extract (70 µl) was transferred into the well and the plates were incubated in Heraeus incubator (Germany) for overnight at 37ºC.

The sensitivity to the individual oils was classified by the diameter of the inhibition zones as follows (Ponce *et al.,* 2003 and Moreira *et al.,* 2005):

• Not sensitive (–) for total diameter smaller than 8 mm
• Sensitive (+) for total diameter is between 9–14 mm
• Very sensitive (++) for total diameter is between 15–19mm
• Extremely sensitive (+++) for total diameter larger than 20 mm

Each assay was performed in triplicates on three separate experimental runs.

Antioxidant activity

Antioxidant activity of the three essential oils was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ((DPPH) was determined based on the method of Ohinishi *et al*., (1994)). Ascorbic acid was used as references or positive controls. All the assay were carried out at four concentrations of each tested essential oil (10, 50, 100 and 200 µl/ml) prepared in methanol having a final DPPH radical concentration. The mixture was shaken vigorously for 1min then left to stand for 15 min in the dark. Scavenging capacity was measured spectrophotometric ally at 517 nm. and results were reported as the average of three replicates. Inhibition (%) was plotted against the sample concentration in the reaction system. The percentage inhibition of the DPPH radical calculated according to the following formula:

% Inhibition of DPPH = (Absorbance of control - Absorbance of sample) x 100/Absorbance of control

Statistical analysis

All the experiments were conducted in triplicate unless stated otherwise**.** Data are presented as mean values ± standard error calculated from triplicate determinations. Analysis of variance (ANOVA) was applied to the data to determine differences ($p < 0.05$). To discover if there were significant differences between the levels of the main factor, contrasts (Tukey's test) between means were made. For the antioxidant activity, ANOVAs with two factors (essential oil and concentration) were applied for each parameter. The statistical analyses were made using Statgraphics 5.1 for Windows.

RESULTS AND DISCUSSION

Essential oils percentage:

The percentage of essential oils from the aerial parts (*Mentha spicata)* and seeds (*Pimpinella anisum* and *Foeniculum vulgare)* shown in Table (2).

Table (2): Essential oils percentage of selected essential oils

Plants	***Pimpinella anisum***	***Mentha spicata***	***Foeniculum vulgare***
Essential oils percentage	0.8 **%**	1.4 **%**	1.8 **%**

Present findings are in close agreement with the findings of Mata *et al*. (2007), who reported the yield of *M. spicata* oil from the

aerial parts to be 0.9%. Kofidis *et al.* (2006), investigated the yield of oil of wild *M. spicata* grown in ranging from 0.1- 1.8%.

Chemical composition of essential oils:

The main volatile components of crude essential oils recovered by GC-MS shown in Table (3).

Table (3): Major chemical compounds percentage of fennel, anise and spearmint oils (by GC-MS analysis)

Component	Anise oil (*Pimpinella anisum*)	Fennel oil (*Foeniculum vulgare*)	Spearmint oil (*Mentha spicata*)
α-Pinene	0.06 **%**	**2.94 %**	1.04 **%**
Camphor	0.00 **%**	**0.40 %**	0.02 **%**
B-Caryophyllene	1.17 **%**	trace	**1.82 %**
1,8-Cineole	0.12 **%**	3.29 **%**	**14.18 %**
Carvon	0.02 **%**	trace	**36.04 %**
Limonene	0.04 **%**	9.34 **%**	**18.01 %**
Zingiberene	**1.02 %**	trace	0.23 **%**
Fenchone	0.00 **%**	**7.58 %**	0.06 **%**
cis-dihydrocarvone	0.26 **%**	0.01 **%**	**1.18 %**
Methyl chavicol	0.19 **%**	**2.36 %**	1.06 **%**
trans-Anethole	**91.80 %**	83.43 **%**	3,62 **%**
Geranyl acetate	**0.15 %**	0.00 **%**	**0.15 %**
α -Terpinol	trace	0.40 **%**	**0.93 %**
Thymol	trace	**0.02 %**	0.01 **%**
Eugenol acetate	**0.91 %**	0.03 **%**	0.15 **%**
Linalool	**0.44 %**	1.32 **%**	0.06 **%**
Monoterpenes	0.26 **%**	**15.04 %**	10.33 **%**

Plant essential oils are complex mixtures of volatile organic compounds, which play indispensable roles in the environment, for the plant itself, as well as for humans. The potential biological information stored in essential oil composition data can provide an insight into the silent language of plants, and the roles of these chemical emissions in defense, communication and pollinator attraction (Sangita, *et.al*., 2014). The majorities of volatile oils components were found in these oils but differs only in the percentage like trans-Anethole, 1, 8-Cineole, cis-dihydrocarvone, etc. This explains the different characteristics of each volatile oil and individually distinctive smell.

Antimicrobial activity:

The MIC (S) of three plant essential oils obtained by the agar dilution method are shown in Table (4). Spearmint oil was exhibited strong activity against the all selected bacterial strains. Spearmint oil was the only essential oil which showed an active antibacterial activity at the lowest used concentration (50µl/ml) against *P. aeruginosa* and S. Typhi. Antimicrobial activities of the essential oils obtained from the

three tested essential oils against nine selected bacterial strains are shown in Table (5). Ceferadine and Tobramycin were used as reference materials or positive control for the antibacterial activity against bacteria.

Table (4): The Minimum inhibitory concentration (MIC) of the Essential oils (µl/ml)

Bacterial Strains	Fennel oil	Anise oil	Spearmint oil
B. cereus	50	50	N T
E.coli enetrotoxigenic strain	12	N T	12
E. coli JM109 DE3	10	12	6
E. coli JM109 DE3 BL21 PLsS	50	100	N T
S. Typhimurium	50	N A	12
Ste. maltophilia	N T	25	25
P. aeruginosa	100	25	12

N A: not active, N T: not test, MIC in µl/ml

Table (5) summarizes the antimicrobial properties of the three essential oils indicated that the all essential oil samples have antibacterial activity against most Gram negative and Gram positive bacteria under study. Bacteria susceptibility to the essential oils, as determined by the agar diffusion method, showed that oils with the highest inhibitory effects produced inhibition zones of 9–20 mm diameter. In the dose response study, the inhibition zone increased with the increasing concentration of essential oil.

The most effective oil against Gram- positive bacteria was anise oil. While the most effective essential oil against Gram -negative bacteria was spearmint oil which gave a larger inhibition zone than TOBRAMYCIN against *E.coli* JM109. At the lowest concentration (50 µl/ml), fennel and anise oils had the same antimicrobial activity at the used concentration against the same used strain *E.coli* JM109DE3. These data coincide with those of LoCantore *et al.* who reported that fennel essential oil displayed a significant antibacterial activity, as determined with the agar diffusion method. The results also showed that anise oil had same antibacterial activity at the different concentration against *B. cereus*, and the same with spearmint oil against *P. aeruginosa*.

Spearmint oil showed compatible activity to TOB against *Ste. maltophilia* at the high test concentration (500µl/ml). Low concentrations (50 µl/ml) of Spearmint essential oils inhibited all selected bacterial strains. At a high concentration (500µl/ml), the essential oil extracts exhibited a marked inhibition activity against tested bacteria, and the inhibition of the essential oil extract of Spearmint was stronger than that of the others, showing inhibition zones ranging from 11–20 mm. Comparatively, *E.coli* JM109 DE3

BL21 PLsS, *E.coli* JM109, *B. cereus, S. Typhimurium* and *P. aeruginosa* were less sensitive to the inhibitory activity of the Fennel essential oils than the other bacterial strains which was more inhibited at the same concentrations of the same essential oils extracts.

Table (5): Antimicrobial activity of different essential oils determined by disc diffusion assay

Bacteria	plants	**Fennel oil**				**Anise oil**				**Spearmint oil**			
	concentrations	50 µl/ml	100 µl/ml	200 µl/ml	500 µl/ml	50 µl/ml	100 µl/ml	200 µl/ml	500 µl/ml	50 µl/ml	100 µl/ml	200 µl/ml	500 µl/ml
m (-	*B. cereus*	-	+	+	+	+	+	+	+	+	+	+	+
	B. lichneformis	+	+	+	+	-	+	+	++	+	++	**+++**	**+++**
Gram (+)ve	*E.coli enetrotoxigenic*	+	+	+	++	+	+	+	+	+	+	+	++
	E.coli JM109	-	+	+	+	+	+	+	+	+	+	+	+
	E.coli JM109 DE3	+	+	+	+	+	+	+	+	+	+	+	**+++**
	E.coli JM109 DE3 BL21 PLsS	-	+	+	+	-	-	+	+	+	+	+	++
	P. aeruginosa	-	-	-	+	-	-	-	-	+	+	+	+
	S. Typhimurium	-	+	+	+	-	+	+	+	+	+	+	+
	Ste. maltophilia	+	+	+	+	+	+	+	+	++	++	++	++

- Not sensitive, + Sensitive, ++ Very sensitive, +++ Extremely sensitive

Table (6): Antibiotic Susceptibility

Strains / Anti. Desc	*E.coli enetrotoxigenic*	*E.coli* JM109	*P. aeruginosa*	*S. Typhimurium*	*Ste. maltophilia*
TOB (10µg/ml)	19 mm	11 mm	15 mm	19 mm	22 mm
CEF (30µg/ml)	16 mm	N A	N A	16 mm	24.5 mm

Anti. : Antibiotic, TOB: tobramycin, CEF: cefradine, mm: mill meter

Antioxidant activity:

Antioxidant activity of the three essential oils was evaluated by the DPPH free radical scavenging and ascorbic acid as references or positive controls. All of the assays were carried out at concentrations of 10, 50, 100 and 200 µl/ml and results were reported as the average of three replicates. The concentrations that inhibited 50 % in each test (IC50 values) are shown in Table (7).

Table (7): Antioxidant activity of the essential oils compared to ascorbic acid as a reference antioxidant

Essential oil	Radical scavenging activity %			
	10 µl/ml	50 µl/ml	100 µl/ml	200 µl/ml
Fennel oil	ND	94	59	31
Anise oil	36.6	11.01	9.2	17.5
Spearmint oil	33.99	82.26	93.16	**280**
Ascorbic Acid	217	230	235	243

ND: not determined

Spearmint oil showed the highest activity even higher than either ascorbic acid at concentration of 200 µl/ml. In another hand, anise oil was the least effective radical scavenger. Spearmint oil showed compatible scavenging activity to ascorbic acid. But fennel oil at 100 mg/ ml showed approximately the same activity but slightly higher than ascorbic acid Based on the data obtained from this study, Spearmint essential oil exhibits ability as a free radical inhibitor or scavenging activity as well as primary antioxidant that reacts with free radicals, which may limit free radicals damage occurring in the human body.

CONCLUSION

Majority of the oils showed antibacterial activity against the tested strains. Spearmint oil has the most potential bactericidal properties (inhibiting both Gram-positive and Gram-negative Bacteria). Spearmint oil can be a good source of antibacterial agents. We believe that the present investigation together with previous studies provide support to the antibacterial properties of spearmint oil. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agents. Additionally, essential oils have a potential to inhibit and inactivate most of microorganisms in agar medium at different concentrations. The inhibitory effects of essential oils increased with increasing concentration. It is suggested to investigate higher essential oils concentrations than were those used in research, and to study the effects over a longer time period to access the potential of plant species essential oils as preservatives.

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تقييم الأنشطة المضادة للميكروبات والنشاط المضادة للأكسدة للزيوت الطيارة المستخلصة من نباتات الينسون والشمر والنعناع البلدى

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الملخص العربى

ثلاثة زيوت عطرية لثلاثة نباتات عطرية طبية تنمو فى مصر وهى الينسون والشمر و النعناع البلدى كمنتجات طبيعية تم اختبارها بمكوناتها الكيميائية كمضادة للنشاط الميكروبى ونشاط مضادات الأكسدة.الزيوت العطرية تم استخلاصها بطريقة التقطير المائى وتم تحليل مكوناتها باستخدام جهاز التحليل الكروماتوجرافى مطياف الكتلة الذى كشف النسبة المئوية للمكونات الرئيسية فى كل زيت عطرى والتى كانت مختلفة للزيوت العطرية المختبرة.نشاط مضادات الأكسدة للزيوت العطرية المختبرة تم تقديرها باسستخدام DPPH.الزيت العطري للنعناع البلدى كان أكثرهم تأثير على النشاط المضاد للأكسدة بالمقارنة بالزيت العطرى للشمر و اليانسون.النشاط المضاد للميكروبات لكل زيت عطرى تم اختبارها كمؤشرعلى بعض سلالات البكتريا الممرضة:

(*Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium, Escherichia coli enterotoxigenic* and *Stenotrophomonas maltophilia*).

وكذلك على بعض البكتريا الغير ممرضة:

(*Bacillus lichneformis*, *Escherichia coli* JM109, *E. coli* JM109 DE3, E. *coli* JM109 DE3 BL21 PLsS)

كل الزيوت المختبرة استخدم منها أربعة تركيزات (50 و100و200 و 500 ميكرولتر / مل) باستخدام طريقة الآجار المخفف.تم تقدير أقل تركيز مثبط لكل زيت عطرى. تم إجراء اختبار الحساسية للمضادات الحيوية ضد الميكروبات المختبرة بواسطة طريقة الانتشار القرصي.استخدم تركيز 70 ميكرولتر لكل الزيوت العطرية المختبرة (قطر 6 مم) فى بيئة آجار موللر هينتون.النتائج أشارت الى أن كل الزيوت العطرية المختبرة لها تأثير مضاد للنشاط الميكروبى بشكل ملحوظ ضد جميع سلالات البكتريا المختبرة عدا *P. aeruginosa* التى لم تتأثر بالزيت العطرى لليانسون و الشمر.زيت النعناع البلدى أظهر نشاط عالى مثبط وقد لوحظ ذلك لكل التركيزات المختبرة.بينما زيت اليانسون كان مثبط ضد سته من البكتريا وزيت الشمر أظهر أقل تأثير على نمو البكتريا.بالرغم من أن بكتريا *Stenotrophomonas maltophilia* *أظهرت أقل حساسية اتجاة الزيوت العطرية المستخلصة.تدعم البيانات المتحصل عليها الى امكانية استخدام الزيوت العطرية اليانسون و الشمر و النعناع البلدى كأسلحة بيولوجية مضادة للبكتريا الممرضة السالبة و الموجبة لجرام.*

كلمات كشافة:*زيوت عطرية ، شمر ،يانسون ،نعناع بلدى،نشاط مضادات أكسدة ، نشاط مضاد للميكروبات ، التحليل الكروماتوجرافى ، الأنيثول ، الفينشون.*