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BIOCHEMICAL STUDIES ON CYTOTOXIC EFFECT OF SOME MEDICINAL PLANTS ESSENTIAL OILS

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ABSTRACT: ***The present study were undertaken to investigate the physical and chemical characteristics of the essential oils produced from rosemary , thyme and clove. These oils were tested for their antimicrobial activity . The physico-chemical properties of such oils which were determined to evaluate their values and application. Rosemary essential oil was distinguished by the highest dextro rotation (+ 11.21º) , while the specific gravity of clove oil was higher than that of rosemary and thyme oils. The highest acid number was recorded for rosemary essential oil (11.84). Chemical composition of essential oils were determined by gas chromatography – mass spectrometry technique. The major compound in rosemary oil was borneol (24.12%), while thymol was the major component in thyme essential oil (39.43%), but for clove essential oil, the major compound was eugenol in relative concentration (83.66%). Essential oils were tested against 6 strains of Gram-negative and Gram-positive bacteria Erwinia carotovora , Pesudomonas fluorescens, serratia marcescens, Bacillus subtilis, Bacillus cereus and Bacillus macerans and three strains of yeast Saccharomyces cervisiae, Candida albicans and Candida sp.. These microorganisms are known as pathogens to human kind and plant, or food spoilage organisms. Increasing the essential oils concentration in all studied plants caused an increment in the inhibition zone for all microorganisms under study.***

Key words: ***Essential oils , rosemary oil , thyme oil , clove oil , physico - chemical properties , essential oils composition , antimicrobial activity.***

INTRODUCTION

Essential oils are of great importance for human activities , i.e. medicinal drugs , antioxidants for keeping quality of oils and fats , antimicrobial agents to some harmful microorganisms (bacteria and yeast) as well as being used for food preservation . In nature , essential oils play an important role in the protection of the plants as antibacterial , antiviral and antifungal agents as well as insecticides and also against herbivores by reducing their appetite for such plants.

The physico-chemical properties of rosemary , clove and thyme essential oils showed the following values +11.42º , - 0.30º and + 5.32º respectively for optical rotation , 0.9178 , 1.1268 and 0.9549 for specific gravity , 1.473 , 1.5449 and 1.4942 for refractive index and 1.30 , 1.69 and 1.50 for acid value respectively (El-Baroty 1988). Chemical composition of rosemary essential

oil was as follows borneol 26.5% , α-pinene 12.7% (Farag *et al.* 1989). The major constituents of rosemary oil were cineole 50% and α-pinene 11% (Maria *et al.* 1996) while camphor they were 20.9% and 1,8 cineole 12.4% as stated by (Quitterie *et al.* 2000). Chemical composition of clove essential oil was as follows : eugenol 80 – 85% , caryophyllene 5.2% , eugenol acetate 3.4% and other compounds as traces (Myint *et al.* 1995) also clove leaf oil contains 85 – 90% eugenol while clove bud oil has 80 – 85% eugenol (Deans *et al.* 1995). The identified compounds in clove essential oil were as follows: eugenol 80 – 87% , eugenol acetate 7.79% , caryophyllene oxide 1.87% and benzyl benzoate 0.40% (Soliman 1999). Chemical composition of thyme essential oil was follows: thymol 39.3% , P-cymene 18.1% and terpinene 13.1% (Adriana *et al.* 1996) also thymol 31.19% , γ terpinene 24.73% and P-cymene 15.79% (Marilena *et al.* 1999) as well as thymol 63.6% , P-cymene 23.5% and γ terpinene 4.3% (Dimitra *et al.* 2000).

The cytotoxic property is of great importance in the application of essential oils not only against certain human or animal pathogens but also for the preservation of agricultural or marine products . Essential oils or their constituents are indeed effective against a large variety of organisms including bacteria , virus , fungi and yeast. Volatile oils of clove and thyme exhibited considerable inhibitory effects against 25 different genera of bacteria included animal and plant pathogens (Dorman and Deans 2000) , while MIC (minimum inhibitory concentration) of the ethanolic solution was 1% for *leuconostoc mesenteroides* , 0.5% for both *Listeria monocytogenes* and *Staphylococcus aureus* , 0.13% for *Streptococcus mutans* , and 0.06% for *Bacillus cereus* (Jose *et al.* 2000).

MATERIALS AND METHODS

Materials

The leaves of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) plants were obtained from the Horticulture Department (Medicinal and Aromatic plants Section) , Ministry of Agriculture , Giza , Egypt while the flower buds of clove (*Eugenia caryophyllus*) were obtained from the Cairo Food Flavour Essences Company.

Methods:

Physical and chemical characteristics:

The extraction of essential oils were carried out by water distillation using special apparatus with general features as devised by Clevenger (1948).

The solubility in 80% ethanol was determined by titrating a known volume (1ml) of essential oil with ethanol 80% to the point of homogeneity and calculated as V/V.

The specific gravity of the oils was determined using a pycnometer (1 ml capacity) as described by Guenther (1960).

The refractive index was determined using Abbe refractometer , Model 60 according to the procedure described in A.O.A.C (1975).

The optical rotation of the oils was determined by a polarimeter at room temperature using sodium lamp. The specific optical rotation of the oils was calculated according to the equation stated by Guenther (1960).
The acid number was determined according to the method described in A.O.A.C (1975).
The saponification number of the various substances encountered in the present investigation was determined according to the standard procedure previously reported by Guenther (1961) . The ester number represents the difference between the saponification number and the acid number.

Chemical composition of essential oils

The chemical composition study was carried out for rosemary , thyme and clove essential oils were studied using Gas chromatography / Mass spectrometry (GC/MS).

Antimicrobial activity of essential oils

The disc-diffusion method was used for detection of the antimicrobial activity of essential oils as described by Conner and Beuchat (1984). Gram positive bacteria *(Bacillus subtilis , Bacillus cereus , Bacillus macerans)* . Gram negative bacteria *(Erwinia carotovora , Pseudomonas fluorescens , Serratia marcescens)* and the yeast *(Saccharomyces cervisiae , Candida albicans and Candida sp.)* were obtained from plant disease department , Agricultural Research Center , Ministry of Agriculture , Giza , Egypt. The essential oils of rosemary , thyme and clove were diluted in ethanol 95% to give solution with different concentrations 0.05% , 0.1% , 0.15% , 0.2% , 0.225% , 0.3% , 1% , 10% , 50% and 100% (V/V) . The inhibitory effect of the 95% ethanol was also tested . The inhibition zones around the discs were measured (mm).

RESULTS AND DISCUSSION:

The physico-chemical properties of essential oils in Table (1) indicated that rosemary essential oil was distinguished by the highest dextro rotation (+11.21°)while thyme oil was the lowest dextro rotation (+5.32°). The specific gravity of clove essential oil (1.0698) was higher than that of rosemary and thyme essential oils which recorded 0.9294 and 0.960 respectively . The refractive index also showed the same trend in which clove had the high value 1.5273 while rosemary and thyme recorded 1.5023 and 1.4710 respectively. The acid number of essential oils under investigation was 1.39 for rosemary and 11.84 , 11.36 for thyme and clove respectively. The higher values indicated the presence of high amount of organic acids , As for ester value, rosemary essential oil was found to have the highest value (16.65) followed by clove oil which recorded 13.5 while thyme oil showed the lowest ester value (2.36).

Table (1): The physico-chemical properties of rosemary , thyme and clove essential oils.

property	Rosemary	Thyme	Clove
Optical rotation	+11.21^o	+5.32^o	-0.30^o
Specific gravity	0.9294	0.9601	1.0698
Refractive index	1.4710	1.5023	1.5273
Solubility in 80% Et	1:1	1:1.5	1:1.5
Acid number	1.39	11.84	11.36
Saponification number	18.04	14.2	24.86
Ester numer	16.65	2.36	13.5

The chemical composition of rosemary essential oil in table (2) showed that the major compound was borneol (24.13%) , while α pinene , α caryophyllene , ledol , camphor , γ terpinene , D-verbenone and eucalyptol were presented in amount less than 10% . The minor compounds in such oil were limonene (2.2%) , methyl jasmonate (2.2%) , α-Muurolol (1.3%) , eugenol (1.1%) , β- pinene (1.4%) , humulene epoxide (1.5%) and borneyl acetate (1.5%), on the other hand , the other compounds were found as traces.

Table (2): Chemical composition of rosemary essential oil.

Component	%	Component	%	Component	%
Borneol	24.126	Humulene epoxide	1.523	α– Himachalene	0.449
α - Pinene	9.717	β - Germacrene	1.43	Copaene	0.27
α- Caryophyllene	8.348	α – Muurolol	1.355	Jasmone	0.218
Ledol	6.423	Eugenol	1.177	Nerolidol	0.235
Eucalyptol	5.115	β – Pinene	1.463	1-Henicosyl formate	0.13
Camphor	5.01	α - Cadinene	0.633	Camphene	0.094
γ – Terpinene	5.005	α – Bisabolol	0.536	1-Xexyen-3-ol	0.026
D- Verbenone	4.168	Farnesyl	0.319	Sabinene hydrate	0.047
Limonene	2.287	Farnesyl acetate	0.505	Myrtenyl	0.052
Methyl jasmonate	2.241	Phellendrene	0.861	(-)-Spathulenol	0.093
α- Muurolene	1.578	Thymol	0.315	Heptacosane	0.032
Borneyl acetate	1.553	Myrtanol acetate	0.325	docosane	0.038
Myrecene	0.204				

Table (3) shows the chemical composition of thyme essential oil, in which data revealed that thyme essential oil contains mainly thymol (39.44%) , P-cymene (23.6%) and γ terpinene (12.52%) in the meanwhile ledol and aromadenrrene were presented in amount less than 10%. The other

compounds occurred as trace materials such as caryophyllene , farnesyl acetate , linalyl acetate , α pinene , α thujone and geranyl acetate.

Table (3): Chemical composition of thyme essential oil.

Component	%	Component	%	Component	%
Thymol	39.44	Geranyl acetate	0.437	Nerolidol	0.077
P-Cymene	23.6	Bisabolene	0.271	(-) – Globulol	0.02
γ – Terpinene	12.515	3-Eicosene	0.261	Hexadecanoic acid	0.019
Ledol	2.243	Farnesyl	0.52	7-Tetradecene	0.032
Aromadenrrene	2.121	Phytol	0.212	Heptacosane	0.056
Caryophyllene	0.937	β- Pinene	0.2	P-menth 2-en-1ol	0.101
Farnesyl acetate	0.633	Thujanol	0.173	5-α-Pregn-16-en-20 one	0.361
Linalyl acetate	0.553	Camphor	0.17	Octadecanoic methyl ester	0.138
α – Pinene	0.682	Ethyl butyrate	0.633	Ethyl chrysanthemumate	0.005
α – Thujone	0.523	Caryophyllene oxide	0.132	2-pentadecanone6,10,14trimethyl	0.07

Table (4) demonstrates the chemical composition of clove essential oil , the major compounds in clove essential oil were eugenol and eugenol acetate in relative concentration of (83.66%) and (12.11% respectively ,as well as caroyophyllene oxide and 2,3,4 trimethoxy acetophenone (1.57% and 1.12% respectively). It is also clear from such data that the following compounds were presented as trace substances : benzyl benzoate , ledol , eucalyptol , linalool α – pinene , camphor and other compounds.

Table (4): Chemical composition of clove essential oil

Component	%	component	%
Eugenol	83.661	5-Heptene-2-one 6 methyl	0.014
Eugenol acetate	12.118	P- Cymene	0.01
Caryophyllene oxide	1.572	D- Limonene	0.013
2,3,4 trimethoxy acetophenone	1.12	2- Hexanone 6-acetyloxy	0.035
Benzyl benzoate	0.185	Methyl salicylate	0.012
Ledol	0.496	Retinol acetate	0.017
Eucalyptol	0.068	(-) – Spathulenol	0.029
Linalool	0.078	3- Methyl cinnamic acid	0.052
Camphor	0.044	Coniferyl alcohol	0.022
2-Nonanone	0.028	Farnesyl acetate	0.008
α - Pinene	0.036	Benzyl salicylate	0.026

Tables (5 and 6) show the effect of the essential oil of rosemary (diameter of inhibition zone in cm) on bacteria and yeast. Generally the concentration of essential oils up to 0.2% exhibited resistance to the inhibitory action of that oils up on the tested bacteria and increasing the essential oil concentration of 1% caused an increment of inhibition zone for all treatments of yeast. The essential oil was more effective on *Bacillus subtilis* and *Bacillus macerans* which produced 2.5 cm followed by *Bacillus cereus* , *Pesudomonas fluorescens* and *serratia marcescens* which recorded 1.8 , 1.7 and 1.3 cm respectively , while *Candida albicans* was more affected by the essentia oil (4.5cm) than *Saccharomyces cervisiae* and *Candida sp.* which were 2.5 and 1.5 cm respectively.

Table (5): Diameter of inhibition zone of bacteria resulted from application of rosemary essential oil

Essential oil concentration %	Diameters of the inhibition zones (cm)					
	Bacillus subtilis	*Bacillus cereus*	*Bacillus macerans*	Erwinia carotovora	*Pesudomonas fluorescens*	*serratia marcescens*
0.05	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.15	0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.225	0.0	0.0	1.1	0.0	0.4	0.0
0.3	0.0	0.0	1.2	0.2	1.1	0.0
1	1	1.25	1.2	0.6	1.2	0.0
10	1.8	1.5	1.7	0.7	1.2	0.0
50	2	1.55	1.8	0.7	1.4	1
100	2.5	1.8	2.5	0.7	1.7	1.3

Table (6): Diameter of inhibition zone of yeast resulted from application of rosemary essential oil

Essential oil concentration %	Diameters of the inhibition zones (cm)		
	Saccharomyces cervisiae	*Candida albicans*	*Candida sp.*
0.05	0.0	0.0	0.0
0.1	0.0	0.0	0.0
0.15	0.0	0.0	0.0
0.2	0.0	0.0	0.0
0.225	0.0	0.0	0.0
0.3	0.0	0.0	0.0
1	1	0.0	1
10	1.5	1.7	1.2
50	1.7	2.5	1.5
100	2.5	4.5	1.5

Tables (7 and 8) show the inhibition effect of thyme essential oil on bacteria and yeast. Increasing the essential oil concentration caused an increment of the inhibition zone for all treatments of all studied microorganisms. The essential oil was more effective on *Bacillus macerans* (5.5 cm) followed by *Bacillus subtilis* (4.3 cm) and *Bacillus cereus* (4 cm) in the meanwhile *Pesudomonas fluorescens* , *serratia marcescens* and *Erwinia carotovora* recorded 3.7 , 3.5 and 3 cm respectively. *Candida albicans* was more affected by thyme essential oil with inhibition zone of (5.9) cm followed by *Saccharomyces cervisiae* (5 cm) and *Candida sp.* (2.2 cm) with Applying the concentration 100% of the essential oil.

Table (7): Diameter of inhibition zone of bacteria resulted from application of thyme essential oil.

Essential oil concentration %	Diameters of the inhibition zones (cm)					
	Bacillus subtilis	*Bacillus cereus*	*Bacillus macerans*	Erwinia carotovora	*Pesudomonas fluorescens*	*serratia marcescens*
0.05	0.0	0.0	1	0.0	0.0	0.0
0.1	0.0	0.0	1.3	0.3	0.6	0.0
0.15	0.0	0.0	1.4	0.5	0.7	0.0
0.2	1.5	1	1.6	0.6	0.8	0.0
0.225	1.5	1.3	1.6	0.6	0.9	0.6
0.3	1.5	1.5	1.6	0.7	1.3	0.7
1	1.7	1.8	1.7	0.7	1.7	1.9
10	2	2	1.9	1.2	2.1	2.1
50	3.4	3.5	4.5	2	2.7	3.4
100	4.3	4	5.5	3	3.7	3.5

Table (8): Diameter of inhibition zone of yeast resulted from application of thyme essential oil

Essential oil concentration %	Diameters of the inhibition zones (cm)		
	Saccharomyces cervisiae	*Candida albicans*	*Candida sp.*
0.05	0.0	0.0	1.2
0.1	0.0	0.0	1.4
0.15	1.3	0.0	1.5
0.2	1.5	0.0	1.7
0.225	1.5	0.0	1.7
0.3	1.5	1.8	1.7
1	1.8	2.1	1.7
10	2	3.1	1.8
50	3.5	3.5	1.9
100	5	5.9	2.2

Tables (9 and 10) demonstrate the effect of the essential oil of clove on bacteria and yeast . Generally increasing the essential oil concentration of 0.2% caused an increment of the inhibition zone for all treatments of all studied bacteria in the same time increasing the oil concentration of 0.3% caused an increment of the inhibition zone for all treatments of all studied yeast. *Pesudomonas fluorescens* was more affected by the essential oil (3.7 cm) followed by *Bacillus macerans* and *Bacillus subtilis* which recorded 2.8 cm and 2.5 cm respectively , in the meanwhile both *Bacillus cereus* and *serratia marcescens* recorded the same inhibition zone (2 cm) , while the lowest inhibition was recorded by *Erwinia carotovora* (1 cm) . In the other hand *Saccharomyces cervisiae* was more affected by clove essential oil (3.4 cm) followed by *Candida albicans* and *Candida sp*. which recorded 2.5 cm and 1.8 cm respectively.

Table (9): Diameter of inhibition zone of bacteria resulted from application of clove essential oil

Essential oil concentration %	Diameters of the inhibition zones (cm)					
	Bacillus subtilis	*Bacillus cereus*	*Bacillus macerans*	Erwinia carotovora	*Pesudomonas fluorescens*	*serratia marcescens*
0.05	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0	0.0
0.15	0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.8	0.5	0.7	0.0
0.225	0.0	0.0	1.3	0.5	1.2	0.0
0.3	0.0	1	1.3	0.5	1.2	0.0
1	1.2	1.7	1.4	0.7	1.2	1
10	1.8	1.9	1.5	0.8	1.4	1.5
50	2.1	2	2.5	0.8	2.2	1.8
100	2.5	2	2.8	1	3.7	2

Table (10): Diameter of inhibition zone of yeast resulted from application of thyme essential oil.

Essential oil concentration %	Diameters of the inhibition zones (cm)		
	Saccharomyces cervisiae	*Candida albicans*	*Candida sp.*
0.05	0.0	0.0	0.0
0.1	0.0	0.0	0.0
0.15	0.0	0.0	0.0
0.2	0.0	0.0	0.0
0.225	0.0	0.0	0.0
0.3	1.5	0.0	0.0
1	1.5	1.5	1.5
10	1.8	2	1.6
50	2.8	2.3	1.6
100	3.4	2.5	1.8

These different inhibition effects of the present studied essential oils may be due to the different constituents of the three plant extracts in which these effects either on bacteria or yeast was increased paralleled with the increasing of the essential oils concentration. These results are agreement with those obtained by Droman and Deans (2000) and Jose et al. (2000) .

REFERENCES

Adriana M. Alippi , Jorge A. Ringuelet , Elsa L. Cerimele , Maria S. Re , Cynthia P. and Henning (1996). Antimicrobial activity of some essential oils against paenibacillus larvae the causal agent of American Foulbrood Disease. J. Herbs , Spices and Medicinal plants , 4 (2) , 9 – 16.

A.O.A.C (1975). "Association of official analytical chemists" 12th ed ., Washington D.C.USA.

Clevenger. (1948). The essential oils . vol. D. Van Nostrand Company , Inc. Canada, C.F. Guenther, E.

Conner, D.E. and L.R. Beuchat (1984). Effect of essential oils from plants on growth of food spoilage yeasts. J. food Science, 49, 429 – 434.

Deans, S.G., R.C. Noble, W. Wuryani and L.G. Penzes (1995). Antimicrobial and antioxidant properties of syzygium aromaticum L. Merr. and Perry: Impact upon bacteria , fungi and fatty acid levels in aging mice. Flavour and Fragrance J. 10 , 323 – 328.

Dimitra, J. D., N. Z. Basil and G. P. Moschos (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on penicillium digitatum. J. Agric. Food Chem. , 48 , 2576 – 2581.

Dorman, H.J.D. and S.G. Deans (2000). Antimicrobial agents from plants : antibacterial activity of plant volatile oils. J. Applied microbiology , 88 , 308 - 316.

El-Baroty , G.S.A.M. (1988). Biochemical studies on some naturally occurring substances and its velation to lipid oxidation. PhD. Thesis , Fac. Agric., Cairo Univ.

Farag, R.S., Z.Y. Daw, F.M. Hewedi and G.S. El-Baroty (1989). Antimicrobial activity of some Egyptian spices essential oils. J. Food protection , 52 (9) : 665 - 667.

Guenther, E. (1960). The essential oils. vol. 1,4th ed .D. Van Nostrand Company, Inc. Princeton, New Jersey, Toronto , New York , London.

Guenther, E.S. (1961). The essential oils. Vol. 4,3th ed. D. Van Nostrand Company, Inc. Princeton, New Jersey, Toronto , New York , London.

Jose, D. C., A. Marie-Josephe and N.T. Christophe (2000). Antimicrobial effect of rosemary extracts. J. Food protection ,. 63 (10) , 1359-1368.

Maria, D. G., C. Nerea and B. Jesus (1996). Characterization of the essential oils of some cultivated aromatic plants of industrial inter est. J. Sci Food Agric., 70, 359 – 363.

Marilena, M., B. Carla and C. Giuseppe (1999). Antimicrobial activity of the essential oils of thymus vulgaris L. measured using a Biomedometeric method . J. Food protection , 62. (9) , 1017 – 1023.

Myint, S., W.R.W. Saud, A.B. Mohamed and A.A.H. Kadhum (1995). Separation and identification of eugenol in ethanol extract of cloves by reversed – phase high – performance liquid chromatography. J. Am. Oil Chem. Soc., 72 (10) 1231 – 1233.

Quitterie, D., G. Virginia, G. R. Christine, M. Georges, M. Cecile and B. Jean-Morie (2000). The antifungal activity of essential oil as determined by different screening methods. J. essential oils., 12 , 256 – 266.

Soliman, B. (1999). Application of cardmon , cinnamon and clove spices and their volatile oils in preservation and flavoring of cookies. PhD. Thesis . Fac. Agric. , Cairo Univ., Egypt.

دراسات كيميائية حيوية على بعض الزيوت العطرية كمضادات ميكروبية

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الملخص العربى:

تم دراسة الخواص الطبيعية والكيميائية للزيوت العطرية المستخلصة من الحصالبان والزعتر والقرنفل والتى اختبر نشاطها ضد الميكروبات وكانت النتائج كما يلى:

وجد أن الزيت العطرى لنبات الحصالبان تميز بأعلى قيمة للدوران الضوئى (+١١.٢١°) فى حين أن الكثافة النوعية للقرنفل كانت أعلى من الكثافة النوعية لكلا من الزعتر والحصالبان. وسجل الزيت العطرى لنبات الحصالبان أعلى قيمة لرقم الحموضة ١١.٨٤ . بدراسة التركيب الكيميائى للزيوت العطرية بواسطة جهاز الكروماتوجرافى الغازى – مطياف الكتلة وجد أن المركب الرئيسى لزيت الحصالبان البورنيول ٢٤.١٢% فى حين أن الثيمول كان المركب الرئيسى بالزيت العطرى للزعتر ٣٩.٤٣% أما بالنسبة للزيت العطرى للقرنفل فالمكون الرئيسى هو الايوجينول ٨٣.٦٦%

تم دراسة تأثير الزيوت العطرية للحصالبان والزعتر والقرنفل ضد ست سلالات بكتيرية منها ثلاث سلالات من البكتريا السالبة لجرام وهى
Erwinia carotovora , *Pesudomonas fluorescens*, *serratia marcescens* هتلاپ زلالإ ة لمإكاقةقيئلك لهجابكجفى هءو ***Bacillus subtilis*, *Bacillus macerans* , *Bacillus cereus*** **كما تم دراسة تأثير الزيوت العطرية الثلاثة ضد الخمائر وهى** ***Saccharomyces cervisiae*, *Candida albicans*, *Candida sp.*** **وتلك الكائنات تسبب أمراض للإنسان والنبات وكذلك تسبب تعفن للاغذية ولذلك فقد تم قياس القدرة التثبيطية لكل زيت على حدة ووجد أن زيادة التركيز للزيوت العطرية تحت الدراسة أدى الى زيادة التثبيط لكل الميكروبات تحت الدراسة.**