Evaluation of the inhibitory effect of a number of medicinal plants fixed oils on a group of pathogenic bacteria invitro

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Abstract

The present study was designed to evaluate the antibacterial effect of 4 of local medicinal plants oils :*Eletteria cardaomum,Nigella sativa,Prunus dulcisvar* and *Brassica alba* in addition to 5 Antibiotics: Ciprofloxacin ,Chloramphenicol, Aztereonam, Sulfamethaxazole /Trimethoprime and Erythromycine as a positive control against 7 gram positive and gram negative pathogenic bacteria:*Micrococcus spp.,Staphylococcus cohnii cohnii ,Enterobacter cloacae,Escherichia coli, Proteus mirabilis,Pseudomonas aeroginosa* and *Klebsiella pneumonia* by using of agar well diffusion method . The result showed that for the oil of *Eletteria cardamomum* the best antibacterial effect was produced against *Proteus mirabilis* followed by *Klebsiella pneumonia* then *Enterobacter cloacae,Pseudomonas aeroginosa,Escherichia coli,Micrococcus spp.* and *Staphylococcus cohnii cohnii* , and for the oil *Nigella sativa* the antibacterial effect was highest against *Micrococcus spp.,* followed by *Proteus mirabilis* then *Escherichia coli* and *Staphylococcus cohnii cohnii. Brassica alba* oil express it is antibacterial effect only against *Pseudomonas aeroginosa* and there was no effect on any other bacteria while *Brassica alba* had no effect on any of the studied bacteria.The inhibition of the growth of *Pseudomonas aeroginosa* was stronger by *Prunus dulcisvar* oil than *Eletteria cardamomum oil* .For *Escherichia coli,Staphylococcus cohnii cohnii and Micrococcus* the wider zone of inhibition were obtained by *Nigella sativa* oil than *Eletteria cardamomum oil,* while for *Proteus mirabilis* the inhibition of the growth was higher for the oil of *Eletteria cardamomum* than *Nigella sativa* oil.The values were given as mean ±Standard deviation and $P< 0.05$ was considered statistically significant.The datad were analyzed by student's *t*-test using SPSS (version 10).

Introduction

Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare.(1).The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in some developing countries, plants are the main medicinal source to treat infectious diseases. Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (2).The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (3). Infectious diseases are the world's major threat to human health and account for almost 50 000 deaths every day (4). The situation has further been complicated with the rapid development of multi drug resistance by the microorganisms to the antimicrobial agents available. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (5). The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America and Africa (6).Production of lactamases is the most common mechanism of resistance among the Gram-negative. The vast majority of strains expressing these

enzymes belong to the family *Enterobacteriacea* like *K. pneumonia* and some *E. coli* strains, lactam antibiotics are the most common treatment for bacterial infections. Extended-Spectrum Lactamases have become a widespread serious problem and several aspects of them are worrying. The potential use of alternative antibiotics in drug-resistant bacteria from various plant extracts have been studied by many researches.(7).Antimicrobial characteristics of the herbs are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue (8), this lead to consider the medicinal plants the strong and widely used alternative to the antibiotics which became no longer able to possess an effect to the pathogenic bacteria ,so we try in this study to examine the antibacterial activity of four medicinal plants(*Eletteria cardamomum,Nigella sativa, Prunus dulcisvar* and *Brassica alba*) against seven gram positive and gram negative pathogenic bacteria (*Pseudomonas aeroginosa,Proteus mirabilis ,Enterobacter cloace, Escherichia coli, Staphylococcus cohnii cohnii ,Klebsiella pneumonia* and *Micrococcus spp.)*in attempt to discover if these tested medicinal plants could be considered as alternative therapy for some drug resistant pathogenic bacteria.

Materials and methods

Medicinal plants oils

In this study we use the fixed oils of four medicinal plants : *Eletteria cardamomum* (Cardamom oil from HEMANI company –IRP*), Nigella sativa* (Black seed oil from EMAD company-Musel-Iraq), *(Prunus dulcisvar* oil from HEMANI company-IRP) and *Brassica alba* (Mustard oil from HEMANI company-IRP) , these oils were obtained from the local market and identified by the National Iraqi Institute for herbs .

Micoorganisms

The antibacterial effect of the previous medicinal plants oils were tested against (7) types of pathogenic bacteria which include the gram positive *(Stahylococcus cohnii cohnii, micrococcus spp.),* and gram negative *(Escherichia coli,Pseudomonas aeroginosa ,Proteus mirabilis , Enterobacter cloacae, Klebsiella pneumonia*). These bacteria isolates were obtained from the laboratory of Microbiology at the College of Veterinary Medicine, Al- Qadisiyia University and all were identified by the Central Laboratory of Health , Baghdad, Iraq.The bacteria were activated in a Broth agar (HIMEDIA-Mumbai-India) that prepared by dissolving 13gm of the agar in (1000)ml of distilled water and after complete dissolving by heating on a Benzen burner we sterilize the broth by autoclave at (15)IP for (15) minute and then we poured the broth in 7 test tubes (10ml)for each test tube and then we made bacterial suspension by taking a swap from the colonies of each bacteria and put it in the broth and later we incubated all these test tubes at (37c□)for (24) hours .

Antibiotics

In this study we use (5) antibiotics to compare there antibacterial effect as appositive control with that of the medicinal plants fixed oils due to there broad spectrum activity and these antibiotics include: ,Aztereonam (ATM) 30 mcg ,Erythromycin(E) 15 mcg , Sulfamethaxazole/ Trimethoprime (SXT) 25mcg (1.25/23.75mcg) Chloramphenicol (C) 30 mcg, and Ciprofloxacin (CIP) 5mcg (Bioanalyse company).

Sensitivity test

After obtaining of all the medicinal plants fixed oils and activation of the pathogenic bacteria the sensitivity test was done according to the method of (9). the Mueller Hinton Agar (HIMEDIA – Mumbai-India) was prepared by dissolve 38 gm from the agar powder in 1000 ml of distilled water in a flask and shaking it well to dissolve the agar and then we start heating it by a Benzen burner in attempt to complete dissolving of all the agar powder and after that the agar was sterilize by using of autoclave at 15 IP for 15 minutes . After preparation of Mueller Hinton agar

we poured it in to Petri plates and after solidification of the agar we made 4 wells (5 mm diameter) in each one of the Petri plates except those of the antibiotics discs , 70 Petri plates containing Mueller Hinton agar were used in this study {(56 plates for the medicinal plant oil study :2 plates for each bacteria with each medicinal plant oil) and (14 Petri plates for antibiotics: 2 plates for each bacteria)}. The study was done by taking swap from each one of the test tubes that contain the bacterial suspension and inoculated it on the Petri plates that contain the Mueller Hinton agar as follow: from the test tube that contain(*Stahylococcucs cohnii cohnii*) we take a swap and inoculated it in 8 Petri plates that contain wells for medicinal plants oil study and 2 Petri plates without wells for antibiotic study, the same procedure is done with the test tube that contain the bacterial suspension of *Pseudomonas aeroginosa ,Escherichia coli ,Micrococcus species, Enterobacter cloacae,Klebsiella pneumonia andProteus mirabillis* and after that we applied (1 ml) of the fixed oil of *Eletteria cardamomum* in each of the wells in the Petri plates that contain the *Stahylococcus cohnii cohnii,*and the same amount of the cardamom oil in each well of the Petri plates that contain the *Pseudomonas aeroginosa* and the same amount of this oil for the Petri plates that contain *Micrococcus species ,Escherechia coli, Proteus mirabilis ,Enterobacter cloacae* and *Klebsiella* , the same procedure was made with the *Nigella sativa* oil , *Brassica alba* oil and *Prunus dulcisvar* oil, and after complete adding each of the oils on each of it is prepared Petri plates then we applied the antibiotics discs in it is places for each of the studied pathogenic bacteria we study the effect of each one of the 5 Antibiotics .After complete applying of all the medicinal plants oils and the Antibiotics at there chosen places we incubated all the Petri plates at 37c for 24 hours .The sensitivity of microorganisms towards the oils was screened by following the agar well –diffusion method .The zone of inhibition (diameter in mm) in triplicates was measured and the mean value (μ) was tabulated (10) .

Results

Invitro antibacterial effect of four medicinal plants oils :*Eletteria cardamomum, Nigella sativa,Prunus dulcisvar and Brassica alba,* and 5 Antibiotic :Erythromycin, Aztereonam ,Sulfamethaxazole/ Trimethoprim, chloramphenicol and Ciprofloxacin were studied in this experiment against 7 pathogenic bacteria :*Klebsiella pneumonia, Pseudomonas aeroginosa,Escherichia coli, Staphylococcus cohnii cohnii,Enterobacter cloacae, Proteus mirabilis* and *micrococcus species.* The medicinal plants oils showed a various antibacterial effect against the pathogenic bacteria according to the type of the plant oil that have been used table (1),*Eletteria cardamomum* oil was the most effective in inhibition the growth of all of the studied pathogenic bacteria ,it showed an inhibition zones measured by millimeter as follow: for *Proteus mirabilis* (21.5±3.55), *Klebsiella pneumonia* (20.16 ± 2.72),Enterobacter cloacae (14.16±5.47), *Pseudomonas aeroginosa* (12.66±2.26) ,*Escherichia coli* (12.08±1.44), *Micrococcus spp.* (11.41±1.16)and for *Staphylococcus cohnii cohnii*(10.83±1.93).*Nigella sativa* oil cause an inhibition in the growth of some of the studied pathogenic bacteria while it had no effect on others as follow: for *Micrococcus spp.*the inhibition zone measured by millimeter was (±16.91±2.39), *Proteus mirabilis* (16.50±2.39), *Escherichia coli* (16.25 ±1.13) and for *Staphylococcus cohnii cohnii* (15.66±2.18)mm, *Prunus dulcisvar* oil had an effect only on *Pseudomonas aeroginosa* with an inhibition zone(27.75±0.86)mm and no effect on the other pathogenic bacteria with no zone of inhibition(0±0)mm,and for *Brassica alba* there was no effect on any of the studied bacteria with no signs of any inhibition of these bacteria growth.The Antibiotics also had a various result in inhibition the growth of the studied bacteria as follow: *Staphylococcus cohnii cohnii* {CIP 27.66±1.2, C 31±0.57, ATM

0±0,SXT 22±0,E 29 ±0.57}, *Klebsiellapneumonia* {CIP 33.66±0.33,C 30.66±0.88, ATM 29±0.57, SXT 24.66 ± 0.4,E 19.33±0.33}, *Micrococcus species* {CIP 29±1,C 25.66±0.33, ATM 0±0,SXT 21±0,E 27±0}, *Escherichia coli* {CIP 23.33±0.88, C 22.66 ± 0.33,ATM 26±0.57,SXT 27.3 ± 0.32,E 0±0}, *Pseudomonas aeroginosa* {CIP 31± o.57,C 10.33±0.4, ATM 9± 0,SXT 0±0,E 0±0},*Proteus mirabilis*{ CIP 33±0.57,C 10±0.57,ATM 0±0,SXT 0±0,E 9±0}, *Enterobacter cloacae* {CIP 30±0,C 20±0.57, ATM 0±0, SXT 25.33 ± 0.4, E 9±0}table(2).

Table(1): The antibacterial effect of the studied medicinal plants oils on a number of gram positive and gram negative pathogenic bacteria.

Medicinal plants oils	*Klebsiella pneumonia*	*Pseudomonas eroginosaa*	*Escherichia coli*	*Staphylococcus cohnii cohnii*	*Proteus Mirabilis*	*Enterobacter cloacae*	*Micrococcus spp* .
Elleteria cardamomum	20.16±2.72 aA	12.66±2.26 bB	12.08±1.44 bB	10.83±1.934 bB	21.5±3.55 aA	14.16±5.47 cA	11.41±1.16 bB
Nigella sativa	0±0 bB	0±0 bC	16.25±1.13 aA	15.66±2.18 aA	16.50±2.39 aB	0±0 bB	16.91±2.39 aA
Prunus dulcisvarr	0±0 bB	27.75±0.86 aA	0±0 bC	0±0 bC	0±0 bC	0±0 bB	0±0 bC
Brassica alba	0±0 aB	0±0 aC	0±0 aC	0±0 aC	0±0 aC	0±0 aB	0±0 aC

- Values were expressed as means ± standard error
- Values with different capital letters are significant differences vertically at ($p < 0.05$).
- Values with different small letters are significant differences horizontally at ($p < 0.05$).

Table (2): The effect of the tested Antibiotics in inhibition the growth of the pathogenic bacteria measured by millimeter.

Antibiotics	*Klebsiella pneumonia*	*Pseudomonas eroginosaa*	*Escherichia coli*	*Staphylococcus cohnii cohnii*	*Proteus Mirabilis*	*Enterobacter cloacae*	*Micrococcus spp*
Aztereonam	29±0.57	9±0	26±0.57	0±0	0±0	0±0	0±0
Erythromycin	19.33±0.33	0±0	0±0	29±0.57	9±0	9±0	27±0
Trimethoprime/ sulfamethaxazole	24.66±0.4	0±0	27.3±0.32	22±0	0±0	25.33±0.4	21±0
Chloramphenicol	30.66±0.88	10.33±0.4	22.66±0.33	31±0.57	10±0.57	20±0.57	25.66±0.33
Ciprofloxacin	33.66±0.33	31±0.57	23.33±0.88	27.66±1.2	33±0.57	30±0	29±1

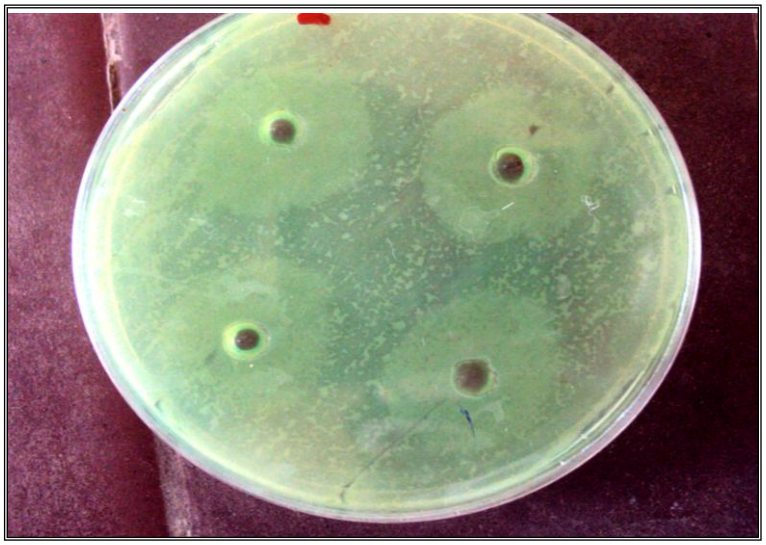

Fig.(1):the Antibacterial effect of *Prunus dulcisvar* on *Pseudomonas aeroginosa*.

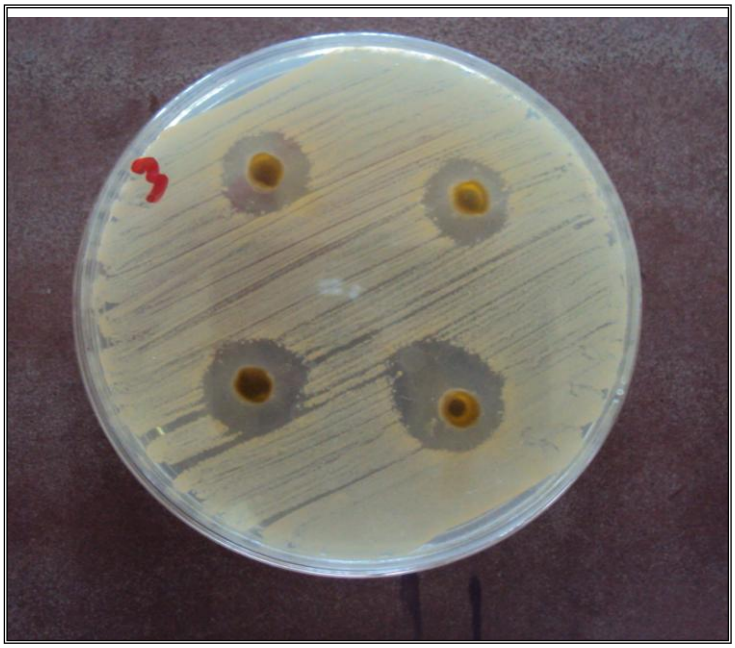

Fig.(2):Antibacterial effect of *Nigella sativa* on *Staphylococcus cohnii cohnii.*

Discussion

Even after introduction of new antimicrobials agents for clinical use ,an alarming increase in bacterial resistance to existing agents demands that renewed effort be made to seek antibacterial agents effective against pathogenic bactaeria resistant to current antimicrobials. (11). *Eletteria cardamomum* is one of the most commonly used medicinal plants the chemical composition of cardamom varies considerably with variety, region and age of the product. The content of volatile oil in the seeds is strongly dependant on storage conditions, but may be as high as8%. The volatile oil contains about 1.5% α-pinene, 0.2%β-pinene, 2.8% sabinene, 1.6% myrcene, 0.2% α-phellandrene, 11.6% limonene, 36.3% 1,8-cineole, 0.7%γ-terpinene, 0.5% terpinolene, 3% linalool, 2.5% linalylacetate, 0.9% terpinen 4-01, 2.6% α-terpineol, 31.3% α-terpinyl acetate, 0.3% citronellol, 0.5% nerd, 0.5%geraniol, 0.2% methyl eugenol and 2.7% trans-nerolidolaroma produced by

acombination of the major components, 1,8-cineole and α-terpinyl acetate (12). Cardamom oil is used in food, perfumery, carminative. In medicine, it is used as a powerful aromatic, antiseptic, stimulant, carminative, stomachic expectorant, anti-spasmodic and diuretic (12).Cardamom oil found to have bactericidal and fungicidal effect (13). Fixed oil of cardamom (*Elettaria cardamomum*) has antimicrobial activity on some pathogens(cardamom 1) , the *Pseudomonas aeruginosa* was found to be inhibited by Cardamom(13)also *K. pneumoniae, S. aureus, E. coli, Enterobacter faecalis.*(8,14).Nigella sativa is a herbaceous plant member of the family of ranunculaceae, used for centuries for treatment of various ailments including infectious disease (15,16). It should be noted that the name of black cumin is sometimes given to entirely unrelated spice *Nigella sativa*.Recently, clinical and animal studies have shown that extract of the black seeds have many therapeutic effects such as immunomodilative(17) antibacterial, hypotensive (18) hepatoprotective and antidiabetic effects (19) . The seeds of black cumin are rich in essential oil, are consumed widely as condiment. In the indigenous system of medicines, seeds are regarded as stimulants and carminatives and found to be useful in diarrhoea and dyspepsia(20) It should be noted that the name of black cumin is given to entirely unrelated spice(7).The crude extracts of N. Sativa were reported to have a promising effect on multi-drug resistant *S. aureus* (21) *Shigella* spp. (22).The black seed(*Nigella sativa)* contains more than 30% of fixed oil and 0.4-0.45 % wt/wt of volatile oil. The volatile oil contains 18.4-24% thymoquinone (TQ) and 46% many monoterpenes such as *p*-cymene and α-piene (23).Crude extract and essential oil of Black cumin possess antibacterial activity against several bacteria (24) The antimicrobial activity of the oil may be attributed to the presence of thymoquinone, thymohydroquinone and thymol in the oil which possessed antimicrobial activity (25).*Nigella sativa* have anti inflammatory effect , *Escherichia coli* are inhibited by black cumin (26),and (27) showed that black cumin oil inhibited the growth of *Staphylococcus epidermidis* as the first record of the inhibitory effect of this oil this was also supported by (11). and (7). The effect of this oil against *Staphylococcus aureus.*was also indicated by(28) , *Pseudomonas aeroginosa* were also found to be sensitive to black cumin oil and it is growth is inhibited by this oil(11) . *Proteus mirabilis ,Klebsiella pneumonia* and *Enterobacter cloacae* was also inhibited by *Nigella sativa* oil in this study while the opposite result to the effect of this oil on these last three bacteria were obtained by (11) how said that these bacteria were not inhibited by black cumin oil. To document the antibacterial effects of these medicinal plants both gram positive and gram negative bacteria were tested.*Prunus dulcisvar* contain a cyanogenic glucose (3-52.5 %) also the bitter almond seed contain Vitamins(A,B) also phosphorus, the antimicrobial effect was proved in this study against *Pseudomonas aeroginosa,* this bacterial inhibitory effect of bitter almond was also supported by(29) and (30).*Brassica alba* seeds contain a glycoside known as Sinalbin and the medicinal effect of this plant are related to this agent.(31)this plant had no inhibitory effect against the studied bacteria in this experiment.In this study there was a significant differences($P<0.05$) in the inhibition of the growth that caused by *Elleteria cardamomum* oil on *Klepsiella pneumonia and Proteus mirabilis* in comparison to *Pseudomonas aeroginosa , Escherichia coli, Micrococcus spp., Staphylococcus cohnii,cohnii* and *Enterobacter cloacae,* also there was a significant difference between Cardamom oil inhibition effect on *Enterobacter cloacae* with *Pseudomonas aeroginosa ,Escherichia coli,Micrococcus aeroginosa and Staphylococcus cohnii,cohnii.*For *Nigella sativa* oil there was no significant differences ($p<0.05$) in the inhibition of the growth between *Escherichia coli, Staphylococcus cohnii cohnii,Micrococcus spp.* and *Proteus mirabilis* while there was

a significant differences between the inhibition of the growth that occur by black seeds oil to the previous bacteria in comparison to *Klebsiella pneumonia, Pseudomonas aeroginosa and Enterobacter cloaca* that this oil express no effect on them ,and for the oil of *Prunus dulcisvar* there was a significant differences ($P<0.05$) between the inhibition of the growth of *Pseudomonas aeroginosa* with all other bacteria where the oil of this plant express no effect on them also there was a significant differences in the inhibition of growth of the bacteria that caused by Cardamom oil on *Klebsiella pneumonia* and *Enterobacter cloacae* in comparison to that obtained by the other tested medicinal plants, also there was a significant differences($P<0.05$) in the effect of *Prunus dulcisvar* oil in comparison with *Eletteria cardamomum* oil on *Pseudomonas aeroginosa* and significant difference between the inhibition effect of Cardamom oil with the effect obtained by Black cumin oil and Mustard oil on *Pseudomonas aeroginosa*, and for *Escherichia coli* , *Staphylococcus cohnii cohnii and Micrococcus spp.* Also there was a significant differences ($P<0.05$) in the effect obtained by Black cumin oil in comparison to that obtained by Cardamom oils and significant differences between the effect of Cardamom oil in comparison to the inhibition effect caused by Mustard oil and Bitter almond oil , for *Proteus mirabilis* there was a significant differences in the inhibition of the growth obtained by Cardamom oil in comparison to inhibition of growth that is caused by Black cumin oil and between Black cumin oil and the inhibition of bacterial growth that caused by Bitter almond and Mustard oils .As a conclusion of this study we tried to found a safe and more secure alternatives for the Antibiotics against the resistant effect that many of the pathogenic bacteria had been developed for many of the commonly used antibiotics and we noticed from the mentioned result the wide spread activity of the used medicinal plants oils against many of these gram positive and gram negative bacteria that had been tested in this experiment.

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تقييم التأثير التثبيطي للزيوت الثابتة لعدد من النباتات الطبية على مجموعة من الجراثيم المرضية مختبريآ

زينب عدنان حاتم العبادي
كلية الطب البيطري / جامعة القادسية

الخلاصة

صممت هذه الدراسة لتقييم الفعل المضاد للبكتريا لأربعة من زيوت النباتات الطبية المحلية وهي:زيت الهيل،زيت الحبة السوداء،زيت اللوز المر وزيت الخردل حيث قيمت فعالية هذه النباتات ضد سبعة من الجراثيم المرضية الموجبة و السالبة لصبغة جرام وهي: *seudomonas aeroginosa,Klebsiella pneumonia,Proteus mirabilis ,Staphylococcus cohnii cohnii, Enterobacter cloacae,Escherichia coli and Micrococcus spp.* بالنسبة الى زيت الهيل فان فعله المضاد للبكتريا ظهر بقوة في تثبيط نمو جرثومة *Proteus mirabillis* يليها جرثومة *Klebsiella pneumonia* ثم جرثومة *Enterobacter cloacae* ثم *Pseudoaeuroginosa* وجرثومة *Escherichia coli* ثم *Micrococcus spp.* و *Staphylococcus cohnii* بأستخدام طريقة الأنتشار في الأكار. اما بالنسبة لزيت الحبة السوداء فان الفعل المضاد للبكتريا كان واضحآ جدآبالنسبة لجرثومة *Micrococcus spp.* ثم *Proteus mirabillis* وجرثومة *Escherichia coli* واخيرآ جرثومة *Staphylococcus cohnii cohnii* .أما زيت اللوز المر فقد احدث تثبيطآ واضحآ فقط في نمو جرثومة *Pseudomon asaeroginosa* بينما لم يظهر أي تأثير على أي جرثومة اخرى. وفيما يخص زيت الخردل فأنه لم يحدث تثبيط في نمو أي من الجراثيم قيد الدراسة.أظهر زيت اللوز تأثير مثبط لنمو جرثومة *Pseudomonas aeroginosa* افضل من التأثير الذي اظهره زيت الهيل ، اما بالنسبة لجراثيم *Micrococcus spp.* *Escherichia coli* ,*Staphylococcus cohnii cohnii* فأن زيت الحبة السوداء احدث تثبيطآ لنمو هذه الجراثيم افضل من التأثير المحدث بواسطة زيت الهيل ، اما لجرثومة. *Proteus mirabillis* فأن نطاق التبيط الذي احدثه زيت الهيل كان افضل من التثبيط الذي احدثه زيت الحبة السوداء.تم احتساب القيم بطريقة المتوسط الحسابي ±الأنحراف القياسي وتحت مستوى احتمالية < ٠,٠٥كفرق معنوي احصائي. حللت النتائج باستخدام اختبار(ت – الطلابي) وبأستعمال البرنامج الأحصائي SPSS (المستوى العاشر).