ANTIMICROBIAL INFLUNCES OF ESSENTIAL OIL EXTRACTED FROM SOME PLANTS FORMULATIONS ON PATHOGENIC BACTERIA

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ABSTRACT

Antimicrobial disk susceptibility tests serve as standard assays for measuring the activity of compounds against pathogenic bacteria. In the current study, some plant-derived proprietary essential oil blends (*Olea europaea , Pimpinella anisum L. , Coriandrum sativum , Matricaria chamomilla L. , Borago officinalis , Cimum basilicum L. , Cuminum cyminum , Thymus vulgaris thymol , Menta xpiperita L. , Rosmarinas officinalis comphora*) were tested for their antibacterial activity against five common strains of pathogenic bacteria using disk susceptibility tests. A formulation intended for topical use (Essential Oil Formulation 1) (EOF1) inhibited the growth of *Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus* as evidenced by zone inhibition diameter measurements when compared to those reported for standard antibiotics. EOF 1 exhibited no activity against *Proteus vulgaris* and *Staphylococcus epidermidis*. The second formulation (Essential Oil Formulation 2) (EOF2), intended for inhalation use, inhibited the growth of all five test bacteria strains with zone inhibition diameters two to three times greater than those reported for standard antibiotics. The growth of all five bacteria strains was inhibited when a cotton swab impregnated with EOF 2 was suspended above the bacterial lawn, indicating a true vapor or fume effect by this formulation.

INTRODUCTION

The antimicrobial properties of some plant-derived essential oils blends (*Olea europaea , Pimpinella anisum L. , Coriandrum sativum , Matricaria chamomilla L. , Borago officinalis , Cimum basilicum L. , Cuminum cyminum , Thymus vulgaris thymol , Menta xpiperita L. , Rosmarinas officinalis comphora*) have been recognized for hundreds of years[1,2] and have been documented in scientific studies[3-7] It has been demonstrated that the antimicrobial activities of

one natural oil, thymol oil obtained, from thymus vulgaris[8]. The purpose of this study was to determine the inhibitory effect of essential oils against five common and clinically significant bacterial pathogens. Antimicrobial activity was assayed by the standard method adopted from the National Committee on Clinical Laboratory Standards (NCCLS) for antibiotic susceptibility testing[9-10]. One of the two proprietary formulations (EOF 1) was developed for topical use and the other (EOF 2) for inhalation. Besides the direct contact disk sensitivity method we devised a technique for the inhalation formulation where a cotton swab was suspended above the agar-based bacterial lawn, mimicking a true vapor or aroma effect.

MATERIALS AND METHODS

A) Culture Preparation

The following strains of gram negative and gram positive bacteria were purchased from biology department of science collage in Basrah university and some other labrotaries.

Each strain was plated out on blood agar plates and incubated for 18 hours at 35°C. Colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 mL of tryptic soy broth (TSB). Turbidity of each bacterial suspension was adjusted with TSB media to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to $2x10^8$CFU/ml. A Wickerham Card (Hardy Diagnostics, Santa Maria, CA) was used for the visual comparison[11] Within 15 minutes after adjusting the turbidity of the inoculum suspension, Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed.

B) Susceptibility Disk Method

The essential oil formulations were acquired from Lane Labs, Allendale, NJ, manufactured and formulated by Bio Excel, Sonoma, CA. Both formulas contain a combination of plant-derived oil extracts (Table 1). Fifteen microliters of either tryptic soy broth (Control), or EOF 1, or EOF 2 was placed on separate 0.25-inch blank filter paper disks (Hardy Diagnostics, Santa Maria, CA.) The disks were dispensed onto the surface of the inoculated agar plates and incubated at 35°C for 18 hours. The diameters of the zones of complete inhibition were recorded and each Petri dish were photographed.

C) Suspended Cotton Swab Method

Sterile cotton swabs were dipped in either sterile water for control plates or in EOF 2 and pressed firmly against the sides of the vessels to prevent dripping. They were then taped to the lid of each Petri dish. The swabs were suspended above the agar to avoid contact with its surface so that only the volatile components of EOF 2 elicited inhibitory activity.

Table 1:- Essential Oil Formulation Igredients.

EOF 1	EOF 2
Olea europaea	*Cimum basilicum L.*
Pimpinella anisum L.	*Cuminum cyminum*
Coriandrum sativum	*Thymus vulgaris thymol*
Matricaria chamomilla L.	*Menta xpiperita L.*
Borago officinalis	*Rosmarinas officinalis comphora*

RESULTS

The zones of bacterial inhibition were measured to the nearest whole millimeter. The results of susceptibility disk tests showed moderate zone inhibition with three of the five bacterial strains in the EOF 1-treated cultures. Inhibition diameters in this group ranged from 11 to 18 mm. All five bacterial cultures treated with EOF 2 exhibited appreciable increases of zones of inhibition ranging from 25–68 mm. Both groups resulted in irregular zone patterns, unlike those typically seen in standard antibiotic susceptibility tests. Antibiotics, for the most part, present a clean circular edge within the bacterial lawn, whereas the essential oil formulations produced an asymmetric jagged or spiked edge (Fig 1). This effect may be a result of the pattern of radial diffusion penetration through troughs of uneven growth distribution within the bacterial lawn, giving rise to micro channels of less dense or sparse bacterial accumulation.

The suspended swab method, incorporating EOF 2, resulted in zones of inhibition ranging from 26–36 mm in diameter in all five bacterial strains studied. An elliptical pattern mimicking the shape of the cotton impregnated with EOF 2 was observed (Fig. 2). There appeared to be a concentration gradient of clear pronounced inhibition in the center, gradually feathering and fading to the periphery of the zone in four of the five bacterial strains tested. Presumably, the highest concentration of vapor molecules was at the center of the swab. This feathered zone edge effect was not prominent with Staphylococcus aureus.

DISCUSSION

The range of sensitivity to a wide variety of antibiotics by strains of bacteria similar to those used in this study (13–29)mm with concentrations of antimicrobials ranging from 1 μg to 350 μg per disk content) is documented in the NCCLS "tables of zone diameter interpretive standards and equivalent minimal inhibitory concentration (MIC) breakpoints.[9]

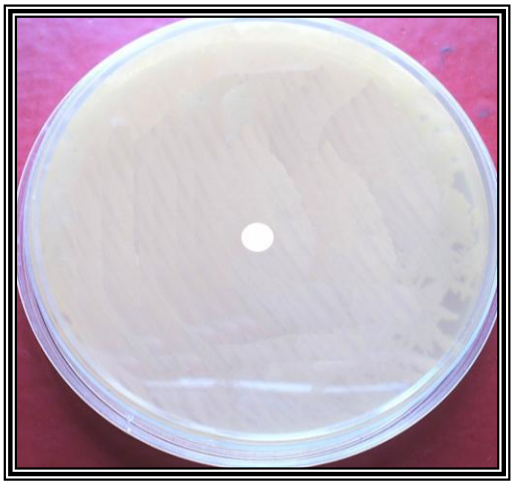
In this screening study, the bacteriostatic and/or bactericidal activities of two combination essential oil formulations (EOF 1, EOF 2) were observed against five ubiquitous strains of both gram negative and gram positive pathogenic bacteria. EOF 1 is intended for topical use and showed minimal to moderate antibacterial action comparable to the lower zone inhibition ranges reported for most antibiotics, 11–18 mm versus (13–29)mm respectively.[3] Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus were more sensitive to EOF 1 as evidenced by 15 mm, 16 mm, and 18 mm inhibited zone diameters respectively.[1-4] Insensitivity was observed with Proteous vulgaris and Staphylococcus epidermidis with 11mm and 12mm zone diameters respectively, when compared to NCCLS-reported antibiotic minimal inhibitory range diameters.[8]

EOF 2 is targeted for inhalation use. When the disk method was utilized, the inhibition zone diameters for all bacterial strains were approximately two to three times the diameter of those of NCCLS-reported antibiotic sensitivity tests, (25–68) mm compared to (13–29)mm respectively.[7] The suspended swab method more closely paralleled the intended inhalation use of EOF 2 by permitting only the gaseous phase of the formulation to make contact with the bacteria hence giving meaning to so called, aromatherapy.[12] The inhibition seen by EOF 2 is greater than twice the reported antibiotic inhibitory diameters (26–36 mm) and is noteworthy since only a small concentration of the vapor molecules would probably come in contact with the growing bacteria. The inhalation application of EOF 2 may prove useful in the prevention and/or treatment of upper respiratory infections caused by some strains of bacteria, or in the treatment of viral-induced secondary bacterial infections.[6]

With the increase of worldwide bacterial resistance of many strains of disease-producing bacteria, there is a need to access new and complementary approaches to antibiotic therapy. This screening study in the laboratory indicates that essential oils may be considered to be used in combination with standard topical and antibiotic therapies. However, to verify clinical utility, it is necessary to extend this research to human applications against similar strains of pathogens and

examine dose responses of both topical and inhalation forms of the oils as well as testing various modes of administration. Because of minimal, if any toxicity [12] and pleasant odor, these oils may have the advantage of greater acceptance by patients and the community.[5]

This initial antimicrobial screen further studies with these formulations on antibiotic-resistant strains and other pathogens such as viruses, fungi, mycoplasma, chlamydia, and yeasts.[11]



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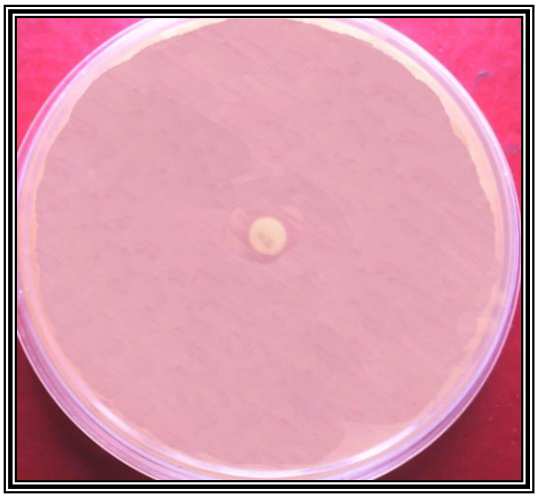
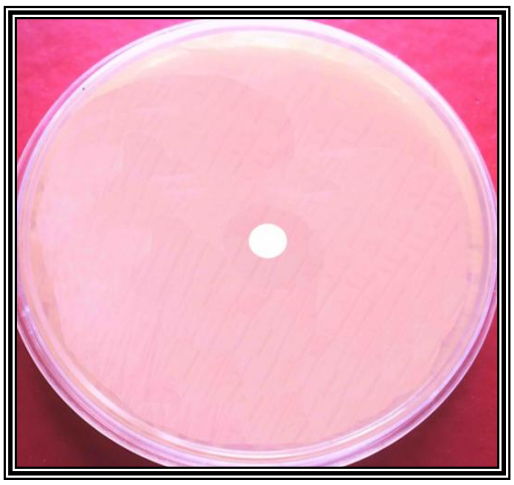


Essential formul A

Essential formul B

Fig 1 :- *Proteus vulgaris*

Each 8mm sensitivity disc was impregnated with 15µl of either stenle TSB media for controls or 15µl of each essential oil formula.



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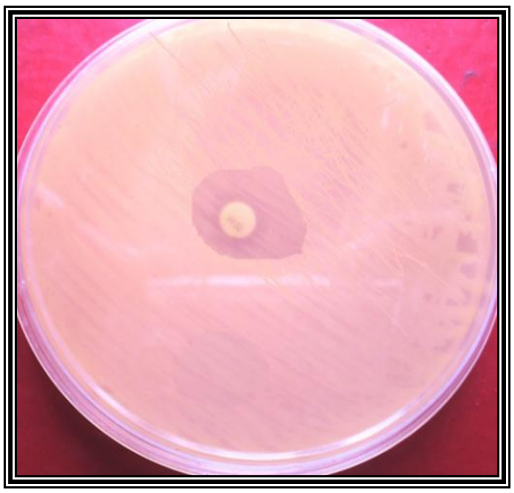
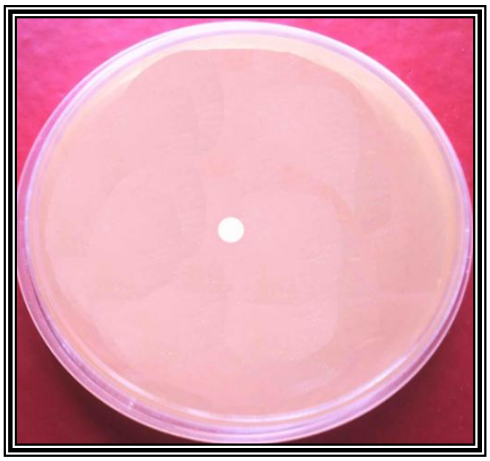


Essential formul A

Essential formul B

Fig 2 :- *Eschenchia coli*

Each 8mm sensitivity disc was impregnated with 15µl of either stenle TSB media for controls or 15µl of each essential oil formula.



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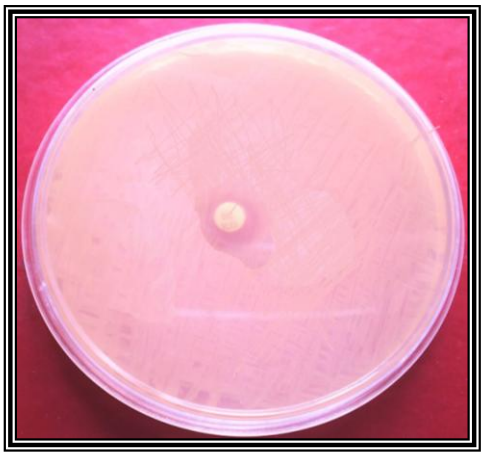
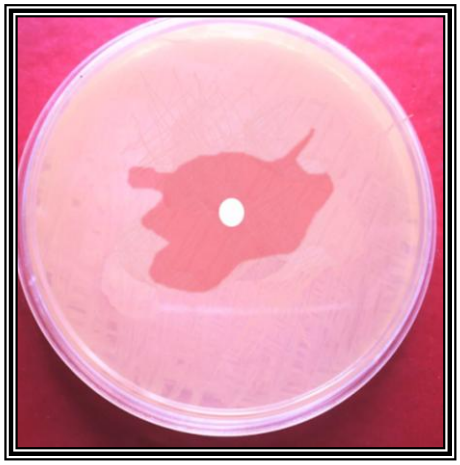


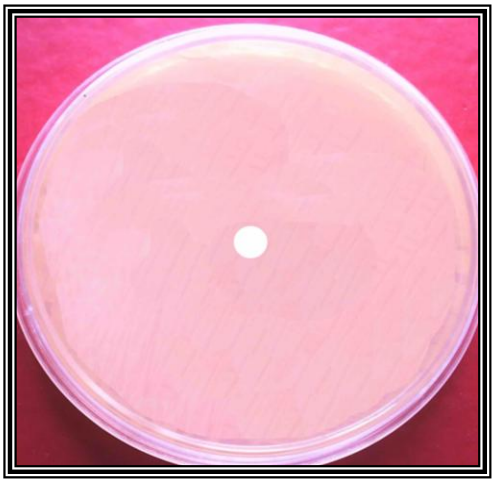
Essential formul A



Essential formul B

Fig 3 :- *Klebsiella pneumoniao*

Each 8mm sensitivity disc was impregnated with 15µl of either stenle TSB media for controls or 15µl of each essential oil formula.



Control

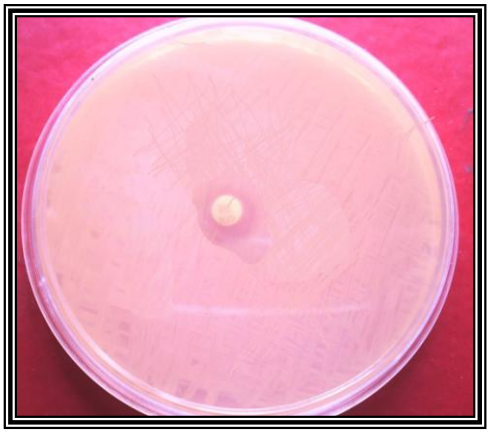
Essential formul A

Essential formul B

Fig 4:- ***Staphylococcus aureus***

Each 8mm sensitivity disc was impregnated with 15µl of either stenle TSB media for controls or 15µl of each essential oil formula.

Control

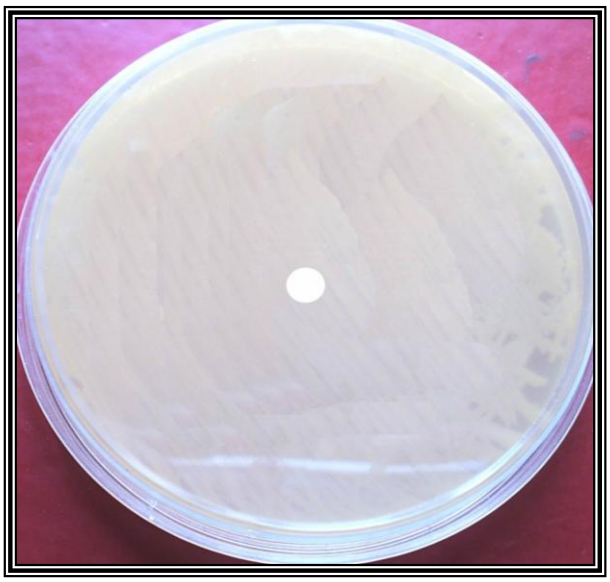


Essential formul A

Essential formul B

Fig 5:- ***Staphylococcus epidermidis***

Each 8mm sensitivity disc was impregnated with 15µl of either stenle TSB media for controls or 15µl of each essential oil formula.



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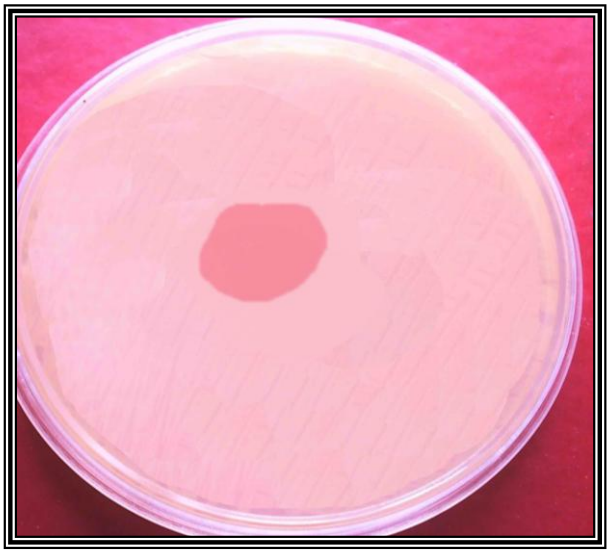
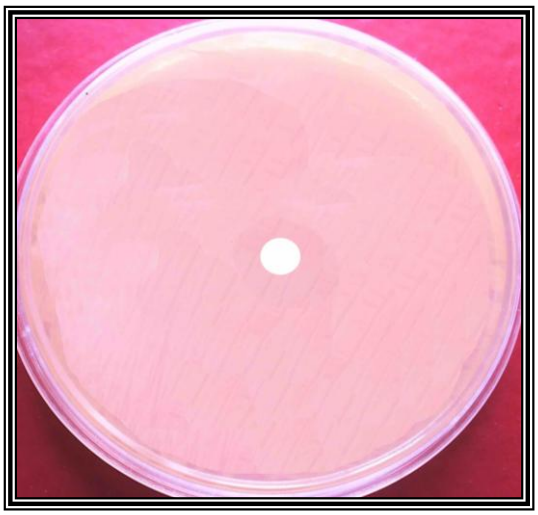


Essential formul 2

Fig 1:- *Proteus vulgaris*

Cotton swabs were diped in either sterile water for control plates or dipped in Essential Oil Formula 2 and taped to the lid of each petry dish. The swabs were suspended above the agar to avoid contact with its surface. The zones of inhibition were a result of vapor from the essential oil.



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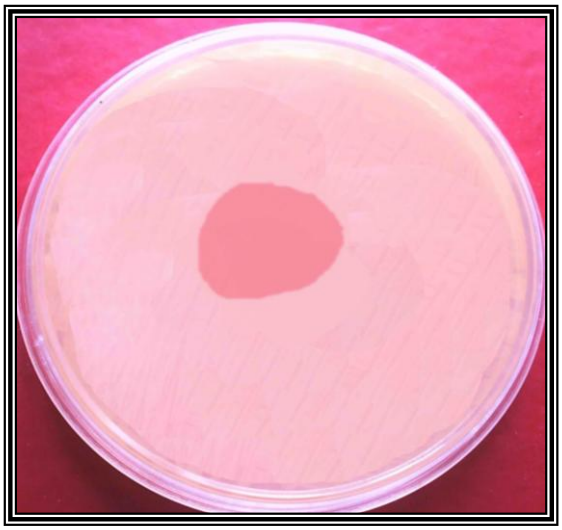

Essential formul 2

Fig 2 :- *Eschenchia coli*

Cotton swabs were diped in either sterile water for control plates or dipped in Essential Oil Formula 2 and taped to the lid of each petry dish. The swabs were suspended above the agar to avoid contact with its surface. The zones of inhibition were a result of vapor from the essential oil.

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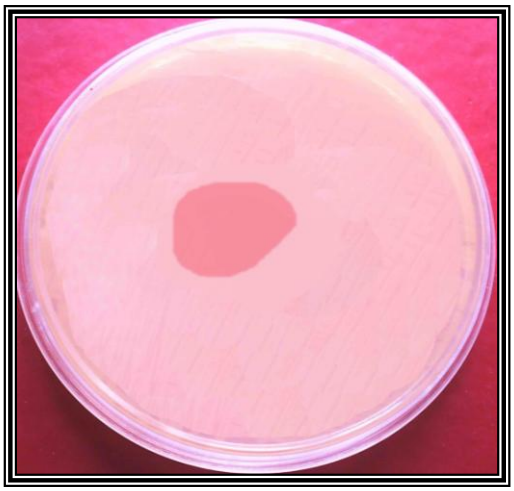

Essential formul 2

Fig 3 :- *Klebsiella pneumoniao*

Cotton swabs were diped in either sterile water for control plates or dipped in Essential Oil Formula 2 and taped to the lid of each petry dish. The swabs were suspended above the agar to avoid contact with its surface. The zones of inhibition were a result of vapor from the essential oil.

Control

Essential formul 2

Fig 4:- *Staphylococcus aureus*

Cotton swabs were diped in either sterile water for control plates or dipped in Essential Oil Formula 2 and taped to the lid of each petry dish. The swabs were suspended above the agar to avoid contact with its surface. The zones of inhibition were a result of vapor from the essential oil.

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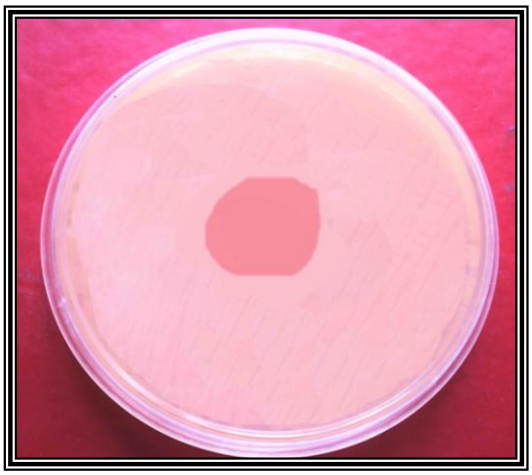

Essential formul 2

Fig 5:- *Staphylococcus epidermidis*

Cotton swabs were diped in either sterile water for control plates or dipped in Essential Oil Formula 2 and taped to the lid of each petry dish. The swabs were suspended above the agar to avoid contact with its surface. The zones of inhibition were a result of vapor from the essential oil.

Table 2: Bacterial growth inhibition zone produce by Essential Oil Formulations (EOF)

Bacterial isolate	Zone Diameter (mm)*	
Bacteria	EOF1	EOF2
Escherichia coli	15	45
Staphylococcus epidermidis	12	25
Staphylococcus ureus	18	47
Klebsiella neumoniae	16	55
Proteous vulgaris	11	68

*nearest whole millimeter

Table 3. Bacterial growth inhibition zone produce by (EOF2) suspended swab test

Bacterial isolate	Zone Diameter
Proteous vulgaris	26
Klebsiella pneumoniae	25
Escherichia coli	32
Staphylococcus epidermidis	25
Staphylococcus aureus	36

*nearest whole millimeter

التأثيرات ضد الميكروبية للزيوت المستخلصة من بعض النبات وتأثيرها على بعض البكتريا المرضية

رنا عدنان فائز * حازم سعد جبار**

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الخلاصة

ان اختبارات الاقراص ضد الجراثيم تعمل كتجارب قياسية لقياس فعالية بعض المركبات ضد البكتريا المرضية. في الدراسة الحالية تم استخلاص الزيوت من بعض النباتات (الزيتون ، اليانسون ، الكزبرة ، بابونك ، الريحان ، كمون ، زعتر البساتين ، نعناع فلفلي و زهرة اكليل الجبل) لاختبا ر فعاليتهما ضد البكتريا وقد استخدمت خمس انواع من البكتريا المرضية باستخدام أقراص تجارب خصصت لهذا الغرض الطريقة التي تستخدم للاستعمال الموضعي (EOF 1) وهي تستخدم لتثبيط نمو *Escherichia coli* ، *Klebsiella pneumoniae*، و*Staphylococcus aureus* ويستدل على منطقة التثبيط بواسطة قطر منطقة الثبيط حيث تقاس عندما تقارن مع تلك الاقراص المضادات الحيوية القياسية. (EOF 1) توضح بأنه لا يوجد فعالية ضد *Staphylococcus epidermidis* و *Proteus vulgaris* . الطريقة الثانية التي استخدمت هي (EOF 2) وهنا تم استخدام الاستنشاق حيث يتم توضيح تثبيط خمسة انواع جميعها من البكتريا بمنطقة تثبيط أقطارها اثنان إلى ثلاثة مرات وهي اكبر من تلك التي تم الاعتماد عليها في أقراص المضادات الحيوية. أن نمو الخمسة أنواع من البكتريا جميعها يتثبط عندما يتم استخدام مسحات القطن التي لحقت بطريقة (EOF 2) وهذا يوضح وجود نمو بكتيري وهذا يشير على وجود بخار أو دخان واضح عند استخدام هذه الطريقة.

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