Antimicrobial Action of Some Types of Homemade and Commercial Oils Extracts Against Bacterial Clinical Isolates

Ali Shaker F. Hadad [1] and Jaleel Samanje *[2]
[(1,2)] College of Health and Medical Techniques, Middle Technical University, Baghdad/Iraq
Correspondence author E-mail (*) : Jaleel.najah@mtu.edu.iq

Abstract

The concentrated natural plant extracts are important sources of oils, which were proven to be good bioactive compound sources with antimicrobial and antioxidative characteristics. The current study aims to study the antimicrobial actions of public extracts of some commercial essential oils sold in market agaisnt a panel of some gram negative and positive clinicaly isolated bacteria. İn our study, fifteen types of commerical extracts were sold from local market and their antimicrobial action was tested against *Pseudomonas. aeruginosa, Klebsiella. pneumoniae, Proteus. vulgaris, Escherichia. coli, Staphylococcus. aureus, Streptococcus. agalactiae and Enterococcus. faecalis*. All the selected pathogens were tested for antibiotic senesitivty profile with 16 types and differet classes of antiobiotics using the Kirby-paur method, and the diffusion method was used for evaluation the antimicrobial action of the selected 15 commercial oil extracts and the inhition zone of each test was recorded in millimetres. Oregano, glycerine and mustard were the most effeccive oils, Shea Butter and Linseed were the less effective against the studied bacteria. The majority of sensitive bacteria were Gram-negative bacilli such as *K. pneumoniae, P. vulgaris and E. coli,* while the *S. auerus* which is Gram-positive cocci bacteria also showed high sensitivity. The current findings suggested the importantce of essential oils as an alternative for using of antibiotics due to their antibacterial activity and this may aid in antimicrobial fight.

Keywords: *Commercial oils, Glycerine, Hommade oils, Mustard, Oregano.*

الفعالية التثبيطية لبعض انواع الزيوت الاساسية المستخلصة محليا وتجاريا ضد مجموعة من العزلات السريرية

تقني طبي علي شاكر فليح[1] وَ أ .م. د. جليل نجاح جليل[2]

الخلاصة

الزيوت الاساسية هي عبارة عن مستخلصات زيتية طبيعية مركزة و مستخلصة من النباتات ومن الاعشاب ، والتي اثبتت انها مصادر جيدة منتجة للمركبات ذات الخصائص المقاومة للبكتريا المرضية وللاحياء الدقيقة من البكتريا والفطريات زغيرها من المكروبات. اجريت هذه الدراسة التي يتوقع منها أن تكشف عن نشاط المضاد الحيوي لخمسة عشر زيت طبيعي ماخوذ من المحال التجارية على ثمانية أنواع مختلفة من البكتريا المأخوذه من عينات سريرية وهذِه الانواع هي:

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(P. aeruginosa. K. pneumoniae, P. vulgaris, S. aureus, S. E.coli, E. faecalis, S .agalactiae, S. epidermidis)

التي كثيرا ما تسبب عدوى و التهاب في البشر، تم استخدام طريقة Kirby-Bauer لاختبار حساسية العزلات المختاره ضد 16 نوع من المضادات الحياتية وكذلك لاختبار قابلية المستخلصات التجارية وعددها 15 زيتًا أساسيًا على تثبيط النشاط البكتيري للعزلات المختارة.من بين 15 زيتًا أساسيًا زيت تبين ان زيت ال Oregano،Glycerine،Nagilla sativa،كان لهم نشاط وفعلية حيوية وتثبيط لبعض العزلات خصوصا ضد معظم العزلات ذات الصبغة سالبة الكرام، بينما اظهر زيت Shea Butter and Linseed اقل نشاط تثبيطي ضد العزلات المدروسة. كذلك برهنت نتائج هذه الدراسة انه جميع العزلات ابدت مقاومة ضد معظم المضادات المستخدمة. خلاصة هذه الدراسة توصلت انه الزيوت الطبيعية المستخلصة قد توفر بدائل لعلاج ومكملات اضافية للمضادات الحيوية ضد انواع البكتريا الشائعة التي تسبب الامراض للانسان.

الكلمات المفتاحية : *الزيوت التجارية ، زيت الكليسرين ، الزيوت المحلية ، زيت الخردل ، زيت الاوريكانو.*

Introduction

The essential oils or the volatile oils are concentrated natural extracts obtained from plants, that are used as alternative medicines since the late 12th century, and became more broadly distributed in the 2nd half of the 16th century. The modern chemistry permitted a deeper access to essential oils resulting in several publications during the 19th century. The essential oils began to be utilized in perfume, food stuff and beverage production [1].The essential oils were shown to be good sources of bioactive compounds with antimicrobial and antioxidative characteristics. Several parts in the plants contain extractable oils including seeds, leaves, roots, barks, resins, berries, flowers and fruits. Steam distillation, hydrodistillation or solvent extraction can be used to purify the essential oils [2]. Their composition is complicated consisting mainly of terpenes (monoterpenes & sesquiterpenes), terpenoids (oxygenated compounds like alcohol, phenols, ketones, aldehydes and ethers) as well as aromatic compounds [2].

Some parts of these compounds are hydrophobic, while others are hydrosoluble. Although terpenes are hydrophobic, they may show water solubility based on their mixing temperatures and structures. The terpenoids show better water solubility than terpenes [3].

The essential oils were shown to be successful in treating many conditions like releasing of pains related to chronic disorders or medical procedures [4] , decreasing postoperative nausea or autonomic responses to pains [5-7] or in possible symptom relief in cancer patients [8-9] or even for treatment of pediculosis in children [10] .Multiple studies have been done on children with no side effects occured. The antibacterial activities of essential oils have demonstrated increasing interests recently and have been presented to be effective even on strains with multidrug resistance [11-13] .The aim of this study was to study the effect of some types of commercial and hommade oil extract against

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different bacteria picked up from different clinical sources using classical method for evaluation the zone of inhibition (ZIH) of each extract.

Materials and methods

Culture media

Nutrient agar, 5% blood sheep agar, MacConkey agar and mannitol salt agar, Muller Hinton agar and nutrient broth were used.

Bacterial strains

Gram-negative and gram-positive bacteria were obtained from Medical Microbiology Department of Baghdad Teaching Hospital /Teaching Laboratories. Strains used included *P. aeruginosa, K. pneumoniae, P. vulgaris, S. aureus, S. agalactiae, E. coli, E. faecalis, S. epidermidis* which were isolated from different clinical samples subjected for assesement of the antimicrobial status against a panel of (15) different commercial oil.

Methods

Reagent and solution preparation

Solutions and culture media were sterilized by autoclaving for 15 minutes at 121^{o}C.

Culture media preparation

All microbiological growth media were prepared in accordance with the manufacturer's guidelines. The desired pH of all media was checked and adjusted prior to autoclaving. The prepared agar dishes were kept at 4 °C and the liquid media were kept at room temperature unless stated otherwise.

Commercial oil extarxt collection

From Al-Shorja market, (15) commercial oil extracts, with 100% concentration, were obtained. Containers of oil extracts were put in sterile plastic bags and transported to the laboratory and kept at 4°C until use as shown in table (1).

Isolation of bacterial strains

The seven strains of bacteria were obtained from Medical Microbiology Department of Baghdad Teaching Hospital /Teaching Laboratories were previously diagnosed with Vitek system and directly used for experiments.

Resistance to antibiotics

The susceptibility to antibiotics for seven strains was assessed by disc diffusion method. The suspension of all bacterial strains was adjusted to a McFarland standard of 0.5, with soaking of a cotton swab into an adjusted suspension and spreading it evenly on Nutrient agar. The selected antibiotic discs of Amikacin (10 MCG), Trimethoprim (5 MCG), Nalidixic Acid (30 MCG), Gentamicin (10 MCG), Ampicillin (25 MCG), Ceftriaxone(30 MCG), Piperacillin (30

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MCG),Gentamicin (30 MCG), Azithromycin(15 MCG), Rifampin (5 MCG), Cefixime (5 MCG), Trimethoprim Sulphamethoxazole (25 MCG), Imipenem (10 MCG), Erythromycin (60 MCG),Cefotaxime (30 MCG), Ceftazidime (30 MCG) and Tetracycline (10) (Bioanalyse, Turkey) were evenly put on the surface of agar then cultures were incubated for 24 hrs. The inhibition zone diameter (IZD) was measured and assessed as shown in table (3).

Evalution of antimicrobial action of selected oils

The method of well diffusion described elsewhere was used with a little modification for the determination of the antimicrobial action of seventeen oil extract strains against some panels of pathogens. All the 17 *strains* were cultured in nutrient agar and incubated at 37°C overnight. Suspensions of all bacterial strains were adjusted to a McFarland standard of 0.5, with soaking of a cotton swab in the adjusted suspensions and spreading them evenly on muller Hinton agar. Wells were punctured in the muller Hinton (MHA) agar plates which have been already seeded with each of the pathogenic bacterial culture adjusted to 0.5 McFarland. To each well 70 to 100 µl of oil extract was transferred and plates were incubated for 24 hours at 37 °C. The inhibition zone of each well was measured and recorded [14].

Statistical analysis

Analysis of data was done using Microsoft Excel software (version 2010).

Results

Table (1): Commercial oil extracts used during study.

No	Name of antibiotic	Origin
1	Aloe vera oil	Hemani, Pakistan
2	Blackseed Oil	Hemani, Pakistan
3	Cress Seed Oil	Hemani, Pakistan
4	Garlic Oil	Hemani, Pakistan
5	Glycerin Oil	Hemani, Pakistan
6	Sesame Oil	Hemani, Pakistan
7	Shea Butter Oil	Hemani, Pakistan
8	Oregano Oil	Hemani, Pakistan
9	Onion Oil	Hemani, Pakistan
10	Linseed Oil	Hemani, Pakistan
11	Thyme Oil	Hemani, Pakistan
12	Taramira Oil	Hemani, Pakistan
13	Myrtle Oil	Hemani, Pakistan
14	Mustard Oil	Hemani, Pakistan
15	Sweet Violet Oil	Hemani, Pakistan

Table (2): Isolation of bacterial species from clinical samples.

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No	Name of bacteria	Sample
1	*P. aeruginosa*	Urine
2	*K. pneumoniae*	Urine
3	*P. vulgaris*	Urine
4	*S. aureus*	wound
5	*S. agalactiae*	Throat
6	*E. coli*	Stool
7	*E. faecalis*	Urine

Table (3): Results of antibiotic sensitivity test of seven clinically isolated bacteria.

No	**Name of bacteria**	***P. aeruginosa***	***K. pneumoniae***	***P. vulgaris***	***S. aureus***	***S. agalactiae***	***E.coli***	***E. faecalis***
		Sample						
		Urine	**Urine**	**Urine**	**Wound**	**Throat**	**stool**	**Urine**
1	TMP (15MCG)	R	R	R	S	R	R	R
2	AK (10MCG)	R	R	R	R	R	R	R
3	NA30(MCG)	R	R	S	R	R	R	R
4	CN(10MCG)	R	R	R	R	R	R	R
5	AM (25MCG)	R	R	R	R	R	R	R
6	CRO (30MCG)	R	R	R	R	R	R	R
7	PRL (30MCG)	R	R	R	R	R	R	R
8	AZM (15 MCG)	R	R	R	S	R	R	R
9	RA (5 MCG)	R	R	R	S	R	R	R
10	CFM (5MCG)	R	R	R	R	R	R	R
11	SXT (25 MCG)	R	R	R	R	R	R	R
12	IPM (10 MCG)	R	R	R	R	R	R	R
13	E (60 MCG)	R	R	R	S	R	R	R
14	CTX (30 MCG)	R	R	R	R	R	R	R
15	CAZ (30MCG)	R	R	R	R	R	R	R
16	TE (10 MCG	R	R	R	R	R	R	R

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TMP; Trimethoprim, AK; Amikacin, NA; Nalidixic Acid (, CN; Gentamicin, AM; Ampicillin CRO; Ceftriaxone PRL;

Piperacillin, AZM; Azithromycin, RA; Rifampin, CFM; Cefixime, SXT; Trimethoprim Sulphamethoxazole, IPM; Imipenem,

E; Erythromycin, CTX; Cefotaxime, CAZ; Ceftazidime, TE; Tetracycline), R, resistance, S; sensitive.

Table (3) and figure (1) revealed that most isolated bacteria were resistant to the 16 selected antibiotic types. *P. aeruginosa*, *K .pneumonia*, *S. agalactiae*, *E. coli* and *E. faeclais* was the most resistance strain. While *P. vulgaris* bacteria were sensitive to some antibiotic like Nalidixic acid (30 MCG), and Gentamicin (30 MCG). *S. auerus* was also sensitive to Trimethoprim ,Gentamicin , Azithromycin, Rifampin and Erythromycin.

Table (4): Results of antimicrobial activity against seven clinically isolated bacteria under the effect of commercial and homemade oil extracts.

No	Name of bacteria	Zone of inhibition (ZHI) millimetre (mm)													
		Aloe vera	Black seed	Cress seed	Garlic	Glycerine	Sesame	Shea Butter	Hommade oregano	Onion	Linseed	Thyme	Tarami ra	Myrtle	mustard	Myrtle
1	*P. aeruginosa*	6	6	5	8	5	5	5	15	5	0	6	6	6	0	5
2	*K. pneumoniae*	0	5	5	5	37	5	0	27	5	0	7	0	0	14	5
3	*P. vulgaris*	5	5	6	5	39	5	0	25	5	5	5	0	0	15	5
4	*S. aureus*	0	26	8	0	37	0	0	42	7	0	8	12	5	0	5
5	*S. agalactiae*	5	5	6	7	5	6	0	8	5	0	8	8	7	8	10
6	*E. coli*	0	8	0	5	38	5	0	25	8	8	7	8	8	0	5
7	*E. faecalis*	0	5	5	5	6	7	5	34	0	0	0	0	0	0	5

Table (4) and figure (1) demonstrated that the commercial glycerine extarct was the most type which exhibited antimicrobial action againt *K. pneumonia, P. vulgaris, S. aureus and E. coli* with a zone of inhibition of 37 mm, 39 mm, 37 and 38 mm respectively. While the homemade oil extracts which were shown to be active against all the isolates in this study included the Oreganol oil. S. auerus was the most effectve one with a ZHI of (42 mm). Most selected isolates showed resistance under the

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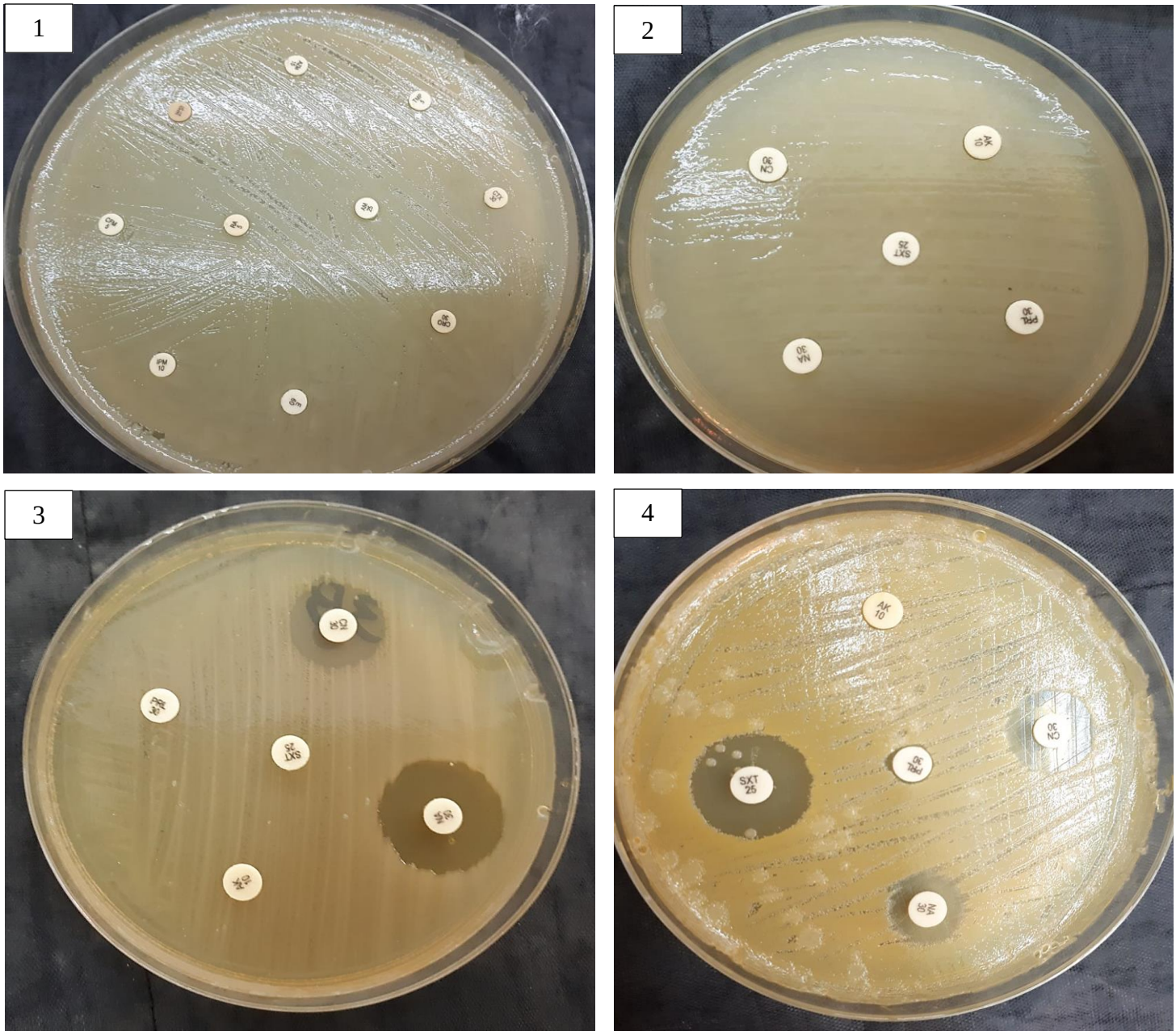
action of commercial Linseed, Shea Butter and Aloe vera. This table also demonstrated that the commercial extracts were more active against gram nagative bacteria than gram positive, selected in this study.



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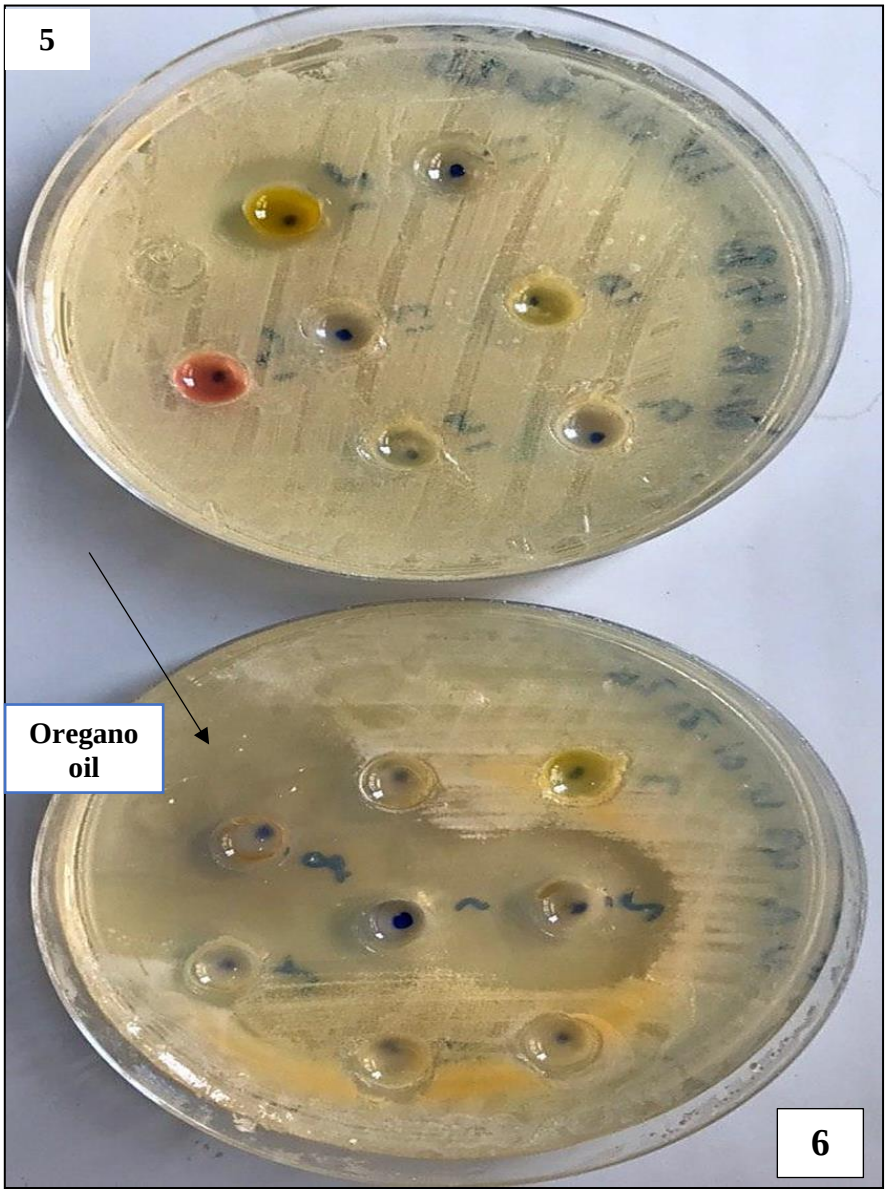

Fig. Results of antibiotic sensitivity and antimicrobial action of commercial oil extract against clinically isolated bacteria, 1-2; *K. pneumoniae*, 3; *E. coli*, 4; *P. vulgaris*, 5-6; *S. auerus*.

Discussion

Essential oils were used from ancient times in aromatherapy and control of disease, with monitorting the observational effect, without knowing the substrate or the micro- or macroorganism interactions. Nowadays, several essential oils are available and widely used in accordance with their local tradation and availability. The results of this study revealed the good effect of some essentail oils against multi-drug resistance bacteria such as hommade oregano oil which showed a big zone of inhibition growth against many most types of selected bacteria of this study, and these results were in agreement with [15] . Oregano (Organum vulgare) is a public popular herb which originates from

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the Mediterraneans, and it is nowadays used worldwide as a food flavouring. From ancient times, the essential oil of Oregano was used in folk medicine and showed antibacterial and antioxidant activities [16,17]. İt was found that nearly 60% of the oils of oregano is carvacrol, a monoterpenoid phenol, which also found in the bergamot and thyme, but with less concentrations [18] . Cavacro has a professional antibacterial action due to it's ability to permeabilization and depolarization of the cytoplasmic membrane. Carvacrol has the ability to inhibit production of microbial toxin, biofilm formation and reduces the production of fimbriae and the motility of swarming of pathogenic E. coli [15]. Our results were also in agreement with [15], who reported that the Thyme (Thymus vulgaris) showed less inhibition against most tested bacteria. Also, this study showed a good antibacterial effect of Nigella sativa oil seed against some selected panel of pathogens, and our result agreed with [19]. The result of this study also demonstrated the good antibacterial effect of glycerin oil against most tested clinical isolates and this result was in a harmony with [20]. Other tested oil extract showed different and little effect against bacteria in this study. In medicine, Myrtle oil was found to show a protective effect against many conditions, like skin and digestive diseases [21]. The composition of Myrtle oil which is considered as a bioactive constituent involved polyphenol, terpene (which is also present as β- pinene-25%), acylphloroglucinols, myrtenyl acetate, limonenes and linalool with antioxidants and antimicrobial actions [22,23] . The thyme or (Thymus vulgaris) is a very popular herb as an essential oil having anti-inflammatory and antimicrobial characteristics [24] .The essential oil of thyme contains large amounts of thymol and carvacrol and aquired the thyme stronge antimicrobial activity [25].

Conclusions

The clinically isolated strains were resistant to most routine antibiotics types. All of the commercial oil extracts had better inhibition effect on clinically bacteria strains more than most antibiotics. Oregano oil was the most active extract against most clinically isolated strains. *E. faecalis* bacteria isolated from urine samples were the most resistant strains when treated with most oil extracts in this study. There is a good possibility to use these commercial oils against many bacterial infections.

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