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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2021 18(3): 2434-2445.

OPEN ACCESS

## Bactericidal and antioxidant effects of Saudi domestic essential oils on certain Pathogenic bacteria.

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Essential oils (EOs) are volatile, liquid substances that come from natural sources such as plants. As secondary metabolites of most plant species, that are typically found in leaf chloroplasts or from the hydrolysis of certain glycosides, their primary function is to combat attacks from predators as well as attract pollinators. Due to their biological activity, EOs have gained popularity in industries such as food processing, cosmetics, and personal care products as well as the pharmaceutical industry. In this study, the antimicrobial nature and antioxidant activity of Saudi Arabian EOs were compared with the antimicrobial activity of known antibiotics. Disc diffusion method was used for the assessment of the bactericidal activity of local EOs. The antioxidant activity was determined using phosphor molybdenum method. Furthermore, RAPD-PCR was used to compare the effects of these EOs on DNA in both untreated and treated pathogenic bacterial strains. Bacterial strains with varying antibiotic resistance potential have been successfully isolated. The inhibition zone measurement revealed that the EOs used had some antibacterial effects on the bacterial strains as well as some selectivity on the bacterial strains that they acted on.

**Keywords:** Essential oils, DNA Analysis, antibiotic resistance, RAPD-PCR, Disc diffusion

### INTRODUCTION

Since antiquity, plants and their metabolites have been used for a variety of purposes in various parts of the world (Ansori et al. 2018; Sandner et al. 2020). Essential oils (EOs) are a type of natural compound made up of volatile aromatic oily/hydrophobic compounds (Heghes et al. 2020; Muneke et al. 2020; Greta, 2020; Bahr et al. 2019; Wińska et al. 2019; Paul et al. 2020). They essentially provide taste and scent to plants, which can act as a repellent for both biotic and abiotic stressors like insects, bacteria, or fungi, as well as a signaling molecule or plant pheromone in ecological interactions like plant pollination (Wińska et al. 2019; Herman et al. 2019; Thirumurugan et al. 2018; Pagare et al. 2015). EO

synthesis is a schizolysigenous process that takes place in specialized secretory tissues of plant organs such as leaf chloroplasts, fruit peels, seeds, and bark (Heghes et al. 2020; Wińska et al. 2019; Paul et al. 2020).

The term "essential oil" is derived from the 16th-century Swiss reformer Paracelsus von Hohenheim's term "quinta essentia" (Wińska et al. 2019). They are bioactive molecules with antibacterial/antimicrobial, antiviral, anthelmintic, antispasmodic, cytotoxic, insecticidal, anti-inflammatory, sedative, and neuroprotective properties that have all been linked to the commercial applications of EOs as flavoring and preservatives in the food and agriculture industries, fragrance additives in the cosmetic

industry, and antibacterial/antimicrobial properties in the pharmaceutical industry. Out of the 3000 plant species known to contain EOs, only 300 have been reported as commercially viable. Terpenoid mixtures (terpenes, monoterpenes, and sesquiterpenes), phenylpropenes, flavonoids, esters, and unsaturated fats have been linked to the bioactive properties of essential oils (Sandner et al. 2020; Greta, 2020; Bahr et al. 2019; Herman et al. 2019). EOs are classified according to their extraction method (steam-distilled oils, cold-pressed/expressed oils, or solvent-extracted oils) or aroma (citrus oils, herbaceous oils, floral oils, and so on). Because extraction methods affect both the number of molecules in the EO and the stereo chemical arrangement of those extracted molecules, extraction methods have an impact on the chemical profile of the EO and how two EOs differ from one another. Because of their growing pharmacological presence, steam-distilled oils are preferred in the pharmaceutical industry (Paul et al. 2020).

Terpenes, such as eugenol, have been linked to the pharmacological properties of EOs such as thyme, and these terpenes are suggested to provide the much-needed stability of EOs during the manufacturing process of different products, as well as the EOs' selective action on specific strains (Greta, 2020). The ability of EOs to reduce the virulence of bacteria strains such as *E. coli* (O15:H7 strain) - a well-known food contaminant that causes serious gastrointestinal problems - demonstrates their antimicrobial activity. (Munketa et al. 2020) demonstrated that EOs, like antibiotics, disrupt intracellular homeostatic conditions in bacterial cells by inducing intracellular component efflux, resulting in cell inactivation and due to the rise in antibiotic resistance, which is caused by genetic changes in bacteria that allow them to adapt to their altered environment while failing to respond to common drugs that are available (Organization, 2020). Saudi Arabia, a country rich in medicinally and economically significant flora, has a number of aromatic compounds with the potential to be used in indigenous medicine systems. This study focused on Saudi Arabian aromatics with the goal of evaluating and characterizing selected EOs from the Jeddah market for their antimicrobial and antioxidant capacities. The goal was to compare the antimicrobial activity of the EOs to that of commonly used antibiotics to see if the bacteria showed the same sensitivity patterns to EOs as they did to antibiotics (Ruangpan and Tendencia, 2004).

## MATERIALS AND METHODS

### 2.1. Essential oils and bacteria strains used in this study:

The essential oils samples were collected from different markets of Jeddah, Saudi Arabia, as shown in Table 1.

**Table 1: List of essential oils used in our study**

No	Commercial oil	Plant Scientific name
1	Pumpkin seed oil	<i>Cucurbita pepo</i>
2	Rose oil	<i>Rosa</i> spp.
3	Cinnamon oil	<i>Cinnamomum verum</i>
4	Peppermint oil	<i>Mentha</i> spp.
5	Thyme oil	<i>Thymus vulgaris</i>
6	Fennel oil	<i>Foeniculum vulgare</i>
7	Violet oil	<i>Viola odorata</i>

For the bactericidal activity, different pathogenic bacteria were used. They are *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* 351218, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* 292123, *Staphylococcus aureus*, *Streptococcus pyogenes*, MRSA, *Staphylococcus epidermidis*, *Shigella sonnei*, *Streptococcus bovis* and *Streptococcus agalactiae*. Microorganisms were inoculated into solid culture and grown at 37°C for 24 hours. The 24 and 48 hr cultures were used in the determination of microbial growth inhibition of essential oils.

### 2.2. Antibiotic sensitivity assay:

The disc diffusion method was used to evaluate the antibiotic profile. About twenty-one different antibiotic disks containing known antibiotics were used for this, as shown in Table 2. The antibiotic disks were placed on nutrient agar plates containing various pathogenic bacterial strains. The Petri dishes with a diameter of 12 cm were incubated at 37°C for 24 to 48 hours. The antibacterial properties of essential oils were studied using pathogenic bacteria strains from nineteen different cultures. King Abdul-Aziz Hospital provided the pathogenic strains used in our study.

**Table 2: Antibiotic disk name and concentration used in this study**

No	Antibiotic	Abbreviation	No	Antibiotic	Abbreviation
1	Ceftazidime (30µg)	CAZ	12	Norfloxacin (10µg)	NOR
2	Imipenem (30µg)	IMI	13	Amikacin (30µg)	AK
3	Pipercillin (100µg)	PRL	14	PenicillinG (10µg)	PG
4	Ciprofloxacin (30µg)	CIP	15	Erythromycin (15µg)	E
5	Aztreonam (10µg)	ATM	16	Clindamycin (2µg)	CD
6	Tobramycin (10µg)	TN	17	Bacitracin (15µg)	BA
7	Nalidixi Acid (30µg)	NA	18	Chloramphenicol (30µg)	C
8	Nitrofurantoin (10µg)	NI	19	PolymyxinB (10µg)	PB
9	Cephalothin (30µg)	KF	20	Gentamicin (30µg)	GM
10	Ampicillin (10µg)	AP	21	Neomycin (30µg)	NE
11	Cotrimoxazole (30µg)	TS			

was transferred to new tubes and stored at 4°C in the dark for immediate analysis.

### 2.3. Antibacterial assay of different used essential oils:

The disk diffusion method was used to screen EOs for antibacterial activity, with 500 µl of the suspensions spread over plates containing approximately 25 ml of Mueller-Hinton agar, using a sterile cotton swab for uniform microbial growth on both control and test plates. Before sterilization, the EOs were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 and filtered through a 0.45 µm membrane filter. Empty sterilized disks (Whatman no. 5, 6 mm dia) were impregnated with 50 µl of the respective EOs and placed on the agar surface under aseptic conditions (Pagare et al. 2015). To prevent test samples from evaporating, all Petri dishes were sealed with sterile laboratory parafilm. The plates were left at room temperature for 30 minutes to allow the oil to diffuse before being incubated at 37°C for 18 hours. Following the incubation period, the inhibition zone was evaluated with a roller. The studies were carried out in triplicate, and the mean value was computed.

### 2.4. Determination of antioxidant activities

#### 2.4.1. Sample preparation

Stock solutions of α-tocopherol and L-ascorbic acid were prepared in hexane, ethanol and water. Exact concentrations were determined with a spectrophotometer at 294 nm using the method described in (Ruangpan and Tendencia, 2004). Extracted oil samples were kept at 4°C before being mixed with hexane (2 mL/g), ethanol (2 mL/g), or water (2 mL/g) and homogenized before being transferred to glass tubes and shaken for 1 hour at 4°C in the dark. Following a 10-minute centrifugation at 6000 g, the supernatant fraction

#### 2.4.2. Determination of total lipid-soluble antioxidant capacity (TLAC) in oils.

The total lipid-soluble antioxidant capacity of hexanic extract samples was determined. Various volumes of solutions (5-200 µL) were supplemented with 1 mL phosphomolybdenum reagent (BQCKit), agitated, and incubated at 95°C for 90 minutes. Absolute ethanol was used in the control reactions. Finally, absorbance at 695 nm (A695) was measured and TLAC was expressed as equivalents of α-tocopherol amounts. Standard curves (A695 vs µM α-tocopherol) were constructed using different amounts of α-tocopherol dissolved in ethanol. For quantitation, an average extinction coefficient of  $\epsilon = 137 \mu\text{M}^{-1} \text{cm}^{-1}$  ( $r^2 = 0.9998$ ) was used. The TLAC/gram of plant material was calculated as follows:

$$\text{TLAC } (\mu\text{mol } \alpha\text{-tocopherol/g}) = A695 \times \epsilon^{-1} \times \text{RV} \times \text{SV}^{-1} \times \text{EV} \times m^{-1}$$

where  $\epsilon^{-1}$  is the inverse of the extinction coefficient ( $137 \mu\text{M}^{-1} \text{cm}^{-1}$ ), RV is the overall reaction volume, SV is the sample volume, EV is the volume of solvent used in the plant material extraction, and m is the amount of fresh plant material extracted (in grams). The assay was performed at 37°C (TLAC37) instead of 95°C, total antioxidant capacity due to strong lipid-soluble antioxidants was obtained (Kubo et al. 1992). All measurements were done in triplicate.



	STRAIN/ ATCC																					
		CAZ	IMI	PRL	CIP	ATM	TN	AK	NA	NI	KF	AP	TS	NOR	PG	E	CD	BA	C	PB	GM	NE
1	<i>E.coli</i> 25922	2.8 ±	3.5 ±	3 ±	3.2 ±	3.3 ±	1.3 ±	2 ±	2.8 ±	2.7 ±	1.6 ±	2.6 ±	1.8 ±	3 ±	R	R	R	R	2.5 ±	1 ±	1.5 ±	
2	<i>Strept pyogens</i> 19615	2 ±	3.5 ±	3 ±	1.5 ±			0.8 ±		2 ±	2.5 ±	3 ±	1 ±	1 ±	2.5 ±	2.7 ±	2.5 ±	1.8 ±	2.5 ±		0.9 ±	0.8 ±
3	<i>K.pnuemonia</i> 700603	R	4 ±	R	1.8 ±	R	R	2.2 ±	1.5 ±	1.7 ±	R	R	R	1.4 ±	R			R	1 ±	1 ±	1 ±	1.1 ±
4	<i>E.coli</i> 35218	3 ±	3.2 ±	1.5 ±	2.5 ±	3.2 ±	1.1 ±	1.9 ±	2.5 ±	2.5 ±	1.5 ±	R	1 ±	2.5 ±	R	R	R	R	R	1 ±	1.5 ±	1.3 ±
5	<i>Prot vulgar</i> 49132	3.2 ±	2.8 ±	2.8 ±	3 ±	3.2 ±	1.5 ±	2.2 ±	2 ±	1.5 ±	2.3 ±	2.3 ±	1.9 ±	2.8 ±	1.8 ±	R	R	R	2.2 ±	1 ±	2 ±	2 ±
6	<i>Strept agalactiae</i>	3 ±	3.5 ±	3 ±	1.5 ±	1 ±				3 ±	3 ±	3 ±			3.5 ±	3 ±	2.5 ±	0.5 ±	2.5 ±			
7	<i>Staph aueros</i> 25923	1.5 ±	4 ±	3.5 ±	3 ±	R	1.6 ±	2 ±	R	2 ±	3.5 ±	4 ±	2 ±	2.5 ±	3.5 ±	3 ±	2.7 ±	1 ±	2 ±	R	2 ±	1.6 ±
8	<i>Shiglla sonna</i> 25931	3 ±	3.2 ±	3 ±	3.2 ±	3.3 ±	1.5 ±	2 ±	2.5 ±	2.2 ±	1.5 ±	2.5 ±	2.2 ±	2.5 ±	R	R	R	R	2.5 ±	1.8 ±	R	R
9	<i>Pratus mirabls</i> 14153	3.3 ±	3.5 ±	3.5 ±	3.2 ±	3.5 ±	1.5 ±	2 ±	2.5 ±	1 ±	2.8 ±	3 ±	R	3 ±	3 ±	R	R	R	2.5 ±	R	2 ±	1.4 ±
10	<i>K.oxytoca</i> 49131	3 ±	3.2 ±	2 ±	3.3 ±	3.3 ±	1.8 ±	2.5 ±	2.3 ±	1.9 ±	R	R	2.5 ±	3 ±	R	R	R	R	2.8 ±	1 ±	2 ±	1.7 ±
11	<i>Strept bovis</i> 49147	3 ±	3.5 ±	3 ±	2 ±	1.5 ±	1 ±	1.2 ±		3 ±	3 ±	3 ±		1.5 ±	3 ±	2.5 ±	2.5 ±					
12	<i>MRSA</i> 43330	1.3 ±	3.8 ±	2.3 ±	2.8 ±	R	R	1.4 ±	R	2.5 ±	2.5 ±	1.5 ±	1.8 ±	2.5 ±	1.2 ±	R	R	1.2 ±	2 ±	R	R	R
13	<i>Staph saproh</i> 15305	2.7 ±	4.2 ±	3.5 ±	2.7 ±	R	2 ±	2.3 ±	R	2.1 ±	3 ±	3.2 ±	2 ±	2.5 ±	3.5 ±	2.8 ±	2.7 ±	1.4 ±	2 ±	0.6 ±	2.1 ±	1.7 ±
14	<i>Staph epidermis</i> 1228	1.8 ±	4.5 ±	2.8 ±	2.2 ±	R	3 ±	2.5 ±	R	2.7 ±	3 ±	2.2 ±	R	2.7 ±	2 ±	3.2 ±		1 ±	2.5 ±	1 ±	2.5 ±	2 ±
15	<i>Psedo aerqinosa</i> 27853	2.7 ±	2.9 ±	2.6 ±	3 ±	2.8 ±	1.8 ±	2.2 ±	R	R	R	R	R	2.8 ±	R	R	R	R	R	1 ±	1.7 ±	1 ±
16	<i>Strept pneamoniae</i> 311432	1.5 ±	3.5 ±	3 ±	1 ±	R	R	R	R	4 ±	2.5 ±	2.5 ±	R	R	2.5 ±	3.5 ±	3.2 ±	1.5 ±	3 ±	R	R	R
17	<i>Entero cloacae</i> 23355	3.5 ±	3.6 ±	3.5 ±	3.5 ±	3.7 ±	2.5 ±	2.5 ±	2.5 ±	3.5 ±	1.7 ±	2.5 ±	2.5 ±	3.5 ±	R	R	R	R	3 ±	2 ±	2.7 ±	2.3 ±
18	<i>Entero faecalis</i> 29212	R	3.5 ±	3 ±	2 ±	R	2 ±	2 ±	R	2 ±	1.5 ±	3 ±	R	1.5 ±	2.1 ±	2.3 ±	1 ±	1 ±	1.7 ±	R	2 ±	1.7 ±
19	<i>Staph aures</i> 29213	1.8 ±	4 ±	2.7 ±	2.5 ±	R	1.7 ±	2.1 ±	R	2.5 ±	3.5 ±	2.5 ±	1.8 ±	2.5 ±	2 ±	2.7 ±	2.7 ±	1.4 ±	2.2 ±	0.7 ±	2 ±	1.8 ±

Table 3: Antibiotic sensitive test Minimum inhibitor concentration (MIC). The inhibition zone was measured in millimeters (mm) 2.5±.

### 2.4.3. Determination of total water-soluble antioxidant capacity (TWAC) in oils.

Water extract samples (5-200 L) were agitated and incubated at 95°C for 90 minutes after being supplemented with 1 mL phosphomolybdenum. In the control reactions, only pure water was used. TWAC was expressed as equivalents of L-ascorbic acid and measured at 695 nm. Standard curves (A<sub>695</sub> vs M L-ascorbic acid) were created by dissolving different amounts of L-ascorbic acid in water. An average extinction coefficient of  $\epsilon$  213  $\mu\text{M}^{-1} \text{ cm}^{-1}$  ( $r^2 = 0.9996$ ) was used for quantitation. Total water-soluble antioxidant capacity per gram of fresh plant material was obtained with the following formula:

$$\text{TWAC } (\mu\text{mol L-ascorbic acid/g}) = A_{695} \times \epsilon^{-1} \times \text{RV} \times \text{SV}^{-1} \times \text{EV} \times \text{m}^{-1}$$

The assays were performed at 37°C (TWAC<sub>37</sub>), instead of 95°C, and total antioxidant capacity due to strong water-soluble antioxidants was obtained (Kubo et al. 1992). All determinations were done in triplicate.

### 2.5. DNA isolation before and after treatment with essential oils:

Total genomic DNA was isolated using the (Azcárate-Peril and Raya, 2001) Protocol, which included mixing 200  $\mu\text{L}$  of TES buffer and 20  $\mu\text{L}$  of lysozyme (10 mg/ml) with an appropriate amount of bacterial pellets for overnight culture. The mixture was incubated in a water bath at 37°C for 20 minutes. Then, 20  $\mu\text{L}$  of proteinase K (10 mg/ml) was added to each sample, which was then incubated in a water bath at 37°C for 20 minutes. The mixture was placed in an ice bath for 5 minutes before adding 250  $\mu\text{L}$  of 4M CH<sub>3</sub>COONa. Following that, 250  $\mu\text{L}$  of chloroform: isoamyl (24:1) was added, and the mixture was stirred to mix before centrifugation at 13000 rpm/2 min. The upper zone was carefully transferred to a new clean Eppendorf tube, and 34  $\mu\text{L}$  of 1v/v isopropanol was added before being stored at -20°C overnight. The solution was centrifuged at 13000 rpm/2 min the next day, after which the liquid zone was completely discarded and the DNA was allowed to dry at room temperature before being re-suspended in 50  $\mu\text{L}$  of distilled water. Following that, 10  $\mu\text{L}$  of isolated DNA was loaded into a 0.5 percent agarose gel in 1x TBE buffer and run at 100 V for 60-90 minutes before being stained with ethidium bromide.

#### 2.5.1 Agarose gel preparation

Agarose was placed in a 1X TBE buffer and boiled in a water bath, then C21H<sub>20</sub>BrN<sub>3</sub> was added to the melted gel at 55°C. The melted gel was poured into the tray of mini-gel apparatus and a comb inserted immediately, before being removed when the gel hardened. The gel was then covered with electrophoresis buffer (1X TBE), and 10  $\mu\text{L}$  of ds DNA and 5  $\mu\text{L}$  of 1kbp DNA ladder were loaded into each well.

## RESULTS

### 3.1. Antibiotic resistance test:

The diameter of the inhibition zone was measured in millimeters (mm). The size of the growth inhibition zone is affected by the depth of the agar media in the plate, as the antimicrobial compound diffuses in three dimensions  $\pm 0.25$ , resulting in a shallow layer of agar producing a larger inhibition zone than a deeper layer of agar. The antibiotic resistance of used pathogenic bacterial strains used in this study was done as illustrated in Table 3 using the antibiotic-disk assay with different types of antibiotics to determine the antibiotic resistance patterns of studied pathogenic bacterial strains. Results show the successful isolation of antibiotic resistance from most used strains (Table 3). Results indicated a different potential for antibiotic resistance among used strains, but with imipenem antibiotic still showing active inhibition against several of bacteria strains.

### 3.2. Evaluation of the antioxidant activity of essential oils.

The determination of antioxidant properties for commercial EOs such as cinnamon (Cin), thyme (Thy), fennel (Fen) and pumpkin (Pum) used a spectrophotometric method developed by (Kubo et al. 1992) based on the formation of a blue-green phosphomolybdenum complex.

#### 3.2.1. Assessing the strength of water-soluble and lipid-soluble antioxidant capacity.

##### 3.2.1.1. The strong antioxidant capacity of TWAC and TLAC at 37°C.

The values of parameter TWAC<sup>37</sup> and TLAC<sup>37</sup> are shown in Figure 1. Data represent the means of three independent determinations  $\pm$  their respective standard deviations. Concentrations are relative to the weight of commercial oils. Strong water-soluble antioxidant capacity (TWAC<sub>37</sub>) is expressed as the equivalent of L-

ascorbic acid in micromoles of L-ascorbic acid per gram of commercial oils. Strong lipid-soluble antioxidant capacity (TLAC<sub>37</sub>) is expressed as the equivalent of  $\alpha$ -tocopherol in micromoles of  $\alpha$ -tocopherol per gram of commercial oils. These parameters represent the strength of water, ethanol and lipid-soluble antioxidant, respectively. The Cin oil has the highest strength of antioxidant capacity compare to Thy, Fen and Pum oil having lower strength, as in Figure 1.

### 3.2.1.2. Total antioxidant capacity of different solvents in TWAC and TLAC at 95°.

The results of four EOs with different solvents - water, ethanol, and hexane in TWAC<sup>95</sup> and TLAC<sup>95</sup> are compared respectively in Figure 2. Data represent the means of three independent

determinations  $\pm$  0.25 their respective standard deviations. Concentrations are relative to the weight of commercial oils. Total water-soluble antioxidant capacity (TWAC) is expressed as the equivalent of L-ascorbic acid in micromoles of L-ascorbic acid per gram of commercial oils. Total lipid-soluble antioxidant capacity (TLAC) is expressed as the equivalent of  $\alpha$ -tocopherol in micromoles of  $\alpha$ -tocopherol per gram of commercial oils. Cin and Fen commercial oils demonstrated high total antioxidant activity with ethanol and then with hexane. Ethanol was identified as the most appropriate solvent of oil antioxidant capacity. Results of Thy and Pum revealed much lower antioxidant activity compared to Cin and Fen's antioxidant capacity

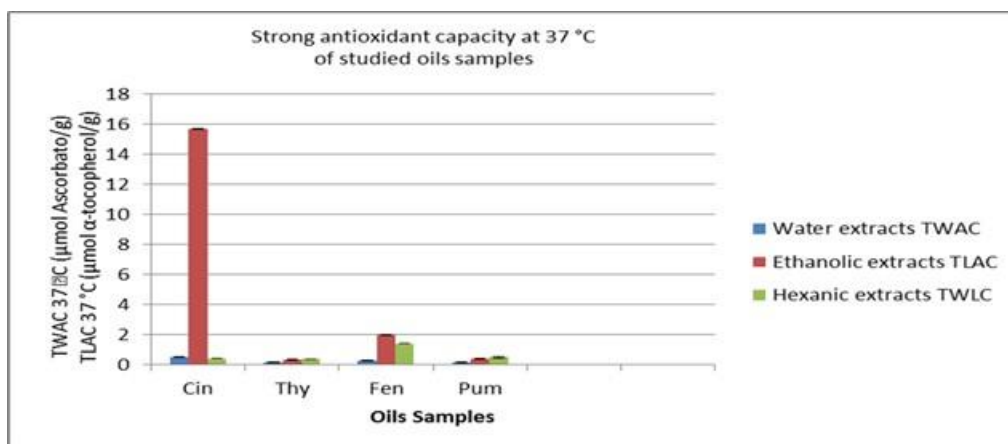


Figure 1: The comparison of strong antioxidant capacity between different commercial essential oils extracts using three different solvents (water, ethanol and hexane) using standard deviation (SD) measures.

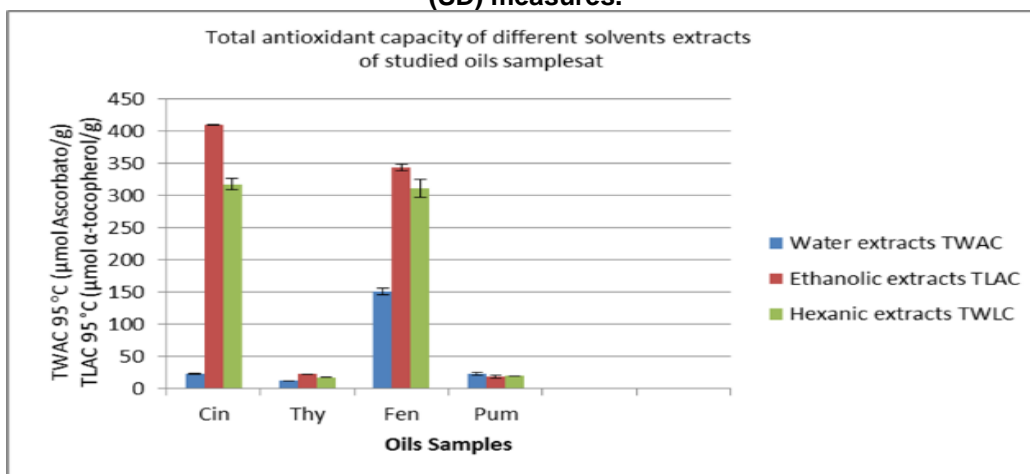


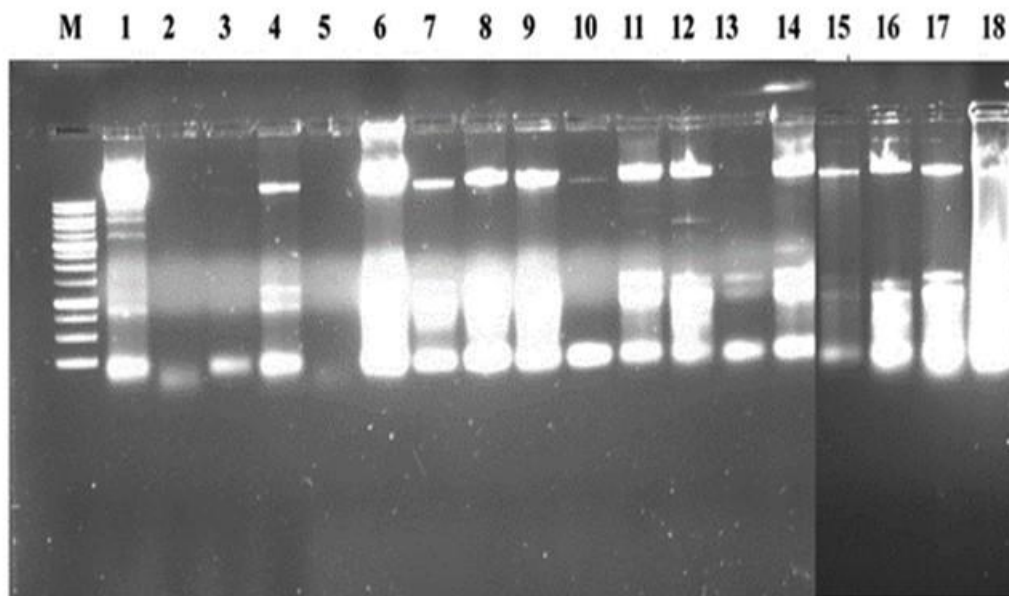
Figure 2: The total of antioxidant capacity between different commercial essential oils extracts using three different solvents (water, ethanol, and hexane) using standard deviation (SD) measures.

### 3.3. Molecular genetics techniques:

#### 3.3.1. DNA isolation from pathogenic microbes before treatment with EOs.

DNA was isolated and determined from 19

used pathogenic strains before treatment with EOs and results indicated successful DNA isolation of all used pathogenic bacterial strains after separation in a 0.7% agarose gel electrophoresis, as shown in Figure 3.



1. *Staphylococcus aureus* 292123
2. *Staphylococcus aureus*
3. *Streptococcus pyogenes*
4. *Klebsiella oxytoca*
5. *Staphylococcus saprophyticus*
6. *Klebsiella pneumoniae*
7. *Staphylococcus epidermidis*
8. *Escherichia coli* 351218
9. *Streptococcus agalactiae*

10. *Streptococcus bovis*
11. *Shigella sonnei*
12. *Proteus mirabilis*
13. *Enterococcus faecalis*
14. *Escherichia coli*
15. MRSA
16. *Proteus vulgaris*
17. *Klebsiella docare*
18. *Pseudomonas aeruginosa*

Figure 3: The DNA band profile of different pathogenic bacteria used for bactericidal activity.



### 3.3.2. DNA isolation of strains after treatment with essential oils:

The use of EOs such as cinnamon, thyme and pumpkin to treat pathogenic microbes in liquid

culture media produces results that indicate that the EOs completely degrade its DNA, as shown in Figures 4, 5 and 6.

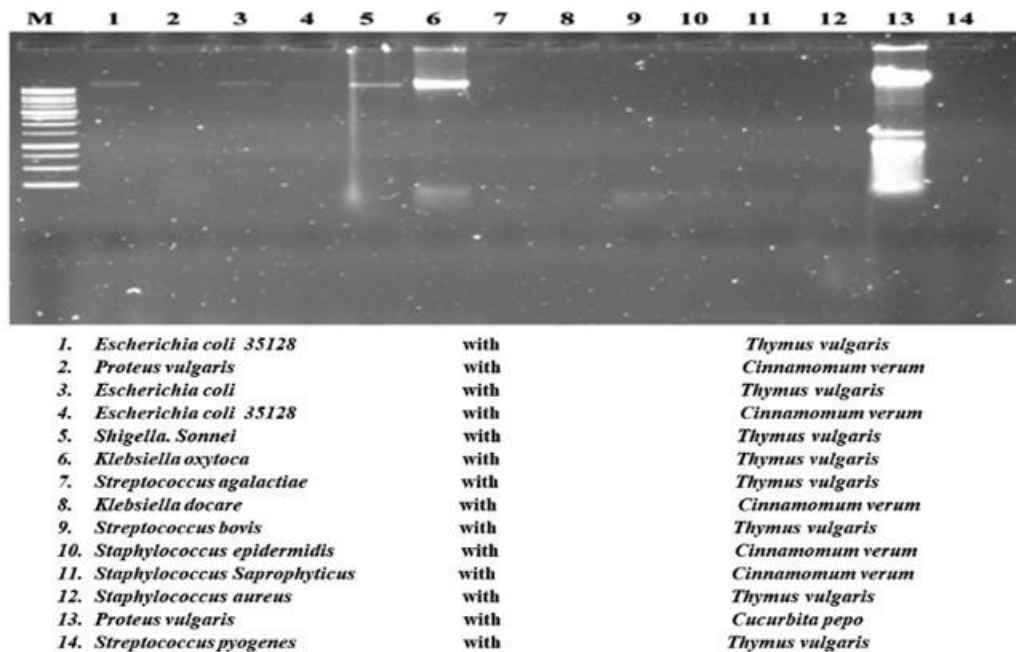


Figure 4: DNA band profile of *Enterococcus faecalis*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* 292123, *Staphylococcus aureus*, *Streptococcus. pyogenes*, *Shigella. Sonna*, *Streptococcus bovis* and *Escherichia coli*.

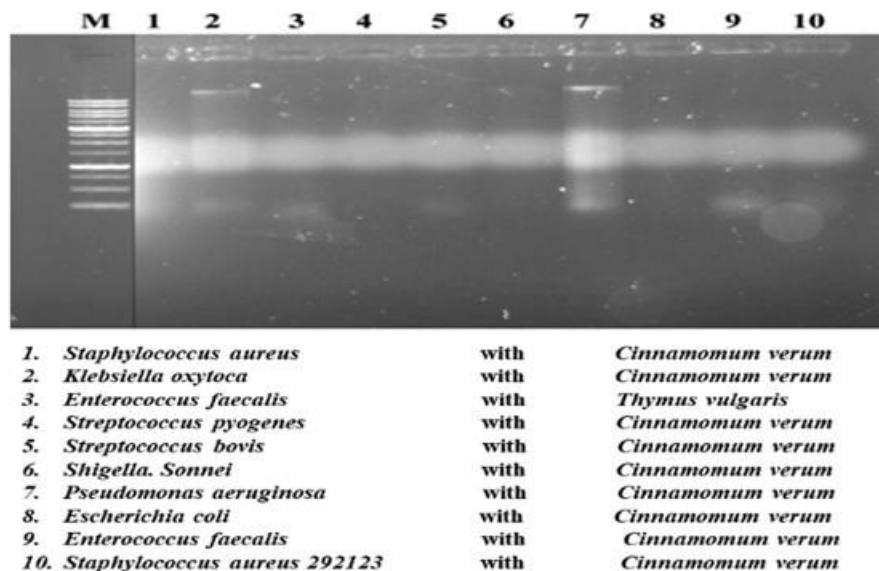
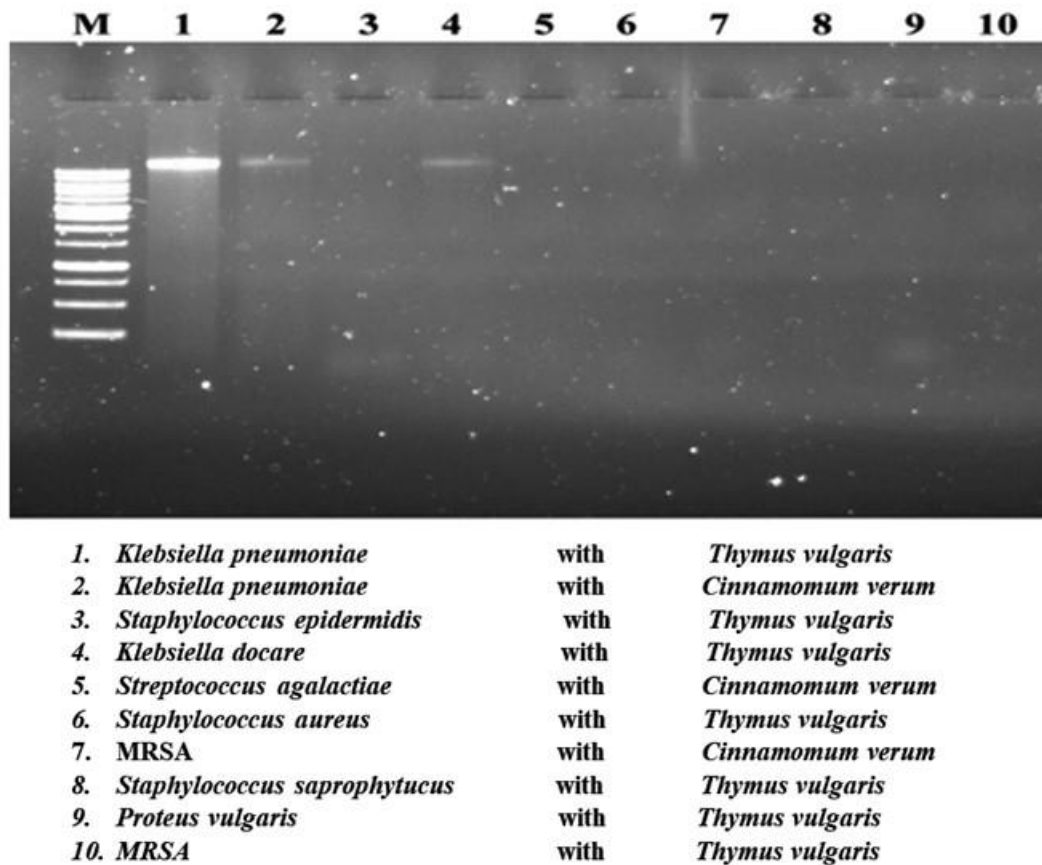


Figure 5: DNA band profile of *Klebsiella Oxytoca*, *Proteus. mirabls*, *Escherichia coli* 35128, *Proteus valgars*, *Staphylococcus Saprophyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus Epidermidis*, *Shigella. Sonnei*, *Staphylococcus agalactiae* and *Escherichia coli*.





**Figure 6:** DNA profiles band of *Klebsiella pneumoniae*, *Klebsiella docare*, *Proteus valgars*, *Staphylococcus Saprophyticus*, *Staphylococcus aureus*, MRSA, *Staphylococcus Epidermidis* and *Staphylococcus agalactiae*.

## DISCUSSION

The antimicrobial effect of essential oils derived from medicinal plants is well-documented (Valero and Salmeron, 2003). Several studies have found that some EOs have the potential to become new antibacterial agents, even against antibiotic-resistant strains (Kone et al. 2004). The disc diffusion method was used in this study to demonstrate that extracts of cinnamon, thyme, and pumpkin oils have antibacterial activity against both gram-negative and gram-positive pathogens, which is consistent with previous findings (Fan and Chen, 2001; Yuste and Fung, 2004). Other researchers who revealed Cin oil's antibacterial effect against *E. coli* found it to be effective against some pathogenic bacterial strains. However, the concentrations used in this study differed from those used in previous studies, with concentrations found in this study exhibiting much higher antibacterial activity than those

mentioned by the preceding authors. The disc diffusion method revealed the antibacterial capacity of thyme oil, corroborating the findings of many authors (Fan and Chen, 2001; Dursun et al. 2003; Nevas et al. 2004). Thyme was found to be effective against *E. coli*, *E. faecalis*, and *E. faecium*, but not against *Salmonella typhimurium*. This variation could be attributed to the use of a lower dose (30mg) rather than the standard 260 mg. There was no antibacterial activity in some extracts against the tested pathogens, and variation may be related to doses, essential oil extraction method, antibacterial study method, genetic variation of plants from which EOs were extracted, as well as plant age or environment.

The antioxidant capacity of strong water-soluble and lipid-soluble Eos, such as ascorbic acid and -tocopherol, was revealed in the determination of antioxidant properties for commercial EOs such as cinnamon (Cin), thyme (Thy), fennel (Fen), and pumpkin (Pum) using spectrophotometric

methods (Kubo et al. 1992). Extracts of commercial EOs from selected herbs, as shown in Figures 2 and 3, demonstrated varying strengths of TWAC37 and TLAC37 antioxidant capacity. The results showed that Cin. Oil had the highest antioxidant activity, particularly when first extracted with ethanol, which was consistent with several previous reports revealing cinnamon's high antioxidant activity (Kubo et al. 1992; Shan et al. 2005; Roussel et al. 2009). Extracts of Thy, Fen, and Pum oils showed lower strong antioxidant activity with the three different solvents (water, ethanol, and hexane), which contradicted previous research (Noorolahi et al. 2013). This indicates that the content of some strong antioxidant agents was lost during oil extraction procedures. As a result, aqueous extracts of EOs demonstrated lower antioxidant capacity for all commercial essential oils studied, indicating that water is unsuitable as a solvent for extracting antioxidant agents from oils. The four commercial oils were extracted with different solvents in terms of total water-soluble and lipid-soluble antioxidant capacities (water, ethanol and hexane, respectively). Commercial oils of Cin and Fen demonstrated high total antioxidant activity when first extracted with ethanol and then with hexane (Kubo et al. 1992; Shan et al. 2005; Roussel et al. 2009). While the results of the four commercial oils extracted with water are still inconsistent with previous reports on these plant materials, they show that ethanol is the best solvent for extracting oil antioxidant capacity. When compared to Cin and Fen antioxidant capacity, Thy and Pum showed significantly lower antioxidant activity (Noorolahi et al. 2013; Mohamed et al. 2007; Sacchetti et al. 2005). The results of previous studies that used molecular genetics methods and found that treating pathogenic strains with selected essential oils resulted in large inhibition zone diameters were confirmed in this study. Furthermore, the isolation of DNA from treated and untreated bacterial strains revealed that all used pathogenic bacterial strains were successfully isolated before treatment, as well as completes DNA degradation of treated strains.

## CONCLUSION

This research reveals that herbal extracts, specifically cinnamon, thyme, and pumpkin may have a similar effect to that of Antibiotic growth promoters. Our research suggests that the selected oils can be used to clean the environment of these pathogenic strains in an environmentally friendly manner, so that these oils

can be used to kill pathogenic bacteria instead of antibiotics. Some of the oils are also high in antioxidants. Cin and Fen oil have the highest antioxidant activity of the four commercial oils. This activity could be attributed to the various alkaloid and phenolic compounds found in Cin and Fen oil. More research is needed to determine the anticancer activity of cinnamon oil extract and fennel oil extract. In addition, high-performance liquid chromatography (HPLC) and gas chromatography (GC) of these oil extracts should be performed to determine the active compounds present.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEDGEMENT

The authors would like to thank the staff from the Medical Microbiology Lab at King Abdul-Aziz University Hospital for the samples. Finally, I would like to thank the staff of the Microbiology lab in Biology Dep. at King Abdul-Aziz University for their help during collecting data.

## AUTHOR CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author Faisal MB. Al-Sarraj designed the Original Article, wrote the protocol, and supervised the work. Author Ahmad M. Qumri carried out all the literature searches and wrote the first and final draft of the manuscript. All authors read and approved the final manuscript.

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