

Bactericidal And Antioxidant Effects of Domestic Essential Oils On Certain Pathogenic Bacteria.

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Abstract

Background: 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.

Methods: The In vitro evaluation of antibacterial activity and antioxidant capacity of EOs collected from Jeddah domestic market for gram-negative and gram-positive pathogenic bacteria was carried out using the disc diffusion method. Disc diffusion method was used for the assessment of bactericidal activity of local EOs. The antioxidant activity was determined using phosphomolybdenum method. Furthermore, RAPD-PCR was used to compare the effects of these EOs on DNA in both untreated and treated pathogenic bacterial strains.

Results and Conclusion: 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.

Introduction

Since antiquity, plants and their metabolites have been used for a variety of purposes in various parts of the world (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; Munketa 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 essential" (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 stereochemical 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 several 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 name	Plant Scientific name
1	Pumpkin seed oil	<i>Cucurbita pepo</i>
2	Rose oil	Rosa spp.
3	Cinnamon oil	<i>Cinnamomum verum</i>
4	Peppermint oil	Mentha 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 a 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	Piperacillin (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			

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 m membrane filter. Empty sterilized disks (Whatman no. 5, 6 mm dia) were impregnated with 50 L 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 10-minute centrifugation at 6000 g, for immediate study, the supernatant fraction was moved to new tubes and deposited at 4°C in the dark.

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

Hexanoic extract samples were tested for total lipid-soluble antioxidant ability. 1 mL phosphomolybdenum reagent (BQCKit) was added to various amounts of solutions (5-200 L), agitated, and incubated at 95°C for 90 minutes. In the control reactions, absolute ethanol was used. Finally, absorbance at 695 nm (A695) was determined, and TLAC was calculated as α -tocopherol equivalents. Different concentrations of α -tocopherol dissolved in ethanol were used to create standard curves (A695 vs M α -tocopherol). An average extinction coefficient of ϵ 137 $\mu\text{M}^{-1} \text{cm}^{-1}$ ($r^2 = 0.9998$) was used for quantification. The following formula was used to quantify the TLAC/gram of plant material:

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

where RV is the overall reaction rate, 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 removed, and ϵ^{-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). Complete antioxidant potential was obtained due to high lipid-soluble antioxidants when the assay was conducted at 37°C (TLAC37) instead of 95°C (Kubo et al. 1992). All tests were made three times.

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

After being supplied with 1 mL phosphomolybdenum, water extract samples (5-200 L) were agitated and incubated at 95°C for 90 minutes. Only distilled water was used in the control reactions. TWAC was calculated at 695 nm and expressed as L-ascorbic acid equivalents. By dissolving different quantities of L-ascorbic acid in the water, standard curves (A695 vs M L-ascorbic acid) were formed. For quantitation, an average extinction coefficient of ϵ 213 $\mu\text{M}^{-1} \text{cm}^{-1}$ ($r^2 = 0.9996$) was used. The following formula was used to calculate the total water-soluble antioxidant potential per gram of fresh plant material:

$$TWAC (\mu - mol L - ascorbic/g) = A695 \times \epsilon - 1 \times RV \times SV - 1 \times EV \times m - 1$$

The assays were carried out at 37°C (TWAC 37) rather than 95°C, and overall antioxidant potential due to high water-soluble antioxidants were determined (Kubo et al. 1992). Each determination was made in triplicate.

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

Total genomic DNA was isolated using (Azcarate-Peril and Raya 2001) Protocol, which included mixing 200 μl of TES buffer and 20 μl of lysozyme (10 mg/ml) with an appropriate number of bacterial pellets for overnight culture. The mixture was incubated in a water bath at 37°C for 20 minutes. Then, 20 μl 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 μl of 4M CH₃COONa. Following that, 250 μl 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 μl 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 μl of distilled water. Following that, 10 μl 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 C21H20BrN3 was added to the melted gel at 55°C. The melted gel was poured into the tray of mini-gel apparatus and a comb was inserted immediately, before being removed when the gel hardened. The gel was then covered with electrophoresis buffer (1X TBE), and 10 μl of ds DNA and 5 μl of 1kbp DNA ladder were loaded into each well.

2.6. Statistical analysis:

All data analyzed by SPSS v.22. One-way ANOVA was used for significant differences between groups. A *P* value < 0.05 was accepted as statistically significant.

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 antibiotics still showing active inhibition against several bacteria strains.

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

	STRAIN/ ATCC	Antibiotics Inhibition (mm)																			
		CAZ	IMI	PRL	CIP	ATM	TN	AK	NA	NI	KF	AP	TS	NOR	PG	E	CD	BA	C	PB	GM
1	<i>E.coli 25922</i>	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 19615</i>	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
3	<i>K.pnuemonia 700603</i>	R	4	R	1.8	R	R	2.2	1.5	1.7	R	R	R	1.4	R			R	1	1	1
4	<i>E.col i 35218</i>	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
5	<i>Prot vulgar 49132</i>	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
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 25923</i>	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
8	<i>Shiglla sonna 25931</i>	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
9	<i>Pratus mirabls 14153</i>	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
10	<i>K.oxytoca 49131</i>	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
11	<i>Strept bovis 49147</i>	3	3.5	3	2	1.5	1	1.2		3	3	3		1.5	3	2.5	2.5				
12	<i>MRSA 43330</i>	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
13	<i>Staph saproh 15305</i>	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
14	<i>Staph epidermis 1228</i>	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
15	<i>Pseodo aerginosa 27853</i>	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	1.5
16	<i>Strept pneamoniae 311432</i>	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
17	<i>Entero cloacae 23355</i>	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	2.5
18	<i>Entero faecalis 29212</i>	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
19	<i>Staph aures 29213</i>	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

3.2. Antibacterial assay of different used essential oils

The inhibition zone measured indicated that some of these essential oils have antibacterial activity against selected bacterial strains as shown in (Table 4). The results also showed that most pathogenic bacteria used in the study were affected by some of the used essential oils varying degrees, our results showed that essential oils of most cultivars exhibited influence on clinical isolates; however, the strongest inhibitory effects were recorded for Cinnamon, Fennel, and Violet oil. The largest inhibition zones were recorded for cinnamon oil with *Shigella sonna* (5.5 cm in diameter).

Table 4: Inhibition zone diameter produced by used Essential oils. The inhibition zone was measured in millimeters (cm) ±.

3.3. 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 (Kubo et al. 1992) developed a technique based on the creation of a blue-green phosphomolybdenum complex.

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

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

The values of parameter TWAC³⁷ and TLAC³⁷ are shown in Figure 1. Three independent determinations ± their respective standard deviations are determined by the data. Compared to the weight of commercial oil, concentrations are quantitative. The equivalent of L-ascorbic acid in micromoles of L-ascorbic acid per gram of commercial oils is expressed as strong water-soluble antioxidant potential (TWAC³⁷). The equivalent of -tocopherol in micromoles of -tocopherol per

Essential Oils / CM									gram of commercial oils is expressed as strong lipid-soluble antioxidant potential (TLAC37). These parameters represent the strength of water, ethanol, and lipid-soluble antioxidant, respectively. Cin oil has the highest strength of antioxidant capacity compared to Thy, Fen, and Pum oil having lower strength, as in (Figure 1).
N.S.	ATCC/strain	Pumpkin seed oil	Rose oil	Cinnamon oil	Peppermint oil	Thyme oil	Fennel oil	Violet oil	
1	<i>Enterococcus. faecalis</i>	-	-	-	-	-	-	1	3.3.1.2. Total antioxidant capacity of different solvents in TWAC and TLAC at 95°. The results of four EOs with different
2	<i>Strept. pneumoniaea</i>	-	-	-	-	1	1.5	3	
3	<i>Klep. oxytoca</i>	-	0.5	-	-	-	-	3.5	
4	<i>Protus. mirablis</i>	-	-	-	4.5	-	-	-	
5	<i>PS. areginosa</i>	-	-	-	-	-	-	-	
6	<i>E. coli 351218</i>	-	-	-	-	-	-	2.5	
7	<i>Klep. pneumoniaea</i>	-	-	-	-	-	0.5	1.5	
8	<i>E. coli 25922</i>	-	-	-	-	-	-	3	
9	<i>Pro. valgars</i>	-	-	-	-	-	1.4	4.5	
10	<i>Staph. saprophytucus</i>	-	-	1	1	0.5	1.2	2.5	
11	<i>Staph. Aurus 292123</i>	1.3	1	0.6	1	0.5	1	3.5	
12	<i>Entero. docar</i>	-	-	-	-	-	0.5	2	
13	<i>Staph. aurus</i>	0.9	-	0.7	0.5	1	0.5	2.2	
14	<i>Strept. pyogenus</i>	-	1.6	1.3	-	-	-	2	
15	<i>MRSA</i>	1.2	-	-	1	-	-	3.5	
16	<i>Staph. epidermidis</i>	-	-	-	1.5	-	0.5	2.4	
17	<i>Shigella. sonna</i>	1	-	-	-	-	0.5	3.5	
18	<i>Strept. bovis</i>	-	-	-	-	2	2	4	
19	<i>Strept. agalactiae</i>	-	-	-	-	1.3	0.7	3	

solvents - water, ethanol, and hexane in TWAC⁹⁵ and TLAC⁹⁵ are compared respectively in (Figure 2). The graph shows three standard variables with independent determinations ± 0.25 . Compared to the weight of commercial oil, concentrations are relative. In micromoles L-ascorbic acid per gram of commercial Oils, the full water-solvable antioxidant potential (TWAC) is indicated to be the inverse of L-ascorbic Acid. Total lipid-soluble antioxidant capacity (TLAC) is expressed as the equivalent of α -tocopherol in micromoles of α -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.

3.4. Molecular genetics techniques:

3.4.1. 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).

3.4.2. 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).

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 1 and 2, 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 complete DNA degradation of treated strains.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Raw data were generated at Biological Department in King Abdulaziz University. Derived data supporting the findings of this study are available from the corresponding author on request. The data that support the findings of this study are available on request from the corresponding author.

Competing interests

The authors declare that they have no conflict of interest and non-financial/financial interests.

Funding

Not applicable.

Authors' 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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References

1. Azcárate-Peril, M. A., & Raya, R. R. (2001). Methods for plasmid and genomic DNA isolation from Lactobacilli. In *Food microbiology protocols* (pp. 135-139): Springer. <https://doi.org/10.1385/1-59259-029-2:135>
2. Bahr, T. A., Rodriguez, D., Beaumont, C., & Allred, K. (2019). The effects of various essential oils on epilepsy and acute seizure: a systematic review. *Evidence-Based Complementary and Alternative Medicine, 2019*. <https://doi.org/10.1155/2019/6216745>
3. Dursun, N., Liman, N., Özyazgan, İ., Güneş, I., & Saraymen, R. (2003). Role of thymus oil in burn wound healing. *The Journal of burn care & rehabilitation, 24*(6), 395-399. <https://doi.org/10.1097/01.BCR.0000095513.67541.0F>
4. Fan, M., & Chen, J. (2001). Studies on antimicrobial activity of extracts from thyme. *Wei sheng wu xue bao= Acta microbiologica Sinica, 41*(4), 499-504.
5. Greta, F. (2020). Plant complexity and cosmetic innovation. *Iscience, 101358*. <https://doi.org/10.1016/j.isci.2020.101358>

6. Heghes, S. C., Filip, L., Vostinaru, O., Mogosan, C., Miere, D., Iuga, C. A., & Moldovan, M. (2020). Essential Oil-Bearing Plants from Balkan Peninsula: Promising Sources for New Drug Candidates for the Prevention and Treatment of Diabetes Mellitus and Dyslipidemia. *Frontiers in Pharmacology*, *11*, 989. <https://doi.org/10.3389/fphar.2020.00989>
7. Herman, R. A., Ayepa, E., Shittu, S., Fometu, S. S., & Wang, J. (2019). Essential Oils and Their Applications-A Mini Review. *Advances in Nutrition & Food Science*, *4*(4), 1-13.
8. Kone, W., Atindehou, K. K., Terreaux, C., Hostettmann, K., Traore, D., & Dosso, M. (2004). Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of ethnopharmacology*, *93*(1), 43-49. <https://doi.org/10.1016/j.jep.2004.03.006>
9. Kubo, I., Muroi, H., & Himejima, M. (1992). Antimicrobial activity of green tea flavor components and their combination effects. *Journal of Agricultural and Food Chemistry*, *40*(2), 245-248. <https://doi.org/10.1021/jf00014a015>
10. Mohamed, R., Pineda, M., & Aguilar, M. (2007). Antioxidant capacity of extracts from wild and crop plants of the Mediterranean region. *Journal of food science*, *72*(1), S059-S063. <https://doi.org/10.1111/j.1750-3841.2006.00207.x>
11. Munekata, P. E., Pateiro, M., Rodríguez-Lázaro, D., Domínguez, R., Zhong, J., & Lorenzo, J. M. (2020). The Role of Essential Oils against Pathogenic *Escherichia coli* in Food Products. *Microorganisms*, *8*(6), 924. <https://doi.org/10.3390/microorganisms8060924>
12. Nevas, M., Korhonen, A.-R., Lindström, M., Turkki, P., & Korkeala, H. (2004). Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. *Journal of food protection*, *67*(1), 199-202. <https://doi.org/10.4315/0362-028X-67.1.199>
13. Noorolahi, Z., Sahari, M., Barzegar, M., Doraki, N., & Naghdi Badi, H. (2013). Evaluation antioxidant and antimicrobial effects of cinnamon essential oil and echinacea extract in Kolompe. *Journal of Medicinal Plants*, *1*(45), 14-28. <http://jmp.ir/article-1-106-en.html>
14. Organization, W. H. (2020). Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020. <http://apps.who.int/iris/bitstream/handle/10665/259744/9789241513449-eng.pdf>
15. Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., & Bansal, Y. (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy*, *9*(3), 293-304.
16. Paul, S., El Bethel Lalthavel Hmar, J. H., & Zothantluanga, H. K. S. (2020). Essential oils: a review on their salient biological activities and major delivery strategies. *microcirculation*, *5*, 9. <https://doi.org/10.33493/scivis.20.02.01>
17. Roussel, A.-M., Hininger, I., Benaraba, R., Ziegenfuss, T. N., & Anderson, R. A. (2009). Antioxidant effects of a cinnamon extract in people with impaired fasting glucose that are overweight or obese. *Journal of the American College of Nutrition*, *28*(1), 16-21. <https://doi.org/10.1080/07315724.2009.10719756>
18. Ruangpan, L., & Tendencia, E. A. (2004). *Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment*. Aquaculture Department, Southeast Asian Fisheries Development Center. <http://hdl.handle.net/10862/1615>
19. Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food chemistry*, *91*(4), 621-632. <https://doi.org/10.1016/j.foodchem.2004.06.031>
20. Sandner, G., Heckmann, M., & Weghuber, J. (2020). Immunomodulatory activities of selected essential oils. *Biomolecules*, *10*(8), 1139. <https://doi.org/10.3390/biom10081139>
21. Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, *53*(20), 7749-7759. <https://doi.org/10.1021/jf051513y>
22. Thirumurugan, D., Cholarajan, A., Raja, S., & Vijayakumar, R. (2018). An introductory chapter: secondary metabolites. *Second metab—sources Appl*, 1-21. <http://dx.doi.org/10.5772/intechopen.79766>
23. Valero, M., & Salmeron, M. (2003). Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. *International journal of food microbiology*, *85*(1-2), 73-81. [https://doi.org/10.1016/S0168-1605\(02\)00484-1](https://doi.org/10.1016/S0168-1605(02)00484-1)
24. Wińska, K., Mączka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A., & Szumny, A. (2019). Essential oils as antimicrobial agents—Myth or real alternative? *Molecules*, *24*(11), 2130. <https://doi.org/10.3390/molecules24112130>
25. Yuste, J., & Fung, D. (2004). Inactivation of *Salmonella typhimurium* and *Escherichia coli* O157: H7 in apple juice by a combination of nisin and cinnamon. *Journal of food protection*, *67*(2), 371-377. <https://doi.org/10.4315/0362-028X-67.2.371>

Figures

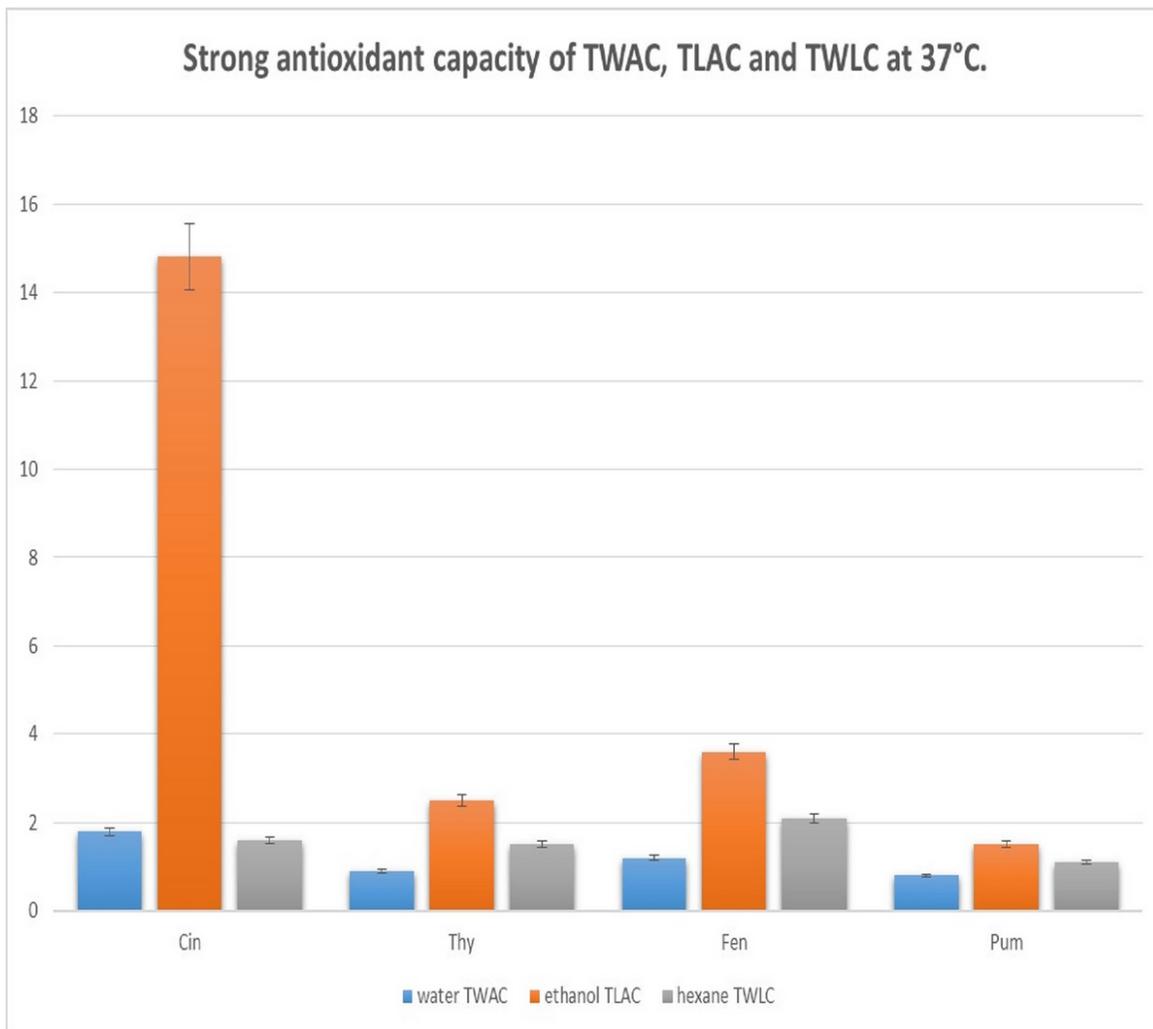


Figure 1

The comparison of strong antioxidant capacity between different commercial essential oil extracts using three different solvents (water, ethanol, and hexane) using standard deviation (SD) measures. A P value < 0.05 was accepted as statistically significant.

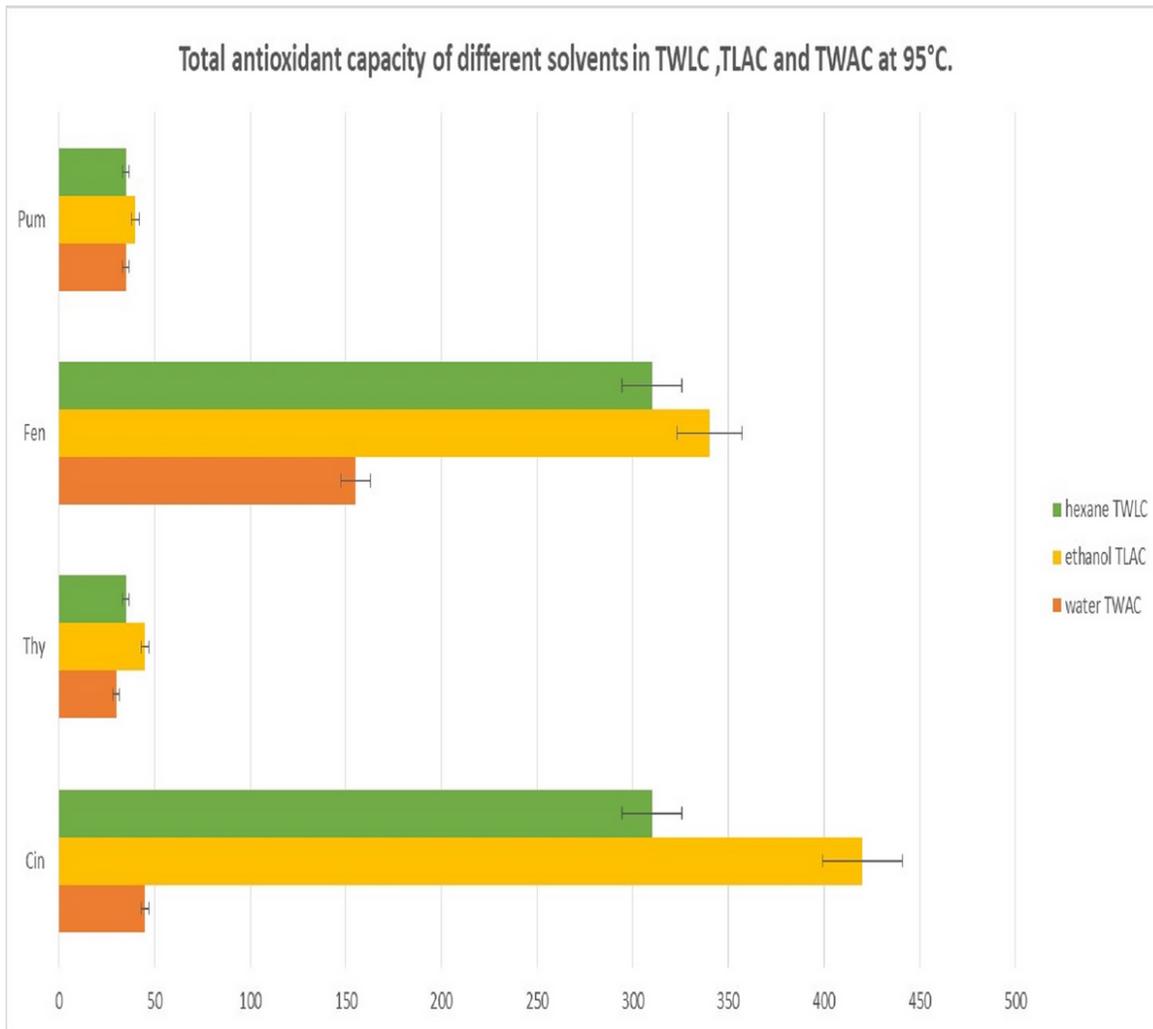


Figure 2
 The total antioxidant capacity between different commercial essential oils extracts using three different solvents (water, ethanol, and hexane) using standard deviation (SD) measures. A P value < 0.05 was accepted as statistically significant.

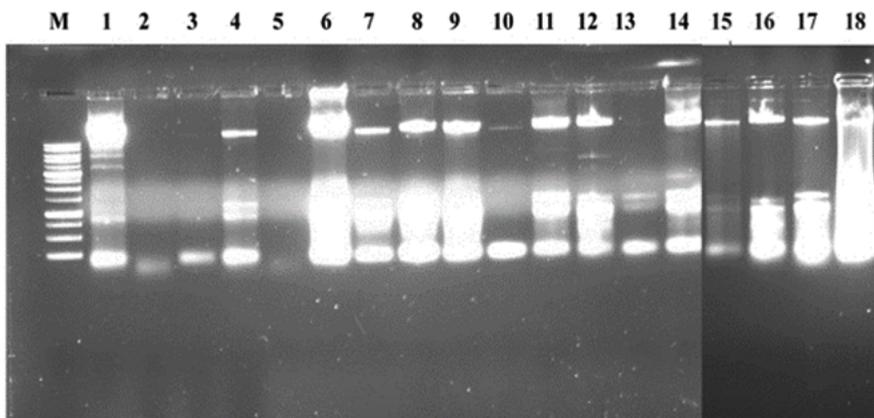


Figure 3
 The DNA band profile of different pathogenic bacteria used for bactericidal activity. M: 100 bp DNA Ladder. Lane 1-18: *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli* 351218, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* 292123, *Staphylococcus aureus*, *Streptococcus pyogenes*, MRSA, *Staphylococcus epidermidis*, *Shigella. sonnei*, *Streptococcus bovis* and *Streptococcus agalactiae*.

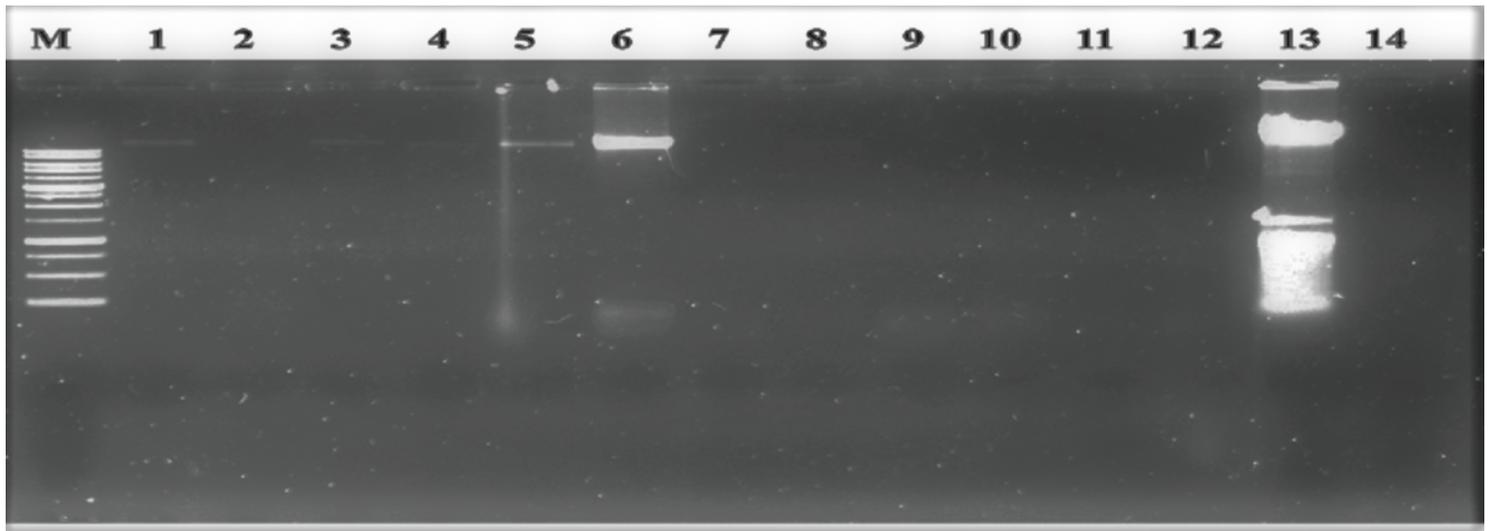


Figure 4
DNA band profile. M: 100 bp DNA Ladder. Lane 1-14: *Klebsiella Oxytoca*, *Protus. mirabls*, *Escherichia coli 35128*, *Proteos valgars*, *Staphylococcus Saprophyticus*, *Staphylococcus aureus*, *Streptococcus pyogenus*, *Staphylococcus Epidermidis*, *Shigella. Sonna*, *Staphylococcus agalactiae*, and *Escherichia coli*.