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MICROBIOLOGY

Determination of chemical compositions and antibacterial effects of selected essential oils against human pathogenic strains

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Abstract: Increasing the rates of drug resistant bacteria, having adverse effects and also high costs of antibiotics lead to essential oils (EOs) with antibacterial properties have gained importance. The present study was predicted to evaluate antibacterial activity of cinnamon, lavender, tea tree, lemon, coconut, oregano, mint, laurel and eucalyptus EOs alone and in combination. Chemical components of effective EOs were examined through gas chromatography/mass spectrometry (GC/MS). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were used to identify antibacterial effects of EOs against bacterial strains. The Fractional Inhibitory Concentration index (FICI) of the binary combinations of EOs was determined by checkerboard method. Carvacrol, linalool, linalyl acetate, 1,8-cineole, cinnamaldehyde, terpinen-4-ol and p-cymene were found main components of EOs. Oregano, cinnamon and tea tree EOs exhibited the strongest antibacterial activity with the MIC range between 0.03125-1.00% (v/v). Tea tree/lavender and cinnamon/lavender mixtures showed a synergistic effect against *Streptococcus pyogenes* and *Streptococcus agalactiae*. Oregano with tea tree and laurel exhibited a synergistic effect against *Staphylococcus aureus*. Oregano showed a synergistic effect when combined with cinnamon, lavender and tea tree against *S.agalactiae*. Our findings indicated that EOs either alone or in combination against pathogens should be preferred as potential antibacterial agents.

Key words: Antibacterial efficiency, essential oils, Fractional Inhibitory Concentration index, gas chromatography/mass spectrometry, Human pathogens, Minimum Inhibitory Concentration.

INTRODUCTION

S. pyogenes asymptomatically colonizes skin and upper respiratory tract of individuals and may lead to outbreaks and causes wide variety of diseases including toxin-mediated diseases [like scarlet fever and streptococcal toxic shock syndrome (STSS)], deep (e.g. bacteremia, myositis, cellulitis, necrotizing fasciitis, puerperal sepsis, pericarditis, meningitis and pneumonia) and superficial infections (e.g. pharyngotonsillitis, impetigo, erysipelas and vaginitis) (Cunningham 2000, Efstratiou 2000). Penicillin is the first preferred antibiotic in the treatment of streptococcal tonsillopharyngitis, and penicillin resistance has not been detected yet. Beta lactam antibiotics (such as macrolides and lincosamides) are used in the case of penicillin allergy or penicillin intolerant (Choby 2009, Brook 2001). However, it has been reported that antibiotic resistance to *S.pyogenes* strains are rapidly increasing all over the world (Richter et al. 2005).

S. aureus is a commensal bacterium that can colonize at the skin, mucosa and nasal vestibule in humans. Moreover, they also cause many infections (like osteomyelitis, nosocomial

and mild superficial skin infections, implant-associated heart valve, endocarditis, severe sepsis and bacteremia) with virulence factors such as toxins and enzymes. Mortality may occurrates will be higher when patients are not treated effectively (Jenul & Horswill 2018, Lowy 1998, Sutcliffe et al. 2019, Mccaig et al. 2006). The treatment of *S.aureus* infections, including soft tissue infections, are becoming more difficult due to increasing resistance to beta-lactam antibiotics among species. Methicillin-resistant *S.aureus* (MRSA) strains can develop not only nozocomial, but also in outpatients (Velasco et al. 2005).

S. agalactiae (group B *streptococcus*) is a commensal bacterium located at the gastrointestinal and genitourinary systems of up to 30% of healthy adults without showing clinical symptoms. On the other hand, this bacteria may colonized during through the passage of the birth canal in newborns which can cause severe neonatal disease like sepsis, meningitis and pneumonia (Nuccitelli et al. 2015). Current applications of Intrapartum Antibiotic Prophylaxis (IAP) are included penicillin, ampicillin and beta lactam antibiotics against *S.agalactiae*. Cefazolin, clindamycin, erythromycin and vancomycin are used as an alternative drugs to penicillin. Antibiotic resistance has been observed since the beginning of the antibiotic age. Increasing the drug resistance and invasive bacteria, and also decreasing in the development and approval of new drugs are threaten human health worldwide (Biasi-Garbin et al. 2015, CDC 2010).

Highly toxic and expensive drugs are used due to increasing resistance to empirical antibiotics (Marasini et al. 2015). In the 21st century, multidrug-resistant bacteria poses a serious threat to health due to increasing prevalence of them. The report was published by the World Health Organization in 2017, it was stated that new antibiotics should be discovered urgently (Martelli & Giacomini 2018, WHO 2017). Although the pharmaceutical industry has produced many new antibiotics in the last decade, microbial resistance to these drugs has increased gradually (Nascimento et al. 2000).

Plants including nature bioactive compounds are preferred to treat bacterial infection as alternative antibacterial agents to antibiotics (Rossiter et al. 2017). Having a great biodiversity (250,000 to 500,000 species) of plants are bioactive phytochemical molecules, in contrast to the limited, non-renewable capacity of current antibiotics, and they can be continuously renewed (Abdallah 2011). Plant-based products have been shown to be effective against different bacterial pathogens compared to antibiotics having serious side effects (Chandra et al. 2017).

EOs or aromatic plant extracts are volatile and fragrant substances, exhibit antibacterial properties and are synthesized by the organs of the plants (flower, root, leaf, stem, etc.) (Balz 1999, Bakkali et al. 2008). To demand for EOs is increasing with the increasing tendency of consumers to the natural treatment. While global market demand was 61.8 kilotons in 2014; it has increased to 226.9 kilotons in 2018 and still continues to rise (Essential Oils Market Size, Share & Trends Analysis Report by Application 2019). The antibacterial effects of many EOs have been shown in studies. EOs containing natural compounds are used to product antibacterial drugs, and this is promising for the treatment of bacterial diseases (Jalal et al. 2015, Marwa et al. 2017). It has been reported that EOs (such as thyme, oregano, mint, cinnamon, cumin, salvia, clove, eucalyptus, bacillus, mandarin, oregano, peppermint and tea tree) showed strong antimicrobial properties (Mourey & Canillac 2002, Solórzano-Santos & Miranda-Novales 2012, Burt 2004, Goni et al. 2009, Turgis et al. 2012,

Gutierrez et al. 2008). Eugenol, carvacrol, thymol, hydrocarbons and oxygenated terpenoids (e.g. alcohols and phenolic terpenes) are the major and minor components of EOs, and formed their antimicrobial properties (Ma et al. 2019, Koroch et al. 2007, Delaquis et al. 2002).

Studies have shown that combinations of EOs or mixtures of purified main constituents are more effective on target bacteria (Shi et al. 2017). Some studies have been reported that combining different EOs were lead to exhibit better antibacterial activity than applied alone as the same concentrations (Gutierrez et al. 2008, Al-Bayati 2008, Clemente et al. 2016). Antagonistic, additive or synergistic effects have been observed between components. It has been reported that very few components showed synergistic activity, but exhibited antagonistic and additive effects (Davidson & Parish 1989, Gill et al. 2002).

The purpose of present study was to evaluate antibacterial activity of 9 EOs (cinnamon, lavender, tea tree, lemon, coconut, oregano, mint, laurel and eucalyptus) against *S .pyogenes*, *S. aureus* and *S. agalactiae in vitro* using agar disc diffusion method. MIC and MBC of effective EOs were determined. Furthermore, the FICI of the binary combinations of EOs against tested human pathogens were performed to detect synergic, additive, no interactive or antagonistic effects using the checkerboard assay.

MATERIALS AND METHODS

Bacterial strains and preparation of cultures

S. pyogenes ATCC 19615, *S.aureus* ATCC 25923 and *S. agalactiae* ATCC 12386 were used in this study and obtained from the American Type Culture Collection (USA). The bacterial cultures were maintained in Tryptic Soy Broth (TSB, Sigma®) containing 20% (v/v) glycerol at -80°C. Stock cultures were inoculated onto Trypticase Soy Agar (TSA, Sigma®) and incubated at 37°C for 24h. After this initial growth cycle, they were subcultured into TSB and grown under the same conditions. In antimicrobial experiments, the bacterial cultures were adjusted to a density of 0.5 McFarland Standard (10^8 CFU/mL) with a sterile 9% aqueous solution of NaCl (Mello et al. 2014).

Preparation of EOs

Cinnamon (*Cinnamomum verum*), lavender (*Lavandula officinalis*), tea tree (*Melaleuca alternifolia*), lemon (*Citrus lemon*), coconut (*Cocos nucifera*), oregano (*Origanum vulgare*), eucalyptus (*Eucalyptus globulus*), mint (*Mentha piperita*) and laurel (*Laurus nobilis*) EOs purified by steam distillation method and were obtained from commercial company in Turkey and stored at 4 °C in dark prior to use. Each EO containig 2.5% Tween 20 sterilized via 0,2 µm syringe filter.

Chemical compositons of EOs by GC-MS

Major and minor chemical components of these EOs were analysed by GC-MS using a Agilent 7697A GC/MSD (Agilent Tehnologies, Santa Clara, CA, USA) system equipped with an Agilent DB-1 MS capillary column (30 m × 320 µm × 0.25 µm). The GC oven was programmed at 50 °C for 1 min, then at 2 °C per minute to 120 °C for 1 min and finally at 4° C per minute to 280 °C for 13 min. Helium (He) was used as carrier gas at a flow rate of 1.8 ml/min. The injector and detector temperature were 250°C and 325°C, respectively. The split ratio was 1:40. The injection volume was 3 µl. The mass spectra (MS) ionization energy was 70 Ev. The various compounds of EO were determined by comparing retention indexes (RI) and recorded mass spectra with the data of standard samples and Wiley library was also consulted (Kivrak et al. 2009).

Antimicrobial assays

Agar disc diffusion test

Antimicrobial activity of 9 EOs was investigated against 3 bacterial strains using the disc diffusion method as described Clinical & Laboratory Standards Institute (CLSI 2012). The bacterial suspensions were inoculated the entire surface of Muller Hinton Agar (MHA, Sigma®) plates. 10 µl of each EO was impregnated on a steril 6mm diameter blank paper disc and aseptically placed onto the surface of the inoculated plates and incubated for 10 minutes at room temperature. Then, all plates were incubated under 5% CO_2 at 37 °C for 24h to avoid evaporation. Vancomycin (30 µg/disc, Oxoid) was used as a positive control; a blank disc (Oxoid) impregnated with sterile distilled water was used as a negative control for bacterial inhibition. After incubation, the diameter of inhibition zones were measured in mm. Each experiment was done in triplicate.

Determination of MIC and MBC

EOs with a large inhibition zones were chosen to examine for their antimicrobial activity against *S.pyogenes* ATCC 19615, *S.aureus* ATCC 25923 and *S.agalactiae* ATCC 12386. The MIC was estimated by microdilution method in 96 well plates according to the National Committee for Clinical Laboratory Standards (NCCLS) with modifications (Wayne 2012, Alizadeh et al. 2017). Firstly, each EO containing 2.5% Tween 20 was serially diluted in Muller Hinton Broth (MHB) with concentrations ranging from 0.03125% to 32% (v/v). The growth control (negative control) consisted of growing the microorganisms in TSB culture medium with 2.5% Tween 20 was put into 12th well. Then 20 µl of each bacterial suspension (containing 10^8 CFU/mL of bacteria) added to each well. After incubation at 37 °C for 24h, MIC was defined as the lowest concentration of EO at which no visible growth (no white pellet) of pathogen compared with control.

To determine MBC values, 10 µl of inoculum was taken aseptic conditions from negative wells that showing absence of visible turbidity and transferred onto TSA. After incubation at 37 °C for 24h, the lowest EO concentration in which tested microorganism eliminated, was declarated as MBC (Bouyahya et al. 2017).

To determine antibacterial effect of EOs, the MBC/MIC ratio was <4, the EO was determined as a bactericidal and when the ratio was >4, it was considered as a bacteriostatic (Levison 2004).

Determination of fractional inhibitory concentration (FIC) by the checkerboard method

The evaluation antibacterial effects of binary combinations of EOs against three bacterial strains (*S. pyogenes* ATCC19615, *S.aureus* ATCC 25923 and *S. agalactiae* 12386) using the checkerboard method described by Moody (2003) and Gutierrez et al. (2008) with some modifications.

Sterile 96-well microtitre plates were used and two-fold serial dilutions [from 32% to 0.03125 (v/v)] of EOs were prepared. First EO was dispensed horizontally, second EO was introduced vertically into the plates according to the MIC values. The final volume in each well was 200 µl (90 µl EO_1+90 µl EO_2+20 µl bacterial suspension). The growth control was put into the 12th-H well. The plates were incubated at 37 °C for 18-24h. The interaction between two EO combinations was obtained by FICI values using the following formula:

$$FIC_1 = MIC_1 \text{ combined}/MIC_1 \text{ alone}$$

$$FIC_2 = MIC_2 \text{ combined}/MIC_2 \text{ alone}$$

$$FICI = FIC_1 + FIC_2$$

The analyse results were interpreted as synergy (FICI≤0.5), addition (0.5<FIC ≤1),

indifference (1<FIC≤4) or antagonism (FIC>4) (Wendakoon & Sakaguchi 1995, Schelz et al. 2006).

Statistical analyses

Statistical calculations were performed by one-way ANOVA and Tukey test using software (SPSS) version 16. Values of $p<0.05$ were considered statistically significant.

RESULTS

Chemical composition of EOs

The qualitative characterizations of EOs were identified by GC-MS. The chemical constituents were presented in Table I. According to the GC-MS analyse results, carvacrol (61.43%), γ-Terpinene (7.43%) and p-cymene (6.71%) in oregano, linalool (35.21%) and linalyl acetate (34.88%) in lavender, 1,8-cineole (48.37%) and α-terpinyl acetate (14.31%) in laurel, cinnamaldehyde (88.12%) in cinnamon, terpinen-4-ol (24.57%), p-cymene (23.8%) and α-pinene (9.63%) in tea tree oil were the most abundant constituents found in EOs.

Screening of EOs antibacterial ability

The antibacterial activity of 9 EOs (oregano, cinnamon, lavender, tea tree, laurel, mint, lemon, coconut and eucalyptus) against *S. pyogenes* ATCC 19615, *S. aureus* ATCC 25923 and *S. agalactiae* ATCC 12386 strains using disc diffusion assay was evaluated. The results obtained regarding the inhibition zones were shown in Table II and Figure 1. Oregano (*O. vulgare)*, cinnamon (*C. verum)*, tea tree (*M. alternifolia)*, laurel (*L. nobilis)* and lavender *(L. officinalis)* are the most effective oils with inhibition zones ranging from 18.25 to 31.50 mm, 22.50 to 28.25 mm, 15 to 29.50 mm, 14 to 18 mm and 27.50 to 31.25 mm, respectively. Mint, eucalyptus and coconut EOs showed no appreciable inhibitory activity against tested human pathogens. Inhibition zone sizes for vancomycin (positive control) showed that the strains were susceptible as expected.

Determination of MIC and MBC Values

According to the disc diffusion test results, 5 effective EOs were evaluated of their MIC and MBC against three strains. MIC and MBC results were shown in Table III. Oregano EO exhibited the strongest antibacterial activity against *S.pyogenes*, *S.aureus* and *S.agalactiae* with MIC values of 0.03125%, 0.125% and 0.125% (v/v); followed by cinnamon 0.50%, 0.25% and 0.50% (v/v) and tea tree 1.00%, 0.125% and 1.00%(v/v), respectively. Five EOs exhibited bactericidal activity (MBC/MIC:2) against *S. aureus*. Oregano, cinnamon and tea tree EOs were found bactericidal (MBC/MIC:2) against *S .pyogenes*. None of the EOs except cinnamon showed bactericidal activity against *S. agalactiae*.

FIC and FICI of binary combinations of EOs against bacteria using checkerboard method

The results of the FIC and FICI of the dual combinations of EOs studied in this study were presented in Table IV. Against *S. agalactiae*, the more effective combinations including oregano/cinnamon (FICI=0.31), oregano/lavender (FICI=0.28), oregano/tea tree (FICI=0.38), tea tree/lavender (FICI=0.38) and cinnamon/lavender (FICI:0.50) showed a synergistic effect, whereas combinations of oregano/laurel (FICI=0.75) exhibited an additive effect and the other EO combinations displayed no interactive effect. Oregano mixed with tea tree (FICI=0.50) and laurel (FICI=0.28) showed a synergistic effect, the other EO combinations exhibited no interactive effect except tea tree/laurel against *S. aureus*. Combination of lavender with tea tree (FIC=0.31) and cinnamon (FIC=0.28) resulted in synergistic effects against *S. pyogenes*. Cinnamon EO showed an additive effect when combined with tea tree and laurel (FICI:0.63) EOs, oregano EO exhibited no interactive effect when combined with cinnamon, tea tree and laurel EOs against *S. pyogenes*.

Table I. The components and percentage composition of EOs analysed by-GC-MS.

Components	Percentage compositions of EOs (%)				
	Origanum vulgare	***Cinnamomum verum***	***Melaleuca alternifolia***	***Laurus nobilis***	***Lavandula officinalis***
α-Pinene	1.27	0.67	9.63	6.86	0.20
α-Thujene	2.61	0.04	-	0.58	-
Camphene	0.68	0.03	0.13	0.86	0.20
β-pinene	0.26	0.26	1.55	5.15	0.18
Sabinene	-	-	0.19	4.82	0.08
cis-Ocimene	-	-	-	-	0.90
β-ocimene	-	-	-	-	1.26
Tricyclene	-	-	0.02	-	0.02
β-Myrcene	3.35	-	0.09	0.57	0.50
α-humulene	-	0.51	0.54	-	-
α-phellandrene	0.38	-	0.51	-	-
α-Terpinene	1.72	-	-	0.98	-
Limonene	0.40	0.10	4.59	1.87	-
delta-cadinene	-	1.03	-	-	-
Cadina-1,4-diene	-	0.13	-	-	-
1,8-cineole	-	0.10	2.65	48.37	3.69
α-amorphene	-	0.12	-	-	-
β-phellandrene	0.41	-	-	-	1.23
γ-Terpinene	7.43	0.04	0.47	1.72	0.02
Calamenene	-	0.55	-	-	-
p-Cymene	6.71	0.45	23.8	2.19	0.04
α-terpinolene	0.20	0.08	0.44	0.43	0.31
α-selinene	-	0.12	-	-	-
δ-3-carene	-	1.41	0.92	1.73	0.05
Cymenene	-	-	0.05	-	-
Benzaldehyde	-	0.12	-	-	-
1-octen-3-ol	0.31	-	-	-	0.04
3-octanol	-	-	-	-	0.16
1,6-octadien-3-ol,3,7-dimethyl-acetate	-	-	-	-	0.04
3-octyl acetate	-	-	-	-	0.17
3-octanone	-	-	-	-	0.82
Phenylmethanol	-	0.03	-	-	-
trans-sabinene hydrate	1.00	-	-	-	0.04
Linalool	4.02	0.03	0.34	-	35.21
Linalool oxide	-	--	-	-	0.32
Epoxylinalol	-	-	-	-	0.03
Linalyl acetate	0.70	-	-	-	34.88
Camphor	-	0.02	1.68	-	4.29
(Z)- 3-Phenylacrylaldehyde	-	0.40	-	-	

Table I. Continuation.

Carveol	-	0.19	0.43	-	-
Carvone	-	0.44	1.44	-	-
Cinnamaldehyde	-	88.13	-	-	-
Carvacrol methyl ether	0.27	-	-	-	-
Terpinen-4-ol	0.76	-	24.57	-	-
β-caryophyllene	1.49	0.15	0.76	0.69	1.30
Caryophyllene-oxide	-	-	5.35	-	0.15
Caryophylla-4(12),8(13)-dien-5-alpha-ol	-	-	0.23	-	-
Aromadendrene	0.18	-	-	-	-
α-terpineol	0.22	-	-	-	-
β-terpineol	-	-	0.29	-	0.07
γ-terpineol	-	-	0.85	-	-
β-Fenchyl alcohol	-	-	8.50	-	-
α-terpinyl acetate	-	-	-	14.31	-
Fenchol	-	-	0.08	-	-
Terpinen-4-ol	-	-	-	3.50	-
Bornyl acetate	-	-	-	0.65	-
1-Hexyl acetate	-	-	-	-	0.98
Cinnamyl acetate	-	1.47	-	-	-
Lavandulyl acetate	-	-	-	-	1.73
β-elemene	-	-	-	0.38	-
Isoborneol	-	-	1.70	-	0.05
Components	**Percentage compositions of EOs (%)**				
	Origanum vulgare	**Cinnamomum verum**	***Melaleuca alternifolia***	***Laurus nobilis***	***Lavandula officinalis***
Isobutyrate	-	-	-	-	0.33
hexyl butyrate	-	-	-	-	2.44
Borneol	1.75	-	0.65	-	2.83
β-bisabolene	1.47	-	-	-	-
Thymol	0.73	-	-	-	-
Carvacrol	61.43	-	-	-	-
Methyl eugenol	-	-	-	2.03	-
Eugenol	-	1.10	-	1.07	-
α-cubebene	-	0.34	-	-	-
Copaene	-	-	0.74	-	-
Longifolene	-	0.16	0.38	-	-
Cryptone	-	-	-	-	0.13
Benzyl benzoate	-	1.62	-	-	-
Acetic acid	-	-	-	-	0.09
Nerol	-	-	-	-	0.19

Table II. Mean, Standard Deviation and Range of Zone Diameters (mm) for EOs against *S. pyogenes*, *S. aureus* and *S. agalactiae*.

	Zone diameters (SD) for EOs										
Bacteria	**Ore**	**Cinna**	**Lav**	**Lau**	**Tea**	**Mint**	**Euca**	**Coco**	**Lem**	**Van**	**P**
S. pyogenes ATCC 19615	18.25 (1.26)	22.50 (1.73)	27.50 (2.08)	14.00 (0.50)	15.00 (2.16)	7.75 (0.50)	-	10.75 (0.96)	11.50 (0.58)	19.75 (1.26)	<0.001*
S. aureus ATCC 25923	27.75 (2.06)	28.25 (3.40)	31.25 (1.27)	18.00 (0.86)	29.50 (3.42)	7.50 (0.58)	-	7.75 (0.50)	12.00 (0.82)	18.75 (0.50)	<0.001*
S.agalactiae ATCC 12386	31.50 (1.91)	26.25 (0.96)	30.75 (0.96)	15.00 (0.86)	26.50 (6.35)	9.0 (0.82)	-	10.50 (1.00)	12.00 (0.82)	17.00 (0.82)	<0.001*

Inhibition zone diameter in mm (Mean±SD: Standard Deviation), Ore: Oregano, Cinna: Cinnamon, Lav: Lavender, Lau: Laurel, Tea: Tea tree, Euca: Eucalyptus, Coco: Coconut, Lem: Lemon, Van: Vancomycin, -: ≤6 (disc zone diameter 6mm), p<0.001: Statistically significant value.

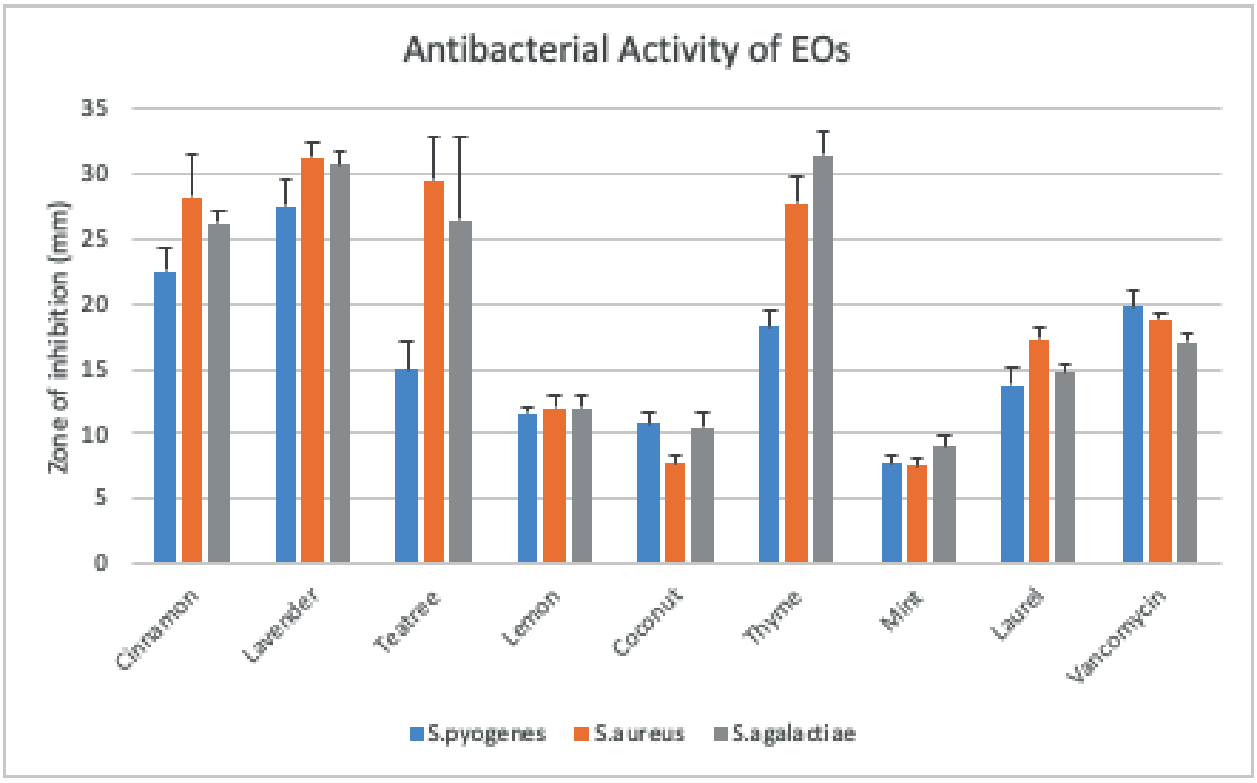

Figure 1. Antibacterial activity of analysed EOs. Vancomycin (30µg/disc) was used as a positive control for inhibition assay. P value less than 0.001 is statistically significant.

Table III. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of EOs (v/v) against *S. pyogenes*, *S. aureus* and *S. agalactiae* strains.

Bacteria	Activity	Oregano	Cinnamon	Lavender	Tea tree	Laurel
S. pyogenes ATCC 19615	MIC	0.03125	0.50	2.00	1.00	8.00
	MBC	0.0625	1.00	Growth	2.00	Growth
S. aureus ATCC 25923	MIC	0.125	0.25	4.00	0.125	4.00
	MBC	0.25	0.50	8.00	0.25	8.00
S. agalactiae ATCC 12386	MIC	0.125	0.50	4.00	1.00	8.00
	MBC	Growth	1.00	Growth	Growth	Growth

Table IV. FIC and FICI of the binary combinations of EOs against *S.pyogenes*, *S. aureus* and *S. agalactiae.*

	***S. pyogenes* ATCC 19615**				***S. aureus* ATCC 25923**				***S. agalactiae* ATCC 12386**			
EO mixtures (1+2)	**FIC_1**	**FIC_2**	**FICI**	**Effect**	**FIC_1**	**FIC_2**	**FICI**	**Effect**	**FIC_1**	**FIC_2**	**FICI**	**Effect**
Oregano+Cinnamon	1.00	0.50	1.50	NI	1.00	0.13	1.13	NI	0.25	0.06	0.31	S
Oregano+Lavender	4.00	0.25	4.25	A	1.00	0.03	1.03	NI	0.25	0.03	0.28	S
Oregano+Tea tree	1.00	2.00	3.00	NI	0.25	0.25	0.50	S	0.25	0.13	0.38	S
Oregano+Laurel	1.00	0.02	1.02	NI	0.25	0.03	0.28	S	0.25	0.50	0.75	AD
Tea tree+Cinnamon	0.13	0.50	0.63	AD	2.00	2.00	4.00	NI	0.25	1.00	1.25	NI
Tea tree+Lavender	0.25	0.06	0.31	S	1.00	0.25	1.25	NI	0.25	0.13	0.38	S
Tea tree+Laurel	1.00	0.02	1.02	NI	8.00	0.25	8.25	A	1.00	0.13	1.13	NI
Cinnamon+Lavender	0.25	0.03	0.28	S	1.00	1.00	2.00	NI	0.25	0.25	0.50	S
Cinnamon+Laurel	0.50	0.13	0.63	AD	0.50	0,50	1.00	NI	1.00	2.00	3.00	NI
Lavender+Laurel	4.00	0.02	4.02	A	1.00	1.00	2.00	NI	1.00	2.00	3.00	NI

Activity: FIC ≤0.5: synergic effect (S); 0.5<FIC ≤1: additive effect (AD); 1<FIC≤4: no interactive effect (NI); FIC>4: antagonistic effect (A).

DISCUSSION

Although a wide range of new antibiotics have been produced in the last thirty years, resistant microorganisms are increased to these drugs. The future of antimicrobial drugs in the treatment of bacterial infections is still uncertain. In traditional medicine, EOs are obtained from plants by various methods (such as hidrodistillation, steam distillation) have been used for a long time. In addition, EOs are used as food preservatives in the food industry, fragrance and pharmaceutical industry (Nascimento et al. 2000, Al-Bayati 2008).

In this study, 5 of 9 EOs, which were oregano, cinnamon, lavender, tea tree and laurel showed strong antimicrobial activity against *S. pyogenes* ATCC 19615, *S. aureus* ATCC 25923 and *S. agalactiae* ATCC 12386 strains. The qualitative chemical compositions of these EOs were determined

by GC-MS. Carvacrol, γ-terpinene and p-cymene in oregano, cinnamaldehyde in cinnamon, terpinen 4-ol, p-cymene and α-pinene in tea tree, linalool and linalyl acetate in lavender, 1,8-cineole, α-terpinyl acetate, α-pinene, β-pinene and sabinene in laurel were found as major components of EOs. Al-Bayati (2008) reported that oregano EO exhibited antibacterial activity with phenolic compounds like carvacrol, thymol, γ-terpinene and p-cymene. It has been reported that EOs, containing aldehydes such as cinnamaldehyde or phenols such as citral, carvacrol, eugenol or thymol showed strong antibacterial effect, on the other hand, containing ketones or esters like β-myrcene, α-thujone or geranyl acetate exhibited weak antibacterial effect. Terpene hydrocarbons were generally ineffective (Dormans & Deans 2000, Inouye et al. 2002, Barros et al. 2009, Nostro et al. 2002, Carson & Riley 1995, Griffin et al. 1999, Tajkarimi et al. 2010, Sachetti et al. 2005, Ait-Ouazzou et al. 2011).

In our study MIC/MBC value of cinnamon EO was 0.50/1.00 (v/v) against *S.pyogenes*, 0.25/0.50 (v/v) against *S. aureus*, 0.50/1.00 (v/v) against *S. agalactiae* were found. A study reported that the MIC values ranged from 0.25 to 0.50 mg/ml against *S. aureus*, The MIC value for the oils and (E)-cinnamaldehyde against *S. pyogenes* was 0.50 mg/ml; MBC values were 0.50-1.00 mg/ml (Firmino et al. 2018). A study by Zhang et al. (2015) was demonstrated the MIC value of 0.25 µl/ml and the MBC value of 0.5 µl/ml were found against *S. aureus*. These results are similar with ours. In this study, oregano and tea tree (MBC/MIC:2) EOs were found bactericidal against *S. pyogenes* and *S. aureus*, and bacteriostatic (MIC: 0.125) against *S. agalactiae*; lavender and laurel EOs were bactericidal (MBC/MIC:2) against *S. aureus*, bacteriostatic against *S. pyogenes* and *S. agalactiae* strains. In another study; however cinnamon (MBC/MIC: 1.32) and oregano (MBC/MIC: 1.08) were found bactericidal against *S. pyogenes*, tea tree ve lavender EO showed moderate inhibitory activity (inhibition zone diameter: 9-13 mm) (Sfeir et al. 2013).

A lot of studies have reported that EOs consist of 20-60 different chemical components and the antimicrobial activity of some are increased and prolonged against pathogens when used together compared to alone (Chouhan et al. 2017, Kumara et al. 2016, Nazzaro et al. 2013, Langeveld et al. 2014). Most of the research have investigated interactions of phenolic monoterpenes (e.g. thymol, carvacrol) and phenylpropanoids (e.g. eugenol) with the other component groups (phenols, phenylpropanoids and monoterpene alcohols, while monoterpenes and sesquiterpenes to a lesser extent) (Bassolé & Juliani 2012). The EOs consist of 76 different components were used in this study. The effects of dual components of EOs on selected human pathogen bacteria were evaluated with FIC index. Some binary EO combinations exhibited a synergistic and an additive effect against strains. Tea tree+lavender (FICI:0.31) and cinnamon+lavender (FICI:0.28) EO mixtures showed a synergistic effect; tea tree+cinnamon (FICI:0.63) and cinnamon+laurel (FICI:0.63) showed an additive effect against *S. pyogenes*. Oregano+tea tree (FICI:0.50) and oregano+laurel (FICI:0.28) EO mixtures exhibited a synergistic effect against *S. aureus*. Oregano+cinnamon (FICI:0.31), oregano+lavender (FICI:0.28), oregano+tea tree (FICI:0.38), tea tree+lavender (FICI:0.38) and cinnamon+lavender (FICI:0.50) EO mixtures showed a synergistic effect and oregano+laurel (FICI:0.75) exhibited an additive effect against *S. agalactiae*. In a study oregano+cinnamon EO mixtures FIC values were 1.08 ve 0.70; tea tree+cinnamon 0.83 ve 0.79; tea tree+oregano 0.53 ve 0.83 against *Paenibacillus amylolyticus* and *Bacillus cereus*, respectively (Ayaria et al. 2020). In this study, the antagonistic

effects of EOs were carried out the mixtures of oregano+lavender and lavender+laurel against *S. pyogenes* ATCC 19615, tea tree+laurel against *S. aureus* ATCC 25923. The FICI values were 4.25, 4.02 and 8.25, respectively. De Rapper et al. (2013) reported that combination of *L. angustifolia* (lavender)+*C.zeylanicum* (cinnamon) EOs exhibited a sinergistic effect (FICI: 0.5) against *S.aureus* ATCC 6538 and mixtures of *L. angustifolia* (lavender)+*O. majorana* (marjoram) EOs showed antagonistic (FICI:4) effect against *S.aureus*. The composition and yield of EOs depending on various factors such as seasonal variations, plant maturity and the organ which is derived from plant, geographical origin and genetics (Anwar et al. 2009).

CONCLUSIONS

In this study, the antibacterial efficacy of phytochemical–rich EOs (oregano, cinnamon, tea tree, laurel and lavender) both alone and binary combinations of them against *S.pyogenes*, *S. aureus* and *S. agalactiae* strains were evaluated. Double combinations of EOs exhibited significant synergy and additive effect. *In vitro, in vivo* (animal studies) and clinical studies are required for the determination of antioxidant and anti-inflammatory activities of EOs and their toxicity. In addition, studies with a large number of clinical isolates need to predict the effective and protective dose (formulation) of EOs. According to the results, various EOs and their binary combinations have shown different effects on different bacterial species.

REFERENCES

ABDALLAH EM. 2011. Plants: An alternative source for antimicrobials. J Appl Pharm Sci 1 (6): 16-20.

AIT-OUAZZOU A, CHERRAT L, ESPINA L, LORÁN S, ROTA C & PAGÁN R. 2011. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. Innov Food Sci Emerg 12: 320-329. https://doi.org/10.1016/j.ifset.2011.04.004.

AL-BAYATI FA. 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J Ethnopharmacol 116: 403-406. https://doi.org/10.1016/j.jep.2007.12.003.

ALIZADEH BEHBAHANI B, SHAHIDI F, YAZDI FT, MORTAZAVI SA & MOHEBBI M. 2017. Use of Plantago major seed mucilage as a novel edible coating incorporated with *Anethum graveolens* essential oil on shelf life extension of beef in refrigerated storage. Int J Biol Macromol 94: 515-526. https://doi.org/10.1016/j.ijbiomac.2016.10.055.

ANWAR F, HUSSAIN AI, SHERAZI STH & BHANGER MI. 2009. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (*Foeniculum vulgare* mill.) fruit at diferent stages of maturity. J Herbs Spices Med Plants 15: 187-202. https://doi.org/10.1080/10496470903139488.

AYARIA S, SHANKARA S, FOLLETTD P, HOSSAINA F & LACROIXA M. 2020. Potential synergistic antimicrobial efficiency of binary combinations of essential oils against *Bacillus cereus* and *Paenibacillus amylolyticus*-Part A. Microb Pathog 141: 104008. https://doi.org/10.1016/j.micpath.2020.104008.

BAKKALI F, AVERBECK S, AVERBECK D & IDAOMAR M. 2008. Biological effects of essential oils-A review. Food Chem Toxicol 46(2): 446-475. https://doi.org/10.1016/j.fct.2007.09.106.

BALZ R. 1999. The Healing Power of Essential Oils, 1st ed.; Lotus Press: Twin Lakes, WI, USA, p. 27-80.

BARROS JC, CONCEIÇÃO ML, GOMES NETO NJ, COSTA ACV, SIQUEIRA JÚNIOR JP & BASILIO JÚNIOR ID. 2009. Interference of *Origanum vulgare* L. essential oil on the growth and some physiological characteristics of *Staphylococcus aureus* strains isolated from foods LWT. Food Sci Technol 42: 1139-1143. https://doi.org/10.1016/j.lwt.2009.01.010.

BASSOLÉ IH & JULIANI HR. 2012. Essential Oils in Combination and Their Antimicrobial Properties. Molecules 17: 3989-4006. https://doi.org/10.3390/molecules17043989.

BIASI-GARBIN RP ET AL. 2015. Effect of eugenol against *Streptococcus agalactiae* and synergistic interaction with biologically produced silver nanoparticles. Evid Based Complement Alternat Med 2015: 861497. https://doi.org/10.1155/2015/861497.

BOUYAHYA A, DAKKA N, TALBAOUI A, ET-TOUYS A, EL-BOURY H, ABRINI J & BAKRI Y. 2017. Correlation between phenological changes, chemical composition and biological activities

of the essential oil from Moroccan endemic Oregano (*Origanum compactum* Benth). Ind Crops Prod 108: 729-737. https://doi.org/10.1016/j.indcrop.2017.07.033.

BROOK I. 2001. Failure of penicillin to eradicate group A beta-hemolytic streptococci tonsillitis: causes and management. J Otolaryngol 30: 324-329. https://doi.org/10.2310/7070.2001.19359.

BURT S. 2004. Essential oils: their antibacterial properties and potential applications in foods-A review. Int J Food Microbiol 94: 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

CARSON CF & RILEY TV. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol 78(3): 264-269. https://doi.org/10.1111/j.1365-2672.1995.tb05025.x.

CDC - CENTERS FOR DISEASE CONTROL AND PREVENTION. 2010. Prevention of Perinatal Group B Streptococcal Disease Revised Guidelines from CDC, November 19, 2010 / Vol. 59 / No. RR-10. https://www.cdc.gov/groupbstrep/about/symptoms.html. Retrieved 21 September 2017.

CHANDRA H, BISHNOI P, YADAV A, PATNI B, MISHRA AP & NAUTIYAL AR. 2017. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials-A Review. Plants 6(2): 16. https://doi.org/10.3390/plants6020016.

CHOBY BA. 2009. Diagnosis and treatment of streptococcal pharyngitis. Am Fam Physician 79(5): 383-390. Erratum in: Am Fam Physician. 2013 Aug 15; 88(4): 222. Dosage error in article text. PMID: 19275067.

CHOUHAN S, SHARMA K & GULERIA S. 2017. Antimicrobial activity of some essential oils—present status and future perspectives. Medicines 4(3): 58. https://doi.org/10.3390/medicines4030058.

CLEMENTE I, AZNAR M, SILVA F & NERIN C. 2016. Antimicrobial properties and mode of action of mustard and cinnamon essential oils and their combination against foodborne bacteria. Innov Food Sci Emerg Technol 36: 26-33. https://doi.org/10.1016/j.ifset.2016.05.013.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2012. "Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard- 11th Edition," Pennsylvania 19087, USA.

CUNNINGHAM MW. 2000. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 13(3): 470-511. https://doi.org/10.1128/CMR.13.3.470.

DAVIDSON PM & PARISH ME. 1989. Methods for testing the efficacy of food antimicrobials. Food Technol 43: 148-155.

DELAQUIS PJ, STANICH K, GIRARDB & MAZZA G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, Int J Food Microbiol 74: 101-109. https://doi.org/10.1016/S0168-1605(01)00734-6.

DE RAPPER S, KAMATOU G, VILJOEN A & VAN VUUREN S. 2013. The in vitro antimicrobial activity of *Lavandula angustifolia* essential oil in combination with other aromatherapeutic oils. Evid Based Complement Alternat Med 2013: 852049. https://doi.org/10.1155/2013/852049.

DORMANS HJD & DEANS SG. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 88(2): 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x.

EFSTRATIOU A. 2000. Group A streptococci in the 1990s. J Antimicrob Chemother 45: 3-12. https://doi.org/10.1093/jac/45.suppl_1.3.

ESSENTIAL OILS MARKET SIZE, SHARE & TRENDS ANALYSIS REPORT BY APPLICATION (CLEANING & HOME, MEDICAL, FOOD & BEVERAGES, SPA & RELAXATION), BY PRODUCT, BY SALES CHANNEL, AND SEGMENT FORECASTS, 2019-2025. GRAND VIEW RESEARCH 2019: 187. https://www.grandviewresearch.com/industry-analysis/essential-oilsmarket. Accessed 2 December 2019.

FIRMINO DF, CAVALCANTE TTA, GOMES GA, FIRMINO NCS, ROSA LD, DE CARVALHO MG & CATUNDA JR FEA. 2018. Antibacterial and antibiofilm activities of *Cinnamomum* sp. essential oil and cinnamaldehyde: Antimicrobial activities. Hindawi Article ID 7405736, 9 pages. https://doi.org/10.1155/2018/7405736.

GILL AO, DELAQUIS P, RUSSO P & HOLLEY RA. 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol 73(1): 83-92. https://doi.org/10.1016/s0168-1605(01)00712-7.

GONI P, LOPEZ P, SANCHEZ C, GOMEZ-LUS R, BECERRIL R & NERIN C. 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem 116: 982-989. https://doi.org/10.1016/j.foodchem.2009.03.058.

GRIFFIN GS, WYLLIE GS, MARKHAM LJ & LEACH DN. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour Fragr J 14: 322-332. https://doi.org/10.1002/(SICI)1099-1026(199909/10)14:5<322::AID-FFJ837>3.0.CO;2-4.

GUTIERREZ J, BARRY-RYAN C & BOURKE P. 2008. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients.

Int J Food Microbiol 124: 91-97. https://doi.org/10.1016/j.ijfoodmicro.2008.02.028.

INOUYE S, YAMAGUCHI H & TAKIZAWA T. 2002. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Inf Chemother 7(4): 251-254. https://doi.org/10.1007/s101560100045.

JALAL Z, ELATKI Y, LYOUSSI B &ABDELLAOUI A. 2015. Phytochemistry of the essential oil of *Melissa officinalis* L. Growing wild in Morocco: Preventive approach against nosocomial infections, Asian Pac J Trop Biomed 6: 458-461. https://doi.org/10.1016/j.apjtb.2015.03.003.

JENUL C & HORSWILL AR. 2019. Regulation of *Staphylococcus aureus* virulence. Microbiol Spectr 6(1). https://doi.org/10.1128/microbiolspec.GPP3-0031-2018.

KIVRAK I, DURU ME, OZTURK M, MERCAN N, HARMANDAR M & TOPCU G. 2009. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chem 116: 470-479. https://doi.org/10.1016/j.foodchem.2009.02.069.

KOROCH AR, RODOLFO JH & ZYGADLO JA. 2007. Bioactivity of Essential Oils and Their Components. In: BERGER RG (Ed). Flavours and Fragrances. Springer, Berlin, Heidelberg, p. 87-115. https://doi.org/10.1007/978-3-540-49339-6_5.

KUMARA SWAMY M, AKHTAR MS & SINNIAH UR. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evid Based Complement Alternat Med 2016: 3012462. http://dx.doi.org/10.1155/2016/3012462. Accessed 2 December 2019.

LANGEVELD WT, VELDHUIZEN EJ & BURT SA. 2014. Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 40(1): 76-94. https://doi.org/10.3109/1040841X.2013.763219.

LEVISON ME. 2004. Pharmacodynamics of antimicrobial drugs. Infect Dis Clin North Am 18(3): 451-465. https://doi.org/10.1016/j.idc.2004.04.012.

LOWY FD. 1998. *Staphylococcus aureus* infections. N Engl J Med 39: 520-532. https://doi.org/10.1056/NEJM199808203390806.

MA X, SHI W & ZHANG Y. 2019. Essential oils with high activity against stationary phase *Bartonella henselae*. Antibiotics 8(4): 246. https://doi.org/10.3390/antibiotics8040246.

MARASINI BP, BARAL P, ARYAL P, GHIMIRE KR, NEUPANE S, DAHAL N, SINGH A, GHIMIRE L & SHRESTHA K. 2015. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. Biomed Res Int 2015: 6. https://doi.org/10.1155/2015/265425.

MARTELLI G & GIACOMINI D. 2018. Antibacterial and antioxidant activities for natural and synthetic dual active compounds. Eur J Med Chem 158: 91-105. https://doi.org/10.1016/j.ejmech.2018.09.009.

MARWA C, FIKRI BENBRAHIM K, OU YAHIA D & FARAH A. 2017. African peppermint (*Mentha piperita*) from Morocco: Chemical composition and antimicrobial properties of essential oil. J Adv Pharm Technol Res 8(3): 86-90. https://doi.org/10.4103/japtr.JAPTR_11_17.

MCCAIG LF, MC DONALD LC, MANDAL S & JERNIGAN DB. 2006. *Staphylococcus aureus*-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis 12: 1715-1723. https://doi.org/10.3201/eid1211.060190.

MELLO S, BITTENCOURT F, FRONZA N, CUNHA A, NEUD G & ROSANA C. 2014. Chemical composition and antibacterial activity of *Laurus nobilis* essential oil towards foodborne pathogens and its application in fresh Tuscan sausage stored at 7 C, LWT. Food Sci Technol 59: 86-93. https://doi.org/10.1016/j.lwt.2014.05.032.

MOODY JA. 2003. Synergism testing: broth microdilution checkerboard and broth microdilution. In: Isenberg HD (Ed), Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, p. 1-28.

MOUREY A & CANILLAC N. 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control 13: 289-292. https://doi.org/10.1016/S0956-7135(02)00026-9.

NASCIMENTO GG, LOCATELLI J, FREITAS PC & SILVA GL. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol 31(4): 247-256. https://doi.org/10.1590/S1517-83822000000400003.

NAZZARO F, FRATIANNI F, DE MARTINO L, COPPOLA R & DE FEO V. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel) 6(12): 1451-1474. https://doi.org/10.3390/ph6121451.

NOSTRO A, CANNATELLI MA, MUSOLINO AD, PROCOPIO F & ALONZO V. 2002. *Helichrysum italicum* extract interferes with the production of enterotoxins by *Staphylococcus aureus*. J Appl Microbiol 35(3): 181-184. https://doi.org/10.1046/j.1472-765x.2002.01166.x.

NUCCITELLI A, RINAUDO CD & MAIONE D. 2015. Group B *Streptococcus* vaccine: state of the art. Ther Adv Vaccines 3: 76-90. https://doi.org/10.1177/2051013615579869.

RICHTER SS, HEILMANN KP, BEEKMANN SE, MILLER NJ, MILLER AL, RICE CL, DOERN CD, REID SD & DOERN GV. 2005. Macrolide-resistant *Streptococcus pyogenes* in the United States,

2002-2003. Clin Infect Dis 41: 599-608. https://doi.org/10.1086/432473.

ROSSITER SE, FLETCHER MH & WUEST WM. 2017. Natural products as platforms to overcome antibiotic resistance. Chem Rev 117: 12415-12474. https://doi.org/10.1021/acs.chemrev.7b00283.

SACHETTI 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 Chem 91(4): 621-632. https://doi.org/10.1016/j.foodchem.2004.06.031.

SCHELZ Z, MOLNAR J & HOHMANN J. 2006. Antimicrobial and antiplasmid activities of essential oils. Fitoterapia 77: 279-285. https://doi.org/10.1016/j.fitote.2006.03.013.

SFEIR J, LEFRANÇOIS C, BAUDOUX D, DERBRÉ S & LICZNAR P. 2013. In Vitro Antibacterial Activity of Essential Oils against *Streptococcus pyogenes*. Hindawi Article ID 269161, 9 pages. http://dx.doi.org/10.1155/2013/269161.

SHI C, ZHANG X, ZHAO X, MENG R, LIU Z, CHEN X & GUO N. 2017. Synergistic interactions of nisin in combination with cinnamaldehyde against *Staphylococcus aureus* in pasteurized milk. Food Control 71: 10-16. https://doi.org/10.1016/j.foodcont.2016.06.020.

SOLÓRZANO-SANTOS F & MIRANDA-NOVALES MG. 2012. Essential oils from aromatic herbs as antimicrobial agents. Curr Opin Biotechnol 23(2): 136-141. https://doi.org/10.1016/j.copbio.2011.08.005.

SUTCLIFFE CG ET AL. 2019. The burden of *Staphylococcus aureus* among native Americans on the Navajo nation. PLoS ONE 14(3): e0213207. https://doi.org/10.1371/journal.pone.0213207.

TAJKARIMI MM, IBRAHIMA SA & CLIVER DO. 2010. Antimicrobial herb and spice compounds in food. Food Control 21(9): 1199-1218. https://doi.org/10.1016/j.foodcont.2010.02.003.

TURGIS M, VU KD, DUPONT C & LACROIX M. 2012. Combined antimicrobial effect of essential oils and bacteriocins against foodborne pathogens and food spoilage bacteria. Food Res Int 48: 696-702. https://doi.org/10.1016/j.foodres.2012.06.016.

VELASCO V, BUYUKCANGAZ E, SHERWOOD JS, STEPAN RM, KOSLOFSKY RJ & LOGUE CM. 2015. Characterization of *Staphylococcus aureus* from humans and a comparison with isolates of animal origin, in North Dakota, United States. PLoS ONE 10: 0140497. https://doi.org/10.1371/journal.pone.0140497.

WAYNE PA. 2012. Clinical and Laboratory Standards Institute (CLSI), Approved Standard M07-A9, Methods for Dilution Antimicrobial Susceptibility, Tests for Bacteria that Grow Aerobically, 9th Ed, Clinical and Laboratory Standarts Institute.

WENDAKOON CN & SAKAGUCHI M. 1995. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J Food Prot 58(3): 280-283. https://doi.org/10.4315/0362-028X-58.3.280.

WHO - WORLD HEALTH ORGANIZATION. 2017. A Global Health Guardian: Climate Change, Air Pollution and Antimicrobial. Geneva, Switzerland: World Health Organization.

ZHANG LQ, ZHANG ZG, FU Y & XU Y. 2015. Research progress of trans-cinnamaldehyde pharmacological efects. Zhongguo Zhong Yao Za Zhi 40(23): 4568-4572. PMID: 27141665.

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