Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2022.055

RESEARCH ARTICLE

In Vitro Effect of Some Essential Oils against Multiple Antibiotic-Resistant Bacteria from Cats and Dogs

Nisa Sipahi*[1], Ayşe Ilgın Kekeç[2] and Barış Halaç[2]

[1]Duzce University, Traditional and Complementary Medicine Applied and Research Center, 81620 Duzce, Turkey
[2]Istanbul University-Cerrahpaşa, Veterinary Medicine Faculty, Department of Microbiology, 34500 Istanbul Turkey
*Corresponding author: sipahi.nisa@gmail.com

ARTICLE HISTORY (22-123)

Received: April 2, 2022
Revised: June 17, 2022
Accepted: July 16, 2022
Published online: August 01, 2022

Key words:
Antimicrobial
Antibiofilm
Multi-drug resistant
Medicinal plants
Essential oils

A B S T R A C T

Multidrug resistant (MDR) bacteria are creating a serious challenge to treat diseases. The present study was aimed to evaluate the antibacterial and antibiofilm activity of four different essential oils (*Melissa officinalis* L., *Nigella sativa* L., *Laurus nobilis* L., and *Origanum onites* L.) against MDR bacteria from cats and dogs. Methicillin-resistant *Staphylococcus aureus* (MRSA), two different multi-drug resistant *S. aureus* (MDR SA1 and MDR SA2), and *Escherichia coli* (ESBL *E. coli*) that contained extended-spectrum beta-lactamases strains were used in the study. Effectiveness of these oils against MDR bacteria was determined through minimum inhibitory concentrations (MIC) test, minimum bactericidal concentrations (MBC) test and antibiofilm assay. Results the inhibitory effect of essential oils determined activity as oregano>melissa>laurel=nigella. Therefore, the most susceptible isolate to essential oils was MRSA. The results also showed significant antibiofilm activity of melissa and oregano against MDR bacteria. In conclusion, the current study declares essential oils from medicinal plants are effective natural products for resistant pathogen bacteria.

To Cite This Article: Sipahi N, Kekeç AI, Halaç B, 2022. In vitro effect of some essential oils against multiple antibiotic-resistant bacteria from cats and dogs. Pak Vet J, 42(4): 561-565. http://dx.doi.org/10.29261/pakvetj/2022.055

INTRODUCTION

In animals (especially domestic animals), the zoonotic nature of most infections also poses a risk to human health. It was reported in previous studies that 61% of 1415 pathogens that were found to cause disease in humans are of animal origin (Cunningham, 2005). One of the most important factors that affect animal health is bacterial infections. However, antibiotics used in treatment can also bring some side effects that affect health negatively. On the other hand, difficulties such as antibiotic resistance and resistant biofilm formation in the treatment of bacterial infections raise concerns for public health. It is already known that the rate of multi-resistance (MDR) of pathogenic bacteria to existing antimicrobials increased considerably in the last decade (Caveney *et al*., 2019; Wu *et al*., 2020). This increase is described as a global and very important healthcare issue by the United States Center for Disease Control and Prevention (CDC), the European Center for Disease Control and Prevention (ECDC), and the World Health Organization (WHO) (Anonymous, 2019). Especially beta-lactam resistant gram-negative like *Escherichia coli* contained extended-spectrum beta-lactamases (ESBL) is a growing problem. Also methicillin-resistant *Staphylococcus aureus* (MRSA), which is the leading cause of nosocomial infections, threaten health to a great extent. Because pathogens such as *S. aureus, Candida albicans, E. coli* are important microbial agents colonizing in various parts of the body in animals and humans, multi-drug resistance is an extremely dangerous condition (Ababneh *et al*., 2022). One of the difficulties that provide resistance in important pathogenic agents is biofilm formation (Craft *et al*., 2019). Biofilms are important barriers limiting drug penetration into the target. For this reason, it is necessary to develop new strategies for biofilm eradication in resistant strains (Jafri *et al*., 2014). Furthermore, infectious diseases caused by bacteria showing MDR cause scientists to seek alternative treatments and therapeutic agents. For this reason, the need for natural products easy to access with no side effects for protection or treatment against such diseases increased considerably on a global scale (Sipahi, 2021). In this regard, the potential of natural products to be effective in the treatment and prophylactic solutions of medicinal plants are the subject matters of recent studies (Sakkas *et al*., 2016; Güceyü *et al*., 2019). In this sense, a

method that involves the use of various medicinal plant extracts and essential oils is used in the prevention and treatment of diseases as well as increasing the efficiency and welfare of animals and is known as "Veterinary Phytotherapy". Although the use of phytopharmaceuticals for therapeutic purposes is more prominent in human health, it is limited for animal health. Natural products could be obtained from medicinal plants can also be effective in infections of animal origin. Studies conducted so far argue that medicinal plants can be used as antitumor, antiparasitic, anti-inflammatory, and antimicrobial agents (Sayyar *et al*., 2021; Dinç *et al*., 2022). In the present study, the inhibitory and antibiofilm effects of essential oils extracted from melissa, laurel, oregano, and nigella were investigated against some bacteria with multiple antibiotic resistance that was isolated from cats and dogs.

MATERIALS AND METHODS

Essential oils: Melissa, nigella, laurel and oregano were collected from Traditional and Complementary Medicine Applied and Research Center in Duzce University. Plant materials were identified by M Fatih Çakır. The essential oils obtained by steam distillation method (by working 125°C steam temperature and 1.5 bar operating pressure), and dilutions of essential oils were prepared with Tween 80 (Merck).

Test agents and culture: MRSA, MDR *S. aureus* 1 & 2, and ESBL *E. coli* strains were used in the study. The strains were obtained from the culture collection of Istanbul University-Cerrahpaşa Veterinary Faculty Microbiology Department Clinic from the animals who applied to treatment. The strains are given in Table 1. Mueller Hinton Agar (MHA), Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB) and Mueller Hinton Broth (MHB) were used for antimicrobial testing and bacterial proliferation (collected from Merck).

Disk diffusion: It was used to compare all four essential oils (CLSI, 2018a). Bacteria in TSA medium were prepared the day before and then adjusted to a turbidity of 0.5 on the McFarland scale in saline. Bacterial solutions spread on MHA. Test samples were absorbed into blank discs (Bioanalyses, blank disc, 6mm) and placed on MHA plaque. Meropenem (Bioanalyses, MEM10, 10µg) was used as a positive control. Test plates were incubated at 37° C for 24 hours. Inhibition zone diameters were calculated. Each formulation was tested 3 times.

Detection of MIC and MBC concentrations: MIC and MBC of the essential oils were determined by the microdilution method. Serial dilutions of essential oils with MHB (from 100 to 0.022 µL/mL and total of 120µL per well) and 5×10^5 cfu/mL bacteria were added to each well and incubated at 37°C. The dilution without growth was determined as MIC. To determine the MBC of essential oils 50µL of each dilution (no growth) was spread on MHA plates. MBC was accepted as the lowest concentration that killed more than 99.9% of bacteria. Experiments were performed in triplicate for each strain on three different days (Elshikh *et al*., 2016; CLSI, 2018b).

Congo red assay: All isolates were inoculated on Congo Red Agar (CRA). The agar plates were incubated at 37°C for 24 hours and then overnight at room temperature. Black colony were defined as strong biofilm-forming, dark-colored colonies as weak biofilm. Red colonies were defined as non-biofilm (Mariana *et al*., 2009; Kord *et al*., 2018).

Antibiofilm assay: Inhibition of biofilm formation were determined with the microplate assay. A hundred µL bacterial culture in TSB (10^6 cfu/mL) supplemented with 1% sucrose was added into each well of 96-well bottom and was incubated at 37°C for 24 h. After that, 100µL of essential oils (at MIC value) added into each well and incubated at 37°C for 24 h. Then the well content was removed and each well washed three times with PBS (phosphate-buffered saline) (Sigma-Aldrich). The adherent bacteria were fixed at 60°C for 45 min. Bacteria were stained with 100µL of 0.1% crystal violet (Himedia) for 15 min at room temperature (RT). Then each well were washed 3 times with PBS again. It was left to dry at RT for 15 min, 100µL of 95% ethanol was added into each well to dissolve bacteria on the bottom. Before the absorbance was measured at OD_{490} nm using a microplate reader (Biotek BT 800, USA), the well plate was left at RT for 10 min and transferred to a clean well. The assay was performed in triplicate (Bazargani and Rohloff, 2016; Chen *et al*., 2020). The inhibition percentage of biofilm formation was calculated using the following formula:

$$\text{Inhibition \%} = \frac{\text{OD in Control - OD in Treated}}{\text{OD in Control}} * 100$$

Statistical analysis: The data are given as mean (± SD). The difference between the MIC of each essential oil in different bacteria and the MIC of different essential oils in a bacterium was evaluated with the Fisher's Exact Test the SPSS 15.0 software was used for the analyses. The statistically significant differences of the reduction of biofilm mass and inhibition zone diameters in groups were determined with Kruskal Wallis and Mann Whitney U by using SPSS 15.0.

RESULTS

Disk diffusion test: In this study, some antimicrobial properties of essential oils were investigated against clinical isolates that are multidrug resistant bacteria. These clinical isolates had been collected from previous studies. Four different resistant isolates were used. The antimicrobial agent potentials of essential oils were first investigated by disk diffusion test. The inhibition zones that were formed by the essential oils on the agar plate were compared. In this respect, it was found that the essential oil that formed the most inhibition zone in the strains was oregano essential oil. For each bacterium, melissa essential oil was found to be more effective after oregano essential oil. Oregano oil continued its effect even with 50% and 30% dilutions. It had quite large inhibition zones, especially against MRSA. However, the difference between effectiveness of oregano and melissa was not significant ($P>0.05$). Nevertheless, each of oregano and melissa were significantly more effective

Table 1: Resistant strains tested in the study

Bacteria	Isolation site	Origin	Antibiotics Resistant to
MRSA	Nasal Swab	Dog	Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Ceftiour, Ceftriaxone, Cefepime Ciprofloxacin, Enrofoxacin, Erythromycin, Gentamicin, Marbofloxacin, Oxytetracycline, Trimethoprim/Sulfamethoxazole
ESBL EC (*E. coli*)	Ear Swab	Dog	Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Ceftiour, Ceftriaxone, Cefepime Ciprofloxacin, Enrofoxacin, Erythromycin, Gentamicin, Marbofloxacin, Rifampin, Trimethoprim/Sulfamethoxazole
MDR SA1 (*S. aureus* 1)	Joint	Cat	Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Ceftiour, Ceftriaxone, Ciprofloxacin, Enrofoxacin, Erythromycin, Gentamicin, Marbofloxacin, Penicillin G, Rifampin, Tetracycline, Trimethoprim/Sulfamethoxazole
MDR SA2 (*S. aureus* 2)	Ear Swab	Cat	Amikacin, Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Ceftiour, Cefixime, Ceftriaxone, Ciprofloxacin, Enrofoxacin, Erythromycin, Gentamicin, Spiramycin Imipenem, Kanamycin, Lincomycin/Spectinomycin, Marbofloxacin, Moropenem, Spiramycin, Tetracycline, Trimethoprim/Sulfamethoxazole, Tobramycin, Levofloxacin, Ofloxacin

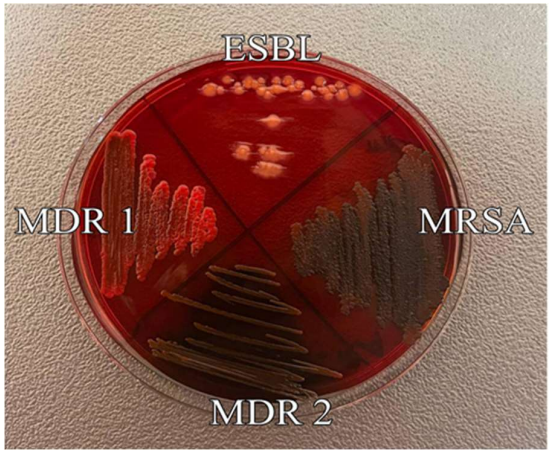

Fig. 1: Biofilm results in CRA.

Table 2: Zone diameters of Essential Oils on Agar Plate

Essential Oil	Zone Diameter (±SD)			
	ESBL EC	MRSA	MDR SA 1	MDR SA 2
Melissa	20(±1)	25(±1)	25(±2.51)	25(±0.57)
Melissa50%	16(±0.57)	20(±1)	20(±2.51)	21(±1)
Melissa30%	13(±0.57)	14(±0.57)	14(±2)	15(±2.51)
Laurel	13(±0.57)	16(±0.57)	14(±2.51)	13(±2.51)
Laurel 50%	R	12(±0.57)	12(±1)	10(±0.57)
Laurel 30%	R	10(±0.57)	R	R
Oregano	25(±0.57)	40(±0.57)	26(±2)	25(±0.57)
Oregano 50%	15(±0.57)	30(±1.52)	17(±1)	17
Oregano 30%	10(±0.57)	19(±0.57)	12(±0.57)	10(±2)
Nigella	12(±0.57)	15(±1.52)	13	10(±1)
Nigella50 %	R	13(±0.57)	R	R
Nigella 30%	R	10	R	R
Positive Control	40	45	35	35

R: Resistant, ± SD: Standard Deviation, Positive Control: Meropenem (10µg)

Table 3: MIC and MBC Values of Essential Oils

	Oregano µL/mL		Melissa µL/mL		Laurel µL/mL		Nigella µL/mL		MEM10 µg/mL	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
ESBL EC	3.12	6.25	6.25	12.5	25	50	25	50	0.5	1
MRSA	1.56	6.25	6.25	25	12.5	50	12.5	50	0.25	0.5
MDR SA1	3.12	6.25	6.25	12.5	25	50	25	50	0.5	1
MDR SA2	3.12	6.25	12.5	25	25	50	25	50	0.5	1

than the other two essential oils ($p<0.05$). Zone diameters formed on resistant isolates did not differ at significant levels between laurel and nigella essential oil ($P>0.05$).

MIC and MBC assays: After determining the zone diameters formed by essential oils, the minimal effective doses were investigated for each essential oil. On each resistant isolate, the effective doses of essential oils were determined with the microdilution method and are shown in Table 3. According to that MIC and MBC values for ESBL EC are 3.12 µL/mL- 6.25 µL/mL oregano, 6.25 µL/mL-12.5 µL/mL melissa, 25 µL/mL-50 µL/mL laurel and 25 µL/mL - 50µL/mL nigella respectively. It for MRSA are 1.56 µL/mL - 6.25 µL/mL oregano, 6.25 µL/mL - 25 µL/mL melissa, 12.5 µL/mL - 50 µL/mL laurel, 12.5 µL/mL - 50µL/mL nigella and for MDR SA1 are 3.12 µL/mL - 6.25 µL/mL oregano, 6.25 µL/mL-12.5 µL/mL melissa, 25 µL/mL-50 µL/mL laurel, 25 µL/mL - 50µL/mL nigella respectively. MIC and MBC values for MDR SA 2 are the same with MDR SA 1 for each essential oil except melissa oils. MIC and MBC of melissa oil for MDR SA 2 were 12.5 µL/mL and 25 µL/mL. In this regard, the effectiveness of essential oils for all isolates was determined as oregano>melissa>laurel= nigella. The most susceptible isolate to essential oils was MRSA.

Antibiofilm assay: Since the isolates were clinical isolates, the biofilm properties were unknown. First, biofilm capabilities were investigated by culture method. The biofilm characteristics of the isolates were evaluated as ESBL EC biofilm negative, MDR SA1 weak, MDR SA2, and MRSA strong biofilm-producing strain in terms of growth patterns in CRA (Fig. 1). Then, biofilm assay were performed with the positive strains to the results of the congo red agar method were confirmed. At the same time, it was also investigated whether essential oils have an antibiofilm effect. The biofilm inhibition rates of essential oils are given in Fig. 2-4. When compared the inhibition rates of nigella and laurel were not significant for all isolates ($P>0.05$), On the other hand, inhibition rates of all other essential oils were significantly different when compared in pairs for each bacterium with Mann Whitney U ($P<0.05$). In terms of statistical significance, antibiofilm activities for MDR SA1 and SA2 were found as oregano>melissa>laurel=nigella and for MRSA, oregano>melissa>laurel>nigella. When all inhibition rates were evaluated collectively with Kruskal Wallis, they were all different and p values were given on the graphs.

DISCUSSION

Antibiotic resistance is one of the global problems of today. The increased prevalence of multidrug resistance entails certain problems. Studies show that bacteria are always looking for a way to survive. For this reason, the biofilm structures formed by bacteria to survive cause some antimicrobial agents to be completely ineffective (Abebe, 2020).

Microbial resistance causes prolongation of the treatment period. Moreover, it causes increased treatment costs and drug side effects. Therefore, it is necessary to develop new therapeutic agents. In this respect, easily

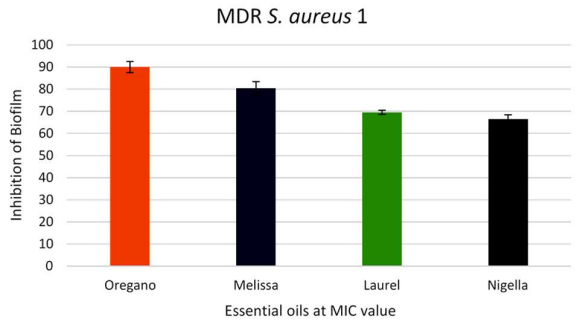

Fig. 2: Inhibitory effect of essential oils on MDR *S. aureus* 1 biofilms: The antibiofilm effect was determined by accepting the control group as 100%. Significant differences were found when all essential oils are compared together with Kruskal Wallis (p=0.019).

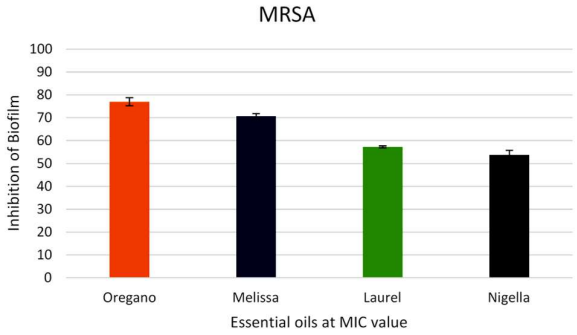

Fig. 3: Inhibitor effect of the essential oils on MRSA biofilms: The antibiofilm effect was determined by accepting the control group as 100%. Significant differences were found when all essential oils are compared together with Kruskal Wallis (p=0.016).

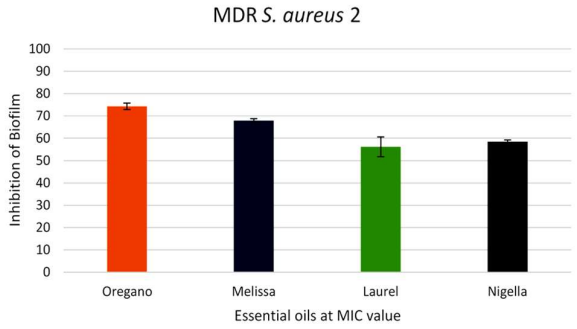

Fig. 4: Inhibitor effect of essential oils on MDR *S. aureus* 2 biofilms: The antibiofilm effect was determined by accepting the control group as 100%. Significant differences were found when all essential oils are compared together with Kruskal Wallis (p=0.024).

accessible phytopharmaceuticals that have low toxic effects are gaining more and more importance. The essential oils tested in the present study showed an effect in the form of oregano > melissa > laurel = nigella against multidrug-resistant microorganisms. It was found that depending on the concentration, it could play important roles in the elimination of biofilms.

The essential oils tested in the study have been used in bacterial infections for hundreds of years with their potential to be effective against multidrug-resistant strains (Tomar *et al*., 2020). *Laurus nobilis* essential oil is used frequently in folk medicine for the treatment of diseases such as dermatitis and rheumatism. It is already known to have antioxidant, antimicrobial, and some pharmacological characteristics (Caputo *et al.,* 2017). *Melissa officinalis* is a perennial herb frequently used as an essential oil with its various pharmacological effects. It was reported to have antimicrobial, antifungal, and anticarcinogenic effects in some studies (Korcan *et al*., 2018). *Origanum sp.* is a genus of the *Lamiaceae* family and it is known as an important aromatic and medicinal plant. On the other hand *Nigella sativa* is often investigated for its use as an antimicrobial drug or food additive with its various pharmacological effects (Georgescu *et al*., 2018). Therefore, this study was conducted in order to determine the potential of solving the problem of drug resistance to conventional antimicrobials in animals with essential oils or their active components. In this study, essential oils were found to have broad-spectrum effects. Also, when the bactericidal and bacteriostatic concentrations of the essential oils were compared with the other studies, similar findings were found. In this study, the lowest MIC values were found as 1.56 and 3.12 µL/mL for oregano. Sakkas *et al*. (2018) found MIC of oregano between 0.6 and 5 µL/mL for MRSA strains and found between 2.5 and 10 µL/mL for vancomycin-resistant *Enterococcus spp.* It was reported in another study that *Thymus vulgaris* and *Origanum vulgare* essential oils showed more effects against all resistant pathogens which are multidrug resistance *E. coli*, *Enterococcus spp*., *Candida spp.* strains isolated from cat and dog urinary system infection than other essential oils (Ebani *et al*., 2018).

Oregano and melissa essential oils were the most effective in the study. This may be due to the fact that they contain more active ingredients than other essential oils. Because essential oils are very rich in phenol components which have been reported as antimicrobial. Especially melissa and oregano have high levels of some compounds (linalool, citronellol, carvone, thymol, carvacrole etc.) and these compounds are quite effective against bacteria. Other previous studies reveal the component of essential oils (Nieto, 2017). Similarly, laurel and nigella also have important components. But maybe there are fewer components of both essential oils cause that they remain less effective in this study. Because the amount of essential oil components can change seasonally and geographically (Camele *et al*., 2019). Also the mechanism of essential oils on bacteria is not completely known. But in general, it is known that the components of essential oils deform lipids in the bacterial cell membrane. Thus, they disrupt the hydrophobic structure and they make it more permeable. Eventually, the bacterial cell structure and cell content deteriorate (Chouhan *et al*., 2017). Such an effect also occurs against the biofilm structure. The essential oil of *Murraya koenigii* from medicinal plants was found to be highly influential in preventing the formation of biofilms in *Pseudomonas spp.* (Bai and Vittal, 2014). In another study, it was reported that some essential oils have a strong antibacterial effect against gram-positive and negative bacteria (El-Tarabily *et al*., 2021). In this study, the MIC of each essential oil was preferred to investigate biofilm inhibition and it was found that oregano and melissa essential oils eliminated biofilms over 75 and 68%, respectively. It seems quite high inhibition of biofilms occurs because of increased concentration. It was

seen that each essential oil has a different potential according to statistical comparison. Especially oregano and melissa are quite different from the other two essential oils. Similarly, Jafri *et al*. (2014) reported that MIC of some essential oils tested in their study destroyed *S. aureus* biofilms effectively. In another study, the effects of 11 different essential oils were investigated against methicillin-resistant and susceptible *Staphylococcus pseudintermedius*-associated pyoderma from dog isolates. It was found essential oils could be important antimicrobials in the treatment of pyoderma in that study (Nocera *et al*., 2020). Especially when the zoonotic potential of resistant strains is considered, alternative therapeutic approaches are needed to prevent the spread of multidrug-resistant strains. Essential oils and medicinal plants can be a very important option in this respect (Nazir *et al*., 2021). The antimicrobial effects of essential oils suggest that they can be employed not only in the therapeutic approach but also as protective additives. Omonijo *et al*. (2018) reported that essential oils can be used instead of antibiotics in feed. For this reason, it can be argued that essential oils which have antimicrobial characteristics are an important product for new antimicrobial strategies in various fields.

Conclusions: the study showed essential oils have inhibitory and antibiofilm effects against resistant isolates. Especially the combined use of oregano and melissa essential oils could be a highly effective agent on pathogen-resistant bacteria. It is possible to obtain effective products for animal health from them. The potentials of essential oils should be further investigated.

Authors contribution: Conceive and designed the experiments: NS, AIK, BH. Performed experiments: NS. Analyzed the data: NS, AIK, BH. Wrote paper: NS, AIK, BH**.**

REFERENCES

Ababneh Q, Jaradat Z and Khanfar M, 2022. Methicillin-resistant Staphylococcus aureus contamination of high-touched surfaces in a university campus. J of Appl Microbiol 2022; doi:10.1111/jam.15526.

Abebe GM, 2020. The role of bacterial biofilm in antibiotic resistance and food contamination. Int J Microbiol doi:10.1155/2020/1705814.

Anonymous (Editorial) 2019. Antibiotic susceptibility diagnostics for the future. Nat Microbiol 4:1603-3.

Bai AJ and Vittal RR, 2014. Quorum sensing inhibitory and anti-biofilm activity of essential oils and their in vivo efficacy in food systems. Food Biotechnol 28:269-92.

Bazargani MM and Rohloff J, 2016. Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and *Escherichia coli* biofilms. Food Control 61:156-64.

Camele I, Elshafie HS, Caputo L, *et al.,* 2019. Anti-quorum sensing and antimicrobial effect of Mediterranean plant essential oils against phytopathogenic bacteria. Front Microbiol 10:2619.

Caputo L, Nazzaro F, Souza L, *et al*., 2017. Laurus nobilis: Composition of essential oil and its biological activities. Molecules 22:930.

Caveney NA, Caballero G, Voedts H, *et al*., 2019. Structural insight into YcbB-mediated beta-lactam resistance in Escherichia coli. Nat Communicat 10:1-11.

Chen Q, Xie S, Lou X, *et al*., 2020. Biofilm formation and prevalence of adhesion genes among Staphylococcus aureus isolates from different food sources. Microbiol Open 9:e00946.

Chouhan S, Sharma K and Guleria S, 2017. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines 4:58.

Clinical and Laboratory Standards Institute (CLSI), 2018. Performance Standards for Antimicrobial Disk Susceptibility Tests; (M02) 13th Edition, 2018a Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI), 2018. Performance standards for antimicrobial susceptibility testing; (M100) 13th Edition, 2018b Wayne, PA.

Craft KM, Nguyen JM, Berg LJ, *et al*., 2019. Methicillin-resistant Staphylococcus aureus (MRSA): antibiotic-resistance and the biofilm phenotype. Med Chem Comm 10:1231-41.

Cunningham AA, 2005. A walk on the wild side--emerging wildlife diseases. BMJ (Clinical research ed.) 331:1214–5.

Dinç H, Yiğin A and Koyuncu İ, 2022. Investigation of the anticancer and apoptotic effect of Micromeria congesta under in vitro conditions and detection of related genes by real-time PCR. Vet Res Forum 13:7-14.

Ebani VV, Nardoni S and Bertelloni F, 2018. Antimicrobial activity of five essential oils against bacteria and fungi responsible for urinary tract infections. Molecules 23:1668.

Elshikh M, Ahmed S, Funston S, *et al*., 2016. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett 38:1015-9.

El-Tarabily KA, El-Saadony MT, Alagawany M *et al*., 2021. Using essential oils to overcome bacterial biofilm formation and their antimicrobial resistance. Saudi J Biol Sci 28:5145-56.

Georgescu M, Tăpăloagă PR, Tăpăloagă D, *et al*., 2018. Evaluation of antimicrobial potential of Nigella sativa oil in a model food matrix. Farmacia 66:1028-36.

Güceyü Ç, Goncagül G and Günaydın E, 2019. Antibacterial effect of *Zingiber officinale*. J Etlik Microbiol 30:44-50.

Jafri H, Husain FM and Ahmad I, 2014. Antibacterial and antibiofilm activity of some essential oils and compounds against clinical strains of Staphylococcus aureus. J Biomed Ther Sci 1:65-71.

Kord M, Ardebili A, Jamalan M, *et al*., 2018. Evaluation of biofilm formation and presence of ica genes in *Staphylococcus epidermidis* clinical isolates. Osong Public Health Res Perspect 9:160.

Korcan SE, Aksoy B, Erdoğmuş SF, *et al*., 2018. Investigation of antimicrobial activity and dna protective capacity of *Melissa officinalis* extracts AKU J Sci Eng 18:011002 (757-62).

Mariana NS, Salman SA, Neela V, *et al*., 2009. Evaluation of modified Congo red agar for detection of biofilm produced by clinical isolates of methicillinresistance *Staphylococcus aureus*. Afr J Microbiol Res 3:330-8.

Nazir I, Rabbani M, Sheikh AA, *et al.,* 2021. Antibacterial activity of medicinal flowers against multi drug resistant *E. coli*. Pak Vet J 41:166-8.

Nieto I, 2017. Biological activities of three essential oils of the lamiaceae family. Medicines 4:1–10.

Nocera F P, Mancini S, Najar B, *et al*., 2020. Antimicrobial activity of some essential oils against methicillin-susceptible and methicillin-resistant *Staphylococcus pseudintermedius*-associated pyoderma in dogs. Animals 10:1782.

Omonijo FA, Ni L, Gong J, *et al*., 2018. Essential oils as alternatives to antibiotics in swine production. Anim Nutr 4:126-36.

Sakkas H, Gousia P, Economou V, *et al*., 2016. In vitro antimicrobial activity of five essential oils on multidrug resistant Gram-negative clinical isolates. J Intercult Ethnopharmacol 5:212.

Sakkas H, Economou V, Gousia P, *et al*., 2018. Antibacterial efficacy of commercially available essential oils tested against drug-resistant gram-positive pathogens. Appl Sci 8:2201.

Sayyar HT, Afroz S and Assad T, 2021. Evaluation of phytochemical screening, antimicrobial and antioxidant activities of ethanol extracts of *Cucumis flexouses* and *Cucumis reticulatus* Seeds. Pak Vet J 41.

Sipahi N, 2021. Investigation of antagonistic effect of probiotic food supplement against different strains of bacteria. IJTCMR, 2:95-100.

Tomar O, Akarca G and Gök V, 2020. Composition and antibacterial effects of Laurus (Laurus nobilis L.) leaves essential oil. J Essent Oil-Bear Plants 23:414-21.

Wu JT, Leung K, Bushman M, *et al*., 2020. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. Nat Med 26:506-10.