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Antibacterial, antibiofilm, and pharmacokinetic properties of some Lamiaceae essential oils against methicillin- and vancomycin-resistant *staphylococcus aureus*

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ABSTRACT

The presence of methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) *Staphylococcus aureus* in food raises a public health concern. This study aimed to investigate the antibacterial and anti-biofilm activity of some Lamiaceae essential oils including *Melissa Officinalis*, *Salvia officinalis*, and *Mentha piperita* against MRSA and for the first time on VRSA strains. For this purpose, the disk diffusion test, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum biofilm inhibitory concentration (MBIC), and minimum biofilm eradication concentration (MBEC) were determined. Then, *M. Officinalis* essential oil compounds were investigated against PBP2a, agrA, and Bap proteins using AutoDocK Vina. Finally, pharmacokinetic properties were investigated using ADMETsar and SwissADME servers. Based on the obtained results, the MIC and MBC values of *M. Officinalis* essential oil against MRSA strain were equal to 0.05 and 0.112 mg/ml, and against VRSA strain were equal to 1.8 and 2.5 mg/ml, respectively. The MBIC and MBEC of *M. Officinalis* essential oil against MRSA strain were equal to 0.03 mg/ml and 0.112 mg/ml, and against VRSA strain were equal to 0.9 mg/ml and 3.2 mg/ml, respectively. The results of molecular docking showed that β-Caryophyllene had a greater binding affinity to PBP2a protein either in the active site or in the allosteric site (-6.6kcal/mol). On the other hand, the effective compounds of this essential oil, especially citronellol, thymol, and citral, were acceptable in terms of pharmacokinetic properties. Since natural antibiotics can be an alternative to conventional antibiotics in the treatment of *Staphylococcus aureus* food-borne diseases, the results of this study showed that *Melissa Officinalis* essential oil is effective on the growth and biofilm of MRSA and VRSA strains, and it can be used as a drug candidate in the prevention and treatment of infections caused by antibiotic-resistant strains of this bacterium.

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1- Introduction

Staphylococcus aureus It can cause serious infections such as bloodstream infections (bacteremia and septicemia), pneumonia, food poisoning, and wound infections. Multiple antibiotic resistance of strains of this bacterium has become a clinical problem worldwide, and researchers are trying to find new drugs against these strains. In Iran, the high presence of methicillin-resistant strains ([1]MRSA) and vancomycin ([2]VRSA) has been reported in various types of raw and processed meat, raw milk, traditional cheese, restaurant foods and hospital foods [1]. Beta-lactam antibiotics, such as penicillin, have been one of the most efficient antibiotics that are effective in the treatment of bacterial wall synthesis.*Staphylococcus aureus*were used. Mechanism of β-lactam antibiotics, irreversible acylation of PBPs[3] or penicillin-binding proteins [2]. PBP proteins can have both transpeptidase and transglycosylase activity. One of the mechanisms of resistance to beta-lactam antibiotics were beta-lactamase enzymes, which cut the beta-lactam ring in the antibiotic structure and made it ineffective for bacteria. With the development of beta-lactam antibiotics resistant to beta-lactamases, such as methicillin and oxacillin, MRSA strains emerged [3]. Resistance in MRSA results mainly from the expression of PBP2a, a penicillin-binding protein that is at least twofold less susceptible to β-lactams [2]. Today, in addition to methicillin-resistant strains, strains have emerged that are resistant to vancomycin, which is used in the treatment of MRSA strains. VanA operon containing Tn1546 transposon acquired from bacteria is one of the causes of vancomycin resistance.*Enterococcus* is. This operon leads to the change of D-alanine-D-alanine pentapeptide peptidoglycan, to D-alanine-D-lactate, and as a result vancomycin binding is no longer possible [4].

Among other causes of antibiotic resistance and also one of the virulence factors resistant strains*Staphylococcus aureus,*Ability to form biofilm Is. Biofilm or biolayer is a collection of microbial cells attached to a surface and covered by extracellular polymeric substances. Compared to free or planktonic bacteria, bacteria that form biofilms are more resistant to cellular stress such as antibiotics, toxic substances, heavy metals, lack of water, increase or decrease in environmental pH, and temperature fluctuations[5]. The benefits of biofilm for its constituent bacteria have made the treatment of infections caused by such bacteria a major challenge, as it is estimated that more than 80% of human infections are caused by biofilms [6;7]. At*Staphylococcus Oreos*Biofilm formation mainly by genes such as fibrinogen-binding proteins (fib), fibronectin-binding proteins (fnbA and fnbB), intercellular adhesion genes (icaA, B, C, D), clamping factors (clfB and clfA), It encodes elastin-binding protein (EBP), laminin-binding protein (Eno), biofilm-associated protein (Bap) and collagen-binding protein (CNA). The above genes encode various surface proteins to induce adhesion*Staphylococcus aureus* to the host, its penetration and colonization and ultimately lead to biofilm formation and pathogenicity. In addition, biofilm formation is usually mediated by the quorum sensing system[4] (Quantity sense) of bacteria is controlled [8].

One of the new approaches of researchers to fight against bacteria with multiple drug resistance is the development of drugs effective on biofilm formation and other virulence factors, as well as drugs effective on bacterial quorum sensing systems. Medicines that target biofilm and quorum sensing bacteria do not create a lot of selective pressure for the bacteria and therefore have a lower chance of developing drug resistance in the bacteria [9]. The review of past studies in recent years on the growth inhibitory effects, biofilm formation and quorum sensing of pathogenic bacteria by herbal products has shown that herbal products (including extracts, essential oils, as well as their effective and pure compounds) have significant effects. and can be considered as drug candidates to replace conventional antibiotics. There are some appropriate review articles in this field that can provide a better view of the mechanism of effect of plant compounds on the quorum sensing system and biofilm of bacteria [10, 11, 12].

Among the plants with significant antimicrobial and antioxidant activity, we can mention the plants of the mint family or Lamiaceae, which has many medicinal and aromatic plants such as lemon balm, mint, oregano, sage, hyssop, thyme, lavender, bramble, etc.

[1] Methicillin-resistant *Staphylococcus aureus* (MRSA)

[2]- Vancomycin-resistant *Staphylococcus aureus* (TRAP)

[3]- Penicillin-binding proteins (PBPs)

[4]- Quorum sensing (QS)

In this plant family, interesting effective compounds such as citronolal, thymol, citral, betacaryophyllene, linalool, carvacrol, alphapinene, etc. have been found. Both the essential oil and the effective compounds of the plant species of this family can be further investigated as new drug candidates[13].

It should be noted that in addition to biological properties, drug candidates should also be investigated in terms of pharmacokinetic properties, because in order to apply herbal products in the food and pharmaceutical industries, it is necessary to investigate the effects of drugs on microbial pathogens and on the human body. Since the laboratory investigation of the effectiveness and pharmacokinetics of drug candidates in the laboratory is time-consuming, costly and has safety risks, in recent years, the use of methods*In silic*o In-computer simulations such as molecular docking (in order to investigate the effectiveness of the drug candidate on the pathogen) and ADME/ADMET (in order to investigate the effects of the drug candidate on the body or drug pharmacokinetics) have been further developed. The meaning of drug pharmacokinetics is the study of things such as drug absorption[5], distribution[6], metabolism[7] and disposal[8] It is called ADME for short. The meaning of drug absorption is the process of entering and transferring the drug from the prescribed route into the bloodstream and plasma. Drug distribution means its penetration and diffusion into tissues and intercellular fluid. The meaning of drug metabolism is its breaking into active and acting components of the drug. The meaning of drug disposal is the process of cleaning and removing the remains of medicinal substances from the body. Sometimes the toxicity potential of the drug is also investigated [2, 14].

As far as the authors know, there has been no report on the effect of the studied essential oils of the mint family (sage, peppermint and lemon balm) on the growth and biofilm formation of the VRSA strain, and the previous studies were mostly on the extracts of these plants and on sensitive strains.*Staphylococcus aureus* have been focused and there are even few reports on the inhibitory effects of the essential oils of mint family plants on the growth and biofilm formation of MRSA strain [15-19]. So The purpose of this study is to evaluate the effect of these essential oils on the growth and biofilm formation of antibiotic resistant strains.*Staphylococcus aureus* in condition*In vitro* It was founded. Also, the mechanism of effect of the effective compounds of these essential oils on proteins effective in creating antibiotic resistance and proteins effective in biofilm formation*Staphylococcus Areos* It was investigated bioinformatically and using molecular docking. Finally, the pharmacokinetics of drug candidates were also investigated.

2- materials and methods

1-2. Materials used

Blank antibiotic disk (empty or without antibiotic) and methicillin, vancomycin and linezolid antibiotic disks were obtained from Padten Teb Company (Iran). The culture media used in this study is Tryptic Soy Broth[9] (TSB), Muller Hinton Brath[10] (MHB) as well as agar-agar, gram staining kit and crystal violet were obtained from Merck (Germany). A 96-well microtitre plate with polystyrene characteristics, with wells with smooth ends and without well treatment, from Biotest (China) was used.

2-2. Bacterial strains, media and culture conditions

Standard strain*Staphylococcus aureus* Methicillin resistant (ATCC 33591) from microbiology laboratory of Shahrood Azad University and resistant clinical strains*Staphylococcus aureus*, It was obtained from Imam Hossein (AS) Shahroud Hospital. Examining the antibiotic resistance tests of clinical strains using E-test showed that some of these strains do not form a halo of non-growth in any vancomycin concentration, and as a result, vancomycin-resistant strain (VRSA) was reported. By definition, strains whose vancomycin MIC is between 4-8 µg/ml are relatively vancomycin-resistant strains (VISA).[11]) and if their vancomycin MIC is above 16 µg/ml, vancomycin-resistant strain (VRSA) should be considered [20]. TSA culture medium was used to recultivate pure bacteria by linear culture method and MHA culture medium was used to check the antibacterial activity of essential oil by disc diffusion method. For long-term storage of strains, bacterial suspension with half McFarland turbidity (equal to the number of CFU[12]/mL $10^8$1x) and 30% glycerol were used and the samples were kept in a freezer at -80°C.

[5]- Absorption
[6]- Distribution
[7]- Metabolism
[8]- Excretion

[9]- Tryptic soy broth or Trypticase soy broth (TSB)
[10] -Mueller Hinton Broth (MHB)
[11]- Vancomycin-intermediate *Staphylococcus aureus* (VISA)
[12]

3-2. Investigated plants and preparation of essential oils

In this study, the studied plants (melon, marjoram, and peppermint) were collected by the Shahrood Agricultural Research Center in the summer of 1401 in the amount of 500 grams. Then the plants (shoots and leaves) were dried at room temperature in a dry and dark place. Preparation of essential oil, using the method of distillation with water and by the Cloninger machine[13] (Shimi Azma Gostar, Iran) according to the previous study protocol. The efficiency of essential oil production of lemon balm (0.74%), sage (0.66%) and peppermint (0.7%) was calculated according to the volume of essential oil obtained per dry weight of the plant (135 grams). The obtained essential oil has a density of about 9 mg/µLThey had 0.All the essential oils were kept in a freezer at -20°C until the experiments [21, 22].

4-2. Investigating the antibacterial activity of essential oil by disc diffusion method

In this test, 1.10, 1.100 and 1.1000 dilutions of essential oils were used, which due to the density of about 9 mg/µL0.0 of essential oils, 10 microliters of essential oils from the studied dilutions in equivalent order900, 90 and 9 micrograms. From tween 80[14] As an emulsifier or emulsifier[15] 3% volume/volume of essential oil and distilled water were used for dilution. The amount of 10 microliters of the obtained essential oil dilutions was placed on a sterile 6 mm antibiogram disc (empty or without antibiotics). Then the discs on the grass culture of the strains*Staphylococcus aureus* resistant to antibiotics, were placed in MHA culture medium with a distance of 1.5 cm from the sides of the plate and 2 cm from each other. The plates were placed in a 37°C incubator for 24 hours, and then the diameter of the non-growth halo was measured using a ruler. The antibiotic linezolid (at a concentration of 30 µg) was also used as a positive control. The amount of antibacterial activity of the studied essential oil, based on the results of the diameter of the halo of non-growth as passive (the diameter of the halo of no growth is less than 7 mm), relatively active (the diameter of the halo of no growth between 7-9 mm), active (the diameter of the halo of no growth between 9-12 mm) and very active (no growth halo diameter more than 12 mm) was reported [23].

5-2. Investigating the minimum growth inhibition concentration (MIC) and bacterial lethality (MBC) by microdilution method

The MIC protocol used in this research was performed according to the method of a previous study [13]. In this test, from MHB medium (50 microliters), dilutions of 1.20, 1.40, 1.80, 1.160, 1.320, 1.640 and 1.1280 essential oil (50 microliters) and the final concentration of bacteria*Staphylococcus aureus* In each well, equivalent to 10 CFU/mL^{5}5× (100 µl of bacterial suspension diluted 100 times with half McFarland turbidity equivalent to 10 CFU/mL^{6}5 × bacteria) were used. Also, in addition to the test wells, positive control or growth control wells (with culture medium and bacteria and without essential oil) in order to ensure the proper conditions for bacterial growth, as well as negative control or sterility control (with only culture medium and without bacteria and essential oil) also in order to confirm The absence of contamination during the work was considered. Finally, the microtitre plate was placed at 37°C for 24 hours and after incubation, the optical absorbance of the wells was read at a wavelength of 630 nm using an ELISA reader (Vira Teb Tehiz, Iran). The minimum concentration of the essential oil that resulted in at least 50% reduction in light absorption compared to the growth control was considered as the minimum inhibitory concentration of bacterial growth (MIC).[16]) Was considered. It should be noted that each essential oil dilution was done in 3 repetitions.

In order to find the minimum concentration of essential oil that causes bacterial death (MBC[17]), after determining the MIC, 10 microliters of the contents of the wells equal to the MIC concentration and higher were taken and cultured on TSA medium. The minimum lethal concentration of essential oil is defined as a concentration in which 99.9% of bacteria are destroyed, in other words, only 0.1% of bacteria survive. Considering that the culture sample volume is 10 microliters (10 mL).$^{-2}$) and the initial number of bacteria was 10 CFU/mL^{5}is 5x, so it is expected that the concentration of the essential oil that caused the formation of a maximum of 5 colonies in the plate will be considered as MBC (Equation 1).

[13]- Clevenger
[14]- Tween80
[15]- Emulsifier
[16]- Minimal inhibitory concentration (MIC)
[17]- Minimal bactericidal concentration (MBC)

Equation 1: $10^{-2}\ mL \times 5 \times 10^{5}\ \frac{CFU}{mL} \times \frac{0.1}{100} = 5\ CFU$

6-2. Investigating the minimum biofilm inhibition concentration (MBIC) by microdilution method

By definition, the minimum biofilm inhibition concentration (MBIC[18]), is the minimum concentration of an antimicrobial substance that prevents the biofilm formation of the studied organism [24]. In order to check MBIC, bacterial suspension with half McFarland turbidity (with optical absorbance of 0.08-0.13 at 630 nm wavelength and equal to 10 CFU/mL^{8}×1), obtained by using Mueller Hinton Broth (MHB) culture medium containing 1% glucose. Similar to the MIC determination method, bacterial suspension (100 microliters), plant essential oil (50 microliters) and sterile culture medium (50 microliters) were added to the microtiter plate wells. Also, wells of positive control or growth control (without adding essential oil) and negative control or sterility (sterile culture medium) were also considered. After the end of the incubation time, the contents of the microtitreplate are drained by inverting it slowly or with the help of a sampler so that only the bacteria forming the biofilm remain in the wells. In order to ensure the removal of planktonic cells, the wells were washed using 100 microliters of sterile physiological serum. Then, 100 microliters of 2% crystal violet was added to the wells and after 10 minutes, the wells were washed again. Finally, 100 microliters of 96-degree ethanol was added to all the wells, and then the optical absorption was read at a wavelength of 630 nm by an ELISA reader [25]. The percentage of inhibition of biofilm formation is calculated from equation 2, where C is the average absorbance of the control (positive control biofilm formation or bacteria in the absence of essential oil), T is the absorbance of the treated (or biofilm formation in the presence of essential oil) and B is the average absorbance of the negative control (or culture medium sterile) [26].

Equation 2: (C – T) / (C – B) × 100 = percent inhibition of biofilm formation

7-2. Investigating the minimum concentration of bacterial lethality in biofilm (MBEC) by microdilution method

In order to investigate the minimum concentration of bacterial lethality in biofilm ([19]MBEC) in the vicinity of plant essential oil, similarly to the MBIC determination method, Mueller Hinton Broth (MHB) culture medium containing 1% glucose was used to obtain a bacterial suspension with half McFarland turbidity. In the MBEC test, bacterial biofilm was first allowed to form in the absence of essential oil, then the tested essential oil was added to the test wells and the rate of growth inhibition or lethality of the bacteria present in the biofilm was studied in the vicinity of the essential oil. For this purpose, the bacterial suspension with half McFarland's standard turbidity by MHB culture medium containing 1% glucose, first diluted to 1.100 dilution and then 100 microliters of it was added to the test wells and positive control (or growth control). A blank microtitreplate column was also considered as a background control in ELISA readings. Then the microtitreplate was placed in an incubator at 37°C for 24 hours without moving or stirring. Finally, the contents of the microtitreplate are drained by inverting it slowly or with the help of a sampler, then 100 microliters of physiological serum is added to each well and the wells of the microtitreplate are drained again so that the planktonic bacteria are removed and not stained with the biofilm-forming bacteria. Then, 100 microliters of MHB medium was added to the wells of the first to ninth columns. Then, 100 microliters of essential oil with different dilutions from 1.10 to 1.640 was added to the wells of the first to seventh columns, and since the same volume of culture medium was also added to the wells, the essential oil dilutions in the said columns were equal to 20. It will be 1 to 1280/1. Column wells 8 and 9 were prepared as positive control (no essential oil) and negative control (no essential oil and bacteria), respectively, similar to the MIC method. Then the microtitreplate was placed in an incubator at 37°C for 24 hours. The method of washing, staining with crystal violet, adding ethanol and reading in the ELISA reader is exactly the same as the MBIC method. Also, the inhibition percentage of bacterial growth in biofilm was obtained using equation 2.

8-2. Investigating the effect of effective essential oil compounds on antibiotic resistance and bacterial biofilm using molecular docking

According to the results of microbial tests, the effective compounds of lemongrass essential oil were used for docking. First, compounds that have a concentration equal to or greater than 1% in the results of mass spectrometry - gas chromatography[20] (GC-MS), were identified [13]. Their molecular structure through the PubChem site (https://pubchem.ncbi.nlm.nih.gov/) were obtained in .sdf format and then converted to .pdb format by the Open Babel online server so that the structures can be used more easily in the docking process. The accession code and molecular formula of these compounds are given in.

[18]- Minimum biofilm inhibitory concentration (MBIC)

[19]- Minimum Biofilm Eradication Concentration (MBEC)

[20]- Gas chromatography–mass spectrometry (GC–MS)

Table 1. All these compounds are terpenoid compounds.

No.	Ligand Name	PubChem Compound identifier (CID)	Molecular Formula	Molecular Weight (g/mol)	Percent in essential oil	able 1 Characteristics of plant-derived natural ligands used in the molecular docking Group
1	Citronellal	7794	$C_{10}H_{18}O$	154.25	37	Aldehyde/Monoterpene
2	Thymol	6989	$C_{10}H_{14}O$	150.22	12	Phenol/Monoterpene
3	Citral	638011	$C_{10}H_{16}O$	152.23	10	Aldehyde/Monoterpene
4	B-Caryophyllene	5281515	$C_{15}H_{24}$	204.35	7	Sesquiterpene
5	1,3,8-P-Menthatriene	176983	$C_{10}H_{14}$	134.22	7	Monoterpene
6	Linalool	6549	$C_{10}H_{18}O$	154.25	3	Alcohol/Monoterpene
7	Carvacrol	10364	$C_{10}H_{14}O$	150.22	1	Phenol/Monoterpene

The structure of antibiotic ligands such as methicillin (PubChem CID=6087) and ceftaroline (PubChem CID=9852981) were also obtained from the PubChem website and converted to .pdb format. Antibiotics were used as a control in the docking process. It should be noted that the studied strains were sensitive to ceftaroline and resistant to methicillin.

In this study, of the 3 PBP2a protein structures*Staphylococcus aureus* It has been used for the docking process to check whether the different determined structures of the proteins can be effective in the docking results or not. The structural features of PBP2a protein are given in Table 2. In this table, the position of residues and key domains of PBP2a protein using UniProt KB server (https://www.uniprot.org/uniprotkb) Is obtained. In addition to PBP2a protein, proteins effective in quorum sensing and biofilm, namely agrA (accession code in PDB = 4g4k) and Bap (accession code in PDB = 7DM0) were also docked with plant and antibiotic ligands. 3D structures of all proteins from the RCSB BDB server (https://www.rcsb.org/) received.

Table 2 Structural characteristics of PBP2a protein effective in MRSA antibiotic resistance

Protein name	PDB code	Method	Structure	Catalytic residue(s)	UniProtKB	Sequence Length	Ligand(s)
PBP2a	5m18	X-RAY diffraction 1.98 Å	2 chains: A, B 26-140: MecA-N		Q7DHH4		beta-muramic acid D-Alanyl-D-Alanine
PBP2a	3zfz	X-RAY diffraction 2.25 Å	140-309: PBP-dimer 346-657: Transpeptidase	S403	A0A0H3JPA5	668aa (PDB:642aa)	Ceftaroline
PBP2a	1vqq	X-RAY diffraction 1. 8 Å			Q93IC2		None

In order to check the interaction between ligands and receptor (protein) in the most stable state in terms of energy and structure, before docking, all molecular structures were optimized in terms of interaction energy using UCSF chimera software (version 1.16) and also Swiss PDB viewer or spdbv (version 4-10). became. Then the structure with the lowest energy was selected and AutoDock Tools software was used for docking studies. The docking process was carried out using AutoDoc Vina, which is faster and more accurate than AutoDoc, in order to investigate the binding affinity of the effective compounds of lemon balm essential oil to proteins effective in antibiotic

resistance and biofilm formation [27]. AutoDock Tools software (version 4.2) initially receives protein structures and plant ligands in pdb format and converts them to pdbqt format by adding charge and atom type information. The characteristics of the grid box in the active site of PBP2a protein have the characteristics of the coordinates of the central points equal to x = 18.123, y = -20.86, z = -17.413 and the number of points in each axis is equal to x = 30, y = 30 and 30 = z is Also, the characteristics of the grid box in the allosteric site of PBP2a protein have the characteristics of the coordinates of the central points equal to x=15.347, y=-19.277, z=-50.318 and the number of points in each axis is equal to x=30, y=30 and z = 30. After the docking process, the best conformation with the lowest binding energy was selected as the result.

9-2. Examining the pharmacokinetic properties of drug candidates

from SwissADME servers (http://www.swissadme.ch/index.php) and admitSAR (http://lmmd.ecust.edu.cn/admetsar2) by using the SMILES code of compounds, the properties of drug candidates were predicted. SwissADME server, six physicochemical properties namely lipophilicity[21] (LIPO), size[22] (SIZE), polarity[23] (POLAR), solubility[24] (INSOLU), flexibility[25] (FLEX) and saturation[26] (INSATU) was considered. One of the unique features of this server is that, to make it easier to see the results, it provides a radar chart in which each axis represents one of these characteristics, and in this chart, the allowed range is shown as a pink area. In the radar diagram, once a drug-like molecule[27] It is considered not to exceed the permissible range. The permissible range of each of these 6 physicochemical properties is as follows: lipophilicity (XLOGP3 between +5 and -0.7), molecular size or weight (between 500-150 g/mol), polarity (range of topological polar surface area[28] or TPSA Ben $Å^2$20 to $Å^2$130), solubility (log S less than 6), saturation (the ratio of carbons in sp3 hybridization is not less than 0.25) and flexibility (no more than 9 rotatable bands) [28]. In addition, the SwissADME server provides an opportunity to evaluate Lipinski's rule of five[29] Provide. Lipinski et al. (1997) provided guidance for predicting orally absorbable compounds through the definition of Rule 5. Rule 5, based on the calculation of the distribution of properties of several thousand drugs, predicts that low absorption or permeation occurs when the target molecule has properties such as molecular weight greater than 500 daltons, lipophilicity factor (ClogP) greater than 5, number of hydrogen bond acceptor atoms greater than of 10 and the number of hydrogen bond donor atoms should be more than 5 [29]. Also, in this study, the admetSAR server was used to check the toxicity of the studied drug candidates.

10-2. Statistical analyses

In this study, the investigated experiments were performed in 3 repetitions. All statistical analyzes were performed using SPSS version 22 software and Kruskal-Wallis non-parametric test.[30] done. the amount of*P-value* Less than 0.05 was considered to be statistically significant.

3- Results

1-3. Antibacterial activity of essential oil

Among the investigated essential oils, lemongrass essential oil was more effective against MRSA and VRSA strains at concentrations of 90 micrograms (0.09 mg) and 900 micrograms (0.9 mg) per disk (Figure 1). Of course, at a concentration of 9 micrograms, the antimicrobial effects of lemongrass essential oil were not observed on any of the investigated strains. The diameter of the non-growth halo of MRSA was more than 12 mm in the presence of 900 μg concentration and

[21]- Lipophilicity
[22]- Molecular weight
[23]- Polarity
[24]- Solubility
[25]- Flexibility
[26]- Saturation
[27]- Drug-like
[28]- Topological polar surface area
[29]- Lipinski's rule of five
[30]- Kruskal–Wallis test

between 9-12 mm in the presence of 90 µg concentration. Therefore, in the investigated concentrations, the effect of lemongrass essential oil is reported to be very active (for a concentration of 900 µg) and active (for a concentration of 90 µg) against the MRSA strain. Also, the diameter of the halo of non-growth of VRSA in the presence of concentrations of 900 micrograms and 90 micrograms of lemon balm essential oil was in the range of 9-12 mm, and thus the effect of lemon balm essential oil against VRSA, in the mentioned concentrations, is active (for the concentration of 900 micrograms) or relatively active (for a concentration of 90 µg) is reported. Peppermint and sage essential oils were not effective on the VRSA strain and did not show growth halo.

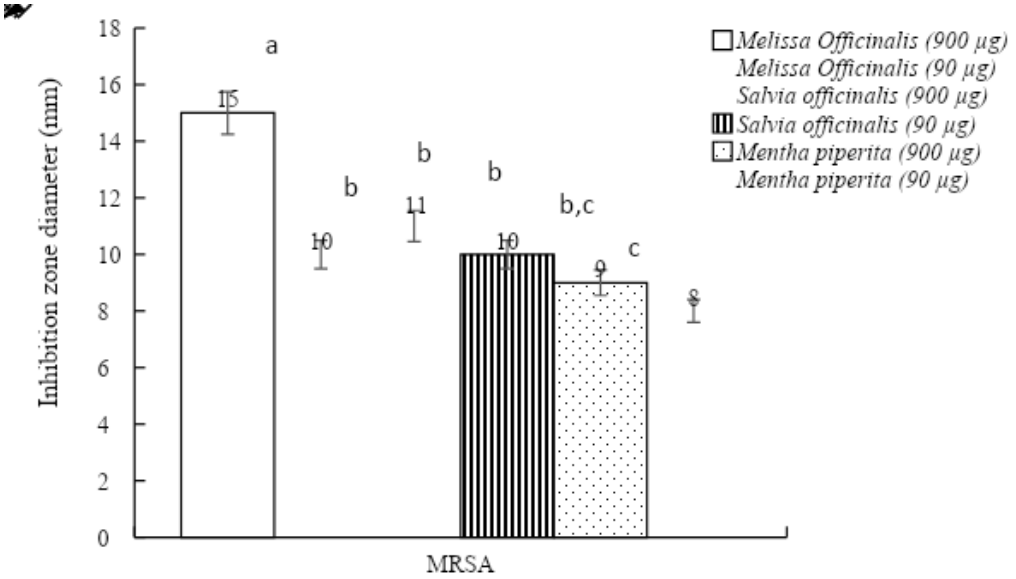


Fig 1 Antibacterial activity of the studied essential oils against antibiotic-resistant *Staphylococcus aureus* strains by disk diffusion method

2-3. Minimum Growth Inhibitory Concentration (MIC) and Bacterial Killing Concentration (MBC)

As shown in Figure 2, the MIC and MBC of VRSA strain are clearly different from MRSA strain. For example, the concentration of lemongrass essential oil that inhibits the growth and death of MRSA strain is 36 times and 22 times lower, respectively, than the concentration that causes the same effects in VRSA strain. Peppermint and sage essential oils had antimicrobial properties in higher concentrations than lemon balm.

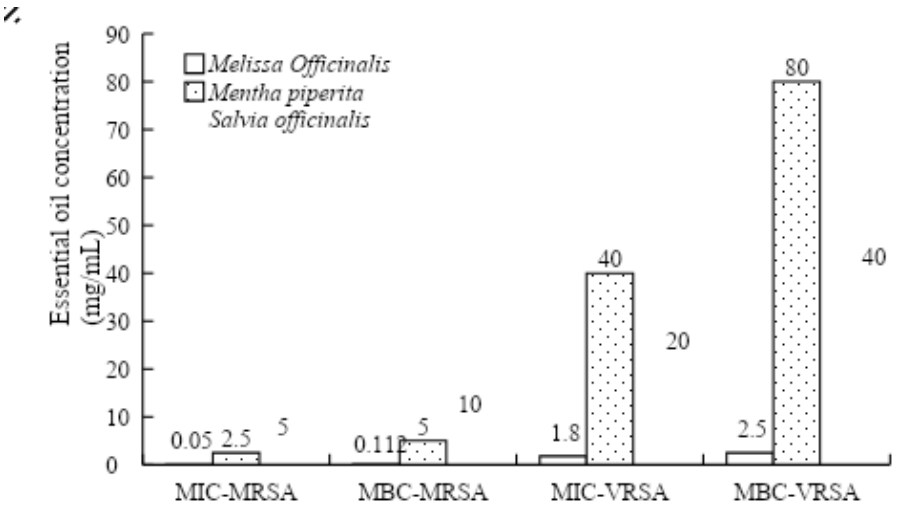


Fig 2 MIC and MBC of the studied essential oils against *Staphylococcus aureus* antibiotic-resistant strains

3-3. Minimum biofilm inhibition concentration (MBIC) and bacterial lethality in biofilm (MBEC)

The results of MBIC and MBEC are shown in Figure 3. The results confirm that the concentration of 30 micrograms (0.03 mg) of lemon essential oil inhibits the formation of MRSA strain biofilm. While, to inhibit the biofilm formation of VRSA strain, a much higher concentration of lemongrass essential oil (30 times), i.e. 900 micrograms (0.9 mg) is needed. Also, to inhibit more than 90% of the bacteria formed in the biofilm, a concentration of 0.1 mg/ml is needed in MRSA and a concentration of 3.2 mg/ml in VRSA strain of lemon essential oil.

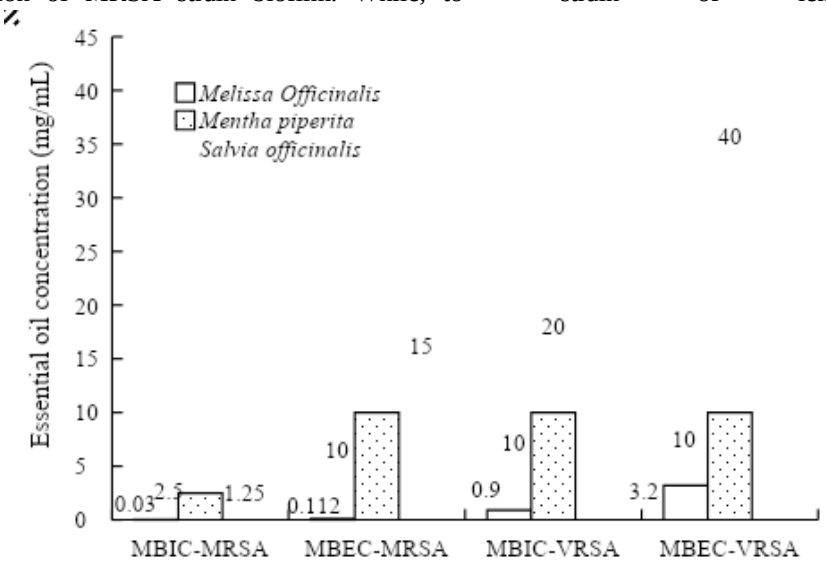


Fig 3 MBIC and MBEC of the studied essential oils against *Staphylococcus aureus* antibiotic-resistant strains

4-3. The effect of effective essential oil compounds on antibiotic resistance and bacterial biofilm using molecular docking

In this study, the main purpose of molecular docking is to investigate the effect of important components of lemongrass essential oil (citronellal, thymol, citral, betacaryophyllene, mentharin, linalool and carvacrol) on proteins.*Staphylococcus aureus* (PBP2a, agrA, Bap proteins). Also, this test is to compare the effects of antibiotics (ceftaroline and methicillin) and herbal ligands on the studied proteins, the active site and the allosteric site of the PBP2a protein, and also to check whether different structural files of the same protein can affect the docking results. It was used to be effective. The results of this test are shown in Figures 4 and 5. According to Figure 4, the effect of antibiotics and plant ligands investigated was

higher on PBP2a>agrA>Bap protein respectively. In other words, the results showed that all investigated ligands have lower binding affinity for agrA and Bap proteins. The docking results of the active site as well as the allosteric site of PBP2a protein using different structures of this protein (1vqq, 3zfz, 5m18) showed that in most cases similar results are obtained and the similarity obtained for the structures of 5m18 and 1vqq was more. Both mentioned structures have similar structural resolution. The obtained result confirms the other docking results of this study.

According to Figure 5, ceftaroline antibiotic has the highest binding energy with all studied proteins among all the studied ligands. Methicillin also ranks second. Therefore, none of the essential compounds had a higher binding energy than the antibiotic ceftaroline and even methicillin. Among the examined compounds, beta-caryophyllene showed the highest binding affinity for both the active site and the allosteric site of PBP2a protein, followed by carvacrol and thymol compounds.

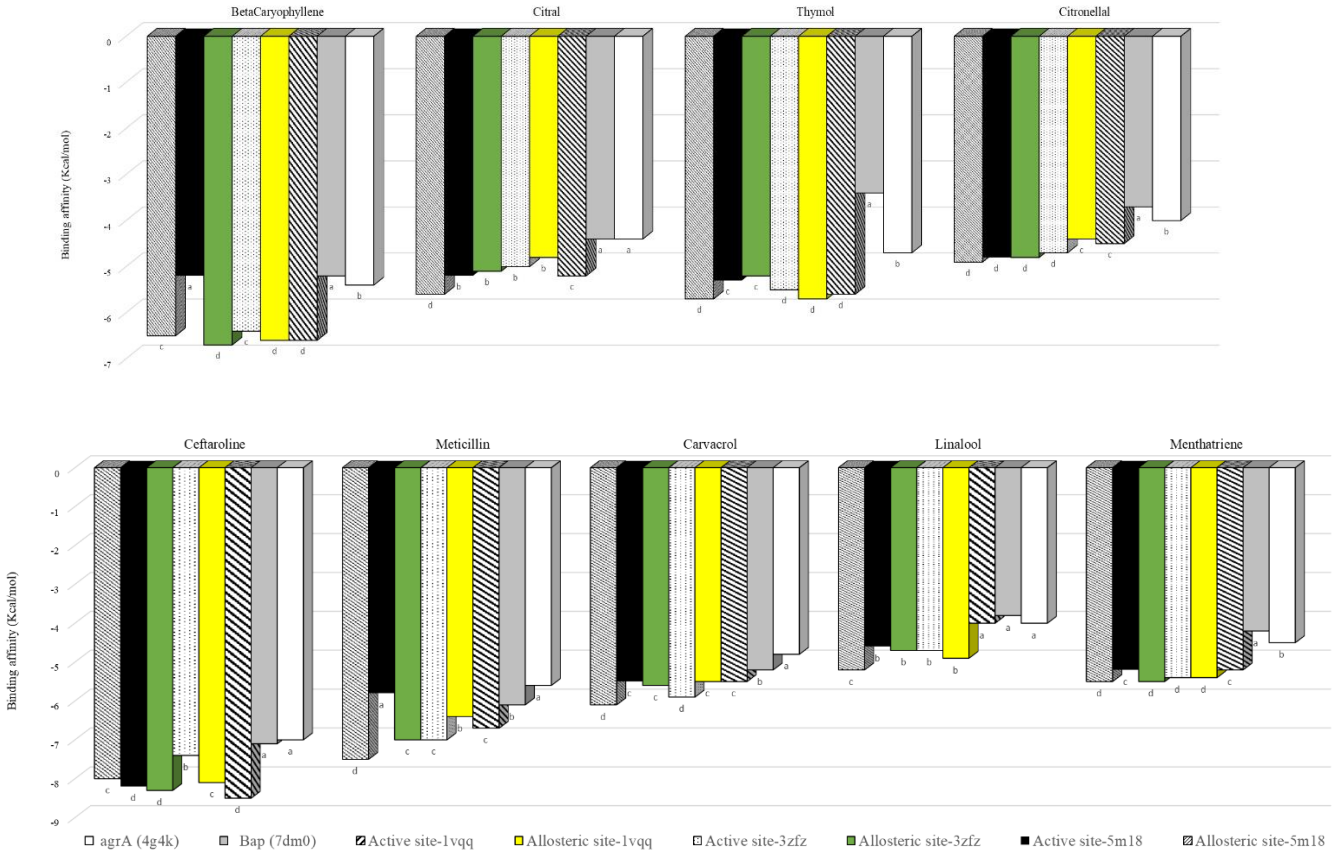


Fig 4 Molecular docking results. Lowercase letters near each column represent statistically significant differences in the results (*p-value* <0.05). Columns having the same letter are not significantly different.

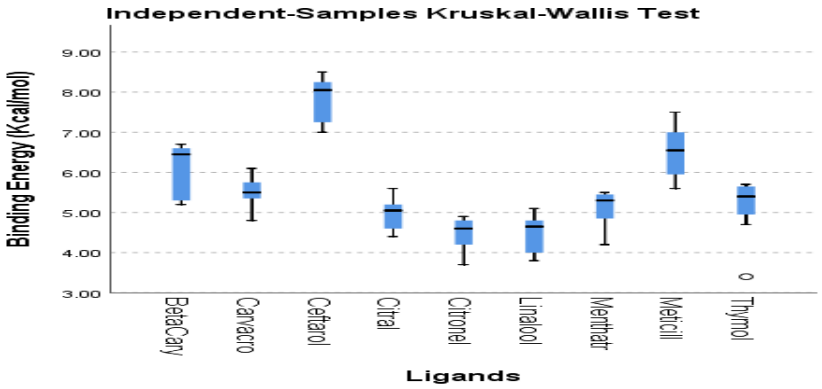
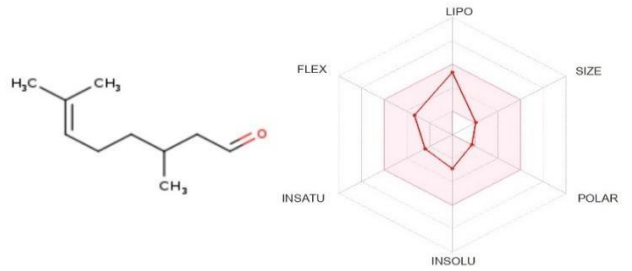


Fig 5 Analysis of molecular docking results using Kruskal-Wallis test

5-3. Pharmacokinetic properties of drug candidates

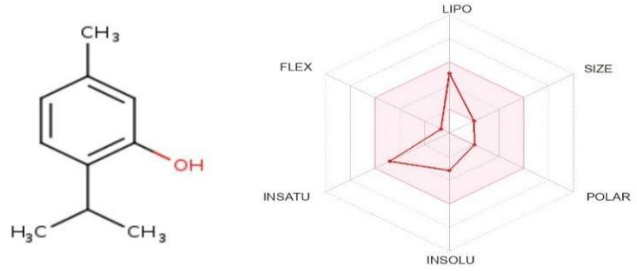
The results of pharmacokinetic properties and drug-likeness of the compounds by the SwissADME server are given in Figure 7. As it can be seen in this figure, all the investigated compounds in this study are placed in the allowed range in the radar diagram and can be considered as medicine-like.

(Citronellal)



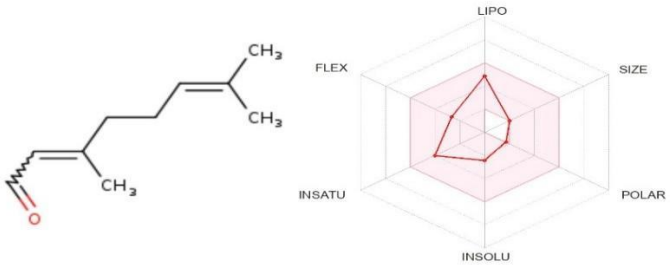
SMILES O=CCC(CCC=C(C)C)C

(Thymol)



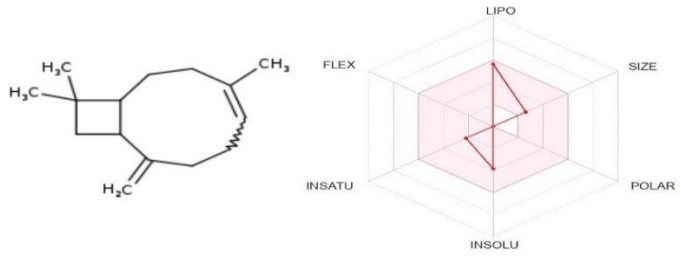
SMILES Cc1ccc(c(c1)O)C(C)C

(Citral)



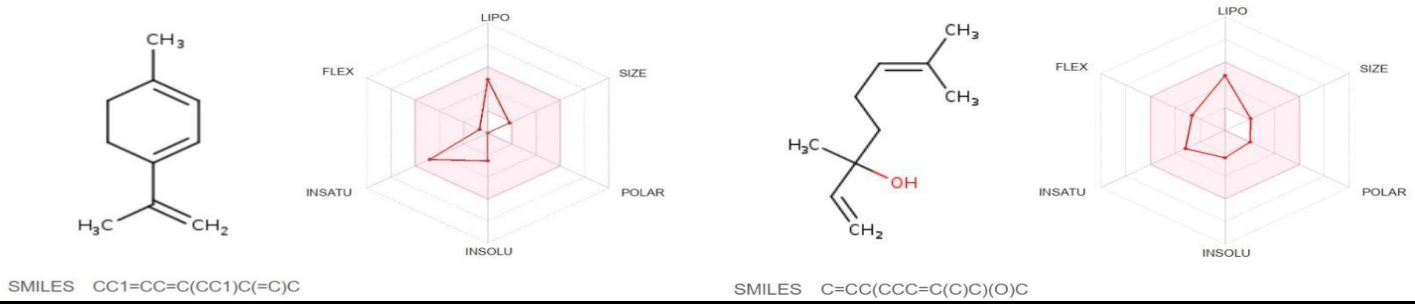
SMILES O=CC=C(CCC=C(C)C)C

(B-Caryophyllene)



SMILES CC1=CCCC(=C)C2C(CC1)C(C2)(C)C

(1,3,8-P-Menthatriene) **(Linalool)**



(Carvacrol)

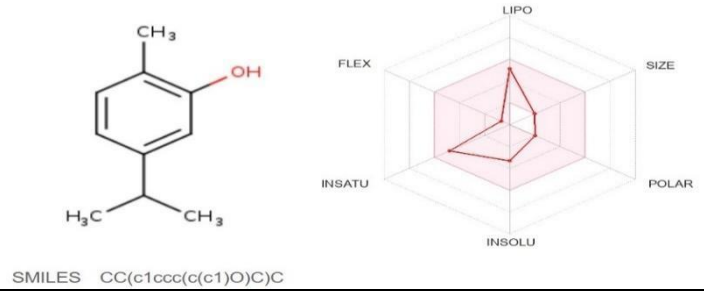


Fig 6 Physicochemical properties of the effective compounds of the studied essential oils by the SwissADME server

Also, the pharmacokinetic characteristics of the studied herbal ligands are given in Table 3. In this table, GI absorption means BBB permeant.[31]), P-gp substrate (glycoprotein P substrate which is a transfer protein and plays an important role in multidrug resistance by active transfer of drugs from inside the cell to outside the cell), CYP inhibitors (various cytochrome P450 (CYP450) inhibitors) which are used to produce several factors including cholesterol and steroids and are necessary for detoxification of foreign chemicals and drug metabolism), Log Kp (a measure to express skin penetration).

Drug toxicity results obtained from serveradmitSAR It is listed in Table 4. In this table, the meaning ofCarcinogenicity (binary) AndCarcinogenicity (trinary) are the investigated models in the classification of the compound's carcinogenic potential [30]. hERG refers to the alpha subunit of the potassium ion channel, which plays an important role in the electrical conduction of the heart and helps coordinate the heartbeat. The meaning of aromatase[32] or estrogen synthetase, is an enzyme responsible for a key step in estrogen biosynthesis [31]. meaning ofPPAR range[33] Peroxisome proliferator activator receptor gamma is a nuclear receptor that acts as a transcription factor and plays a role in fatty acid storage and glucose metabolism. PPAR family of nuclear receptors play a major regulatory role in energy homeostasis and metabolic function [32].

[31]- Blood-brain barrier penetration (BBB)

[32] -Aromatase

[33]- Peroxisome proliferator- activated receptor gamma (PPAR-γ or PPARG)

Table 3 Pharmacokinetic characteristics of the effective compounds of the studied essential oils by SwissADME server

Pharmacokinetics	Citronellal	Thymol	Citral	B-Caryophyllene	1,3,8-P-Menthatriene	Linalool	Carvacrol
GI absorption	High	High	High	Low	Low	High	High
BBB permeant	Yes	Yes	Yes	No	Yes	Yes	Yes
P-gp substrate	No	No	No	No	No	No	No
CYP1A2 inhibitor	No	Yes	No	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	Yes	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No
Log Kp (skin permeation)	-4.52 cm/s	-4.87 cm/s	-5.08 cm/s	-4.44 cm/s	-4.97 cm/s	-5.13 cm/s	-4.74 cm/s
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4 Medicinal toxicity characteristics of the effective compounds of the studied essential oils by the admetSAR server

Admet predicted profile	Citronellal	Thymol	Citral	B-Caryophyllene	1,3,8-P-Menthatriene	Linalool	Carvacrol
Carcinogenicity (binary)	-	-	-	-	-	-	-
Carcinogenicity (trinary)	Non-required	Non-required	Non-required	Warning	Warning	Non-required	Non-required
Eye corrosion	+	+	+	-	+	-	+
Eye irritation	+	+	+	+	+	+	+
Ames mutagenesis	-	-	-	-	-	-	-
hERG (the human Ether-à-go-go-Related Gene) inhibition	-	-	-	+	-	-	-
Micronuclear	-	-	-	-	-	-	-
Hepatotoxicity	+	+	+	-	+	-	-
skin sensitization	+	+	+	+	+	+	+
Respiratory toxicity	-	-	-	-	-	-	-
Reproductive toxicity	-	-	-	-	+	-	-
Mitochondrial toxicity	-	+	-	-	-	-	+
Nephrotoxicity	+	-	+	+	+	+	-
Acute Oral Toxicity (c)	III	III	III	III	III	III	III
Estrogen receptor binding	-	-	-	-	-	-	-
Androgen receptor binding	-	-	-	-	-	-	-
Thyroid receptor binding	-	-	-	-	-	-	-
Glucocorticoid receptor binding	-	-	-	-	-	-	-
Aromatase binding	-	-	-	-	-	-	-
PPAR range	-	-	-	-	-	-	-
Biodegradation	+	-	+	-	+	+	-

4- Discussion

Reports on the presence of antibiotic resistant strains*Staphylococcus aureus* And especially MRSA from various food sources, including processed meats, as well as the spread of life-threatening hospital infections, including pneumonia, bacteremia, and surgical wound infections caused by this bacterium, is a serious alarm that can cause major clinical problems in the coming years if antibiotic alternatives are not found. have a clue Due to the widespread antibiotic resistance of beta-lactams, the first line of treatment for serious infections of MRSA strains is vancomycin (a glycopeptide antibiotic). Both beta-lactam antibiotics and vancomycin eventually weaken the integrity of the bacterial cell wall and lead to bacterial lysis. However, reports of increased vancomycin resistance have encouraged researchers to find more suitable drug candidates [33]. However, in the development of modern drugs, in addition to evaluating their efficiency, the ability of drugs to achieve their goals in bioactive form should also be investigated. These investigations include clinical trials at the cellular, animal, and human levels, which are often costly and fraught with safety risks. In this regard, the use of bioinformatics tools that can help by saving time and money will be a way forward [34]. In this study, to investigate the effects of some essential oils of the mint family (sage, peppermint and lemongrass) on antibiotic resistant strains.*Staphylococcus aureus* paid. Among the examined essential oils, lemongrass essential oil showed better results than sage and peppermint essential oils. Preliminary results using disk diffusion test showed that lemongrass essential oil at a concentration of 9 micrograms had no inhibitory effect on either MRSA or VRSA strains. In the concentration of 90 µg and 900 µg, its effect on MRSA strain (10 and 15 mm) was more than VRSA (9 and 11 mm). However, it has led to the creation of an aura of lack of growth in both directions. Disc diffusion results on susceptible strains*Staphylococcus aureus*, it was equal to 23 mm by lemon balm extract in the study of Ahmadi et al. (2016), equal to 15 mm by lemon essential oil in the study of Alizadeh et al. (2014), and in the study of Arjang et al. [15, 16, 17]. Comparison of the results of the present study with the results obtained from previous studies on sensitive strains*Staphylococcus aureus* have worked, shows that lemon balm can inhibit the growth of strains with antibiotic resistance to methicillin and vancomycin*Staphylococcus aureus* also be successful. Also, comparing the disc diffusion results of the present study with previous studies that worked on the MRSA strain, such as the study by Abu-Shanab et al. The study of Sipahi et al. (2022), who investigated the essential oil (25 mm) of lemongrass on MRSA strain, has been interesting [18, 19]. The reason for the difference in the results obtained in different studies, in addition to the unknown concentration of lemongrass extract or essential oil in the disk diffusion test of previous studies, can be due to different test conditions, differences in the concentration and type of plant compounds and MRSA strains, but stillIt confirms that lemongrass essential oil can have antibacterial effects on antibiotic resistant strains.*Staphylococcus aureus*have The MIC and MBC of lemongrass essential oil on MRSA strain in the present study were 0.05 and 0.112 mg/ml, respectively. While MIC and MBC of lemongrass essential oil on VRSA strain were equal to 1.8 and 2.5 mg/ml, respectively. In the MIC and MBC tests, at concentrations close to 9 micrograms of essential oil, no inhibitory effect was observed on the investigated strains, which is in agreement with the results of the disk diffusion test. In the study of Erjang et al. (2014) MIC and MBC of lemon balm essential oil on sensitive strains*Staphylococcus aureus* Above 200 micrograms or 0.2 mg/ml in both tests and in Alizadeh et al.'s study (2014), the MIC level was 0.25 mg/ml and the MBC level was reported as 2.5 mg/ml [16, 17]. Therefore, it can be said that lemon balm essential oil in the present study has a more inhibitory or lethal effect than the mentioned studies; Despite the fact that in the mentioned studies of sensitive strains*Staphylococcus aureus* were used and the results of the disc diffusion test of the present study were similar to them. Therefore, it can be concluded that the comparison of antibacterial effects between different studies should not be done based on the disk diffusion test, but the microtitreplate test, in which specific and different concentrations of the antimicrobial substance are examined, is more preferable for comparison. In both MRSA and VRSA strains, the concentration of essential oil that kills planktonic bacteria (MBC) was 2 times higher than the concentration that inhibits the growth of planktonic bacteria (MIC). Since antimicrobial compounds are considered bactericidal if their MIC and MBC do not differ by more than 4 times, it can be said that lemongrass essential oil is effective against strains with antibiotic resistance.*Staphylococcus aureus* It has shown bactericidal properties [35]. In addition to inhibiting bacterial growth, lemongrass essential oil showed positive results on biofilm formation (MBIC) and also on inhibiting the growth of bacteria in biofilm (MBEC). The results of MBIC and MBEC tests in MRSA strain

are 0.03 mg/ml and 0.112 mg/ml, respectively. Also, the results of MBIC and MBEC tests in VRSA strain are equal to 0.9 mg/ml and 3.2 mg/ml, respectively. Comparing these results with the results of MIC and MBC of lemon balm essential oil, it can be said that in each of the two methods studied, lemon balm essential oil at a lower concentration leads to the inhibition of biofilm formation (MBIC) and at a higher concentration leads to the inhibition of bacterial growth (MIC) (Figures 2 and 3). Interestingly, similar concentrations of essential oil are required to kill planktonic bacteria (MBC) and biofilm bacteria (MBEC) of MRSA strain. But in the case of VRSA strain, a higher concentration of lemongrass essential oil was required to inhibit the growth of bacteria in biofilm (MBIC = 3.2 mg/mL) than planktonic bacteria (MBC = 2.5 mg/mL). This result shows that biofilm formation in VRSA strain had an effect on the resistance of this strain to essential oil. Due to the observation of the bactericidal property of lemongrass essential oil, one of the antimicrobial mechanisms of compounds that can have bactericidal effects is the effect on the synthesis of the cell wall of bacteria [36]. Therefore, in molecular docking, to investigate the interaction of the effective compounds of lemon balm essential oil on the protein involved in wall synthesis.*Staphylococcus aureus* resistance, namely PBP2a, was investigated, in order to possibly determine the mechanism of action of lemongrass essential oil on this bacterium. In addition to investigating how lemongrass essential oil compounds interact with the active site of PBP2a, their interaction with the allosteric site of PBP2a was also studied, because[34] et al. (2017) reported that the allosteric site of PBP2a, which is distant from the active site of this enzyme, when properly occupied by a β-lactam antibiotic or a substrate (such as a neighboring peptidoglycan strand), gatekeeper residues[35] opens in the active site and rearranges the conformation of key residues to enable catalysis. Thus, PBP2a will not be inhibited by beta-lactam antibiotics. Also, this hypothesis is proved by observing that ceftaroline (one of the two effective cephalosporins against MRSA) binds covalently to the active site and non-covalently to the allosteric site. In addition, point mutations in the allosteric site of PBP2a have been observed in recently discovered ceftaroline-resistant clinical strains. Allosteric regulation of PBP2a activity seems to be of considerable evolutionary importance [2]. The results of this study have also shown (Figure 5) that antibiotics Ceftaroline has a higher binding energy than methicillin both in the active site and in the allosteric site. This result can clarify the reason for the sensitivity of MRSA strains to this antibiotic.Among the compounds of lemongrass essential oil, beta-caryophyllene showed the highest binding energy with the active site and allosteric site of PBP2a protein. The binding energy of beta-caryophyllene was comparable to methicillin both in the allosteric site and in the active site, while the rest of the analyzed compounds of lemon essential oil showed lower binding energy in both PBP2a protein sites. Beta-caryophyllene, or trans-caryophyllene, is a bicyclic sesquiterpene found in several plants, including cinnamon, rosemary, and red pepper. This molecule is produced from the precursor molecule acetyl-CoA during the mevalonate pathway in plants. Betacaryophyllene has attracted the attention of researchers both because of its unique structure that is unusual in nature and because of its pleasant sweet aroma [37, 38, 39]. It should be noted that betacaryophyllene is a selective phytocannabinoid agonist of type 2 receptors ([36]CB2-R), but due to its lack of affinity for cannabinoid receptor type 1 (CB1), it is not considered a psychoactive compound. This compound has anticonvulsant, analgesic, sedative and anti-depressant effects. Among various biological activities, β-caryophyllene exerts anti-inflammatory effects by inhibiting key inflammatory mediators, such as inducible nitric oxide synthase (iNOS), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor (TNF). has shown [38]. In addition, the results of other studies on the effectiveness of beta-caryophyllene in infections*streptococcus* have shown [40].

Nevertheless, the docking results of this study showed that none of the effective compounds of the studied lemongrass essential oil had a higher binding affinity than the studied beta-lactam antibiotics either in the active site or in the allosteric site. These results can seem in contradiction with the results of laboratory tests that lemon essential oil was effective on MRSA and VRSA strains. But it should be kept in mind that in essential oil, groups of effective compounds are

[34]- It's great

[35]- Gatekeeper residues

[36]- Cannabinoid receptor type 2 (CB2-R)

examined together, but in the docking process, each compound is analyzed separately. In other words, possible synergistic effects of combinations should also be considered. On the other hand, the functional mechanism of the effective compounds of the essential oil can not affect the wall synthesis, in other words, the PBP2a protein. Therefore, in order to find out the functional mechanism of these compounds, more research is needed. Therefore, in this study, the effect of lemon balm essential oil compounds on proteins effective in biofilm formation, including biofilm-associated protein (Bap) and agrA protein, was investigated. agrA protein is one of the components The classical two-component signaling system is the quorum sensing system (response regulation protein) and, when activated, leads to a large increase in transcription from promoters.P2 AndP3The agr operon is at the end of the log phase of the growth curve, that is, when the concentration of signal molecules in the environment is high[41]. Among the studied plant ligands, betacaryophyllene and carvacrol have the highest binding affinity to proteins.Bap and agrA showed. However, their binding affinity for the mentioned proteins is lower than PBP2a protein.

Considering the positive effects of lemongrass essential oil on resistant strains*Staphylococcus aureus*, the compounds of this essential oil were examined by ADMET analysis servers in terms of different properties and the possibility of being drug-like (Tables 3 and 4). In general, it can be said that all the investigated compounds showed significant characteristics in this analysis and obeyed the 5-law of Lippisenki, and therefore, if used as medicine, they can be orally absorbed. Also, the results of this study showed that all compounds (except beta-caryophyllene) were not inhibitors of different types of cytochrome p450, so these compounds do not cause a disturbance in the detoxification of foreign chemicals and the metabolism of drugs in the body. Inhibition of various cytochrome P450 isoforms (most notably, CYP3A4) by beta-caryophyllene has also been reported by previous studies [38]. Also, the examined compounds, in carcinogenic potential tests (modelbinary, Modeltrinary and Ames test) did not show carcinogenic properties. It should be noted that many studies*In vitro* And*In vivo*, show that beta-caryophyllene even has antitumor properties and the phenotype of animals used to model tumors (colon, breast, pancreas, lymphoma, melanoma and glioma cancer) as well as nervous system diseases (Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, stroke)) improves [38]. Also, since none of the compounds were P-gp or P-glycoprotein substrates, if they are used as drugs, multi-drug resistance will not be observed.

5. Conclusion

In general, according to the results obtained in this study, it can be said that lemon essential oil has good antimicrobial and anti-biofilm effects against MRSA and VRSA strains of bacteria.*Staphylococcus aureus* shows. Also, In-Silco investigations of the compounds of this essential oil against methicillin resistance protein (PBP2a), quorum sensing (agrA) and biofilm production (Bap) showed that these compoundsprobably act synergistically. Among the components of lemongrass essential oil, betacaryophyllene and carvacrol showed better results against PBP2a, agrA and Bap proteins. On the other hand, the results obtained from the ADMET analysis of the effective compounds of this essential oil were also acceptable. Therefore, lemongrass essential oil can be used as a natural antimicrobial agent with few side effects to prevent as well as treat infections.*Staphylococcus aureus*including infections caused by food.

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6- Resource

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خصوصیات ضدمیکروبی، ضد بیوفیلمی و فارماکوکینتیکی برخی از اسانس های خانواده نعناعیان علیه *استافیلوکوکوس اورئوس* مقاوم به متی سیلین و ونکومایسین

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چکیده

وجود *استافیلوکوکوس اورئوس* مقاوم به متی‌سیلین (MRSA) و ونکومایسین (VRSA) در مواد غذایی، یک نگرانی جدی برای سلامت عمومی ایجاد کرده است. هدف از این مطالعه، بررسی فعالیت ضدمیکروبی و ضدبیوفیلمی برخی از اسانس‌های خانواده نعناعیان شامل بادرنجبویه، مریم گلی و نعنا فلفلی، بر سویه‌های MRSA و برای اولین بار بر VRSA است. بدین منظور، تست‌های دیسک دیفیوژن، تست حداقل غلظت مهاری رشد (MIC)، حداقل غلظت باکتریسیدالی (MBC)، حداقل غلظت مهار تشکیل بیوفیلم (MBIC) و حداقل غلظت موردنیاز برای مهار باکتری‌های موجود در بیوفیلم (MBEC) تعیین شد. سپس ترکیبات مؤثره اسانس بادرنجبویه علیه پروتئین‌های PBP2a، agrA و Bap با استفاده از اتوداک وینا موردبررسی قرار گرفت و درنهایت، خصوصیات فارماکوکینتیک آن‌ها با استفاده از سرورهای ADMETsar و SwissADME بررسی شد. بر اساس نتایج به‌دست‌آمده، میزان MIC و MBC اسانس بادرنجبویه بر روی سویه MRSA به ترتیب برابر با ۰/۰۵ و ۰/۱۱۲ میلی‌گرم/میلی‌لیتر و بر روی سویه VRSA به ترتیب برابر با ۱/۸ و ۲/۵میلی‌گرم/میلی‌لیتر به دست آمد. میزان MBIC و MBEC اسانس بادرنجبویه در سویه MRSA به ترتیب برابر با ۰/۰۳میلی‌گرم/میلی‌لیتر و ۰/۱۱۲میلی‌گرم/میلی‌لیتر و در سویه VRSA به ترتیب برابر با ۰/۹میلی‌گرم/میلی‌لیتر و ۳/۲ میلی‌گرم/میلی‌لیتر شد. نتایج داکینگ مولکولی نشان داد که ترکیب بتاکاریوفیلن چه در جایگاه فعال و چه در جایگاه آلوستریک پروتئین PBP2a انرژی اتصالی بالاتری از بقیه ترکیبات موردبررسی نشان می‌دهد (۶/۶- کیلوکالری بر مول). از طرفی، ترکیبات مؤثره این اسانس، مخصوصاً سیترونلال، تیمول و سیترال ازنظر خصوصیات فارماکوکینتیک قابل‌قبول بودند. از آنجایی که آنتی‌بیوتیک‌های طبیعی می‌توانند جایگزین مناسبی برای آنتی‌بیوتیک‌های معمولی در درمان بیماری‌های ناشی از موادغذایی *استافیلوکوکوس اورئوس* باشند، نتایج این مطالعه نشان داد که اسانس بادرنجبویه بر رشد و بیوفیلم سویه‌های MRSA و VRSA مؤثر است و می تواند به عنوان یک کاندید داروئی در پیشگیری و درمان عفونت‌های ناشی از سویه‌های دارای مقاومت آنتی‌بیوتیکی این باکتری مورد استفاده قرار گیرد.