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RESEARCH ARTICLE

Crude Extracts of Three *Iris* Species as Sources of MRSA Antimicrobial Compounds

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ABSTRACT

Objective: *Iris* species are widely used in pharmaceutical and cosmetic applications owing to their high content of bioactive compounds with anti-inflammatory and antimicrobial properties. This study aimed to investigate the potential antibacterial effect of crude extracts (aqueous, 50% and 80% ethanol) of three *Iris* species (*I. pumila*, while *I. reichenbachii* and *I. illyrica* are endemic) from Bosnia and Herzegovina against the multiresistant bacterial strain methicillin-resistant *Staphylococcus aureus subsp. aureus* ATCC 33591 (MRSA strain).

Materials and Methods: The antimicrobial compounds in the crude extracts were identified using High-performance liquid chromatography (HPLC), and their effects on the MRSA strain were tested using agar well diffusion and broth microdilution method. The binding affinities were analysed using molecular docking simulations.

Results: We identified bioactive targeted compounds in these extracts, mainly flavonoids named isorhamnetin, hesperidin, quercetin, fisetin, genistein, and kaempferol. Antibacterial assays showed that extracts of all three *Iris* species inhibited MRSA. The binding affinity analysis showed that isorhamnetin and hesperidin had the highest affinity scores, stronger (isorhamnetin) or the same (hesperidin) as the positive control ceftobiprole.

Conclusion: This*in vitro* and *in silico* study showed that *Iris* species represent a valuable source of bioactive compounds that can be used against multidrug-resistant strains such as MRSA. The potential use of these agents in multiple drugs is warranted, and further evaluation for human application is needed.

Keywords: Plant bioactive compounds, Methicillin-resistant *Staphylococcus aureus*, Molecular docking, Minimum inhibitory concentration.

INTRODUCTION

Staphylococcus aureus is a Gram-positive facultative anaerobic bacterium commonly found in the body as part of its microbiota.[1] During the production of virulence factors, such as extracellular toxins and enzymes or cell surface protein expression, *S. aureus* can promote infections by becoming a pathogenic strain.[2] These high lethality rates can include infections of the skin and soft tissues, endovascular infections, pneumonia, endocarditis, sepsis, and similar.[3] It is one of the most important human bacterial pathogens that can cause infection in almost any human tissue. The treatment of these infections can be problematic due to the presence of antibiotic-resistant *S. aureus*. A subgroup that has developed drug resistance primarily to β-lactams is methicillin-resistant *S. aureus* (MRSA). Decreased affinity for β-lactams or β-lactams resistance of *S. aureus* has been developed due to the presence of *mecA*, which encodes penicillin-binding protein 2a (PBP2a).[4] MRSA has become one of the greatest threats in clinical medicine, resulting in difficult prediction and expensive treatment.

Despite intensive research and development of a new broad range of antibiotics, there is still a need for their effectiveness confirmation.[5] The progress in combating antimicrobial resistance is greatly reduced because of the effects of the pandemic.[6] Thus, the incidence of the increased number of drug-resistant

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pathogens is highlighted as a primary investigation field by various pharmaceutical and scientific communities; they recognised the potential in alternative treatments with the usage and drug repurposing of plant-derived substances.[3] Because natural compounds constitute the majority of currently used clinical antibiotics, numerous plant extracts are continuously important sources of antimicrobials.[3,7]

Worldwide, many endemic plant species have shown significant antimicrobial activity.[8-11] These studies highlight the potential of endemic plants as sources of new antimicrobial agents. Within the Iridaceae family, *Iris spp.* represent the largest genus and one of the most significant families of flowering plants, ranging throughout Eurasia and North America, with a vast diversity of species.[12] Many secondary metabolites that have been identified from *Iris* species have demonstrated various biological properties, including antimicrobial, antioxidant, anti-inflammatory, antitumor, and immunomodulatory effects.[13] The unique chemical compounds that endemic plants often produce are potential sources of novel antimicrobial agents that may be effective against resistant strains of bacteria and fungi.[14] Research on the medicinal properties of endemic plants also highlights their ecological importance and promotes conservation efforts that are crucial for maintaining biodiversity and protecting ecosystems.[15]

This study explored the antimicrobial *in vitro* activity of different *Iris* plant extracts from Bosnia and Herzegovina, including dwarf iris, *Iris pumila* L., *Iris reichenbachii* Heuff., and Illyrian iris, *Iris illyrica* Tomm., against methicillin-resistant *S. aureus*, as well as the confirmation of their potential antimicrobial properties using molecular docking simulation studies.

MATERIALS AND METHODS

Preparation of Plant Extracts

Plant material from three different *Iris* species was collected in the period of April-June 2021 in different regions of Bosnia and Herzegovina (*I. pumila*-Kalinovik region, *I. reichenbachii*-Kladanj region, and *I. illyrica*-Neretva Canyon). Clean and air-dried rhizomes were macerated and pulverised using an IKA mill and dissolved in a 1:1 volume ratio of the solvents (distilled water, 50% ethanol, or 80% ethanol). Following evaporation, the extracts were dissolved in absolute ethanol and filtered through a PTFE syringe sterile filter (LabExpert, Slovenia, 0.45 µm) under sterile conditions. The final concentration of the crude extract was 330 mg/mL.

Componential Analysis of Target Compounds

High-performance liquid chromatography (HPLC) was used to identify potential antimicrobial compounds in *Iris* species plant extracts using an Agilent Infinity II 1260 HPLC system. Analysis was performed at a constant temperature of 40°C during a 55 min run. HPLC standards for the targeted compounds (fisetin, quercetin, kaempferol, isorhamnetin, genistein, and hesperidin) were acquired from Sigma-Aldrich, USA.

Evaluation of Antibacterial Activity

The antimicrobial properties of *Iris* species extracts were tested against the multi-drug-resistant bacteria *Staphylococcus aureus* subsp. *aureus* ATCC 33591 (MRSA strain) using agar well diffusion and broth microdilution. The MRSA strain was cultivated on Mueller–Hinton (MH) medium overnight at 37°C as part of the agar well diffusion method.[16] The cultured MRSA strain was used at 1-2 x 10^8 CFU/mL as the inoculum, obtaining uniform homogeneous turbidity corresponding to 0.5 McFarland.[17] Then, 50 µL of each extract was added to the wells made by drilling the plates with a sterile borer. Tests were performed in triplicate. Clear inhibition zones were measured after incubation at 37°C for 24 h. We used several antibiotics as positive controls: Colistin (10 µg), Streptomycin (10 µg), Ampicillin (10 µg), and Amoxicillin (25 µg), all made by Oxoid™, Great Britain. The broth microdilution method[18] was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The 50 µL of liquid Mueller–Hinton Broth (Sigma Aldrich) was added to every well in a sterile microtiter plate, except in the wells of the first column. Subsequently, serial two-fold dilutions of the tested Iris species extracts were added in the 165-0.32 mg/mL range. After that, 50 µL of the MRSA suspension was added to the first eleven columns, at approx. 5x10^5 CFU/mL concentration. The eleventh column contained only the standard inoculum used as the positive control, while the twelfth column contained the negative control sterile liquid MHB. After incubation for 16-18 h at 37°C, 30 µl of 0.015% resazurin (Sigma-Aldrich, USA) was added to each well and left for another 2-4 h of incubation. At the end of the analysis, the wells without visible change represented the concentration of the extract above the MIC value.[19] MBCs were determined by plating well contents with concentrations higher than the MIC and incubating at 37°C for 24 h aerobically. Each test was performed twice.

Receptor-Ligand Binding Evaluation

To elucidate the potential antibacterial effect against multidrug bacteria, we analysed the binding affinity of determined bioactive compounds in *Iris* species extracts (fisetin, quercetin, kaempferol, isorhamnetin, genistein, and hesperidin) to the SauPBP2a active site—the protein responsible for MRSA resistance to β-lactams. The obtained affinity values were compared against ceftobiprole, which is a high-affinity reference molecule, and methicillin, which has a low affinity to the SauPBP2a active site.[20] The three-dimensional (3D) crystal structure of SauPBP2a was retrieved from the Protein Data Bank (PDB) database (RCSB Protein Data Bank) (PDB

ID: 1MWT). The structure data files (SDF) were obtained from the PubChem database (isorhamnetin CID:5281654; hesperidin CID:10621; ceftobiprole CID: 135413542; quercetin CID: 5280343; fisetin CID: 5281614; genistein CID:5280961; kaempferol CID:5280863; methicillin CID:6087). The SDF ligand files were converted to PDB 3D format using PyMOL 2.4. (https://pymol.org/2/), whereas the preparation of target proteins and ligands and their conversion to the PDBQT format was performed using AutoDock Tools software (http://mgltools.scripps.edu/downloads). Selection and determination of the catalytic binding site of SauPBP2a (amino acid residues within chain B named Ser403, Lys406, Tyr446, Ser462, Asn464, Ser598, Gly599, and Thr600) were based on reference data, and also PrankWeb.[21] Grid box dimensions were based on data from the literature. Molecular docking followed standard procedures that require AutoDock Vina 1.1.2. software.[22] To identify the molecular interactions, we used PyMOL 2.4.

Statistical Analysis

Mean values ± standard deviation (SD) were calculated using Microsoft Office 2019 (Excel (Microsoft Corporation, USA). One-way ANOVA ($P<0.05$ and $P<0.01$) and Tukey's multiple comparison test were calculated using software STATISTICA 10; StatSoft. Inc.

RESULTS

In Vitro Antimicrobial Activity of Identified Bioactive Compounds

HPLC revealed that targeted bioactive compounds in *I. pumila, I. reichenbachii*, and *I. illyrica* extracts (aqueous, 50% ethanol, and 80% ethanol) were present in *Iris* species extracts at different concentrations, in a range from 0.223647 to 292.6555, expressed in $mg.g^{-1}$ DW. Fisetin was not present in all *I. pumila* extracts, kaempferol, and hesperidin in *I. illyrica* extracts, whereas genistein was identified only in the aqueous and 50% ethanolic extracts of *I. pumila* (Table 1).

The results of the antibacterial assay (agar well diffusion method) showed that all tested extracts of *Iris* spp. had antibacterial effects against the MRSA strain. The aqueous extract of *I. pumila* had the greatest inhibitory effect against this multidrug-resistant strain (20.00±1.73), although the other values of the effects of the extracts were also not negligible. The potential antimicrobial properties of the extracts were also evaluated using MIC and MBC as parameters (Table 2). None of the four antibiotics tested (colistin, ampicillin, streptomycin, and amoxicillin), did not cause the growth inhibition, whereas most extracts had MIC values between 10.31 and 20.63 mg/mL.

In Silico Molecular Docking Simulation

A molecular docking study revealed that the best binding affinity (rmsd l.b. 0.000; rmsd u.b. 0.000) to the SauPBP2a site was for isorhamnetin (-8.3), following the hesperidin (-8.1) as well as the positive control of binding ceftobiprole (-8.1), followed by quercetin (-7.9), fisetin (-7.8), genistein (-7.5), kaempferol (-7.3) and methicillin (-5.3), all expressed in kcal/mol. It was noted that all compounds had better scores than the negative control of binding affinity methicillin.

After acquiring binding affinity results, we analysed intermolecular interactions of ligands with better or the same binding scores as the positive controls ceftobiprole (isorhamnetin and hesperidin) and the target protein SauPBP2a. These data revealed interactions between amino acid residues and selected compounds in terms of the formation of good hydrogen bonds. Isorhamentin formed five hydrogen bonds in total, including three with the amino acid residue Ser403 (2.9 Å; 2.2 Å; 2.0 Å), one with Asn464 (3.2 Å), and Ser462 (2.5 Å). The bioactive component hesperidin exhibited hydrogen bonding with the amino acid residues: Ser494 (2.2 Å), Asn500 (2.3 Å), and Gly282 (2.4 Å). The positive control for binding affinity, ceftobiprole, formed two hydrogen bonds with the amino acid residues Ser598 (2.7 Å) and Thr600 (2.0 Å) (Figure 1).

DISCUSSION

Iris species are well known for their use in the pharmaceutical, cosmetic, and food industries, but they also have various potential applications as antioxidant, anticancer, hepatoprotective, anti-inflammatory, and antimicrobial agents.[13,23] Some are widely used in traditional medicine for inflammation, bacterial, and viral infections, and in some cases as adjuvant therapy for cancer treatment.[24] These biological activities are the result of numerous bioactive compounds present, such as xanthones, quinones, flavonoids and their derivates, terpenes, and simple phenolics.[23-25] In the present study, we examined the potential antibacterial effect of crude extracts of *Iris* species (*I. pumila, I. reichenbachii*, and *I. illyrica*) obtained using different solvents against the MRSA strain. Using HPLC, we identified bioactive targeted compounds (isorhamnetin, hesperidin, quercetin, fisetin, genistein, and kaempferol). Isorhamnetin, quercetin, fisetin, and kaempferol belong to the class of flavonoids named flavanols; hesperidin is in the flavanone group of flavonoids, while genistein is an isoflavone. To our knowledge, the results from our research represent the first report on the phytochemical composition of three *Iris* species crude extracts from the Bosnia and Herzegovina area, characterised via HPLC technique. Other phytochemical studies of *Iris* spp. have revealed the presence of different flavonoids in their composition.[26,27]

Table 1. Compound contents of the examined extracts were determined using HPLC.

		Compound content (mg/1 g DW)					
Plant species	**The type of extraction**	**Fisetin**	**Quercetin**	**Kaempferol**	**Isorhamnetin**	**Genistein**	**Hesperidin**
Iris reichenbachii Heuff.	aqueous	81.31115	48.4339	0	6.97005	0	10.35255
	50 % ethanol	218.0028	18.08235	0.1995659	0.469365	0	46.45055
	80 % ethanol	292.65555	22.1808	0.87237	0.933755	0	76.9613
Iris pumila L.	aqueous	0	53.02175	10.16585	25.4444	0	15.16575
	50 % ethanol	0	12.9293	0.922625	2.075675	0.113647	58.0301
	80 % ethanol	0	9.2454	0.894715	2.20035	0.132236	48.0050
Iris ilyirica Tomm.	aqueous	65.8556	15.81085	0	14.5912	0	0
	50 % ethanol	84.4289	7.905	0	2.651465	0	0
	80 % ethanol	168.878735	14.96365	0	8.1085	0	0

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tested extracts and control and diameter of inhibition zones* (mm) obtained through the agar well diffusion method.

		***Staphylococcus aureus* subsp. *aureus* ATCC 33591 (MRSA strain)**		
Plant species	**The type of extraction**	**MIC• (mg/mL)**	**MBC• (mg/mL)**	**Diameter of inhibition zones (mm)**
Iris reichenbachii Heuff.	aqueous	10.31	20.63	18.00±0.00
	50 % ethanol	10.31	20.63	19.00±1.00 $^{* c, f}$
	80 % ethanol	10.31	20.63	16.30±0.57 $^{* b,h,i \ **d}$
Iris pumila L.	aqueous	10.31	20.63	20.00±1.73 $^{** c,e.f}$
	50 % ethanol	10.31	20.63	16.70±0.57 $^{** d}$
	80 % ethanol	10.31	20.63	16.30±0.57 $^{* b,h,i \ **d}$
Iris illyrica Tomm.	aqueous	10.31	20.63	18.00±0.00
	50 % ethanol	10.31	20.63	19.00±1.00 $^{* c,f}$
	80 % ethanol	20.63	41.25	19.00±0.00 $^{* c,f}$
Control (mg/mL)	Amoxicillin, initial concentration = 0.512 mg/mL	NI	NI	N/A

•Same values obtained through duplicates
* Note: The data are given as mean ± standard deviation (SD) of triplicate experiments. Results were subjected to One-way ANOVA, and mean comparisons were performed using Tukey's multiple comparison test. Different superscripts in the column indicate significant differences (**P<0.01 and *P<0.05).
NI=No inhibition

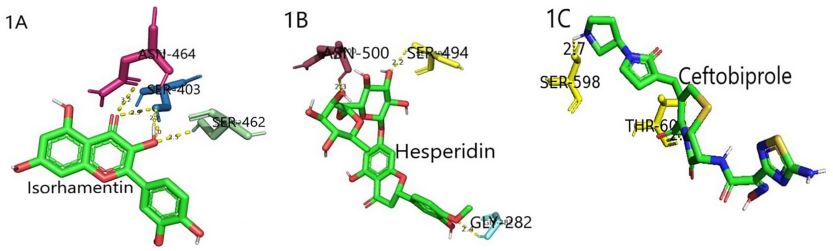

Figure 1. Results of intermolecular bonding (H-bond yellow dots) between ligands (1A-isorhamnetin; 1B-hesperidin; positive control 1C-ceftobiprole) and amino acid residues of the SauPBP2a active site.

Many *in vitro* or *in vivo* studies have pointed out that these flavonoids have prominent antimicrobial effects[28,29], whereas some of those studies emphasised the antibacterial effect against MRSA. For instance, some studies indicated the inhibitory effects of kaempferol and quercetin against MRSA[30], hesperidin inhibited biofilm formation of MRSA[31], genistein was highlighted as a promising drug for MRSA-induced osteomyelitis.[32] These are just a few of the studies that are being conducted to explore the potential of research-targeted bioactive compounds against multiresistant MRSA. They are positively correlated with our findings; potential antimicrobial effects of compounds mentioned above were found in the *Iris* species examined in this study.

A molecular docking analysis of binding affinity showed that isorhamnetin had the highest affinity score, even higher than the positive control ceftobiprole, whereas hesperidin had the same binding affinity as ceftobiprole. The binding energies of the analysed ligands were ranged from -5.3 to -8.3 kcal/mol. Alhadrami et al. analysed the antimicrobial effect of certain flavonoids against MRSA, i.e., the PBP2a receptor. Their results and data obtained through *in silico* analysis, followed by *in vitro* studies on the bacterial MRSA strain, suggested that, apart from the other compounds, quercetin, kaempferol, and hesperidin had antimicrobial potential, whereas hesperidin showed a synergistic effect.[27] In the present study, kaempferol had higher binding affinity than negative control methicillin. However, compared to the other ligands, it had the lowest score. Ceftobiprole had a slightly better binding score than quercetin. The study of Kalalo et al. showed that quercetin (-8,5 kcal/mol) and kaempferol (-8,3 kcal/mol) had the potential to inhibit MRSA.[33] Both ligands, isorhamnetin, and hesperidin, interacted within residues that are in the transpeptidase domain of PBP2a protein (residues 327-668)[34], which suggests that these compounds have the potential to bind to the PBP2a active site, which could ultimately have an impact on cell wall synthesis.

According to the results of the agar well diffusion test, all extracts showed strong inhibitory activity against MRSA.[35] The highest antibacterial activity with an inhibition zone value of 20 mm was observed for the aqueous extract of *I. pumila*, and the statistical analyses revealed that it was significantly greater ($P<0.01$) than the inhibition zone values for 50% and 80% ethanol extracts of *I. pumila* and 80% ethanol extract of *I. reichenbachii*. Additionally, positive effects against the MRSA strain were recorded for the 50% ethanol extract of *I. reichenbachii* and 50% and 80% ethanol extracts of *I. illyrica* (19 mm), with statistically significant differences ($P<0.05$) when compared with the 50% and 80% ethanol extracts of *I. pumila*. According to Gold et al., absolute ethanol has no microbiocidal effect; therefore, its antimicrobial effect cannot be attributed to the solvent but mostly to the components that possess antimicrobial potential, as recorded in the literature.[36] In the existing literature, there are no data on the effects of these three species of the genus *Iris* on multiresistant MRSA, but studies are available on other *Iris* species. A study by Hoang et al. (2020) showed that *Iris* spp. of the Iridaceae family have antimicrobial potential against *S. aureus*, whereby the methanol extracts showed also the anti-biofilm formation effect.[37]

CONCLUSION

Our study using *in vitro* and *in silico* methods showed that *Iris* species from Bosnia and Herzegovina have the potential to be a valuable source of antimicrobial compounds, particularly against multiresistant bacterial strains such as MRSA. However, further studies are needed to confirm the mechanism of action and possible cytotoxic and genotoxic effects for initial use in pharmacotherapy. This study can serve as a basis for exploring the health-based properties of endemic plants from the Balkan peninsula.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer Review: Externally peer-reviewed.

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REFERENCES

1. Masimen MAA, Harun NA, Maulidiani M, Ismail WIW. Overcoming methicillin-resistant *Staphylococcus aureus* (MRSA) using antimicrobial peptides-silver nanoparticles. *Antibiotics (Basel)*. 2022;11(7):951. doi: 10.3390/antibiotics11070951
2. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med.* 1998;339(8):520-532.
3. Ahmad-Mansour N, Loubet P, Pouget C, et al.

Staphylococcus aureus toxins: An update on their pathogenic properties and potential treatments. *Toxins (Basel)*. 2021;13(10):677. doi: 10.3390/toxins13100677

4. Baek KT, Gründling A, Mogensen RG, et al. β-Lactam resistance in methicillin-resistant *Staphylococcus aureus* USA300 is increased by inactivation of the ClpXP protease. *Antimicrob Agents Chemother*. 2014;58(8):4593-4603.
5. Brown NM, Goodman AL, Horner C, Jenkins A, Brown EM. Treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): Updated guidelines from the UK. *JAC-Antimicrob Resist*. 2021;3(1):dlaa114. doi: 10.1093/jacamr/dlaa114
6. CDC. Special report: COVID-19 U.S. Impact on antimicrobial resistance. 2022; Available: https://www.cdc.gov/drugresistance/pdf/covid19-impact-report-508.pdf
7. Johnston CW, Badran AH. Natural and engineered precision antibiotics in the context of resistance. *Curr Opin Chem Biol*. 2022;69:102160. doi: 10.1016/j.cbpa.2022.102160
8. Rakotofina HME, Donno D, Tombozara N, et al. Chemical composition, antimicrobial activity, and antioxidant capacity of *Micromeria flagellaris* Baker and *M. madagascariensis* Baker: Two endemic species from Madagascar as sources of essential oils. *Heliyon*. 2024;10(5):e26865. doi: 10.1016/j.heliyon.2024.e26865
9. Dini S, Chen Q, Fatemi F, Asri Y. Phytochemical and biological activities of some Iranian medicinal plants. *Pharm Biol*. 2022;60(1):664-689.
10. İnanir M, Uçar E, Tüzün B, Eruygur N, Ataş M, Akpulat HA. The pharmacological properties of *Gypsophila eriocalyx*: The endemic medicinal plant of northern central Turkey. *Int J Biol Macromol*. 2024;266(Pt2):130943. doi: 10.1016/j.ijbiomac.2024.130943
11. Alam K, Ahmad N, Ahmad I, Nafees M. Pharmacological activities of *Rhododendron afghanicum*; An endemic species of Khyber Pakhtunkhwa, Pakistan. *Chem Biodivers*. 2023;20(12):e202301273. doi: 10.1002/cbdv.202301273
12. Fan L, Gao Y, Hasenstein KH, Wang L. 'Flower Angel': A new *Iris sanguinea* cultivar. *HortSci*. 2021;56:617-618.
13. Khatib S, Faraloni C, Bouissane L. Exploring the use of *Iris* species: Antioxidant properties, phytochemistry, medicinal and industrial applications. *Antioxidants (Basel)*. 2022;11(3):526. doi: 10.3390/antiox11030526
14. Abdallah EM, Alhatlani BY, de Paula Menezes R, Martins CHG. Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*. 2023;12(17):3077. doi: 10.3390/plants12173077
15. Volenzo T, Odiyo J. Integrating endemic medicinal plants into the global value chains: The ecological degradation challenges and opportunities. *Heliyon*. 2020;6(9):e04970. doi: 10.1016/j.heliyon.2020.e04970
16. Nigussie D, Davey G, Legesse BA, Fekadu A, Makonnen E. Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complement Med Ther*. 2021;21(1):2. doi: 10.1186/s12906-020-03183-0
17. European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. EUCAST, Basel, Switzerland (2017).
18. CLSI, Clinical and Laboratory Standards Institute. MO7: Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 11th ed. Wayne, PA: USA (2018).
19. Elshikh M, Ahmed S, Funston S, et al. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnol Lett*. 2016;38(6):1015-1019.
20. Masumi M, Noormohammadi F, Kianisaba F, Nouri F, Taheri M, Taherkhani A. Methicillin-Resistant *Staphylococcus aureus*: Docking-based virtual screening and molecular dynamics simulations to identify potential inhibitors of penicillin-binding protein 2a in natural flavonoids. *Int J Microbiol.* 2022;9130700. doi: 10.1155/2022/9130700
21. Jendele L, Krivak R, Skoda P, Novotny M, Hoksza D. PrankWeb: A web server for ligand binding site prediction and visualization. *Nucleic Acid Res.* 2019;47(1):W345-W349.
22. Trott O, Olson A. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem.* 2010;31(2):455-461.
23. Kaššák P. Secondary metabolites of the choosen genus *Iris* species. *Acta Univ Agric et Silvic Mendel Brun.* 2012;60(8):269-280.
24. Amin HIM, Hussain FHS, Najmaldin SK, et al. Phytochemistry and biological activities of Iris species growing in Iraqi Kurdistan and phenolic constituents of the traditional plant *Iris postii. Molecules*. 2021;26(2):264. doi: 10.3390/molecules26020264
25. Periferakis A, Periferakis K, Badarau IA, et al. Kaempferol: Antimicrobial properties, sources, clinical and traditional applications. *Int J Mol Sci*. 2022;23(23):15054. doi: 10.3390/ijms232315054
26. Unver T, Uslu H, Gurhan I, Goktas B. Screening of phenolic components and antimicrobial properties of *Iris persica* L. subsp. *persica* extracts by *in vitro* and *in silico* methods. *Food Sci Nutr*. 2024; 00:1–17. doi:10.1002/fsn3.4251
27. Alhadrami HA, Hamed AA, Hassan HM, Belbahri L, Rateb ME, Sayed AM. Flavonoids as potential anti-MRSA agents through modulation of PBP2a: A computational and experimental study. *Antibiotics* (Basel). 2020;9(9):562. doi: 10.3390/antibiotics9090562
28. Pyrzynska K. Hesperidin: A Review on extraction methods, stability and biological activities. *Nutrients*. 2022;14(12):2387. doi: 10.3390/nu14122387
29. Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother Res*. 2001;15(1):39-43.
30. Yin N, Yang X, Wang L, et al. Kaempferol inhibits the expression of α-hemolysin and protects mice from methicillin-resistant *Staphylococcus aureus*-induced lethal pneumonia. *Microb Pathog*. 2022;162:105336. doi: 10.1016/j.micpath.2021.105336
31. Vijayakumar K, Muhilvannan S, Vignesh MA. Hesperidin inhibits biofilm formation, virulence and staphyloxanthin synthesis in methicillin resistant *Staphylococcus aureus* by targeting SarA and CrtM: an *in vitro* and *in silico* approach. *World J Microbiol Biotechnol*. 2022;38(3):44. doi: 10.1007/s11274-022-03232-5
32. Guo X. Antibacterial and anti-inflammatory effects of genistein in *Staphylococcus aureus* induced osteomyelitis in rats. *J Biochem Mol Toxicol*. 2023;37(4):e23298. doi: 10.1002/jbt.23298
33. Kalalo MJ, Fatimawali, F, Kalalo, T, Rambi CIJ. Tea bioactive compounds as inhibitor of MRSA penicillin binding protein 2a (PBP2a): A molecular docking study. *J Farm Medica.* 2020; 3(2):70-75. Doi: 10.35799/pmj.3.2.2020.32878
34. Fishovitz, J, Hermoso, JA, Chang, M, Mobashery, S. Penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus. IUBMB Life.* 2014; 66(8):572-7. doi: 10.1002/iub.1289
35. Iwashina T, Mizuno T. Flavonoids and Xanthones from the genus

Iris: Phytochemistry, relationships with flower colors and taxonomy, and activities and function. *Nat Prod Commun*. 2020;15(10). doi:10.1177/1934578X20937

36. Gold, NA, Mirza TM, Avva U. Alcohol sanitizers. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. PMID: 30020626
37. Hoang L, Beneš F, Fenclová M, et al. Phytochemical composition and *in vitro* biological activity of *Iris spp.* (Iridaceae): A new source of bioactive constituents for the inhibition of oral bacterial biofilms. *Antibiotics (Basel)*. 2020;9(7):403. doi: 10.3390/antibiotics9070403

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