Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.08221

Antimicrobial activity of *Rhus Coriaria* L. and *Salvia Urmiensis* bunge against some food-borne pathogens and identification of active components using molecular networking and docking analyses

Maryam GHANE[1]*, Laleh BABAEEKHOU[1], Masumeh SHAMS[1]

Abstract

This research aimed to evaluate the *in vitro* antibacterial activity of methanol extracts of *Rhus coriaria* L. (sumac) and *Salvia urmiensis* Bunge against some food-borne pathogens, survey the phytochemical constituents of the extracts, and their activity against some drug targets in pathogens. The antibacterial activity of the extracts was evaluated against six pathogens using agar well diffusion and broth microdilution methods. Both extracts exhibited antibacterial activity and the highest activity was obtained by sumac against *Staphylococcus aureus* (27.7 ± 0.8 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sumac against tested bacteria were in the range of 0.125-0.5 and 0.25-1 mg/mL, respectively. *S. urmiensis* showed weaker antimicrobial activity with a MIC range of 2-4 mg/mL. A molecular networking analysis of extracts resulted in annotation of 18 and 12 compounds from the methanol extracts of *S. urmiensis* and sumac respectively, which mainly were related to flavonoids. Molecular docking analysis could effectively characterize some glycosylated flavonoids with a high binding affinity to the studied enzymes. This study confirmed the efficacy of sumac and *S. urmiensis* extracts as natural antimicrobial agents and introduced a wide range of compounds, which can be used in food preservation to control foodborne pathogens in food.

Keywords: *Rhus coriaria* L.; *Salvia urmiensis* Bunge; molecular docking; anti-bacterial agents; *Staphylococcus aureus*.

Practical Application: Application of medicinal plants or their derivatives as a food preservative.

1 Introduction

Food safety is one of the major concerns for both consumers and food producers. Bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., *Klebsiella pneumoniae,* etc. that can be present in food may cause food spoilage and incidence of food-borne disease (Bintsis, 2017; Zhang et al., 2018). Over the past decades, synthetic antimicrobial agents have been extensively used to prevent the growth of food pathogenic and food spoilage microorganisms. However, the emergence of microbial resistance to antimicrobial agents has posed a major health concern due to the indiscriminate use of chemical preservatives in the food industry (Kiessling et al., 2002). Today, consumers are well aware of the long-term harmful effects of chemical preservatives on human health. Therefore, food manufacturers should focus on applying natural compounds as safe alternatives and satisfy the consumer preferences for "green foods" (Bondi et al., 2017).

Due to the increased microbial resistance to antimicrobial agents and the side effects of chemical preservatives (Negi, 2012; Sharma, 2015), there is an urgent need for an alternative antimicrobial agent with fewer side effects. Herbs are rich sources of phytochemicals with antibacterial activity that can solve this problem (Negi, 2012). They are rich resources of a wide variety of phytochemical compounds such as tannins, terpenoids, alkaloids, phenolics, and flavonoids, with destructive effects on different parts of bacterial pathogens as well as food-borne or food spoilage bacteria. A large number of medicinal plants have also been considered by researchers for the treatment of infectious diseases (Gonelimali et al., 2018; Górniak et al., 2019). Also, there are different reports in the literature indicating that the extracts of Iranian herbs can effectively inhibit the growth of bacterial strains (Abedini et al., 2014; Ghasemi et al., 2010). Considering this respective, effort for the discovery of new and effective antimicrobial natural agents is of interest. Sumac (*Rhus coriaria* L.) is a medicinal plant that is widely used as a drug in Persian folk medicine, as Rhazes and Avicenna utilized the fruit of this plant for the treatment of two infectious ailments, namely, purulent ear and diarrhea (Aahia, 1993; Al-Razi, 2013). Furthermore, a literature survey indicated the antibacterial activity of extracts of sumac against both Gram-positive and Gram-negative bacteria (Ahmadian-Attari et al., 2016; Nasar-Abbas & Halkman, 2004). Therefore, this is rational to suppose sumac as a source of antibacterial substances. *Salvia* species have also been used in traditional medicine all around the world since ancient times (Topçu, 2006). Many reports focused on the biological activities of extracts, purified compounds, and essential oils of these plants such as antimicrobial, cytotoxicity, anti-malarial, anti-tumor, cardiovascular, and anti-inflammatory activities (Tohma et al., 2016; Zengin et al., 2018). The endemic species *Salvia urmiensis* Bunge grows in the West Azerbaijan

Received 17 Feb., 2021
Accepted 03 Nov., 2021
[1] *Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*
*Corresponding author: ghane@iiau.ac.ir

province of Iran. Some phytochemical analyses have been performed on this plant so far (Farimani et al., 2015; Farjam, 2012; Moridi Farimani & Abbas-Mohammadi, 2016). However, the antimicrobial activity of the extracts and components of this species remains unclear.

In natural products (NPs) research, the active components of the crude extracts with thousands of metabolites have to be characterized for drug discovery purposes or biomarker identification. Different bioinformatics programs have been developed to rapidly identify known metabolites by comparison of experimental spectral data to databases. Among these new approaches, molecular networking (MN) is a particularly effective one to dereplicate the chemical components by providing the MS/MS fragmentation spectra of samples and compare them with databases (Allard et al., 2016). Today, computer-aided drug discovery (CADD) tools are also getting a lot of attention in the pharmaceutical industry and academia (Acúrcio et al., 2019). CADD has played an important role in the discovery of several pharmaceutical drugs that have obtained FDA approvals (Sliwoski et al., 2013). Most of the effects of medicines are based on the interaction between therapeutic chemical compounds (drugs) and proteins (targets). Molecular docking (MD) consists of accurate prediction of the structure of a ligand within the constraints of a receptor binding site and correctly estimation of the binding strength (Meng et al., 2011).

In this research, the antibacterial activity of the methanol extract of *R. coriaria* and *S. urmiensis* was evaluated against some food-borne pathogenic strains by an *in vitro* method and a molecular networking technique was carried out to annotate the chemical components of both extracts. Then, a molecular docking analysis was used to assay the antibacterial activity of the dereplicated compounds.

2 Materials and methods

2.1 Bacterial strains

Six human pathogenic strains *K. pneumoniae* ATCC 700603, *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *Sh. dysenteriae* ATCC 13313, *Sh. sonnei* ATCC 25931, and *Sh. flexneri* ATCC 12022 were prepared from the Iranian Research Organization for Science and Technology (IROST) and Pasteur Institute of Iran. All the strains were cultured in trypticase soy agar (TSA) (Merck, Germany) plates and incubated at 37 °C for 24 h.

2.2 Plant materials and extraction

The roots of *S. urmiensis* were collected at the full flowering stage in Takab, West Azarbaijan province. A voucher specimen with the number of MPH-1220 has been deposited at the Herbarium of Medicinal Plants and Drug Research Institute (MPH) of Shahid Beheshti University, Tehran, Iran. The fruit of *Rhus coriaria* L. was provided from a local market and authenticated by a botanist at Shiraz University of Medical Sciences. A voucher specimen (PM 533) has been deposited in the Shiraz School of Pharmacy herbarium. 200 g of plants were separately ground into a fine powder in a mechanical grinder and extracted with methanol (3 × 200 mL) using a maceration method at room temperature. The extracts were then combined and evaporated to dryness under reduced pressure to obtain solventless extracts. Stock solutions of extracts were prepared by dissolving in 1% DMSO (Dimethyl sulfoxide) (Sigma Aldrich, USA).

2.3 Determination of bacteriostatic (MIC) and bactericidal (MBC) concentrations

The MIC value of sumac and *S. urmiensis* extracts was evaluated against bacterial strains using the broth dilution method in the microtiter plates (Javidnia & Miri, 2003). Briefly, serial dilutions of the extracts were prepared from stock solutions and used in this study. The overnight culture of each bacterial strain was diluted to get 0.5 McFarland turbidity (10^8 CFU/ml). Thereafter, 1:100 dilution of this suspension was prepared in fresh Trypticase Soy Broth (TSB) (Merck, Germany) medium and 50 µl of diluted suspension was added to each well. The plates were then sealed and incubated at 35 °C for 24 h. The lowest concentration of the extract that prevented visible growth was considered the MIC. To determine MBC, dilutions with no visible growth were sub-cultured on TSA plates. After incubation, the lowest concentration that yielded no bacterial growth on solid medium was considered as MBC (Cos et al., 2006).

2.4 Agar-well diffusion method

To assess the susceptibility of the bacterial strains against extracts, the agar-well diffusion method was applied as described previously (Albayrak et al., 2010). A suspension of bacteria was provided in 0.5 McFarland concentrations and spread on a Mueller Hinton agar (Merck, Germany) plate using a sterile swab. Thereafter, wells of 6 mm diameter were dug into agar medium using a sterilized cork borer and filled with 100 µl (1 mg/mL) of each filter-sterilized extract and allowed to diffuse at room temperature for 2 h. Wells containing 1% DMSO and 10 µg/mL Penicillin (AppliChem, Germany) were used as negative and positive control respectively. After incubation at 35 °C for 24 h, the diameter of the inhibition zone was measured in millimeters.

2.5 Molecular networking

To perform molecular networking (MN) analysis, the LC-MS/MS spectra of the methanol extract of sumac and *S. urmiensis* were firstly recorded on a Waters Acquity UPLC system by positive mode according to the UPLC and MS conditions of our previous report (Babaeekhou & Ghane, 2021). Then, a molecular network was generated using the online workflow on the GNPS website (Global Natural Products Social Molecular Networking, 2021). The precursor and MS/MS fragment ion mass tolerances were selected as 0.2 and 0.08 Da, respectively. For network creation, multiple MS/MS spectra of the same component were combined to give a consensus spectrum. Each consensus spectrum is considered as a node in the created network. A similarity score threshold of 0.6 and a minimum of 4 equal signals were employed between nodes to connect and generate clusters through edges. After network construction, nodes were matched to the GNPS library and dereplicated with the compound data. They were filtered as the same as the input data. All matches kept between network spectra and

library spectra were required to have a score above 0.60 and at least 5 matched peaks (Wang et al., 2016). The results of the molecular network were visualized on Cytoscape 3.7.2 using the Circular layout plug-in.

2.6 Molecular docking

A molecular docking (MD) study was performed by Glide application using Schrodinger package 2016-2 (Schrodinger, LLC) (Vasavi et al., 2017). The structure of DNA gyrase and wild-type penicillin-binding protein 5 from *E. coli*, DNA gyrase from *S. aureus* and topoisomerase IV, and MrkH in apo state of *K. pneumoniae* (PDB IDs: 1KZN, 1NZO, 3G7B, 5EIX, and 5KEC respectively) was downloaded from protein data bank and edited using protein preparation on Maestro 10.6. The geometry optimization of all enzymes was applied with the optimized potentials for liquid simulations (OPLS3) force field. All water molecules, ions, and heteroatoms were deleted and hydrogen atoms were also added to the physiological pH value. The structure of enzymes was finally minimized within the selected force field with a cut-off RMSD of 0.3 Å. To define the binding site, the grid center of enzymes was specified by the box center coordinates using the online DEPTH server. The structure of the ligands was drawn by the molecule sketching program ChemDraw and saved in an SDF format. They were imported to the workspace of the Schrodinger package. Different parameters including desalting, chirality specification, structure optimization, and partial charge calculations were performed on structures in the Ligprep module to obtain clean 3D structures from all of the ligands. Finally, docking analysis was established on a glide-dock module at an extra precision level. Penicillin G was used as the positive control.

3 Results and discussion

Agar well diffusion method was applied to evaluate the antimicrobial activity of the extracts. As shown in Table 1, the methanol extract of *R. coriaria* was found to be effective against all bacterial strains. The largest inhibition zone was observed against *S. aureus* with an inhibition zone of 27.7±0.8 mm ($P < 0.05$). The extract showed also appreciable inhibition zones against other strains (ranging from 22.7-25.2 mm). Whereas the methanol extract of *S. urmiensis* represented a moderate antimicrobial activity against *Shigella* strains with the inhibition zone values of 12-13 mm, no growth inhibition was observed for other microorganisms at a tested concentration of extract (1 mg/mL).

The MIC and MBC values of *R. coriaria* and *S. urmiensis* extracts were measured against pathogenic strains as well. The extracts exhibited a varying degree of antimicrobial activity against all tested microorganisms (Table 1). It was observed that *R. coriaria* was more effective than *S. urmiensis*. The lowest MIC value was obtained against *S. aureus* (0.125 mg/mL). The extract of *R. coriaria* was also effective against Gram-negative strains (MIC values of 0.25-0.5 mg/mL). The antimicrobial activity obtained by the extract of *R. coriaria* against these isolates indicates that it may be an important alternative agent to control foodborne pathogens in food. Previous studies on sumac have also shown antibacterial effects of its different extracts and purified compounds against Gram-positive and Gram-negative bacteria. In a study performed by Ahmadian-Attari et al. (2016), the ethyl acetate extract of sumac showed strong antibacterial activity against both *S. aureus* and *E. coli* strains. The isolated compound 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid (a quinone derivative) was also indicated high antibacterial activity against the Gram-positive bacteria of *S. aureus* (Ahmadian-Attari et al., 2016). In another study, the ethanolic and methanolic extracts of *R. coriaria* exhibited a wide spectrum of antibacterial activity against Gram-positive bacteria including Methicillin-resistant *S. aureus* and Gram-negative bacteria including *Pseudomonas aeruginosa*, *E. coli* O157, *Proteus vulgaris*, and *K. pneumoniae* (Abu-Shanab et al., 2005). Their results showed that *R. coriaria* extracts was more effective against Gram-positive bacteria than Gram-negative, which was in line with our results.

The methanol extract of *S. urmiensis* indicated a weaker antimicrobial activity against studied bacteria. As it is shown in Table 1, the MIC and MBC values of this extract were recorded at a range of 2-4 mg/ml for all tested strains. Compare to our results, Farjam found a greater antimicrobial activity by ether extract of *S. urmiensis* against *K. pneumoniae* (MIC value of 10.7 µg/ml). This difference may be due to the difference in the solvent system used in his study (Farjam, 2012).

The metabolite profile of the methanol extract of two plants *S. urmiensis* and *R. coriaria* was determined by UHPLC-HRMS. After acquiring the LC-MS/MS spectra of both extracts, they were used to generate a network to annotate the chemical compounds

Table 1. Antimicrobial activity of methanol extracts of *S. urmiensis* and *R. coriaria* against bacterial strains.

Microorganisms	MIC and MBC values (mg/mL)				Zone of inhibition (mm)			
	S. urmiensis		*R. coriaria*		*S. urmiensis*	*R. coriaria*	Penicillin	DMSO
	MIC	MBC	MIC	MBC				
K. pneumoniae ATCC 700630	4	-	0.50	1	-	22.7 ± 0.8^{c}	10.3 ± 0.8 ^{b}	-
E. coli ATCC 35218	2	4	0.250	0.50	-	23 ± 0.3 ^{bc}	8.3 ± 0.6^{c}	-
S. aureus ATCC 25923	4	4	0.125	0.25	-	27.7 ± 0.8^{a}	25.5 ± 0.5 ^{a}	-
Sh. dysenteriae ATCC 13313	2	2	0.250	0.50	12.8 ± 0.8 ^{a}	24.2 ± 1^{bc}	9.3 ± 0.8 ^{bc}	-
Sh. sonnei ATCC 25931	2	2	0.250	0.50	12.8 ± 0.8 ^{a}	25.2 ± 1^{b}	9.33 ± 0.3 ^{bc}	-
Sh. flexneri ATCC12022	2	2	0.250	0.50	11.5 ± 0.3 ^{a}	23.7 ± 0.3 ^{bc}	9.5 ± 0.5 ^{bc}	-

Data of the inhibition zone are expressed as mean ± standard deviation (n = 3). Means within the same column with different superscript letters are statistically different based on Tukey's test ($P < 0.05$).

of extracts comparing the spectral data with databases. Analysis of the MS/MS data led to the identification of 1154 parent ions, which were visualized as nodes in a molecular network. In this network, the data of *S. urmiensis* and *R. coriaria* extracts are represented by pink and blue circles, respectively (Figure 1). The edge width depicts the cosine similarity between different MS/MS spectra. Network screening against the GNPS spectral database led to the identification of 18 compounds from the methanol extract of *S. urmiensis*, mainly related to the flavonoids. In addition, a search of the LC-MS/MS spectra of the methanol extract of *R. coriaria* against the GNPS library resulted in the dereplication of twelve compounds. Full details of dereplicated compounds have been shown in Table 2. Most dereplicated compounds were classified to the flavonoid derivatives, which the antibacterial activity of extracts may be related to the presence of some of these polyphenols. Our results obtained from molecular networking analysis indicated a good agreement with previously published reports about the chemical composition of both plants (Abu-Reidah et al., 2015). Numerous studies have been performed on *Salvia* species from a phytochemical viewpoint confirming that they are rich resources of different biologically active components such as essential oils, flavonoids, and terpenoids (Jassbi et al., 2016; Šulniūtė et al., 2017). However, a few phytochemical reports are found on *S. urmiensis* species in the literature. Previous studies afforded the isolation and identification of several polyhydroxylated triterpenoids with rare carbon skeletons, flavonoids, and different aliphatic esters and ketones (Farimani et al., 2015; Moridi Farimani & Abbas-Mohammadi, 2016).

Molecular docking analysis was carried out to evaluate the binding affinities and interaction modes of dereplicated ligands

Table 2. Name, molecular weight and cosine score of dereplicated compounds.

No.	Compound name	Spec MZ	Cosine score
SU-1	Syringic acid	0.8	199.06
SU-2	Adenosine	0.94	268.1
SU-3	Kaempferol	0.89	287.06
SU-4	Quercetin	0.95	303.04
SU-5	Isorhamnetin	0.75	317.07
SU-6	Sucrose	0.66	325.12
SU-7	Chlorogenic acid	0.73	377.09
SU-8	Isoquercitrin	0.95	465.11
SU-9	myricetin-3-O-hexoside	0.6	479.19
SU-10	5,4-dihydroxy -5-(hydroxyl methyl)- 3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxolan-2-yl]oxy-2-(4-hydroxy phenyl)-7-methoxy chromen-4-one	0.76	579.29
SU-11	3-[4,5-dihydroxy-6-(hydroxyl methyl)-3-[3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl] oxy-5,7-dihydroxy-2-(4-hydroxy phenyl)chromen-4-one	0.94	611.16
SU-12	3-[3,4-dihydroxy-6-(hydroxyl methyl)-5-[3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl] oxy-2-(3,4-dihydroxy phenyl)-5,7-dihydroxy chromen-4-one	0.93	627.14
SU-13	Quercetin 3,4'-diglucoside	0.77	627.62
SU-14	7-[4,5-dihydroxy-6-(hydroxyl methyl)-3,4,5-trihydroxy-6-methyl oxan-2-yl]oxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxy-3-methoxy phenyl)-2,3-dihydrochromen-4-one	0.7	628.17
SU-15	3-[4,5-dihydroxy-6-(hydroxyl methyl)-3-[3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl] oxy-5,7-dihydroxy-2-(4-hydroxy phenyl)chromen-4-one	0.76	633.13
SU-16	3-[4,5-dihydroxy-6-(hydroxyl methyl)- 3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl]oxy-2-(3,4-dihydroxy phenyl)-5-hydroxy-7-methoxy chromen-4-one	0.92	641.18
SU-17	3-[3,4-dihydroxy-6-(hydroxyl methyl)-5-[3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl] oxy-2-(3,4-dihydroxy phenyl)-5,7-dihydroxy chromen-4-one	0.79	649.13
SU-18	3-[3-[4,5-dihydroxy-6-(hydroxyl methyl)-3-[3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]oxyoxan-2-yl]oxy-4,5-dihydroxy-6-(hydroxyl methyl) oxan-2-yl]oxy-2-(3,4-dihydroxy phenyl)-5,7-dihydroxy chromen-4-one	0.8	789.21
RC-1	2-(Hydroxymethyl)-1,4-naphthoquinone	0.82	189.78
RC-2	Coriariaoic acid	0.75	195.17
RC-3	4-Methoxy-1-naphthalenemethanol	0.9	211.23
RC-4	3-Caryolanol	0.84	223.38
RC-5	Kaempferol	0.72	287.06
RC-6	Quercetin	0.9	303.04
RC-7	3'-O-Methyl quercetin	0.80	317.06
RC-8	Myricetin	0.94	319.23
RC-9	Coriarianthracenyl ester	0.63	453.52
RC-10	Querciturone	0.87	479.08
RC-11	4',4''',5,5'',6'',7,7''-Heptahydroxy-3,8'''-biflavone	0.92	554.469
RC-12	beta-sitosterol-beta-D-glucoside	0.67	577.84

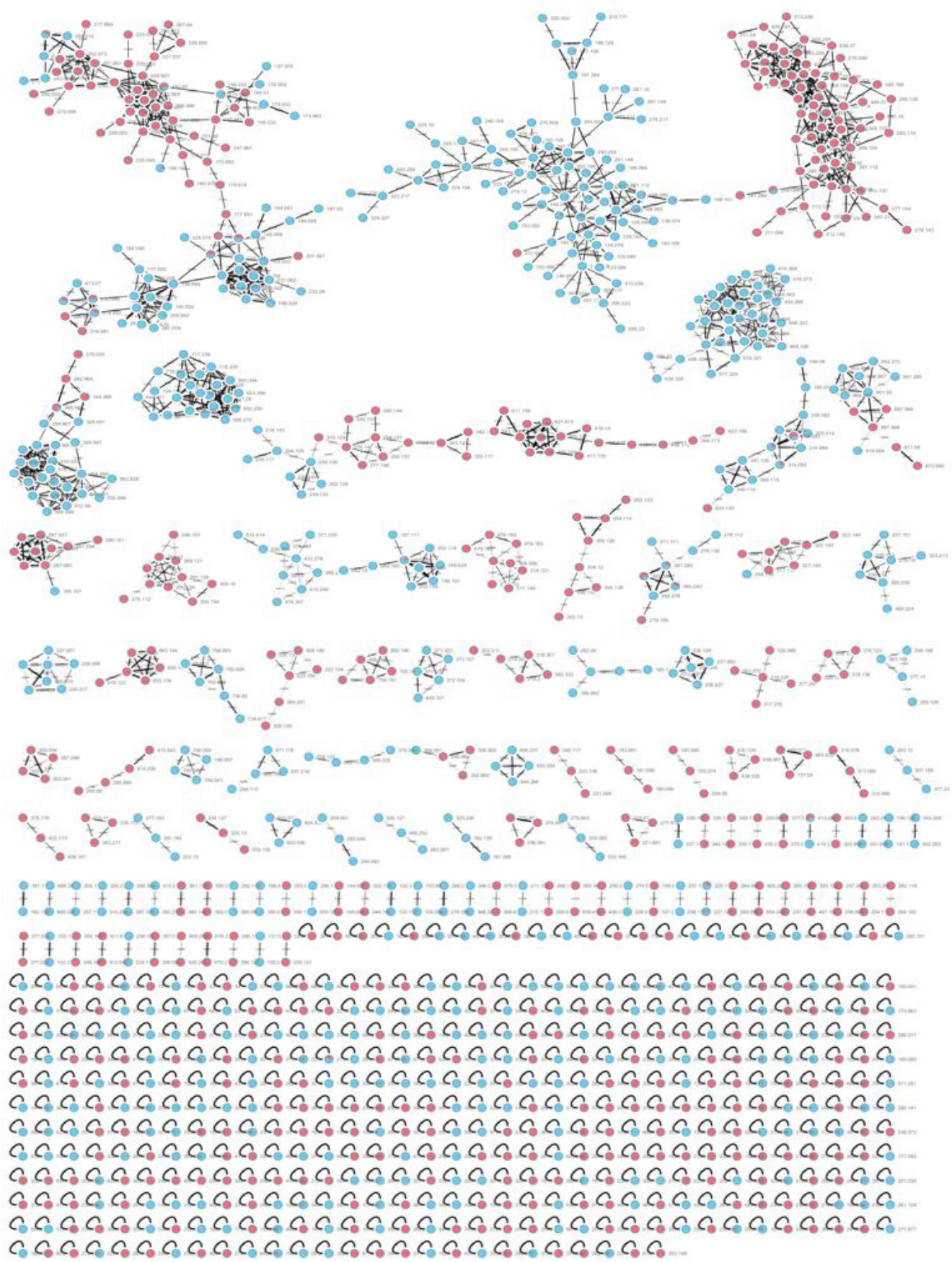

Figure 1. The molecular network of MeOH extract of *R. coriaria* and *S. urmiensis* with a cosine score cut off of 0.6.

against targets DNA gyrase and penicillin-binding protein 5 from *E. coli* (PDB IDs: 1KZN and 1NZO, respectively), DNA gyrase from *S. aureus* (PDB ID: 3G7B) and topoisomerase IV and MrkH in apo state of *K. pneumoniae* (PDB IDs: 5EIX and 5KEC, respectively). The results of docking analysis have been shown in Table 3, given by docking scores in kcal/mol, where polyhydroxylated compounds such as flavonoids indicated the high affinity to enzymes.

The screening results revealed that glycosylated flavonoids **SU-17**, **SU-18,** and **SU-12** from *S. urmiensis* extract and flavonoid myricetin (**RC-8**) from sumac extract had a high binding affinity to the 1KZN enzyme with docking scores of -9.58, -8.87, -8.80, and -7.16 kcal/mol, respectively. Among the identified compounds of both extracts, quercetin 3,4'-diglucoside (**SU-13**) and querciturone (**RC-10**) showed the highest affinity to the 1NZO enzyme with the values of -11.73 and -10.61 kcal/mol, respectively. Two flavonoids **SU-18** and **SU-17** were additionally predicted to dock to this enzyme with low binding energy (-10.31 and -10.28 kcal/mol). The most active components against the 3G7B enzyme from the MeOH extract of *S. urmiensis* were identified to be three glycosylated flavonoids **SU-18**, **SU-12,** and **SU-17** with docking values of -10.18, -9.38, and -8.93 kcal/mol, respectively. The glycosylated flavonoid querciturone (**RC-10**) exhibited a high binding affinity to the 3G7B amongst the compounds of *R. coriaria* with a value of -7.01 kcal/mol. Evaluation of the binding energies of docked ligands with 5EIX enzyme exhibited a high affinity of **SU-18**, **SU-16,** and **SU-17** from *S. urmiensis* and 3'-O-methyl quercetin (**RC-7**) from sumac to enzyme with docking scores of -10.27, -8.75, -8.04, and -6 kcal/mol, respectively. The highest binding affinity to 5KEC was obtained for querciturone (**RC-10**), dereplicated from sumac with a binding energy of -10.66 kcal/mol and followed by **SU-18**, **RC-7,** and **SU-5** with scores of -10.26, -10.16, and -10.15 kcal/mol. The binding energy of positive control (penicillin G) with enzymes 1KZN, 1NZO, 3G7B, 5EIX, and 5KEC were found to be -2.04, -3.89, -3.78, -3.65, and -4.20 kcal/mol, respectively. The high antibacterial property of the methanol extract of *R. coriaria* may be assigned to the presence of polyphenols. This can be concluded from the strong interaction of flavonoid querciturone (**RC-10**) with 1NZO and 5KEC, 3'-O-Methyl quercetin (**RC-7**) with 5KEC, and myricetin (**RC-8**) with 1KZN. In the case of *S. urmiensis* glycosylated flavonoids Quercetin 3,4'-diglucoside (**SU-13**), exhibited the strongest interaction with 1NZO. Furthermore, other glycosylated flavonoids **SU-17** and **SU-18** showed strong interaction with all studied enzymes. The antimicrobial activity of flavonoids reported in the literature supports our results (Farhadi et al., 2019). Wang et al. (2018) showed the bacteriostatic effect of quercetin on *S. aureus*, *Salmonella enterica* Typhimurium, *E. coli*, and *P. aeruginosa* (S. Wang et al., 2018). In another study, flavonoids Quercetin 3-methyl ether, myricetin 3-O-rhamnoside isolated from *Inga fendleriana* extracts displayed antibacterial activity against *S. aureus* and *Staphylococcus epidermidis* with MICs in the range from 31 to 250 μg/ml (Pistelli et al., 2009). Furthermore, MD analysis has also revealed high interaction of flavonoids with biologically important targets in bacteria. For instance, Plaper and co-workers showed that flavonoid quercetin binds to the 24 kDa fragment of *E. coli* gyrase B with a K_D value of 15 μM and inhibits its ATPase activity (Plaper et al., 2003). A high binding affinity of quercetin with Topoisomerase IV of *E. coli* was also reported previously (Alves et al., 2014) and in a study conducted by Rani et al. Quercetin 3-o-rutinoside (Rut) in allosteric sites showed active site inhibition of PBP2a (Rani et al., 2016). In the present study, Quercetin 3,4'-diglucoside revealed a better binding efficiency (11.73) with PBP 5 than Rut (-7.79). Based on the results obtained from the interaction of the ligands with all studied enzymes in molecular docking analysis, it can be deduced that polyphenols SU-13, SU-17, SU-18, and RC-10 have high antimicrobial activity, which are suggested for further

Table 3. Molecular docking analysis of the chemical compounds of *S. urmiensis* and *R. coriaria* against studied targets

Title	docking score (kcal/mol)					Title	docking score (kcal/mol)				
	1KZN	1NZO	3G7B	5EIX	5KEC		1KZN	1NZO	3G7B	5EIX	5KEC
SU-1	N.A	-4.26	-2.53	-3.03	-4.46	SU-17	**-9.58**	**-10.28**	**-8.93**	**-8.04**	-9.17
SU-2	-6.37	-5.83	-3.37	-4.54	-4.60	SU-18	**-8.87**	**-10.31**	**-10.18**	**-10.27**	**-10.26**
SU-3	-5.12	-5.06	-4.12	-4.69	-3.68	RC-1	-5.05	-5.07	-3.39	-5.13	-3.04
SU-4	-6.15	-6.16	-5.49	-5.51	-9.52	RC-2	N.A	-4.12	-1.55	-2.33	-4.46
SU-5	-5.18	-5.50	-4.26	-5.26	**-10.15**	RC-3	-4.00	-3.65	-3.67	-3.56	-2.64
SU-6	-6.55	-8.05	-5.96	-6.77	-7.16	RC-4	N.A	-3.73	-1.44	-2.43	-2.44
SU-7	-5.57	-7.44	-5.79	-5.82	-7.86	RC-5	-5.12	-5.06	-4.12	-4.69	-3.68
SU-8	-6.48	-7.30	-6.61	-7.20	-7.24	RC-6	-6.15	-6.16	-5.49	-5.51	-9.52
SU-9	-8.06	-5.78	-5.84	-6.10	-10.01	RC-7	-6.16	-5.44	-4.97	**-6.00**	**-10.16**
SU-10	-8.00	-8.69	-6.27	-5.89	-6.28	RC-8	**-7.16**	-6.16	-5.51	-5.17	-9.23
SU-11	-7.35	-9.37	-8.55	-6.80	-10.05	RC-9	0.08	-3.83	-3.87	-3.53	-4.35
SU-12	**-8.80**	-9.61	**-9.38**	-7.36	-10.00	RC-10	-6.22	**-10.61**	**-7.01**	-5.16	**-10.66**
SU-13	-8.06	**-11.73**	-7.94	-7.70	-8.93	RC-11	N.A	-6.38	-4.90	-5.01	-3.53
SU-14	-6.79	-9.22	-7.33	-7.62	-7.61	RC-12	N.A	-5.50	-4.39	-2.58	-2.16
SU-15	-6.70	-9.45	-7.67	-5.22	-9.17	penicillin G	-2.04	-3.89	-3.78	-3.65	-4.20
SU-16	-7.60	-9.24	-7.50	**-8.75**	-8.78						

Bold values signifies high docking scores.

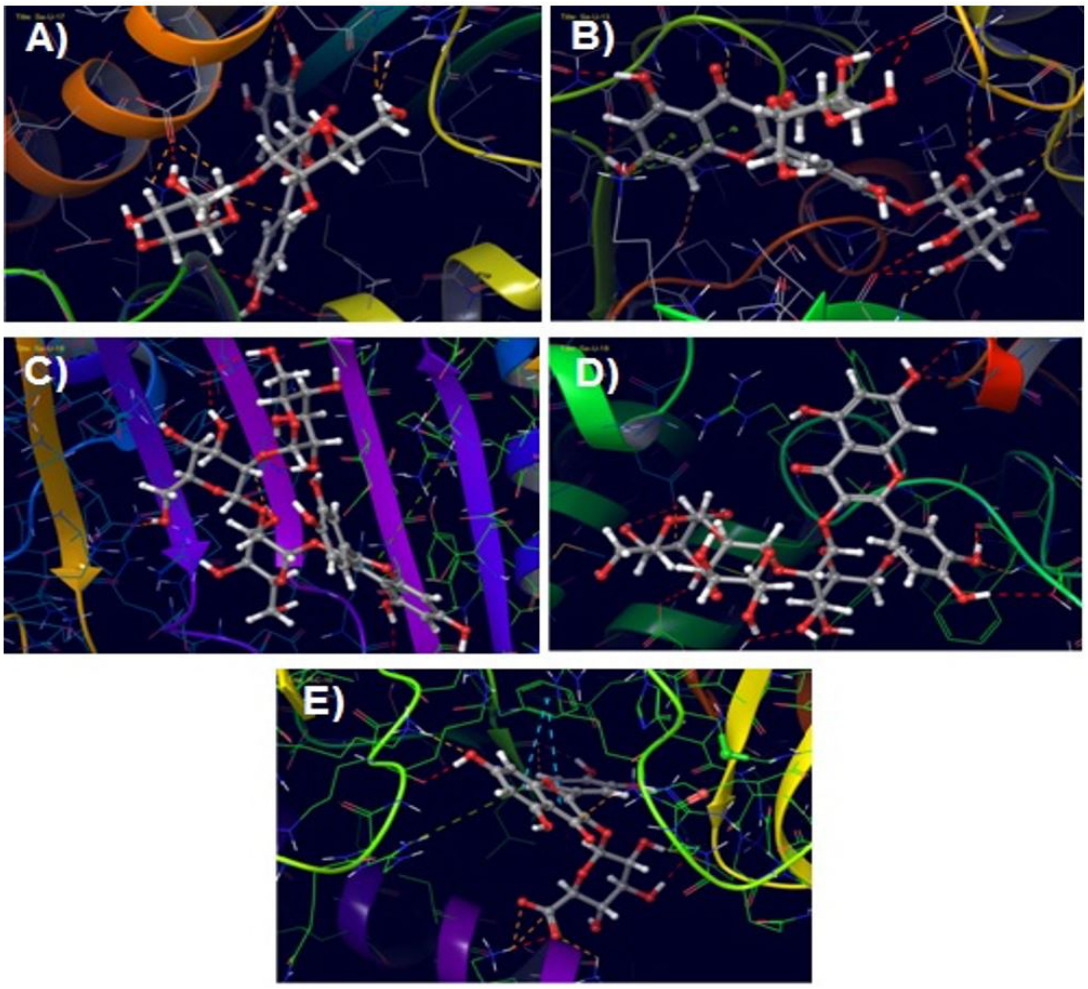

Figure 2. Diagram of the best docked ligand-protein complexes: (A) **SU-17** with PDB 1KZN (B) **SU-13** with PDB 1NZO (C) **SU-18** with PDB 3G7B (D) **SU-18** with PDB 5EIX (E) **RC-10** with PDB 5KEC.

biological studies. In molecular docking analysis, the binding affinity is strongly dependent to the different type of binding interactions between ligands and active pocket of proteins. Force-field-based scoring functions estimate the binding energy by summing the contributions of bonded and non-bonded interactions including: hydrogen and covalent bonds as well as ionic, polar, electrostatic and van der Waals interactions (Eriksson et al., 2017). Figure 2 shows the interactions of best docked ligands with each studied enzyme, where the most important interactions were H-bond of hydroxyl groups and π- π staking of phenyl rings of ligands with the amino acid residues of the active site of studied enzymes.

4 Conclusion

The present study indicated that sumac and *S. urmiensis* are valuable sources of active compounds with antibacterial activity against pathogens. Dereplication of different flavonoids and their glycosylated derivatives, which were in agreement with the chemical structures of both studied genus reported in the literature, confirmed that molecular networking can hopefully carry out in phytochemical analyses for the identification of natural compounds. Results from molecular docking analysis indicate that polyphenols SU-13, SU-17, SU-18, and RC-10 which exhibited high interaction with studied enzymes could be introduced as potential candidates for natural drug or food preservative development against pathogenic bacteria.

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