Original article

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ANTIMICROBIAL ACTIVITY OF FRACTIONS AND THE EXTRACT FROM *GENTIANA ASCLEPIADEA* L. UNDERGROUND PARTS WITH MOLECULAR DOCKING ANALYSIS

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The willow gentian (*Gentiana asclepiadea* L.) is a valuable source of secoiridoids, *C*-glycosylated flavones and xanthones used empirically in the treatment of liver and gastrointestinal disorders. Guided by ethnopharmacological data on the use of *G. asclepiadea* underground parts in the treatment of diarrhea, antimicrobial activity against selected pathogens of gastrointestinal significance was examined. Presented study was aimed to evaluate antimicrobial activity of the aqueous-ethanolic extract of *G. asclepiadea* underground parts and its petroleum ether, ethyl acetate, butanol and water fractions. A molecular docking analysis was performed as well. The antimicrobial activity against pathogens related to gastrointestinal disorders was tested by a microdilution method. The ethyl acetate fraction showed the greatest antimicrobial activity. The lowest MIC of 0.78 mg/ml was observed against *Bacillus cereus* and *Staphylococcus aureus*, achieved by the petroleum ether and ethyl acetate fractions, respectively. The greatest bactericidal activity (MBC of 0.78 mg/ml), achieved by the ethyl acetate fractions, was recorded against *Enterococcus faecalis*. The yeast *Candida albicans* was the most resistant against the fractions and the extract. *C*-glycosylated flavones isoorientin and isovitexin showed the best binding affinity on *Enterococcus faecalis* lipoate-protein ligase A as determined by a molecular docking analysis. Considering the results of our study, underground parts of *G. asclepiadea* could be used as a valuable natural source of secondary metabolites with promising antimicrobial activity.

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Key words: *Gentiana asclepiadea, antimicrobial activity, extracts, fractions, molecular docking*

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Introduction

Infectious diseases are still a major cause of morbidity and mortality and a leading public health problem worldwide, especially in developing countries. According to the World Health Organization (WHO), there are approximately 1.7 billion cases of diarrhoeal disease among child populations every year, of which over half a million with a lethal outcome. Diarrhea most often appears with symptoms of gastrointestinal infection caused by various pathogens (1). Although many of these infections are treatable, the need for research and development in this area remains due to the existence of side effects of the drugs, its irrational use and the consequent emergence of resistant strains of pathogens (2).

A widely accepted approach in pharmaceutical research of drug candidates or new indications for already existing drugs is the harvesting of ethnopharmacological data on indigenous drugs and their experimental valorization (3). One of the ethnopharmacologically valuable plant sources, which is traditionally used among the population of south-eastern Serbia for the treatment of diarrhea, is the underground part of the willow gentian (*Gentiana asclepiadea* L., *Gentianaceae*). For that purpose, tea or macerate from the pulverized underground parts of willow gentian is used orally (4). Sarić (1989) noticed that the roots and rhizomes of this plant are used empirically in the treatment of infectious

hepatitis type A, while Milojević and Mihajlović (1966) reported its use among the local population of Prokletije Mountain in the treatment of cough and tuberculosis (5, 6). Recent studies have confirmed the hepatoprotective effect of this plant species (7).

The *G. asclepiadea* L. belongs to a large cosmopolitan genus *Gentiana* L. The genus *Gentiana* comprises about 400 plant species, of which 11 species occur in the central part of the Balkan Peninsula (9). Plants of this genus are chemotaxonomically characterized by the biosynthesis of bitter secoiridoid compounds gentiopicroside, sweroside and/or swertiamarin as secologanin derivatives (8, 9). Due to their bitterness, plants of this genus are traditionally used as appetite stimulants and tonics (*amara pura*) (10). In addition to secoiridoids, the main secondary metabolites of *G. asclepiadea* are *C*-glycosylated flavones (isoorientin and isovitexin) and *C*-glycosylated xanthones (isogentisin, mangiferin). Mangiferin is present only in the aboveground part of this plant (9).

Some recent studies (11, 12) confirmed an antimicrobial activity of underground parts of *G. asclepiadea*. Ballast materials such as biopolymers and organic acids can be co-extracted simultaneously with the target compounds, therefore it is necessary to fractionate the primary (crude) extract (13). The aim of this study was to evaluate the *in vitro* antimicrobial properties of the primary aqueous-ethanolic extract of the underground parts of *G. asclepiadea* and its fractions using a set of pathogens with gastrointestinal significance. Additionally, an *in silico* molecular docking analysis of selected secondary metabolites on the target protein of the most sensitive bacterial strain (*Enterococcus faecalis* lipoate-protein ligase A) was carried out to examine their binding affinities and interaction patterns.

Materials and methods

Plant material

Pulverised underground parts (rhizomes with roots) of willow gentian (*G. asclepiadea* L., Gentianaceae) were donated from the Institute for Medicinal Plants Research "Dr. Josif Pančić", batch number 26841019. Until the experiment, the plant material was stored in a dark and dry place at room temperature.

Extraction and fractionation procedure

The primary extract was obtained by a maceration method at room temperature. Powdered underground parts (100 g) were measured in an Erlenmeyer flask, inside of which 70% aqueous ethanol (1 L) was added and was left on a laboratory shaker (100 rpm) for 14h. After filtration, the collected liquid extract was evaporated to dryness on a laboratory Buchi rotavapor R-114. A portion of the obtained primary extract was separated for further antimicrobial testing, while the residue was dissolved in 100 ml of distilled water and further used for fractionation. Fractionation was performed in a funnel by successive liquid-liquid re-extraction using organic solvents of increasing polarity (petroleum ether, ethyl acetate and n-butanol). The liquid fractions were evaporated to dryness on a vacuum evaporator yielding 1.42, 2.01, 10.01, and 70.42% of primary extract for petrolether, ethyl acetate, n-butanol, and water fraction, respectively (14).

Antimicrobial activity

Samples were tested against pathogens related with foodborne poisoning and gastrointestinal disorders. A set of eight microbial strains were used:

- three Gram-positive bacterial strains - *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 19433), and *Bacillus cereus* (ATCC 11778);
- four Gram-negative bacterial strains - *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), *Enterobacter aerogenes* (ATCC 13048), and *Pseudomonas aeruginosa* (ATCC 9027); and
- one yeast strain - *Candida albicans* (ATCC 24433). Overnight cultures (18h) were prepared on a Mueller Hinton Agar (MHA) for bacterial strains or a Sabouraud Dextrose Agar (SDA) for yeast strain.

The antimicrobial activities of the crude extract and its fractions were examined *in vitro* by microdilution method according to CLSI (2012), with slight modification (15). Overnight cultures of selected pathogens were used to prepare the suspensions of 0.5 McFarland turbidity which corresponds to a density of 10^8 CFU/mL. Stock solutions of the crude extract and the collected fractions were prepared in 10% dimethylsulfoxide (DMSO) at a concentration of 400 mg/ml. Testing samples in concentrations ranging from 0.01 to 200.00 mg/ml were prepared by a series of two-fold dilutions of stock solutions. Inoculated Mueller Hinton broth and prepared testing samples were poured in 96-well microtiter plates to a final volume of 100 µL and a microorganism density of 10^6 CFU/mL. Microtiter plates were incubated at 37 ^{O}C for 18 hours. Antimicrobial activity is expressed as the values of minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC). The MIC was defined as the concentration of the sample in which there is no visible growth of microorganisms. Visualization of cell growth was determined using a 0.5% aqueous solution of triphenyltetrazolium chloride (TTC). MMC is the sample concentration at which 99.9% of the cells of the microorganisms are killed. MMC was determined by transferring the contents of the wells without visible growth of microorganisms onto Petri dishes with MHA for bacteria, or SDA for yeasts and counting the grown colonies. The experiment was conducted in the Microbiological Laboratory of the Department of Biology, Faculty of Science and Mathematics - University of Niš. All of the tests were performed three times.

Molecular docking

Molecular binding simulation of gentiopicroside, swertiamarin, sweroside, isovitexin, isoorientin and isogentisin, as main ingredients of underground parts of *G. asclepiadea* and potential antimicrobial ligands, was conducted on the *Enterococcus faecalis* lipoate-protein ligase A (lplA-1) as the target. A molecular docking analysis was performed using an AutoDock Vina 1.1.2 and AutoDock Tools 1.5.6 software. The three-dimensional structures of gentiopicroside, swertiamarin, sweroside, isovitexin, isoorientin and isogentisin were acquired from PubChem (CID number: 88708, 442435, 161036, 162350, 114776, 5281640, respectively), while the lplA-1 was obtained from the Protein Data Bank (PDB ID: 5IJ6). The binding pocket of lplA-1 was prepared based on the reference complexed ligand (lipoic acid). After removing all water molecules and the ligand, as well as adding polar hydrogen, the PDBQT format of the target protein and the selected compounds were prepared by AutoDock Tools. The dimension of the grid box was 20 × 20 × 20 Å with the coordinates of the center being x = 63.38, y = 72.83, z = 127.86. The amino acid residues involved in the interaction and binding affinity, expressed as binding free energy, were determined by the AutoDock Vina analysis. The visualization of the compound-receptor docked complex was performed using the Discovery Studio 2020 Client (Biovia Corp. of San Diego, USA).

Results

In vitro antimicrobial activity

The antimicrobial activity of the primary 70% ethanol extract and its petroleum ether, ethyl acetate, butanol and water fractions against a set of eight selected pathogens (three Gram-positive bacteria, four Gram-negative bacteria and one strain of yeast) are summarized in Table 1. Doxycycline and nystatin were used as a positive control to compare the antibacterial and antifungal activity, respectively, with the examined extract and fractions.

Table 1. Antimicrobial activity of extract of *Gentiana asclepiadea* underground parts and its fractions

Pathogen	70% ethanolic extract	Petrolether fraction	Ethyl acetate fraction	Butanol fraction	Water fraction	Positive control†
	MIC / MMC* (mg/ml)					MIC / MMC (μg/ml)
Gram (+) bacteria						
Staphylococcus aureus (ATCC 6538)	25.00 / 50.00	25.00 / 50.00	0.78 / 25.00	6.25 / 12.50	6.25 / 25.00	7.81 / 15.61
Bacillus cereus (ATCC 11778)	3.13 / 25.00	0.78 / 12.50	1.56 / 12.50	3.13 / 25.00	25.00 / 50.00	0.90 / 15.61
Enterococcus faecalis (ATCC 19433)	3.13 / 3.13	6.25 / 6.25	1.56 / 0.78	3.13 / 1.56	6.25 / 6.25	0.90 / 1.90
Gram (-) bacteria						
Escherichia coli (ATCC 25922)	6.25 / 12.50	12.50 / 25.00	6.25 / 25.00	6.25 / 12.50	6.25 / 25.00	15.61 / 15.61
Salmonella enteritidis (ATCC 13076)	12.50 / 25.00	25.00 / 25.00	6.25 / 25.00	12.50 / 12.50	12.50 / 25.00	0.90 / 1.90
Enterobacter aerogenes (ATCC 13048)	50.00 / 50.00	50.00 / > 200.00	3.13 / 12.50	12.50 / 50.00	50.00 / > 200.00	7.81 / 15.61
Pseudomonas aeruginosa (ATCC 9027)	25.00 / 50.00	25.00 / 50.00	6.25 / 25.00	12.50 / 25.00	25.00 / 50.00	15.61 / 15.61
Yeast						
Candida albicans (ATCC 24433)	50.00 / > 200.00	50.00 / 50.00	12.50 / 12.50	12.50 / 25.00	25.00 / 50.00	3.91 / 7.81

*MIC - minimum inhibitory concentration,
MMC - minimum microbicidal concentration;
†Positive control - doxycycline for bacteria or nystatin for yeast

The examined extract of *G. asclepiadea* underground parts and its fraction inhibited growth of all tested pathogen strains. The values of minimum inhibitory concentrations (MIC) for Gram-positive strains varied from 0.78 to 25 mg/ml. These values were slightly higher (ranged from 3.13 to 50 mg/ml) for Gram-negative strains, clearly indicating the selectivity of antimicrobial activity depending on

the structure of the bacterial cell wall. This has also been confirmed by the values of minimum bactericidal activity (MBC) which were mostly lower for Gram-positive strains (in the range of 0.78 - 50 mg/ml compared to 3.13 - > 200 mg/ml for Gram-negative strain). The lowest MIC values in all of the tested strains were related to the ethyl acetate fraction, with the exception of *B. cereus* strain where the most prominent antimicrobial activity (MIC of 0.78 mg/ml) was achieved by the petrolether fraction.

The obtained results indicate the separation of the basic 70% EtOH extract by liquid-liquid re-extraction can strongly affect antimicrobial activities. This effect was emphasized in the case of *S. aureus* where the MIC of the primary extract was about 32-fold higher than that of the MIC of its ethyl acetate fraction. This confirms that fractionation could improve the inhibitory activity of the extract. On the other hand, this discrepancy was mitigated in the case of *E. coli* where the MIC of the extract and its fractions were almost equal. *B. cereus* and *E. faecalis* showed the highest sensitivity to the extract and fractions in relation to all investigated strains. The greatest bactericidal activity (MBC of 0.78 mg/ml) was achieved by the ethyl acetate fraction against *E. faecalis*. Interestingly, the increase in polarity of the solvents used to prepare the extract fractions was associated with reducing *B. cereus* sensitivity.

Regarding yeast, the tested extract and fractions of *C. albicans* showed a moderate antifungal activity with a MIC ranging from 12.5 to 50 mg/ml, and a minimum fungicide concentration (MFC) ranging from 12.5 to > 200 mg/ml.

Molecular docking

A molecular docking analysis on lplA-1 was performed in order to predict extract ingredients with potential antimicrobial activity and to elucidate the possible ligand-protein interaction. The docking results of six dominant compounds of *G. asclepiadea* underground parts (representatives of the three main groups of compounds) and lipoic acid as the referent ligand are listed in Table 2. All examined compounds showed a significant binding affinity with the binding free energy in the range from 6.0 to -7.4 kcal/mol (lower free energy corresponds to higher binding affinity). The greatest binding affinity was observed in the case of isoorientin and isovitexin with values of -7.4 and -7.3 kcal/mol, respectively. The 2D structures of the lplA-1 active site complexed with potentially active ingredients are illustrated in Figure 1. The active site of the lplA-1 enzyme with isoorientin (3D) as the compound with the best docking score is illustrated in Figure 2.

Table 2. Binding affinity and amino acid residues involved in interaction of examined molecules as ligands and *Enterococcus faecalis* lipoate-protein ligase A (lp1A-1)

Ligand		Binding free energy (kcal/mol)	Residues involved in **H-bond** interactions		Residues involved in **hydrophobic** interactions	
			Amino acid	Distance (Å)	Amino acid	Distance (Å)
Reference ligand	Lipoic acid	-6.0	Asn135 Gly73 Asn123	2.11 2.04 2.70	Gly72 Tyr35 Ile42 His147	3.69 5.08 5.19 4.18
Secoiridoids	Gentiopicroside	-6.3	Asn135 Gly73 Lys131 Gly134	2.71 2.21 2.67 2.76	Asp124 Gly72	3.37 3.54
	Swertiamarin	-6.0	Ala74 Lys131	3.03 2.26	Ala74	3.43
	Sweroside	-6.0	Ala74 Lys131	3.03 2.29	Ala74 Lys131	3.39 3.50
C-glycosylated flavones	Isovitexin	-7.3	Asn123 Tyr35 Asn123 Asn123	2.79 3.04 2.91 2.07	Gly72 Gly72 Arg68 Gly73 Thr149 His147 Gly134 Asn135 Gly134 Asn135 Val75 Ala136 Val75	3.25 3.62 3.65 2.97 3.90 4.56 4.23 4.23 5.29 5.29 5.00 4.52 4.56

Ligand		Binding free energy (kcal/mol)	Residues involved in **H-bond** interactions		Residues involved in **hydrophobic** interactions	
			Amino acid	Distance (Å)	Amino acid	Distance (Å)
C-glycosylated flavones	Isoorientin	-7.4	Asp286 Asn123 Asn123 Asn123	1.86 2.88 2.94 2.23	Gly72 Arg68 His147 Gly134 Asn135 Val75 Ala136 Val75 Val75 Ala136	3.16 3.75 4.50 4.28 4.28 4.97 4.71 5.42 4.66 5.05
C-glycosylated xanthone	Isogentisin	-6.8	Lys131 Lys131	2.48 2.65	Asp286 Gly72 Asp124 Asp286 Gly73 Gly73 Asn123 Gly134 Asn135 Ala136	3.53 3.13 4.95 4.22 2.43 3.02 2.31 4.40 4.40 5.23

Discussion

The results of this study indicate that the extract of *G. asclepiadea* underground parts shows the potential to inhibit the growth of pathogens related with foodborne poisoning and gastrointestinal disorders.

A high sensitivity of *Bacillus* species (*B. subtilis*, *B. cereus*, and *B. subtilis* ATCC 6633) and the selectivity against Gram-positive bacterial strains, were similarly noted by Stefanović et al. (2018) and Mihajlović et al. (2011) (10, 11). In a comparative study of aqueous, ethanolic, acetone and ethyl acetate extracts from the root of *G. asclepiadea*, ethyl acetate extract showed the greatest antimicrobial activity (11). These findings were also in agreement with ours, indicating that the antimicrobial active ingredients have an affinity for distribution in an ethyl acetate solvent. Therefore, ethyl acetate can be efficiently used for extraction, purification and separation of active compounds from examined plant drug that have a potential antimicrobial activity.

The improvement in antimicrobial activity using extract fractionation can be explained by the different concentrations of the active ingredients. The ballast compounds of the extract contribute to the increase of the mass of the dry extract and cause dilution of the active ingredients, thus interfering with the antimicrobial activity. Using the re-extraction process, ballast compounds can be removed from extract. The drastic difference of activities between the primary extract and its fraction (as in the case of *S. aureus*) may be due to the presence of antagonistic compounds separated by fractionation. Such discrepancy in activities, points out the ability of the used extract fractionation procedure to elucidate the potential biological activity of the trace compounds and/or compounds whose activity is influenced by other ingredients of the mixture.

The antimicrobial potential of other plant species of the genus *Gentiana* showed a close pattern of antimicrobial activity. Olennikov et al. (2015) reported about potent antimicrobial activity of bitter herb tea decoctions of four *Gentian* species (*G. algida*, *G. triflora*, *G. macrophylla* and *G. decumbens*), as well as gentiopicroside and loganic acid-6-O-β-D-glucoside as major bitterness compounds against six gastrointestinal pathogens (16). Gentiopicroside, a major secondary metabolite of *G. asclepiadea* underground parts (17), showed an antimicrobial activity with a MIC ranging from 0.1 to 0.4 mg/ml. In accordance with our results, in all tested samples the yeast *C. albicans* was the most resistant. This is not unexpected, given that plants of genus *Gentiana*, especially the underground parts, are rich sources of carbohydrates (18, 19). The presence of carbohydrates as source of carbon in the microenvironment of *C. albicans*, significantly affects the level of yeast growth, survival and tolerance to antifungal drugs (20).

Root extract of *G. kuroo* showed a similar antimicrobial spectrum directed towards Gram-positive bacteria (21). Yin et al. (2017) determined an antibacterial activity of *G. macrophylla* roots extract against bacteria isolated from infected burn wounds that are predominantly Gram-positive strains (22). On the other hand, a broad-spectrum (non-selective) antimicrobial activity of *G. lutea* leaves and flowers extracts was observed (23). This non-selectivity of antimicrobial activity is probably due to differences in the phytochemical profile of the underground and aboveground organs. Namely, in the aboveground part of the *G. lutea*, the major compounds are flavonoids and xanthones, unlike

roots where secoiridoids are dominant (9). A similar antimicrobial spectrum of the root extracts of plants belonging to the genus *Gentiana* that target Gram-positive strains may indicate that ingredients with antimicrobial activity are secondary metabolites that are universally distributed in all or most plant species of this genus, such as secoiridoids, xanthones, and/or flavonoids. In order to explore the possible interactions of the six main compounds from underground parts of *G. asclepiadea* and the selected target protein, the research was further extended by a molecular docking analysis.

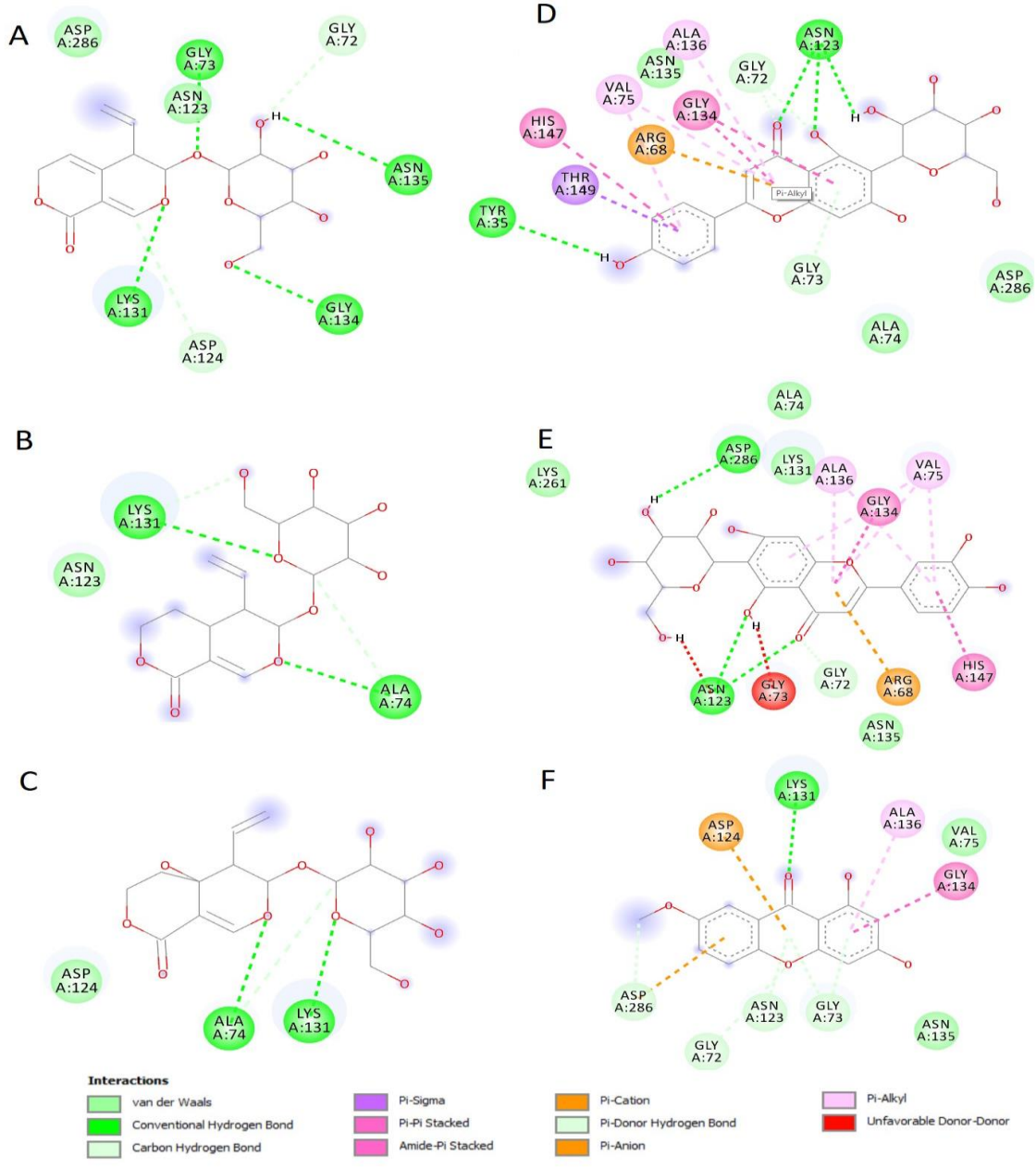

Figure 1. 2D structures of the lipoate-protein ligase A (lp1A-1) complexed with gentiopicroside (A), swertiamarin (B), sweroside (C), isovitexin (D), isoorientin (E), and isogentisin (F).

Enterococcus faecalis lipoate-protein ligase A (lp1A-1) was chosen as the target enzyme, because the lowest MBC (0.78 mg/ml) was recorded on this bacterial strain, which indicates its sensitivity to the constituents of the examined extract and fractions. lp1A-1, as one of the pivotal enzymes involved in

lipoic acid metabolism (24), is proved to be a potential target of *E. faecalis* directed antimicrobial compounds (25). The lowest negative value of the binding free energy of isoorientin (-7.4 kcal/mol) indicates that this ligand can bind more strongly and establish a more stable complex with the catalytic part of the enzyme compared to other compounds.

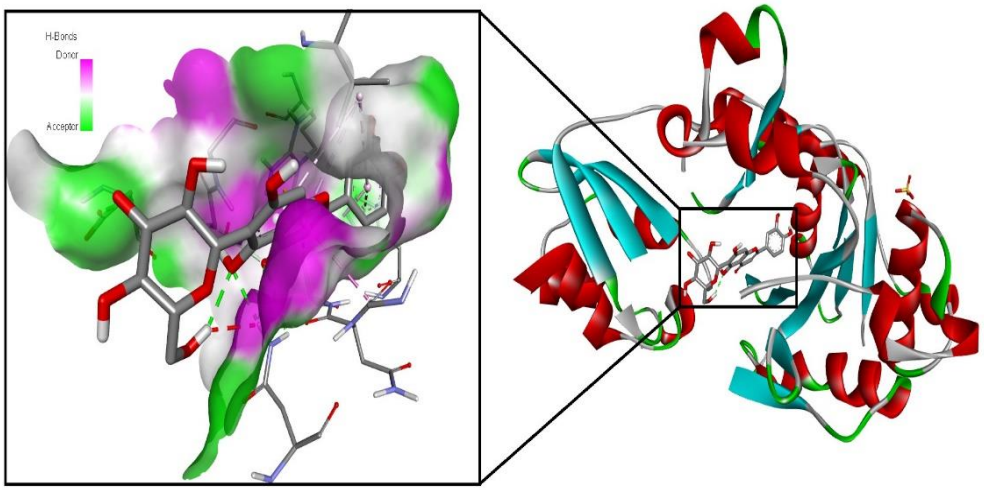

Figure 2. 3D structures of the lipoate-protein ligase A (lp1A-1) with an emphased binding pocket in interaction with isoorientin

An analysis of the interaction profile (Figure 1E) showed that isoorientin formed four conventional hydrogen bonds with Arg123 and Asp286 amino acid residues. It is well known that the ability to form hydrogen bonds between ligands and binding pockets is the primary factor influencing binding affinity (26). Isovitexin also formed four conventional hydrogen bonds via the two amino acid residues Asn123 and Tyr35 (Figure 1D). Although the number of hydrogen bonds is the same, the slightly lower binding free energy of isoorientin (-7.4 kcal/mol) compared to isovitexin (-7.3 kcal/mol) can be explained by the shorter bond length (distance, Å). Studies have shown that a higher hydrogen bond strength and a consequently higher complex stability is associated with a shorter bond length (26). Both mentioned compounds form a multitude of hydrophobic bonds, which are characterized by lower strength compared to the interactions via hydrogen bonds. The contribution of the hydrophobic binding to the docking score was highlighted in the case of genciopiroside. Although it formed four conventional hydrogen bonds (Figure 1A), a significantly lower binding affinity (-6.3 kcal/mol) was observed compared to isovitexin and isoorientin, which is a consequence of weaker hydrophobic binding. Amino acid residues involved in the interactions (hydrogen and hydrophobic) and the interatomic distance between selected compounds and lp1A-1 are listed in Table 2.

Conclusion

The results of the conducted study confirm that the underground parts of *Gentiana asclepiadea* are a valuable plant drugs with a significant antimicrobial potential against pathogens related with gastrointestinal diseases. Fractionation of the primary extract was a suitable method to improve the antimicrobial activity. The ethyl acetate fraction was particularly active, indicating that the active ingredients of the extract have an affinity for distribution in this solvent. The activity of the extract and the fractions was selective, mostly towards Gram-positive bacterial strains. The molecular docking and interaction profile analysis of the six major secondary metabolites of the examined drug on lipoate-protein ligase A (lp1A-1) show that the *C*-glycosylated flavones isoorientin and isovitexin have the best binding affinity. Further *in vivo* studies and detailed phytochemical analysis of extract and fractions are required to determine their active compounds and evaluate possible uses in the treatment of gastrointestinal disorders.

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References

1. World Health Organisation. Diarrhoeal disease. Fact Sheets Detail 2017 May 2 "cited 2021 Jun 22". Available from: https://www.who.int/en/news-room/fact-sheets/detail/diarrhoeal-disease
2. Bulteel AJB, Larson EL, Getahun H. Identifying global research gaps to mitigate antimicrobial resistance: A scoping review. Am J Infect Control 2021;49(6):818-24. [CrossRef] [PubMed]
3. Tekwu EM, Pieme AC, Beng VP. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastro-intestinal relevance. J Ethnopharmacol 2012;142(1): 265-73. [CrossRef] [PubMed]
4. Matejić JS, Stefanović N, Ivković M, Živanović N, Marin PD, Džamić AM. Traditional uses of autochthonous medicinal and ritual plants and other remedies for health in Eastern and South-Eastern Serbia. J Ethnopharmacol 2020;261:113186. [CrossRef] [PubMed]
5. Sarić M, editor. Medicinal Plants of SR Serbia. Belgrade: Serbian Academy of Sciences and Art; 1989.
6. Milojevic B, Mihajlov M, editors. Project report about medicinal plants investigations in the area of Prokletije Mountains and mountains Komovi. Belgrade: Institute for Medicinal Plants Research; 1966.
7. Mihailović V, Mihailović M, Uskoković A, Arambašić J, Mišić D, Stanković V, et al. Hepatoprotective effects of Gentiana asclepiadea L. extracts against carbon tetrachloride induced liver injury in rats. Food Chem Toxicol 2013;52:83-90. [CrossRef] [PubMed]
8. Pan Y, Zhao YL, Zhang J, Li WY, Wang YZ. Phytochemistry and Pharmacological Activities of the Genus Gentiana (Gentianaceae). Chem Biodivers 2016; 13(2):107-50. [CrossRef] [PubMed]
9. Šavikin K, Aljančić I, Vajs V, Milosavljević S, Jadranin M, Đordević I, et al. Bioactive secondary metabolites in several genera of Gentianaceae species from the central regions of the Balkan Peninsula. In: Rybczyński JJ, Davey MR, Mikuła A, editors. The Gentianaceae - Volume 2: Biotechnology and Applications. Berlin: Springer; 2015. p. 319-47. [CrossRef]
10. Menković N, Savikin K, Tasić S, Zdunić G, Stesević D, Milosavljević S, et al. Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). J Ethnopharmacol 2011; 133(1):97-107. [CrossRef] [PubMed]
11. Stefanović O, Ličina B, Vasić S, Radojević I, Čomić L. Bioactive extracts of *Gentiana asclepiadea*: antioxidant, antimicrobial, and antibiofilm activity. Bot Serb 2018;42(2):223-9. [CrossRef]
12. Mihailović V, Vuković N, Nićiforović N, Solujić S, Mladenović M, Mašković P, et al. Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea* L. J Med Plants Res 2011;5(7):1164-74.
13. Šavikin K, Živković J, Alimpić A, Zdunić G, Janković T, Duletić-Laušević S, et al. Activity guided fractionation of pomegranate extract and its antioxidant, anti-diabetic and antineurodegenerative properties. Ind Crops Prod 2018;113:142-9. [CrossRef]
14. Zdunić G, Alimpić-Aradski A, Gođevac D, Živković J, Duletić-Laušević S, Krstić-Milošević D, et al. *In vitro* hypoglycemic, antioxidant and antineurodegenerative activity of chokeberry (*Aronia melanocarpa*) leaves. Ind Crops Prod 2020;148:112328. [CrossRef]
15. Clinical and Laboratory Standards Institute (CLSI). M07-A9. 2012;29(2):1-63.
16. Olennikov DN, Kashchenko NI, Chirikova NK, Koryakina LP, Vladimirov LN. Bitter Gentian Teas: Nutritional and Phytochemical Profiles, Polysaccharide Characterisation and Bioactivity. Molecules 2015; 20(11):20014-30. [CrossRef] [PubMed]
17. Popović Z, Krstić-Milošević D, Marković M, Vidaković V, Bojović S. *Gentiana asclepiadea* L. from Two High Mountainous Habitats: Inter- and Intrapopulation Variability Based on Species' Phytochemistry. Plants (Basel) 2021;10(1):140. [CrossRef] [PubMed]
18. Cheng Z, Zhang Y, Song H, Zhou H, Zhong F, Hu H, et al. Extraction optimization, characterization and antioxidant activity of polysaccharide from *Gentiana scabra* bge. Int J Biol Macromol 2016;93(Pt A):369-80. [CrossRef] [PubMed]
19. Zou YF, Fu YP, Chen XF, Austarheim I, Inngjerdingen KT, Huang C, et al. Polysaccharides with immunomodulating activity from roots of Gentiana crassicaulis. Carbohydr Polym 2017;172:306-14. [CrossRef] [PubMed]
20. Van Ende M, Wijnants S, Van Dijck P. Sugar Sensing and Signaling in *Candida albicans* and *Candida glabrata*. Front Microbiol 2019;10:99. [CrossRef] [PubMed]
21. Baba SA, Malik SA. Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle. Saudi J Biol Sci 2014; 21(5):493-8. [CrossRef] [PubMed]
22. Yin C, Xie L, Guo Y. Phytochemical analysis and antibacterial activity of Gentiana macrophylla extract against bacteria isolated from burn wound infections. Microb Pathog 2018;114:25-8. [CrossRef] [PubMed]
23. Šavikin K, Menković N, Zdunić G, Stević T, Radanović D, Janković T. Antimicrobial activity of Gentiana lutea L. extracts. Z Naturforsch C J Biosci 2009;64(5-6): 339-42. [CrossRef] [PubMed]
24. Cronan JE, Zhao X, Jiang Y. Function, attachment and synthesis of lipoic acid in Escherichia coli. Adv Microb Physiol 2005;50:103-46. [CrossRef] [PubMed]
25. Arulmozhi P, Vijayakumar S, Praseetha PK, Jayanthi S. Extraction methods and computational approaches for evaluation of antimicrobial compounds from *Capparis zeylanica* L. Anal Biochem 2019;572:33-44. [CrossRef] [PubMed]
26. Khayrani AC, Irdiani R, Aditama R, Pratami DK, Lischer K, Ansari MJ, et al. Evaluating the potency of Sulawesi propolis compounds as ACE-2 inhibitors through molecular docking for COVID-19 drug discovery preliminary study. J King Saud Univ Sci 2021; 33(2):101297. [CrossRef] [PubMed]

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ANTIMIKROBNA AKTIVNOST FRAKCIJA I EKSTRAKTA PODZEMNIH DELOVA BILJNE VRSTE *GENTIANA ASCLEPIADEA* L. SA ANALIZOM MOLEKULARNOG DOKINGA

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Trava od žutice (*Gentiana asclepiadea* L.) vredan je izvor sekoiridoida, *C*-glikoziliranih flavona i ksantona, koja se u tradicionalnoj medicini upotrebljava za lečenje bolesti jetre i gastrointestinalnog trakta. Cilj ovog istraživanja bio je ispitati antimikrobnu aktivnost vodeno-etanolnog ekstrakta podzemnih delova trave od žutice i njegovih frakcija (petrol-etarska, etil-acetatna, butanolna i vodena frakcija). Takođe, sprovedena je analiza molekularnog vezivanja. Antimikrobna aktivnost na patogene gastrointestinalnog trakta testirana je mikrodilucionom metodom. Generalno, najbolju aktivnost ispoljila je etil-acetatna frakcija. Najniža minimalna inhibitorna koncentracija od 0,78 mg/ml zabeležena je kod soja *Bacillus cereus* pomoću petrol-etarske frakcije, odnosno *Staphylococcus aureus* pomoću etil-acetatne frakcije. Najbolja baktericidna aktivnost (minimalna baktericidna koncentracija od 0,78 mg/ml) ostvarena je etil-acetatnom frakcijom za soj *Enterococcus faecalis*. *Candida albicans* bila je najotpornija na dejstvo ispitivanog ekstrakta i njegovih frakcija. Analizom molekulskog vezivanja, utvrđeno je to da *C*-glikozilirani flavoni izoorijentin i izoviteksin pokazuju najveći afinitet vezivanja prema *Enterococcus faecalis* lipoat-protein ligazi A. Na osnovu rezultata sprovedenog istraživanja, može se zaključiti da bi podzemni delovi trave od žutice mogli biti vredan izvor sekundarnih metabolita sa obećavajućom antimikrobnom aktivnošću.

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Ključne reči: *Gentiana asclepiadea, antimikrobna aktivnost, ekstrakti, frakcije, molekularni doking*