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In silico and in vitro antibacterial evaluation of eight Anatolian *Salvia* species with chemical profiling by LC-HRMS

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Salvia **species play a significant role in the pharmaceutical and nutraceutical industries due to their rich chemical profiles and notable biological activities. This study aimed, for the first time, to comprehensively investigate the phytochemical profile and antibacterial properties of eight selected *Salvia* species. Ethanol and water extracts were prepared from the dried aerial parts, and their chemical compositions were analyzed via LC-HRMS. Rosmarinic acid was the major compound in almost all the prepared extracts. Following rosmarinic acid, caffeic acid, fumaric acid, salvianolic acid B, and hispidulin-7-*O*-glucoside were also identified as principal constituents. The antibacterial potential of the relevant metabolites was evaluated in silico, and their receptor binding profiles targeting dihydrofolate reductase and dihydropteroate synthase were elucidated. Additionally, the in vitro antibacterial efficacy of all the extracts was determined against various bacterial strains using a modified microbroth dilution method. Among all tested extracts, both the infusion and decoction of *Salvia albimaculata* exhibited the strongest antibacterial activity against *Staphylococcus aureus* and MRSA. Additionally, the ethanol extracts of *S. albimaculata*, *S. viridis*, *S. verticillata*, and *S. pilifera* demonstrated moderate activity against MRSA. These findings contribute valuable insights into the development of natural-based antibacterial agents for the pharmaceutical and food industries.**

Keywords *Salvia*, LC-HRMS, Rosmarinic acid, Antibacterial activity, Molecular docking

Abbreviations

LC-HRMS	Liquid chromatography - high resolution accurate mass spectrometry
MRSA	Meticillin-resistant Staphylococcus aureus
DMSO	Dimethyl sulfoxide
ADME	Absorption, distribution, metabolism, and excretion
LOD	Limit of detection
LOQ	Limit of quantification
ATCC	American Type Culture Collection
QSAR	Quantitative Structure Activity Relationship

The use of plants in folk medicine has been a longstanding practice, shedding light on the development of drugs[1,2]. Türkiye, with its rich plant diversity, plays a key role in the discovery of plant-derived bioactive molecules and standardized extracts[3–6]. The Lamiaceae family, particularly the *Salvia* genus, stands out due to its

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extensive use and 50% endemism rate in Türkiye. Ethnopharmacological records highlight *Salvia*'s applications in traditional medicine, where its herbal teas, infusions, and decoctions are valued for antibacterial, antiseptic, diuretic, and wound-healing effects. *Salvia* is also widely used in the pharmaceutical and industrial sectors due to its aromatic and medicinal properties. Fresh leaves are used in dairy products, vinegars, and salads, and the dried form is used as a flavoring in many dishes, preserves, and sausages[7–9]. Research on natural resources that minimize harm to human health and ecosystems has revealed that essential oils and polar extracts from *Salvia* possess notable insecticidal properties[10]. Studies from various research groups have demonstrated a wide range of bioactivities of *Salvia* species, based on their traditional and folk uses. These include anticholinesterase, antioxidant, hypotensive, hypoglycemic, hypolipidemic, and anticancer activities. A review of the literature reveals that these plants also possess antibacterial properties, which are often attributed to bioactive constituents such as phenolic compounds, essential oils, terpenic molecules, and flavonoids[11–21]. These antibacterial properties are of great interest for potential uses and applications in various fields, such as the food, pharmaceutical, and cosmetic industries, where natural antibacterial substances are sought for their preservative effects. In recent years, the use of synthetic preservatives has been associated with adverse effects on human health, particularly cancer and allergic reactions[22]. For this reason, to mitigate these risks, reduce contamination by pathogenic microorganisms, extend shelf life, and improve overall product safety, the application and integration of natural products has significantly increased. Moreover, in sectors where sustainability is also a key concern, naturally based products are increasingly favored due to the antimicrobial properties of polyphenolic compounds derived from fruit peels, seeds, and other plant parts[23–26].

Furthermore, phenolic-rich plant extracts have been shown to offer significant health benefits, particularly because they coexist with probiotic lactic acid bacteria during both the preparation and subsequent stages of product processing. These extracts play a critical role in inhibiting the production of microbial toxins and preventing biofilm formation. The species from which natural-based antibacterial secondary metabolites are frequently obtained in the industry include mainly *Salvia* species, *Camellia sinensis* (L.) Kuntze, *Calendula officinalis* L., *Mentha piperita* L., *Lavandula angustifolia* Miller, and *Cinnamomum zeylanicum* J. Presl[27–29]. Phenolic compounds with diverse antimicrobial mechanisms, especially at the cellular level, have been reported to be more effective when included in the formulation of perishable products, external products that may cause contact contamination, and edible coating materials[30,31].

Antibacterial activity is significant in many areas, supporting human health, safety, and the general welfare of society[18,32]. Globally, microbial agents are one of the main sources of morbidity and mortality[33,34]. The rise in infection-related diseases worldwide, along with the resistance pathogens have developed against synthetic products, has increased interest in natural products. The resources available for handling microbial issues are expanded by the ongoing investigation of natural sources, such as *Salvia* species, for their antibacterial characteristics[35].

The usage of in silico drug design technology significantly reduces the time and financial expenses involved in drug discovery. Advances in computational technology have led to substantial progress in pharmaceutical research[36]. This technology enables the execution of 2D / 3D QSAR (Quantitative Structure Activity Relationship) and molecular docking studies[37]. Additionally, the pharmacodynamic parameters, pharmacokinetic properties, and toxicity of molecules can be assessed and analyzed through the use of this technology[38]. Consequently, these methods collectively enhance the efficiency and selectivity of the drug discovery process[36].

Structure-based and ligand-based computational approaches have a significant impact on the discovery of new antibacterial natural agents. Thus, it is a valuable step in researching specific potential drug molecules for use in treating diseases[16,39]. In recent years, in silico research has become increasingly valuable in drug discovery studies, offering significant advantages in terms of cost reduction and time efficiency[33,40].

Although numerous studies have been conducted on the antibacterial properties of *Salvia* species, their comparisons in multiple platforms, such as in vitro and in silico approaches, have not been sufficiently focused on. However, the integration of these methodologies will certainly hold significant potential to advance understanding and bring new perspectives to this field.

This study aimed to characterize the polar extracts of eight selected *Salvia* species using LC-HRMS, assess their in vitro antibacterial activity, investigate binding interactions of the detected compounds with target enzymes via molecular docking (PDB IDs: 3SRW and 2VEG), and evaluate their drug-like properties through in silico ADME analysis[41,42].

Materials and methods

Plant material

Table 1 lists the voucher specimens, endemism data, collection dates, and locations of the plant materials, which were gathered from 5 provinces throughout Türkiye (Southeastern Anatolia, Mediterranean region of Anatolia, Eastern Anatolia Region). All species were collected under legal permits. The plant materials were identified by Prof. Emine Akalın, Assoc. Prof. Yeter Yeşil and Assoc. Prof. Münevver Bahar Gürdal Abamor from the Department of Pharmaceutical Botany, Istanbul University. The voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University, Türkiye (ISTE).

No	Herbarium Number	Plant name	Endemism	Collection dates	Locations
1	107526	*Salvia pilifera* Montbret & Aucher ex Benth.	Endemic	02.05.2015	Kahramanmaraş (Southeastern Anatolia Region)
2	107527	*Salvia verbenaca* L.	-	02.05.2015	Kahramanmaraş (Southeastern Anatolia Region)
3	107528	*Salvia viridis* L.	-	02.05.2015	Kahramanmaraş (Southeastern Anatolia Region)
4	107530	*Salvia aramiensis* Rech.f.	-	03.05.2015	Hatay (Southeastern Anatolia Region)
5	107540	*Salvia verticillata* L.	-	31.05.2015	Hatay (Southeastern Anatolia Region)
6	107552	*Salvia pisidica* Boiss. & Heldr. ex Benth.	Endemic	27.06.2015	Antalya (Mediterranean region of Anatolia)
7	107570	*Salvia albimaculata* Hedge & Hub.-Mor.	Endemic	25.06.2015	Karaman (Mediterranean region of Anatolia
8	107574	*Salvia caespitosa* Montbret & Aucher ex Benth.	-	18.07.2015	Malatya (Eastern Anatolia Region)

Table 1. Information on the plant materials.

Chemicals

Ethanol (Merck), p-Coumaric acid (≥98%, Sigma-Aldrich), caffeic acid (≥98%, Sigma-Aldrich), (-)-epicatechin Gallate (>97%, TRC Canada), (-)-epigallocatechin gallate (>97%, TRC Canada), apigenin (>97%, TRC Canada), acacetin (>97%, TRC Canada), luteolin (95%, Sigma-Aldrich), isosakuranetin (>97%, Phytolab), hispidulin (>97%, TRC Canada), (+)-trans taxifolin (>97%, TRC Canada), (-)-epigallocatechin (>97%, TRC Canada), nepetin (98%, Supelco), orientin (>97%, TRC Canada), luteolin 7-glucoside (>97%, TRC Canada), nepetin-7-glucoside (>97%, Phytolab), verbascoside (86.31%, HWI ANALYTIK GMBH), quercetin (≥95%, Sigma-Aldrich), naringenin (≥95%, Sigma-Aldrich), chrysin (≥96%, Sigma-Aldrich), rutin (≥94%, Sigma-Aldrich), rosmarinic acid (≥96%, Sigma-Aldrich), dihydrocapsaicin(≥97%, Sigma-Aldrich), fumaric acid (≥99%, Sigma-Aldrich), chlorogenic acid, caffeine (≥99%, Sigma-Aldrich), 3,4-hydroxy benzoic acid (≥99%, Sigma-Aldrich), vanillic acid (≥97%, Sigma-Aldrich), salicylic acid (≥98%, Sigma-Aldrich), ascorbic acid (≥99%, Sigma-Aldrich), hesperidin (≥98%, J&K), homogentisic acid (≥97%, Biyosynth Carbosynth), 2,5-dihydroxybenzoic acid (≥%99, J&K), salvianolic acid B (≥97%, Biyosynth Carbosynth), pinocembrin (≥97%, Biyosynth Carbosynth), genkwanin (≥97%, Biyosynth Carbosynth), carnosic acid (≥97%, Biyosynth Carbosynth), hispidulin-7-*O*-glucoside (≥97%, Biyosynth Carbosynth) were used. Purity assessment of the isolated compounds sclareol (≥90%, NMR), 6-OH-luteolin-7-*O*-glucoside (≥90%), 6-*O*-Me-luteolin-7-*O*-glucoside (≥90%), apigenin-7-*O*-acylglucoside (≥94%), luteolin-7-*O*-acylglucoside (≥97%), chrysoeriol (≥92%), cirsimaritin (≥90%), cirsilineol (≥92%), apigenin-7-*O*-methylate (≥95%), dihydro caffeic acid (≥97%), luteolin-7-*O*-rutinoside (95%), apigenin-7-*O*-glucoside (94%) was carried out by quantitative NMR as described in the literature with the internal standards of maleic acid and trimethoxybenzene[43]. Ciprofloxacin (Bayer Türk Kimya San. Istanbul/Türkiye), Dimethylsulfoxide (DMSO- Merck) Tryptic soy agar (TSA) from GBLaboratory, Istanbul/Türkiye, Mueller Hinton broth (MHB), Mueller Hinton Agar (MHA) TitanBiotek, Istanbul/Türkiye were purchased.

Extraction

The shaded-dried and coarsely powdered aerial parts of *Salvia* species (25 g) were extracted with ethanol (250 mL) using a continuous extraction technique in a Soxhlet apparatus. Following the completion of the extraction procedure, samples were prepared by removing the organic solvent under reduced pressure using a rotary evaporator. Infusion and decoction were prepared separately by extracting the dried aerial parts (25 g) of all *Salvia* varieties with distilled water (250 mL). The collected infusion and decoction extracts were lyophilized to obtain crude extracts. All extracts were stored in a refrigerator at 4 °C until the time of biological activity analysis, additionally, 250 mg of each extract was allocated for chemical analysis and stored in a freezer at -35°C[16]. The extraction yields (%) of the different *Salvia* species using ethanol, infusion, and decoction methods are summarized in Table 2.

Salvia Species	Ethanol Extract (%)	Infusion Extract (%)	Decoction Extract (%)
Salvia pilifera	19.60	3.28	7.61
Salvia verbenaca	17.22	6.33	6.04
Salvia viridis	42.40	2.29	5.23
Salvia aramiensis	30.80	31.20	32.00
Salvia verticillata	23.60	3.90	1.16
Salvia pisidica	13.69	1.88	6.70
Salvia albimaculata	32.40	6.20	6.31
Salvia caespitosa	11.18	3.80	3.92

Table 2. Extract yields (%) of *Salvia* Species.

Gradient Time	Flow (mL/min)	% A (1% Formic Acid-H_2O)	%B (1% Formic Acid-MeOH)
0.00	0.35	50	50
1.00	0.35	50	50
3.00	0.35	0	100
6.00	0.35	0	100
7.00	0.35	50	50
15.00	0.35	50	50

Table 3. The mobile phase (LC-HRMS) with gradient program of *Salvia* Extracts.

LC-HRMS analysis

The secondary metabolite profiling of *Salvia* extracts was conducted using a previously established and validated LC-HRMS method developed by our research group. Chromatographic separation was achieved on a Troyasil C18 column (150 × 3 mm, 5 µm) using a Thermo Ultimate 3000 HPLC system coupled with a Thermo Orbitrap Q-Exactive mass spectrometer in Bezmialem Vakif University Drug Application and Research Center (DARC). The identification of the compounds was carried out by comparing the retention times of standard substances and the high-resolution mass spectrometry (HRMS) data obtained from the Drug Application and Research Center Library (ILMER) of Bezmialem Vakif University. To minimize variability caused by external factors such as ionization repeatability in mass spectrometry measurements, dihydrocapsaicin (purity 95%) was used as an internal standard during LC-HRMS analyses. Analyses were performed by using the mobile phase system with gradient programs of 50% A and 50% B for 0–1 min, 100% B for 1–6 min, and 50% A and 50% B for 6–10 min. Laboratory ambient conditions were recorded as 22.0 ± 5.0 °C and 50 ± 15% relative humidity. MS parameters included a sheath gas flow rate of 45, auxiliary gas flow rate of 10, spray voltage of 3.80 kV, capillary temperature of 320 °C, auxiliary gas heater temperature of 320 °C, and an S-lens RF level of 50. Compound identification was performed by comparing retention times and characteristic ions with those of reference standards. The method's LC and Mass conditions (Table 3, Tables S1 and S2) were given in detail in Supplementary material. The validation parameters of this study were linearity, recovery, repeatability, limits of the detection (LOD), and limits of the quantification (LOQ) (Tables S3-S5)[44–47].

Sample Preparation for LC-HRMS

Approximately 50 mg of each extract was weighed and dissolved in 2 mL of methanol. The mixture was sonicated in an ultrasonic bath until a clear solution was obtained. The solution was filtered using a 0.45 µm Millipore Millex-HV syringe filter. To each filtered extract solution, 30 µL of dihydrocapsaicin internal standard (prepared from a 1000 ppm stock solution in methanol) was added. Subsequently, 1 mL of the final mixture was transferred into vials for LC-HRMS analysis and injected to the LC-HRMS system as 5 µL. In order to measure the components present at very low concentration in the extract within the detection limits of the quantified secondary metabolites, the injection volume was repeated as 50 µL instead of 5 µL[48].

Standard solutions

Standard solutions were prepared by dissolving the compounds in methanol to obtain ten different concentrations: 0.01, 0.05, 0.1, 0.3, 0.5, 1, 3, 5, 7, and 10 mg/L. A stock solution of dihydrocapsaicin (purity 97%) at a concentration of 1000 mg/L in methanol was utilized as the internal standard.

Method validation of LC-HRMS

The LC-HRMS method was validated based on EURACHEM CITAC Guide and previous reports of our group. Method validation parameters were selected as specificity, accuracy, linearity, LOD and LOQ[49–51].

Specificity The specificity of the developed LC-HRMS method was assessed by the direct (blind) analysis of various prepared solvent samples, *Salvia* extracts, and samples spiked with the target analytes. Specificity was defined as the ability to clearly detect the target analyte at its specific retention time without interference from impurities, matrix components, or degradation products. The LC-HRMS technique was chosen to ensure high selectivity and sensitivity within the complex matrix and to effectively minimize matrix-related interferences.

Accuracy Accuracy represents the degree of closeness between the measured values and the true concentration of the analyte. In this study, accuracy was evaluated by calculating the percentage recovery (% recovery) for each analyte, based on LC-HRMS data. The % recovery was determined using the following formula (Tables S3-S5).

Recovery % = Recovered concentration /injected concentration x 100.

Linearity To enable the quantitative determination of the identified secondary metabolites via LC-HRMS, a calibration curve was established. For this purpose, six replicate measurements were performed using standard solutions at various concentrations. The calibration curve was constructed based on the nominal concentrations of the analytes. From this curve, the linear regression equation and correlation coefficient (R^2) were calculated to evaluate the linearity of the method.

Detection and quantitation limits Limits of detection (LOD), limit of quantification (LOQ), reproducibility, recovery, and linearity were the characteristics used to validate the method. Using the following equation, the

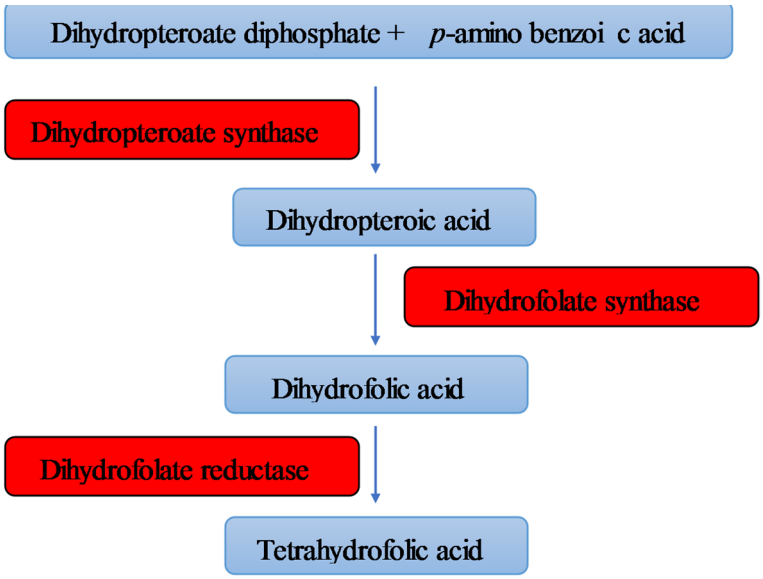


Fig. 1. Biosynthesis pathways of tetrahydrofolic acid.

LODs of the approach for distinct substances were determined: LOD or LOQ = κSDa/b, where LOQ is 3 and κ = 3 for LOD[49–51].

In Silico studies

Selection and identification of target crystal structures

The inhibition of dihydropteroate synthase is a significant enzyme that can explain the antibacterial activity. Enzyme inhibition inhibits 7,8-dihydropteroic acid formation, thus bacterial folic acid synthesis. Similarly, dihydrofolate reductase inhibitors inhibit the formation of tetrahydrofolic acid from dihydrofolic acid, thus affecting DNA / RNA biosynthesis[52] (Fig. 1).

The crystal structure of dihydropteorate synthase from *Streptococcus pneumoniae*: complex with 6-hydroxymethyl-7,8-dihydropterin monophosphate (PDB ID: 2VEG)[41] was retrieved from RCSB Protein Data Bank. The resolution of 2VEG was 2.40 Å. The organism was *Streptococcus pneumoniae* and the expression system was *Escherichia coli*. No mutation was reported for the structure.

The crystal structure of *Staphylococcus aureus* Dihydrofolate Reductase complexed with 7-aryl-2,4-diaminoquinazolines (PDB ID: 3SRW)[42] was retrieved from RCSB Protein Data Bank. The resolution of 3SRW was 1.70 Å. The organism was *Staphylococcus aureus* and the expression system was *Escherichia coli*. No mutation was reported for the structure.

Inhibiting dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) is a vital strategy for antibacterial development, as these enzymes are essential for bacterial folate biosynthesis, which is critical for DNA and protein production. Humans lack DHPS and rely on dietary folate, and bacterial DHFR differs structurally from the human enzyme, enabling selective targeting. Sulphonamides inhibit DHPS, while trimethoprim inhibits DHFR, and their combination (e.g., co-trimoxazole) disrupts two consecutive steps in the pathway, providing enhanced antibacterial effects. Despite the emergence of resistance, the folate pathway remains a key antibacterial target, with ongoing research focused on designing dual-action and novel inhibitors to overcome resistance and improve treatment efficacy[53–55].

Docking procedure

The protein preparation process was performed with Protein Preparation Wizard Module of Schrödinger Software Suite (Schrödinger Version 2020-3). Optimization and minimization processes were performed with the module. Ligands were prepared, optimized and minimized using LigPrep module of the software. The binding sites were determined by generating grids around the co-crystallized ligands of the crystal structures. Standard precision was used in docking studies.

In Silico ADME studies

Qikprop module of the molecular modelling software was utilized for this part (Version 2020-3). The analyzed descriptors related to ADME properties of the title compounds were given in Table 4.

No.	Descriptor	Optimal ranges of the values
1.	Molecular Weight (MW)	150–500
2.	Octanol/water partition coefficient (Log Po/w)	−2 to 5
3.	Hydrogen bond donor (donorHB)	< 5
4.	Hydrogen bond acceptor (accptHB)	< 10
5.	Aqueous solubility (Log S)	−6.5 to 0.5
6.	Percent of human oral absorption (HOA%)	≥ 80% is high, ≤ 25 is poor

Table 4. The analyzed descriptors related to ADME properties of the title compounds.

In vitro antibacterial activity study

The extracts were tested against Gram-positive and Gram-negative reference strains; *Staphylococcus aureus* ATCC 25923, MRSA (methicillin-resistant *S. aureus*) ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633 from the Institute of Microorganisms Culture Collection of Istanbul Medical Faculty/ Istanbul/Türkiye.

Microbroth Dilution method

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of each extract were determined on the basis of the microbroth dilution method in 96 multi-well microtitre plates[56–58]. The bacterial cultures were prepared from overnight cultures on Tryptic Soy Agar (TSA) (bacteria at 37 °C for 24 h) by diluting in Mueller Hinton Broth (MHB) from approx.10^8 CFU/mL to 2×10^6 CFU/mL. The 50 µL Tween 80 + Mueller Hinton Broth (MHB) was added to the wells starting from the first well and continuing up to the twelfth. The extracts were prepared 1% in DMSO. Two-fold serial dilutions were made for final concentration ranging from 5.000 to 30 µg/mL. One row of positive controls for Ciprofloxacin (final concentrations from 8 to 0.01 µg/mL) was added to each plate. In addition, an extra row of DMSO was used as a vehicle control to determine its possible inhibitory activity. Finally, 25 µL of bacterial suspensions and 0.001% resazurin solution were added to each well.

After incubation at 37 °C for 24 h, the microtiter plates were examined visually for microbial growth, which appeared as pink colored by resazurin dye. In each row, the well containing the least concentration and showed no visible growth was considered the MIC. MBC's were determined by taking samples from the wells that showed no visible growth. The bacterial samples were inoculated on TSA plates and incubated at 37 °C for 24 h.

Results

Compound detection using LC-HRMS

Supplementary Tables S3-S5 and Tables 5 and 6 present the LC-HRMS analysis results for the polar extracts of *Salvia* species, including their retention times. Chromatograms are also provided in supplementary material (Figures S1-S132). In the present study, LC-HRMS analysis of ethanol and water (infusion and decoction) extracts from 8 *Salvia* species revealed that rosmarinic acid was the major constituent in all extracts which has strong antibacterial, antioxidant, anticancer, and anti-inflammatory activity[16,59,60].

A total of 48 compounds were identified in the ethanol extracts, while 43 and 44 compounds were found in the decoction and infusion water extracts, respectively. These include phenolic acids and derivatives, phenylpropanoids, flavonoids, terpenoids, and other compounds.

Among the ethanol extracts, rosmarinic acid, caffeic acid, fumaric acid, salvianolic acid B, and hispidulin-7-*O*-glucoside were detected as the major molecules in almost all of the 8 extracts. However, salvianolic acid B was present in minor amounts in *S. viridis* ethanol extract.

Salvianolic acid B was found as the principal substance in decoction extracts of *S. pilifera*, *S. verbenaca*, *S. aramiensis*, *S. pisidica*, *S. albimaculata*, and *S. caespitosa*, while it was observed as a minor compound in *S. viridis*. Whereas salvianolic acid B was not detected in *S. aramiensis* infusion extract, it was found in low amounts in *S. viridis* extract and was detected in major concentrations in the other 6 extracts.

Hispidulin-7-*O*-glucoside was determined as the main component among the water extracts of the overall *Salvia* species.

Verbascoside was identified as the principal compound in *S. viridis* ethanol, infusion, and decoction extracts, whereas it was either undetected or present in low levels in the other extracts.

Homogentisic acid appeared in minor amounts in *S. viridis*, and *S. verticillata* infusions from water extracts, while it was found in high concentrations in other extracts. It was detected at a major rate in *S. pilifera*, *S. pisidica*, and *S. caespitosa* decoctions, and at a minor rate in the other 5 extracts. In ethanol extracts, it was detected at a low amount in *S. viridis* extract, while it was found at high levels in the other 7 ethanol extracts.

Salicylic acid was found to be the principal in the infusions of *S. viridis*, *S. pisidica*, and *S. albimaculata* from water extracts; it was detected at a low rate in the other 5 extracts. While salicylic acid was found in major amounts in *S. pilifera*, *S. aramiensis*, *S. pisidica*, and *S. albimaculata* decoction extracts, it was detected in low concentrations in the other 4 extracts. Among the prepared ethanol extracts, salicylic acid was observed in major amounts in all extracts.

	S. pilifera			*S. verbenaca*			*S. viridis*			*S. aramiensis*		
Compounds	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**
Ascorbic acid	405.27	< LOD	129.17	272.34	< LOD	694.59	233.16	< LOD	257.39	332.98	< LOD	253.57
(-)-Epigallocatechin	177.27	95.64	210.75	69.62	4.08	86.48	132.91	46.12	50.59	290.17	36.77	242.66
(-)-Epigallocatechin gallate	15.90	23.76	19.48	< LOD	0.00	< LOD	< LOD	< LOD	< LOD	134.74	80.04	83.97
Chlorogenic acid	2053.63	2851.40	3949.13	267.10	46.64	173.57	1608.34	5307.68	4635.99	285.20	695.03	1602.74
Fumaric acid	4513.27	111413.72	71169.48	6572.48	79237.24	58351.15	2967.57	70865.16	70559.34	1915.17	14591.28	46706.39
Verbascoside	568.46	34.56	45.44	70.34	< LOD	< LOD	23992.18	8811.92	3868.82	44.83	28.95	11.19
Orientin	37.40	8.60	< LOD	14.48	4.12	< LOD	35.65	14.08	< LOD	0.46	1.34	< LOD
Caffeic acid	4404.55	3389.80	5156.19	5504.38	1482.96	6919.47	669.14	1506.80	1764.78	2484.34	1427.14	8474.37
(+)-*trans*taxifolin	8.59	1.76	1.59	2.28	0.28	< LOD	1.39	0.08	0.29	17.19	6.11	2.74
Luteolin-7-*O*-rutinoside	177.66	20.20	25.44	20.28	7.40	< LOD	2463.14	1429.48	1543.75	249.30	110.97	81.87
Vanillic acid	442.24	< LOD	< LOD	1142.72	< LOD	< LOD	246.40	< LOD	< LOD	788.87	< LOD	< LOD
Luteolin-7-*O*-glucoside	977.30	40.32	53.29	44.48	4.76	26.31	3265.96	898.68	947.87	1730.83	785.36	760.12
p-Coumaric acid	2580.46	< LOD	< LOD	3036.55	< LOD	< LOD	643.73	< LOD	< LOD	772.85	< LOD	< LOD
Hesperidin	2180.39	227.12	231.59	3.10	< LOD	< LOD	2.56	< LOD	0.26	6.82	< LOD	6.23
Rutin	433.59	35.04	29.92	232.59	262.04	104.34	257.84	57.56	57.50	1.03	< LOD	< LOD
Rosmarinic acid	58451.28	32763.76	27291.90	42186.97	22332.24	37919.92	3762.52	3484.40	2989.08	35663.77	29649.23	31327.82
Hyperoside	373.07	25.08	35.75	90.41	34.20	70.61	180.07	3.04	25.11	44.14	< LOD	41.55
Apigenin-7-*O*-glucoside	1224.42	42.96	59.68	63.38	7.08	11.89	95.90	13.20	13.57	144.50	31.32	28.06
Nepetin-7-*O*-glucoside	15.77	< LOD	3.06	< LOD	< LOD	< LOD	7.53	26.44	27.94	3143.01	2514.83	2014.29
Quercetin	66.86	5.24	9.80	7.97	5.60	4.51	25.37	0.32	5.07	31.52	7.97	10.12
Salicylic acid	4667.97	760.80	779.68	1086.90	99.72	578.81	2820.80	1888.96	535.48	1963.68	277.40	822.42
Naringenin	61.11	12.60	6.23	12.90	5.36	< LOD	9.25	1.80	0.92	57.58	7.61	14.37
Luteolin	752.20	58.84	29.96	224.52	24.76	80.16	772.98	575.28	598.24	572.32	161.89	558.10
Nepetin	19.35	0.48	< LOD	7.93	1.24	< LOD	8.45	0.32	0.00	1012.48	336.09	346.67
Apigenin	616.13	104.48	89.25	90.79	7.64	12.42	189.51	32.80	57.54	67.95	8.64	57.46
Hispidulin	340.52	31.92	24.01	31.83	3.12	0.49	220.59	48.92	58.64	296.75	62.21	88.93
Isosakuranetin	21.79	13.20	6.19	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Caffeic asit phenethyl ester	3.22	0.52	0.71	1.28	0.08	0.12	0.11	0.04	0.04	0.70	0.16	0.20
Chrysin	1.27	0.48	0.60	1.31	0.96	19.75	1.76	0.52	0.77	0.89	0.32	1.47
Acacetin	307.25	42.44	26.03	55.90	4.60	4.96	16.64	5.12	3.75	32.68	8.76	4.84
Dihydro Caffeic Acid	57.27	50.24	51.59	27.52	11.60	46.97	22.34	52.28	20.04	35.83	21.54	89.56
6-OH-Luteolin-7-*O*-glucoside	36.75	< LOD	< LOD	< LOD	< LOD	< LOD	34.08	44.60	41.29	335.13	255.74	188.41
6-OMe-Luteolin-7-*O*-glucoside	30.11	30.76	30.52	28.24	30.76	44.96	65.16	75.68	93.27	678.54	440.20	470.36
Apigenin-7-*O*-acylglucoside	95.32	< LOD	< LOD	0.00	< LOD	< LOD	28.88	< LOD	< LOD	9.87	< LOD	< LOD
Luteolin-7-*O*-acylglucoside	< LOD	420.12	< LOD	< LOD	< LOD	< LOD	< LOD	1081.48	< LOD	< LOD	118.34	< LOD
Chrysoeriol	150.24	24.24	18.33	18.79	4.60	4.02	109.14	24.64	33.01	192.75	45.92	68.77
Cirsimaritin	390.28	144.84	116.27	23.24	1.56	2.34	< LOD	< LOD	< LOD	80.17	76.06	46.55
Cirsilineol	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	204.90	199.88	102.70
Apigenin-7-*O*-methylate	40.00	5.48	3.41	22.38	0.60	0.66	2.41	0.68	0.51	4.24	1.14	0.63
Sclareol	1.11	< LOD	< LOD	58.52	32.68	37.54	1.17	2.08	< LOD	0.36	< LOD	0.91
Homogentisic acid	1695.87	649.24	810.83	903.90	244.28	797.62	222.56	268.68	134.67	1076.99	362.68	870.99
3,4-Dihydroxybenzaldehyde	147.71	28.84	24.13	198.34	9.44	152.54	19.45	11.92	< LOD	100.70	7.53	66.67
2,5-Dihydroxybenzoic acid	65.72	64.16	56.75	30.76	12.12	31.56	28.52	62.48	23.16	33.18	21.62	53.97
Salvianolic acid B	13366.41	5349.44	3716.15	7027.34	1950.76	3046.97	57.07	145.24	158.71	4687.91	< LOD	4325.00
Hispidulin-7-*O*-glucoside	3668.03	972.60	991.47	833.86	1227.24	1545.82	6672.69	4548.36	4696.54	6080.20	5332.74	5865.83
Pinocembrin	0.13	< LOD	< LOD	1.03	< LOD	< LOD	0.37	< LOD	< LOD	0.33	< LOD	< LOD
Genkwanin	30.89	2.68	0.95	4.83	< LOD	< LOD	0.37	< LOD	< LOD	1.95	< LOD	< LOD
Carnosic acid	69.27	1.60	0.99	13.79	2.72	0.29	25.23	4.48	1.03	0.03	0.87	0.36

Table 5. Compounds detected in *Salvia* species (*S. pilifera*, *S. verbenaca*, *S. viridis*, *S. aramiensis*) in ethanol extract, infusion, and decoctions (g/kg extract).

Luteolin-7-*O*-glucoside and Luteolin-7-*O*-rutinoside compounds were found at lower rates in infusion and decoction extracts compared to ethanol extracts. On the other hand, these molecules were detected at the highest rates in ethanol extracts of *S. albimaculata* and *S. viridis* (respectively).

Quercetin was detected in low amounts in all infusion extracts.

	S. verticillata			*S. pisidica*			*S. albimaculata*			*S. caespitosa*		
Compounds	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**	**EtOH**	**Infusion**	**Decoction**
Ascorbic acid	264.00	< LOD	37.64	633.93	< LOD	407.90	790.16	< LOD	299.30	1951.74	< LOD	399.11
(-)-Epigallocatechin	100.80	31.16	18.80	117.54	169.92	131.63	142.56	77.02	231.40	130.31	43.67	102.49
(-)-Epigallocatechin gallate	< LOD	< LOD	< LOD	< LOD	26.04	3.73	67.99	82.27	134.19	0.00	< LOD	< LOD
Chlorogenic acid	15.76	24.00	1.69	55.33	76.32	91.74	336.61	708.19	1014.19	103.27	78.92	99.40
Fumaric acid	9430.16	44158.44	13052.31	2869.46	12221.16	49517.99	5052.42	10079.92	98020.66	4217.83	13232.06	52106.68
Verbascoside	35.80	1.00	< LOD	1.80	< LOD	< LOD	3.12	< LOD	3.31	8.65	< LOD	0.57
Orientin	42.76	17.60	1.84	152.13	19.24	38.10	1049.39	229.90	320.81	40.83	2.71	< LOD
Caffeic acid	6873.28	4219.48	369.46	4813.19	4542.08	9298.17	6451.89	3049.30	5042.54	12047.03	5642.16	7337.87
Caffeine	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	7.03	< LOD	< LOD
(+)-*trans*taxifolin	3.96	2.80	0.04	4.70	0.32	< LOD	8.26	1.95	< LOD	20.18	0.72	< LOD
Luteolin-7-*O*-rutinoside	22.24	7.04	< LOD	521.59	22.64	25.52	651.98	108.31	207.98	258.32	9.02	4.87
Vanillic acid	1017.56	< LOD	< LOD	2159.44	< LOD	< LOD	1194.26	196.30	< LOD	9991.90	< LOD	< LOD
Luteolin-7-*O*-glucoside	380.32	46.08	3.04	1166.94	17.88	10.64	3415.78	227.16	633.46	2147.13	72.57	52.90
p-Coumaric acid	3865.08	< LOD	< LOD	1924.29	< LOD	< LOD	1756.52	< LOD	< LOD	2046.73	< LOD	< LOD
Hesperidin	3.28	1.16	< LOD	1191.51	44.56	95.98	1584.43	657.02	1233.13	375.38	22.12	13.50
Rutin	271.96	94.68	5.55	254.62	15.88	11.52	9.51	< LOD	< LOD	70.18	4.91	< LOD
Rosmarinic acid	50568.40	26360.76	2371.52	41536.30	12761.84	20615.54	42091.44	20943.50	27868.79	39343.49	7453.93	2270.34
Hyperoside	108.60	11.32	0.53	297.39	14.08	40.33	1013.88	162.47	233.49	153.09	14.25	5.36
Apigenin-7-*O*-glucoside	124.60	8.00	1.24	134.40	4.80	1.54	142.11	6.40	14.56	157.13	2.99	3.77
Nepetin-7-*O*-glucoside	1.72	5.68	< LOD	669.80	< LOD	1.68	1241.22	< LOD	450.18	18.50	< LOD	< LOD
Quercetin	51.80	1.52	0.53	66.56	8.56	1.28	71.08	10.46	2.54	6.94	1.40	0.82
Salicylic acid	2125.12	323.76	54.18	3351.88	833.96	1055.21	4794.29	739.01	1079.26	1056.18	266.11	395.17
Naringenin	8.24	2.12	< LOD	10.33	4.24	4.46	30.79	12.88	8.57	26.88	6.11	8.38
Luteolin	1076.24	187.80	16.62	873.56	195.20	188.85	976.14	371.85	241.69	501.53	115.13	231.23
Nepetin	97.92	7.00	< LOD	18.13	1.48	0.91	32.27	< LOD	< LOD	16.64		0.46
Apigenin	995.60	120.80	9.42	161.12	56.40	54.44	336.08	43.90	31.07	314.80	16.33	35.56
Hispidulin	753.80	64.92	1.16	965.54	421.60	555.90	1429.06	196.78	158.82	383.21	33.69	90.87
Isosakuranetin	< LOD	< LOD	< LOD	< LOD	< LOD	6.98	33.16	3.58	< LOD	0.00	< LOD	< LOD
Caffeic asit phenethyl ester	2.88	0.40	0.08	0.25	0.36	0.77	0.80	0.32	0.07	0.28	0.04	0.07
Chrysin	5.36	0.80	0.19	4.88	1.84	1.76	1.75	0.95	1.29	0.95	0.48	1.88
Acacetin	139.32	12.24	0.71	74.84	41.16	46.87	198.51	30.42	16.65	100.00	7.43	13.96
Dihydro Caffeic Acid	49.48	24.00	7.35	41.34	32.40	61.97	57.47	26.48	28.97	127.34	66.31	76.16
6-OH-Luteolin-7-*O*-glucoside	11.28	22.92	5.18	348.15	9.96	< LOD	974.53	73.24	197.90	17.95	5.63	< LOD
6-OMe-Luteolin-7-*O*-glucoside	23.96	30.52	4.35	95.30	14.36	61.10	77.21	64.85	109.34	60.49	29.34	20.92
Apigenin-7-*O*-acylglucoside	106.96	< LOD	< LOD	12.60	< LOD	< LOD	13.49	< LOD	< LOD	11.41	< LOD	< LOD
Luteolin-7-*O*-acylglucoside	< LOD	1131.52	< LOD	< LOD	825.16	< LOD	< LOD	386.32	< LOD	< LOD	40.16	< LOD
Chrysoeriol	331.56	39.12	4.28	276.92	185.84	174.08	568.59	98.09	62.46	139.85	18.92	43.87
Cirsimaritin	340.08	83.84		40.90	16.04	31.55	155.07	46.68	48.90	117.13	2.40	< LOD
Cirsilineol	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	36.08	2.74	< LOD	42.29	< LOD	< LOD
Apigenin-7-*O*-methylate	20.56	1.64	0.11	9.83	5.32	6.14	25.62	3.94	2.17	13.12	1.00	1.85
Sclareol	0.60	< LOD	< LOD	9.18	< LOD	0.99	1.16	< LOD	< LOD	0.86	< LOD	< LOD
Homogentisic acid	1680.20	817.56	94.11	1820.22	648.60	1346.87	1486.27	632.09	799.01	3514.86	1395.13	3818.40
3,4-Dihydroxybenzaldehyde	171.04	48.60	< LOD	113.09	13.88	72.76	181.58	26.72	44.49	268.38	47.39	70.69
2,5-Dihydroxybenzoic acid	31.72	15.84	7.39	38.26	28.68	118.03	58.10	48.19	56.07	44.68	29.90	44.26
Salvianolic acid B	15068.04	2689.36	160.15	5371.76	854.40	2578.24	8089.51	2119.13	2511.51	8732.11	1539.60	1359.08
Hispidulin-7-*O*-glucoside	4143.56	1125.64	102.25	7540.25	1095.60	1882.56	13563.15	2002.39	4081.29	6339.45	884.87	798.08
Pinocembrin	3.60	< LOD	< LOD	0.31	< LOD	< LOD	0.30	< LOD	< LOD	0.61	< LOD	< LOD
Genkwanin	12.80	< LOD	< LOD	6.53	2.52	3.36	19.61	1.47	0.11	7.03	< LOD	< LOD
Carnosic acid	5.20	1.16	0.19	33.28	1.28	0.29	17.83	0.72	0.59	242.20	4.31	2.06

Table 6. Compounds detected in *Salvia* species (*S. verticillata*, *S. pisidica*, *S. albimaculata*, *S. caespitosa*) in ethanol extract, infusion, and decoctions (g/kg extract).

Hyperoside was detected in all ethanol extracts compared to the prepared water extracts.

Whereas ascorbic acid was found in the decoction and ethanol extracts, no ascorbic acid was found in the prepared infusion extracts.

Hesperidin was detected as the major substance in *S. albimaculata* infusion, decoction, and ethanol and *S. pilifera* ethanol extracts while it was detected as minor in all other ethanol extracts.

Rutin was detected as minor or not detected in all extracts.

Chlorogenic acid was found in the major proportion in *S. pilifera* and *S. viridis* infusion, decoction, and ethanol extracts, while it was detected as a minor substance in all other ethanol extracts. In addition, chlorogenic acid was detected as the main substance in *S. aramiensis* and *S. albimaculata* decoction extracts.

Vanillic acid was detected at a higher rate in all ethanol extracts compared to water extracts, and the highest vanillic acid content among ethanol extracts was determined in *S. caespitosa* extract. It was also determined that apigenin and luteolin were found at higher rates in all ethanol extracts compared to water extracts.

Apigenin-7-*O*-glucoside, acacetin, apigenin 7-methylate, apigenin, and chrysoeriol were found to be minor substances in infusion and decoction extracts. In addition, naringenin, luteolin, 6-*O*-me-luteolin-7-*O*-glycoside, and hispidulin were also detected as minor substances in the infusion extract.

Carnosic acid, (-)-epigallocatechin, dihydro caffeic acid, caffeic acid phenethyl ester, 2,5-dihydroxybenzoic acid, and chrysin were detected as minor components in all ethanol, infusion, and decoction extracts.

In Silico studies

Validation of the Docking procedure

To validate the performance of Glide, we modeled the interactions between the co-crystallized ligands, Pterin-6-yl-methyl-monophosphate and 7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine, with their respective proteins, dihydropteroate synthase (PDB ID: 2VEG) and dihydrofolate reductase (PDB ID: 3SRW). We then superimposed the experimentally observed (co-crystallized) conformations of these ligands onto those predicted by Glide. The results showed that Glide accurately reproduced the experimental binding conformation of Pterin-6-yl-methyl-monophosphate within the binding pocket of 2VEG, with a root-mean-square deviation (RMSD) of 0.248 Å. Similarly, for 3SRW, the RMSD was 0.506 Å, a value within the acceptable range, confirming the validity of the docking model generated by Glide.

Docking results

Table 7 presents the docking scores of the compounds on DHPS (2VEG) and DHFR (3SRW).

The docking score of Pterin-6-yl-methyl-monophosphate was determined as -8.426 kcal/mol (Table 7). The phosphate moiety of Pterin-6-yl-methyl-monophosphate formed two diverse salt bridge interactions with ARG 282, one salt bridge interaction with LYS 237, and one hydrogen bond interactions with ASN 17. The pterin structure formed one pi-cation interaction with ARG 282, one Pi-Pi stacking interaction with PHE 206, one salt bridge with ASP 201, one hydrogen bond interaction with ASP 201, one hydrogen bond interaction with ASN 110, and one hydrogen bond interaction with ASH 91. The compound also formed hydrophobic contacts with ILE 15, PHE 206, VAL 133, MET 135, PHE 231 and ILE 112 (Fig. 2).

The docking score of salvianolic acid B in the active site of 2VEG was determined as -8.188 kcal/mol. The hydroxyl groups of the compound formed two diverse hydrogen bond interactions with GLU 55 and one hydrogen bond interaction with ASH 91. The phenyl moiety of the compound formed one Pi-Pi stacking interaction with PHE 206. The hydroxyl groups of the compound formed two diverse hydrogen bond interactions with ASP 201 and one hydrogen bond interaction with ASN 110. The negative charged oxygen of the compound formed one hydrogen bond interaction with ASN 17 and one salt bridge interaction with ARG 282. The carbonyl groups of the compound formed one hydrogen bond interaction with ARG 282 and one hydrogen bond interaction with ARG 238. The OH groups of the compound formed three diverse hydrogen bond interactions with ARG 238. The compound formed hydrophobic contacts with ILE 241, LEU 232, PHE 231, ILE 15, ILE 112, VAL 133, MET 135, PHE 206, PHE 151, PRO 152, PHE 154 and TRP 93 (Fig. 3).

The docking score of verbascoside in the active site of 2VEG was determined as -6.154 kcal/mol. The OH groups of the compound formed two diverse hydrogen bond interactions with ARG 238, one hydrogen bond interaction with LYS 237, two diverse hydrogen bond interactions with ASP 201, one hydrogen bond interaction with ASN 17 and two diverse hydrogen bond interactions with GLU 55. Moreover, the carbonyl group of the compound formed one hydrogen bond interaction with ASN 17 and another hydrogen bond interaction with ARG 282. The compound formed hydrophobic contacts with PHE 151, PHE 206, PHE 231, ILE 112, VAL 133, MET 135 and ILE 241 (Fig. 4).

The docking score of rosmarinic acid in the active site of 2VEG was determined as -6.129 kcal/mol. The hydroxyl groups of the compounds formed one hydrogen bond interaction with ASH 91, one hydrogen bond interaction with GLY 54, two diverse hydrogen bond interactions with GLU 55, one hydrogen bond interaction with ARG 236, and one hydrogen bond interaction with ARG 238. The phenyl groups of the compound formed one pi-cation interaction with ARG 282 and another pi-cation interaction with LYS 237. The negative charged oxygen of the compound formed two diverse salt bridges with ARG 282 and LYS 237. The carbonyl group of the compound formed a hydrogen bond interaction with LYS 237. Moreover, the compound formed hydrophobic contacts with ILE 15, ILE 241, PHE 206, PHE 151 and ILE 112 (Fig. 5).

The docking score of 7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine in the active site of 3SRW was determined as -9.503 kcal/mol. The compound formed one salt bridge interaction and two diverse hydrogen bond interactions with ASP 28. Besides, it formed hydrogen bond interactions with LEU 6 and PHE 93. The compound also formed hydrophobic contacts with LEU 55, LEU 29, VAL 32, ALA 8, VAL 7, LEU 6, PHE 93, LEU 21, ILE 15, PHE 99 and ILE 51 (Fig. 6).

Compound	Docking Score (kcal/mol) 2VEG	Docking Score (kcal/mol) 3SRW
Rosmarinic acid	-6.129	-7.230
Caffeic acid	-4.305	-4.181
Fumaric acid	-3.374	-2.908
Salvianolic acid B	-8.188	-8.656
Hispidulin-7-*O*-glucoside	-5.688	-7.123
Verbascoside	-6.154	-8.295
Homogentisic acid	-5.346	-5.759
Salicylic acid	-4.445	-4.598
Luteolin-7-*O*-glucoside	-6.072	-8.414
Luteolin-7-*O*-rutinoside	-5.818	-8.448
Quercetin	-5.687	-8.531
Hyperoside	-5.277	-6.921
Ascorbic acid	-3.699	-4.271
Hesperidin	-5.429	-9.243
Rutin	-5.744	-7.170
Chlorogenic acid	-5.453	-5.796
Vanillic acid	-4.302	-4.955
Apigenin-7-*O*-glucoside	-5.572	-8.693
Acacetin	-5.735	-8.408
Apigenin 7-*O*-methylate	-4.459	-8.387
Apigenin	-5.406	-8.123
Chrysoeriol	-5.436	-7.668
Naringenin	-5.679	-7.563
Luteolin	-5.529	-8.438
6-*O*-Me-Luteolin-7-*O*-glycoside	-5.741	-8.449
Hispidulin	-5.370	-7.035
Carnosic acid	-3.964	-6.550
(-)-Epigallocatechin	-5.191	-8.021
Dihydro caffeic acid	-4.686	-5.638
Caffeic acid phenethyl ester	-3.919	-7.700
2,5-Dihydroxybenzoic acid	-5.303	-5.031
Chrysin	-5.252	-7.995
Pterin-6-yl-methyl-monophosphate	-8.426	-
7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine	-	-9.503
Sulfamethoxazole	-4.287	-
Trimethoprim	-	-7.063

Table 7. The docking scores of the detected compounds on 2VEG and 3SRW.

The docking score of hesperidin in the active site of 3SRW was determined as -9.243 kcal/mol. The OH groups of the compound formed one hydrogen bond interaction with THR 47, two diverse hydrogen bond interactions with ASP 28 and one hydrogen bond interaction with GLN 20. Moreover, the compound formed hydrophobic contacts with PRO 56, LEU 55, ILE 51, PHE 99, PHE 93, ILE 15, LEU 21, TRP 23, VAL 32, LEU 29, LEU 6, VAL 7, and ALA 8 (Fig. 7).

The docking score of apigenin-7-*O*-glucoside in the active site of 3SRW was determined as -8.693 kcal/mol. The OH groups of the compound formed one hydrogen bond interaction with GLN 20 and one hydrogen bond interaction with ASN 19. Moreover, the carbonyl group of the compound formed another hydrogen bond interaction with ALA 8. The compound formed hydrophobic contacts with LEU 6, VAL 7, ALA 8, LEU 21, PHE 99, ILE 15, PHE 93, ILE 51, LEU 29, LEU 55, and VAL 32 (Fig. 8).

The docking score of salvianolic acid B in the active site of 3SRW was determined as -8.656 kcal/mol. The negative charged oxygen of the compound formed a salt bridge with ARG 58, the OH groups of the compound formed one hydrogen bond interaction with THR 122 and two diverse hydrogen bond interactions with SER 50. The compound formed hydrophobic contacts with LEU 55, VAL 32, LEU 29, PRO 26, PHE 99, LEU 21, PHE 93, ILE 15, and ILE 51 (Fig. 9).

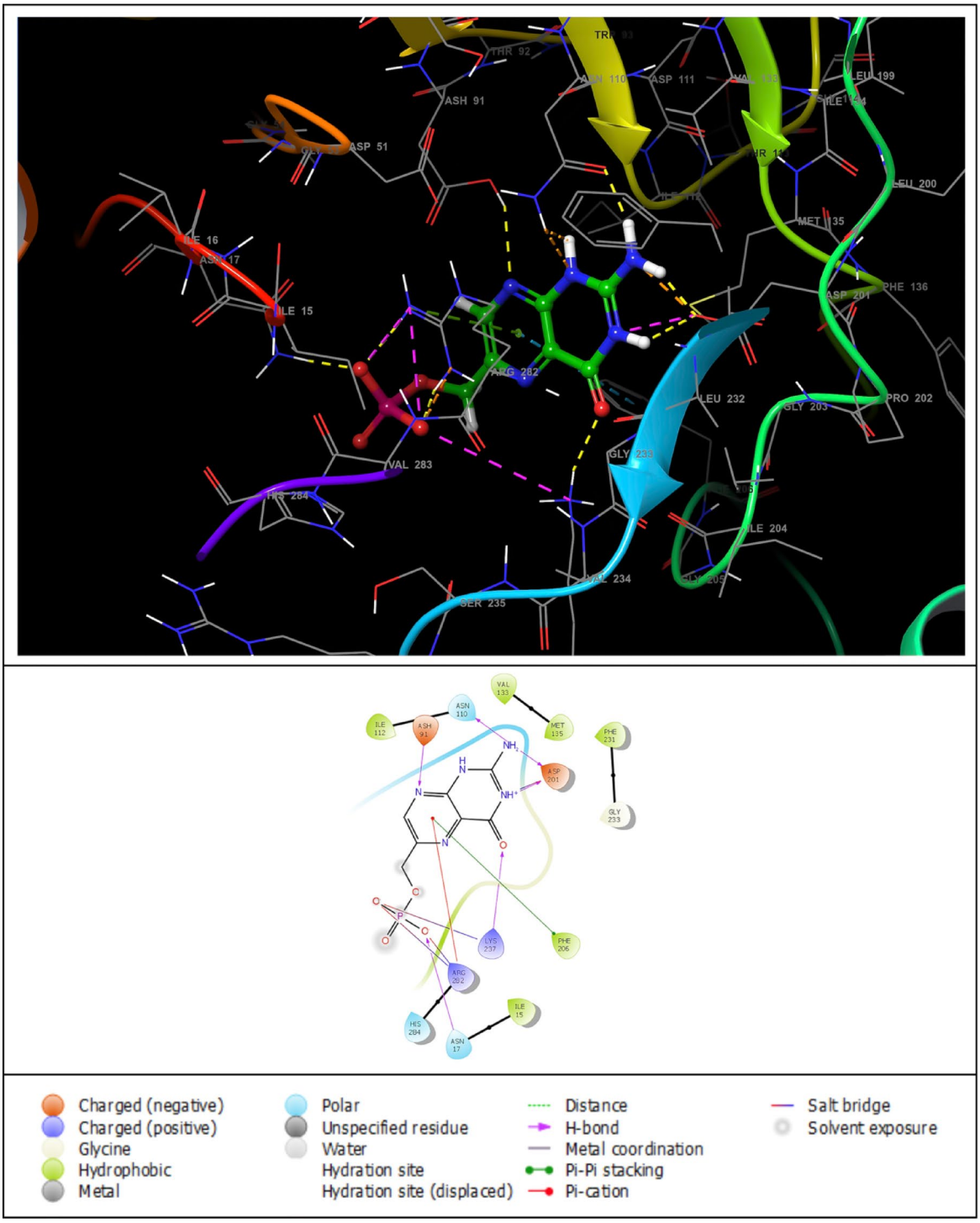


Fig. 2. Pterin-6-yl-methyl-monophosphate and its interactions with the crystal structure of dihydropteroate synthase (PDB ID: 2VEG)-Schrödinger Software Suite (Schrödinger Version 2020-3).

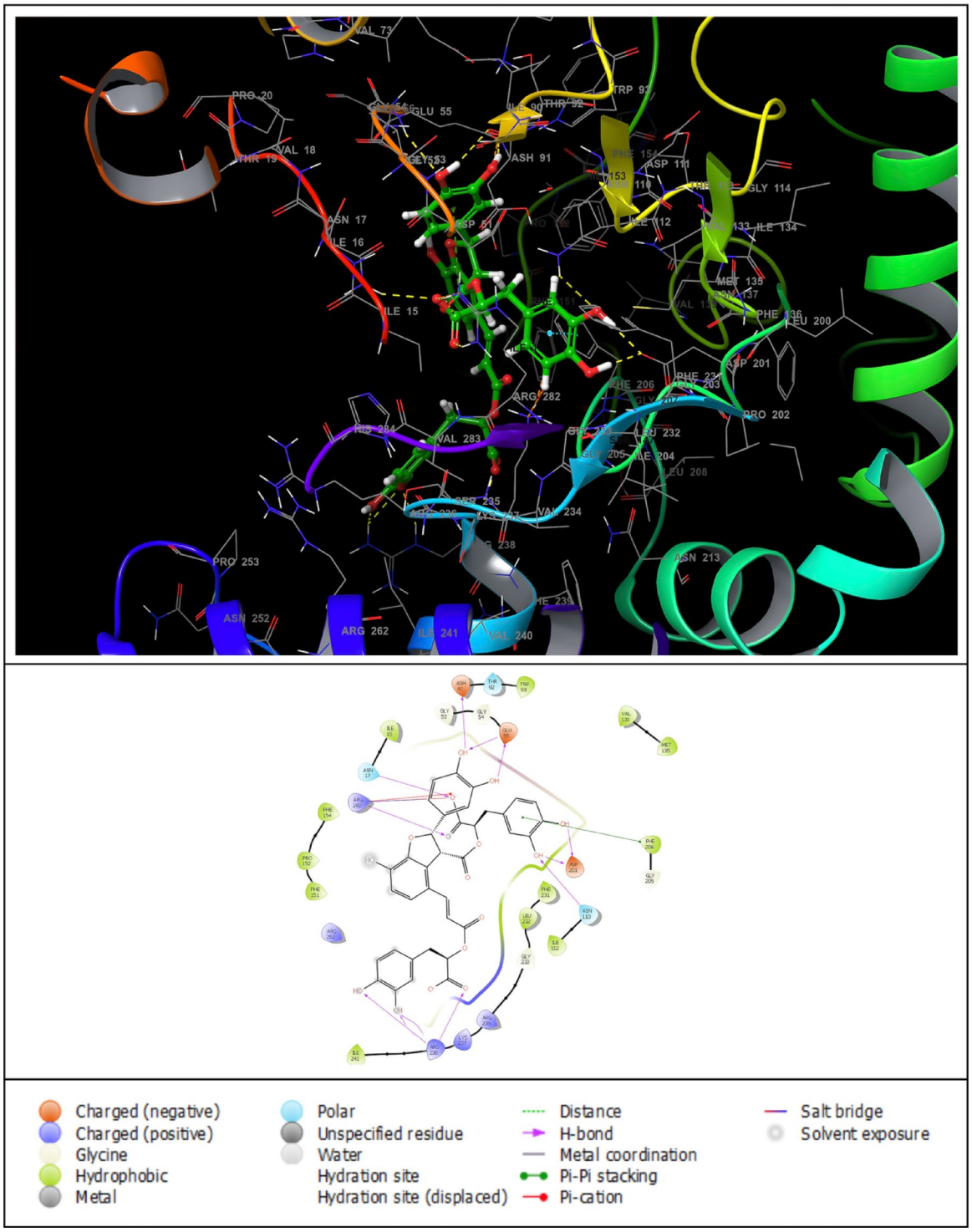


Fig. 3. Salvianolic acid B and its interactions with the crystal structure of dihydropteroate synthase (PDB ID: 2VEG)-Schrödinger Software Suite (Schrödinger Version 2020-3).

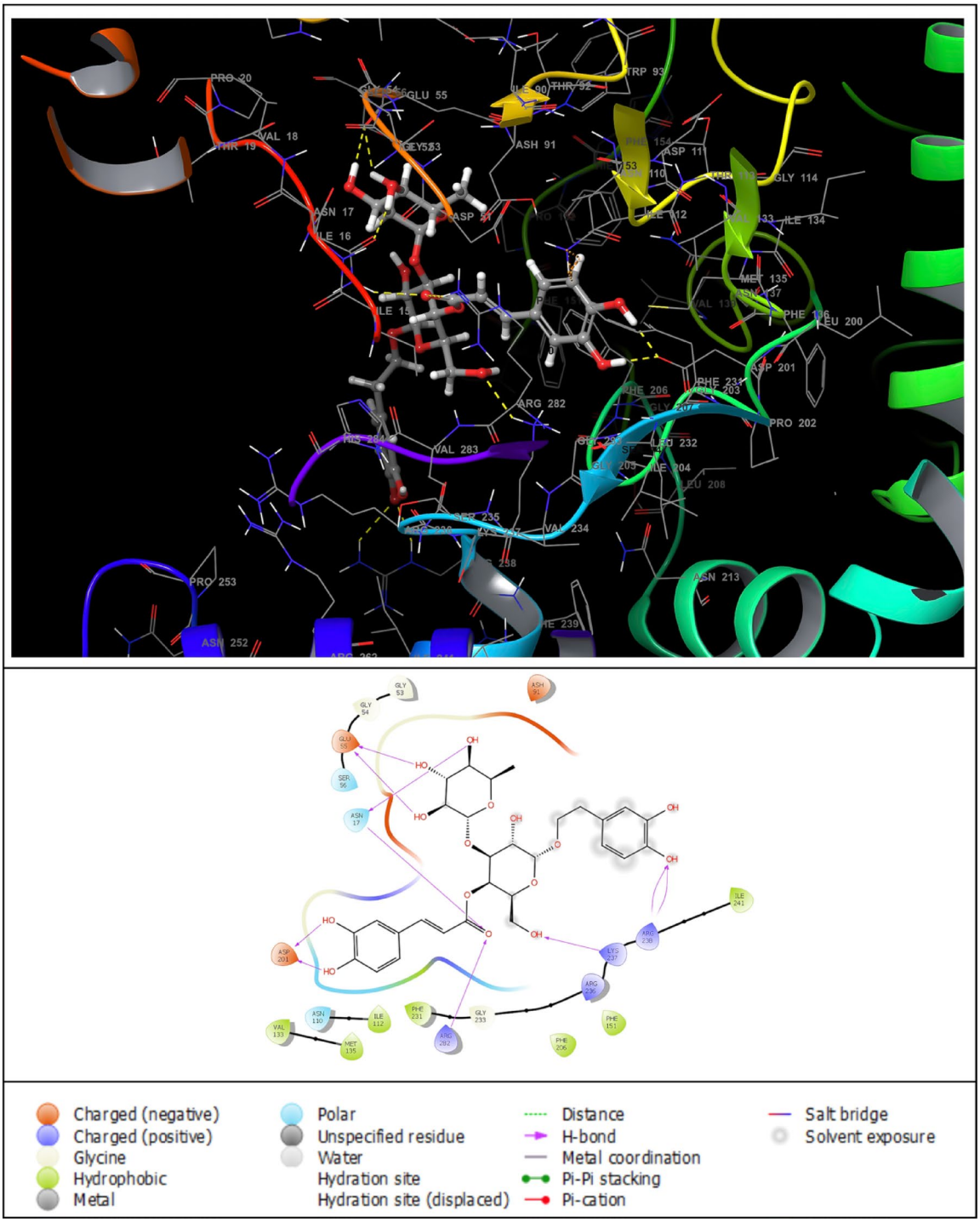


Fig. 4. Verbascoside and its interactions with the crystal structure of dihydropteroate synthase (PDB ID: 2VEG)-Schrödinger Software Suite (Schrödinger Version 2020-3).

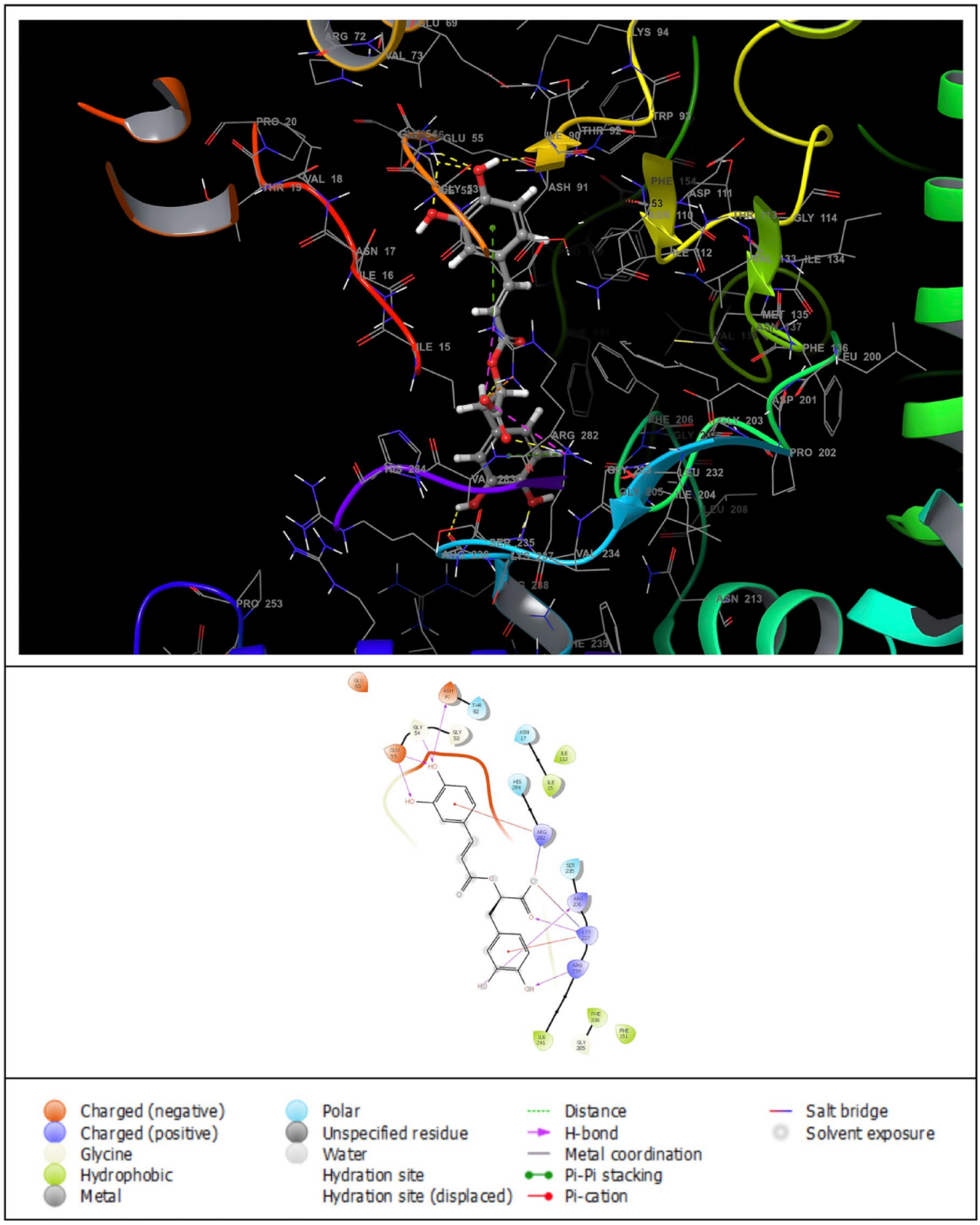


Fig. 5. Rosmarinic acid and its interactions with the crystal structure of dihydropteroate synthase (PDB ID: 2VEG)-Schrödinger Software Suite (Schrödinger Version 2020-3).

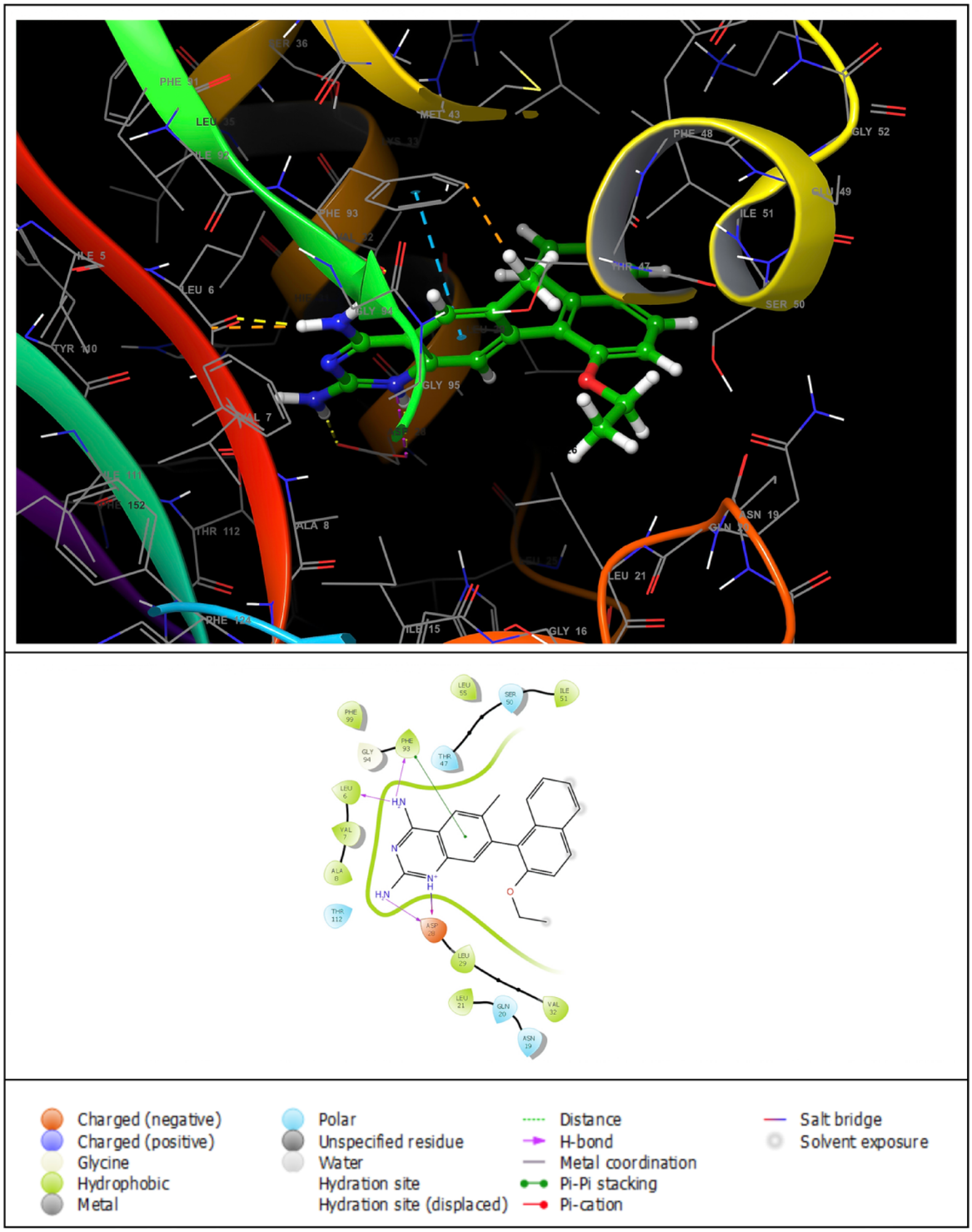


Fig. 6. 7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine and its interactions with the crystal structure of dihydrofolate reductase (PDB ID: 3SRW)-Schrödinger Software Suite (Schrödinger Version 2020-3).

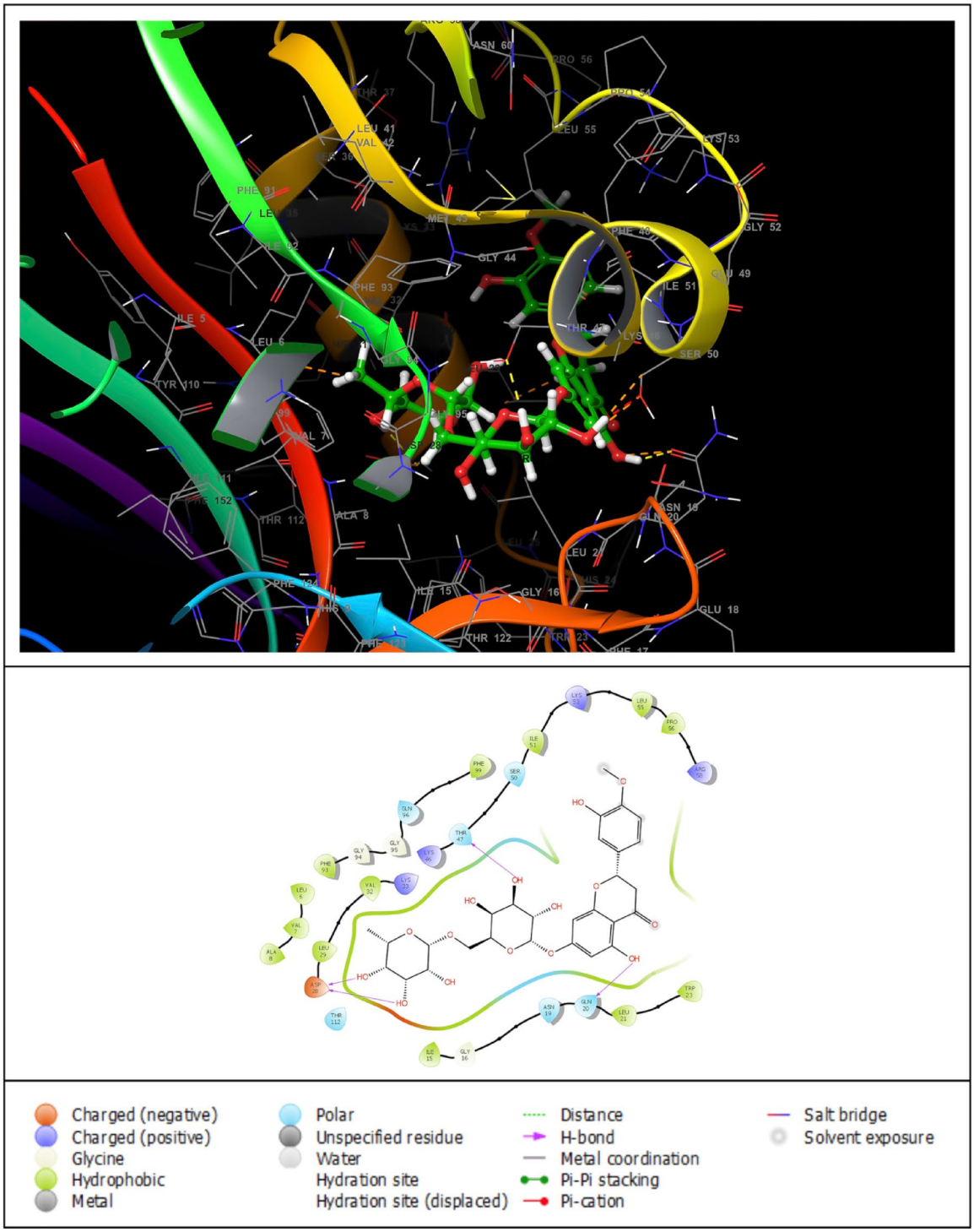


Fig. 7. Hesperidin and its interactions with the crystal structure of dihydrofolate reductase (PDB ID: 3SRW)-Schrödinger Software Suite (Schrödinger Version 2020-3).

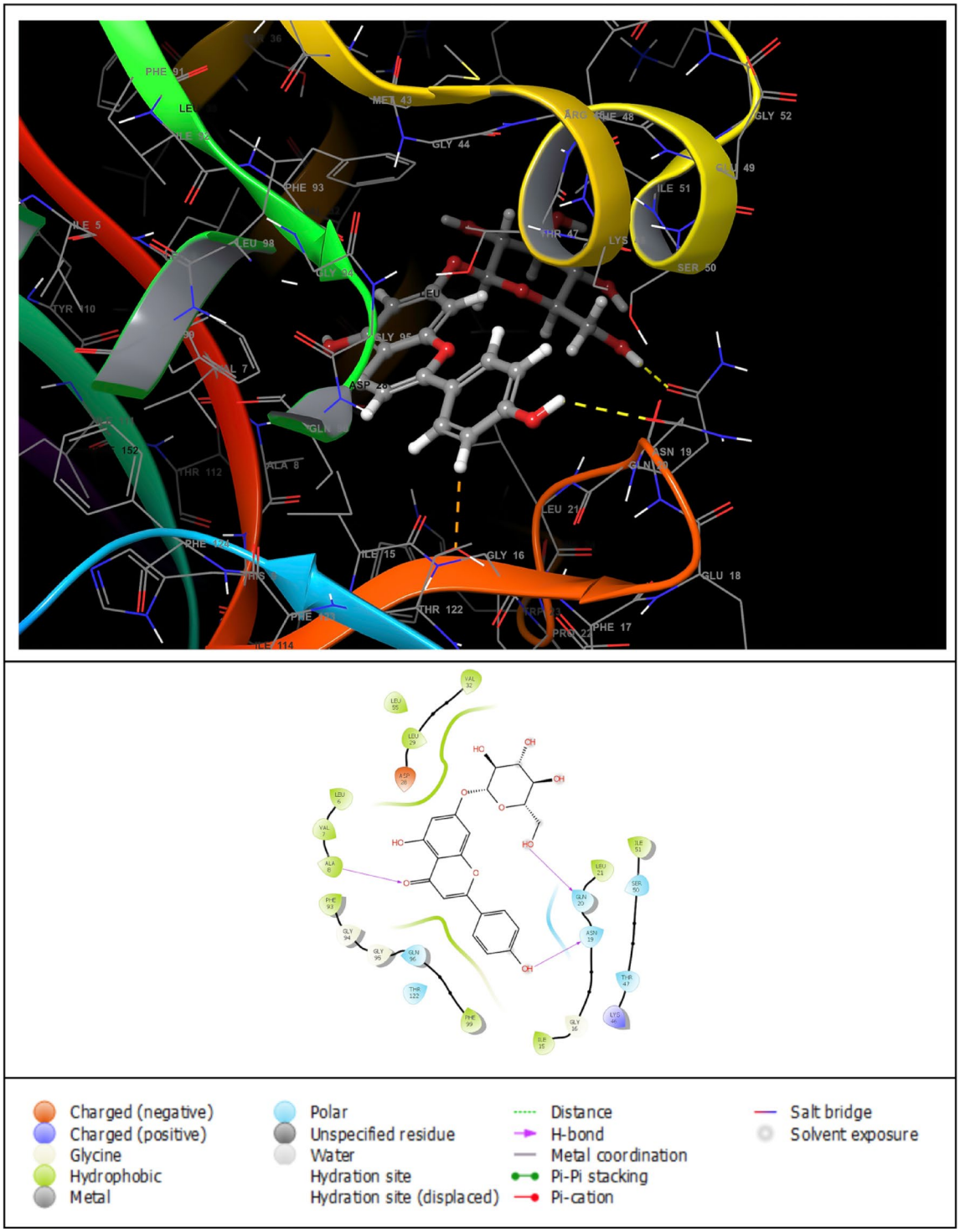


Fig. 8. Apigenin-7-*O*-glucoside and its interactions with the crystal structure of dihydrofolate reductase (PDB ID: 3SRW)-Schrödinger Software Suite (Schrödinger Version 2020-3).

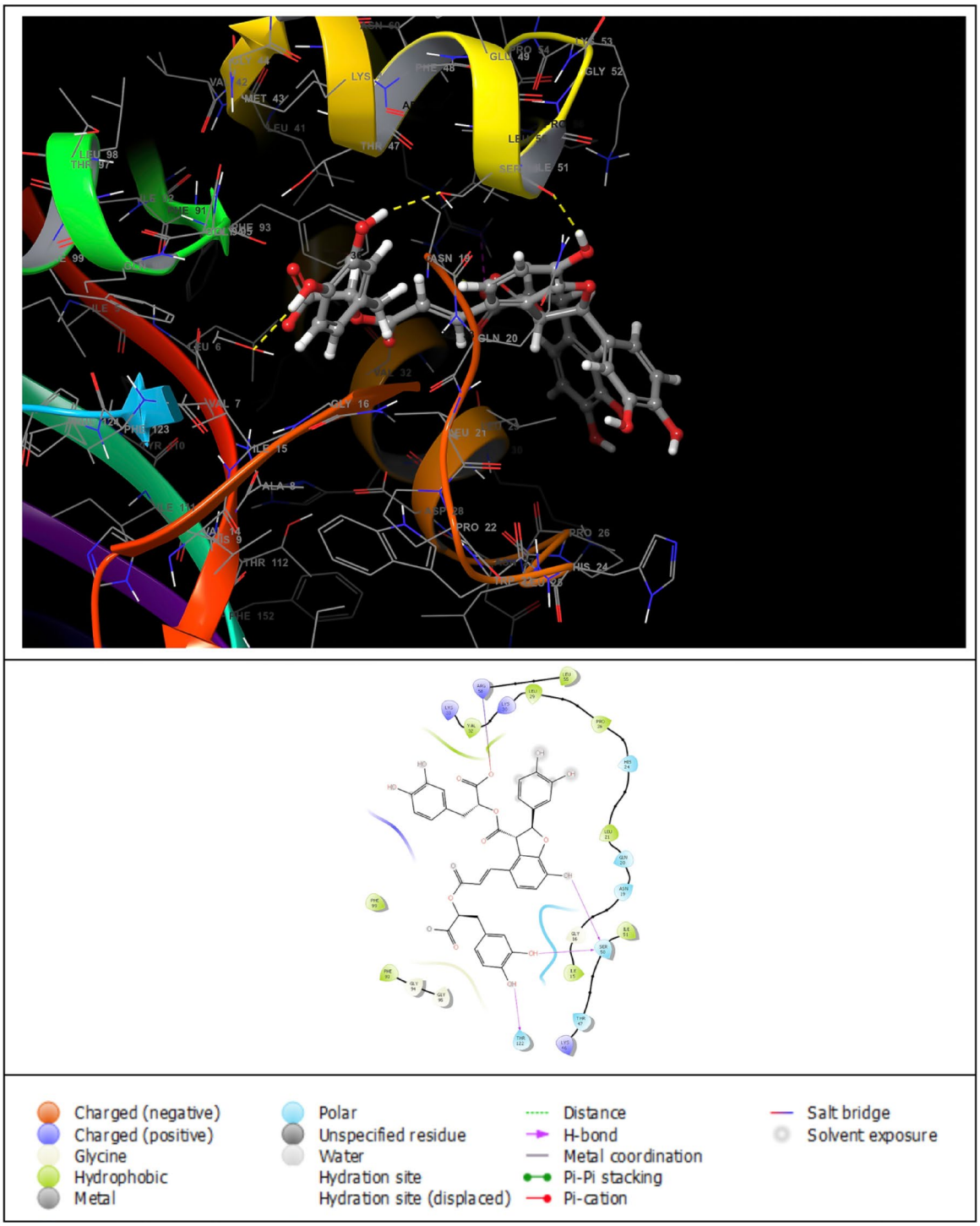


Fig. 9. Salvianolic acid B and its interactions with the crystal structure of dihydrofolate reductase (PDB ID: 3SRW)-Schrödinger Software Suite (Schrödinger Version 2020-3).

In Silico ADME studies

In silico ADME properties of the compounds were stated in Table 8.

Using the QikProp module from the Schrödinger Software Suite (Schrödinger 2020-3: QikProp, Schrödinger, LLC, New York, NY, 2020), six ADME-related descriptors for the identified compounds were calculated. These properties, along with their ideal ranges, are outlined in Table 4, while Table 8 presents the predicted ADME descriptor values for the compounds studied. The first four descriptors align with Lipinski's rule of five, which includes molecular weight (MW) < 500, partition coefficient < 5, donorHB < 5, and accptHB < 10. While most compounds met these criteria for molecular weight, hesperidin (MW: 610.568 g/mol), luteolin-7-*O*-rutinoside

Compound	MW	Log Po/w	donorHB	acceptHB	Log S	HOA%	Rule of five violation
(-)-Epigallocatechin	306.271	-0.203	6.000	6.200	-2.298	36.486	1
2,5-Dihydroxybenzoic acid	154.122	0.771	2.000	2.500	-1.043	58.696	0
Acacetin	284.268	2.449	1.000	3.750	-3.791	88.038	0
Apigenin	270.241	1.624	2.000	3.750	-3.317	73.955	0
Apigenin-7-*O*-glucoside	432.383	-0.311	5.000	12.250	-3.378	30.111	1
Ascorbic acid	176.126	-1.852	4.000	7.900	-0.639	44.781	0
Caffeic acid phenethyl ester	284.311	3.086	2.000	3.500	-4.143	90.850	0
Caffeic acid	180.160	0.526	3.000	3.500	-1.255	54.337	0
Carnosic acid	332.439	3.699	3.000	3.500	-4.346	89.701	0
Chlorogenic acid	354.313	-0.298	6.000	9.650	-2.347	17.131	1
Chrysin	254.242	2.368	1.000	3.000	-3.590	87.567	0
Chrysoeriol	300.267	1.810	2.000	4.500	-3.513	76.672	0
Dihydrocaffeic acid	182.176	0.655	3.000	3.500	-1.399	56.069	0
Fumaric acid	116.073	-0.151	2.000	4.000	-0.072	42.142	0
Hesperidin	610.568	-1.324	7.000	20.050	-2.704	0.000	3
Hispidulin	300.267	1.881	2.000	4.500	-3.472	78.851	0
Hispidulin-7-*O*-glucoside	462.409	-0.105	5.000	13.000	-3.507	20.157	2
Homogentisic acid	168.149	0.396	3.000	3.500	-1.043	56.505	0
Hyperoside	464.382	-1.260	7.000	13.750	-2.423	6.969	2
Luteolin	286.240	0.941	3.000	4.500	-3.039	62.050	0
Luteolin-7-*O*-glucoside	448.382	-0.948	6.000	13.000	-3.204	5.524	2
Luteolin-7-*O*-rutinoside	610.524	-2.693	9.000	21.500	-2.151	0.000	3
Naringenin	272.257	1.549	2.000	4.000	-3.146	74.380	0
Quercetin	302.240	0.362	4.000	5.250	-2.830	52.348	0
Rosmarinic acid	360.320	1.190	5.000	7.000	-3.136	42.607	0
Rutin	610.524	-2.637	9.000	20.550	-1.772	0.000	3
Salicylic acid	138.123	2.253	1.000	1.750	-1.605	76.601	0
Salvianolic acid B	718.623	1.973	9.000	14.000	-5.486	0.000	3
Vanillic acid	168.149	1.058	2.000	3.500	-1.274	68.424	0
Verbascoside	624.594	-1.537	9.000	20.300	-2.842	0.000	3
Apigenin 7-*O*-methylate	284.268	2.425	1.000	3.750	-3.742	87.746	0
6-*O*-Me-luteolin-7-*O*-glycoside	478.409	-0.872	6.000	13.750	-2.786	8.826	2

Table 8. Prediction of ADME properties of the title compounds using QikProp module of Schrödinger.

(MW: 610.524 g/mol), rutin (MW: 610.524 g/mol), salvianolic acid B (MW: 718.623 g/mol), and verbascoside (MW: 624.594 g/mol) were exceptions.

The Log Po/w values of the determined natural compounds were between carnosic acid (Log Po/w: 3.699) and luteolin-7-*O*-rutinoside (Log Po/w: -2.693). Excluding luteolin-7-*O*-rutinoside and rutin, the Log Po/w values of all compounds were between −2 and 5.

DonorHB values of (-)-epigallocatechin, apigenin-7-*O*-glucoside, chlorogenic acid, hesperidin, luteolin-7-*O*-rutinoside, rutin, salvianolic acid B, verbascoside and 6-*O*-me-luteolin-7-*O*-glycoside were determined as higher than 5. AccptHB values of apigenin-7-*O*-glucoside, hesperidin, hispidulin-7-*O*-glucoside, rutin, salvianolic acid B, verbascoside and 6-*O*-me-luteolin-7-*O*-glycoside were higher than 10 and did not follow Lipinski's rule of five.

Aqueous solubility (Log S) of overall determined compounds were between −6.5 and 0.5 and followed the drug-likeness criteria.

HOA% values of chlorogenic acid, hesperidin, hispidulin-7-*O*-glucoside, hyperoside, luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside, rutin, salvianolic acid B, verbascoside, 6-*O*-Me-luteolin-7-*O*-glycoside were determined as poor (< 25).

No Lipinski's Rule of Five Violations were determined for 2,5-Dihydroxybenzoic acid, acacetin, apigenin, ascorbic acid, caffeic acid phenethyl ester, caffeic acid, carnosic acid, chrysin, chrysoeriol, dihydrocaffeic acid, fumaric acid, hispidulin, homogentisic acid, luteolin, naringenin, quercetin, rosmarinic acid, salicylic acid, vanillic acid and apigenin 7-*O*-methylate.

In vitro antibacterial activity results

The antibacterial effect results of extracts against the tested strains were summarized in Tables 9 and 10. The prepared *Salvia* extracts showed antibacterial activity at concentrations of 312.5–5000 µg/mL. The results were compared to the antibacterial drug ciprofloxacin.

	Salvia pilifera				*Salvia verbenaca*				*Salvia verticillata*				*S. pisidica*			
	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin
	MIC µg/mL															
S.aureus	> 5000	> 5000	625	0.5	> 5000	2500	625	0.5	> 5000	> 5000	625	0.5	> 5000	> 5000	625	0.5
MRSA	> 5000	> 5000	625	0.5	> 5000	5000	1250	0.5	> 5000	> 5000	625	0.5	> 5000	> 5000	1250	0.5
E.faecalis	> 5000	> 5000	1250	1	> 5000	5000	1250	1	> 5000	> 5000	1250	1	> 5000	> 5000	1250	1
K. pneumoniae	> 5000	> 5000	1250	0.5	> 5000	5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5
B.subtilis	> 5000	> 5000	1250	0.25	> 5000	5000	1250	0.25	> 5000	> 5000	625	0.5	> 5000	> 5000	1250	0.5
P.aeruginosa	> 5000	> 5000	625	0.5	> 5000	2500	625	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.25
P.mirabilis	> 5000	> 5000	625	0.5	> 5000	5000	1250	0.25	> 5000	> 5000	1250	0.25	> 5000	> 5000	625	0.5
	Salvia viridis				*Salvia aramiensis*				*S. albimaculata*				*S. caespitosa*			
	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin
	MIC µg/mL															
S.aureus	> 5000	> 5000	625	0.5	625	625	1250	0.06	625	625	312.5	0.5	> 5000	> 5000	625	0.5
MRSA	> 5000	> 5000	625	0.5	312.5	312.5	625	0.25	312.5	312.5	312.5	0.5	> 5000	> 5000	1250	0.5
E.faecalis	> 5000	> 5000	1250	1	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	1	> 5000	> 5000	1250	1
K. pneumoniae	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5
B.subtilis	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.25	1250	1250	625	0.5	> 5000	> 5000	1250	0.5
P.aeruginosa	> 5000	> 5000	625	0.5	> 5000	> 5000	625	0.25	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.5
P.mirabilis	> 5000	> 5000	625	0.5	5000	> 5000	1250	0.25	> 5000	> 5000	625	0.5	> 5000	> 5000	1250	0.06

Table 9. Minimum inhibitory concentration (MIC µg/mL) values of *Salvia* extracts.

	Salvia pilifera				*Salvia verbenaca*				*Salvia verticillata*				*S. pisidica*			
	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin
	MBC µg/mL															
S.aureus	> 5000	> 5000	625	0.5	> 5000	2500	625	0.5	> 5000	> 5000	625	0.5	> 5000	> 5000	1250	0.5
MRSA	> 5000	> 5000	625	0.5	> 5000	5000	1250	0.5	> 5000	> 5000	625	0.5	> 5000	> 5000	2500	0.5
E.faecalis	> 5000	> 5000	1250	1	> 5000	5000	2500	1	> 5000	> 5000	1250	1	> 5000	> 5000	1250	1
K. pneumoniae	> 5000	> 5000	1250	0.5	> 5000	5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5
B.subtilis	> 5000	> 5000	1250	0.25	> 5000	5000	2500	0.25	> 5000	> 5000	2500	0.5	> 5000	> 5000	2500	0.5
P.aeruginosa	> 5000	> 5000	625	0.5	> 5000	2500	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	2500	0.25
P.mirabilis	> 5000	> 5000	625	0.5	> 5000	5000	1250	0.25	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.5
	Salvia viridis				*Salvia aramiensis*				*S. albimaculata*				*S. caespitosa*			
	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin	Infusion	Decoction	Ethanol extract (EtOH)	Cipro-floxacin
	MBC µg/mL															
S.aureus	> 5000	> 5000	625	0.5	625	625	1250	0.06	625	625	625	0.5	> 5000	> 5000	625	0.5
MRSA	> 5000	> 5000	625	0.5	312.5	312.5	250	0.25	312.5	312.5	625	0.5	> 5000	> 5000	1250	0.5
E.faecalis	> 5000	> 5000	1250	1	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	1	> 5000	> 5000	1250	1
K. pneumoniae	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.5
B.subtilis	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.25	1250	1250	2500	0.5	> 5000	> 5000	1250	0.5
P.aeruginosa	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.5
P.mirabilis	> 5000	> 5000	625	0.5	5000	> 5000	1250	0.25	> 5000	> 5000	1250	0.5	> 5000	> 5000	1250	0.06

Table 10. Minimum bactericidal concentration (MBC µg/mL) values of *Salvia* extracts.

The infusion and decoction extracts were examined, and *S. aramiensis* and *S. albimaculata* exhibited the most potent effects at a concentrations of 312.5 µg/mL against MRSA. Additionally, ethanol extracts of both showed good activity against MRSA.

The ethanol extracts showed good effects against *Staphylococcus aureus* except *S. aramiensis* and against MRSA except *S. caespitosa* and *S. pisidica*.

The MIC/MBC values of extracts from other plants were within the concentration range of 1250–2500 µg/mL.

The ethanol extracts of *S. pilifera*, *S. verbenaca*, *S. aramiensis*, and *S. viridis* were the most effective species against *P. aeruginosa*, with MIC/MBC values ranging from 625 to 1250 µg/mL concentration. The ethanol extracts of *Salvia pilifera*, *S. viridis*, *S. pisidica*, and *S. albimaculata* exhibited relatively higher antibacterial activity against *Proteus mirabilis*, with MIC/MBC values ranging from 625 to 1250 µg/mL. It was determined that the extracts of other plants exhibited similar efficacy.

The ethanol extracts of *S. verticillata*, *S. viridis* and *S. pilifera* (having MIC/MBC values of 0.6 mg/mL) showed good activity against MRSA and additionally *S. verticillata* against *Staphylococcus aureus*.

Discussion

The eight *Salvia* species growing in Southeastern Anatolia, the Mediterranean region of Anatolia, and the Eastern Anatolia Region were comprehensively investigated for phytochemical composition by LC-HRMS, integrating molecular docking analyses against 3SRW and 2VEG, ADME predictions, and in vitro antibacterial activity assessments. The chemical compositon of water and ethanol extracts of *S. verbenaca*, *S. aramiensis*, *S. pisidica*, *S. albimaculata*, and *S. caespitosa* was characterized for the first time in this study. According to phytochemical analysis, rosmarinic acid had the highest concentration among all compounds in all extracts. The ethanol extracts were found to be richer than water-based extracts in terms of compound diversity and density. The phytochemical profile of *Salvia* species is thought to vary depending on both the species and the extraction method used, and this chemical diversity plays a significant role in the outcomes of biological activity studies conducted on their extracts[61].

The antibacterial activity evaluation demonstrated that *Salvia* extracts exhibited varying degrees of efficacy against different bacterial strains. Considering their in vitro antibacterial properties, especially ethanol extracts have been observed to be more effective compared to other extracts against many strains. Similar to our study, many *Salvia* ethanol extracts have been mentioned to have antibacterial effects in literature reviews. In a study, ethanol extract prepared from *Salvia halophila* was shown to be effective against various Gram-positive bacterial strains at different concentrations[62]. A recent study by Balaşoiu et al., three *Salvia* species, particularly focusing on their polar extracts, were investigated against both Gram-positive, Gram-negative bacterial strains and *Candida albicans* as fungi. Their findings revealed that alcohol extracts of *Salvia glutinosa* (MIC: 20 mg/mL) and *Salvia splendens* (MIC: 20 mg/mL) were the most effective[63]. Another study on the genus *Salvia* reported the antibacterial potential of various species, highlighting that *Salvia virgata* exhibited moderate inhibitory effects on the growth of Gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*, at a concentration of 250 µg/mL[64]. Additionally, the antimicrobial activity of *Salvia pilifera* extract was assessed against various bacterial and fungal strains, demonstrating low to moderate antibacterial effects with MIC values of 250 µg/mL against *Staphylococcus aureus* and *Bacillus subtilis*[65]. Askun et al. exhibited the antibacterial of *Salvia fruticosa* Mill., *Salvia tomentosa* Mill., *Sideritis albiflora* Hub.-Mor. (endemic), *Sideritis leptoclada* O. Schwarz & P.H. Davis, (endemic), and *Origanum onites* L. against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae*. The best antibacterial activity (MIC 640 µg/mL) was shown against S. *typhimurium* and *E. aerogenes* by *S. fruticosa*; *E. coli*, and *S. typhimurium*, *E. aerogenes* by *S. tomentosa; S. typhimurium*, and *E. aerogenes* by *S. leptoclada* and *S. typhimurium*, *E. aerogenes* and *S. epidermidis* by *O. onites*, respectively[66].

On the basis of previous in vitro activity studies, the antibacterial efficacy of polar extracts has been suggested to originate from salvianolic acid B, rosmarinic acid, and hispidulin-7-*O*-glucoside[14,67,68]. LC-HRMS analysis revealed that decoction extracts of *S. pilifera*, *S. verbenaca*, *S. aramiensis*, *S. pisidica*, *S. albimaculata*, and *S. caespitosa* are rich in salvianolic acid B. Among them, *S. aramiensis* and *S. albimaculata* decoction extracts showed the better in vitro activity against MRSA. Interestingly, despite the absence of salvianolic acid B in the infusion extract of *S. aramiensis*, the extract exhibited high anti-MRSA activity, possibly due to the synergistic effects of other polar compounds. According to the literature survey, aqueous and other polar extracts prepared from both aerial and root parts of *Salvia* species were found to be more effective, especially against Gram-positive bacteria[69]. Although studies on the antimicrobial activity of the *Salvia aramiensis* species have focused mostly on its essential oils, the fact of its water extracts also having this feature, presents an important step in evaluating this species in this field[70].

Verbascoside was identified as the major compound in all extracts of the *S. viridis* species, and it has been suggested that the efficacy, particularly against *S. aureus* and *P. mirabilis* strains, exhibited by the ethanol extract may be associated with this compound (docking score: -8.295 kcal/mol). In their comprehensive review, Alipieva et al. emphasized the notable in vitro antibacterial activity of verbascoside, underscoring its potential as a bioactive phytochemical[71]. Furthermore, in silico studies have provided additional insights into the compound's mechanism of action, revealing its potential to modulate various antibacterial biological pathways[72]. In a separate study, verbascoside was found to exhibit moderate binding affinity to both Gram-positive and Gram-negative bacteria, with binding energies of – 5.95 and – 6.30 kcal/mol, respectively[73].

Some in silico and in vitro studies have revealed that hesperidin has antibacterial properties[74,75]. In our study, hesperidin was detected as one of the major components in all extracts of *Salvia albimaculata*. In silico analyses showed that hesperidin exhibited a strong effect against the 3SRW target protein with a binding energy of -9.243 kcal/mol, which is thought to support the in vitro biological activity observed in polar extracts of *S.*

albimaculata. In another study involving 51 compounds including hesperidin, hesperidin exhibited the highest binding score (-11.4 kcal/mol) against citrate synthase, highlighting its potential antimicrobial activity[76].

The in silico molecular docking results provided important insights into the antibacterial potential of the major compounds identified in the *Salvia* species. The binding affinity of rosmarinic acid was found to be very strong against both dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), which are key enzymes in bacterial folate biosynthesis. These findings suggest that rosmarinic acid may play a central role in the observed antibacterial activity. Other compounds, such as caffeic acid, fumaric acid, and salvianolic acid B, also exhibited favorable binding affinities and interactions, supporting their potential as multi-target inhibitors.

Conclusion

In this study, the chemical composition and pharmacological potential of eight *Salvia* species were evaluated for the first time through LC-HRMS-based phytochemical profiling, in silico molecular docking, and in vitro antibacterial assays, providing valuable insights into their potential use in antibacterial drug discovery and development. The findings that the antibacterial activities observed, particularly in the ethanol and decoction extracts, are considered to be strongly associated with key phytochemicals such as salvianolic acid B, rosmarinic acid, verbascoside, hesperidin, and salicylic acid. Molecular docking analyses corroborated these findings by revealing high binding affinities of rosmarinic acid and related compounds to DHFR and DHPS, two key bacterial enzymes. In addition, the obtained data demonstrate that this selected *Salvia* species may also be beneficial in infectious diseases. Overall, the potential applications of *Salvia* species, which play a significant role in the food, drug, and cosmetic industries, have expanded to different areas, offering a novel perspective for future research. More extensive studies on additional resistant organisms, along with animal testing and toxicological evaluation, are necessary to provide more definitive evidence of their antibacterial activity.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Declarations

Competing interests

The authors declare no competing interests.

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