JOURNAL of the MEXICAN CHEMICAL SOCIETY

https://www.jmcs.org.mx/

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J. Mex. Chem. Soc. **2026**, *70(1):e2525*

Received July 20th, 2025
Accepted November 25th, 2025

http://dx.doi.org/10.29356/jmcs.v70i1.2525
e-location ID: 2525

Keywords:
β-lactamases, *Sedum sediforme,* molecular docking, HPLC, Swiss ADME

Palabras clave:
β-lactamasas, *Sedum sediforme*, acoplamiento molecular, HPLC, Swiss ADME

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ISSN-e 2594-0317

Phytochemical Characterization and Evaluation of *Sedum sediforme* Extracts as Potential β-lactamase Inhibitors: An *In vitro* and *In Silico* Study

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Abstract. This study investigated the flavonoid content and β-lactamase inhibitory activity of three *Sedum sediforme* extracts: crude (CrE), chloroform (ChE), and ethyl acetate (EAe). Total flavonoids were quantified using $AlCl_3$ complexation, and HPLC analysis revealed quercetin (36.52 %) and gallic acid (24.11 %) as the predominant compounds in CrE. Enzymatic assays showed that CrE exhibited the highest β-lactamase inhibition, followed by ChE and EAe. In addition, an *in silico* analysis was conducted to explore the molecular interactions between phenolic compounds from *S. sediforme* and various β-lactamase enzymes. Seventeen phenolic constituents were identified by HPLC, with notable levels of caffeic acid (6.65 %), hesperetin (6.17 %), syringic acid (5.47 %), kaempferol (4.05 %), and rutin (3.83 %). Three-dimensional structures of these compounds were obtained from PubChem, optimized using Avogadro, and docked against four β-lactamase targets—TEM-1 (PDB: 1NYM), NDM-1 (PDB: 4EXS), AmpC (PDB: 1C3B), and OXA-48 (PDB: 7KHQ)—via AMDock. Docking results revealed

J. Mex. Chem. Soc. **2026**, *70(1):e2525*
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ISSN-e 2594-0317

strong binding affinities, including quercetin with TEM-1 (–8.9 kcal/mol), rutin with AmpC (–9.3 kcal/mol) and NDM-1 (–6.79 kcal/mol), and gallic acid with OXA-48 (–7.45 kcal/mol). Interaction profiling using BIOVIA Discovery Studio confirmed hydrogen bonding, hydrophobic interactions, and steric complementarity. A significant correlation was found between compound concentration and binding energy for TEM-1 ($p = 0.023$) and AmpC ($p = 0.010$). Pharmacokinetic predictions from Swiss ADME showed that quercetin and gallic acid satisfy Lipinski's Rule of Five, indicating good oral bioavailability, whereas rutin does not. BOILED-Egg analysis predicted blood–brain barrier permeability for quercetin and gallic acid. Toxicity predictions using ProTox-II revealed potential organ-specific toxicities among top ligands.

Resumen. En este estudio se investigó el contenido de flavonoides y la actividad inhibidora en β-lactamasas de tres extractos de *Sedum sediforme*: crudo (CrE), cloroformo (ChE) y acetato de etilo (EAe). Los flavonoides totales se cuantificaron mediante la formación de complejos con $AlCl_3$, y su análisis mediante HPLC reveló que la quercetina (36.52 %) y el ácido gálico (24.11 %) fueron los compuestos predominantes en CrE. Los ensayos enzimáticos mostraron que el extracto CrE presentó la mayor inhibición de β-lactamasas, seguida por los extractos de ChE y EAe. Además, se realizó un análisis *in silico* para explorar las interacciones moleculares entre los compuestos fenólicos de *S. sediforme* y diversas enzimas β-lactamasas. Se identificaron diecisiete componentes fenólicos mediante HPLC, con concentraciones notables de ácido cafeico (6.65 %), hesperetina (6.17 %), ácido siríngico (5.47 %), kaempferol (4.05 %) y rutina (3.83 %). Las estructuras tridimensionales de estos compuestos se obtuvieron de PubChem, se optimizaron con Avogadro y se acoplaron a cuatro blancos de β-lactamasa: TEM-1 (PDB: 1NYM), NDM-1 (PDB: 4EXS), AmpC (PDB: 1C3B) y OXA-48 (PDB: 7KHQ) mediante AMDock. Los resultados del acoplamiento revelaron fuertes afinidades de unión, incluyendo la quercetina con TEM-1 (–8.9 kcal/mol), la rutina con AmpC (–9.3 kcal/mol) y NDM-1 (–6.79 kcal/mol), y el ácido gálico con OXA-48 (–7.45 kcal/mol). El perfil de interacción con BIOVIA Discovery Studio confirmó la formación de enlaces de hidrógeno, las interacciones hidrofóbicas y la complementariedad estérica. Se determinó que existe una correlación significativa entre la concentración del compuesto y la energía de enlace para TEM-1 ($P = 0.023$) y AmpC ($P = 0.010$). Las predicciones farmacocinéticas de Swiss ADME mostraron que la quercetina y el ácido gálico cumplen la regla del cinco de Lipinski, lo que indica una buena biodisponibilidad oral, a diferencia de la rutina. El análisis de huevo cocido predijo la permeabilidad de la barrera hematoencefálica para la quercetina y el ácido gálico. Las predicciones de toxicidad con ProTox-II revelaron posibles toxicidades órgano-específicas entre los ligandos principales.
En general, estos hallazgos resaltan el potencial de los fenólicos derivados de *S. sediforme*, particularmente la quercetina y el ácido gálico, como prometedores inhibidores multiobjetivos de β-lactamasa para combatir la resistencia a los antibióticos.

Introduction

Bacterial resistance to antibiotics is a major public health threat, complicating the monitoring, treatment, and prevention of bacterial infections. This resistance has become increasingly serious over time due to the excessive or inappropriate use of antimicrobials [1]. Several causes are responsible for the emergence and development of bacterial resistance to antibiotics, such as improper storage of antibiotics and failure to follow the prescribed dosage [2]. β-lactam antibiotics, which account for 60% of global use, are widely prescribed due to their broad spectrum of antibacterial activity. In contrast, β-lactamases, which are enzymes produced by bacteria and can destroy β-lactam antibiotics, are classified into four groups (A, B, C, and D) based on primary sequence homology and differences in their mechanisms of action. Phytochemicals, especially those from medicinal plants, influence bacterial resistance [3]. In addition, these organic substances can influence the absorption of drugs by bacteria, which reinforces the inhibition of antibiotics [4].

In silico methods and computer-assisted molecular modeling have become indispensable in organic and pharmaceutical chemistry. They allow the reactivity and interactions of molecules to be studied virtually. Molecular docking, a technique used to analyze protein-ligand interactions, and molecular dynamics to assess the energetic stability of complexes. These theoretical methods are used for their efficiency, speed, and environmental aspects in predicting potential interactions between biomolecules and drug candidates [5,6].

http://dx.doi.org/10.29356/jmcs.v70i1.2525

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ISSN-e 2594-0317

In this context, *in silico* approaches provide an efficient alternative for predicting the binding affinity of natural compounds with β-lactamase enzymes, significantly reducing the time and resources required for experimental screening. The present study aims to computationally assess the interaction potential of bioactive ligands derived from *Sedum sediforme* against various clinically relevant β-lactamases, including TEM-1, OXA-48, NDM-1, and AmpC. Specifically, the objectives are: (i) to evaluate the binding affinities of these natural ligands through molecular docking and identify the compounds with the most favorable binding energies; (ii) to characterize the molecular interactions involved, such as hydrogen bonding, hydrophobic interactions, and steric effects; and (iii) to investigate the correlation between ligand concentration and binding affinity, thereby providing insights into their potential as multitarget enzyme inhibitors. In addition to the *in silico* investigations, an *in vitro* study was conducted to evaluate the inhibitory effect of *Sedum sediforme* extract on a penicillinase-type β-lactamase enzyme.

Experimental

Plant material

Sedum sediforme (SS) was collected from N'Gaous, Batna (Algeria), at the end of March 2012 and identified by Professor Oudjhih Bachir, Department of Agronomy, Batna University, Batna, Algeria. A voucher specimen was deposited in an official herbarium of the same department (no. I.A.B./990) for future reference.

Extraction procedure

The extraction procedure was performed using solvents of increasing polarity to obtain fractions enriched in compounds of varying chemical nature. Following the method described by [7]. The plant material was extracted with methanol, and the crude extract (CrE) was fractionated using solvents of increasing polarity-hexane, chloroform, and ethyl acetate-yielding three fractions: Crude extract (CrE), chloroform (ChE), ethyl acetate (AcE). Each fraction was concentrated under reduced pressure.

Determination of total flavonoid

Total flavonoid content of each extract was determined by a colorimetric method as described by [8].

HPLC conditions

High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1260 series system equipped with a Zorbax Eclipse Plus C8 column (4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of water (solvent A) and acetonitrile containing 0.05 % trifluoroacetic acid (solvent B), delivered at a flow rate of 0.9 mL/min. A linear gradient elution was applied as follows: 0–1 min, 82 % A; 1–11 min, 75 % A; 11–18 min, 60 % A; 18–24 min, 82 % A. Detection was carried out using a multi-wavelength detector set at 280 nm. The injection volume was 5 µL, and the column temperature was maintained at 40 °C throughout the analysis.

β-latamase inhibition assays

Tannin removal from crude extract

To eliminate tannins, crude plant extracts were treated with bovine serum albumin (BSA) following the method described by [9]. Briefly, 2 mL of a BSA solution was mixed with 1 mL of plant extract solution (1 mg/mL). The mixture was incubated at 4 °C for 24 hours. After incubation, the samples were centrifuged at 3,000 × g for 15 minutes. The resulting precipitate was discarded, and the supernatant was collected for further enzymatic assays.

β-lactamase inhibition assay

The inhibitory activity of the plant extracts against β-lactamase was assessed using nitrocefin as a chromogenic substrate. Extracts at different concentrations (1.25, 2.5, 5, and 10 mg/mL) were tested. In a 96-well microplate, 80 µL of each extract solution was added, followed by 10 µL of diluted β-lactamase enzyme. After incubation at room temperature for 15 minutes, the reaction was initiated by adding 10 µL of nitrocefin (250 µM). Enzymatic activity was monitored at 482 nm using a microplate reader for a minimum of 20 minutes. The percentage of β-lactamase inhibition was calculated using the following formula: Inhibition (%) = 100 × (Ac − Ae) / Ac. Where Ac represents the absorbance of the control reaction (enzyme without inhibitor), and Aeis the absorbance in the presence of plant extract.

http://dx.doi.org/10.29356/jmcs.v70i1.2525

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Molecular docking study

Molecular docking study of the components of SS extract was performed to predict the inhibitory effect of β-lactamases using the AutoDock Vina program. All ligands used in this study were downloaded from PubChem and then optimized using Avogadro software to achieve a stable geometry with minimal energy.The proteins used were 1NYM, 7KH, 4EXS, and 1C3B [11].The receptors (proteins) were prepared using Discovery Studio to remove water molecules and heteroatoms, as well as adding polar hydrogen atoms and Kollman charges [10]. Then, the AutoDock Vina program 1.1.2 was used to perform molecular docking simulations. Finally, Discovery software was used to visualize protein-ligand interactions in 2D and 3D formats.

Swiss ADME and toxicity

We used Lipinski's rule to evaluate the medicinal capacity of Phyto-compounds, characterized by five parameters: no more than 5 hydrogen bond donors (-OH and -NH groups), must not exceed 10 hydrogen bond acceptors (O and N atoms), a molecular mass less than 500 Da, an octanol-water partition coefficient (milogP) ≤ 5, no more than one rule may be violated [12].

For the best ligands (the highest scores), the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profile was used: online software (swiss ADME website) for the prediction of physicochemical and pharmacokinetic parameters [13]. Pro-tox 3.0 was used for the evolution of toxicity risks.

Results and discussion

Determination of flavonoids content

As one of the most diverse and widespread groups of natural compounds, flavonoids are probably the most important natural phenolic compounds. These compounds possess a broad spectrum of chemical and biological activities, including free radical scavenging properties [14].

This method allows for the determination of total flavonoid content by forming a stable complex with AlCl3, even in the presence of other polyphenolic compounds that do not form such complexes [15]. The flavonoid content was quantified using this assay and expressed as micrograms of quercetin equivalents (µg EQ/mg extract) and rutin equivalents (µg ER/mg extract), as shown in Table 1.

Table 1. Flavonoid assay of SS fractions.

Extract	µg EQ/mg extract	µg ER/mg extract
CrE	63.86 ± 1.224	122.51 ± 2.95
ChE	76.431 ± 12.84	152.85 ± 31.01
EAe	63.73 ± 4.86	122.20 ± 11.73

Values are mean ± SD (n = 3).

The order of flavonoid content is EAe, ChE and CrE. In other words, the flavonoids present in EAe are almost of the same concentration in ChE.

Determination of the composition of the crude extract of SS by HPLC

The composition of the CrE was determined by HPLC analysis. 17 molecules were detected. The principal constituents identified were Querectin (36.52 %) and gallic acid (24.11 %), as detailed in (Table 2 and Fig. 1). Coffeic acid, Hesperetin and Syringic acid were also predominant compounds, with 6.65 %, 6.17 % and 5.47 %, respectively. Followed by Kaempferol (4.05 %), rutin (3.83 %).

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ISSN-e 2594-0317

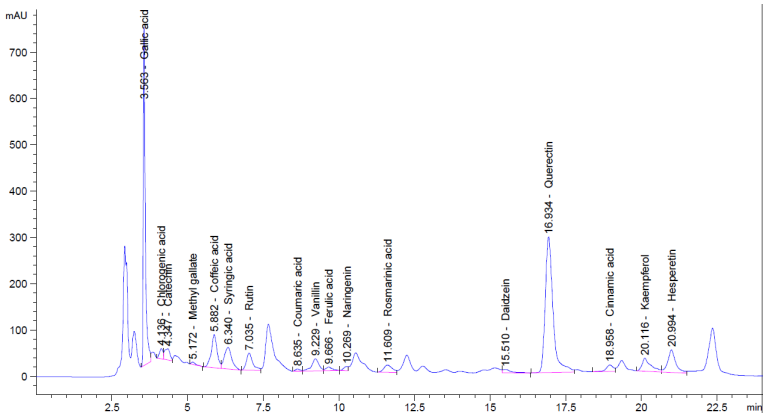

Fig. 1. HPLC profile of phenolic components from SS.

Table 2. Identification and quantification of the molecules present in SS.

Sample (SS)			
	Area	**Conc. (µg/mL)**	**Conc. (µg/g)**
Gallic acid	3452,02	252,75	12637,46
Chlorogenic acid	176,14	24,54	1227,23
Catechin	298,43	64,11	3205,52
Methyl gallate	51,89	2,90	145,16
Coffeic acid	952,25	48,85	2442,68
Syringic acid	783,88	46,11	2305,36
Rutin	547,45	81,95	4097,45
Ellagic acid	-	-	-
Coumaric acid	47,37	1,70	85,12
Vanillin	435,00	15,78	788,85
Ferulic acid	118,92	6,90	345,17
Naringenin	89,27	8,24	411,94
Rosmarinic acid	292,97	28,45	1422,67
Daidzein	150,65	8,63	431,28
Querectin	5228,39	651,16	32558,17
Cinnamic acid	223,56	4,33	216,64
Kaempferol	580,71	14,60	729,94
Hesperetin	883,97	41,39	2069,64

http://dx.doi.org/10.29356/jmcs.v70i1.2525

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ISSN-e 2594-0317

In contrast to previous literature on SS, our study aligns with [16] in Turkey, who identified quercetin, rutin, naringenin, protocatechuic, p-coumaric, caffeic, and chlorogenic acids as the dominant compounds. However, [17] in Austria, they presented a different SSprofile, with gallic acid and myricitrin as dominant constituents.

Enzymatic inhibition of β-lactamases

The inhibitory effects of SS extracts on a penicillinase-type β-lactamase from *Bacillus cereus* were evaluated using a nitrocefin-based enzymatic assay. The results showed a dose-dependent inhibition for the CrE and EAe fractions. CrE exhibited the most pronounced activity at 1.25 mg/mL, with inhibition ranging from 33% to 71%, suggesting a strong concentration-effect relationship. The absence of detectable inhibition for CrE at 10 mg/mL could indicate enzyme saturation, while EAe showed weaker but increasing activity (0.69–26.09 %). The ChE showed moderate inhibition (30.86–46.00%) with a less marked dose-dependence. These results support the potential of polyphenol-rich fractions as β-lactamase inhibitors (Table 3).

Experimental results further suggest that the structure of the flavonoid compounds may play a more critical role in β-lactamase inhibition than concentration alone. In particular, statistical analysis using Spearman's correlation indicated no significant relationship between flavonoid concentration and enzyme inhibition for the crude (CrE) and chloroform (ChE) extracts ($p = 0.057$ and $p = 0.196$, respectively). However, a significant correlation was observed for the ethyl acetate extract (EAe), with $p = 0.033$, indicating that certain solvent-extracted fractions may enrich more potent flavonoid inhibitors.

Table 3. SS extracts inhibition percentages of β-Lactamase activity

Extracts	**Inhibition %**			
	1.25 mg/mL	**2.5 mg/mL**	**5 mg/mL**	**10 mg/mL**
CrE	32.97 ± 3.15	49.09 ± 14.55	71.20 ± 4.85	Nd
ChE	30.86 ± 2.20	31.60 ± 2.18	31.71 ± 9.16	46.00± 0.24
EAe	0.69 ± 0.07	6.57± 0.73	22.86 ± 0.01	26.09 ± 0.93

The increasing prevalence of β-lactamase-producing bacterial pathogens represents a significant global health concern, as these enzymes undermine the effectiveness of widely used β-lactam antibiotics. Recent studies have demonstrated that plant-derived flavonoids possess promising inhibitory activity against β-lactamases, offering a potential strategy to restore the efficacy of these antibiotics. For example, extracts from *Terminalia superba*, *Annona senegalensis*, and *Psidium guajava* have shown significant inhibitory effects on β-lactamase activity, with *T. superb* achieving up to 75.7 % inhibition in *Bacillus cereus* β-lactamase and improving the efficacy of ceftriaxone by 64.9 % [18]. Similarly, flavonoid-rich extracts from *Ocimumtenui florum* and *Terminalia chebula* have demonstrated β-lactamase inhibitory effects comparable to clavulanic acid, especially in ethyl acetate fractions [19].

Molecular docking study

Following molecular docking analysis between 17 compounds identified by HPLC from the SS extract and four β-lactamase enzymes (Table 3). The molecular docking results revealed that quercetin exhibited a strong binding affinity with TEM-1 β-lactamase (-8.9 kcal/mol). Rutin demonstrated notable interactions with two targets: NDM-1, with a binding affinity of -6.79 kcal/mol, and AmpCβ-lactamase, with a binding affinity of -9.3 kcal/mol. Additionally, gallic acid showed a significant interaction with OXA-48 β-lactamase, yielding a binding affinity of -7.45 kcal/mol. These findings highlight the potential of *Sedum sediforme* as a source of multi-target β-lactamase inhibitors. Binding energy represents the strength of the interaction between a ligand and its protein target; more negative values indicate stronger and more stable interactions [20]. These results suggest that the identified secondary metabolites may serve as promising inhibitors of β-lactamases, potentially offering new avenues to overcome bacterial resistance to antibiotics.

http://dx.doi.org/10.29356/jmcs.v70i1.2525

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Table 3. Docking binding energies and inhibition constants of docked compounds in the active site of 4 proteins

Molecules	TEM1 (1NYM)		NDM (4EXS)		AMPc(1C3B)		7KHQ (OXA48)	
	Energy (kcal/mol)	Ki (μM)	Energy (kcal/mol)	Ki (μM)	Energy (kcal/mol)	Ki (μM)	Energy (kcal/mol)	Ki (μM)
Caffeic acid	-6,7	12,27	-5,51	91,44	-6,4	20,36	-5,19	156,93
Ferulic acid	-6,3	24,10	-5,3	130,34	-6,1	33,78	-5,11	179,62
Chlorogenic acid	-8,1	1,16	-6,12	32,66	-8,2	0,98	-6,32	23,30
Cinnamic acid	-6,3	24,10	-4,76	324,26	-5,8	56,05	-4,9	256,02
Coumaric acid	-6,1	33,78	-4,79	308,25	-5,6	78,55	-4,31	693,02
Vanillin	-5,3	130,34	-3,95	1272,42	-4,9	256,02	-3,81	1611,57
Methyl gallate	-6	39,99	-4,05	1074,80	-5,4	110,10	-4,3	704,82
Rosmarinic acid	-7,4	3,76	-6,09	34,36	-8,4	0,70	-6,99	7,52
Gallic acid	-8	1,37	-5,11	179,62	-8,2	0,98	-7,45	3,46
Syringic acid	-5,9	47,34	-3,56	2457,54	-5,5	93,00	-5,02	209,08
Hesperetin	-8,2	0,98	-6,57	15,28	-8	1,37	-6,98	7,65
Kaempferol	-8,3	0,82	-5,99	40,67	-7,3	4,46	-5,95	43,51
Catechin	-8	1,37	-6,55	15,81	-7,6	2,69	-6,33	22,91
Naringenin	-8	1,37	-6,31	23,70	-7,5	3,18	-6,48	17,79
Quercetin	**-8,9**	0,30	-6,05	36,76	-8	1,37	-5,94	44,25
Daidzein	-7,1	6,25	-5,87	49,80	-7,2	5,28	-6,36	21,78
Rutin	-8,7	0,42	-6,79	10,54	-9,3	0,15	-5,38	113,88

Table 4 and Figures 2, 3, 4, and 5 present the molecular interactions between the ligands—quercetin, rutin, and gallic acid—and the target β-lactamase enzymes: TEM-1 β-lactamase (PDB ID: 1NYM), New Delhi metallo-β-lactamase NDM-1 (PDB ID: 4EXS), AmpCβ-lactamase (PDB ID: 1C3B), and class D OXA-48 β-lactamase (PDB ID: 7KHQ). Numerous studies have employed molecular docking approaches to identify β-lactamase inhibitors derived from natural products. Phenolic compounds, including those utilized in the present study, have been extensively investigated for this purpose. A key aspect of these investigations involves analyzing the molecular interactions that underlie specificity and inhibitory capacity. Hydrogen bonding, in particular, plays a pivotal role in molecular recognition due to its intermediate strength between covalent and van der Waals interactions. These bonds are especially significant in biological systems, facilitating ligand binding and enzymatic catalysis, where both specificity and reversibility are essential.

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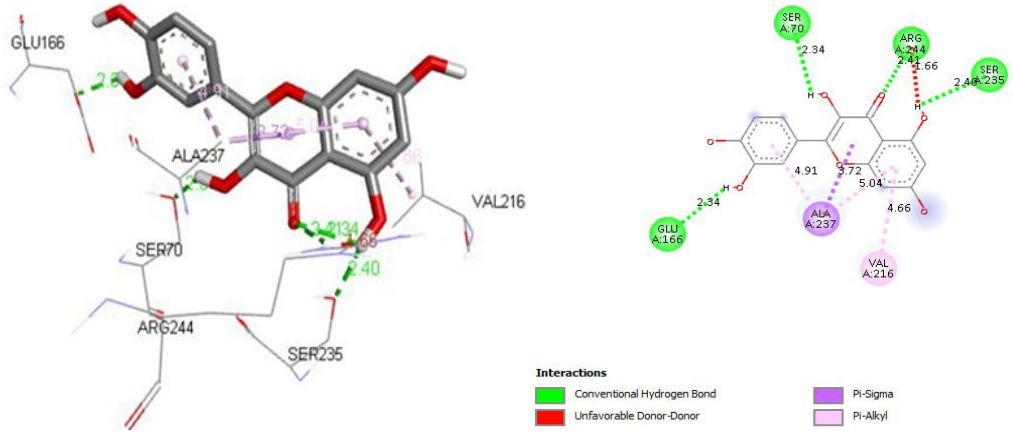

Fig. 2. Interaction of quercetin with the TEM1 protein in 2D and 3D form.

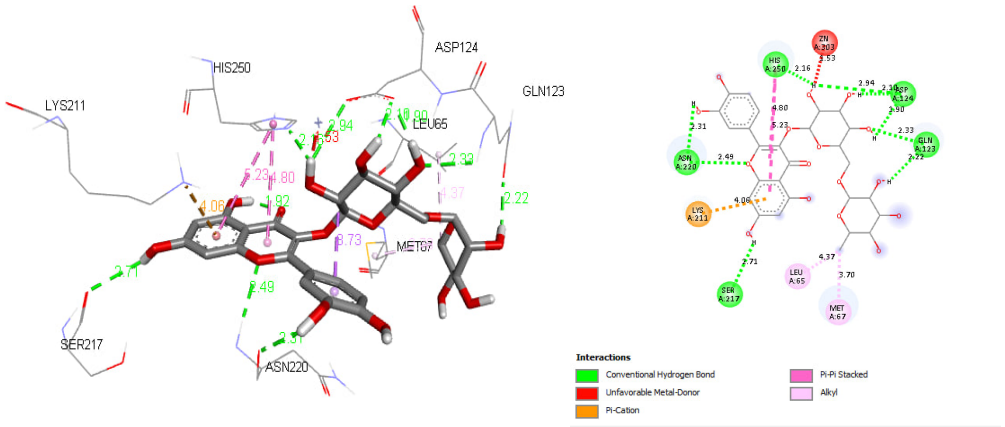

Fig. 3. Interaction of rutin with NDM-1 in 2D and 3D form.

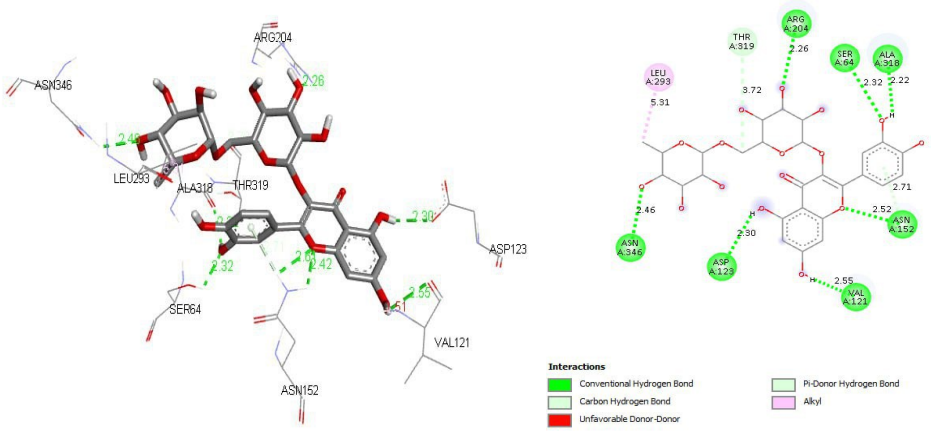

Fig. 4. Interaction of rutin with the AMPc protein in 2D and 3D form.

http://dx.doi.org/10.29356/jmcs.v70i1.2525

J. Mex. Chem. Soc. **2026**, *70(1):e2525*
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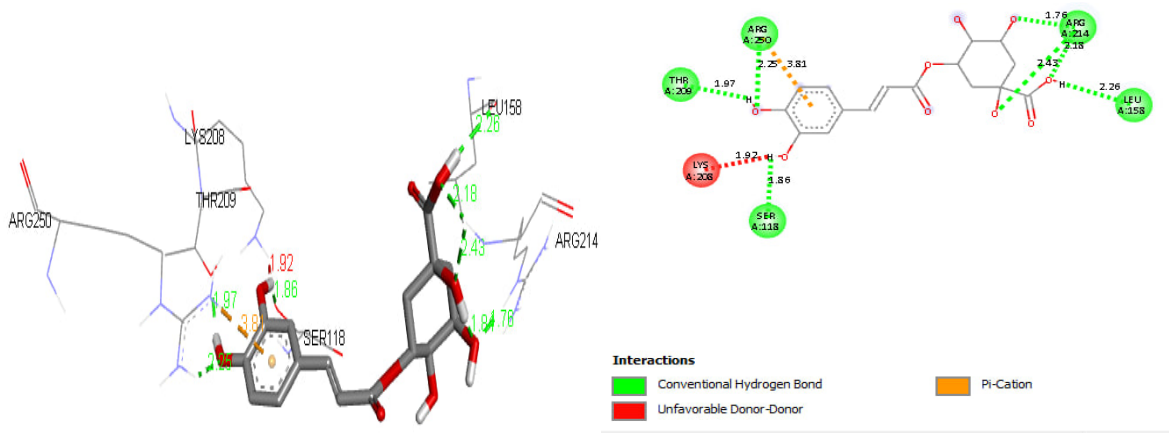

Fig.5. Interaction of gallic acid with OXA-48 protein in 2D and 3D forms.

Table 4. Number and type of bonds participating in protein-ligand interactions.

		Hydrogen bond	Carbon hydrogen bond	Pi-donor Hydrogen bond	Alkyl	Pi- alkyl	Pi- cation	Pi- stacked	Pi- sigma
Interaction of quercetin with TEM1	Number	04	/	/	/	01	/	/	01
	Aminoacid	SER A :70 ARG A :244 SER A :235 GLU A :166	/	/	/	VAL A :215	/	/	ALA A :237
Interaction of rutin with NDM-1	Number	05	/	/	01	/	01	01	/
	Aminoacid	HIS A:250 ASP A:124 GLN A:123 ASN A: 220 SER A: 217	/	/	LEU A: 65 MET A: 67	/	LYS A: 211	HIS A: 250	/
Interaction of rutin with AMPc	Number	07	01	01	01	/	/	/	/
	Aminoacid	ASP A: 123 ARG A: 204 SER A: 64 ALA A: 318 ASN A: 152 VAL A: 121 ASN A: 346	THR A :319	THR A: 319	LEU A: 293	/	/	/	/
Interaction of gallic acid with OXA-48	Number	05	/	/	/	/	01	/	/
	Aminoacid	ARG A: 250 THR A: 209 SER A: 118 ARG A: 214 LEU A: 158	/	/	/	/	ARG A: 250	/	/

http://dx.doi.org/10.29356/jmcs.v70i1.2525

J. Mex. Chem. Soc. **2026**, *70(1):e2525*
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In this study, quercetin, rutin, and gallic acid were selected as candidate inhibitors against various β-lactamases, a choice supported by findings from previous research. Quercetin, a flavonoid characterized by five hydroxyl groups and three aromatic rings, binds within the enzyme's active site, a large polar cavity spanning β-sheet structures-where it adopts a compact conformation. The presence of negatively charged and hydrophobic residues within this cavity enhances the ligand's binding affinity [21].

In a comparative study aiming to identify novel alternatives to conventional β-lactamase inhibitors, quercetin, which lacks a β-lactam ring, was evaluated using molecular docking and molecular dynamics simulations. Docking results ranked quercetin as the top ligand, exhibiting stronger predicted affinity than known inhibitors. However, as noted by [22], molecular docking provides only preliminary insights into ligand binding positions and energetics, requiring further validation.

Additional docking analyses have shown that gallocatechin gallate, isolated from green tea and recognized for its β-lactamase inhibitory activity, demonstrates high affinity toward TEM-1 from *Escherichia coli* and NDM-1 [23]. Similarly, [24] identified quercetin 3-(6'-O-caffeoyl)-β-D-glucopyranoside (M2), an O-glucoside of quercetin, as the most effective ligand against 1C3B, supported by favorable hydrophobic interactions. [11] reported that quercetin, a widely distributed bioflavonoid in marine algae, displays a broad spectrum of biological activities, including antibacterial properties, which may be attributed to such interactions. Moreover, gallic acid hexoside, a phenolic compound naturally present in honey, has demonstrated greater inhibitory efficacy than avibactam, a clinically used β-lactamase inhibitor [25]. These findings suggest that's uch natural compounds may act as potent competitive inhibitors of β-lactamases. Continued research on these bioactive molecules could contribute to the development of effective strategies to restore the therapeutic potential of β-lactam antibiotics against resistant pathogens.

Recent studies have highlighted the potential of flavonoids as natural inhibitors of β-lactamases, key enzymes responsible for bacterial resistance to β-lactam antibiotics. [26] demonstrated that quercetin acts as a reversible, non-competitive inhibitor of OXA-48 β-lactamase. Enzyme kinetics and molecular docking confirmed this inhibition, which is stabilized by hydrogen bonding between the hydroxyl groups at positions 3′, 4′, and 7 and residues Thr209, Ala194, and Gln193 of the enzyme. *In vivo*, the combination of quercetin with piperacillin significantly reduced bacterial burden in a murine infection model. Complementarily, [27] identified quercetin-3-O-rutinoside and luteolin as potent inhibitors of SHV-1 and AmpCβ-lactamases, respectively, with binding affinities exceeding that of avibactam. These findings suggest that flavonoid glycosylation, particularly in the form of rutinoside derivatives, may enhance binding via additional hydrogen bonds or hydrophobic contacts within the enzyme's active site. [28] Through screening of 180 plants extracts, identified *Ficus religiosa* bark extract (FRAE) as a potent β-lactamase inhibitor. HR-LCMS analysis revealed major constituents including quercetin, luteolin, myricetin, taxifolin, and miquelianin. Combined docking and molecular dynamics (MD) simulations showed that these flavonoids interact with Glu166 of the Ω-loop in class A β-lactamases (SHV-1, TEM-1, KPC-2, CTX-M-27), there by disrupting the deacylation step essential for enzymatic activity. Myricetin and miquelianin formed the most stable complexes with SHV-1/KPC-2 and TEM-1/CTX-M-27, respectively, indicating structure-dependent variations in inhibitory potency. [29] focused on methicillin-resistant *Staphylococcus aureus* (MRSA), using *in silico* screening to identify flavonoid glycosides (rutin, isoquercitrin, nicotiflorin, quercetin-3-rhamnoside, vicenin-2, quercitrin, and orientin) exhibiting high binding affinities ($\Delta G < -10$ kcal/mol) toward MRSA β-lactamase. Rutin showed inhibition constants (Ki) in the picomolar range.

Application of Spearman's test assessed the existence of a possible correlation between the concentration of ligands and their binding energy with the target β-lactamases (Table 5). The results showed a significant correlation with TEM-1 ($p = 0.023$) and AMPc ($p = 0.010$).

Table 5. Correlation between energy and concentration.

Protein /Concentration	**TEM-1**	**NDM**	**Ampc**	**OXA-48**
The p-value	0.023	0.117	0.010	0.076

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ISSN-e 2594-0317

Quercetin, rutin, and gallic acid are the predominant compounds. This strong correlation indicates that concentration dependence influences binding energy, with ligands present at higher concentrations exhibiting more negative binding energies. This observation could be explained by: (1) better occupancy of the active site at higher concentration, (2) potentiation of weak interactions (cooperative effect), (3) saturation of secondary binding sites [20].

Toxicity and Swiss ADME

The compounds exhibiting the most favorable binding energies were subjected to further analysis to assess their pharmacokinetic properties and toxicity profiles, including key physicochemical and pharmacokinetic parameters [13]. As shown in Table 6, which summarizes these findings in comparison with Lipinski's Rule of Five, both gallic acid and quercetin comply with the established criteria, supporting their potential as promising candidates for oral administration. Conversely, rutin violates Lipinski's rule due to its high molecular weight and excessive number of hydrogen bond donors and acceptors.

The polar surface area (PSA) is a widely recognized molecular descriptor for evaluating drug transport properties, particularly gastrointestinal absorption (HIA) [30] and blood-brain barrier (BBB) permeability [31]. PSA represents the sum of the van der Waals surface areas of polar atoms—primarily oxygen, nitrogen, and their attached hydrogens.

To assess human intestinal absorption and BBB permeability of the selected compounds, the BOILED-Egg predictive model was employed via the Swiss ADME web tool. This model correlates the total polar surface area (TPSA) with lipophilicity (WLogP), calculated using the Wildman-Crippen method [13], offering a graphical representation of passive gastrointestinal absorption and brain penetration capabilities.

Table 6. Physicochemical parameters of quercetin, rutin, and gallic acid in the BOILED-Egg model.

	TPSA (˚A²)[a]	**PM[b]**	**MLOGP[c]**	**NHD[d]**	**NHA[e]**	**NRB[f]**	**Lipenski violation**
Quercetin	131.36	302.24	-0.56	5	7	1	0
Rutin	269.43	610.52	-3.89	10	16	6	3
Gallicacid	97.99	170.12	-0.16	4	5	1	0
Reference	<500	<500	<5	≤5	≤10	≤10	≤1

a Topological polar surface, b Molecular mass g/mol, c Partition coefficient, d Number of donor hydrogen bonds, e Number of acceptor hydrogen bonds, f Number of rotatory bonds.

Quercetin and gallic acid are located in the albumen zone, meaning that these molecules can cross the BBB (potential effect on the central nervous system). Rutin is located outside these zones, suggesting low intestinal absorption and/or low BBB permeability. An alternative route of administration could be envisaged for this compound. Despite its BBB penetration, rutin may have limited oral bioavailability due to its high molecular weight (>500 Da).

Fig.6 shows that quercetin and gallic acid belong to the P-gp category, meaning that they are not eliminated from the CNS via P-gp. These analyses of pharmacokinetic and physicochemical parameters confirm that the molecules studied have promising potential for pharmaceutical applications [32].

J. Mex. Chem. Soc. **2026**, *70(1):e2525*
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ISSN-e 2594-0317

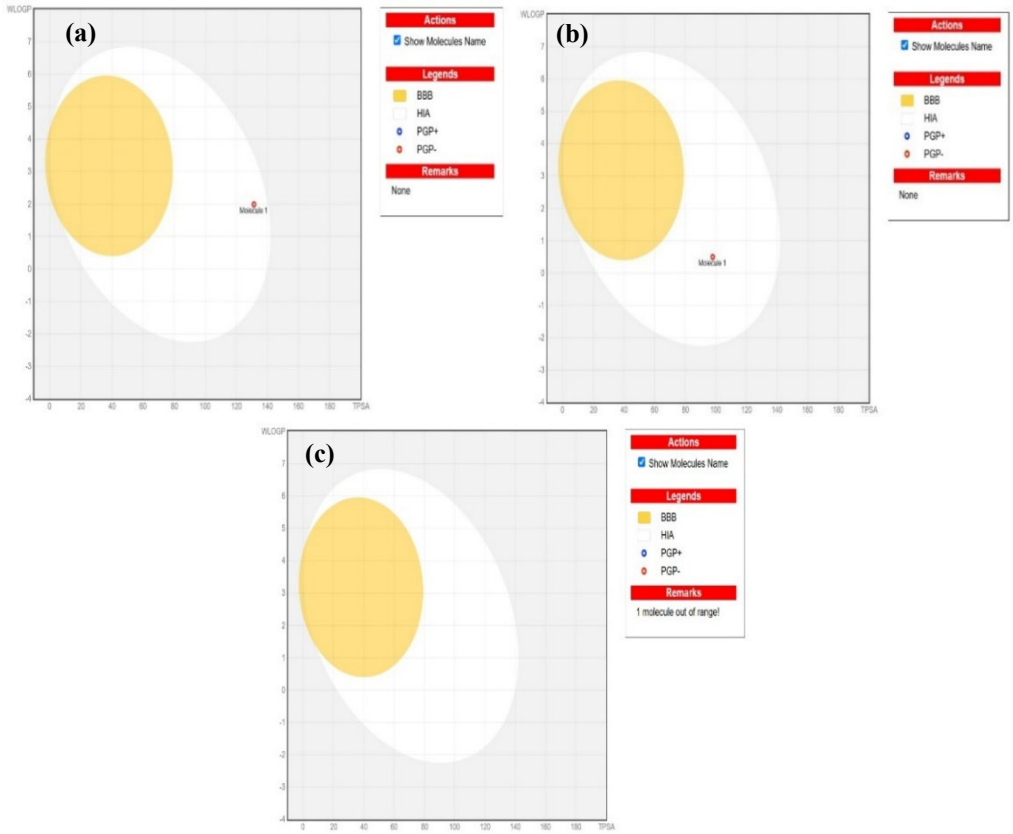

Fig. 6. BOILED-Egg model for quercetin **(a)**, gallic acid **(b)**, and rutin **(c)**.

The toxicological risk assessment showed that quercetin, rutin, and gallic acid have certain toxic effects on specific organs (Table 7). Rutin has the highest LD_{50} of the compounds tested, suggesting a lower toxic risk than the others.

Table 7. Toxicological parameters for quercetin, rutin, and gallic acid.

	Quercetin	**Rutin**	**Gallicacid**
DL_{50} (mg/Kg)	159	5000	2000
Toxicity class	3	5	4
Hepatotoxicity	Non	Non	Non
Neurotoxicity	Non	Non	Non
Nephrotoxicity	Oui	Oui	Oui
Respiratorytoxicity	Oui	Oui	Oui
Cardiotoxicity	Non	Non	Non

http://dx.doi.org/10.29356/jmcs.v70i1.2525

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Regular Issue

ISSN-e 2594-0317

Conclusions

Molecular docking saves time and reduces costs associated with practical work, while providing valuable structural information for the development of anti-resistance agents. The primary goal of this *in silico* study was to identify the ligand with the best inhibitory capacity among 17 natural compounds from *Sedum sediforme* against different classes of β-lactamases (TEM-1, NDM-1, AMPc, and OXA-48). The results show that quercetin and gallic acid exhibit higher affinities than clavulanic acid (the reference inhibitor), with binding energies reaching -8.9 kcal/mol (TEM-1) and -7.46 kcal/mol (OXA-48). Rutin as the best inhibitor of AMPc and NDM-1 proteins. Structural analyses identified key interactions (hydrogen bonds, hydrophobic effects) explaining these strong affinities. A significant correlation was observed between ligand concentration and their binding energy with TEM-1 and AMPc, suggesting a concentration-dependent effect. Gallic acid and quercetin stand out as promising candidates for orally administered drugs, unlike rutin, which exhibits absorption limitations according to Lipinski's rule. In conclusion, the *in vitro* and *in silico* approaches are highly complementary. While *in vitro* assays confirm biological efficacy and offer direct evidence of β-lactamase inhibition, *in silico* methods elucidate the molecular basis of this inhibition and enable the screening of multiple targets with high precision. Together, they strengthen the case for *Sedum sediforme* phenolics, particularly quercetin and gallic acid as promising natural β-lactamase inhibitors.

Acknowledgements

The authors acknowledge the Algerian Ministry of Higher Education and Scientific Research (MESRS), and the Thematic Research Agency in Health and Life Sciences (ATRSSV), for the financing of this work.

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