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Phytochemical profiling and *in silico* evaluation of *Mentha pulegium* L. compounds against monkeypox virus methyltransferase VP39

F. Boudou[1], A. Belakredar[2]

[1] University of Science and Technology of Oran,
BP 1505, Bir El Djir 31000, Oran, Algeria
[2] University of Mostaganem,
Route Nationale N 11, Kharouba, Mostaganem 27000

E-mail: farouk.boudou@univ-usto.dz

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This study explores the potential of bioactive compounds from *Mentha pulegium* L. as inhibitors of the monkeypox virus methyltransferase VP39. High-Performance Liquid Chromatography (HPLC) was employed to identify and characterize phenolic compounds in the plant extract. Computational methods were used to predict drug-likeness and toxicity and to evaluate binding interactions through molecular docking and dynamics simulations.

The aim. To investigate the antiviral potential of *M. pulegium* compounds against monkeypox virus VP39 by evaluating their drug-likeness, toxicity, and interaction stability through computational approaches.

Materials and methods. Various phenolic compounds in *M. pulegium* were identified using HPLC. Drug-likeness and toxicity were predicted using SwissADME, ProTox 3.0 Tool, and OSIRIS Property Explorer tools. Molecular docking studies assessed the binding affinity of selected compounds with VP39, and molecular dynamics simulations evaluated the stability of these interactions over time.

Results. Luteolin and rosmarinic acid exhibited the highest binding affinities to VP39, with docking scores of −9.3 kcal/mol and −8.7 kcal/mol, respectively, and formed multiple hydrogen bonds with key amino acid residues including Ile94, Gly96, Phe115, Val139, Ala158, Lys186, and Tyr189 for luteolin, and Gly68, Ile94, Asp95, Val112, Phe115, Val141, and Asn156 for rosmarinic acid. Molecular dynamics simulations showed that these compounds interacted with moderately flexible regions of the enzyme (residues 67–79 and 243–246), with the RMSD stabilizing at around 3.91 Å after 5000 ps, enhancing binding stability and suggesting a strong potential for inhibitory activity.

Conclusion. The findings underscore the potential of luteolin and rosmarinic acid from *M. pulegium* as promising antiviral agents against the monkeypox virus. This research provides a foundation for further exploration and development of novel therapeutic strategies based on these natural compounds.

Keywords: *Mentha pulegium* L.; monkeypox virus; methyltransferase VP39; molecular docking; molecular dynamics; drug-likeness; toxicity assessment

Abbreviations: HPLC — High-Performance Liquid Chromatography; VP39 — viral protein 39; RMSD — root mean square deviation; RMSF — root mean square fluctuation; MD — molecular dynamics; ADME — absorption, distribution, metabolism, and excretion; LD_{50} — median lethal dose; MW — molecular weight; WLogP — Wildman–Crippen LogP; MR — molar refractivity.

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Фитохимическое профилирование и оценка *in silico* соединений *Mentha pulegium* L. против метилтрансферазы VP39 вируса оспы обезьян

Ф. Буду[1], А. Белакредар[2]

[1] Университет науки и технологий Орана,
BP 1505, Бир-эль-Джир 31000, Оран, Алжир
[2] Университет Мостаганема,
Национальный маршрут № 11, Харуба, Мостаганем, 27000, Алжир

E-mail: farouk.boudou@univ-usto.dz

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В исследовании изучен потенциал биологически активных соединений *Mentha pulegium* L. в качестве ингибиторов метилтрансферазы VP39 вируса оспы обезьян. Для идентификации и характеристики фенольных соединений в растительном экстракте была использована высокоэффективная жидкостная хроматография (ВЭЖХ). Вычислительные методы были использованы для прогнозирования сходства и токсичности лекарственных средств, а также для оценки энергий связывания с помощью молекулярного докинга и моделирования динамики.

Цель. Изучить противовирусный потенциал соединений *M. pulegium* против вируса оспы обезьян VP39 путем оценки их сходства с лекарственными препаратами, токсичности и стабильности связывания с помощью вычислительных методов.

Материалы и методы. Различные фенольные соединения *M. pulegium* идентифицированы с помощью ВЭЖХ. Сходство с лекарственными препаратами и токсичность предсказаны с помощью SwissADME, ProTox 3.0 и OSIRIS Property Explorer. С помощью молекулярного докинга оценивали сродство выбранных соединений к VP39, а моделирование молекулярной динамики позволило оценить стабильность этих связей с течением времени.

Результаты. Лютеолин и розмариновая кислота продемонстрировали наилучшую степень связывания с VP39, составив –9,3 и –8,7 ккал/моль соответственно, а также образовывали множественные водородные связи с ключевыми аминокислотными остатками: Ile94, Gly96, Phe115, Val139, Ala158, Lys186 и Tyr189 для лютеолина; Gly68, Ile94, Asp95, Val112, Phe115, Val141 и Asn156 для розмариновой кислоты. Моделирование молекулярной динамики показало, что лютеолин и розмариновая кислота взаимодействуют с умеренно гибкими участками фермента (остатки 67–79 и 243–246), при этом RMSD стабилизируется на уровне около 3,91 Å после 5000 пс, что повышает стабильность связывания и свидетельствует о сильном потенциале ингибирующей активности.

Заключение. Полученные данные подчёркивают потенциал лютеолина и розмариновой кислоты *M. pulegium* в качестве перспективных противовирусных веществ в борьбе с вирусом оспы обезьян. Данное исследование является основой для дальнейшего изучения и разработки новых терапевтических стратегий, основанных на этих природных соединениях.

Ключевые слова: *Mentha pulegium* L.; вирус оспы обезьян; метилтрансфераза VP39; молекулярный докинг; молекулярная динамика; сходство с лекарственными препаратами; токсичность

Список сокращений: ВЭЖХ — высокоэффективная жидкостная хроматография; VP39 — вирусный белок 39; RMSD — среднеквадратичное отклонение; RMSF — среднеквадратичные колебания; MD — молекулярная динамика; ADME — всасывание, распределение, метаболизм и выведение; LD_{50} — полулетальная доза; MW — молекулярная масса; WLogP — логарифм Уайлдмена–Криппена; MR — молярная преломляемость.

INTRODUCTION

The Monkeypox is a member of the orthopoxvirus family, the most notable member of which is smallpox. The double-stranded DNA virus was first noted in monkeys in the 1950s and has a wide host range, notably including rodents. Human cases first came to attention during the smallpox eradication campaign. Handling bushmeat, an animal bite or scrape, bodily fluids, contaminated objects, or intimate contact with an infected human can all transmit monkeypox [1]. In recent years, monkeypox outbreaks have been identified in 11 African countries, with the most cases

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reported in the Democratic Republic of the Congo. The increase in monkeypox outbreaks in recent years may be related to diminished population immunity to smallpox over time. Smallpox is closely related to monkeypox, and vaccination programs for smallpox ended decades ago [2]. This virus causes an infectious illness that can harm humans and other animals. The initial symptoms include fever, headache, muscular aches, enlarged lymph nodes, and exhaustion. A skin rash with blisters and crusts appears after these symptoms [3]. The usual time between exposure and disease onset is 7 to 14 days, and symptoms typically last two to four weeks. Severe cases are conceivable, especially in youngsters, pregnant women, and people with weakened immune systems [4, 5]. Complications can occur, including involvement of the central nervous system and airway compromise from lymphadenitis. Mortality has been reported to range from 1 % to 10 % but may differ in settings where advanced medical care is available [2]. Smallpox antivirals having poxvirus activity, such as cidofovir, brincidofovir, and tecovirimat, have activity against monkeypox. The latter two medications have been approved for use in smallpox therapy by the United States Food and Drug Administration (FDA). Such treatments would most likely be reserved for severe instances or in immunocompromised individuals and would be obtained through a public health department or the Centers for Disease Control and Prevention (CDC) [2, 6]. In addition, smallpox vaccines are useful for monkeypox prevention and post-exposure prophylaxis. A preventive vaccine given as soon as possible after possible exposure can prevent or greatly reduce the disease. Immunoglobulin vaccination can be used as an alternative to post-exposure prophylaxis in cases where the smallpox vaccine is contraindicated (7, 8).

The study **AIMS** to explore the potential of *Mentha pulegium* L. compounds as inhibitors of the monkeypox virus methyltransferase VP39.

The specific objectives are:

1. To identify and characterize the bioactive compounds in *M. pulegium* using High-Performance Liquid Chromatography (HPLC);
2. To predict the drug-likeness and potential toxicity of these compounds to assess their suitability as antiviral agents;
3. To evaluate the binding affinity of the compounds to VP39 through molecular docking analysis;
4. To investigate the stability and interaction dynamics of the compounds with VP39 using molecular dynamics simulations. This approach aims to identify promising antiviral candidates and contribute to the development of new therapeutic strategies against monkeypox virus infections.

MATERIALS AND METHODS

Plant Extraction and Phytochemical Profiling

The dried aerial parts of *M. pulegium* were purchased from an herbal supplier (Jardin d'Épices, Saïda, Algeria) and authenticated by a botanist before use. Dried and powdered *M. pulegium* (10 g) was subjected to extraction by maceration in methanol (100 mL) for 48 hours. The extraction process involved renewing the solvent after 24 hours and employing magnetic stirring throughout. Following extraction, the macerate was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated to dryness using a rotary evaporator. The dry residue was then stored in hermetically sealed containers at 4°C until further analysis. For phytochemical profiling, the residue was dissolved in methanol and filtered through a 0.45 µm membrane filter. The filtrate was analyzed using a Thermo Scientific Dionex UltiMate 3000 HPLC system equipped with a UV/Visible multiwavelength detector. Separation was achieved on a Hypersil ODS C18 reversed-phase column (250 × 4.6 mm, 5 µm) using a mobile phase composed of acetonitrile and water with 0.2% sulfuric acid. The gradient elution program varied the percentages of solvents A (acetonitrile) and B (water) over 28 minutes. Data acquisition and processing were performed using Chromeleon™ 7.2 software (Thermo Fisher Scientific, USA), with the detector scanning the 200–400 nm range. Phenolic compounds were identified based on their retention times and UV spectra relative to analytical standards.

Selection of Potential Ligands

The selection of potential ligands for further computational analysis was based on the bioactive compounds identified from *M. pulegium* through HPLC. The three-dimensional (3D) structures of these compounds were retrieved in SDF format from the PubChem database[1] [9], which provides comprehensive molecular information necessary for accurate computational studies. Each compound's 3D structure

[1] PubChem. Available from: https://www.pubchem.ncbi.nlm.nih.gov/

was verified and energy-minimized using Open Babel software (version 3.1.1) before being prepared for molecular docking and dynamics simulations to assess their potential as inhibitors of the monkeypox virus methyltransferase VP39.

Drug-Likeness Prediction

The drug-likeness of the selected compounds was evaluated using the SwissADME[2] web tool. The compounds' SMILES notations were input into the database to analyze their physicochemical and pharmacokinetic characteristics. This assessment aimed to verify compliance with Lipinski's Rule of Five [10] and the Ghose filter [11]. Lipinski's Rule of Five sets criteria for drug-like molecules, including a maximum of 5 hydrogen bond donors, 10 hydrogen bond acceptors, a molecular weight (MW) below 500, and an octanol-water partition coefficient (log P) of 5 or less (or MlogP > 4.15). The Ghose filter, which extends these guidelines, considers additional parameters such as a Log P between –0.4 and +5.6, a molar refractivity ranging from 40 to 130, a molecular weight between 180 and 480, and a total atom count between 20 and 70 (including hydrogen bond donors and acceptors).

Toxicity Risk Assessment

The potential toxicity of the natural compounds was predicted using computational methods such as the OSIRIS Property Explorer tool [12]. This tool specifically examined the natural compounds for mutagenic (MUT), tumorigenic (TUM), irritant (IRR), and reproductive effect (RE) toxicity. The OSIRIS Property Explorer validates chemical structures and rapidly calculates various drug-related properties. These predictions are recorded and color-coded, with red signifying high-risk properties, while green indicates properties suitable for drug use [13]. Additionally, the ProTox 3.0 Tool was used to predict the median lethal dose (LD_{50}) in mg/kg for each compound [14].

Selection of Target Protein and Molecular Docking

Phytochemicals that complied with Lipinski's Rule of Five (with a maximum of one allowable violation) and met the Ghose filter criteria (with no violations) were selected for molecular docking studies, provided they also demonstrated favorable drug-like properties. The target protein for this study was methyltransferase VP39, a critical enzyme involved in the replication and pathogenicity of the monkeypox virus (15). The crystal structure of the enzyme (PDB: 8B07) was sourced from the RCSB Protein Data Bank[3] (Fig. 1). Enzyme preparation was carried out using Molegro Virtual Docker 2.5 software, and molecular docking simulations were performed with PyRx-Python 0.8 (16). The docking results were visualized using BIOVIA.

Molecular Dynamics simulations

Normal Mode Analysis (NMA)

The protein structure was analyzed using iMODS to explore its conformational dynamics through Normal Mode Analysis (NMA) and morphing simulations [17]. This provided insights into the protein's flexibility by simulating movements along normal modes and visualizing transitions between states. Rigid cluster decomposition, color-coded with the largest cluster in blue, and animations of trajectory motions were generated. Additionally, deformability and B-factor data assessed conformational changes and flexibility, highlighting regions of high deformability and potential chain 'hinges.'

Protein Structure Simulation Using NMSIM

The protein structure corresponding to PDB ID 8B07 was prepared for Normal Mode Simulation (NMSIM) using the NMSIM server [18]. The structure was initially obtained from the Protein Data Bank (PDB) and processed with specific parameters: a hydrogen bond cutoff of -1 kcal/mol, a hydrophobic cutoff distance of 0.35 Å calculated using method 3, and a Radius of Gyration (ROG) guided motion simulation. The Rigid Cluster Normal-Mode Analysis (RCNMA) method was used with a 10 Å cutoff for C-alpha atoms, considering normal modes from 1 to 50, and ROG mode set to 1. NMSIM simulations were conducted to explore the protein's conformational flexibility. The simulations involved generating 1 trajectory over 1 NMSIM cycle, applying a side-chain distortion factor of 0.3, running 500 simulation cycles with an output frequency of 1 per cycle, and using a step size of 0.5. Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) data were generated to assess the protein's conformational changes and flexibility.

RESULTS AND DISCUSSION

HPLC Phenolic Compounds Identification

Table 1 displays the HPLC analysis of *Mentha pulegium*'s methanolic extract, revealing a profile rich in bioactive phenolic compounds. Rosmarinic acid was

[2] SwissADME. Available from: http://www.swissadme.ch/index.php

[3] RCSB Protein Data Bank. Available from: https://www.rcsb.org/

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identified as the predominant constituent (11.30 %), followed by luteolin (5.97 %) and ferulic acid (5.47 %). Other notable compounds included *p*-coumaric acid (4.01 %), rutin (2.80 %), vanillic acid (2.76 %), apigenin (2.47 %), quercetin (1.62 %), and caffeic acid (1.63 %). This profile is largely consistent with, but quantitatively distinct from, previous reports on *M. pulegium*. For instance, our finding of rosmarinic acid as the major compound aligns with A.M. Al-Rajhi et al. [20], who also reported it as a dominant component. However, the specific percentages vary, which can be attributed to differences in plant origin, harvest time, and extraction methodologies. Furthermore, our study identified a broader spectrum of flavonoids, including luteolin and apigenin, at quantifiable levels, complementing the findings of N.K. Alharbi et al. [19] and adding depth to the known phytochemical repertoire of this species. The collective presence of these compounds is significant, as they are well-documented in the literature for their potent antioxidant, anti-inflammatory, and antiviral activities [19, 20]. The high concentration of rosmarinic acid, in particular, is noteworthy due to its recognized efficacy against viral infections, suggesting it may be a key contributor to the potential therapeutic properties of this extract.

Results of Drug-Likeness Prediction

The results of drug-likeness predictions are presented in Table 2.

Results of toxicity risk assessment

The results presented in Table 3 highlight the predicted toxicity risks and LD_{50} values of various natural compounds, evaluated using the OSIRIS Property Explorer and ProTox 3.0 tools. Compounds such as caffeic acid, ferulic acid, sinapic acid, chlorogenic acid, rutin, and luteolin exhibit low toxicity risk across all categories, as indicated by green color-coding in mutagenicity, tumorigenicity, irritancy, and reproductive effects. These compounds also demonstrate high LD_{50} values, with chlorogenic acid, rutin, and rosmarinic acid showing an LD_{50} of 5000 mg/kg, indicating their low acute toxicity and favorable safety profiles.

In contrast, certain compounds raise potential safety concerns. Quercetin, for instance, presents a notable risk with red color coding for both mutagenicity and tumorigenicity, coupled with a relatively low LD_{50} of 159 mg/kg. This suggests that quercetin, despite its therapeutic potential, may pose significant toxicity risks, necessitating careful consideration in drug development. This finding aligns with previous studies that have identified quercetin as a compound with both therapeutic and toxicological profiles, warranting careful consideration in its use as a therapeutic agent [21, 22]. Vanillic acid, although largely non-toxic, shows a mutagenic risk, similar to the findings for apigenin, which, despite being generally safe, also exhibits a moderate LD_{50} of 1000 mg/kg, underscoring the need for dose management.

The presence of reproductive effect risks in compounds like 3,4,5-trimethoxycinnamic acid and p-coumaric acid, despite their otherwise low toxicity, highlights the complexity of evaluating natural compounds for therapeutic use.

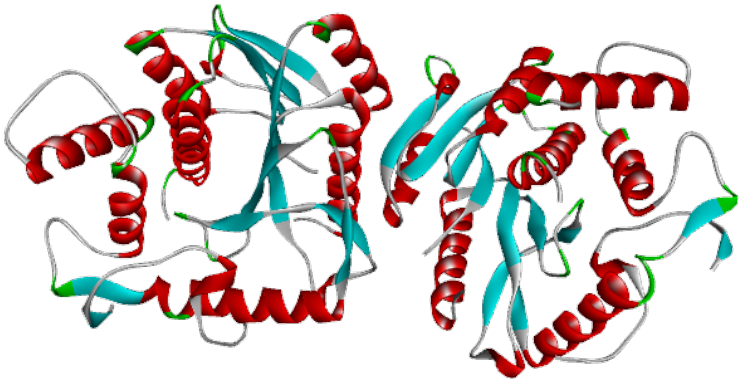

Figure 1 — 3D Structure of the Target enzyme methyltransferase VP39 (PDB ID: 8B07).

Table 1 — Phytochemical profiling of the methanolic extract of *M. pulegium* using HPLC analysis

Retention Time (min)	Lambda max (nm)	Compounds	%
22.383	325 / 284	Caffeic acid	1.628
23.136	223 / 283	Ferulic acid	5.470
25.606	283	Vanillic acid	2.764
25.862	223 / 320	Sinapic acid	1.828
27.407	345 / 265 / 312	Chlorogenic acid	2.599
28.802	331	Quercetin	1.622
29.505	223	Unidentified	1.093
30.539	223 / 326 / 313	Rosmarinic acid	11.300
33.675	221 / 344	3,4,5-Trimethoxycinnamic acid	1.344
34.315	261 / 343 / 353	Rutin	2.800
35.272	303 / 223 / 282 / 256 / 353	P-coumaric acid	4.010
36.837	342 / 291	Luteolin	5.967
37.815	226 / 302	Apigenin	2.468

Table 2 – Drug-likeness prediction of selected plant compounds using SwissADME

Ligands	Lipinski rule	Ghose filter
Caffeic acid	Yes; 0 violation	Yes
Ferulic acid	Yes; 0 violation	Yes
Vanillic acid	Yes; 0 violation	Yes
Sinapic acid	Yes; 0 violation	Yes
Chlorogenic acid	Yes; 1 violation: NHorOH>5	No; 1 violation: WLogP<-0.4
Quercetin	Yes; 0 violation	Yes
Rosmarinic acid	Yes; 0 violation	Yes
3,4,5-Trimethoxycinnamic acid	Yes; 0 violation	Yes
Rutin	No; 3 violations: MW>500, NorO>10, NHorOH>5	No; 4 violations: MW>480, WLogP<-0.4, MR>130, #atoms>70
P-coumaric acid	Yes; 0 violation	Yes
Luteolin	Yes; 0 violation	Yes
Apigenin	Yes; 0 violation	Yes

Note: MW — Molecular Weight; MR — Molar Refractivity; NH or OH — Number of Hydrogen Bond Donors; WLogP — Wildman–Crippen LogP.

Table 3 – Toxicity risk predicted and LD_{50} by OSIRIS property explorer and ProTox 3.0.

Ligand	MUT	TUM	IRR	RE	LD50 (mg/kg)
Caffeic acid	green	green	green	green	2980
Ferulic acid	green	green	green	green	1772
Vanillic acid	Red	green	green	green	2000
Sinapic acid	green	green	green	green	1772
Chlorogenic acid	green	green	green	green	5000
Quercetin	Red	Red	green	green	159
Rosmarinic acid	green	green	green	green	5000
3,4,5-Trimethoxycinnamic acid	green	green	green	Red	300
Rutin	green	green	green	green	5000
P-coumaric acid	green	green	green	Red	2850
Luteolin	green	green	green	green	2000
Apigenin	Red	green	green	green	1000

Note: MUT — mutagenic effect; TUM — tumorigenic effect; IRR — irritant effect; RE — reproductive effect; LD_{50} — median lethal dose.

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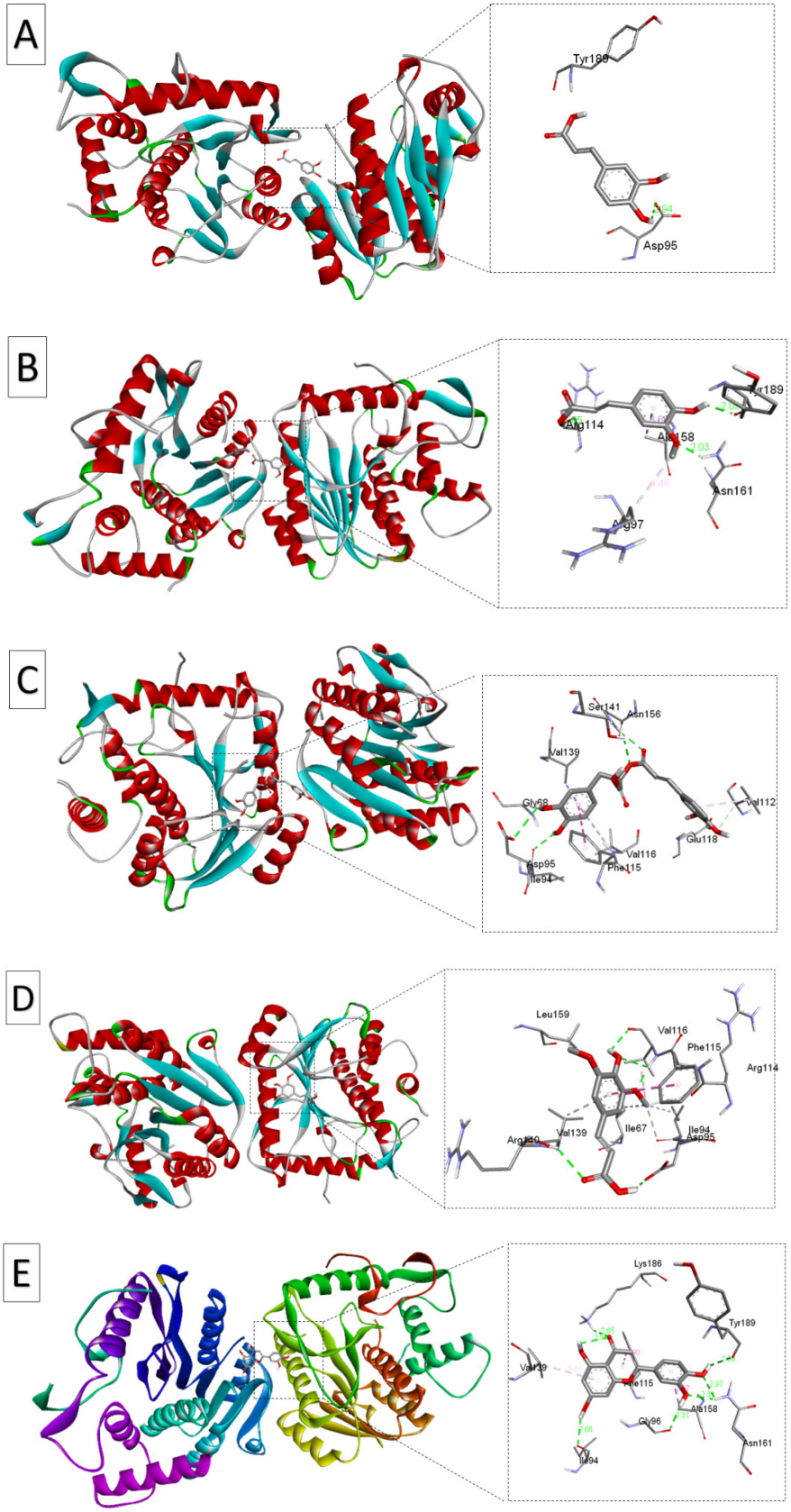

Figure 2 — Ribbon Views and Active Site Interactions of methyltransferase VP39-Ligands Complexes.
Note: Ribbon views (left) and active site details (left box) highlighting enzyme-ligand interactions (right box).
(A) Enzyme-Caffeic acid Complex, (B) Enzyme — Ferulic acid Complex, (C) Enzyme–Sinapic acid Complex,
(D) Enzyme-Rosmarinic acid Complex, and (E) Enzyme Luteolin complex.

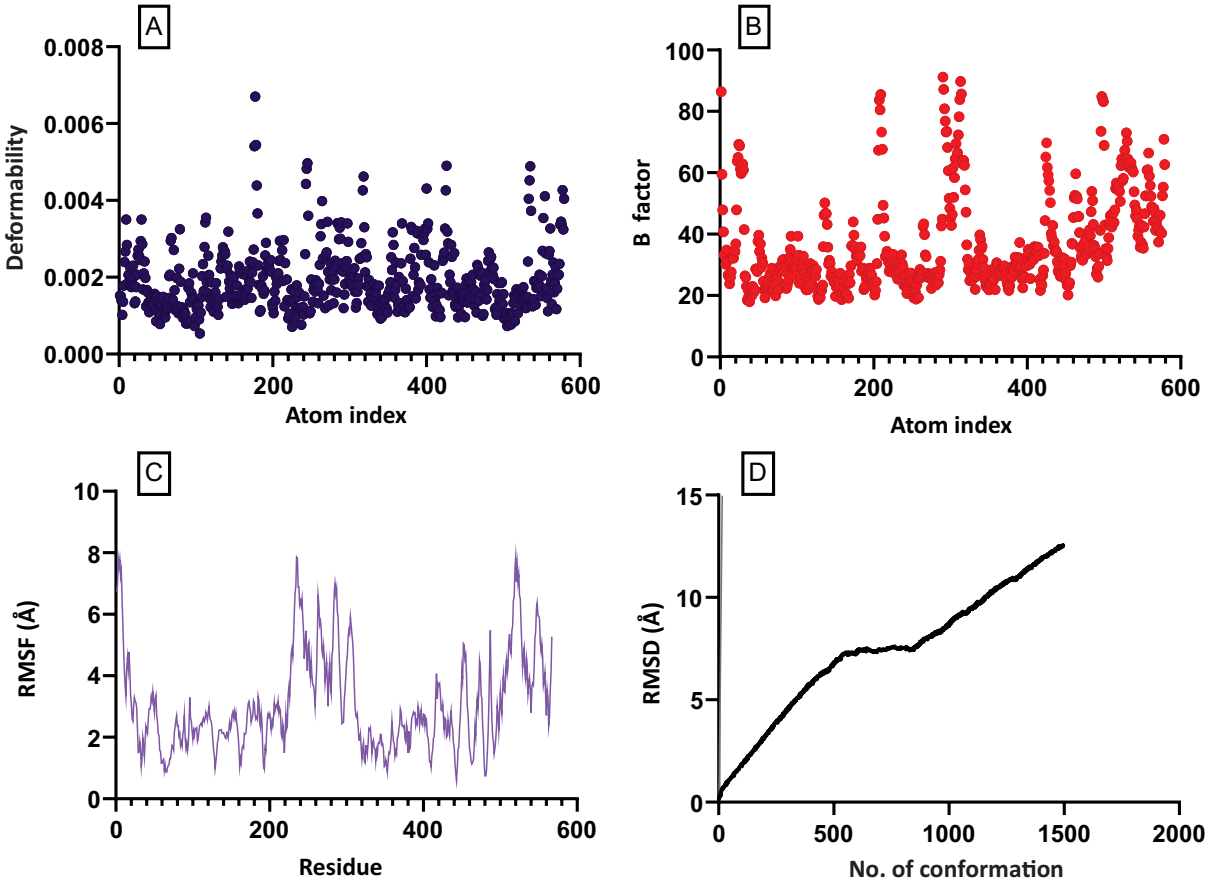

Figure 3 — Molecular Dynamics Analysis of Methyltransferase VP39.
Note: A — Deformability Plot, (B) B-factor Analysis, (C) RMSF Profile, (D) RMSD Trajectory.

Table 4 – The binding energies of the identified molecules and their molecular interactions with the active site of the target protein

Ligand	PubChem ID	Chemical Formula	Molecular Weight (g/mol)	Estimated Free Energy of Binding (kcal/mol)	Residue Interactions
Caffeic acid	689043	$C_9H_8O_4$	180.16	–6.5	Ala A:158, Tyr A:189
Ferulic acid	445858	$C_{10}H_{10}O_4$	194.18	–6.6	Arg B:97, Arg A:114, Asn A:161, Tyr A:189, Ala A:158
Sinapic acid	637775	$C_{11}H_{12}O_5$	224.21	–6.4	Ile A:67, Ile A:94, Asp A:95, Arg A:114, Phe A:115, Val A:116, Val A:139, Arg A:140, Leu A:159
Rosmarinic acid	5281792	$C_{18}H_{16}O_8$	360.31	–8.7	Gly A:68, Ile A:94, Asp A:95, Val B:112, Phe A:115, Val A:141, Asn A:156
Luteolin	5280445	$C_{15}H_{10}O_6$	286.24	–9.3	Ile B:94, Gly B:96, Phe B:115, Val B:139, Ala A:158, Lys A:186, Tyr A:189

Molecular docking results

Table 4 presents the results of molecular docking, highlighting the binding energies of various active compounds. The findings show that luteolin and rosmarinic acid exhibit the highest binding affinities to the target enzyme, with binding energies of –9.3 kcal/mol and –8.7 kcal/mol, respectively. The analysis of molecular interactions reveals that luteolin forms hydrogen bonds with residues such as Ile 94, Gly 96, and Phe 115, and interacts with Val 139, Ala 158, Lys 186, and Tyr 189 through additional hydrophobic interactions. Rosmarinic acid interacts with Gly 68, Ile 94, Asp 95, Val 112, Phe 115, Val 141, and Asn 156 via both hydrogen bonding and hydrophobic interactions. These compounds are followed by ferulic acid (–6.6 kcal/mol) and caffeic acid (–6.5 kcal/mol), which also demonstrate strong binding interactions with residues such as Arg 97,

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Arg 114, Asn 161, Ala 158, and Tyr 189 for ferulic acid, and Ala 158 and Tyr 189 for caffeic acid. These results are consistent with other research highlighting the antiviral potential of phenolic compounds, which often act by disrupting viral enzyme activity or interfering with the viral replication process [23, 24]. Fig. 2, created using the BIOVIA Discovery Studio Visualizer, visually represents the docking of these ligands at the enzyme's active site, including a ribbon view that illustrates the binding pocket and the visualization of the interactions between the ligands and the enzyme's amino acid residues.

Molecular dynamic results

The MD simulation results provide a comprehensive analysis of the protein-ligand complex's dynamics and stability, as illustrated in the associated figures. The deformability plot (Fig. 3A) and B-factor analysis (Fig. 3B) highlight regions of varying flexibility within the protein structure. Low deformability and B-factor regions (indices 1-10, 35-60, 120-140) indicate stable areas crucial for ligand binding, while high deformability peaks and B-factors (indices 7-10, 29-30, 67-79, 243-246) suggest flexible regions that may influence binding stability. Strong-binding ligands, like luteolin and rosmarinic acid, interact with moderately flexible regions, enhancing stable binding, while weaker-binding ligands, such as ferulic and caffeic acids, bind to more stable but less dynamic regions. The RMSF analysis (Fig. 3C) reveals that different regions of the protein exhibit varying degrees of flexibility. The initial and terminal residues show high flexibility, likely corresponding to loops or termini, while the core region (residues 31–80) remains stable, indicating a structured core. The central and terminal flexible regions suggest areas involved in ligand binding or conformational changes. Finally, the RMSD plot (Fig. 3D) demonstrates the overall structural stability of the complex, with initial fluctuations stabilizing around 3.91 Å after 5000 ps, indicating that the system has reached equilibrium. This consistent stability throughout the simulation suggests that the protein maintained a stable conformation, with no major structural disruptions, underscoring its robustness.

CONCLUSION

The study successfully identified and characterized bioactive compounds from *M. pulegium* with potential inhibitory effects on the monkeypox virus methyltransferase VP39. The combination of HPLC profiling, drug-likeness evaluation, and toxicity risk assessment provided a comprehensive understanding of the suitability of these compounds as antiviral agents. Molecular docking analysis revealed that luteolin and rosmarinic acid demonstrate the highest binding affinities and favorable interactions with VP39, while molecular dynamics simulations confirmed their stable binding. These findings suggest that *M. pulegium* compounds, particularly luteolin and rosmarinic acid, hold promise as effective inhibitors of VP39 and potential candidates for further antiviral drug development. Future research should focus on validating these results in biological systems and exploring their therapeutic potential *in vivo*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Farouk Boudou — conceptualization, investigation, formal analysis, writing—original draft, writing—review & editing; Amal Belakredar — conceptualization, investigation, visualization, writing—original draft, writing—review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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AUTHORS

Farouk Boudou — Doctor of Philosophy, Department of Applied Molecular Genetics, Faculty of Natural and Life Sciences, University of Science and Technology of Oran (Algeria). ORCID ID: 0000-0002-6008-3325. E-mail: farouk.boudou@univ-usto.dz

Amal Belakredar — Doctor of Biotechnology, Assistant Professor, Department of Biotechnology, Faculty of Natural Sciences and Life, University of Mostaganem (Algeria). ORCID ID: 0000-0003-0997-690X. E-mail: belakredar-amel@hotmail.fr