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Research Article/Özgün Araştırma

Computational analysis of eupatorin binding to human neutrophil elastase: Therapeutic potential in inflammatory foot diseases

Eupatorin'in insan nötrofil elastazına bağlanmasının hesaplamalı incelenmesi: İnflamatuar ayak hastalıklarında terapötik potansiyeli

Mehmet Veysi ÇETİZ¹ , Şükrü AKMEŞE¹ , Ramazan TUTUŞ²

¹Harran University, Faculty of Medicine, Department of Medical Biochemistry, 63290, Şanlıurfa-Türkiye

²Uşak University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, 64200, Uşak-Türkiye

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Abstract

Aim: This study aims to investigate the inhibitory effect of eupatorin, a flavonoid, on human neutrophil elastase (HNE), which plays a role in chronic inflammatory foot conditions such as diabetic foot ulcers.

Materials and Methods: Binding interactions between eupatorin and HNE were examined using molecular docking simulations performed in AutoDock Vina. Next, 100-nanosecond molecular dynamics simulations and MM/GBSA binding free energy analyses were performed.

Results: Docking analysis showed that eupatorin binds to HNE with a binding affinity of -7.9 kcal/mol. Dynamic simulations revealed that the ligand retained its position within the binding site and exhibited a non-covalent interaction pattern involving 0-3 hydrogen bonds per frame, with an average of one. SASA values indicated that the ligand resides within a solvent-accessible pocket. MM/GBSA analysis further supported favorable binding energetics throughout the simulation period.

Conclusion: These findings suggest that eupatorin establishes a reversible, non-covalent interaction pattern with HNE. Eupatorin can be considered a promising natural scaffold that warrants experimental evaluation for the modulation of HNE-associated inflammatory processes.

Keywords: Diabetic foot; Docking; Eupatorin; Human neutrophil elastase; Molecular dynamics.

Öz

Amaç: Bu çalışma, bir flavonoid olan eupatorin'in, diyabetik ayak ülseri gibi kronik inflammatuar ayak hastalıklarında görev alan İnsan nötrofil elastazı (HNE) üzerindeki inhibitör etkisini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Eupatorin'in HNE ile olası bağlanma etkileşimleri, AutoDock Vina ile yürütülen moleküler yerleştirme simülasyonları ile incelenmiştir. Ardından, 100 nanosaniyelik moleküler dinamik simülasyonlar ve MM/GBSA bağlanma serbest enerjisi analizleri gerçekleştirilmiştir.

Bulgular: Moleküler yerleştirme analizleri eupatorin'in HNE'ye -7,9 kcal/mol bağlanma afinitesi ile bağlandığını göstermiştir. Dinamik simülasyonlar, ligandın konumunu bağlanma cebinde koruduğunu ve 0-3 arasında değişen, ortalama 1 hidrojen bağı içeren bir etkileşim örüntüsü sergilediğini ortaya koymuştur. SASA değerleri, ligandın çözücüye açık, yarı gömülü bir cepte yer aldığını göstermektedir. MM/GBSA analizleri ise bağlanmanın enerjetik olarak elverişli olduğunu desteklemiştir.

Sonuç: Bu bulgular, eupatorin'in HNE ile tersinir ve kovalent olmayan bir etkileşim örüntüsü kurabileceğini düşündürmektedir. Eupatorin, HNE ile ilişkili inflammatuar süreçlerin modülasyonu için deneysel olarak değerlendirilmesi gereken umut vadeden doğal bir yapı iskelesi olarak değerlendirilebilir.

Anahtar Kelimeler: Diyabetik ayak; Docking; Eupatorin; İnsan nötrofil elastazı; Moleküler dinamik.

Yazışma Adresi/Address for Correspondence: Ramazan TUTUŞ, Uşak University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, 64200, Uşak -Türkiye, E-mail: ramazan.tutus@usak.edu.tr

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Introduction

Chronic wounds of the lower extremities, particularly those affecting the foot, such as diabetic foot ulcers (DFUs), remain a major cause of morbidity and disability among people with diabetes worldwide. These lesions are characterized by prolonged inflammation, extracellular matrix (ECM) degradation, and impaired tissue repair. This often leads to delayed healing and, in severe cases, amputation of the limb.^{1,2} One central molecular contributor to this pathological environment is human neutrophil elastase (HNE), a serine protease released by activated neutrophils. Excessive elastase activity degrades ECM components, inactivates growth factors, and perpetuates inflammatory cycles within non-healing wounds.³

Therefore, pharmacological inhibition of HNE is considered a promising therapeutic approach to enhance wound healing and restore tissue integrity in diabetic foot ulcers and other chronic foot-related inflammatory conditions.^{4,5} Although synthetic elastase inhibitors exist, there is a growing interest in finding naturally derived, bioavailable, and low-toxicity alternatives. Eupatorin, a naturally occurring polymethoxyflavone found in several medicinal plants, including *Orthosiphon stamineus*, *Salvia connivens*, and *Artemisia anomala*, has attracted considerable attention because of its antioxidant, anti-inflammatory, and anticancer activities. Recent studies have shown that eupatorin can modulate key inflammatory mediators and signaling pathways associated with inflammatory responses.¹⁵ However, its interaction with HNE and potential as an elastase inhibitor have not yet been investigated at the molecular level.^{6,7}

This study employs molecular docking, 100-ns molecular dynamics (MD) simulations, and MM/GBSA-based binding free energy analysis to investigate the interaction of eupatorin with HNE. These complementary *in silico* approaches provide valuable information regarding binding affinity, interaction stability, conformational dynamics, and energetic determinants of ligand–protein recognition under near-physiological conditions. By characterizing the molecular basis of the

Eupatorin–HNE interaction, this work aims to provide preliminary insights into the potential of eupatorin as a natural modulator of elastase-associated inflammatory processes relevant to chronic foot disorders.

Materials and Methods

To evaluate the binding potential of eupatorin toward HNE, molecular docking simulations were first conducted. The crystal structure of HNE (PDB ID: 1HNE, resolution 1.84 Å) was obtained from the RCSB Protein Data Bank. The 3D conformation of eupatorin was sourced from PubChem and subsequently optimized in Avogadro software, where Gasteiger charges were applied. Preparation of the protein, including the assignment of partial atomic charges and hydrogen atoms, was executed using AutoDockTools v4.2.6 and BIOVIA Discovery Studio. AutoDock Vina v1.1.2 was employed for docking runs, using an exhaustiveness parameter of 32 to ensure adequate conformational sampling. The docking grid was centered at X = 35.958, Y = 15.029, and Z = 53.690, with dimensions of 40 × 40 × 40 Å, covering the binding pocket occupied by the co-crystallized inhibitor. Validation of the docking protocol was achieved by re-docking the co-crystallized inhibitor and comparing the root-mean-square deviation (RMSD) between experimental and predicted poses.^{8,9} Protein–ligand interactions were profiled using PLIP, while molecular graphics and binding site representations were created using PyMOL v2.5.8.^{10,11}

Following the docking analysis, molecular dynamics (MD) simulations were conducted to evaluate the conformational behavior and dynamic stability of the Eupatorin–HNE complex. The system preparation steps—which include complex assembly, solvation, ion addition, and force field parameterization—were performed using the CHARMM-GUI interface.^{12,13} Protein structures were parameterized with the CHARMM36m force field, while the ligand topologies were generated using CGenFF.^{12,13,14} Each complex was placed in a periodic cubic box filled with explicit TIP3P water molecules, maintaining a minimum 10 Å distance from the solute to the box boundary. Sodium and chloride ions were added to

neutralize the system and mimic physiological ionic strength (0.15 M). Energy minimization was carried out using the steepest descent algorithm until the system reached a maximum force threshold below $1000 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-1}$. Equilibration was conducted in two phases: 500 ps under NVT conditions, followed by 1 ns under the NPT ensemble at 310 K, applying LINCS constraints to all bonds. Electrostatic interactions were treated using the Particle Mesh Ewald (PME) method with a 12 Å cutoff, and short-range non-bonded interactions were calculated using the Verlet scheme. Production MD simulations were finally run for 100 ns using GROMACS v2023.3.

To further evaluate the energetic characteristics of ligand binding, MM/GBSA calculations were performed using snapshots extracted from the 100-ns MD trajectory. Binding free energy profiles were calculated throughout the simulation period to assess the energetic stability of the Eupatorin–HNE complex. In addition, per-residue energy decomposition analysis was conducted to identify amino acid residues contributing to ligand stabilization and to characterize residue-specific energetic contributions to binding. The total binding free energy was estimated by combining molecular mechanics energy terms with polar and non-polar solvation contributions.

Results

Molecular docking

To evaluate the potential inhibitory interactions of eupatorin (Figure 1), a naturally occurring methoxylated flavonoid with reported anti-inflammatory activity, molecular docking simulations were carried out targeting HNE, a serine protease implicated in the pathogenesis of chronic inflammatory foot disorders. Validation of the docking protocol was achieved by re-docking the co-crystallized inhibitor and comparing the root-mean-square deviation (RMSD) between the predicted and experimental poses. The resulting RMSD value of 0.01 demonstrated excellent agreement between the experimental and predicted binding poses, supporting the suitability of the docking protocol for subsequent analyses.

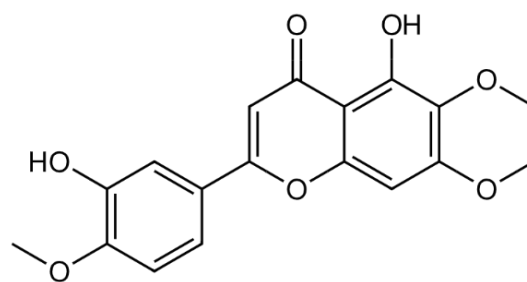


Figure 1. 2D Structure of eupatorin.

This targeting strategy ensured that docking was constrained to the functionally validated pharmacophore region of HNE, thereby enhancing biological relevance and mechanistic interpretability. Eupatorin exhibited a best binding affinity of $-7.9 \text{ kcal}\cdot\text{mol}^{-1}$, suggesting a favorable and moderately strong non-covalent interaction with the protein.

The top-ranked pose revealed a well-defined interaction network involving both polar and non-polar residues. Notably, hydrogen bonds were observed with LEU130, SER166, and GLN233, with dual polar contacts formed at both LEU130 and SER166, indicative of stable anchoring interactions along the substrate-binding channel. In addition, hydrophobic contacts were identified with ARG129 and ARG178, located on the periphery of the ligand pocket, which likely contribute to complex stabilization via van der Waals interactions (Figure 2).

Taken together, these findings suggest that eupatorin engages the HNE active site through a spatially coordinated, non-covalent binding mode that mimics the occupancy of known inhibitory scaffolds. The observed binding mode is consistent with a potential reversible, non-covalent interaction with the HNE binding region, warranting further enzymatic validation.

Molecular dynamics simulations

A 100-nanosecond molecular dynamics simulation was performed to evaluate the dynamic behavior of the Eupatorin–HNE complex over an extended timescale. The ligand's RMSD showed moderate fluctuations during the simulation, indicating continued association with the binding region despite conformational adjustments over time (Figure 3A). Residue-level fluctuations of HNE, as

measured by RMSF, revealed localized mobility in several solvent-exposed regions while preserving the overall structural framework of the enzyme (Figure 3B). This finding suggests that ligand binding did not substantially perturb the structural integrity of the enzyme. The hydrogen-bond profile exhibited a moderate and somewhat transient interaction pattern, with typically 0–3 hydrogen bonds observed throughout the trajectory (Figure 3E). Concurrently, the solvent-accessible surface area (SASA) of eupatorin exhibited modest fluctuations

throughout the simulation period (Figure 3C). In addition, the minimum distance between eupatorin and HNE remained relatively constant during the simulation, supporting persistent ligand retention within the binding region (Figure 3D). Collectively, the RMSD, RMSF, SASA, hydrogen-bond, and minimum-distance analyses indicate sustained association of eupatorin with HNE throughout the 100-ns simulation period while highlighting the dynamic nature of the interaction.

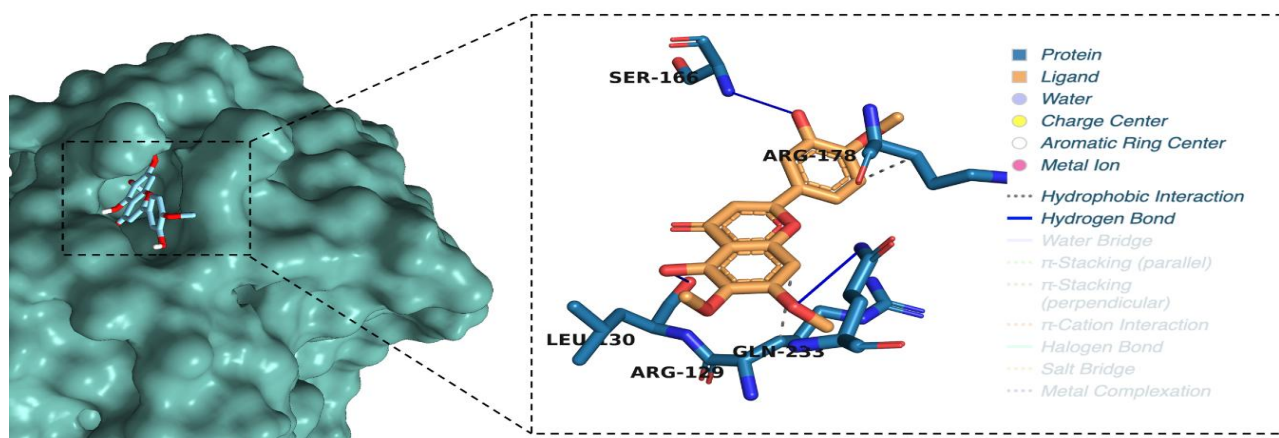


Figure 2. Binding pose of eupatorin in the human neutrophil elastase.

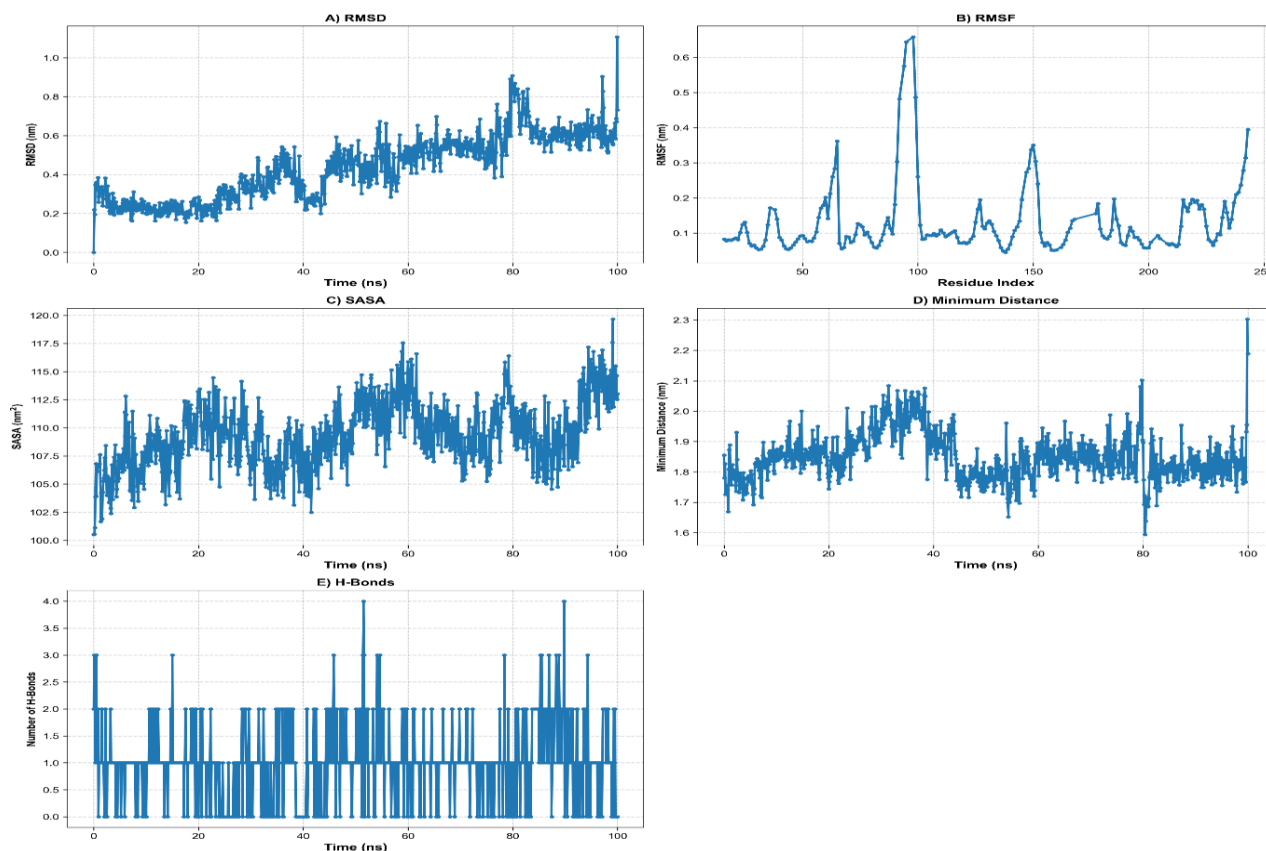


Figure 3. Structural stability and interaction dynamics of the Eupatorin–HNE complex during a 100-nanosecond molecular dynamics simulation. **A)** RMSD of eupatorin. **B)** RMSF of HNE residues. **C)** SASA of eupatorin. **D)** Minimum distance between eupatorin and HNE. **E)** Hydrogen bond count between eupatorin and HNE over time.

MM/GBSA binding free energy analysis

To further characterize the energetic basis of the Eupatorin–HNE interaction, MM/GBSA calculations were performed using snapshots extracted from the 100-ns molecular dynamics trajectory. The binding free energy profile remained consistently negative throughout the simulation, indicating a favorable interaction between eupatorin and HNE (Figure 4A). Although moderate fluctuations were observed during the trajectory, the moving-average trend suggested sustained energetic stability of the complex over time.

Per-residue energy decomposition analysis identified several amino acid residues as important contributors to ligand binding, including ARG129, LEU130, ARG178, SER179, VAL181, and PRO230 (Figure 4B). Notably, ARG129, LEU130, and ARG178 were also involved in the docking interaction profile, supporting the persistence of key protein–ligand contacts during the simulation. These findings indicate that eupatorin binding is maintained through favorable residue-specific energetic contributions and dynamic non-covalent interactions throughout the simulation period.

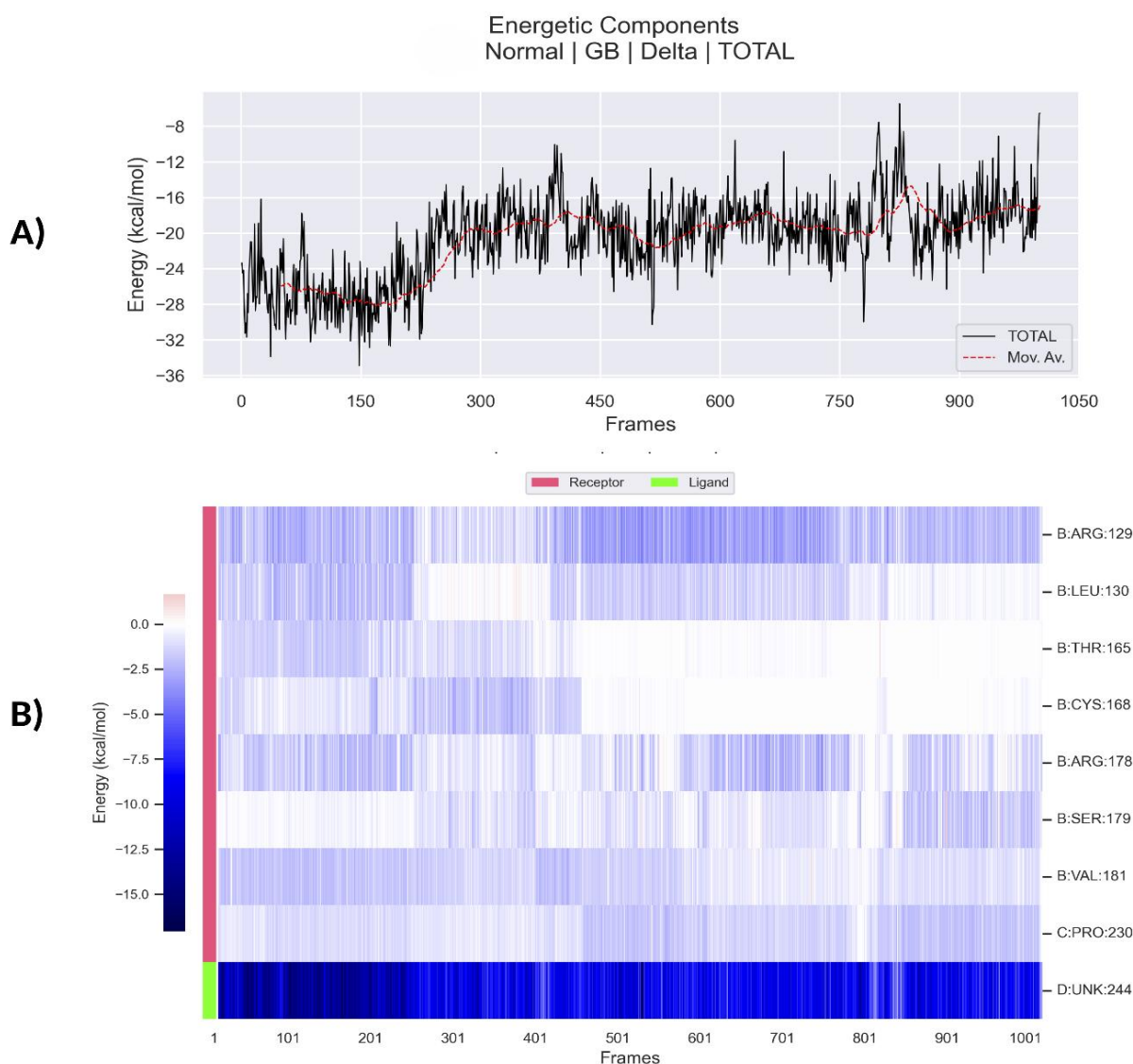


Figure 4. MM/GBSA-based energetic analysis of the Eupatorin–HNE complex during the 100-ns molecular dynamics simulation. A) Temporal profile of binding free energy (ΔG_{bind}). B) Per-residue energy decomposition analysis of key amino acid residues contributing to ligand stabilization.

Discussion

This study provides initial molecular-level insight into the interaction between eupatorin and human neutrophil elastase (HNE), a protease implicated in chronic inflammatory tissue damage and impaired wound healing. Molecular docking demonstrated that eupatorin can occupy a functionally relevant binding region of HNE with a favorable binding affinity ($-7.9 \text{ kcal} \cdot \text{mol}^{-1}$) and establish multiple hydrogen-bonding and hydrophobic interactions involving residues such as ARG129, LEU130, SER166, ARG178, and GLN233. The observed interaction pattern suggests that eupatorin can engage the HNE binding region through reversible non-covalent interactions, supporting its potential as a naturally derived bioactive compound for further investigation.

The 100-ns molecular dynamics simulation further supported the persistence of the Eupatorin–HNE interaction. RMSD, RMSF, SASA, hydrogen-bond, and minimum-distance analyses collectively indicated that eupatorin remained associated with the binding region despite conformational fluctuations during the simulation. Furthermore, MM/GBSA analysis revealed consistently favorable binding free energies throughout the trajectory, while per-residue energy decomposition identified ARG129, LEU130, ARG178, SER179, VAL181, and PRO230 as important contributors to ligand stabilization. Notably, ARG129, LEU130, and ARG178 were also identified in the docking interaction profile, supporting the persistence of key protein–ligand contacts under dynamic conditions. Taken together, these findings support eupatorin as a candidate molecule for further investigation in the context of HNE modulation.

Conclusion

The present in silico investigation suggests that eupatorin can interact favorably with human neutrophil elastase through a combination of hydrogen-bonding, hydrophobic, and residue-specific energetic contributions. Molecular docking, 100-ns molecular dynamics simulations, and MM/GBSA analyses collectively indicated

that eupatorin maintained a persistent but dynamic association with the HNE binding region during the simulation period. These findings support eupatorin as a promising natural scaffold for further investigation in the context of HNE modulation. However, enzymatic inhibition assays, cellular studies, and additional experimental validation are required to confirm its biological activity and therapeutic relevance.

Ethical Committee Approval

In the current work, there was no animal or human experiments conducted.

Author Contributions

All authors contributed equally to the design of the study, the analysis of the data, and the writing of the manuscript. The results were jointly discussed, and the final version of the manuscript was approved by all authors.

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Conflict of Interest

The authors declare that there is no conflict of interest for this article.

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