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# Molecular Docking and Pharmacokinetic Profiling of Dimethyl ellagic acid from *Eucalyptus globulus* as a Broad-Spectrum Inhibitor of Microbial and Insecticidal Virulence Toxins

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## Abstract

**Background**: Antimicrobial resistance and toxin-mediated virulence are significant health issues of concern worldwide. **Aim**: This study set out to computationally refurbish Dimethyl ellagic acid as a pan-specific toxin protein inhibitor of bacteria, insects, arachnids, and marine organisms by evaluating binding affinity and ADMET. **Methods**: The ligand was minimized and optimized in PDBQT format with rotatable bonds and Gasteiger charges. The toxin structures were pre-processed by using four toxins: *Clostridium botulinum* (3BTA), *Apis mellifera* (1POC), *Agelenopsis aperta* (1OAV), and Conus magus (1OMG) to remove the water molecules, add the polar hydrogen atoms, and Kollman charges. AutoDock Vina (v1.2.0) under a rigid receptor flexible ligand (grid box: 20x20x20A; exhaustiveness:4) was used to carry out molecular docking. The poses with the highest ranking were chosen by binding energy and orientation. Prediction of ADMET properties was done through ADMET-AI. **Results**: *C. botulinum* toxin (−7.712 kcal/mol) was found to bind with the highest affinity, with the assistance of hydrogen bonding with Ser, Thr, and Asn, and hydrophobic interactions with Leu, Ile, Val, and Ala. The affinities observed between moderate were between *A. mellifera* (−5.163 kcal/mol) and A. aperta (−5.158 kcal/mol), and C. magus exhibited the lowest affinity (−4.424 kcal/mol). The ADMET analysis showed that the drug possessed good drug-like properties such as moderate molecular weight, balanced lipophilicity, good intestinal absorption, and low risk of carcinogenicity, but hepatotoxicity may need to be evaluated further. **Conclusion**: Dimethyl ellagic acid selectively binds well-defined toxin pockets, indicating its potential as a natural anti-virulence scaffold that needs to be experimentally confirmed.

**Keywords:** Dimethyl ellagic acid; *Eucalyptus globulus;* anti-virulence; toxin inhibition; molecular docking; AutoDock Vina.

## 1. Introduction

The recent development of antimicrobial resistance and the ongoing threat of toxin-mediated virulence of microbes and venomous organisms qualify as significant concerns to global human health and biosecurity. This is in contrast to the traditional antimicrobial approaches that seek to suppress microbial growth, which have been increasingly considered as an alternative means of therapy, focusing on virulence factors, especially those that are secreted in the form of toxins. Virulence toxins are essential in host colonization, evasion of the immune system, tissue damage, and severity of the disease, and their inhibition can reduce pathogenicity and not cause strong selection pressure to develop resistance [1,2].

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Examples of the most powerful biological toxins known are bacterial exotoxins, which include those of *Clostridium botulinum*, and cause severe neuromuscular paralysis and life-threatening clinical outcomes [3]. Equally, insect, arachnid, marine toxins, and venom-associated proteins, like Apis mellifera, Agelenopsis aperta, and Conus magus, have their biological action through very specific molecular interactions with ion channels, receptors, or enzyme targets [4-6]. Although the biological origin and physiological functionality of most of these toxins differ, most of them have conserved structural motifs and active-site characteristics that can be harnessed to find a wealth of potential common-spectrum inhibition.

Natural products are known to be an excellent source of bioactive compounds that possess a wide range of pharmacological properties. Eucalyptus globulus is a medicinal product with a strong potential for application in traditional and modern phytotherapy that is abundant in polyphenolic compounds, such as ellagic acid derivatives, flavonoids, and tannins, which were correlated with the antimicrobial and toxin-modulating activity [7,8]. Dimethyl ellagic acid is a methylated form of ellagic acid, and it is more lipophilic and more permeable to the membrane than its parent molecule, which might provide a better bioavailability and accessibility of the target [9]. The research in the past has already shown antimicrobial and enzyme-inhibitory properties of ellagic acid and its derivatives, but the actual mechanisms of their interaction with virulence-associated toxins have not been described in detail [10]. Particularly, extensive in silico studies that have combined both molecular docking with pharmacokinetic profiling are few.

Computational repurposing methods, especially molecular docking, offer a cost-effective and efficient mechanism of predicting ligand protein interactions and in the identification of promising inhibitors of a variety of biological targets [11,12]. Docking studies can provide insight into the mechanism of interference with toxin action by small molecules by assessing both the binding affinity, interaction geometry, and residue-level contacts, before experimental validation. These in silico analyses are particularly useful with structurally different toxin families, in which experimental screening can be hampered due to toxicity, availability, or ethical reasons.

In this regard, the current research investigated the use of Dimethyl ellagic acid in computing its potential as a broad-spectrum virulence-related toxin inhibitor of bacteria, insects, arachnids, and marine sources. The Dimethyl ellagic acid from *Eucalyptus globulus* was evaluated with the help of molecular docking simulations through the use of the protein structures of the selected toxins in *Clostridium botulinum*, Apis mellifera, Agelenopsis aperta, and Conus magus toxins. The aim of the study was a comparative binding affinity study, key interaction amino acid residues, and the architecture of the binding sites to clarify the structure-interaction relationships. The results suggest the viability of Dimethyl ellagic acid as a multitarget anti-virulence agent repurposed and add to the developing body of knowledge on the use of natural-product-based approaches to toxin inhibition.

## 2. Methodology

An integrated in silico workflow was used in this experiment to evaluate the potential of Dimethyl ellagic acid from *Eucalyptus globulus* as an inhibitor of microbial and insect-associated virulence toxins. Dimethyl ellagic acid was extracted out of PubChem database and optimized structurally before analysis. The virulence-associated proteins (3BTA, 1POC, 1OAV, and 1OMG) were put into docking using the normal protein processing protocols. The molecular docking was conducted in order to determine the binding affinity and interaction pattern of Dimethyl ellagic acid with each target. Docking poses were further studied with the help of BIOVIA Discovery Studio to find out essential molecular interactions. Lastly, ADMET profiling was carried out in order to forecast the pharmacokinetic behavior and safety properties of Dimethyl ellagic acid, as shown in Figure 1.

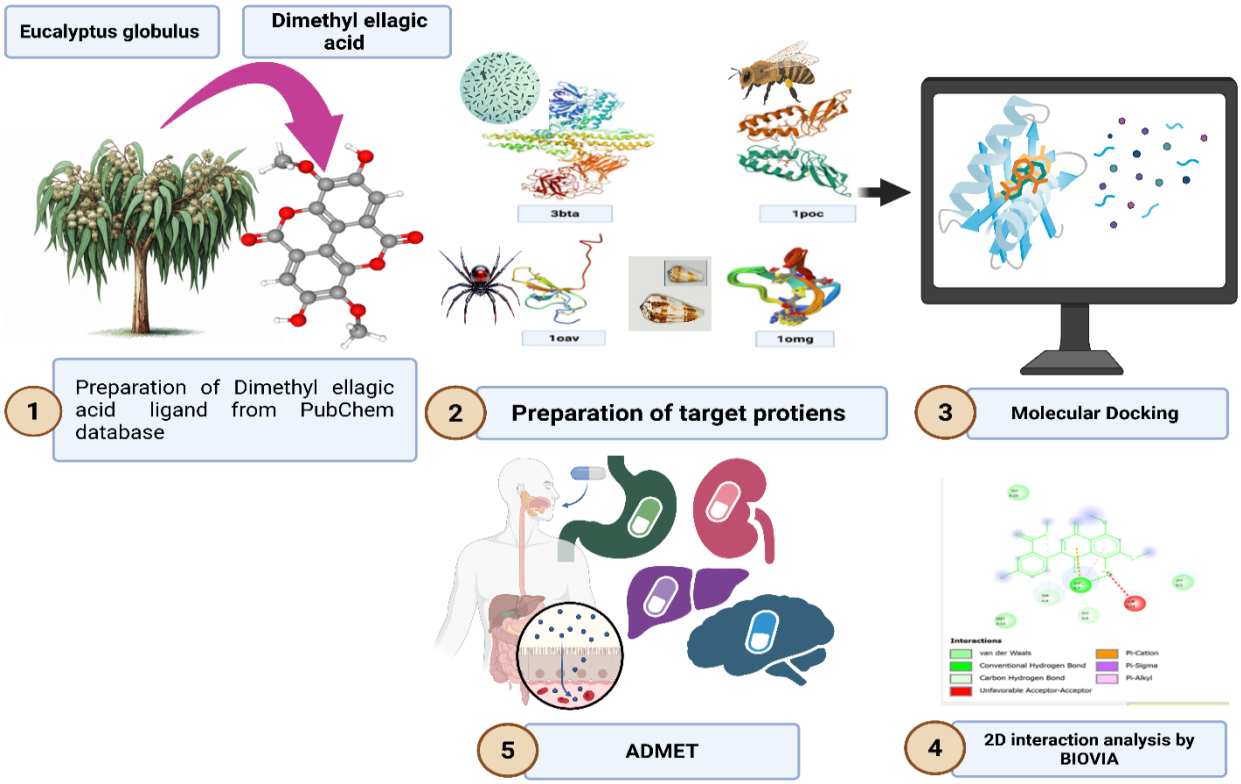


Figure 1: Workflow of this study. This figure was created by Biorender.

### 2.1. Ligand Preparation

The chemical structure of the ligand of interest, Dimethyl ellagic acid, PubChem CID: 5488919, (COC(=O) C1=C(C=CC(=C1) O) C2=CC(=O) C3=C(C2=O) C(=C(C3OC) OC) O was built and energy minimized before docking. AutoDock Tools was used to convert the optimized ligand structure to PDBQT format. In this step, the Gasteiger partial charges were designated, and all rotatable bonds were distinguished in order to permit flexible ligand conformation during docking.

### 2.2. Protein Preparation

The 3bta_modified.pdb, 1poc_modified.pdb, 2mlt modified.pdb, and 1omg modified.pdb target protein structures were subject to docking by eliminating crystallographic water molecules and any other co-crystallized ligands (Table 1 and Figure 2). At-oms polar hydrogen were added, and the protein structures were assigned Kollman charges. All the prepared proteins were then stored in PDBQT format to be used later in docking [7,11].

**Table** 1. Target protein structures (PDB IDs) and their corresponding source organisms used in the docking study.

| Protein Structure (PDB ID) | Organisms |
|---|---|
| 3bta_modified.pdb | *Clostridium botulinum* |
| 1poc_modified.pdb | *Apis mellifera* |
| 1oav_modified.pdb | ***Agelenopsis aperta*** |
| 1omg_modified.pdb | *Conus magus* |

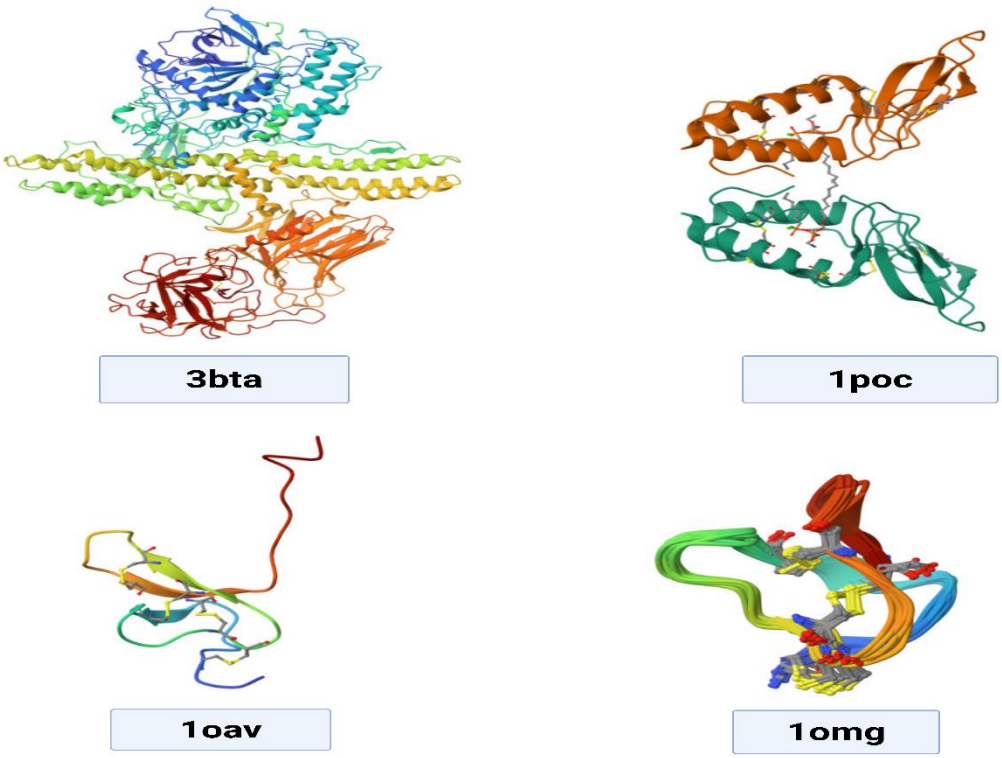


Figure 2: Chemical structure of target proteins.

### 2.3. Molecular Docking Protocol

The simulations of molecular docking were performed with the AutoDock Vina (1.2.0) software [14,15]. An inflexible strategy of receptor and flexible ligand docking was used for all targets. The grid boxes were defined to dock the active sites of each protein with a grid box size of 20 x 20 x 20 A. The grid box centers were individually placed on each target 3bta (38, -47, 56), 1poc (46, 29, 26), 1oav ( -1, -1, -5), and 1omg ( -1, 1, 0).

**Table 2.** Grid box parameters used for molecular docking of target proteins.

| Protein Target (PDB ID) | Grid Box Size (Å) | Grid Box Center (X, Y, Z) |
|---|---|---|
| 3bta | 20 × 20 × 20 | (38, −47, 56) |
| 1poc | 20 × 20 × 20 | (46, 29, 26) |
| 1oav | 20 × 20 × 20 | (−1, −1, −5) |
| 1omg | 20 × 20 × 20 | (−1, 1, 0) |

The parameter of exhaustiveness was 4 to get sufficient conformational sampling and a certain level of computational efficiency. In every protein-ligand system, a number of binding poses were obtained, and the docking product was ranked by the predicted binding affinities in kcal/mol.

### 2.4. Docking Analysis

The binding pose with the lowest predicted binding free energy (reliable predictive binding energy) and visual inspection of the binding orientation at the active site was chosen as the optimal binding pose in each case, according to the target protein. A comparative analysis was made to determine the trends in binding affinity of the various targets, and the docking poses ranked highest were taken to further interpretation and discussion.

### 2.5 Two-dimensional (2D) interaction between ligands and target proteins

Discovery Studio Visualizer was used to perform post-docking visualization, and BIOVIA Draw was used to perform analysis of interaction (BIOVIA, Dassault Systems; available at https://discover.3ds.com/discovery-studio-visualizer-download, accessed on 15 January 2026). The docked complexions were also studied to determine some important intermolecular interactions, such as hydrogen bonds, pi -pi stacking, and hydrophobic interactions between the ligand and the active site molecules [17].

### 2.6. ADMET-AI Analysis Procedure

The ADMET-AI web platform was used to predict the ADMET properties of Dimethyl ellagic acid. Canonical SMILES of Dimethyl ellagic acid were input directly to the ADMET-AI interface, and default prediction settings were used. The platform made physicochemical parameter, absorption, distribution, metabolism, excretion, and toxicity endpoint in silico predictions using validated machine-learning models. The predicted values were tabulated, and the percentile scores in DrugBank were tabulated to assess the pharmacokinetic behavior and safety profile of Dimethyl ellagic acid.

### 2.6 Ethics approval:

The Study is purely computational and does not entail human subjects, animals, or biological specimens; hence, ethical approval was not necessary.

## 3. Results

Table 3 and Figure 3 present the molecular interactions of Dimethyl ellagic acid and four toxin proteins as a summary of the binding affinity values and the interaction features of the residues and the interaction characteristics of the binding-sites. Clostridium botulinum toxin (3bta_modified.pdb) had the highest binding affinity (−7.712 kcal/mol), and this is reflected in the existence of an extended and deep pocket filled with polar residues (Ser, Thr, Asn) and hydrophobic residues (Leu, Ile, Val, Ala). The combination allows stable ligand anchoring, as well as effective pocket enclosure.

As compared to this, the toxins of Apis mellifera (1poc_modified.pdb) and Agelenopsis aperta (1oav_modified.pdb) exhibited intermediate and almost similar binding affinities (−5.163 and -5.158 kcal/mol, respectively). Dimethyl ellagic acid also reacts with fewer polar residues and partially exposed hydrophobic or aromatic residues in more flexible and less enclosed binding regions in these targets. The Conus magus toxin (1omg_modified.pdb) had the weakest interaction ( -4.424 kcal/mol), which was aligned with an open and solvent-accessible binding area that had no structured hydrophobic cavity. Generally, the table shows that a stronger binding with Dimethyl ellagic acid would be linked with well-defined pockets with balanced polar and hydrophobic residues, and a shallow or exposed binding site would lead to a weaker binding. As compared to this, the toxins of Apis mellifera (1poc_modified.pdb) and Agelenopsis aperta (1oav_modified.pdb) exhibited intermediate and almost similar binding affinities (−5.163 and -5.158 kcal/mol, respectively). Dimethyl ellagic acid also reacts with fewer polar residues and partially exposed hydrophobic or aromatic residues in more flexible and less enclosed binding regions in these targets. The Conus magus toxin (1omg_modified.pdb) had the weakest interaction ( -4.424 kcal/mol), which was aligned with an open and solvent-accessible binding area

that had no structured hydrophobic cavity. Generally, the table shows that a stronger binding with Dimethyl ellagic acid would be linked with well-defined pockets with balanced polar and hydrophobic residues, and a shallow or exposed binding site would lead to a weaker binding.

**Table 3.** Molecular interaction between Dimethyl ellagic acid and different toxins.

| Target (PDB ID) | Organism | Best Binding Affinity (kcal/mol) | Key Polar Residues Involved | Key Hydrophobic / Aromatic Residues Involved |
|---|---|---|---|---|
| 3bta_modified.pdb | *Clostridium botulinum* | −7.712 | Ser, Thr, Asn | Leu, Ile, Val, Ala |
| 1poc_modified.pdb | *Apis mellifera* | −5.163 | Gly, Ser | Phe, Tyr |
| 1oav_modified.pdb | *Agelenopsis aperta* | −5.158 | Gln, Asn | Leu, Pro |
| 1omg_modified.pdb | *Conus magus* | −4.424 | Gly, Ser, Asp | Limited hydrophobic residues |

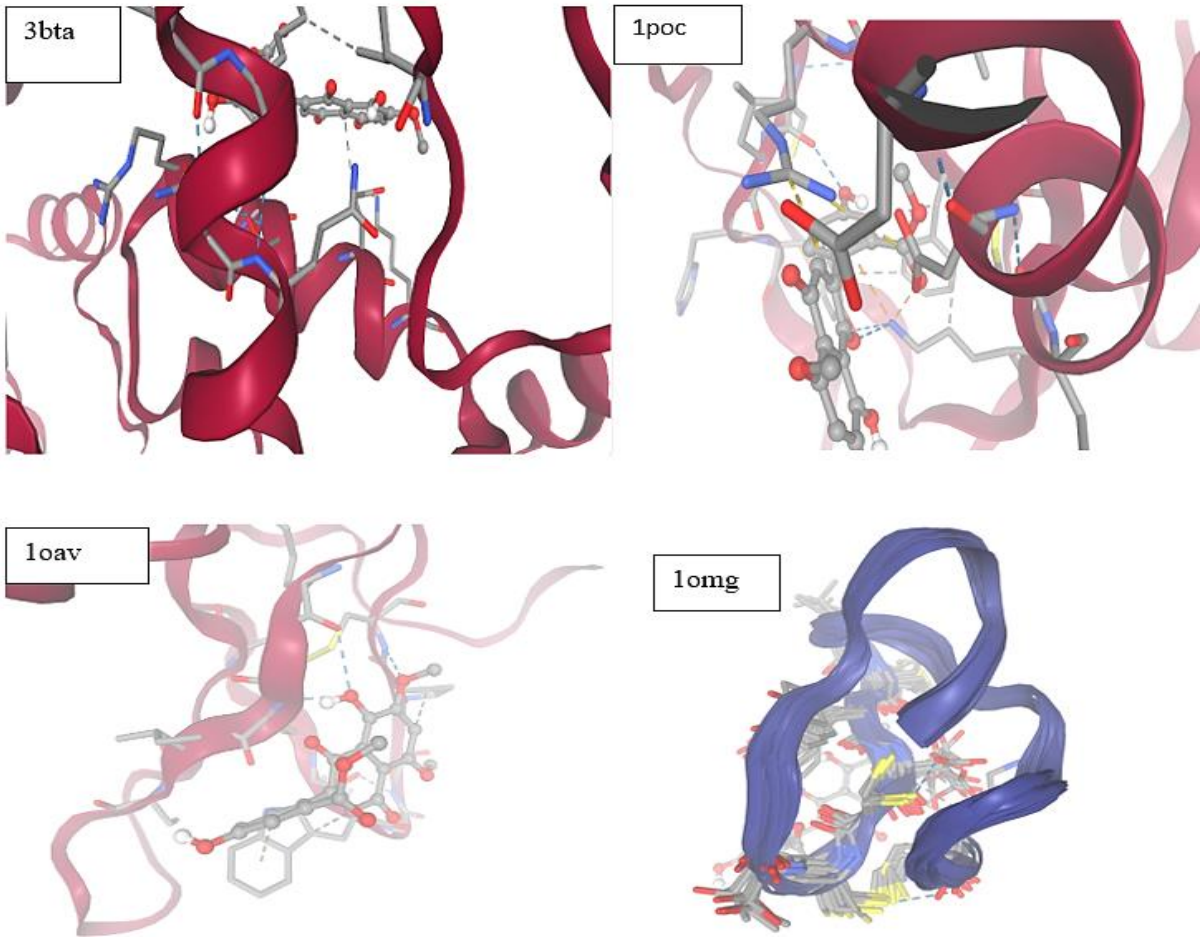


**Figure 3. Docking between** Dimethyl ellagic acid and Different toxins C. botulinum, A. mellifera, A. aperta, and C. magus.

Figure 4 and Table 4 show the molecular contacts of Dimethyl ellagic acid in the binding site of the Clostridium botulinum toxin protein (3bta_modified.pdb). The stabilization of Dimethyl ellagic acid is by hydrogen bond interactions between polar amino acid residues, mainly serine, threonine, and asparine which interact with the hydroxyl and ester functional groups of the ligand. Further

stabilization is achieved by hydrophobic contacts with surrounding residues, e.g., leucine, isoleucine, valine, and alanine, which decorate the interior of the binding pocket and facilitate van der Waals interactions. Affective interactions, which are aromatic and alkyl-associated, also assist in the positioning of perfringing within the cavity. Together, the engagement of polar and hydrophobic amino acids into a

Clearly defined binding hole is the reason why the predicted binding affinity of the Dimethyl ellagic acid toxin complex is so strong.

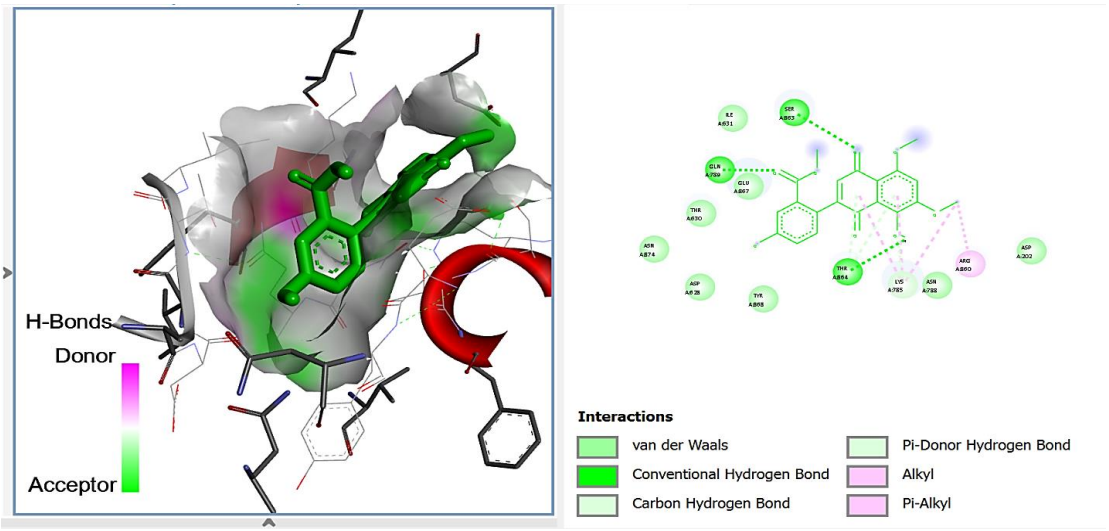


**Figure 4.** Dimethyl ellagic acid (COC(=O) C1=C(C=CC(=C1) O) C2=CC(=O) C3=C(C2=O) C(=C(C=C3OC) OC) O) of (3bta_modified.pdb, Clostridium botulinum) toxin.

Figure 5 and Table 4 are a representation of the binding mode around Dimethyl ellagic acid in the toxin protein of Apis mellifera (1poc_modified.pdb). Through the hydroxyl and ester groups, the ligand stabilizes hydrogen bond interactions with the polar amino acids, primarily Ser and Gly. Further non-covalent stabilization is afforded by aromatic residues Phe and Tyr, which are involved in π cation interactions with the aromatic moieties of Dimethyl ellagic acid and πalkyl interactions with both aromatic moieties of Dimethyl ellagic acid. The presence of van der Waals contact with the surrounding hydrophobic residues also helps in positioning some ligand at the binding interface. The presence of surface-exposed polar and aromatic amino acids indicates a fairly stable interaction within a flexible and partially open binding region.

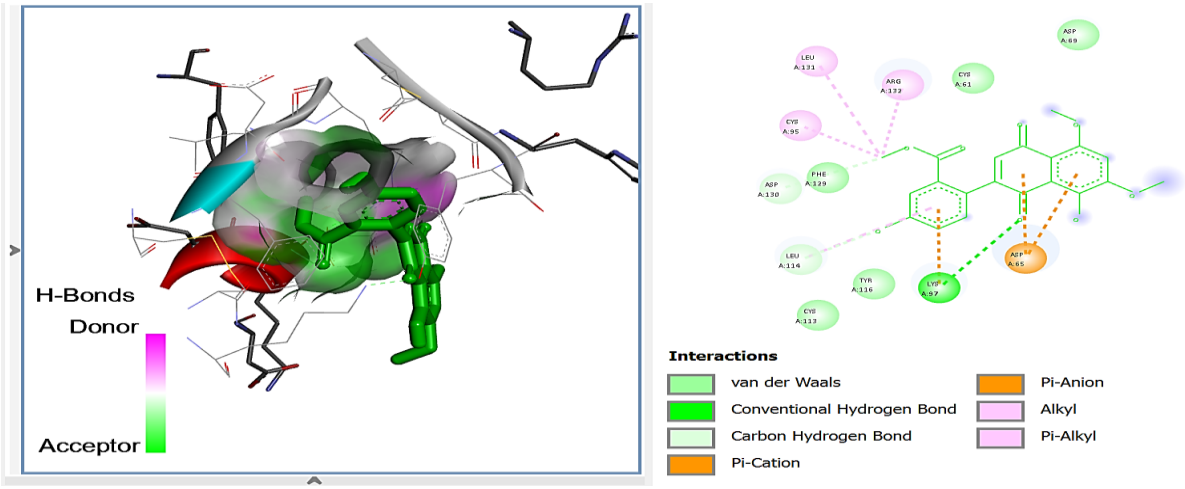


**Figure5.** Dimethyl ellagic acid and (1poc_modified.pdb, Apis mellifera) toxin.

The binding conformation of Dimethyl ellagic acid in the toxin protein of the Agelenopsis aperta (1oav_modified.pdb) is shown in the figure. The ligand is held together by the standard hydrogen bond interaction of polar amino acids, especially Cys and Thr, which bind to the hydroxyl and ester functional groups of Dimethyl ellagic acid. Further carbon-hydrogen bonding helps in the fixation of the ligand in the binding site. Aromatic residues are π -piled and π -alkyl-interacting, which contributes to additional stabilization of the Dimethyl ellagic acid aromatic rings. Hydrophobic amino acids such as Ile, Gly, and Pro surrounding it give van der Waals contacts, which help in preserving the orientation of the ligand. The total interaction profile shows a somewhat stable complex developed in a shallow and partially enclosed binding site, which is in line with the intermediate affinity of binding to the toxin target, as shown in Figure 6 and Table 4.

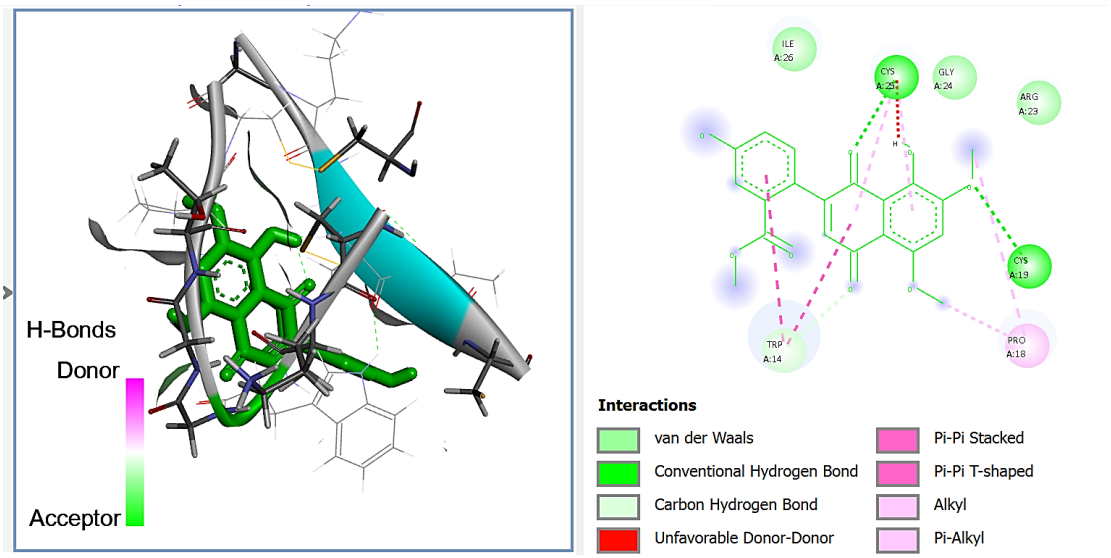


**Figure 6.** Dimethyl ellagic acid with (1oav_modified.pdb, Agelenopsis aperta ) toxin.

Figure 7 and Table 4 are used to demonstrate the binding mode of Dimethyl ellagic acid in the toxin protein of Conus magus (1omg_modified.pdb). Dimethyl ellagic acid is located in an open and flexible binding region, and polar amino acid residues are the major mediators of stabilization. With Ser and Gly, conventional hydrogen bond interactions are apparent between the hydroxyl and ester group of the ligand. Other carbon-hydrogen bonds help in the partial binding of Dimethyl ellagic acid in the interaction face. Hydrophobic and van der Waals contacts are restricted and include residues like Met and Ala, which means that the nonpolar enclosure of the ligand is weak. The association between aromatic groups is low, and an adverse acceptor interaction is observed with an acidic residue (Asp), indicating that this region is not well compatible with binding. In general, the interaction profile indicates loosely stabilized ligand orientation in a solvent-accessible binding site, as is expected of the lower predicted binding affinity of the Conus magus toxin.

### 3.1. Pharmacokinetics of Dimethyl ellagic:

Table 5 and Figure 8 are a detailed in silico analysis of the physicochemical properties, pharmacokinetics, and other toxicity properties of Dimethyl ellagic. The physicochemical values show that Dimethyl ellagic acid has an intermediate molecular weight (384.34 Da) and a balanced lipophilicity (LogP = 2.36), which explains its suitability with drug-like chemical space. Eight hydrogen bond acceptors and two hydrogen bond donors, together with a fairly large polar surface area (topological) of 119.36 A 2, indicate that the compound is strongly polar interacting and still does not exceed the reasonable limits of the Lipinski rule of five. Its good physicochemical profile is also supported by the fact that its estimate of drug-likeness (QED = 0.77) is also positive.

Predictions involving absorption indicate that there is sufficient absorption in the intestines of human beings, though the oral bioavailability is intermediate, which could be affected by the low aqueous solubility of the compound. The membrane transport potential is reasonable based on the Lipophilicity and permeability parameters, such as PAMPA permeability and cell effective permeability. The P-glycoprotein inhibition is predicted to possibly cause interaction with other efflux transport mechanisms that could influence systemic exposure.

The distribution analysis demonstrates a minimal penetration of the blood-brain barrier, which means that there is a low chance of a central nervous system accumulation. On the contrary, Dimethyl ellagic acid has a high plasma protein binding rate, and its volume of distribution is quite large, which indicates that it is widely distributed in tissues after systemic exposure.

Metabolic profiling anticipates significant inhibitory capacity to various of the cytochrome P450 isoforms, specifically CYP1A2, CYP2C9, and CYP3A4; the substrate likelihood to these enzymes is low to moderate. These results imply that Dimethyl ellagic acid does not have to be degraded by the most important CYPs to alter hepatic metabolism.

Parameters that are related to excretion show that it has a moderate half-life and a high rate of its clearance in both the hepatocyte and microsomal systems, meaning that it is actively eliminated through metabolism. The predictions of toxicity indicate a low carcinogenic and clinical toxicity risk, moderate acute toxicity risk, and a restricted hERG channel blocking risk. Nonetheless, the fact that this score strongly predicted drug-induced liver injury also raises the issue of the necessity of interpretation and additional experimental confirmation. In general, the built-in ADMET profile indicates that Dimethyl ellagic acid has a number of positive drug-like characteristics, and the al-so offers pharmacokinetic and safety concerns that should be further investigated.

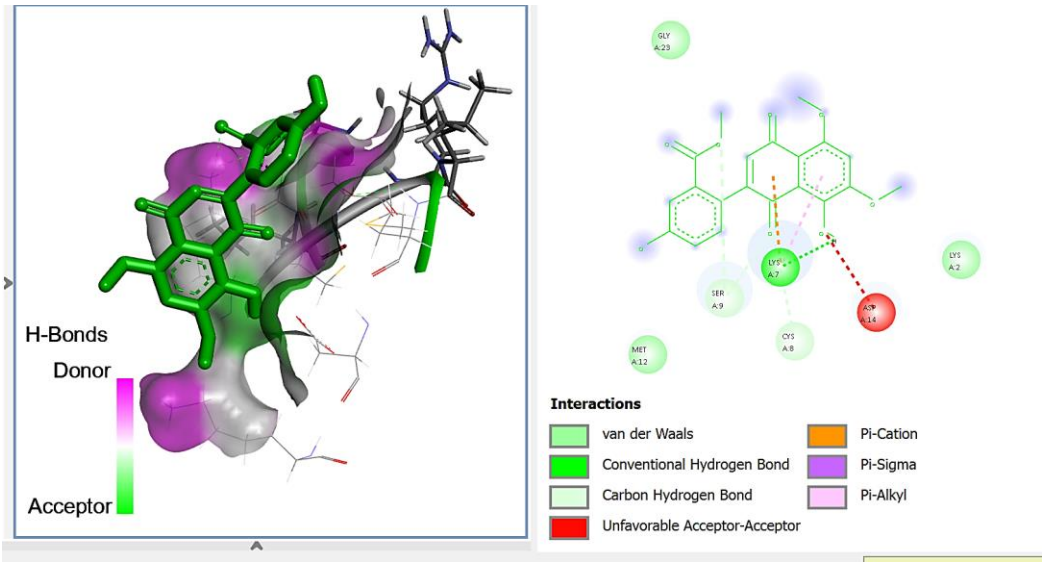


**Figure 7.** Dimethyl ellagic acid Dimethyl ellagic acid with conus magus) 1omg_modified.pdb toxin.

**Table 4.** Amino acid–level interaction profile of Dimethyl ellagic acid with different toxin proteins.

| Target (PDB ID) | Organism | Key Polar Amino Acids Involved | Key Hydrophobic / Aromatic Amino Acids Involved | Dominant Interaction Types |
|---|---|---|---|---|
| 3bta_modified.pdb | *Clostridium botulinum* | Ser, Thr, Asn | Leu, Ile, Val, Ala | Conventional hydrogen bonds, carbon–hydrogen bonds, van der Waals, alkyl and aromatic-associated interactions |
| 1poc_modified.pdb | *Apis mellifera* | Ser, Gly | Phe, Tyr | Hydrogen bonding, π–alkyl, π–cation, van der Waals interactions |
| 1oav_modified.pdb | *Agelenopsis aperta* | Cys, Thr | Ile, Gly, Pro | Hydrogen bonding, carbon–hydrogen bonds, π–π stacking, π–alkyl, van der Waals interactions |
| 1omg_modified.pdb | *Conus magus* | Ser, Gly, Asp | Met, Ala | Limited hydrogen bonding, carbon–hydrogen bonds, weak van der Waals interactions; unfavorable acceptor–acceptor contact |

**Table 5.** Integrated physicochemical, pharmacokinetic, and toxicity profile of Dimethyl ellagic.

| Category | Property | Value / Prediction | DrugBank Percentile | Units |
|---|---|---|---|---|
| **Physicochemical** | Molecular Weight | 384.34 | 61.54 | Dalton |
| | LogP | 2.36 | 50.79 | log-ratio |
| | Hydrogen Bond Acceptors | 8.00 | 82.36 | # |
| | Hydrogen Bond Donors | 2.00 | 60.86 | # |
| | Lipinski Rule of Five | 4.00 | 63.80 | # of 4 |
| | QED | 0.77 | 84.92 | – |
| | Stereo Centers | 0.00 | 22.49 | # |
| | TPSA | 119.36 | 77.86 | Å$^2$ |
| **Absorption** | Human Intestinal Absorption | 1.00 | 66.89 | – |
| | Oral Bioavailability | 0.51 | 19.89 | – |
| | Aqueous Solubility | −5.11 | 12.95 | log(mol/L) |
| | Lipophilicity | 1.85 | 57.08 | log-ratio |
| | Hydration Free Energy | −10.69 | 39.55 | kcal/mol |
| | Cell Effective Permeability | −4.78 | 62.89 | log($10^{-6}$ cm/s) |
| | PAMPA Permeability | 0.86 | 60.37 | – |
| | P-glycoprotein Inhibition | 0.63 | 81.04 | – |
| **Distribution** | Blood–Brain Barrier Penetration | 0.27 | 17.02 | – |
| | Plasma Protein Binding | 96.01 | 84.41 | % |
| | Volume of Distribution (SS) | 6.20 | 81.00 | L/kg |
| **Metabolism** | CYP1A2 Inhibition | 0.96 | 97.44 | – |
| | CYP2C19 Inhibition | 0.48 | 83.02 | – |
| | CYP2C9 Inhibition | 0.70 | 94.77 | – |
| | CYP2D6 Inhibition | 0.28 | 77.78 | – |

| | | | | |
|---|---|---|---|---|
| | CYP3A4 Inhibition | 0.84 | 92.09 | – |
| | CYP2C9 Substrate | 0.06 | 22.18 | – |
| | CYP2D6 Substrate | 0.04 | 30.32 | – |
| | CYP3A4 Substrate | 0.38 | 40.02 | – |
| **Excretion** | Half-life | 28.31 | 74.56 | h |
| | Hepatocyte Clearance | 99.51 | 90.19 | μL/min/$10^6$ cells |
| | Microsomal Clearance | 74.78 | 88.99 | μL/min/mg |
| **Toxicity** | hERG Blocking | 0.51 | 64.09 | – |
| | Clinical Toxicity | 0.04 | 33.31 | – |
| | Mutagenicity | 0.44 | 78.52 | – |
| | Drug-Induced Liver Injury | 0.98 | 98.22 | – |
| | Carcinogenicity | 0.02 | 8.03 | – |
| | Acute Toxicity ($LD_{50}$) | 2.33 | 37.73 | log(1/(mol/kg)) |
| | Skin Reaction | 0.71 | 77.20 | – |
| | Androgen Receptor (FL) | 0.02 | 41.99 | – |
| | Androgen Receptor (LBD) | 0.03 | 77.12 | – |
| | Aryl Hydrocarbon Receptor | 0.85 | 98.60 | – |
| | Aromatase | 0.15 | 80.46 | – |
| | Estrogen Receptor (FL) | 0.24 | 83.79 | – |
| | Estrogen Receptor (LBD) | 0.17 | 92.44 | – |
| | PPAR-γ | 0.05 | 82.40 | – |
| | Nrf2/ARE Pathway | 0.73 | 94.07 | – |
| | ATAD5 | 0.26 | 95.85 | – |
| | Heat Shock Factor Response | 0.16 | 90.23 | – |
| | Mitochondrial Membrane Potential | 0.94 | 98.76 | – |
| | Tumor Protein p53 | 0.48 | 95.62 | – |

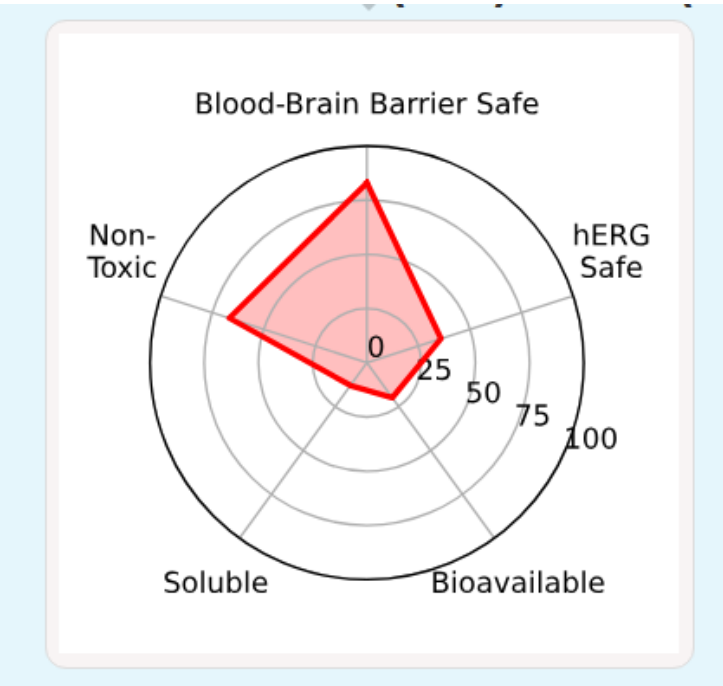


**Figure 8.** ADMET of Dimethyl ellagic acid.

## 4. Discussion

The research question that was undertaken in this study was to determine the potential of Dimethyl ellagic acid, a tetranortriterpenoid from *Eucalyptus globulus,* as a broad-spectrum inhibitor of virulence-associated toxins through an integrated in silico strategy. Through a mixture of molecular docking, residue-level interaction study, and ADMET prediction, the study aimed to evaluate the feasibility of target-binding as well as pharmacokinetic constraints in a comparative and mechanistic context.

The docking outcome revealed that the Dimethyl ellagic acid has different binding affinities to toxin proteins of bacterial, insect, arachnid, and marine sources. The binding with the Clostridium botulinum toxin (3bta_modified.pdb) was observed the most that can be explained by the structural aspects of its binding site. The toxin has a deep and well-structured pocket that has the capacity to accommodate the large and highly functionalized Dimethyl ellagic acid scaffold. Directional hydrogen bonds with serine, threonine, and asparagine residues and massive hydrophobic interactions with leucine, isoleucine, valine, and alanine stabilized it. These cooperative polar hydrophobic patterns of interaction have been found to be typical of high-affinity ligand-binding, and have been widely reported with toxin-inhibitor complexes, where ligand burial in a defined cavity contributes greatly to binding stability [3,18].

Conversely, the toxins of Apis mellifera and Agelenopsis aperta had moderate binding affinities. In such instances, Dimethyl ellagic acid reacted mainly with surface exposed or partially enclosed areas, leading to a smaller number of stabilizing contacts and an increased access of solvents. In the case of A. mellifera toxin, hydrogen bonding with small polar residues like serine and glycine dominated the interactions, and this was complemented with π-alkyl and π-cation interactions between the aromatic residues. Though these interactions do play a role in the association of a ligand, in most cases, they are not enough to generate a high binding affinity without a recessed binding site [19]. The same trend was followed with

the A. aperta toxin, in which the hydrogen bonding and the aromatic stacking came into play, but in a shallow binding region, thus restricting overall stabilization.

The poorest interaction was found with the Conus magus toxin, which does not have a specific hydrophobic cavity and which is defined by a loose and accessible binding surface to solvents. The binding of Dimethyl ellagic acid here was largely based on polar contacts with serine and glycine residues, very little hydrophobic enclosure, and the occurrence of an undesirable acceptor contact involving an acidic residue. These types of interaction environments are usually known to decrease the binding affinity and are commonly related to transient or non-specific association of the ligand, instead of the effective association [20]. The results highlight the significance of the topology of binding-sites and the composition of residues in the identification of the inhibitory capacity of Dimethyl ellagic acid.

In a mechanistic approach, the selectivity observed for the bacterial toxin indicates that Dimethyl ellagic acid would be more adapted to highly rigid and defined active or binding sites. This is in line with past studies, which have shown that complex natural products tend to show target preference due to structural compatibility and not universal binding capacity [21]. Notably, the capacity of Dimethyl ellagic acid to react with several classes of toxins, even at moderate affinity, justifies its standing as an anti-virulence scaffold, which is multi-target as opposed to a highly specific toxin antagonist.

The ADMET analysis provided the necessary background for interpreting the docking results. Dimethyl ellagic acid demonstrated physicochemical characteristics that were in line with those of bioactive compounds, such as moderate molecular weight, balanced lipophilicity, and a good quantitative measure of the drug-likeness. The high polar surface area and hydrogen bonding ability can be attributed to the high polar interactions seen in docking, especially when compared to the C. botulinum toxin. Yet, the same characteristics probably make it insoluble in water and have medium oral bioavailability, the drawbacks that are typical of structurally complex natural products [22].

Distribution projections showed that blood brain bar verses are not well penetrated, and this could decrease the risk of toxicity to the central nervous system. The binding by high plasma proteins and the large volume of distribution imply a widespread association in tissues, which can prove beneficial in the local action of toxins, but would complicate systemic pharmacokinetic properties. Metabolic forecasts had high inhibitory potential of various cytochrome P450 isoforms, especially CYP1A2, CYP2C9, and CYP3A4. Although this increases the chance of drug-drug interactions, it also implies the presence of metabolic persistence that can be useful in protracted anti-virulence action [23].

The outcomes of the toxicity predictions were mostly positive, with the carcinogenicity and the clinical toxicity being low. However, the probability score of drug-induced liver injury is very high, and it should be taken into consideration. Many bioactive natural products are known to cause hepatotoxicity, especially those that undergo high levels of hepatic metabolism [24]. Thus, no translational conclusions will be drawn without experimental validation of the system in vitro in a hepatocyte model in vitro and in vivo toxicity studies.

Comprehensively, this paper demonstrates the notion that Dimethyl ellagic acid can be repurposed as an anti-virulence agent, which can be computationally repurposed with specific potential against bacterial toxins with well-defined binding pockets. The results underline that the activity of Dimethyl ellagic acid depends on the structure and identify its advantages and shortcomings as a therapeutic building block. Notably, the work does not purport clinical efficacy, but instead, it offers a justification to carry out experimental research. The

balanced analysis of docking and ADMET provides a realistic and balanced evaluation, which has been in line with the recent strategies of natural-product-based drug discovery and anti-virulence studies.

## 5. Conclusions

This study has shown that Dimethyl ellagic acid has structure-specific binding behavior with virulence-associated toxins of various biological origins. The strongest interaction was found with the Clostridium botulinum toxin, which was assisted by a clear binding pocket that facilitates polar and hydrophobic interactions, but weaker toxins of insect, arachnid, and marine toxins were found to bind with more open binding pockets. At the level of residues, it was found that binding strength is correlated with pocket depth, complementarity of the residues, and enclosure of the ligand. Additional in silico ADMET profiling also revealed that Dimethyl ellagic acid has a number of desirable drug-like and pharmacokinetic characteristics despite average bioavailability and possible metabolic and hepatic issues. On the whole, the results justify the use of Dimethyl ellagic acid as a potential natural scaffold in antivulence approaches and give a logical reason for the next experimental validation aiming at toxin-mediated pathogenicity.

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