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Molecular Docking Assessment of Limonoids from Cameroonian *Entandrophragma* Species as Potential Inhibitors of *Anopheles gambiae* Acetylcholinesterase (AChE)

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Abstract: Malaria remains one of the great killers in tropical regions of the world due to the transmission of the *Plasmodium* parasite by the bites of the female mosquito *Anopheles*. The resistance of this species to synthetic insecticides contributes to an increase in the incidence of malaria and therefore necessitates the development of new potent and eco-friendly insecticides. In this study, twelve previously reported limonoids from four *Entandrophragma* species collected in Cameroon have been computationally evaluated for their *Anopheles gambiae* AChE inhibitory activity. The docking procedure was carried out through Molecular Operating Environment 2019.01 (MOE), while the UCSF Chimera program was used to model the docking results based on interactions between proteins and ligands, and molecular dynamics trajectories were analyzed using the GROMACS 2021.1 tool. Entandrophragmin and encandollens B and C with docking scores ranging from −6.45 to −7.28 kcal/mol were the most promising hits compared to the reference azadirachtin (−6.22 kcal/mol) and were further evaluated for their mechanism of action. Subsequent evaluation classified encandollen C as the best candidate for the development of new potent eco-friendly insecticides based on its lower average RMSD and RMSF and its compactness over a 150 ns duration with acetylcholinesterase.

Keywords: *Anopheles gambiae* AChE; *Entandrophragma* species; limonoids; molecular docking; molecular dynamics

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1. Introduction

Malaria is an infectious disease caused by the *Plasmodium* parasite and transmitted to humans by *Anopheles* mosquitoes [1–4]. It poses a significant threat, especially in tropical regions, where children under five and pregnant women are most vulnerable [5]. According to the World Health Organization (WHO), there were 241 million recorded cases of malaria worldwide in 2021, with Africa bearing 96% of the global malaria burden and accounting for 80% of malaria-related deaths in children under 5 [1,5]. The fight against malaria faces two main challenges: the resistance of the *Plasmodium* parasite to antimalarial drugs and the increasing resistance of *Anopheles* mosquitoes to commonly used insecticides such as organophosphates, carbamates, and pyrethroids [6]. As a result, higher doses of synthetic insecticides have been necessary, leading to increased human exposure to these chemicals and their associated health risks [7]. This emphasizes the need for urgent research into new, naturally derived insecticidal candidates that are safe, environmentally friendly, and effective. In many regions of Cameroon, plant extracts have been utilized by local populations for grain storage and pest management due to their repellent, antifeedant, or insecticidal properties, with minimal or no harmful effects on humans or the environment [8].

It is well reported that limonoids are highly oxygenated and modified triterpenoids that serve as the chemomarkers of Meliaceae plants. They represent a major class of compounds with a wide range of biological activities in medicinal and agricultural fields [1,9–11]. Five *Entandrophragma* species have been identified in Cameroon including *E. angolense*, *E. candollei*, *E. congoënse*, *E. cylindricum*, and *E. utile* [5,12,13]. During the last decade, our investigations of antiplasmodial constituents from *Entandrophragma* plants collected in Cameroon led to the isolation and identification of several classes of compounds including triterpenoids, limonoids, and steroids as the major and bioactive ones [5,10,13,14]. Due to their well-known insecticidal activities with diverse action mechanisms, limonoids from *Entandrophagma* plants (Meliaceae) are thus of great interest in research on new potent insecticides.

Acetylcholinesterase (AChE) is the primary target of several organophosphate and carbamate insecticides which inhibit the breakdown of acetylcholine to acetic acid and choline, causing the accumulation of acetylcholine in the synapses, which keeps acetylcholine receptors permanently open and leads to muscle paralysis, coma, and death of the insect [15,16]. Several classes of natural compounds like alkaloids, xanthones, or triterpenes are reported to have good AChE inhibitory activity [17]. As a continuity of our investigations of Meliaceae plants from Cameroon and the screening of their contribution to fighting malaria [1], we have computationally studied twelve previously reported limonoids from the genus *Entandrophragma* as potential inhibitors of the AChE of *Anopheles gambiae*, the vector of *Plasmodium falciparum*.

2. Material and Methods

2.1. The Limonoids Involved in This Study

The twelve limonoids (**1**–**12**, Figure 1) evaluated in this study have been isolated from our previous investigations of four *Entandrophragma* species collected in Cameroon [5,10,12–14]. This includes five phragmalin-type limonoid orthoesters, namely *β*-dihydroentandrophragmin-ethyl acetate (**1**), entandrophragmin (**2**), and encandollens A–C (**3**–**5**) from *E. cylindricum* and *E. candollei* [10,18]; four andirobin-type limonoids identified as methyl angolensate (**6**), angolensic acid (**7**), and mollucensins N and O (**8** and **9**) from *E. congoënse* [5,13]; and three heptanortriterpenoids viz entilins A–C (**10**–**12**) from *E. congoënse* and *E. utile* [13,19,20].

Briefly, the stem barks of *E. cylindricum*, *E. candollei*, and *E. congoënse*, as well as the roots of *E. congoënse*, were separately extracted using a mixture of dichloromethane–methanol (1:1) at room temperature for 48 h and 24 h, respectively. The solvent was removed using a rota-evaporator under reduced pressure to afford the crude extracts for each plant. Part of the crude extract was dissolved in double-distilled water and partitioned with *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol to obtain four main fractions for each extract. The LC-MS profiles of each fraction showed that the *n*-hexane-soluble fractions of the stem bark extracts of *E. cylindricum*, the roots of *E. congoënse*, and the dichloromethane-soluble fractions of the stem bark extracts of *E. candollei* and *E. congoënse* were rich in limonoids and were further purified using column chromatography on silica gel, eluting with a gradient of the mixture of ethyl acetate in *n*-hexane to afford compounds **1** and **2** from the *n*-hexane-soluble fraction of the *E. cylindricum* stem bark extract; compounds **3**–**5** and **6**–**9** from the dichloromethane-soluble fractions of the *E. candollei* and *E. congoënse* stem bark extracts, respectively [5,13,18]; and compounds **10**–**12** from the *n*-hexane-soluble fraction of the *E. congoënse* root extract [13].

Additionally, azadirachtin (Figure 2), selected as the standard compound in this study, exhibits significant antifeedant activity against various insects, with IC_{50} values ranging from 20 to 50 μM depending on the protocol of the bioassay. It is derived from the seeds of *Azadirachta indica* (neem), a plant widely utilized as a natural bio-pesticide in grain storage [21]. Azadirachtin is the primary active component of the neem plant and is effective against approximately 200 different insect species by primarily disrupting their reproductive cycle and growth [22,23]. Specifically, the limonoid azadirachtin has demonstrated larvicidal activity against *Anopheles* mosquito larvae [24–26], indicating its potential to eliminate arthropods responsible for numerous diseases, including malaria.

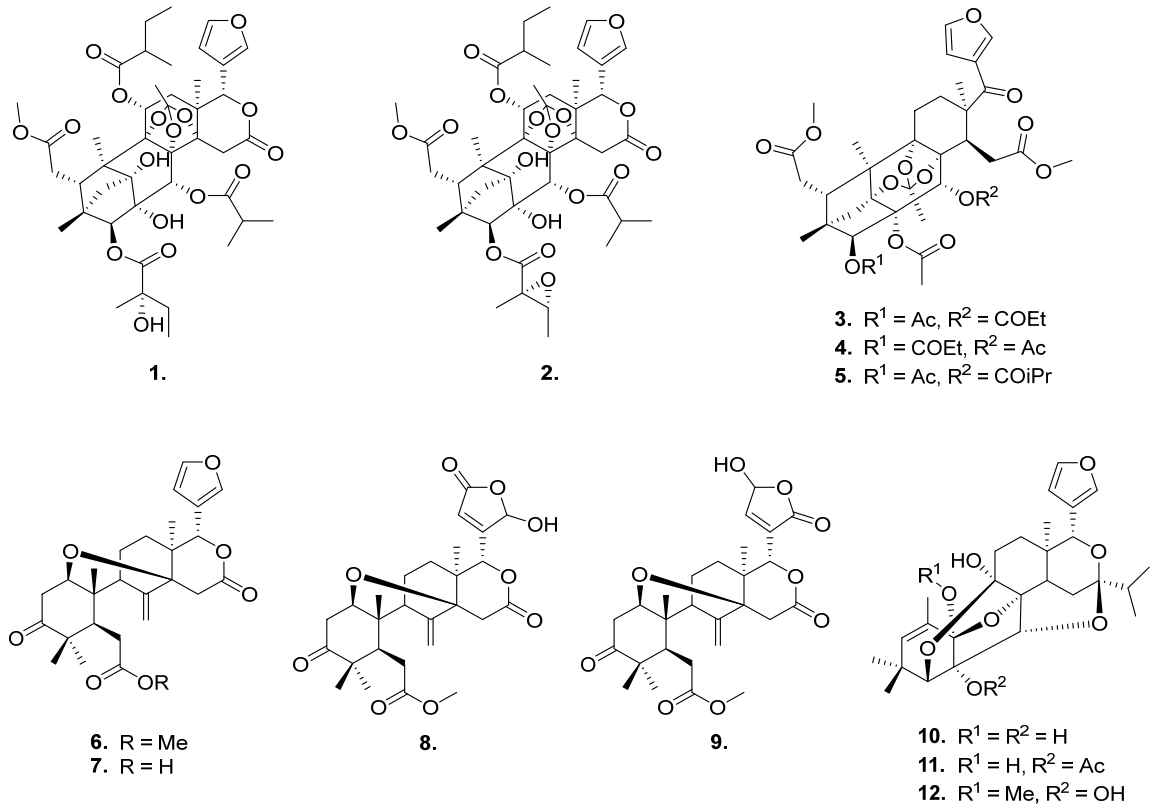


Figure 1. Limonoids (**1**–**12**) isolated from genus *Entandrophragma*.

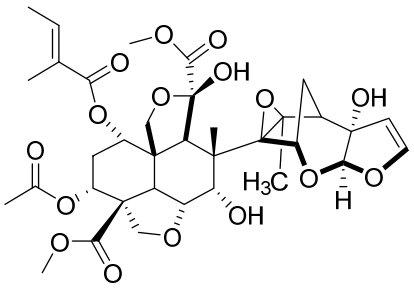


Figure 2. Structure of azadirachtin (reference compound).

2.2. Geometry Optimization

The thirteen compounds involved in this study including limonoids **1**–**12** and the standard compound azadirachtin were subjected to geometry optimization using the Gaussian 09W software version 9.0. The compounds were optimized to obtain the lowest ground-state energy using the tight convergence option with the B3LYP/3-21G** basis set of Density Functional Theory (DFT) [27].

2.3. Molecular Docking

Molecular Docking Simulation is a powerful technique that indicates the key interactions underlying ligand binding to a protein target via introducing a molecule of concern into the binding site of the protein [28]. The binding interactions between limonoids and AChE were determined by applying all docking algorithms within the Molecular Operating Environment version 2019.01 (MOE) using the default parameters. The 2D chemical structures of each particular limonoid were obtained using ChemDraw Professional version 16.0.1.4. Molecular Operating Environment version 2019.01 (MOE) software employs MMFF94 force field to optimize the structures, protonation, and energy minimization of each limonoid compound to obtain the most stable conformation [29,30]. The AChE protein

with PDB ID 5X61 was extracted from the Protein Data Bank and utilized as a template for the docking study [31]. The docking procedure was carried out through MOE to put all twelve limonoid compounds along with the reference compound within the binding cavity of AChE. For each molecule, a maximum of 50 conformations were produced, and the best conformation was chosen for further analysis based on the docking score. The UCSF Chimera program version 1.14 was used to model the docking results based on interactions between proteins and ligands [32].

2.4. Molecular Dynamics Simulation

Molecular dynamics simulation (MD) was conducted to evaluate the robustness of the obtained hits along with a comparison study utilizing the corresponding ligand for the protein. Molecular dynamics simulation of 150 ns was conducted for the selected compounds in a complex with their respective protein, as well as for the protein in its ligand-free (Apo protein) state. GROMACS version 2021.1® was implemented to run these simulations [33]. AMBER99SB force field was used to estimate the atomic charges, and the Extended Simple Point Charge (SPCE) water model was employed to solvate the systems for MD simulations. Each of the systems was simulated within a cubic box, with careful attention paid to maintaining ample spacing between the system and the box boundaries [34,35]. The systems were rendered electro-neutral by adding an appropriate number of counter ions. The steepest descent algorithm was utilized for energy minimization, enabling the gradual relaxation of the systems through a series of 5000 steps. The systems were equilibrated to 300 K using a Berendsen thermostat with a coupling time of 100 ps, and the pressure was maintained using coupling to a reference pressure of 1 atm, following 1000 ps of equilibration with position constraints on the protein and ligand molecule using NVT and NPT ensembles [36,37].

2.5. Trajectory Analysis

The results of the MD trajectories were analyzed using the GROMACS 2021.1® tool. The root mean squared deviation (RMSD) and Root Mean Squared Fluctuation (RMSF) of the studied protein's backbone atoms in the ligand-free state and in docked complexes were calculated using the gmx_rms and gmx_rmsf tools, respectively. The compactness of all systems was determined by calculating the radius of gyration (Rg) using gmx_gyrate tools.

3. Results and Discussion

3.1. Geometry Optimization

Compounds **1**–**12** and the standard azadirachtin were optimized using the Gaussian 09W software version 9.0 to obtain the lowest ground-state energy using the tight convergence option with the B3LYP/3-21G** basis set of Density Functional Theory (DFT). The results of the summarized optimized energy were obtained from the GaussView modelling package version 5.0 and are displayed in Table 1.

Table 1. Optimized 3D structures and ground-state energies of the compounds.

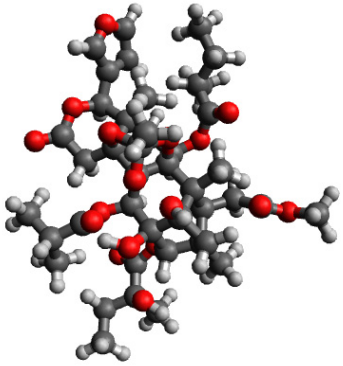
Compounds	Optimized 3D Structure	Energy (Hartree)
1		−1.186023

Table 1. *Cont.*

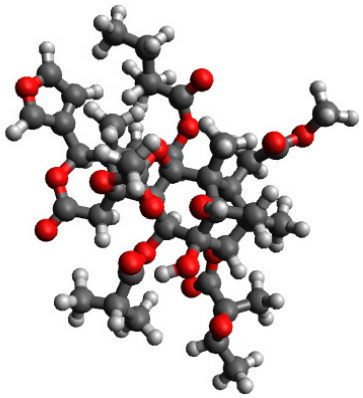
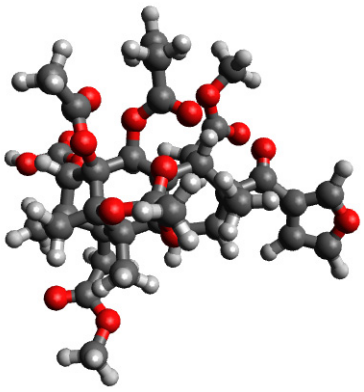
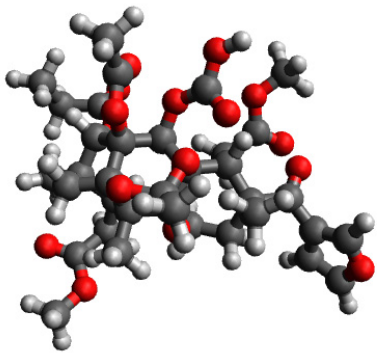
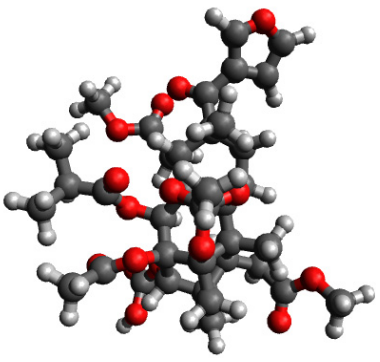
Compounds	Optimized 3D Structure	Energy (Hartree)
2		−1.116389
3		−1.038584
4		−1.035614
5		−1.046825

Table 1. *Cont.*

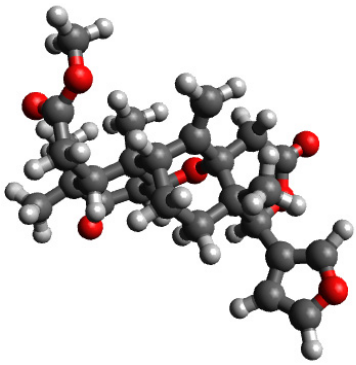
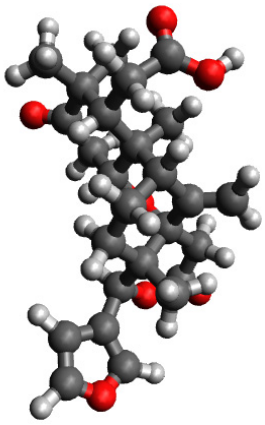
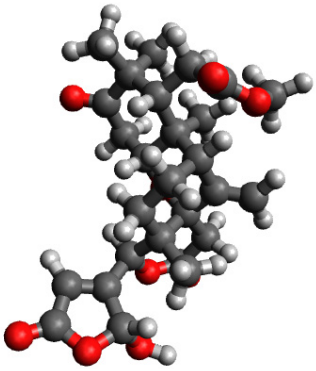
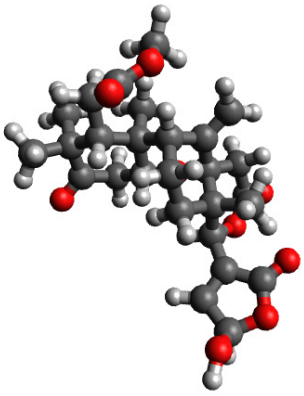
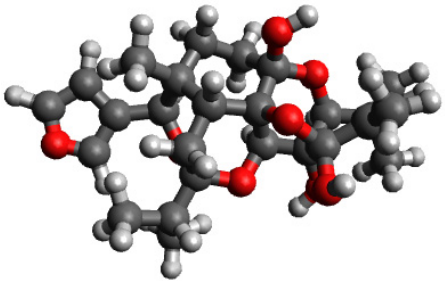
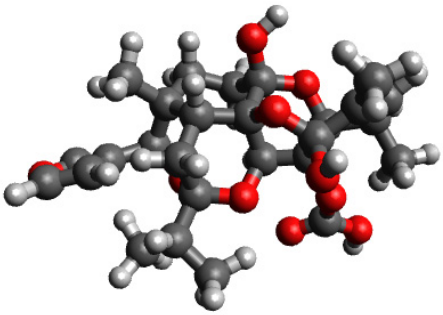
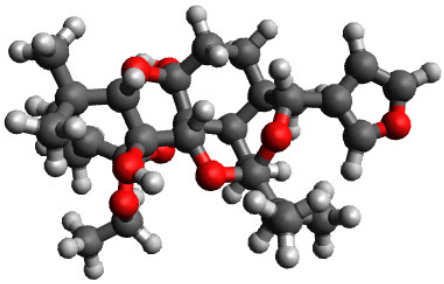
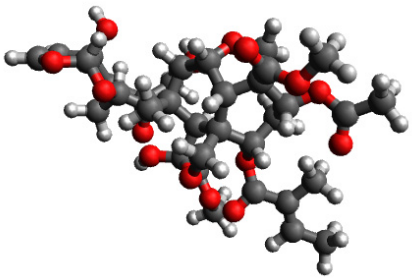
Compounds	Optimized 3D Structure	Energy (Hartree)
6		−0.442500
7		−0.448302
8		−0.584515
9		−0.582419

Table 1. *Cont.*

Compounds	Optimized 3D Structure	Energy (Hartree)
10		−0.551982
11		−0.671810
12		−0.549421
Azadirachtin		−0.845361

3.2. Molecular Docking Simulation

The results of the docking study indicated that the limonoids effectively occupied the AChE binding site and helped to stabilize the enzyme. Three top compounds, with docking scores ranging from −6.45 to −7.28 kcal/mol, were chosen for further investigation and compared to the standard azadirachtin (−6.22 kcal/mol), as shown in Table 2. The docking results were visualized using VMD 1.9.3® (Visual Molecular Dynamics) [38] to assess the potential interactions between the protein and the ligands. The binding poses of all of the limonoid compounds, along with reference ligands in AChE, are depicted in Figure 3.

Based on their docking scores compared to azadirachtin (the reference sample), entandrophragmin (**2**), encandollen B (**4**), and encandollen C (**5**) were selected as the most promising hits for further in-depth mechanistic study.

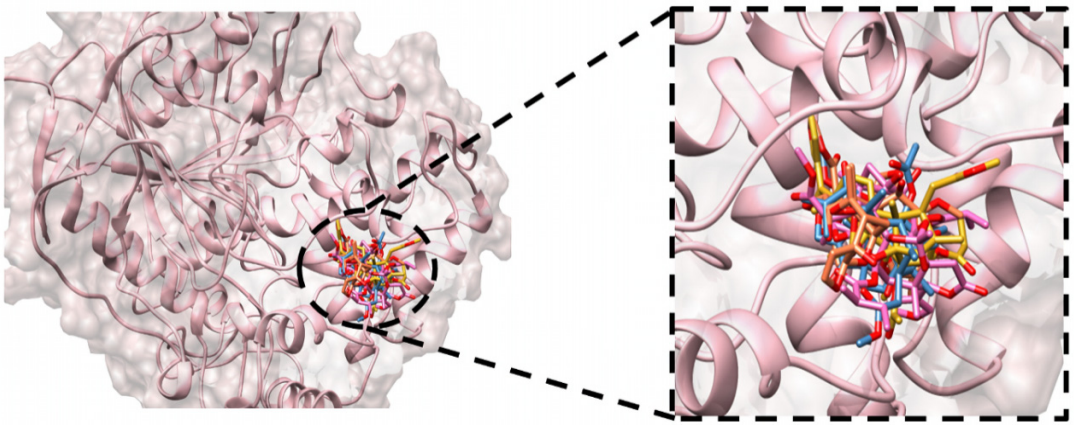


Figure 3. The assembled dock poses of compounds **2**, **4**, and **5** and the reference compound (azadirachtin) against the binding pocket of acetylcholinesterase depicted in 3D format.

Table 2. Docking scores of limonoids (**1**–**12**) from *Entandophragma* and azadirachtin.

Compound	Docking Score (kcal/mol)
1	−6.18
2	−6.45
3	−5.76
4	−7.00
5	−7.28
6	−5.24
7	−5.41
8	−6.10
9	−5.40
10	−5.68
11	−6.00
12	−5.16
Azadirachtin	−6.22

Azadirachtin binds to the binding site of AChE by forming two hydrogen bonds with the active site residues Cys447 and Glu448. Additionally, hydrophobic interactions involve Trp441, Leu444, and Tyr494 residues. Entandrophragmin (**2**) forms two hydrogen bonding interactions with the residues Leu444 and Tyr493 and also exhibits hydrophobic interactions with the residues Ile231, Trp441, Tyr494, and Thr496 in the active site of AChE. Encandollen B (**4**) forms two hydrogen bonds with the residues Tyr282 and Thr496, while the most prominent hydrophobic interactions with the active site of the receptor involve Val235, Trp441, Leu444, and Thr496. Similarly, encandollen C (**5**) also forms two hydrogen bonding interactions with the residues Cys447 and Glu448. Additionally, Ile231, Val235, Tyr282, Trp441, and Tyr493 are involved in the hydrophobic interactions with AChE.

The interactions of all of the ligands and AChE are depicted in Figure 4. Finally, three potential hits with strong affinities and interactions with the respective protein proceed to undergo a stability assessment in the dynamic state, as provided by molecular dynamics (MD) simulations.

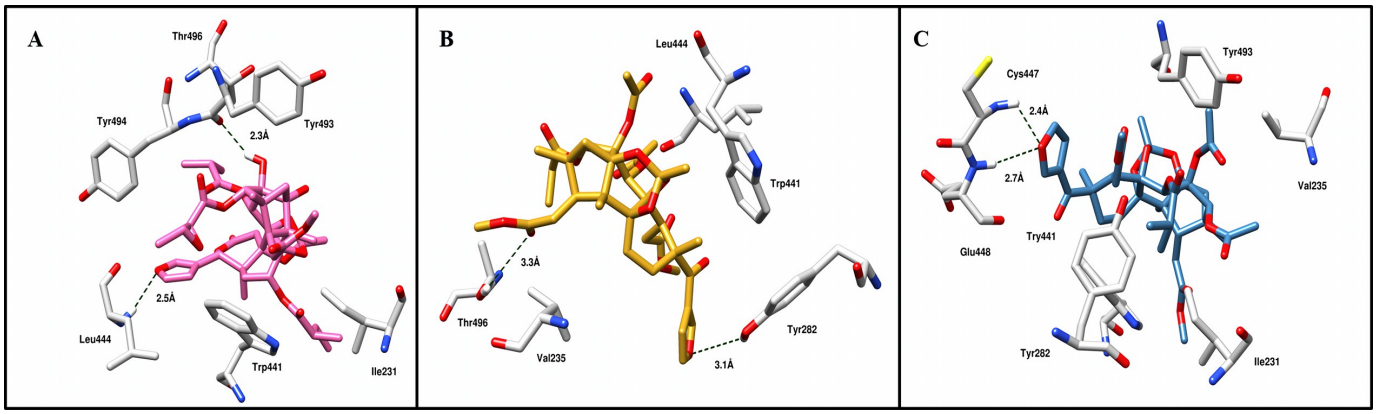


Figure 4. Three-dimensional graphical structures representing the binding interactions of compounds **2** (**A**), **4** (**B**), and **5** (**C**) in the active pocket of acetylcholinesterase.

3.3. Molecular Dynamics Simulation

The molecular dynamics (MD) simulation has proven to be a valuable tool for studying both internal and external motions, as well as conformational changes, with respect to the average positions of all atoms. In this study, simulation analyses were conducted to observe the time-dependent accessibility of specific ligands within the binding pocket. Throughout the simulation process, five systems were examined, including four complex systems and one ligand-free system. Various parameters such as RMSD, RMSF, and Rg were scrutinized to gain a comprehensive understanding of the stability of the complexes and the dynamic behavior of the selected compounds within the protein's binding pocket.

3.3.1. Root Mean Square Deviation (RMSD)

The root mean square deviation (RMSD) serves as a crucial parameter for assessing the dynamic stability of a complex by quantifying the deviation of protein backbone atoms over time. In the field of computer-aided drug design, a lower RMSD value signifies a more stable system, whereas higher RMSD values suggest a lack of stability [39]. Analysis of the protein's backbone atoms revealed that the Apo protein experienced fluctuations for the initial 60 ns, followed by a period of stability for the remainder of the simulation. Encandollens B (**4**) and C (**5**) initially exhibited fluctuations over the first 20 ns, followed by stabilization, showing behavior similar to the reference compound. Entandrophragmin (**2**) displayed fluctuation patterns comparable to the Apo-form, indicating minimal impact on the protein's backbone. The average RMSD values for the reference compound azadirachtin, entandrophragmin (**2**), encandollen B (**4**), encandollen C (**5**), and the free state of the protein were found to be 0.22 ± 0.02, 0.28 ± 0.03, 0.24 ± 0.01, 0.22 ± 0.01, and 0.30 ± 0.04 nm, respectively, as illustrated in Figure 5. These results indicate that compound **5** exhibits a lower average root mean square deviation (RMSD) value compared to the reference compound, suggesting that encandollen C (**5**) is the most promising inhibitor among the candidates.

3.3.2. Root Mean Square Fluctuations

The Root Mean Square Fluctuation (RMSF) is a useful tool for studying the average fluctuations in the positions of individual residues or atoms in a dynamic system. It helps to assess the flexibility of residues within protein–ligand complexes compared to their mean spatial arrangement as they change over time. Similar to the RMSD measurement, higher RMSF values in a biomolecular system indicate lower residual stability, and vice versa [40,41]. According to the results shown in Figure 6, all of the shortlisted compounds exhibit a very similar RMSF pattern to the standard compound. The average RMSF values for the Apo protein, azadirachtin, entandrophragmin (**2**), encandollen B (**4**), and encandollen C (**5**) were found to be 0.14 ± 0.14, 0.12 ± 0.09, 0.13 ± 0.11, 0.11 ± 0.07, and

0.11 ± 0.08 nm, respectively. Once again, the lower average RMSF value of encandollen C (**5**) indicates that it is a more effective inhibitor compared to the other two compounds.

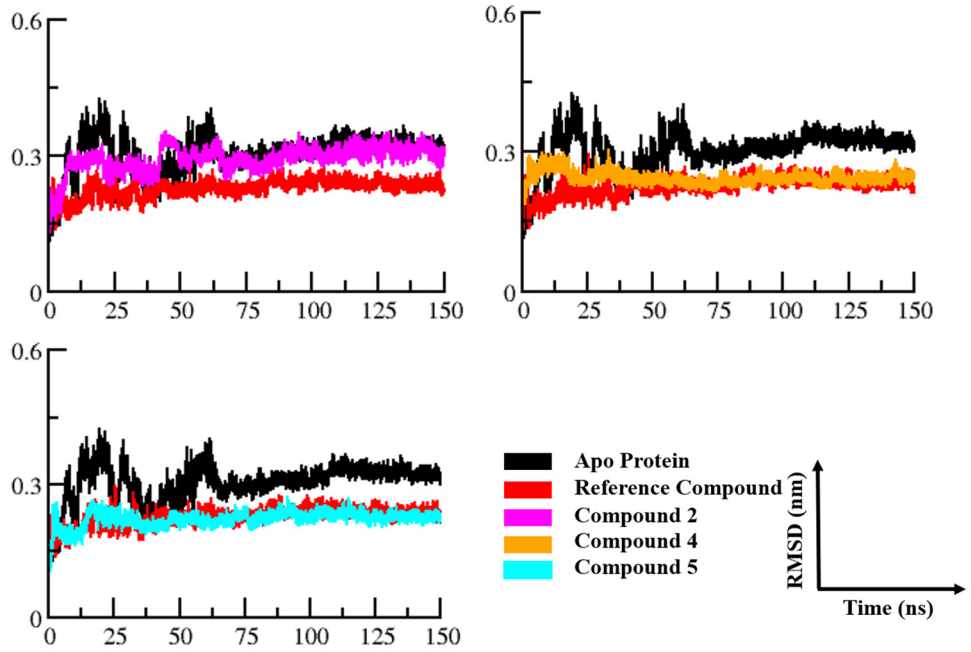


Figure 5. The RMSD spectrum of the Apo protein, the reference compound (azadirachtin), and compounds **2**, **4**, and **5** during the MD simulation of 150 ns.

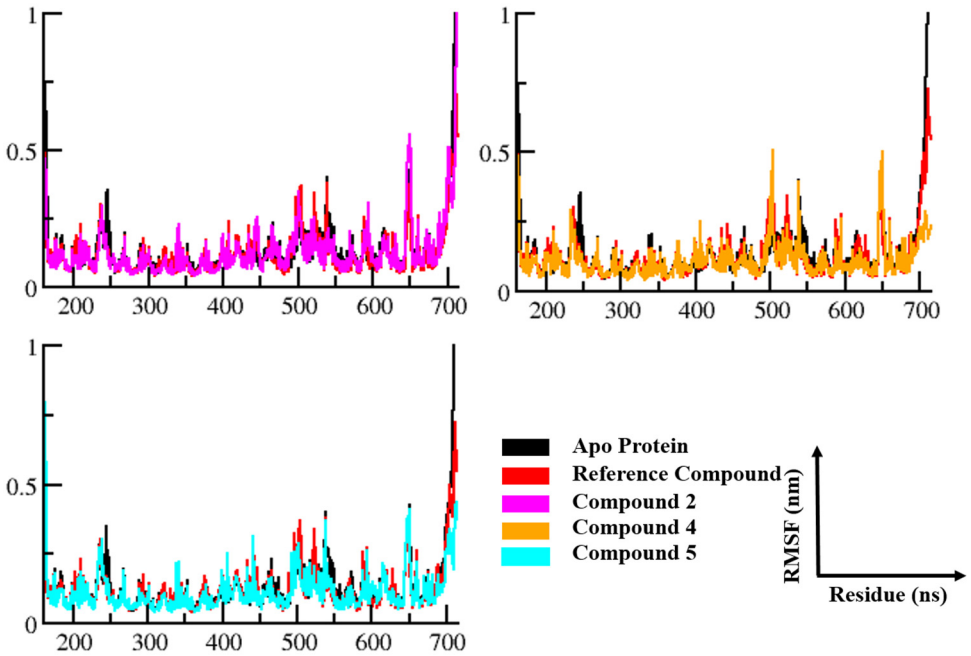


Figure 6. The RMSF spectrum of the Apo protein, the reference compound (azadirachtin), and compounds **2**, **4**, and **5** during the MD simulation of 150 ns.

3.3.3. Radius of Gyration

The radius of gyration (Rg) was used to assess the overall compactness of the protein in the absence and presence of virtual hits. For a well-folded, stable molecular structure, the Rg value is usually constant, while the Rg value of an unfolded structure changes over time [42]. The average Rg values, as shown in Figure 7, were determined for the Apo protein, azadirachtin, entandrophragmin (**2**), encandollen B (**4**), and encandollen C

(**5**), resulting in respective values of 2.40 ± 0.01, 2.40 ± 0.01, 2.30 ± 0.01, 2.40 ± 0.01, and 2.30 ± 0.01 nm. The analysis of the Rg plot revealed that during the 150 ns simulation, the protein maintained its compactness after binding to compounds **2**, **4**, and **5**.

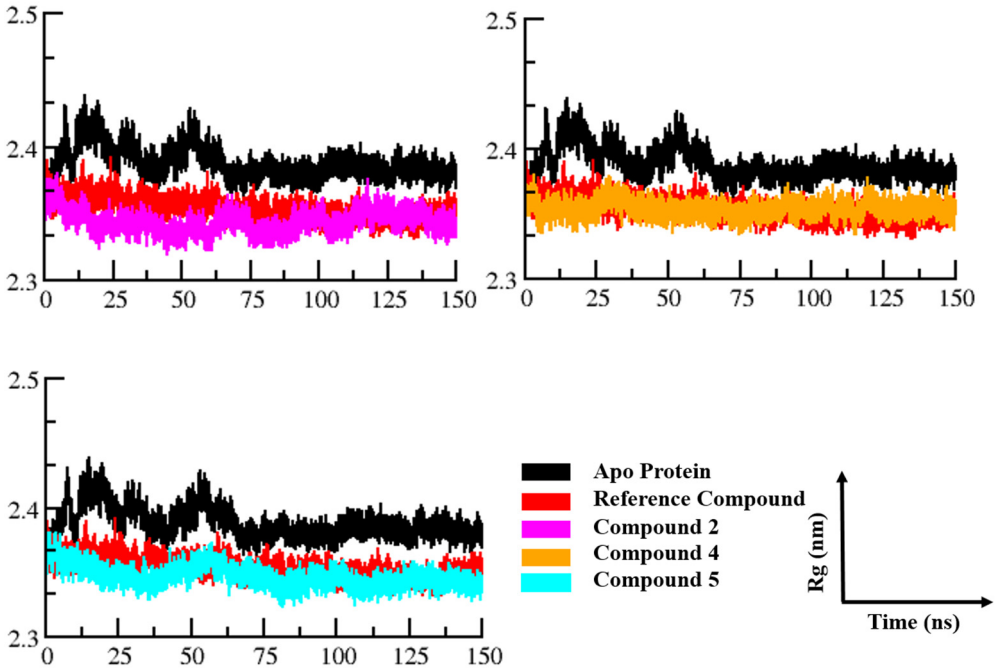


Figure 7. The Rg spectrum of the Apo protein, the reference compound (azadirachtin), and compounds **2**, **4**, and **5** during the MD simulation of 150 ns.

4. Conclusions

A total of twelve limonoids previously reported from four *Entandrophragma* species collected in Cameroon have been computationally evaluated for their potential inhibitory activity against acetylcholinesterase from the mosquito *Anopheles gambiae*. The docking scores of the evaluated compounds, in comparison with the well-known insecticidal limonoid azadirachtin, allowed us to select the phragmalin-type limonoid orthoesters entandrophragmin (**2**), encandollen B (**4**), and encandollen C (**5**) for in-depth mechanistic analysis. The analysis of the radius of the gyration plot showed that AChE kept its compactness over 150 ns of simulation with all three hits, while encandollen C (**5**) demonstrated more promising effectiveness based on its lower average RMSD and RMSF values compared to the reference. Therefore, it might be classified as a good candidate for the development of new potent eco-friendly insecticides. This is the first in silico evaluation of limonoids from *Entandrophragma* for the computational validation of their AChE inhibitory activity that could significantly help fight malaria. This study further indicates that phragmalin-type limonoids with their characteristic orthoester bridges are the more potent insecticidal compounds amongst the limonoids with open rings found in *Entandrophragma* plants. However, despite the promising in silico results obtained in this study, further analyses of pharmacological activities, including the in vitro AChE inhibitory activity of compounds **2**, **4**, and **5**, as well as their insecticidal or larvicidal activities against *A. gambiae* (or its larvae), are required to confirm their effectiveness in killing the mosquito and for the efficient development of potent and less toxic insecticides that will significantly contribute to fighting malaria in Africa and tropical regions of the world.

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