

Molecular Docking And In Silico Analysis Of Parkia Biglobosa Bioactive Compounds For Inhibition Of Acetylcholinesterase And L-Amino Acid Oxidase In Snake Venom-Induced Systemic Toxicity

Iyiola Aanuoluwa Temitayo

aanuoluwaigiola94@gmail.com

Department of Biochemistry, School of Life Sciences, Federal University of Technology, Minna, Nigeria
<https://orcid.org/0009-0005-8708-6365>

Orjiewulu Vivian Chidera

Department of Pharmacy, Madonna University, River State, Nigeria <https://orcid.org/0009-0004-4709-4582>

Charles-Ukeagu Love

Department of Microbiology, Immunology and Pharmacology, St George's University Grenada
<https://orcid.org/0009-0004-5899-368X>

Adefemi Oluwasegun Temitope

Department of Biomedical Technology, Federal University of Technology, Akure, Nigeria.
<https://orcid.org/0009-0009-8226-1434>

Fashina Latifat Tolulope

Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, P.M.B. 12003, Lagos, Nigeria. <https://orcid.org/0009-0000-1701-3690>

Obialor Akachukwu Precious

Department of Biotechnology, Ural Federal University, Russia <https://orcid.org/0000-0003-3995-5333>

Oyeyinka Barnabas Oluwatomide

Biomedicinal Research Centre (BMRC), Forestry Research Institute of Nigeria, Ibadan- Oyo State, Nigeria <https://orcid.org/0000-0003-1191-9365>

Mahmud Habib

Department of Pharmaceutics and Industrial Pharmacy, Ahmadu Bello University, Zaria, Nigeria.
<https://orcid.org/0009-0006-0601-6611>

Iyiola Emmanuel Bukola

Department of microbiology, Faculty of life sciences, University of Ilorin, Ilorin, Kwara state, Nigeria.
<https://orcid.org/0009-0009-3205-3979>

Research Article

Keywords: Parkia biglobosa, Snakebite envenomation, Molecular docking, Acetylcholinesterase, L-amino acid oxidase, ADMET profiling

Posted Date: May 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9632624/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations:

The authors declare no competing interests.

Ethics approval, consent to participate, consent to publish: Not applicable

Consent for Publication: All the authors approved the manuscript for publication

Data and/or code availability: All data generated or analysed during this study are included in this published article. Further enquiries can be directed to the corresponding author

Competing interest: The authors declare that we have no competing interests

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions: Study concept and design: I.A, O.C, C.L. Data collection: O.C, A.O, F.T. Methodology: I.A, O.A, O.O, M.H, I.E. Software: I.A, I.E, M.H, O.C. Supervision: I.A, C.L, O.O; Validation: A.O, O.O and I.A. Writing- original draft: F.T, C.L, I.A. Writing- review & editing: I.A, I.EM.H and O.V. All authors have read, edited, and contributed to the content of this manuscript. This work has not been previously published and has not been considered for publication elsewhere.

Acknowledgements: We would like to express our sincere gratitude to all authors for their invaluable contributions and intellectual support during the preparation of this manuscript.

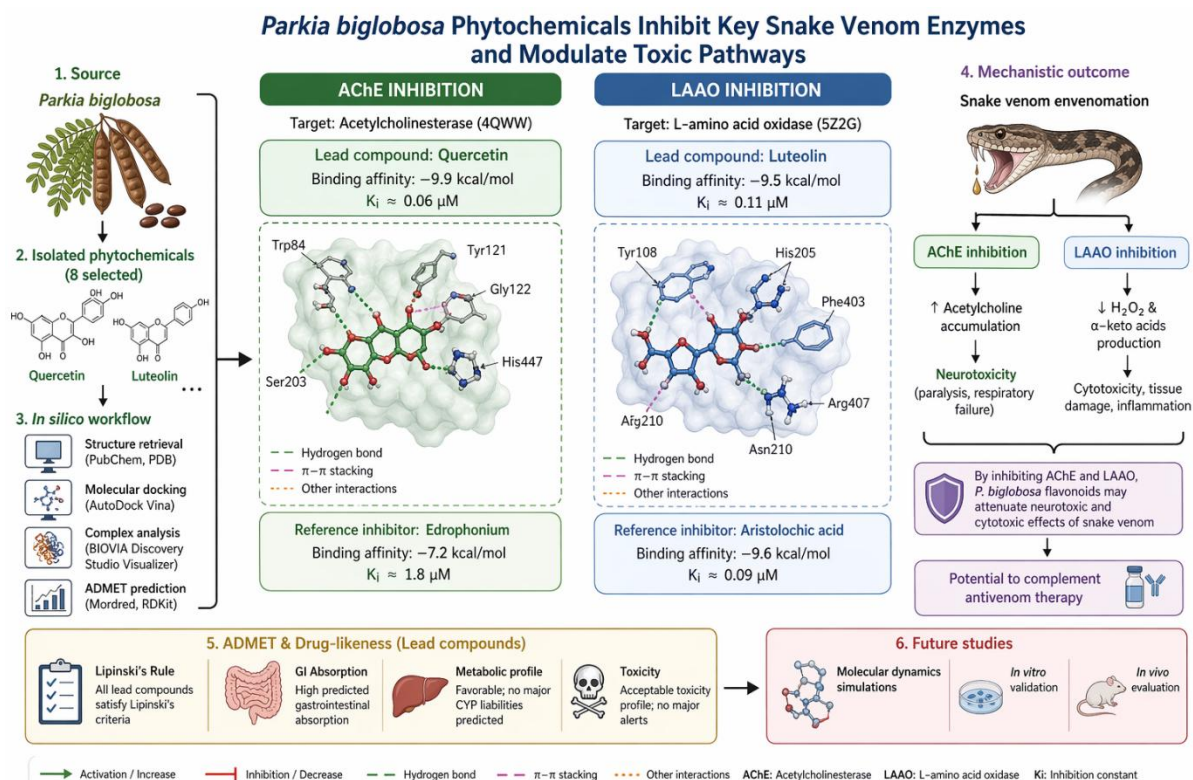
Molecular Docking and In silico analysis of *Parkia biglobosa* bioactive compounds for inhibition of acetylcholinesterase and L-amino acid oxidase in snake venom-induced systemic toxicity

ABSTRACT

Snakebite envenoming is a significant neglected tropical disease with a high mortality and morbidity burden, especially in resource-constrained areas, in which the systemic toxicity is mediated by enzymes like acetylcholinesterase (AChE) and L-amino acid oxidase (LAAO). Despite the ethnomedicinal use of *Parkia biglobosa* in managing envenomation, its bioactive constituents lack detailed molecular-level validation and integrated pharmacokinetic characterisation. This study investigated the inhibitory potential of selected *P. biglobosa* phytochemicals against AChE and LAAO using an integrative molecular docking and in silico pharmacokinetic approach. Eight bioactive compounds were selected from the plant, while edrophonium and aristolochic acid were used as control inhibitors in this study. The 3D structures of the bioactive compounds and the control inhibitors were retrieved from the PubChem database. Additionally, the crystal structures of acetylcholinesterase (4QWW) and L-amino acid oxidase (5Z2G) were obtained from the Protein Data Bank (PDB). Using the RDKit cheminformatics package, the drug-likeness of the bioactive compounds was assessed, and AutoDock Vina was employed for molecular docking with the target proteins. The docked complexes were analysed with BIOVIA Discovery Studio Visualizer. Additionally, the Mordred and RDKit libraries were used to identify the ADMET properties of the ligands. Quercetin and luteolin were the best candidates with desirable binding affinities, with quercetin strongly binding AChE (−9.9 kcal/mol; $K_i \approx 0.06 \mu\text{M}$) outperforming the reference inhibitor edrophonium, and luteolin displaying a competitive affinity towards LAAO (−9.5 kcal/mol; $K_i \approx 0.11 \mu\text{M}$). Each of the lead compounds met Lipinski requirements, exhibited high predicted gastrointestinal absorption and acceptable toxicity profiles. These results imply that flavonoid scaffolds of *P. biglobosa* might engage with important catalytic sites in both enzymes, and thereby may mediate neurotoxic and cytotoxic pathways of envenomation, which are molecular-level-supported indicators of the ethnopharmacological significance of *P. biglobosa*, and may be useful as a complement to antivenom therapy in snakebite envenomation. Further, molecular dynamics, in vitro and in vivo studies are required to validate these results.

Keywords: *Parkia biglobosa*, Snakebite envenomation, Molecular docking, Acetylcholinesterase, L-amino acid oxidase, ADMET profiling

Graphical Abstract



1.0 INTRODUCTION

Snakebite envenoming (SBE) is a worldwide health emergency, which has recently been classified by the World Health Organization as a priority neglected tropical disease because of its shocking death and disability statistics [5, 40]. Globally, snakebites cause an estimated 5.4 million cases and lead to as many as 138,000 deaths, and hundreds of thousands are permanently physically handicapped or psychologically traumatized [17,41]. This is especially acute in the rural areas of sub-Saharan Africa and the Indian subcontinent, where farming activities expose humans to more interactions with snakes and where health services are inadequate [18, 42]. SBE is a significant cause of mortality in Nigeria, especially in the northeastern and north-central savanna, where the venomous elapids and vipers like *Naja nigricollis* and *Echis ocellatus* are common [18, 39].

Envenomation pathophysiology is complicated, with a rapid neurotoxic paralysis and intense local cytotoxicity [22]. Neurotoxicity commonly results from the blockade of nicotinic acetylcholine receptors at the neuromuscular junction, leading to respiratory failure, whereas cytotoxic components contribute to tissue necrosis, haemorrhage, and systemic inflammation [18, 29].

The traditional approach to treatment of SBE involves administration of animal-derived antivenom only through intravenous means [18]. Although it is effective against systemic changes, antivenoms have serious clinical and

logistical challenges that include: high cost, need to keep at cold-chain conditions, and inability to counteract local tissue injury that starts as soon as one is bitten [5,31]. Moreover, the possibility of hypersensitivity, including anaphylaxis and serum sickness, requires clinical attention, which is, in most cases, not possible in remote envenomation settings [18]. Such restrictions have perpetuated a traditional dependence on ethnomedicinal plants in the treatment of snakebites in Africa [2]. One of these is *Parkia biglobosa* (Jacq.). R.Br. ex G. Don, which is also referred to as the African locust bean, has gained a lot of popularity in neutralizing the effects of cobra and viper venoms [3, 28]. Past pharmacological experiments on *P. biglobosa* extracts have shown antioxidant, anti-inflammatory, and anti-hemorrhagic effects, which are commonly explained by the high concentration of tannins, flavonoids, and phenols in the extracts [17, 28]. The antibodies used in antivenom studies include the acetylcholinesterase (AChE), a vital modulatory target in neurotoxicity, and L-amino acid oxidase (LAAO), a flavoenzyme that causes oxidative stress, apoptosis, and tissue necrosis [7, 32].

Despite the established ethnomedicinal use and preliminary *in vitro* efficacy of *P. biglobosa*, there is a major research gap on molecular-based interactions of its secondary metabolites with venom enzymes. Additionally, while the success of traditional practitioners with the use of bark and leaf infusions is reported, the types of bioactive scaffolds that produce those effects are not well characterized in regard to their binding properties and pharmacokinetic profile [3, 21]. A significant part of the available literature is based on crude extract studies without the use of any modern computational drug discovery methods, including molecular docking and ADMET profiling, which are necessary in the process of drug development [12, 43]. In addition, no strict comparative study has been done on these natural phytochemicals and common pharmacological inhibitors. Such a lack of mechanistic and pharmacokinetic validation prevents the development of traditional remedies into standardized adjunct treatment, which can be used to complement current protocols of antivenom treatment [5, 17].

These limitations are overcome by employing a computational biology method in this study that assesses the therapeutic potential of eight bioactive phytochemicals of *P. biglobosa* against systemic toxicity pathways mediated by AChE and LAAO. *In silico* molecular docking is a fast and cost effective technique used to determine the binding affinity and orientation of ligands in the active sites of enzymes, giving information that would otherwise be hard to determine via wet-lab studies only [8, 43]. The analysis of ADMET profiling also evaluates the drug-likeness and safety of such compounds, a crucial measure in limiting late-stage attrition in drug discovery [26, 45]. The selection of targets like AChE and LAAO was based on the centrality of these targets in lethal neurotoxic and cytotoxic cascades of elapid envenomation [33, 34]. The compounds of interest, such as flavonoids quercetin, luteolin, catechin, among others, were chosen on the basis of their established presence in *P. biglobosa* and structural complexity that enables them to anchor on multi-point receptors [28, 32].

The main objective of the experiment is to determine the observed binding affinities, interactions at the residue level, and pharmacokinetic properties of the *Parkia biglobosa* secondary metabolites as potential snake venom-implicated enzyme inhibitors. The research uses the comparative docking to edrophonium and aristolochic acid as the standard inhibitors, which meet the rule of five presented by Lipinski, to determine the promising bioactive leads that may be orally bioavailable [11, 12]. These discoveries can help in the continued identification of inexpensive, natural antivenom adjuncts that can be effective in counteracting the tragic effects of snakebite envenomation of the susceptible populations [19, 24, 36] by offering a molecular framework of AChE and LAAO activity modulation.

2.0 MATERIALS AND METHODS

2.1. Ligand Selection and Structural Retrieval

A library of eight bioactive phytoconstituents from *Parkia biglobosa* was curated by identifying secondary metabolites; specifically phenols, flavonoids, and terpenoids, which are documented within the Nigerian Medicinal Plants and Phytochemicals (NMPP) Nigerian Medicinal Plants and Phytochemicals (NMPP)(<https://nmppdb.com.ng/species-details?specy=%20parkia-biglobosa&hl=en-GB>). The 2D chemical structures (Canonical SMILES), 3D structures (SDF) and unique PubChem identification numbers (CIDs) of the NCBI PubChem web server (<https://pubchem.ncbi.nlm.nih.gov/>) were retrieved to make high-throughput analysis possible. The reason why these natural compounds were chosen in this study is that they have different scaffold structures that provide them with a variety of hydrogen-bond donor/acceptor sites that are critical in exhibiting competitive inhibition at the catalytic pocket of the enzymes in the venom. To establish a rigorous comparative baseline, Edrophonium (CID: 3202) and Aristolochic acid (CID: 2236) were selected as the control inhibitors for AChE and LAAO, respectively, with their structural data acquired through the PubChem database to ensure methodological consistency across the entire in silico library. Table 1 presents bioactive compounds from the African plants and the control inhibitors. Figure 2 presents the flowchart of the study.

2.1 1 Ligand Preparation and Optimisation

All ligand preparations were conducted within a Python 3.10- based Linux environment to ensure strict methodological reproducibility.. Using the open-source cheminformatics toolkit RDKit (v2023.09.1), hydrogen atoms were programmatically added to simulate physiological pH (7.4) protonation states [23]. Spatial coordinates (3D) were then constructed using Experimental-Torsion Knowledge Distance Geometry (ETKDG) algorithm, and the structure was individually relaxed and minimized with the Universal Force Field (UFF) to obtain the lowest-energy bioactive conformer of every ligand.. Finally, the optimized 3D structures were converted into the AutoDock Vina-compliant .pdbqt format using the Meeko Python module [14]. Meeko was strictly employed to calculate partial Gasteiger charges, assign AutoDock atom types, and define torsional degrees of freedom by embedding precise Vina-compliant topological tags (ROOT, BRANCH, and ENDBRANCH), thereby yielding a standardized, fully prepared ligand library ready for molecular docking simulations.

2.2 Protein Target Selection

The three-dimensional (3D) crystallographic structures of the target proteins were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (<https://www.rcsb.org/>). To evaluate the inhibition of venom-induced neurotoxicity, the crystal structure of Bungarus fasciatus Acetylcholinesterase (BfAChE) was obtained under PDB ID: 4QWW (2.70 Å). For the assessment of systemic hemorrhage and apoptosis, the crystal structure of L-amino acid oxidase (LAAO) from the venom of *Naja atra* was retrieved using PDB ID: 5Z2G (2.68 Å). These specific targets were prioritized as they represent well-characterized experimental models for studying the enzymatic mechanisms of elapid systemic toxicity. The high-resolution X-ray diffraction

data for both proteins ensure the structural integrity of the catalytic sites required for precise molecular docking simulations. Graphical representations of these target proteins are presented in Figure 1a & figure 1b.

2.2.1 Protein Target Preparation

The 3D structures of the target proteins obtained from the protein data bank (PDB) were prepared and cleaned within the Linux-based interactive environment (Ubuntu 24.04.1 LTS) environment to ensure structural suitability for molecular docking [6]. Essential chains were retained while all non-standard residues, water molecules, and co-crystallized ligands were computationally removed to isolate the refined receptors. To simulate physiological conditions, OpenBabel (v3.1.1) was employed for protonation at pH 7.4, where polar hydrogens were added to establish the necessary hydrogen-bonding networks [30]. Non-polar hydrogens were subsequently merged using the United-Atom model to optimize the scoring efficiency of the AutoDock Vina engine. During the intermediate refinement phase, Gasteiger-Marsili partial charges were assigned to ensure accurate electronegativity perception across the protein scaffolds. The resulting protein structures were translated into the Vina-compatible PDBQT format, with Gasteiger charges assigned and a specific type of AutoDock atom typing to characterise the aromaticity and hydrogen-bond donor/acceptor sites.

2.3 Screening for Drug - Likeness

To evaluate the oral bioavailability and drug-likeness of the selected ligands, we performed a programmatic screening using the RDKit (v2023.09.1) (<https://www.rdkit.org/>) cheminformatics package within a Python 3.x environment. The Simplified Molecular Input Line Entry System (SMILES) of bioactive compounds and the control inhibitors, obtained from the PubChem online web server, were utilized for the screening process. The selected compounds, including the natural leads and positive control inhibitors, were subjected to Lipinski's Rule of Five (Ro5) filters. Key physicochemical descriptors, including Molecular Weight (MW), Octanol-water partition coefficient (LogP), Hydrogen Bond Donors (HBD), and Hydrogen Bond Acceptors (HBA), were calculated. In our study, any bioactive compounds exhibiting fewer than two violations were prioritized for further molecular docking simulations, ensuring the identified hits possess viable pharmacokinetic scaffolds for downstream drug development.

2.4 Receptor Grid Generation

To facilitate site-specific molecular docking, the search space for each target receptor was defined to cover the critical catalytic and regulatory residues identified through literature-driven coordinate mapping and co-crystallized ligands coordinates. For Acetylcholinesterase (4QWW), a grid box of 28.4 x 31.6 x 25.8 Å was centered at coordinates X: 12.302, Y: -38.322, and Z: -40.812. This box was large enough to cover both the catalytic triad (Ser203, His446, and Glu333) at the bottom of the 20 Å deep gorge and the peripheral anionic site (PAS) at the entrance. For the L-amino acid oxidase (5Z2G), the grid size was set to 55.8 x 64.0 x 46.3 Å, centered at X: -48.405, Y: 7.443, and Z: -18.915. This region was used to monitor the ligand movement through the 25 Å hydrophobic funnel, starting with the Arg322 gatekeeper, to the FAD isoalloxazine ring. The mapping of these

coordinates was done relative to established active residues, to ensure that the docking search was done across the entire binding domain of the venom enzymes.

2.5 Molecular Docking

The binding affinity of the bioactive compounds in *Parkia biglobosa* and the target, snake venom acetylcholinesterase and L-amino acid oxidase, against the bioactive compounds of the facilities was assessed by molecular docking simulations. AutoDock Vina (v1.2.5) [37] was used to run the simulations. The molecular docking software was uploaded with the 3D structure of the downloaded bioactive compounds and the standard inhibitor. The preparation of ligands was done through OpenBabel, which included the addition of polar hydrogens and Gasteiger partial charges to standardize the molecules at physiological pH. Literature-validated residues were used to mathematically define target search spaces that cover critical binding regions, such as the catalytic triad and peripheral anionic site of 4QWW, and the FAD-binding hydrophobic funnel of 5Z2G. Conformational searches were run with an exhaustiveness setting of 16, and the resulting poses were ranked by the lowest empirical binding free energy (ΔG , kcal/mol) to find the most stable ligand-receptor complexes.

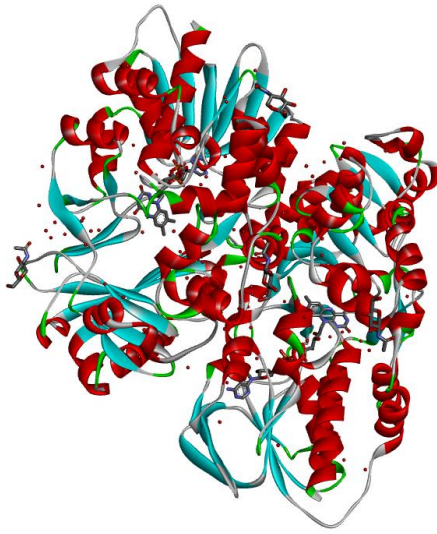
2.6 Assessment of Binding Affinity and Inhibition Potential

The estimated inhibition constant (K_i) was calculated to translate computational binding scores into standardized pharmacological values. The K_i value represents the dissociation constant of the enzyme-inhibitor complex; a lower K_i indicates stronger binding and greater inhibitory potential, as it reflects a reduced likelihood of complex dissociation. This constant is calculated using the equation, as described by Morris et al. [27]:

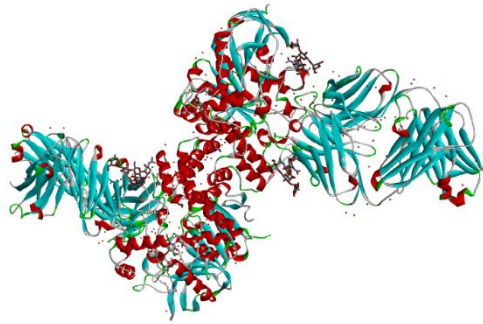
$$K_i = e^{RT\Delta G} \times 1000$$

Where, K_i =inhibition constant (in micromolar, $\mu M=10^{-6}M$), ΔG =binding free energy (in kcal/mol), R =universal gas constant ($1.987 \text{ cal K}^{-1} \cdot \text{mol}^{-1}$), and T =absolute temperature (298.15 K for 25 °C)

a



b



c.



Fig.1 Plant source and The target receptors: a. L –amino acid oxidase(PDB ID: 5Z2G); b. Acetylcholinesterase (PDB ID: 4QWW); c. *P. biglobosa* Plant(source: <https://nmppdb.com.ng/>).

2.7 Molecular Interaction Analysis

Molecular interaction analysis was performed to evaluate the binding stability and spatial orientation of the ligand-receptor complexes. The complexes generated were saved in PDB format and stored for further analysis. The optimal docking poses were visualized in three-dimensional (3D) and two-dimensional (2D) formats using the BIOVIA Discovery Studio Visualizer (v21.1) (<https://discover.3ds.com/discovery-studio-visualizer-download>). This analysis involved mapping the non-bonded interactions, such as hydrogen bonding, hydrophobic contacts, and electrostatic forces, between the bioactive compounds and the specific amino acid residues of the target proteins. The software was utilized to measure bond distances and define the chemical nature of the contacts within the active site, ensuring the observed binding affinities were supported by specific molecular geometries and inter-atomic distances. All 2D interaction maps and 3D surface visualizations were exported to characterize the structural basis of the inhibition.

2.8 In Silico ADMET Profiling and Lead Prioritization

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of the bioactive compounds and reference standards were predicted using a computational pipeline executed in Jupyter Lab. Molecular descriptors were calculated using the RDKit (v2023.09.1) and Mordred libraries (<https://github.com/mordred-descriptor/mordred>) within the Python 3.10 environment to characterize the pharmacokinetic behaviour of the ligands. The analysis was aimed at predicting critical parameters; the Gastrointestinal (GI) absorption and Blood-Brain Barrier (BBB) permeance were predicted based on TPSA and LogP thresholds, whereas susceptibility to P-glycoprotein (P-gp) efflux and CYP3A4 enzymatic inhibition were predicted through fragment-based toxicophore identification [12]. Moreover, a multi-parameter safety test was performed, including acute oral toxicity (LD 50 estimation), hERG-mediated cardiotoxicity risk and AMES genotoxicity screen. The compounds were prioritized by a Multi-Parameter Optimization approach (MPO) with the priority of "Hit" to the ligands with zero Ro5 violations and a better safety profile in comparison to the existing standards.

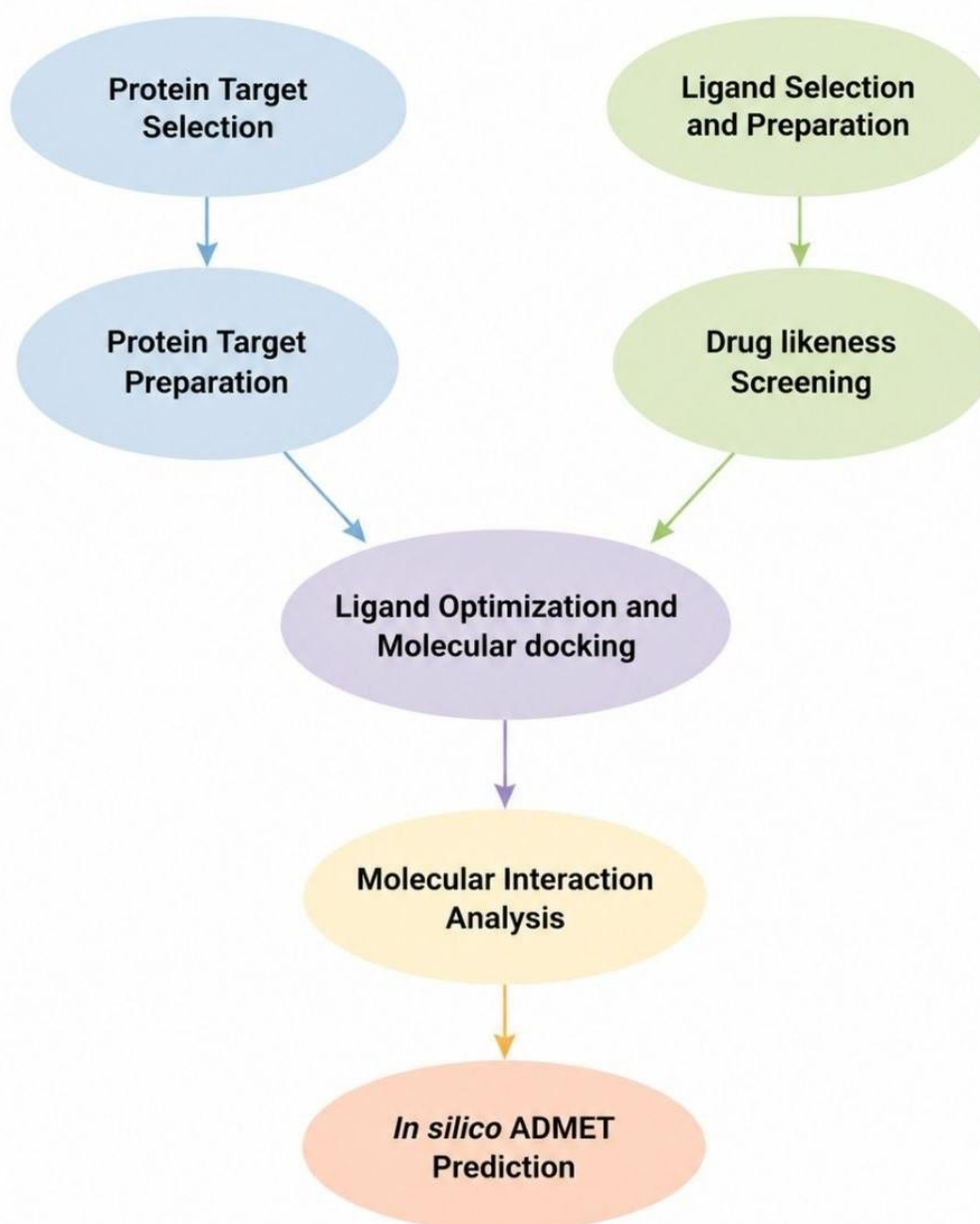


Fig 2: Stepwise flowcharts Outlining the methods employed in this *in-silico* study. This workflow involves protein target selection and preparation, alongside ligand selection and drug-likeness screening. These pathways converge at ligand optimization and molecular docking, followed by molecular interaction analysis and *in silico* ADMET prediction to evaluate pharmacokinetic and toxicity profiles.

3.0 RESULTS

Table 1: Lipinski's drug-likeness screening of the bioactive compounds and the control Inhibitors

	Compound_Name	Chemical formula	PubChem_CID	Molecular weight (MW)	LogP	HBD	HBA	nRotB	RO5_Violations
0	2,6-Dimethoxyphenol	C ₈ H ₁₀ O ₃	7041	154.16	1.41	1.0	3.0	2.0	0.0
1	4-hydroxy-3-methoxy-benzaldehyde (Vanillin)	C ₈ H ₈ O ₃	1183	152.15	1.21	1.0	3.0	2.0	0.0
2	Beta-amyrin	C ₃₀ H ₅₀ O	73145	386.66	7.24	1.0	1.0	0.0	1.0
3	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	7311	206.33	3.99	1.0	1.0	0.0	0.0
4	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	3026	278.35	3.60	0.0	4.0	8.0	0.0
5	Quercetin	C ₁₅ H ₁₀ O ₇	5280343	302.24	1.99	5.0	7.0	1.0	0.0
6	Luteolin	C ₁₅ H ₁₀ O ₆	5280445	286.24	2.28	4.0	6.0	1.0	0.0
7	Catechin	C ₁₅ H ₁₄ O ₆	9064	290.27	1.55	5.0	6.0	1.0	0.0
8	Edrophonium (STD)	C ₁₀ H ₁₆ NO ⁺	3202	166.24	1.98	1.0	1.0	2.0	0.0
9	Aristolochic acid I (STD)	C ₁₇ H ₁₁ NO ₇	2236	341.28	3.34	1.0	6.0	3.0	0.0

3.1 Drug-likeness screening of selected compounds

The drug-like properties of the promising bioactive compounds of the *Parkia biglobosa* were assessed considering important physicochemical descriptors, such as the molecular weight (MW), lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), number of rotatable bonds (nRotB), and a violation of the Lipinski rule of five (RO5). As observed in Table 1, all the lead compounds, quercetin, luteolin, catechin, vanillin, and 2,6-dimethoxyphenol, in general, adhered to the Lipinski rule, with the only cases of zero violation. Their molecular weights fell within the acceptable range (less than 500 Da), and their LogP values were medium lipophilicity, which is a good indication of membrane permeability. Moreover, the amount of hydrogen bond donors and acceptors of these compounds stayed within the acceptable range, which is an indication of their

possible good oral bioavailability. The number of hydrogen bond donors and acceptors of quercetin, luteolin and catechins was relatively higher than that of other compounds, and this could facilitate the formation of stable interactions with the target proteins. Nevertheless, these values were still at acceptable levels, which means that there was no compromise in drug-likeness. The molecular weights of vanillin and 2, 6- dimethoxyphenol were lower, and their structure was less complex; their physicochemical properties further justify their use as drug candidates. Conversely, beta-amyrin had a single violation of the Lipinski rule mainly because its lipophilicity was high (LogP > 5), hence limiting its bioavailability. Nevertheless, other compounds like dibutyl phthalate and 2,4-di-tert-butylphenol had acceptable profiles with no violation of rules. The standard inhibitors, edrophonium (AChE inhibitor) and aristolochic acid I (LAAO inhibitor), also complied with Lipinski's criteria, validating the screening approach used in this study. These results indicate that the selected lead compounds possess favourable drug-like properties and are suitable for further molecular docking and interaction studies.

Table 2a: Molecular docking scores and binding interaction analysis of selected phytochemicals and standard inhibitor against AChE.

Compound s	Bindin g score (kcal/m ol)	Inhibit ion affinity (μ M)	Hydrog en bond (Numbe rs)	Hydrog en bond (Interac ted residues)	Hydroph obic bond (Number s)	Hydroph obic bond (Interacte d residues)	Others (Numbe rs)	Others (Interac ted residues)
Quercetin	-9.9	0.06	5	MET68, ASN 83, SER 120, TYR 128, UNK 426	3	Tyr 328, TRP 82	1	MET 68
Luteolin	-9.7	0.07	5	GLN 67, TRP 82, GLY 115, GLU 197, UNK 426	2	TRP 82	-	-
Catechin	-8.2	0.91	3	ASN 83, GLY 116, GLU 197	3	Tyr 119, TRP 82	1	GLU 197

Compound s	Bindin g score (kcal/m ol)	Inhibit ion affinity (μM)	Hydrog en bond (Numbe rs)	Hydrog en bond (Interac ted residues)	Hydroph obic bond (Number s)	Hydroph obic bond (Interacte d residues)	Others (Numbe rs)	Others (Interac ted residues)
Vanillin	-5.9	40.91	2	Tyr 128, SER 120	1	TRP 82	-	-
2,6- dimethoxyph enol	-5.6	60.82	3	SER 120, ASN 83, GLU 197	1	TRP 82	-	-
Standard (Edrophoni um)	-6.5	15.50	4	Trp 82, SER 130, GLY GLY 116, GLU A197	5	TYR 328, TRP 82, GLY 115	1	GLU 197

Table 2b: Molecular docking scores and binding interaction analysis of selected phytochemicals and standard inhibitor against LAAO.

Compound s	Bindin g score (kcal/m ol)	Inhibit ion affinity (μM)	Hydrog en bond (Numbe rs)	Hydrog en bond (Interac ted residues)	Hydroph obic bond (Number s)	Hydroph obic bond (Interacte d residues)	Others (Numbe rs)	Others (Interac ted residues)
Quercetin	-9.2	0.17	3	GLU 3, GLU 8, GLU 12	3	His 90, TYR 11	1	ARG89

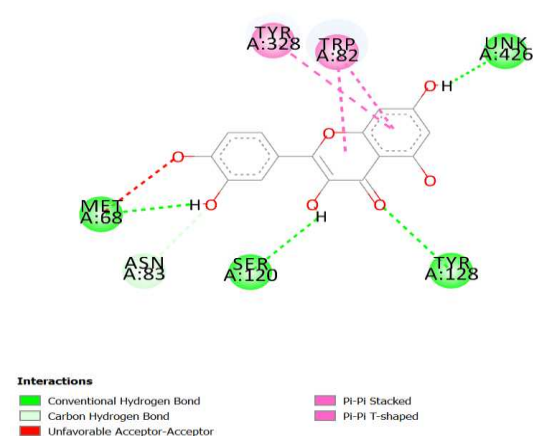
Compound s	Bindin g score (kcal/mol)	Inhibit ion affinity (μM)	Hydrog en bond (Numbe rs)	Hydrog en bond (Interacted residues)	Hydroph obic bond (Number s)	Hydroph obic bond (Interacted residues)	Others (Numbe rs)	Others (Interacted residues)
Luteolin	-9.5	0.11	4	LYS 338, ARG 85, SER 215, GLU 204,	5	PHE 222, LEU 202, TYR 152, SER 218	3	LYS 338, SER 218, ASP 219
Catechin	-8.5	0.61	2	ASN 203, GLN 109	3	LEU 367, PHE 222, ASN 203	1	TYR 365
Vanillin	-5.9	49.75	3	GLU 8, ARG 91, HIS 90	1	TYR 11	1	PHE 6
2,6-dimethoxyphenol	-5.7	63.12	2	GLU 8, GLN 7	1	TYR11	-	-
Standard (Aristolochic acid)	-9.8	0.07	4	ASN 203, ARG 85, ARG 315	4	PHE 222, LEU 202	-	-

3.2 Molecular Docking, Inhibition Affinity, and Molecular Interaction Analysis

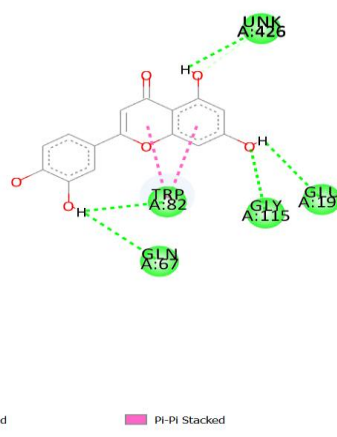
The binding pockets of snake venom acetylcholinesterase (AChE) and L-amino acid oxidase (LAAO) were identified based on literature-validated residues and co-crystallized inhibitor. This data helped ensure that the bioactive compounds from *Parkia biglobosa* were correctly placed in the enzymes' active sites. In this study, the reference inhibitors, Edrophonium (for AChE) and Aristolochic acid (for LAAO), were docked to their respective proteins first to determine their binding strength and which parts of the enzymes they interact with. Following the screening of the bioactive compounds of the plant, a few phytochemicals showed a better or equal affinity to these standard inhibitors. During the docking analysis with AChE, Quercetin had the highest score with a binding energy of -9.9 kcal/mol and an estimated inhibition constant (K_i) of 0.06 mM. Five hydrogen bonds with MET68, ASN83, SER120, TYR128, and UNK426 supported this high affinity, and hydrophobic interactions with TYR328 and TRP82. Luteolin was the second-best with a binding energy of -9.7 kcal/mol and K_i of 0.07 μM. It created

five hydrogen bonds with GLN67, TRP82, GLY115, GLU197 and UNK426, as well as hydrophobic contacts with TRP82 (Table 1; Fig. 1a, b). Catechin was the next to bind with a binding score of -8.2 kcal/mol, with hydrogen bonds formed with ASN83 and GLY116 and GLU197, and hydrophobic interactions with TYR119 and TRP82. Conversely, the conventional drug Edrophonium exhibited a much lower binding energy of -6.5 kcal/mol, with hydrogen bonding of TRP82, SER130, GLY116 and GLU197, and hydrophobic interactions of TYR328, TRP82 and GLY115. The standard inhibitor Aristolochic acid gave a binding energy of -9.8 kcal/mol and K_i of 0.07 μ M in the docking analysis against the target- LAAO with hydrogen bonds formed with ASN203, ARG85 and ARG315. Luteolin demonstrated a highly competitive affinity of -9.5 kcal/mol (K_i : 0.11 μ M), anchoring itself through four hydrogen bonds with LYS338, ARG85, SER215, and GLU204, and five hydrophobic interactions with PHE222, LEU202, TYR152, and SER218. Quercetin followed with a binding score of -9.2 kcal/mol, establishing hydrogen bonds with GLU3, GLU8, and GLU12, as well as hydrophobic bonds with HIS90 and TYR11 (Table 1; Fig. 2a, b). Catechin also showed significant potential with a binding energy of -8.5 kcal/mol, interacting with ASN203 and GLN109 through hydrogen bonding and forming hydrophobic contacts with LEU367, PHE222, and ASN203. Smaller phenolic compounds, Vanillin and 2,6-dimethoxyphenol, exhibited binding affinities of -5.9 and -5.7 kcal/mol, respectively, with Vanillin forming hydrogen bonds with GLU8, ARG91, and HIS90. Overall, the lead flavonoids showed a distinct ability to saturate the catalytically active sites more effectively than the reference standards across both venom-implicated enzymes. The 3D visualizations confirmed that these compounds, particularly the flavonoids, fit better within the enzyme cavities and formed more stable interactions than the reference standards.

A



B



C

D

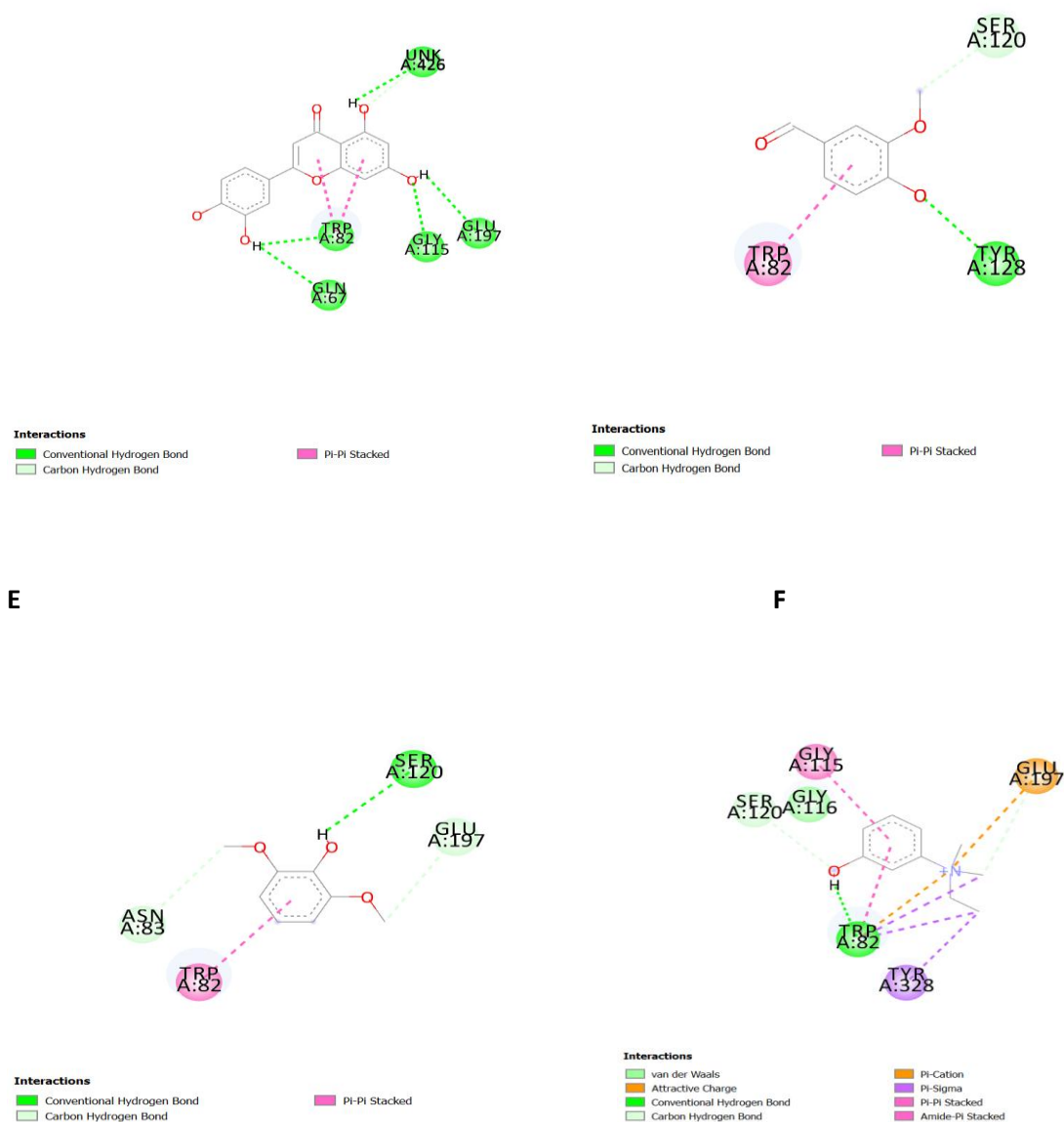
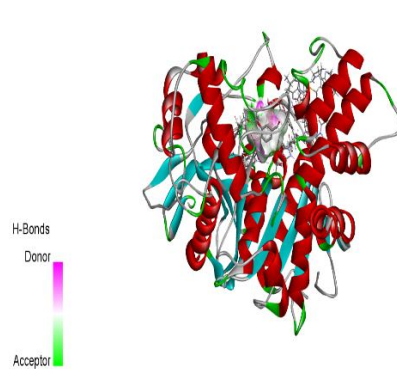
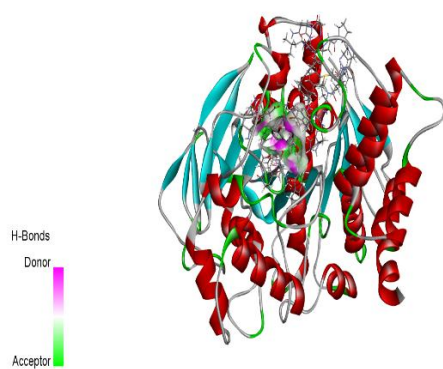
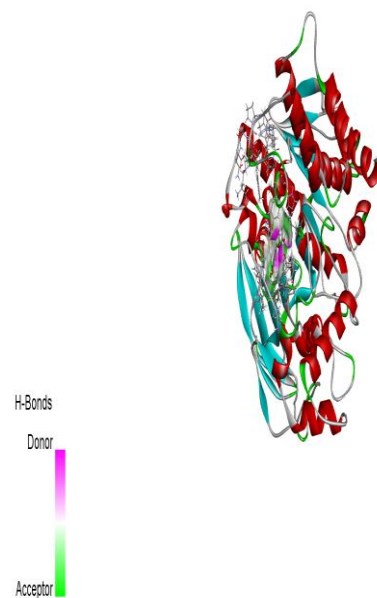
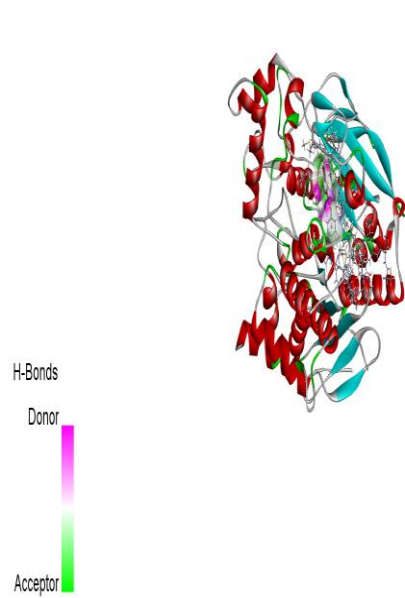


Fig. 3a 2D molecular contacts profiling of docked compounds with amino acid residues at the active site of AChE: (A) Quercetin (B) Luteolin (C) Catechin (D) 4-hydroxy-3-methoxy-benzaldehyde (E) 2,6-dimethoxyphenol (F) Standard (Edrophonium)



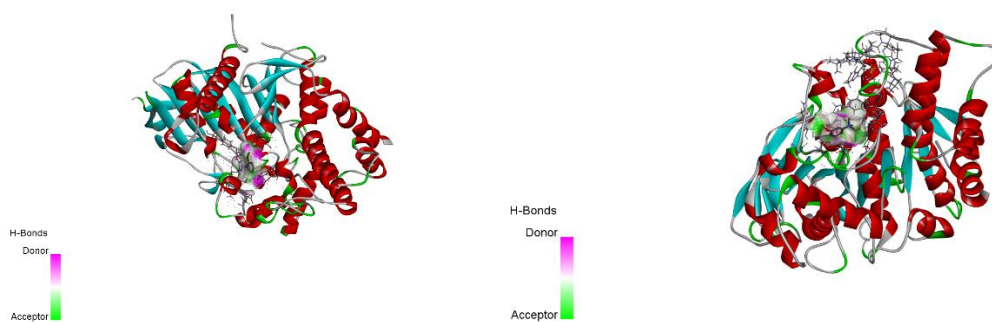
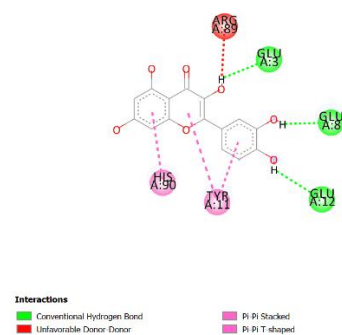
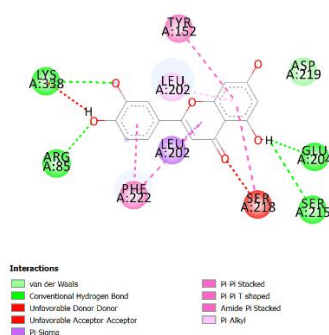


Fig. 3b 3D molecular contacts profiling of docked compounds with amino acid residues at the active site of AChE: (A) Quercetin (B) Luteolin (C) Catechin (D) 4-hydroxy-3-methoxy-benzaldehyde (E) 2,6-dimethoxyphenol (F) Standard (Edrophonium)

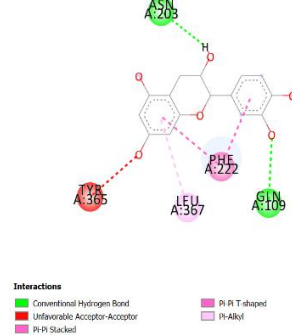
A



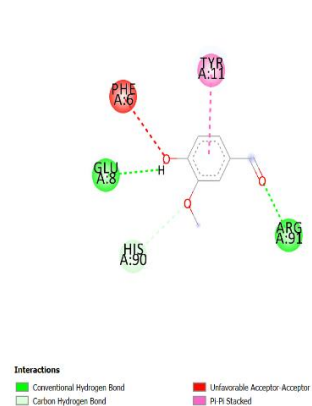
B



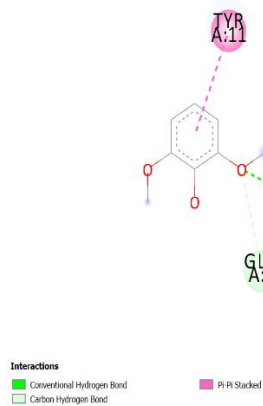
C



D



E



F

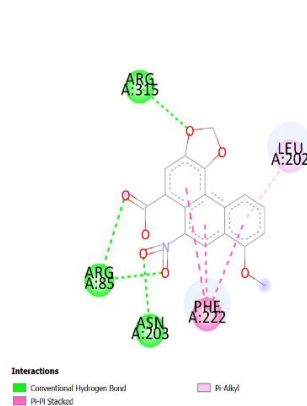
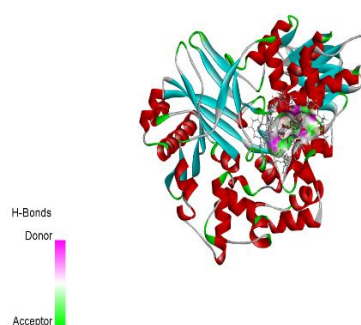
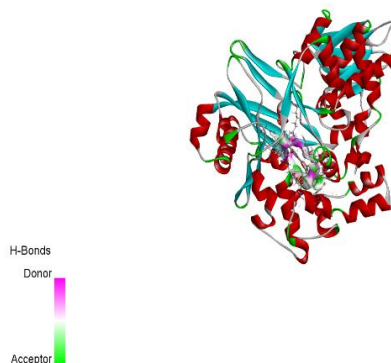


Fig. 4a 2D molecular contacts profiling of docked compounds with amino acid residues at the active site of LAAO: (A) Quercetin (B) Luteolin (C) Catechin (D) 4-hydroxy-3-methoxy-benzaldehyde (E) 2,6-dimethoxyphenol (F) Standard (Aristocholic acid)

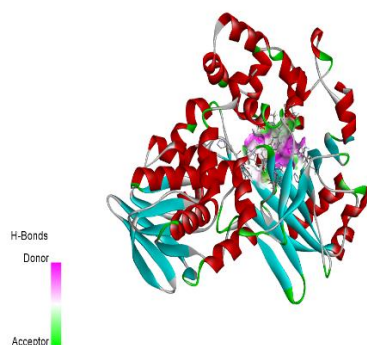
A



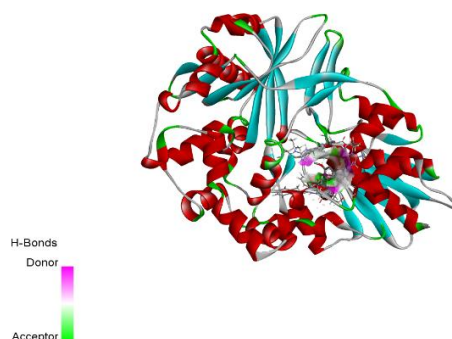
B



C



D



E



F



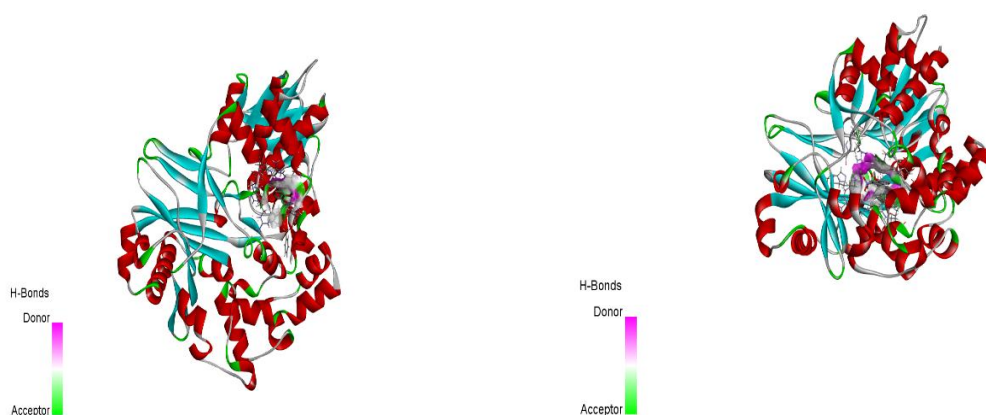


Fig. 4b 3D molecular contacts profiling of docked compounds with amino acid residues at the active site of LAAO: (A) Quercetin (B) Luteolin (C) Catechin (D) 4-hydroxy-3-methoxy-benzaldehyde (E) 2,6-dimethoxyphenol (F) Standard (Aristocholic acid)

Table 3: Predicted ADMET and pharmacokinetic profiles of Selected bioactive compounds and standard Inhibitors For Antivenom Therapy

	Compound_Name	Solubility	GI_Abs	B B	P-gp_Sub	CYP3 A4_Inh	Bio_Score	LD50_mgkg	Tox_Class	hERG_Risk	AME S_Tox	Skin_Sensitization
1	2,6-Dimethoxyphenol	Low	High	Yes	No	No	0.55	1141.0	4	Low Risk	Negative	Yes
2	Vanillin	Low	High	Yes	No	No	0.55	1121.0	4	Low Risk	Negative	Yes
3	Quercetin	Low	High	No	No	No	0.55	1199.0	4	Low Risk	Negative	Yes
4	Luteolin	Low	High	No	No	No	0.55	1228.0	4	Low Risk	Negative	Yes
5	Catechin	Low	High	No	No	No	0.55	1155.0	4	Low Risk	Negative	Yes
6	Edrophonium (STD)-L8	Low	High	Yes	No	No	0.55	1198.0	4	Low Risk	Negative	Yes
7	Aristolochic acid I (STD)-L9	Low	High	No	No	Yes	0.55	1334.0	4	Low Risk	Negative	Yes

3.3 Pharmacokinetics and Toxicity Profiling

Table 3 shows a comparison of the pharmacokinetic properties of the investigated compounds. Parameters such as Ames mutagenesis, blood-brain barrier, P-glycoprotein, and CYP3A4 were studied, though not all the compounds were able to penetrate the blood-brain barrier (BBB). Pharmacokinetic evaluation of the identified bioactive compounds of *Parkia biglobosa* and the reference standards exhibited a promising absorption and safety profile in the use of these compounds in antivenom therapy. All the compounds were found to be highly gastrointestinally absorbed, including the standards Edrophonium and Aristolochic acid, meaning that they would be readily absorbed into the body on oral intake. Vanillin, 2,6-dimethoxyphenol, and the standard Edrophonium could cross the blood-brain barrier (BBB), whereas the primary flavonoids, Quercetin, Luteolin, and Catechin, and Aristolochic acid were predicted to be non-permeable. This non-penetration of the flavonoids in the BBB is good because it would diminish the possibility of side effects in the central nervous system. Moreover, none of the bioactive compounds was substrates to P-glycoprotein, which further diminishes the chances of the body actively pumping the compounds out, which maintains steady therapeutic concentrations. The metabolic profile demonstrated that the bioactive compounds had no generally inhibitory effect on the CYP3A4 enzyme, with the exception of Aristolochic acid, meaning that the plant-derived compounds have a low likelihood of drug interactions. Toxicity analysis showed that the toxicity of all the compounds is toxicity class 4 and the LD50 of 1121.0 mg/kg to 1334.0 mg/kg, indicating that they are relatively safe in biological applications. The LD 50 of Quercetin, Luteolin, and Catechin were 1199.0 mg/kg, 1228.0 mg/kg and 1155.0 mg/kg, respectively, which are close to the standards. All the compounds were negative for Ames toxicity, which proves that they are not mutagenic. Although the risk of hERG inhibition was minimal in all the compounds, the majority of them exhibited the possibility of skin sensitization, which is a typical feature of a great deal of phenolic bioactive compounds. The bioactivity score of all the compounds is 0.55, which further confirms the possibility of the compounds being active in physiological activity. The evidence above indicates that the bioactive compounds of *Parkia biglobosa* possess the required pharmacokinetic stability and safety margin to be a promising solution in the treatment of snake venom-related toxicity (Table 3).

4.0 DISCUSSION

The findings of this computational study give an in-depth molecular understanding of the possibilities of secondary metabolites of *Parkia biglobosa* to interrelate with important enzymes involved in snake venom-related systemic toxicity. The identified leads, quercetin, luteolin, and catechin, were predicted to bind acetylcholinesterase (AChE) and L-amino acid oxidase (LAAO) and demonstrated the predicted binding profiles in accordance with the modulation of the neurotoxic and cytotoxic pathways related to elapid envenomation. These results offer a scientific richness to the long-standing ethnomedicinal status of the plant in West Africa in treating snake bites like *Naja nigricollis* [2, 3, 28]. Nonetheless, since it is a molecular docking and ADMET study, it is important to reiterate that the findings are still predictive and are more of a theoretical foundation as opposed to a validation of clinical effectiveness [43]. The positive predicted pharmacokinetic characteristics and

binding affinities are indicative of the presence of bioactive scaffolds in *P. biglobosa*, which can be used to supplement the existing antivenom-based treatments [5, 21].

The estimated compliance of the lead phytochemical library with the Rule of Five presented by Lipinski is a crucial initial reading of the possible applicability of the phytochemicals to oral delivery. Having zero violations on quercetin, luteolin, catechin, vanillin, and 2,6-dimethoxyphenol, these compounds have the desired molecular weight, lipophilicity, and hydrogen-bonding potential that can support favourable oral bioavailability. This is consistent with larger surveys of FDA-approved oral medications, in which about 66% comply with these regulations to guarantee gastrointestinal absorption [9, 12]. In rural areas, where the use of conventional intravenous antivenom is prone to delay, the discovery of bioavailable scaffolds of local flora is crucial in the development of available adjunct therapies [5].

There is, however, a critical difference when examining the blood-brain barrier (BBB) permeability. Larger phenols, such as quercetin, luteolin, and catechin, were too big to cross the BBB, whereas smaller ones, such as vanillin, were able to cross. This is in line with observations by Daina et al. [12] on the restrictive effect of the BBB on bulkier, highly hydroxylated scaffolds. Although this could restrict its application to central neuroprotection, it may provide a safety benefit in that interactions are concentrated on peripheral neuromuscular junctions, the major location of elapid neurotoxicity, without causing any central nervous system side effects [26, 32]. The moderate predicted lipophilicity ((LogP 1.21–2.28)) of these further indicates the ability of the compound to be membrane permeable, unlike the "molecularly obese" synthetic leads that frequently fail because of too much hydrophobicity [9, 38].

The docking studies showed that there is a clear order in the expected binding affinity with flavonoids performing better than smaller phenolic compounds and conventional inhibitors. Both quercetin (-9.9 kcal/mol) and luteolin (-9.7 kcal/mol) had binding affinities scores that were significantly more negative than that of the standard inhibitor edrophonium (-6.5 kcal/mol) against AChE. This is consistent with the studies of Orhan [32] and Taqui et al. [36], who reported the high inhibitory ability of quercetin against cholinesterases. The exceptional performance of flavonoids compared to edrophonium can be scientifically attributed to the complex aromatic structure that enables them to have multiple interactions with residues such as Trp82 and Tyr328 through the interaction of their structure, which is absent in the case of the simple edrophonium molecule [19, 32]. We also observed more desirable affinities when compared to other natural products such as boeserngin A (-6.07 kcal/mol), which further indicates that the flavone scaffold is naturally predisposed to occupy AChE active sites and not by chance [1].

In LAAO targeting, our findings indicated that luteolin (-9.5 kcal/mol) and quercetin (-9.2 kcal/mol) had the most competitive binding affinities to the standard inhibitor, aristolochic acid (-9.8 kcal/mol). This point is important since, although aristolochic acid is a reported potent binder, it has been linked with serious genotoxicity, which restricts its feasibility in clinical practice [7]. These large scores in the LAAO models are consistent, indicating that flavonoids are binding to one or more of the conserved structural elements of the enzyme, including the FAD-binding funnel, as in the case of pentagalloylglucopyranose (PGG) of Thai mango seed extracts [24]. Conversely, the smaller phenolics, including vanillin, were significantly less sensitive to predicted affinity, suggesting that at least molecular complexity and multi-point receptor anchoring play a crucial role in the attainment of robust complex stability in these deep catalytic channels [9, 36].

The predicted interactions of quercetin and luteolin through this study are explained by the functional groups of these compounds. The leads in the BfAChE complex formed hydrogen bonds with Met68, Asn83, and Tyr128. This is consistent with the results of Azmal et al. [4] and Cheung et al. [10], who found these residues to play a role in the stabilization of high-affinity complexes in the catalytic triad and peripheral anionic site (PAS) of the enzyme. The aromatic rings of the flavonoids are planar, and this allows the π -interaction with aromatic residues in the catalytic gorge stabilizing the quaternary ammonium group of acetylcholine. The *P. biglobosa* leads by settling in these areas can theoretically disrupt the entrance of acetylcholine or adjust the adherence of venom neurotoxins [11, 32].

In the case of LAAO, the postulated mechanism is the blockage of the FAD-binding hydrophobic funnel. The interaction of luteolin with Lys338 and Arg85 by hydrogen bonding with the substrate and the hydrophobic interactions of the luteolin with Phe222 and Leu202 are as expected by the substrate trajectory models put forward by Pawelek et al. [33] and Zhang et al. [44]. Blocking this pathway through docking simulations, these phytochemicals could be used to inhibit the oxidative deamination reaction that produces reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂). This agrees with the structural analysis of LAAO in different snake species, with the FAD funnel being a conserved inhibitory target in these studies [7, 35]. The C₂=C₃ double bond and structural flexibility of flavonoids enable a more effective adjustment to this funnel as opposed to smaller and more rigid compounds [16].

The interactions that are predicted have far-reaching effects on mitigating the systemic impact of elapid venoms. The mechanism of AChE implicated in neurotoxicity is somewhat complicated; although the enzyme is a host protein, the competitive inhibition of nicotinic acetylcholine receptors by the neuromuscular junction with alpha-neurotoxins is the hallmark of elapid neurotoxicity [22, 29]. We have found that the supportive or modulatory action of *P. biglobosa* could lead to their ability to sustain a higher concentration of synaptic acetylcholine, perhaps counteracting venom toxin effects, and causing neuromuscular collapse delay [21, 32].

The applicability to LAAO interaction is associated with the suppression of oxidative stress and tissue necrosis. LAAO is a flavoenzyme that plays a role in cytotoxic phenomena by generating H₂O₂ that leads to proinflammatory cascades and local tissue damage [20, 34]. These events could be less severe by the capability of the lead flavonoids to theoretically counter the LAAO activity. This is backed by in vitro research conducted on *P. biglobosa* aqueous extracts that have shown inhibitory concentration-dependent effects on these particular enzymatic processes [21, 31]. This multi-target strategy is consistent with the history of the plant to reduce the symptoms of local and systemic envenomation [3, 28].

4.1 Conclusion

In this study, we found that the identified bioactive compounds of *Parkia biglobosa*, especially quercetin, luteolin, and catechin, passed the Lipinski rule of five with zero violations, meaning good oral drug-likeness and pharmacokinetic properties. The molecular docking analysis findings of this study show that *Parkia biglobosa* has bioactive scaffolds that could be used to interact with the key enzymatic targets that are involved in the snake venom-induced neurotoxicity and cytotoxicity. It is notable that the capacity of the said flavonoid to interact with both AChE and LAAO suggests a dual-target interaction pattern, which can provide a more holistic approach to envenomation pathways modulation compared to single-target-based modulation. These compounds can stabilize

enzymatic activity by multi-point interactions in the catalytic gorge of AChE and in the FAD-binding funnel of LAAO, which offer a molecular explanation of their binding stability. Nevertheless, these results are informative, but molecular dynamics simulations and experimental validation, such as in vitro enzyme inhibition studies and in vivo experiments, are necessary to verify the stability, efficacy, and safety of such compounds.

4.2 Limitations

There are a number of considerations that are important to note as constraints of this study. To begin with, the research is limited by a fixed molecular docking analysis, in which the ligand - protein interactions were simulated with a rigid receptor model in AutoDock Vina. Although informative, it might not be able to describe the intricacies of biological interactions in living beings comprehensively. Moreover, binding affinities and estimated Ki values are estimates of docking scores, and not a guarantee of enzymatic inhibition. Although RDKit- and Mordred-based ADMET profiling offered preliminary data on drug-likeness and pharmacokinetic characteristics, metabolic transformation pathways, more extensive interactions with cytochromes, and drug-drug interactions were not assessed. Another limitation of the study is its narrow selection of the ligand, which only included eight reported phytochemicals in *Parkia biglobosa*, and may have missed other active constituents or synergies in the plant matrix. These studies were only compared with edrophonium and aristolochic acid, which prevented the overall benchmarking with other types of inhibitors. The possible off-target effects were also not evaluated, especially considering that acetylcholinesterase is physiologically relevant in host systems. The absence of mutational analysis also does not allow for assessing the performance of the ligands against structurally variable venom enzymes. The molecular dynamics simulations will include the flexibility of proteins and the influence of solvents, and the calculation of free energies to refine the binding estimates in the future. To ensure biological activity, experimental validation will be required by enzyme inhibition assays and in vivo studies, as well as an expanded compound screening and mutagenesis modelling will enhance the translational significance of these results.

REFERENCES

1. Abdelwahab, S. I. (2013). In vitro inhibitory effect of Boeserngin A on human acetylcholinesterase: Understanding its potential using in silico ADMET studies. *Journal of Applied Pharmaceutical Science*, 3(03), 030–035.
<https://doi.org/10.7324/JAPS.2013.30306>
2. Abubakar, M. S., Balogun, E., Abdurahman, E. M., Nok, A. J., Shok, M., Mohammed, A., & Garba, M. (2006). Ethnomedical treatment of poisonous snakebites: Plant extract neutralized *Naja nigricollis* venom. *Pharmaceutical Biology*, 44(5), 343–348.
<https://doi.org/10.1080/13880200600746253>

3. Asuzu, I. U., & Harvey, A. L. (2003). The antisnake venom activities of *Parkia biglobosa* (Mimosaceae) stem bark extract. *Toxicon*, 42(7), 763–768.
<https://doi.org/10.1016/j.toxicon.2003.10.004>
4. Azmal, M., et al. (2024). A computational approach to identify phytochemicals as potential inhibitor of acetylcholinesterase: Molecular docking, ADME profiling and molecular dynamics simulations. *PLoS ONE*, 19(6), e0304490.
<https://doi.org/10.1371/journal.pone.0304490>
5. Bala, A. A., Mohammed, M., Umar, S., Ungogo, M. A., Hassan, M. A. K., Abdussalam, U. S., ... & Chedi, B. A. Z. (2023). Pre-clinical efficacy of African medicinal plants used in the treatment of snakebite envenoming: A systematic review. *Toxicon*, 224, 107035.
<https://doi.org/10.1016/j.toxicon.2023.107035>
6. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., & Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research*, 28(1), 235–242.
<https://doi.org/10.1093/nar/28.1.235>
7. Bhattacharjee, P., Bera, I., Chakraborty, S., Ghoshal, N., & Bhattacharyya, D. (2017). Aristolochic acid and its derivatives as inhibitors of snake venom L-amino acid oxidase. *Toxicon*, 138, 1–17. <https://doi.org/10.1016/j.toxicon.2017.08.003>
8. Bultum, L. E., Tolossa, G. B., & Lee, D. (2022). Combining empirical knowledge, in silico molecular docking and ADMET profiling to identify therapeutic phytochemicals from *Brucea antidysentrica* for acute myeloid leukemia. *PLoS ONE*, 17(7), e0270050.
<https://doi.org/10.1371/journal.pone.0270050>
9. Caminero Gomes Soares, A., et al. (2023). Absorption matters: A closer look at popular oral bioavailability rules for drug approvals. *Molecular Informatics*, 42(11), e202300115.
<https://doi.org/10.1002/minf.202300115>
10. Cheung, J., et al. (2012). Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *Journal of Medicinal Chemistry*, 55(22), 10282–10286. <https://doi.org/10.1021/jm300871y>
11. Cortes, N., Zea-Perez, M. L., Glossman-Mitnik, D., Alzate-Guarin, F. A., & Osorio, E. (2015). Neuroprotective activity and acetylcholinesterase inhibition of five Amaryllidaceae species: A comparative study. *Life Sciences*, 122, 42–50.
<https://doi.org/10.1016/j.lfs.2014.12.011>
12. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7(1), 42717. <https://doi.org/10.1038/srep42717>

13. Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology*, 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19
14. Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. *Journal of Chemical Information and Modeling*, 61(8), 3891–3898. <https://doi.org/10.1021/acs.jcim.1c00203>
15. Forli, S., et al. (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols*, 11(5), 905–919. <https://doi.org/10.1038/nprot.2016.051>
16. Fu, M., et al. (2021). Essential moieties of myricetins, quercetins and catechins for binding and inhibitory activity against α -Glucosidase. *Bioorganic Chemistry*, 115, 105235. <https://doi.org/10.1016/j.bioorg.2021.105235>
17. Gbolade, A. A. (2020). Nigerian medicinal plants with anti-snake venom activity: A review. *Journal of Malaria Research and Phytomedicine*, 4(1), 39–44. <https://doi.org/10.4314/ajbr.v5i3.54000>
18. Gutiérrez, J. M., Calvete, J. J., Habib, A. G., Harrison, R. A., Williams, D. J., & Warrell, D. A. (2017). Snakebite envenoming. *Nature Reviews Disease Primers*, 3, 17079. <https://doi.org/10.1038/nrdp.2017.79>
19. Herrera-Acevedo, C., Perdomo-Madrigal, C., Herrera-Acevedo, K., Coy-Barrera, E., Scotti, L., & Scotti, M. T. (2021). Machine learning models to select potential inhibitors of acetylcholinesterase activity from Sistemax: a natural products database. *Molecular Diversity*, 25(3), 1553–1568. <https://doi.org/10.1007/s11030-021-10245-z>
20. Hiu, J. J., & Yap, M. K. K. (2020). Cytotoxicity of snake venom enzymatic toxins: phospholipase A2 and L-amino acid oxidase. *Biochemical Society Transactions*, 48(2), 719–731. <https://doi.org/10.1042/BST20200110>
21. Idakwoji, P. A., Abdullahi, M. J., Ezeizu, V. C., Ahmed, H., Osikhena, U. K., Banwo, F. M., ... & Muhammed, H. (2025). Pharmacological screening of aqueous extract of *Parkia biglobosa* leaves: In vitro inhibitory activity against key snake venom enzymes. *Research Square (Preprint)*. <https://doi.org/10.21203/rs.3.rs-7886338/v1>
22. Kini, R. M. (2019). Toxins for decoding interface selectivity in nicotinic acetylcholine receptors. *Biochemical Journal*, 476(10), 1515–1520. <https://doi.org/10.1042/BCJ20190137>
23. Landrum, G. (2016). RDKit: Open-source cheminformatics. <https://doi.org/10.5281/zenodo.1147366>

24. Leanpolchareanchai, J., Pithayanukul, P., Bavovada, R., & Saparpakorn, P. (2009). Molecular docking studies and anti-enzymatic activities of Thai mango seed kernel extract against snake venoms. *Molecules*, 14(4), 1404–1422.
<https://doi.org/10.3390/molecules14041404>
25. Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1-3), 3–25.
[https://doi.org/10.1016/s0169-409x\(96\)00423-1](https://doi.org/10.1016/s0169-409x(96)00423-1)
26. Maliehe, T. S., Tsilo, P. H., & Shandu, J. S. (2020). Computational evaluation of ADMET properties and bioactive score of compounds from *Encephalartos ferox*. *Pharmacognosy Journal*, 12(6), 1357–1362. <https://doi.org/10.5530/pj.2020.12.187>
27. Morris, Garrett M., et al. (1998). Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function. *Journal of Computational Chemistry*, 19(14), 1639–62. [https://doi.org/10.1002/\(SICI\)1096-987X\(19981115\)19:14<1639::AID-JCC10>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B)
28. Muhammed, M., Yusuf, A. A., Odey, B. O., Alawode, A. R., Adegbola, G. A., & Agboola, R. A. (2021). A systematic review of domestication, ethnopharmacological use, phytochemistry, nutritional composition, and biological activities of *Parkia biglobosa*. *BIOMED Natural and Applied Science*, 1(1), 20–31.
<https://doi.org/10.5281/zenodo.4725032>
29. Nirthanan, S., Awal, W., & Niranjana, N. R. (2017). Snake α -neurotoxins and the nicotinic acetylcholine receptor. In *Snake Venoms* (pp. 215–244). Springer.
https://doi.org/10.1007/978-94-007-6410-1_11
30. O’Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, 3(1), 33. <https://doi.org/10.1186/1758-2946-3-33>
31. Okafor, A. I., & Onyike, E. (2020). Inhibition of key enzymes linked to snake venom induced local tissue damage by kolaviron. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(5), 785–794. <https://doi.org/10.1515/jbcpp-2019-0286>
32. Orhan, I. E. (2021). Cholinesterase inhibitory potential of quercetin towards Alzheimer’s disease - A review. *Current Neuropharmacology*, 19(12), 2205–2213.
<https://doi.org/10.2174/1570159X18666201119153807>
33. Pawelek, P. D., Cheah, J., Coulombe, R., Macheroux, P., Ghisla, S., & Vrielink, A. (2000). The structure of L-amino acid oxidase reveals the substrate trajectory into an

- enantiomerically conserved active site. *EMBO Journal*, 19(16), 4204–4215.
<https://doi.org/10.1093/emboj/19.16.4204>
34. Samel, M., Vija, H., Rönholm, G., Siigur, J., Kalkkinen, N., & Siigur, E. (2006). Isolation and characterization of an apoptotic and platelet aggregation inhibiting L-amino acid oxidase from *Vipera berus berus* venom. *Biochimica et Biophysica Acta*, 1764(4), 707–714. <https://doi.org/10.1016/j.bbapap.2006.01.021>
 35. Stabeli, R. G., et al. (2009). Snake Venom L-Amino Acid Oxidases. *Protein & Peptide Letters*, 16(8), 910–912. <https://doi.org/10.2174/092986609788923338>
 36. Taqui, R., Debnath, M., Ahmed, S., & Ghosh, A. (2022). Advances on plant extracts and phytocompounds with acetylcholinesterase inhibition activity for possible treatment of Alzheimer's disease. *Phytomedicine Plus*, 2(1), 100184.
<https://doi.org/10.1016/j.phyplu.2021.100184>
 37. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461. <https://doi.org/10.1002/jcc.21334>
 38. Usin, S., et al. (2025). Molecular docking and dynamics profiling of bioactive compounds from *Echinacea purpurea* against AXL receptor kinase. *Discover Oncology*, 16, 1954.
<https://doi.org/10.1007/s12672-025-02102-4>
 39. Warrell, D. A. (2010). Snake bite. *The Lancet*, 375(9708), 77–88.
[https://doi.org/10.1016/S0140-6736\(09\)61754-2](https://doi.org/10.1016/S0140-6736(09)61754-2)
 40. World Health Organization. (2019). National Policy on Traditional Medicine and Regulation of Herbal Medicines: Report of WHO global survey February. Geneva: WHO.
 41. World Health Organization. (2022). WHO Fact Sheet on Prevalence of Snakebite Envenoming. Retrieved from WHO.
 42. Yusuf, P. O., Mamman, M., Ajagun, E., Suleiman, M. M., Kawu, M. U., Shittu, M., Isa, H. I., Tauheed, M., & Yusuf, A. (2015). Snakes responsible for bites in Norths-Eastern Nigeria—a hospital based survey. *Journal of Environmental Science*, 9, 118–121.
 43. Zhang, B., Li, H., Yu, K., & Jin, Z. (2022). Molecular docking-based computational platform for high-throughput virtual screening. *CCF Transactions on High Performance Computing*, 4(1), 63–74. <https://doi.org/10.1007/s42514-021-00086-5>
 44. Zhang, H., et al. (2004). Purification, structural determination of AHPLAAO from *Agkistrodon halys pallas* venom. *Acta Crystallographica Section D*, 60(5), 974–977.
<https://doi.org/10.1107/S090744490400346X>

45. Zhang, J., Zhang, Y., & Zhang, C. (2025). Computational toxicology in drug discovery: applications of artificial intelligence in ADMET and toxicity prediction. *Briefings in Bioinformatics*, 26(5), bbaf533. <https://doi.org/10.1093/bib/bbaf533>