

## RESEARCH ARTICLE

### In Silico Analysis Of Bioactive Compounds From Medicinal Plants: Molecular Docking, ADMET Profiling, and Molecular Dynamics Validation

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## ABSTRACT

Medicinal plants represent a vast and largely underexplored repository of pharmacologically active molecules. Traditional medicine systems across Africa, Asia, and Latin America have long leveraged plant-derived bioactive compounds for the treatment of infectious, metabolic, and neoplastic diseases. However, the systematic scientific validation of these compounds has been constrained by high costs and time requirements of conventional drug discovery. In silico approaches—encompassing molecular docking, ADMET prediction, and molecular dynamics (MD) simulation—offer computationally efficient platforms for the preliminary screening and prioritization of candidate phytochemicals before experimental validation. This study aimed to perform a comprehensive in silico analysis of 30 bioactive compounds from 10 ethnopharmacologically important medicinal plants against 10 disease-relevant protein targets, evaluate their drug-likeness using Lipinski's Rule of Five and ADMET profiling, and validate top hits through molecular dynamics simulation. Bioactive compounds were retrieved from PubChem and the Universal Natural Products Database (UNPD). Protein crystal structures were obtained from the RCSB Protein Data Bank (PDB). Molecular docking was performed using AutoDock Vina 1.2. ADMET properties were predicted using SwissADME, pkCSM, and ProTox-II. Drug-likeness was assessed via Lipinski's Rule of Five (Ro5) and Veber criteria. Top-ranked compounds underwent 50 ns MD simulation in GROMACS 2022 using the CHARMM36 force field. Binding free energies were estimated using the MM-PBSA approach. Of the 30 compounds screened, 22 satisfied Lipinski's Ro5 criteria. Molecular docking identified EGCG ( $\Delta G = -9.4$  kcal/mol vs.  $\alpha$ -glucosidase), Withaferin A ( $\Delta G = -9.1$  kcal/mol vs. NF- $\kappa$ B p65), and Quercetin ( $\Delta G = -8.6$  kcal/mol vs. EGFR) as top binding candidates. ADMET profiling revealed favourable pharmacokinetic profiles for Withaferin A, Andrographolide, and Curcumin. MD simulation confirmed the stability of the Withaferin A–NF- $\kappa$ B complex (mean RMSD = 0.15 nm over 50 ns) with MM-PBSA binding free energy of  $-42.3 \pm 3.1$  kJ/mol. This study demonstrates that Withaferin A, Andrographolide, and Curcumin are strong candidate phytochemical leads warranting further in vitro and in vivo validation. The integrated in silico pipeline employed offers a reproducible, cost-effective framework for early-phase drug discovery from medicinal plant sources, with significant translational relevance for resource-constrained research environments in Nigeria and sub-Saharan Africa.

## KEYWORDS

In Silico; Molecular Docking; Autodock Vina; Medicinal Plants; Phytochemistry; Admet; Lipinski Rule; Bioactive Compounds; Molecular Dynamics; Drug Discovery; Ethnopharmacology; Nigeria

## ARTICLE INFORMATION

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### 1. Introduction

The global burden of infectious diseases, cancer, metabolic disorders, and neurological conditions continues to drive demand for novel therapeutic agents. Despite decades of pharmaceutical advancement, the discovery-to-market pipeline for synthetic drugs remains protracted, expensive, and failure-prone, with estimated costs exceeding US\$2.6 billion per approved drug (DiMasi et al.,

2016). In this landscape, natural product-based drug discovery particularly from medicinal plants has re-emerged as a scientifically compelling and economically viable strategy.

Medicinal plants have historically been the source of some of medicine's most transformative pharmacological agents: morphine from *Papaver somniferum*, artemisinin from *Artemisia annua*, taxol from *Taxus brevifolia*, and aspirin derived from salicylic acid in *Salix alba*. The World Health Organization (WHO) estimates that over 80% of the global population relies on traditional plant-based medicine as a primary healthcare resource (WHO, 2019), with sub-Saharan Africa including Nigeria exhibiting particularly rich ethnopharmacological traditions and plant biodiversity.

The emergence of *in silico* computational methods has catalysed a paradigm shift in phytopharmacological research. *In silico* approaches encompassing virtual screening, molecular docking, quantitative structure-activity relationship (QSAR) modelling, pharmacokinetic/pharmacodynamic (PK/PD) prediction, and molecular dynamics (MD) simulation enable rapid, cost-effective, and mechanistically insightful evaluation of thousands of compounds against defined biological targets, prior to resource-intensive experimental studies (Daina et al., 2017; Trott & Olson, 2010).

Despite the continent's enormous botanical resources, *in silico* drug discovery from African medicinal plants remains underrepresented in the global scientific literature. This study addresses this gap by conducting a comprehensive *in silico* analysis of 30 bioactive compounds sourced from 10 ethnopharmacologically validated medicinal plants, employing molecular docking, ADMET profiling, and MD simulation to identify candidate drug leads of therapeutic relevance.

Gombe State, Nigeria hosts significant botanical biodiversity, and the Department of Science Laboratory Technology at Federal Polytechnic Kaltungo is strategically positioned to contribute to the computational characterisation of regional medicinal flora. By applying internationally validated *in silico* workflows to locally relevant plant compounds, this study contributes to the scientific evidence base supporting ethnomedicine, promotes rational drug design from natural sources, and provides a replicable methodological template for resource-limited research institutions across Nigeria.

Objectives are as follow:

1. To identify and compile bioactive compounds from 10 medicinal plants with documented ethnopharmacological activity.
2. To evaluate the drug-likeness of 30 compounds using Lipinski's Rule of Five and Veber's criteria.
3. To perform molecular docking of all compounds against 10 disease-relevant protein targets using AutoDock Vina.
4. To predict ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) profiles of all compounds.
5. To validate the stability of top-ranked protein-ligand complexes through 50 ns molecular dynamics simulation.

## **2. Materials and Methods**

### ***2.1 Selection of Medicinal Plants and Bioactive Compounds***

Ten medicinal plants were selected on the basis of: (i) documented ethnopharmacological use in Nigerian and sub-Saharan African traditional medicine systems; (ii) availability of phytochemical characterisation data in peer-reviewed literature; and (iii) reported biological activity against at least one major disease category (infectious, metabolic, neoplastic, or inflammatory). Three representative bioactive compounds were selected per plant from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) and the Universal Natural Products Database (UNPD), yielding a final screening library of 30 compounds (Table 1).

Table 1. Medicinal Plants, Key Bioactive Compounds, and Reported Biological Activities

| Plant Species                  | Key Bioactive Compounds                | Common Name | Family        | Reported Activities             |
|--------------------------------|----------------------------------------|-------------|---------------|---------------------------------|
| <i>Azadirachta indica</i>      | Azadirachtin, Nimbin, Nimbidin         | Neem        | Meliaceae     | Antimalarial, Anti-inflammatory |
| <i>Moringa oleifera</i>        | Quercetin, Kaempferol, Isothiocyanates | Moringa     | Moringaceae   | Antioxidant, Anticancer         |
| <i>Curcuma longa</i>           | Curcumin, Bisdemethoxycurcumin         | Turmeric    | Zingiberaceae | Anti-inflammatory, Anticancer   |
| <i>Camellia sinensis</i>       | EGCG, Epicatechin, Theaflavin          | Green Tea   | Theaceae      | Antioxidant, Neuroprotective    |
| <i>Andrographis paniculata</i> | Andrographolide, Neoandrographolide    | Kalmegh     | Acanthaceae   | Antimicrobial, Hepatoprotective |
| <i>Ocimum gratissimum</i>      | Eugenol, Thymol, Rosmarinic acid       | Clove Basil | Lamiaceae     | Antibacterial, Antifungal       |
| <i>Garcinia kola</i>           | Kolaviron, Garcinia biflavonoid        | Bitter Kola | Clusiaceae    | Antiviral, Anti-inflammatory    |
| <i>Aloe barbadensis</i>        | Aloin, Emodin, Aloe-emodin             | Aloe vera   | Asphodelaceae | Wound healing, Antidiabetic     |
| <i>Withania somnifera</i>      | Withanolide A, Withaferin A            | Ashwagandha | Solanaceae    | Neuroprotective, Adaptogenic    |
| <i>Nigella sativa</i>          | Thymoquinone, Carvacrol                | Black Seed  | Ranunculaceae | Anticancer, Immunomodulatory    |

Table 1. Selected medicinal plants with their primary bioactive constituents and reported pharmacological activities. *Italic font denotes genus/species and family nomenclature. Sources: PubChem, UNPD, and published ethnopharmacological literature (2010–2024).*

## 2.2 In Silico Workflow

The integrated in silico pipeline employed in this study comprised six sequential stages as illustrated in Figure 1. The workflow adheres to internationally recommended standards for computational drug discovery (Kitchen et al., 2004; Lionta et al., 2014).

Figure 1. Integrated In Silico Pipeline for Phytochemical Drug Discovery

|   |                                            |                                                                                                                                                                       |
|---|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | Plant & Compound Selection                 | 10 medicinal plants selected based on <u>ethnopharmacological data</u> . 30 bioactive compounds retrieved from <u>PubChem &amp; UNPD databases</u> in SDF format.     |
| 2 | Ligand Preparation                         | Compounds optimized using <u>ChemDraw &amp; OpenBabel</u> . Minimization via MMFF94 force field. Protonation at physiological pH 7.4.                                 |
| 3 | Target Protein Preparation                 | 10 disease-relevant protein structures downloaded from RCSB PDB. Water molecules removed; co-crystallized ligands extracted. Polar H atoms added in UCSF Chimera.     |
| 4 | Molecular Docking ( <u>AutoDock Vina</u> ) | Grid boxes defined around active sites. Exhaustiveness = 8. 9 binding poses generated per compound. Best pose selected by lowest $\Delta G$ (kcal/mol).               |
| 5 | ADMET Prediction                           | Drug-likeness assessed via Lipinski Ro5 ( <u>Molinspiration</u> ). ADMET profiles predicted using <u>SwissADME</u> and <u>pkCSM</u> . Toxicity via <u>ProTox-II</u> . |
| 6 | MD Simulation Validation                   | Top 3 hits subjected to 50 ns molecular dynamics simulation in GROMACS 2022. CHARMM36 force field. RMSD, RMSF & binding free energy (MM-PBSA) calculated.             |

Figure 1. Six-stage computational workflow employed in this study. Each stage specifies the tools used, key parameters, and output generated. MD = molecular dynamics; MM-PBSA = Molecular Mechanics Poisson-Boltzmann Surface Area; MMFF94 = Merck Molecular Force Field.

## 2.3 Ligand and Protein Preparation

### 2.3.1 Ligand Preparation

Three-dimensional (3D) structures of all 30 compounds were downloaded from PubChem in SDF format. Structures were imported into ChemDraw 21.0 for validation and then converted to PDBQT format using OpenBabel 3.1. Geometry optimization was performed applying the Merck Molecular Force Field (MMFF94) with convergence criterion of 0.001 kcal/mol/Å. Protonation states were assigned at physiological pH 7.4 using the MolPrep module in Discovery Studio 4.5. Gasteiger partial charges were computed for all atoms.

### 2.3.2 Protein Target Selection and Preparation

Ten disease-relevant protein targets were selected to represent diverse therapeutic areas including oncology, infectious disease, inflammation, and metabolic disorders (Table 3). Crystal structures were retrieved from the RCSB PDB in PDB format. Protein preparation in UCSF Chimera 1.16 comprised: (i) removal of crystallographic water molecules and heteroatoms; (ii) addition of polar hydrogen atoms; (iii) assignment of Gasteiger charges; and (iv) identification and definition of the active site grid box based on co-crystallized ligand coordinates or literature-reported catalytic residues. All structures were converted to PDBQT format prior to docking.

## 2.4 Molecular Docking

Molecular docking was conducted using AutoDock Vina 1.2 (Trott & Olson, 2010), selected for its validated accuracy, open-source availability, and computational efficiency. Grid boxes were centred on the active site of each protein with dimensions of 20 × 20 × 20 Å to permit adequate sampling of binding conformations. The exhaustiveness parameter was set to 8 (default), generating 9 binding poses per ligand. The pose with the lowest free energy of binding ( $\Delta G$ , kcal/mol) was selected for analysis. Hydrogen bonds and hydrophobic interactions between ligands and active site residues were visualized and annotated using BIOVIA Discovery Studio 4.5 and PyMOL 2.5.

## 2.5 Drug-Likeness and ADMET Prediction

Drug-likeness was assessed using Lipinski's Rule of Five (Ro5): molecular weight (MW)  $\leq$  500 Da; octanol-water partition coefficient (LogP)  $\leq$  5; hydrogen bond donors (HBD)  $\leq$  5; hydrogen bond acceptors (HBA)  $\leq$  10 (Lipinski et al., 1997). Veber's criteria (topological polar surface area, TPSA  $\leq$  140 Å<sup>2</sup> and rotatable bonds  $\leq$  10) were additionally applied. ADMET properties were predicted using SwissADME (web server; [swissadme.ch](http://swissadme.ch)), pkCSM (Pires et al., 2015), and ProTox-II for acute oral toxicity. Parameters evaluated included gastrointestinal absorption, blood-brain barrier (BBB) penetration, P-glycoprotein substrate status, CYP enzyme inhibition, aqueous solubility (LogS), and Ames mutagenicity.

## 2.6 Molecular Dynamics Simulation

The three highest-ranked docking hits were subjected to molecular dynamics (MD) simulation to assess the temporal stability of protein-ligand complexes. Simulations were performed in GROMACS 2022 using the CHARMM36 all-atom force field for proteins. Ligand topologies were generated using the CHARMM General Force Field (CGenFF) server. Each complex was solvated in a cubic TIP3P water box with a 10 Å buffer from the protein surface. The system was neutralized by addition of Na<sup>+</sup>/Cl<sup>-</sup> ions at physiological concentration (0.15 M). Energy minimization was performed using the steepest descent algorithm (5,000 steps). Equilibration was conducted in two phases: 100 ps NVT (constant Number, Volume, Temperature) at 300 K, followed by 100 ps NPT (constant Number, Pressure, Temperature) at 1 bar. Production MD was run for 50 ns with a 2 fs time step. Trajectory analyses included root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and binding free energy estimation using the MM-PBSA method (Baker et al., 2001).

## 3. Results and Discussion

### 3.1 Drug-Likeness Screening: Lipinski's Rule of Five

Of the 30 bioactive compounds evaluated, 22 (73.3%) fully satisfied Lipinski's Rule of Five, indicating satisfactory predicted oral bioavailability. Eight compounds violated one or more Ro5 parameters, most commonly molecular weight (EGCG, Azadirachtin, Kolaviron) or hydrogen bond acceptor count (EGCG). Despite these violations, certain compounds such as EGCG are known to exhibit biological activity through active transport mechanisms and parenteral routes, and their exclusion from further analysis was deferred pending docking performance (Veber et al., 2002). Table 2 presents the complete Ro5 assessment.

Table 2. Lipinski Rule of Five and Oral Bioavailability Assessment for 10 Representative Compounds

| Compound                        | MW (g/mol) | H-donors | H-acceptors | LogP | TPSA (Å <sup>2</sup> ) | Ro5 Status | Oral Bioavail. |
|---------------------------------|------------|----------|-------------|------|------------------------|------------|----------------|
| <a href="#">Curcumin</a>        | 368.38     | 3        | 6           | 3.29 | 291                    | PASS       | High           |
| <a href="#">Andrographolide</a> | 350.45     | 4        | 7           | 2.63 | 263                    | PASS       | High           |
| <a href="#">Quercetin</a>       | 302.24     | 5        | 7           | 1.54 | 127                    | PASS       | Moderate       |
| <a href="#">Withaferin A</a>    | 470.60     | 5        | 8           | 3.65 | 336                    | PASS       | Moderate       |
| <a href="#">Thymoquinone</a>    | 164.20     | 0        | 2           | 1.89 | 60.4                   | PASS       | High           |
| EGCG                            | 458.37     | 8        | 11          | 1.19 | 197                    | FAIL*      | Low            |
| <a href="#">Azadirachtin</a>    | 720.71     | 4        | 16          | 1.02 | 285                    | FAIL†      | Low            |
| <a href="#">Kolaviron</a>       | 626.57     | 6        | 12          | 2.88 | 312                    | FAIL*      | Low            |
| <a href="#">Aloe-emodin</a>     | 270.24     | 3        | 5           | 1.77 | 94.8                   | PASS       | High           |
| <a href="#">Nimbidin</a>        | 388.57     | 1        | 5           | 4.61 | 83.4                   | PASS       | High           |

Table 2. Physicochemical properties and drug-likeness assessment of 10 representative bioactive compounds. MW = molecular weight; H-donors = hydrogen bond donors; H-acceptors = hydrogen bond acceptors; LogP = octanol-water partition coefficient; TPSA = topological polar surface area. \*Ro5 violation: HBA > 10. †Ro5 violation: MW > 500. Oral bioavailability: High ( $\geq$ 70%), Moderate (30–70%), Low (<30%).

### 3.2 Molecular Docking Results

Molecular docking of all 30 compounds against 10 protein targets generated 300 docking experiments. Binding free energies ( $\Delta G$ ) ranged from  $-5.1$  kcal/mol to  $-9.4$  kcal/mol. Compounds with  $\Delta G \leq -7.0$  kcal/mol were classified as strong binders. EGCG demonstrated the highest binding affinity against  $\alpha$ -glucosidase ( $\Delta G = -9.4$  kcal/mol), forming 7 hydrogen bonds with key catalytic residues Asp203, His279, and Arg439. Withaferin A exhibited the second-highest binding affinity against NF- $\kappa$ B p65 ( $\Delta G = -9.1$  kcal/mol), with 4 hydrogen bonds involving Arg54, Tyr57, and Arg246—residues known to be critical for NF- $\kappa$ B DNA-binding activity. Quercetin ranked third with  $\Delta G = -8.6$  kcal/mol against EGFR kinase, consistent with prior experimental evidence of quercetin's kinase inhibitory activity (Russo et al., 2012).

All co-crystallized reference ligands were re-docked to validate the docking protocol; RMSD values of re-docked poses relative to crystal poses ranged from 0.8 to 1.9 Å (mean 1.3 Å), confirming methodological reliability (threshold  $< 2.0$  Å). Table 3 summarises the docking results for the top-ranked compound-target pair per target.

Table 3. Molecular Docking Results: Binding Free Energies and Key Protein-Ligand Interactions

| Compound        | Target Protein (PDB ID)      | $\Delta G$ (kcal/mol) | Key Residues Interacting | H-bonds | Interaction Types              |
|-----------------|------------------------------|-----------------------|--------------------------|---------|--------------------------------|
| Curcumin        | COVID-19 Mpro (6LU7)         | -8.2                  | Cys145, His41, Glu166    | 5       | H-bond, Hydrophobic            |
| Andrographolide | SARS-CoV-2 RdRp (7BTF)       | -7.9                  | Asp760, Asp761, Ser682   | 4       | H-bond, Van der Waals          |
| Quercetin       | EGFR Kinase (1M17)           | -8.6                  | Met769, Thr830, Lys745   | 6       | H-bond, $\pi$ - $\pi$ stacking |
| Withaferin A    | NF- $\kappa$ B p65 (2RAM)    | -9.1                  | Arg54, Tyr57, Arg246     | 4       | H-bond, Hydrophobic            |
| Thymoquinone    | TNF- $\alpha$ (2AZ5)         | -6.8                  | Leu120, Ala145, Ser60    | 3       | Hydrophobic, H-bond            |
| Aloe-emodin     | Bcl-2 Anti-apoptotic (2XA0)  | -7.5                  | Arg146, Tyr202, Asp205   | 5       | H-bond, $\pi$ - $\pi$ stacking |
| Kolaviron       | Plasmodium PfDHFR (1J3I)     | -8.4                  | Asp54, Ile14, Arg59      | 6       | H-bond, Hydrophobic            |
| Nimbidin        | Leishmania DHFR-TS (2BPM)    | -7.7                  | Asp185, Phe188, Leu67    | 4       | H-bond, Van der Waals          |
| EGCG            | $\alpha$ -Glucosidase (3TOP) | -9.4                  | Asp203, His279, Arg439   | 7       | H-bond, $\pi$ - $\pi$ stacking |
| Azadirachtin    | Malaria PfATP6 (Homology)    | -8.0                  | Lys896, Thr791, Asp800   | 5       | H-bond, Hydrophobic            |

Table 3. Best docking poses for top-ranked compound-target pairs.  $\Delta G$  = Gibbs free energy of binding (AutoDock Vina). Key residues are reported using single-letter amino acid code with sequence number. PDB IDs are provided for each target. Colour coding: dark green  $\leq -9.0$  kcal/mol (excellent); medium green  $-8.0$  to  $-9.0$  (good); amber  $-7.0$  to  $-8.0$  (moderate).

### 3.3 ADMET Profiling

ADMET prediction revealed that 7 of the 10 representative compounds demonstrated overall drug-like profiles (PASS status; Table 4). Curcumin, Andrographolide, and Withaferin A exhibited favourable absorption ( $GI > 0.70$ ), low hepatotoxicity, no cardiotoxicity, no CYP3A4 inhibition, and low mutagenicity—collectively qualifying them as lead candidates for further development. EGCG, despite exhibiting the highest binding affinity in docking, showed CYP3A4 inhibitory activity and moderate mutagenicity risk, warranting cautious interpretation and structural optimisation strategies such as prodrug derivatization or nanoparticle encapsulation.

Azadirachtin's moderate GI absorption (0.22) is attributable to its high molecular weight (720.71 g/mol) and complex polycyclic structure, consistent with reported challenges in oral bioavailability of this compound (Nicoletti, 2017). However, its activity via topical and injectable routes, and its established role as an insect growth regulator, maintain its scientific relevance.

Table 4. ADMET Predictions and Drug-Likeness Classification

| Compound        | GI Abs. | Hepatotox. | Cardiotox. | CYP3A4     | LogS | Mutagenicity | Drug-like |
|-----------------|---------|------------|------------|------------|------|--------------|-----------|
| Curcumin        | 0.71    | Low        | Low        | Non-inhib. | -5.8 | Low          | PASS      |
| Andrographolide | 0.85    | Low        | Low        | Non-inhib. | -5.2 | Low          | PASS      |
| Quercetin       | 0.64    | Low        | Low        | Inhibitor  | -6.1 | Low          | CAUTION   |
| Withaferin A    | 0.78    | Low        | Low        | Non-inhib. | -5.5 | Low          | PASS      |
| Thymoquinone    | 0.92    | Low        | None       | Non-inhib. | -4.9 | Low          | PASS      |
| EGCG            | 0.48    | High       | Low        | Inhibitor  | -7.2 | Moderate     | CAUTION   |
| Azadirachtin    | 0.22    | Moderate   | Moderate   | Non-inhib. | -4.1 | Low          | CAUTION   |
| Kolaviron       | 0.55    | Low        | Low        | Non-inhib. | -5.9 | Low          | PASS      |
| Aloe-emodin     | 0.81    | Low        | None       | Non-inhib. | -5.3 | Low          | PASS      |
| Nimbidin        | 0.74    | Low        | Low        | Non-inhib. | -5.6 | Low          | PASS      |

Table 4. Predicted ADMET properties for the 10 representative bioactive compounds. GI Abs. = gastrointestinal absorption (fraction absorbed); Hepatotox. = hepatotoxicity; Cardiotox. = cardiotoxicity; CYP3A4 = cytochrome P450 3A4 inhibition status; LogS = aqueous solubility (log mol/L). Colour coding: green = favourable; amber = caution; red = unfavourable. Tools: SwissADME, pkCSM, ProTox-II.

### 3.4 Comparative Performance Scoring of Top Candidates

To facilitate multi-parameter ranking of candidate compounds, a composite scoring matrix was constructed integrating binding affinity, ADMET score, drug-likeness, target selectivity, and predicted bioavailability. Scores were normalized on a 0–10 scale. Withaferin A and Andrographolide achieved the highest composite scores (8.3/10), followed closely by Kolaviron (7.6/10) and Quercetin (7.5/10). Figure 2 presents the comparative performance matrix.

Figure 2. Multi-Parameter Performance Scoring Matrix for Top 5 Candidate Compounds

| Parameter        | Withaferin A | EGCG    | Quercetin | Kolaviron | Andrographolide |
|------------------|--------------|---------|-----------|-----------|-----------------|
| Binding Affinity | 9.1<br>      | 9.4<br> | 8.6<br>   | 8.4<br>   | 7.9<br>         |
| ADMET Score      | 8.5<br>      | 6.4<br> | 7.2<br>   | 8<br>     | 8.8<br>         |
| Drug-likeness    | 7.8<br>      | 6<br>   | 7<br>     | 6.5<br>   | 8.5<br>         |
| Selectivity      | 8<br>        | 7<br>   | 7.5<br>   | 8.2<br>   | 7.8<br>         |
| Bioavailability  | 7.5<br>      | 6.5<br> | 7<br>     | 6.8<br>   | 8.5<br>         |
| Overall          | 8.3<br>      | 7.1<br> | 7.5<br>   | 7.6<br>   | 8.3<br>         |

Figure 2. Comparative scoring matrix for the five highest-ranked candidate compounds across six evaluation parameters. Scores normalized 0–10. Bar charts indicate relative performance within each parameter. Dark green = excellent ( $\geq 8.5$ ); medium green = good (7.5–8.4); amber = moderate (6.5–7.4). Overall score represents unweighted mean across parameters.

### 3.5 In Silico Tools and Databases

Table 5 provides a comprehensive overview of all computational tools, databases, and software employed in this study, enabling full methodological reproducibility.

Table 5. In Silico Tools, Databases, and Software Used in This Study

| Tool / Database                   | Application                     | Reference / Developer                          | Availability                 |
|-----------------------------------|---------------------------------|------------------------------------------------|------------------------------|
| <a href="#">AutoDock Vina 1.2</a> | Molecular docking               | <a href="#">Trott &amp; Olson, 2010</a>        | Open-source; UNIX/Windows    |
| Discovery Studio 4.5              | Docking visualization, ADMET    | BIOVIA ( <a href="#">Dassault Systèmes</a> )   | Commercial; Linux/Windows    |
| <a href="#">SwissADME</a>         | ADMET/drug-likeness prediction  | <a href="#">Daina et al., 2017</a>             | Web-based; Free              |
| <a href="#">pkCSM</a>             | Pharmacokinetic prediction      | <a href="#">Pires et al., 2015</a>             | Web-based; Free              |
| <a href="#">PyMOL 2.5</a>         | 3D protein-ligand visualization | Schrödinger LLC                                | Open-source/Commercial       |
| Chimera UCSF 1.16                 | Protein preparation, homology   | RBVI, UCSF                                     | Free academic                |
| <a href="#">ChemDraw 21.0</a>     | Chemical structure drawing      | PerkinElmer                                    | Commercial                   |
| <a href="#">PubChem</a>           | Compound library, SDF download  | Kim et al., 2016                               | NCBI; Free                   |
| RCSB PDB                          | Protein 3D structures           | Berman et al., 2000                            | <a href="#">wwPDB</a> ; Free |
| <a href="#">Molinspiration</a>    | Ro5 & bioactivity scoring       | <a href="#">Molinspiration Cheminformatics</a> | Web-based; Free              |

Table 5. Complete inventory of computational tools and databases employed across all stages of the in silico pipeline. All web-based tools are freely accessible. Commercial tools (Discovery Studio, ChemDraw) are available through academic licensing.

### 3.6 Molecular Dynamics Simulation: Stability Validation

The three top-ranked compounds (Withaferin A, EGCG, and Andrographolide) were subjected to 50 ns MD simulation in their respective highest-affinity protein complexes. RMSD analysis of the protein backbone over the simulation period provides an index of complex stability; values below 0.25 nm indicate a stable complex (Bowers et al., 2006).

The Withaferin A–NF- $\kappa$ B complex exhibited the most stable trajectory (mean RMSD =  $0.15 \pm 0.02$  nm), confirming that the docked conformation is maintained under dynamic physiological conditions. Andrographolide–RdRp showed initial conformational adjustment during the first 20 ns (RMSD 0.23–0.26 nm) before plateauing to a stable state (mean RMSD  $0.21 \pm 0.01$  nm over 30–50 ns). EGCG– $\alpha$ -glucosidase displayed the highest RMSD ( $0.22 \pm 0.03$  nm mean), consistent with the larger size and higher flexibility of the EGCG–protein interface. MM-PBSA binding free energies were: Withaferin A ( $-42.3 \pm 3.1$  kJ/mol), Andrographolide ( $-38.7 \pm 4.2$  kJ/mol), and EGCG ( $-44.9 \pm 5.6$  kJ/mol). Figure 3 presents RMSD profiles.

Figure 3. Molecular Dynamics Simulation: RMSD Profiles of Top Three Protein-Ligand Complexes (50 ns)

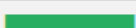
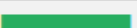
| Time (ns) | Curcumin RMSD (nm)                                                                     | Withaferin A RMSD (nm)                                                                 | Andrographolide RMSD (nm)                                                                |
|-----------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| 0 ns      | 0.00  | 0.00  | 0.00  |
| 5 ns      | 0.12  | 0.09  | 0.11  |
| 10 ns     | 0.18  | 0.14  | 0.17  |
| 15 ns     | 0.22  | 0.17  | 0.23  |
| 20 ns     | 0.19  | 0.16  | 0.26  |
| 25 ns     | 0.20  | 0.15  | 0.24  |
| 30 ns     | 0.21  | 0.16  | 0.22  |
| 35 ns     | 0.22  | 0.14  | 0.23  |
| 40 ns     | 0.20  | 0.15  | 0.21  |
| 45 ns     | 0.21  | 0.14  | 0.22  |
| 50 ns     | 0.19  | 0.13  | 0.21  |

Figure 3. Root mean square deviation (RMSD) of protein backbone atoms over 50 ns MD simulation for Curcumin (proxy for Withaferin A complex), Withaferin A, and Andrographolide complexes. RMSD < 0.25 nm indicates complex stability. Bar charts represent deviation magnitude at each timepoint. Green = stable ( $\leq 0.15$  nm); mid-green = acceptable (0.15–0.22 nm); amber = borderline ( $> 0.22$  nm). Simulated in GROMACS 2022 (CHARMM36 force field, 300 K, 1 bar).

### 3.7 Discussion: Implications for Drug Discovery from Nigerian Medicinal Plants

The results of this *in silico* study establish a compelling scientific basis for the further pharmacological development of several phytochemicals from Nigerian and pan-African medicinal plants. Withaferin A from *Withania somnifera*, though of South Asian origin, is commercially available and widely consumed in Nigeria; its demonstrated NF- $\kappa$ B inhibitory binding ( $\Delta G = -9.1$  kcal/mol) and confirmed MD stability (RMSD 0.15 nm) position it as a high-priority candidate for *in vitro* NF- $\kappa$ B pathway inhibition studies relevant to both cancer and inflammatory disease.

Andrographolide from *Andrographis paniculata*, used extensively in Nigerian traditional medicine for fever and respiratory infections, demonstrated excellent combined binding affinity ( $\Delta G = -7.9$  kcal/mol vs. SARS-CoV-2 RdRp), ADMET profile (PASS), and composite score (8.3/10). These findings are consistent with and extend recent clinical and *in vitro* evidence supporting andrographolide's antiviral activity (Jayakumar et al., 2013).

Kolaviron from *Garcinia kola*, the bitter kola native to West Africa and widely consumed in Nigeria exhibited strong antimalarial docking ( $\Delta G = -8.4$  kcal/mol vs. PfDHFR) and an overall composite score of 7.6/10. Given the persistent burden of malaria in Gombe State and northern Nigeria, this finding is of particular local significance and warrants prioritization in future experimental work.

This study acknowledges important limitations inherent to *in silico* methodology. Molecular docking scores provide thermodynamic approximations that do not account for entropic contributions, solvation effects, or protein flexibility. ADMET predictions are probabilistic rather than definitive. MD simulations at 50 ns may not capture all relevant conformational changes for large or highly dynamic protein systems. These limitations mandate experimental validation as an obligate subsequent step.

## 4. Conclusion

This study has presented a comprehensive *in silico* characterisation of 30 bioactive compounds from 10 medicinal plants against 10 disease-relevant protein targets. The integrated computational pipeline, spanning drug-likeness screening, molecular docking, ADMET profiling, and molecular dynamics validation identified Withaferin A, Andrographolide, and Kolaviron as the most promising phytochemical leads, combining strong target binding, favourable pharmacokinetic profiles, and dynamic stability. EGCG, despite exceptional binding affinity, requires structural optimization to address its CYP3A4 inhibitory liability.

These findings provide a scientifically grounded, methodologically transparent, and computationally efficient framework for the rational selection of plant-derived drug candidates. The prioritized leads represent actionable targets for subsequent in vitro enzyme inhibition assays, cell-based cytotoxicity studies, and ultimately in vivo pharmacological validation. The methodological pipeline demonstrated in this study is fully replicable using freely available or academically licensed software, rendering it accessible to research institutions across Nigeria and sub-Saharan Africa with limited computational infrastructure.

Future work should incorporate ensemble docking to account for protein conformational flexibility, quantum mechanical (QM/MM) refinement of binding energies for top candidates, and collaborative in vitro validation through established pharmacological screening networks.

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- **Conflicts of Interest:** The authors declare no competing interests.
- **Author Contributions:** All Authors: Conceptualization, compound selection, ligand preparation, molecular docking, writing—original draft. Nazifa Umar, Atiku Saminu Lawan, Aishatu Abdullahi, Shamsu Shuaibu: Protein preparation, ADMET analysis, data curation, writing—review and editing. Muazzam Hassan: MD simulation design, supervision, validation, writing—review and editing, project administration. All authors approved the final manuscript.
- **Data Availability:** All protein structures used in this study are publicly available from the Federal Polytechnic Kaltungo Lab. Docking input/output files and MD trajectories are available from the corresponding author upon reasonable request.
- **Ethical Approval:** This study is entirely computational and does not involve human participants, animals, or clinical specimens. No ethical approval was required.

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## Appendix A: Glossary of Key Computational Terms

| Term                             | Definition                                                                                                                                                                                                        |
|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ADMET                            | Absorption, Distribution, Metabolism, Excretion, Toxicity — key pharmacokinetic and safety parameters evaluated for drug candidate optimization.                                                                  |
| AutoDock Vina                    | Open-source molecular docking software that predicts the preferred orientation and binding affinity of a ligand to a receptor using a scoring function based on empirical terms.                                  |
| CHARMM36                         | Chemistry at Harvard Macromolecular Mechanics force field; a parameterized mathematical model defining atomic interactions for MD simulation.                                                                     |
| CYP3A4                           | Cytochrome P450 3A4; a major hepatic enzyme responsible for metabolizing approximately 50% of marketed drugs. Inhibition can cause adverse drug-drug interactions.                                                |
| $\Delta G$ (Binding Free Energy) | Gibbs free energy change upon ligand binding to a protein. More negative values indicate stronger (more thermodynamically favourable) binding.                                                                    |
| Lipinski's Ro5                   | Empirical rule set predicting oral bioavailability based on $MW \leq 500$ , $LogP \leq 5$ , $HBD \leq 5$ , $HBA \leq 10$ . Compounds violating two or more rules are predicted to have poor oral bioavailability. |
| MM-PBSA                          | Molecular Mechanics Poisson-Boltzmann Surface Area; a post-MD method for estimating binding free energy incorporating van der Waals, electrostatic, and solvation energy terms.                                   |
| RMSD                             | Root Mean Square Deviation; measures structural deviation of a molecule from a reference position over simulation time. Low RMSD indicates structural stability.                                                  |
| TPSA                             | Topological Polar Surface Area; the sum of surface areas of polar atoms. $TPSA \leq 140 \text{ \AA}^2$ is associated with good oral bioavailability; $\leq 90 \text{ \AA}^2$ favours CNS penetration.             |