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ORIGINAL RESEARCH

In Silico Evaluation of Phytochemicals from *Acacia nilotica, Moringa oleifera,* and *Curcuma longa* as Potential Inhibitors of AmpC β-Lactamase in *Escherichia coli*

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Abstract:

Introduction: Antibiotic resistance driven by β-lactamases, particularly AmpC β-lactamase in *Escherichia coli*, poses a significant threat to global health.

Aims: This study investigates the inhibitory potential of phytochemicals from *Acacia nilotica*, *Moringa oleifera*, and *Curcuma longa* against AmpC β-lactamase using computational methods.

Materials and Methods: The 3D structure of *E. coli* AmpC β-lactamase was prepared by removing heteroatoms, while antibacterial phytochemicals from PubChem were energy-minimized and converted to PDBQT format. Molecular docking utilized a grid-box-focused active site to predict ligand-receptor binding affinities and conformations. Interactions were visualized, and drug-likeness and ADMET profiles (ADMETLab3.0) were computationally assessed.

Results: β-amyrin exhibited the strongest binding affinity (-9.9 kcal/mol), surpassing the control compound ceftazidime (-8.5 kcal/mol), with critical interactions involving Tyr221 and Lys315. Sitosterol also showed promising binding energies (-9.1 kcal/mol), forming hydrogen bonds and π-π interactions with key residues. Drug-likeness screening via DruLiTo confirmed compliance with Lipinski's Rule of Five for all compounds, except minor violations in molecular weight (curcumin) and hydrogen bond donors (β-amyrin). ADMET profiling using ADMETLab 3.0 revealed favourable pharmacokinetic properties, including high gastrointestinal absorption for β-amyrin and sitosterol. However, toxicity risks such as hepatotoxicity (β-amyrin, betulin) and genotoxicity (AMES test positivity) were noted. Molecular interaction analysis highlighted targeting of critical active-site residues (Tyr150, Tyr221, and Ser64), suggesting disruption of catalytic activity

Conclusion: These findings position β-amyrin as a lead compound for further development, despite requiring structural optimization to mitigate toxicity. The study underscores the potential of plant-derived compounds to combat AmpC-mediated resistance, offering a promising avenue for novel inhibitor design

Keywords: AmpC β-lactamase, Phytochemicals, Antibiotic Resistance.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Antibiotic resistance, driven by the rapid evolution of bacterial pathogens, has become a global health crisis. Among the most concerning mechanisms of resistance is the enzymatic hydrolysis of β-lactam antibiotics by β-lactamases, which degrade the β-lactam ring, rendering these drugs ineffective. AmpC β-lactamase, a Class C enzyme primarily found in *Escherichia coli* and other Gram-negative bacteria, is particularly problematic due to its ability to hydrolyse cephalosporins and cephamycins, limiting treatment options for severe infections [1, 2]. The spread of AmpC-mediated resistance, exacerbated by plasmid-mediated transfer and inducible overexpression, has intensified the need for novel inhibitors to restore β-lactam efficacy [3].

Current β-lactamase inhibitors, such as clavulanic acid and avibactam, face challenges including narrow spectra, toxicity, and the emergence of resistance [4. 5]. Natural products, particularly phytochemicals from plants, offer a promising alternative due to their diverse bioactive properties and potential for multi-target inhibition. Among these, *Moringa oleifera*, *Curcuma longa*, and species of the Genus *Acacia nilotica* have garnered attention for their antimicrobial, antioxidant, and enzyme-inhibitory activities [6, 7, 8].

Moringa oleifera contains isothiocyanates, flavonoids, and phenolic acids, which exhibit antibacterial and antifungal effects by disrupting bacterial membranes and inhibiting enzymatic pathways [9]. *Curcuma longa* (turmeric) is rich in curcuminoids, known for their broad-spectrum antimicrobial activity and ability to modulate bacterial protein synthesis [10]. Meanwhile, *Acacia nilotica* species are a source of terpenoids, flavonoids, and phytosterols, which have demonstrated antibacterial and enzyme-inhibitory properties in previous studies [11].

This study evaluates the inhibitory potential of phytochemicals from *Moringa oleifera*, *Curcuma longa*, and *Genus Acacia nilotica* against *E. coli* AmpC β-lactamase using in silico methods, including molecular docking and molecular dynamics simulations. By analysing binding affinities, protein-ligand interactions, and pharmacokinetic properties, this work aimed to identify lead compounds with high inhibitory activity and drug-like characteristics. The findings could pave the way for the development of novel, plant-derived inhibitors to combat AmpC-mediated resistance, addressing a critical gap in current antimicrobial strategies.

2. MATERIAL AND METHODS

2.1 Protein Target Selection and Preparation

The three-dimensional (3D) crystal structure of *E. coli* AmpC β-lactamase in complex with ceftazidime (PDB code: 1IEL, resolution 2.00 Å) was retrieved from the Protein Data Bank (PDB, https://www.rcsb.org/). The protein was prepared for docking using Notepad++ (version 8.6.9.0) by removing heteroatoms (co-crystallized ligand and water molecules) to generate a receptor-ready structure. The processed protein file was saved in PDB format for subsequent analyses.

2.2 Ligand Preparation

Three-dimensional structures in Simple Data Format (SDF) of phytochemicals from the three selected plants previously reported to have antibacterial activity were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SDF files were converted to PDB format using OpenBabel (version 2.4.1) (https://sourceforge.net/projects/openbabel/). Molecular optimization was performed via energy minimization using the Merck Molecular Force Field (MMFF94) in PyRx (version 0.8) to ensure geometric stability.

2.3 Molecular Docking

Molecular docking was conducted using PyRx (version 0.8), a suite incorporating AutoDock Vina, following a flexible docking protocol [12, 13]. The receptor (processed PDB file) and ligands (optimized PDBQT files) were input into the software. A grid box centered on the active site residues was defined to restrict docking to the binding pocket. Multiple configurations were generated for each ligand-protein complex, and binding affinities (in kcal/mol) were calculated.

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2.5 Molecular Interaction Analysis

Visualization and analysis of protein-ligand interactions were performed using Biovia Discovery Studio (version 24.1, 2024) (https://discover.3ds.com/discovery-studio-visualizer-download). The software was used to map hydrogen bonds, π-π stacking, and hydrophobic interactions between ligands and critical residues, and generate 3D snapshots of ligand-receptor complexes for interpretive analysis.

2.5 Drug-Likeness Screening

The DruLiTo software (version 1.0) (https://drulito.software.informer.com/) was employed to evaluate drug-like properties of the ligands. Molecular descriptors (logP, molecular weight, hydrogen bond donors/acceptors) were computed and screened against Lipinski's Rule of Five [14]. Ligands violating more than one rule were excluded from further analysis.

2.7 ADMET Profiling

Pharmacokinetic and toxicological properties of top-performing ligands (based on docking results) were predicted using ADMETLab 3.0 web server (https://admetlab3.scbdd.com/). Parameters analysed included absorption, distribution, metabolism, and excretion/toxicity.

3. Results And Discussion

3.1. Crystal Structure of and mechanism of its resistance

The crystal structure of AmpC beta-lactamase from *E. coli* in Complex with Ceftazidime [15] is given in **Fig, 1**. Ceftazidime is a third-generation cephalosporin antibiotic widely used in clinical settings to treat infections caused by Gram-negative bacteria, including *Escherichia coli* [3]. It exerts its antibacterial effects by inhibiting cell wall synthesis through binding to penicillin-binding proteins (PBPs), essential enzymes for peptidoglycan cross-linking [16]. However, the efficacy of ceftazidime is compromised by β-lactamases, a family of enzymes that hydrolyse the β-lactam ring, rendering antibiotics ineffective. Ceftazidime has been shown to possess some intrinsic ability to inhibit certain β-lactamases, particularly those belonging to Class A, by competing with their natural substrates for active-site binding [17, 18]. Despite this, it remains susceptible to hydrolysis by extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases, which are increasingly prevalent in multidrug-resistant pathogens [19]. Therefore, combining ceftazidime with specific β-lactamase inhibitors, such as avibactam or clavulanic acid, enhances its therapeutic potential by protecting it from enzymatic inactivation. In this study, ceftazidime serves as a reference compound to evaluate the inhibitory activity of phytochemicals against AmpC β-lactamase, providing a benchmark for comparing their efficacy.

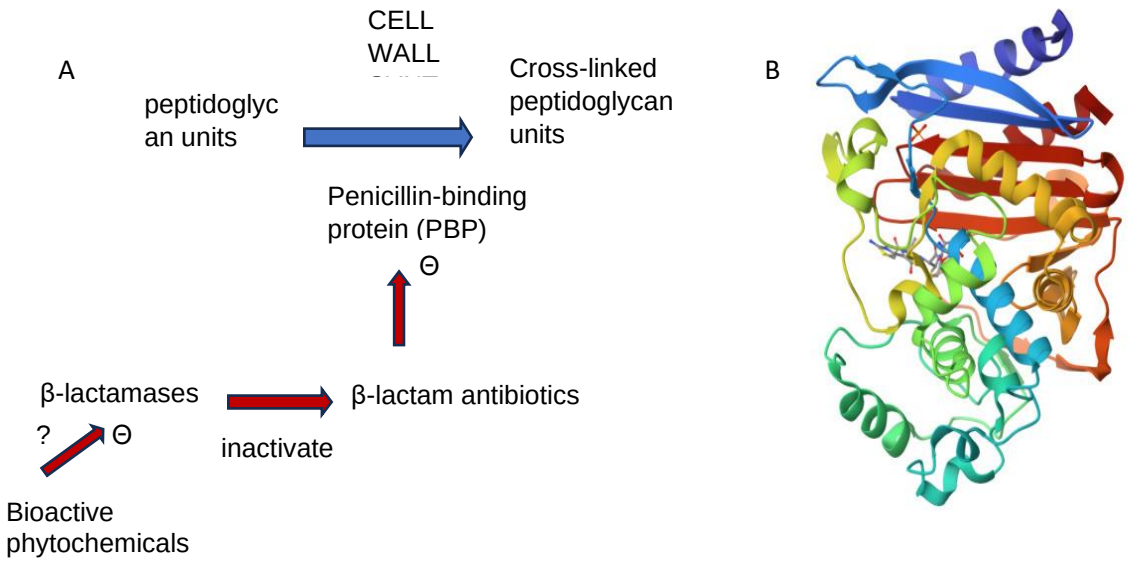


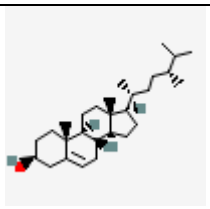
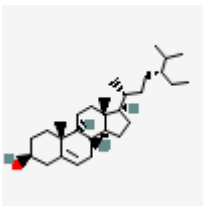
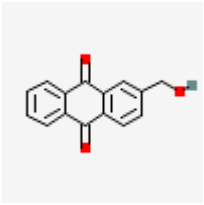
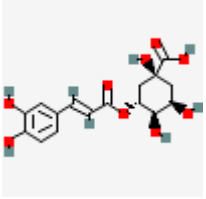
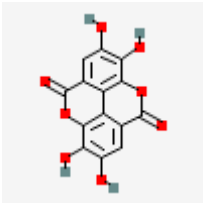
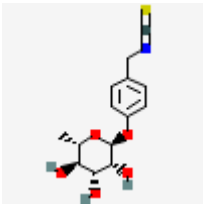
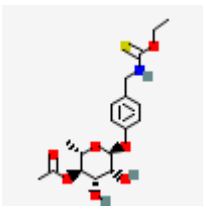
Fig. 1. Crystal Structure of AmpC beta-lactamase and mechanism of its resistance. (A) Crystal Structure of AmpC beta-lactamase from *E. coli* in complex with Ceftazidime. (B) Resistance of lactamases to available drugs leads to inactivation of lactam antibiotics

To address resistance, ceftazidime is often combined with specific β-lactamase inhibitors such as avibactam or clavulanic acid, enhancing its therapeutic potential by protecting it from enzymatic inactivation [20]. Avibactam, a non-β-lactam inhibitor, can inhibit a broader spectrum of β-lactamases, including some Class A, Class C, and even certain Class D enzymes, through reversible binding without forming a stable acyl-enzyme complex [18]. This combination (ceftazidime-avibactam) is particularly effective against multidrug-resistant organisms.

3.2. Drug likeness screening

Phytochemicals selected from the three different plants known for their antibacterial activities for docking studies are presented in shown in **Table 2**. Drug-likeness screening via DruLiTo confirmed compliance with Lipinski's Rule of Five for all compounds, though minor violations were noted. β-amyrin exceeded the hydrogen bond donor limit (4 donors vs. ≤5), while curcumin marginally exceeded molecular weight (368.37 g/mol vs. ≤500 g/mol) (**Table 3**). These deviations suggest minimal synthetic modifications may be required to enhance drug-like properties.

Table 1: Structures of bioactive compounds in *M. oleifera*, *C. longa*, and *Acacia nilotica*

S/N	Plant Source	Ligand	Molecular Weight (g/mol)	Chemical Formula	Chemical Structure
1	*C. longa*	Campesterol	400.7	$C_{24}H_{48}O$	
		β-Sitosterol	414.7	$C_{29}H_{50}O$	
		2-Hydroxylmethl anthraquinone	238.24	$C_{15}H_{10}O_3$	
2	*M. oleifera*	Chlorogenic acid	354.31	$C_{16}H_{18}O_9$	
		Ellagic acid	302.19	$C_{14}H_6O_8$	
		Moringin	311.36	$C_{14}H_{17}NO_5S$	
		Niaziminin	399.5	$C_{18}H_{25}NO_7S$	

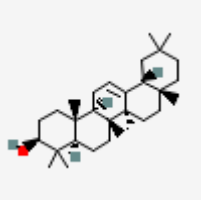
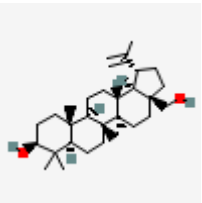
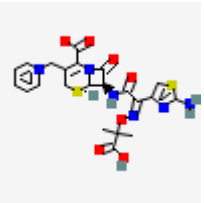
3	*Acacia nilotica*	β-Amyrin	426.7	$C_{30}H_{50}O$	
		Betulin	442.7	$C_{30}H_{50}O_2$	
		Ceftazidime	546.6	$C_{22}H_{22}N_6O_7S_2$	

Table 2: Physicochemical properties of compounds calculated based on Lipinski's rule of 5

Plant	Compound	Molecular Weight	LogP	No of HB Donor	No. of HB Acceptor	No of violations
C. longa	Campesterol	400.70	7.633	1.0	1.0	1
	Beta-sitosterol	414.70	8.000	1.0	1.0	1
	2-hydroxylmethyl anthraquinone	238.24	2.494	1.0	3.0	0
M. oleifera	Chlorogenic acid	354.31	1,036	6.0	9.0	1
	Ellagic acid	302.19	0.951	4.0	8.0	0
	Moringin	311.36	1.661	3.0	6.0	0
	Niaziminin	399.50	0.144	2.0	7.0	0
Acacia nilotica	β-Amyrin	426.70	5.989	1.0	1.0	1
	Betulin	442.70	5.204	1.0	1.0	1
Synthetic drug	Ceftazidime	546.6	-0.865	4.0	13.0	2

3.3. Molecular Docking and Interactions between *E. coli* AmpC beta-lactamase and phytochemicals

The AmpC β-lactamase structure was evaluated for interactions with phytochemicals derived from *Acacia nilotica* species, *Moringa oleifera*, and *Curcuma longa*. Molecular docking revealed significant binding affinities for compounds from all three plants, with β-amyrin from *Acacia nilotica* demonstrating the strongest interaction (binding energy = -9.9 kcal/mol), surpassing the control compound ceftazidime (-8.5 kcal/mol) (**Table 3**). β-amyrin formed critical π-σ interactions with Tyr221 and hydrogen bonds with Lys315 and Glu272, stabilizing its position within the enzyme's active site. This interaction likely disrupts the oxyanion hole critical for β-lactam hydrolysis, thereby inhibiting enzymatic activity.

Table 3: Molecular interactions between bioactive compounds and *E. coli* AmpC β-lactamase

Plant	Compound	No of Hydrogen bonds	Residues involved in hydrogen bond formation	Residues involved in hydrophobic interaction	Residues involved in π-stacking	Binding energy (kcal/mol)
C. longa	Campesterol	2	Arg 296, Arg 148	-	Tyr150, Tyr221	-8.5
	B-Sitosterol	2	Arg 296, Arg 148	-	Tyr221	-9.2
	2-hydroxylmethyly Anthraquinone	4	Tyr150, Ser64, Lys315, Ala318	Tyr150, Ala318	Tyr150	-8.8
M. oleifera	Chlorogenic acid	0	-	Tyr150	Yyr150	-8.5
	Ellagic acid	2	Tyr316, Lys315	Tyr150	Tyr150	-9.9
	Moringin	1	Gly286	-	-	-7.3
	Niaziminin	0	-	Tyr150	Tyr150	-7.3
Acacia nilotica	β-Amyrin	-	-	-	Tyr221	-9.9
	Betulin	1	Thr316	-	-	-8.6
Synthetic drug	Ceftazidime	3	Ile291, Thr316, Ala318	Tyr150	Tyr221	-8.5

3.4. ADMET Properties of selected phytochemicals

ADMET profiling using ADMETLab 3.0 highlighted nuanced pharmacokinetic and toxicity profiles (**Table 4**). β-amyrin displayed high gastrointestinal absorption (HIA score = 0.8) but exhibited potential hepatotoxicity (positive *CYP450* inhibition) and genotoxicity (AMES test positive). Curcumin, despite its strong binding affinity, faced challenges with low Caco-2 permeability (0.45 μm/s) and weak plasma protein binding, limiting oral bioavailability. However, its non-genotoxic profile aligns with prior studies on curcumin's antioxidant properties [21].

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Table 4: ADMET properties of hit compounds from *M. oleifera*, *C. longa*, and *Acacia nilotica*

Class	Properties	Campesterol	β-Sitosterol	2-hydroxylmethylanthraquinone	Chlorogenic acid	Ellagic acid	Moringin	Niazimin	β-Amyrin	Betulin
Absorption	Caco-2 permeability	No	Yes	Yes	No	No	No	No	Yes	Yes
	Gastrointestinal absorption	High	High	High	High	Moderate	Moderate	Moderate	Moderate	Moderately
	Pgp-inhibitor	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	Pgp-substrate	Yes	Yes	Yes	No	No	No	No	Yes	Yes
Distribution	BBB	Yes	Yes	Yes	No	No	No	No	No	No
	PPB	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

	Subcellular localisation	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria	Mitochondria
Metabolism	CYP1A2 inhibitor	Yes	Yes	Yes	No	Yes	No	No	No	No
	CYP1A2 substrate	Yes	Yes	Yes	-	-	-	-	No	No
	CYP2C19 inhibitor	Yes	Yes	Yes	No	Yes	Yes	No	No	No
	CYP2C19 substrate	Yes	Yes	Yes	-	-	-	-	Yes	Yes
	CYP2C9 inhibitor	Yes	Yes	Yes	No	No	No	No	No	No
	CYP2C9 substrate	Yes	Yes	Yes	No	-	No	No	Yes	No
	CYP2D6 substrate	Yes	Yes	Yes	No	No	No	No	No	Yes

	CYP2D6 inhibitor	Yes	Yes	YEs	No	No	No	No	Yes	No
	CYP3A4 inhibitor	Yes	Yes	Yes	No	No	No	No	No	Yes
	CYP3A4 substrate	No	No	No	No	No	Yes	No	No	No
Toxicity	Acute oral toxicity	Yes	Yes	Yes	No	Yes	No	No	No	No
	Human Mutagenicity	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
	Human hepatoxicity	Yes	Yes	Yes	Yes	Yes	No	No	No	No
	Carcinogenicity	No	Yes	Yes	No	Yes	No	No	No	No
	Genotoxicity	Yes	Yes	Yes	-	-	-	-	-	-

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BBB: blood brain barrier; PPB: Plasma protein binding

Clinically, these compounds offer promising alternatives to traditional β-lactamase inhibitors like clavulanate and sulbactam, which face limitations in spectrum and toxicity. β-amyrin's strong binding suggests it could synergize with β-lactams such as ceftazidime, restoring antibiotic efficacy against AmpC-producing *E. coli*. However, its ADMET challenges necessitate structural optimization. For instance, reducing β-amyrin's hydrophobicity via polar group addition could mitigate hepatotoxicity while preserving binding affinity. Curcumin's poor bioavailability might be addressed through nanoparticle formulations or piperine co-administration to inhibit efflux pumps [22].

The observed interactions with critical residues (for example, TYR221) underscore the multi-target potential of phytochemicals, which may reduce the likelihood of resistance emergence compared to single-target inhibitors. This aligns with studies highlighting natural products' ability to modulate multiple pathways [23].

Limitations of this study include reliance on *in silico* methods, which assume rigid protein conformations. Molecular dynamics simulations could better assess ligand stability over time. Additionally, ADMET predictions require experimental validation (e.g*., in vitro* toxicity assays and pharmacokinetic studies in animal models). Despite these constraints, the findings provide a robust foundation for advancing these compounds toward preclinical testing.

4. CONCLUSION

This study demonstrates the potential of phytochemicals from *Acacia nilotica*, *Moringa oleifera*, and *Curcuma longa* as AmpC β-lactamase inhibitors. β-amyrin (*Acacia nilotica*) exhibited the strongest inhibitory profile, while curcumin and isothiocyanate showed context-dependent efficacy. Despite ADMET challenges such as hepatotoxicity and poor bioavailability, strategic structural modifications and delivery systems could enhance their therapeutic potential. These findings highlight natural products as a viable strategy to combat AmpC-mediated resistance, addressing a critical gap in antibiotic development. Future work should prioritize experimental validation of binding affinity, toxicity, and synergistic activity with β-lactams to advance these compounds toward clinical applications.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Fatai A designed and supervised the study and wrote the manuscript. Yusuf-Esilokun H, Oluokun F, Osisami E, Jokosanya A, Abdulkabir H, Adeyeye H and Komi D carried out the analyses. Bankole H and Kazeem I read the manuscript and approved the manuscript.

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