Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

June 2026
Volume 6
Issue 1

Natural Inhibitors from Polyalthia longifolia Target NDM-1 and DNA Gyrase B in Multidrug-Resistant Klebsiella pneumoniae and Acinetobacter baumannii: An Integrated Experimental and Computational Study

Olumide Oluyele*[1], Ruth Ayomipo Loto[2], Gladys Egunjobi[3]

[1,2,3] Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Emails: olumide.oluyele@aaua.edu.ng, lotoayomipo@gmail.com, diekonifeoluwagladys774@gmail.com

Received 18 November 2025 - Accepted 10 February 2026 - Published 05 May 2026

ABSTRACT:

Background: *Acinetobacter baumannii and Klebsiella pneumoniae are WHO critical-priority pathogens with widespread multidrug resistance, posing a significant threat to global health. This study evaluated the antibacterial activity of solvent fractions of Polyalthia longifolia leaves and examined the molecular docking, pharmacokinetic, and toxicity profiles of its phytochemicals against NDM-1 and DNA gyrase B.*

Results: *All fractions exhibited antibacterial activity, with zones of inhibition ranging from 18.33 ± 0.33 to 23.00 ± 0.58 mm. The aqueous fraction was most active (A. baumannii: 22.33 ± 0.33 mm; K. pneumoniae: 23.00 ± 0.58 mm) and showed the lowest minimum inhibitory concentration (6.25 mg/mL). High-performance liquid chromatography identified Quercetin, Apigenin, and catechin as major bioactive constituents. Docking revealed that mangiferin (-9.263 kcal/mol), myricetin (-8.703 kcal/mol), and Baicalin (-8.411 kcal/mol) bound NDM-1 more strongly than reference drugs, while Quercetin (-7.400 kcal/mol), catechin (-7.262 kcal/mol), and Apigenin (-7.216 kcal/mol) showed high affinity for DNA gyrase B. ADME profiling indicated good oral bioavailability, moderate lipophilicity, and minimal CYP450 interference. Toxicity predictions confirmed low hepatotoxicity, cardiotoxicity, and mutagenic risks.*

Conclusion: *P. longifolia leaves possess potent antibacterial metabolites. Mangiferin, Quercetin, and myricetin showed strong inhibitory potential with favorable pharmacokinetic and safety profiles, supporting their promise as leads against multidrug-resistant K. pneumoniae and A. baumannii.*

KEYWORDS:

Polyalthia longifolia, antibiotic resistance, Klebsiella pneumoniae, Acinetobacter baumannii, NDM-1, DNA gyrase B, phytomedicine.

1. Introduction

Antimicrobial resistance (AMR) is widely recognized as one of the greatest threats to global health, with the World Health Organization describing it as a "silent pandemic" projected to cause 10 million deaths annually by 2050 if left unaddressed (1). Multidrug-resistant (MDR) bacteria, defined as organisms resistant to at least one agent in three or more antimicrobial classes, pose particular challenges to modern medicine (2). Among these, *Klebsiella pneumoniae* and *Acinetobacter baumannii* are classified as critical priority pathogens due to their high resistance levels and their association with severe clinical infections, including pneumonia, bloodstream infections, urinary tract infections, and wound sepsis (3–8).

Understanding the factors that drive resistance in these pathogens is essential for developing effective interventions. Mechanisms such as indiscriminate antibiotic use, horizontal gene transfer, biofilm formation, and efflux pump activity collectively limit the effectiveness of conventional therapies (5,9,10). Consequently, the rise of MDR pathogens has placed substantial strain on healthcare systems worldwide, particularly in low- and middle-income countries where access to novel antimicrobials is limited (11). The slowdown in new antibiotic development, due to high costs, long development timelines, and low returns on investment, further underscores the urgent need

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

for alternative therapeutic strategies (12).

Medicinal plants offer a promising source of structurally diverse bioactive compounds with antimicrobial potential. Historically, natural products have provided the foundation for many frontline antibiotics, and contemporary research continues to validate their importance in drug discovery (13–15). Phytochemicals such as alkaloids, flavonoids, terpenoids, phenolics, and tannins exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant properties, highlighting their potential as templates for novel antimicrobial agents (16,17). These characteristics make plant-derived compounds particularly attractive for combating MDR pathogens and addressing the limitations of conventional antibiotics.

Among the many medicinal plants with antimicrobial potential, *Polyalthia longifolia* (family Annonaceae), commonly known as the Ashoka tree, has emerged as a particularly promising candidate. Its selection is based on both its rich phytochemical composition and its historical use in managing infections. Traditionally used across Asia and Africa, various plant parts—including leaves, bark, and seeds—have been employed to treat infections, fever, wound healing, and gastrointestinal disorders (18,19). Ethnobotanical surveys further highlight that leaf decoctions are widely used to treat bacterial infections (20), reflecting both cultural relevance and practical accessibility in resource-limited settings.

2. Materials and Methods

Test Microorganisms

Multidrug-resistant clinical strains of *Klebsiella pneumoniae* and *Acinetobacter baumannii* were used, selected due to their classification as critical-priority pathogens by the World Health Organization. Bacterial inocula were standardized to ~1 × 10^6 CFU/mL using a 0.5 McFarland turbidity standard prepared by mixing 0.05 mL of 1% barium chloride dihydrate with 9.95 mL of 1% sulfuric acid. Strains were subcultured on nutrient agar, incubated at 37 °C for 18 hours, and suspended in sterile saline to match the McFarland standard visually (21).

Plant Material, Extraction, and Fractionation

Air-dried leaves of *Polyalthia longifolia* (syn. *Monoon longifolium*), Annonaceae, were collected and authenticated, and a voucher specimen was prepared. Crude ethanolic extraction was performed using maceration (22). Briefly, 520 g of powdered leaves were soaked in 3 L of ethanol for three days with occasional shaking, filtered through muslin and Whatman No. 1 paper, concentrated, and stored at −20 °C. Fractionation was conducted via liquid–liquid extraction (23). The crude Extract was dissolved in water (1:10, w/v) and sequentially partitioned with n-hexane, a 1:1 n-hexane: ethyl acetate mixture, ethyl acetate, and water to yield four fractions: F1 (n-hexane), F2 (n-hexane: ethyl acetate), F3 (ethyl acetate), and F4 (aqueous). All fractions were concentrated, freeze-dried, and stored at 4 °C for further analysis. This polarity-gradient approach facilitates the enrichment of metabolites according to their solubility and chemical characteristics, enabling targeted evaluation of bioactivity.

Antibacterial Assays

The agar well diffusion method was used to evaluate antibacterial activity (23). Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions. Wells (6 mm) were loaded with 100 µL of Extract (100 mg/mL prepared in 5% DMSO), while amoxicillin or Ofloxacin served as positive controls based on the confirmed susceptibility of the multidrug-resistant (MDR) isolates to these antibiotics. Plates were allowed to pre-diffuse for 15 minutes at room temperature and subsequently incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method followed by subculturing. Serial dilutions of the extracts (100–3.125 mg/mL) were prepared in Mueller–Hinton broth, inoculated with 0.5 mL of standardized bacterial suspension, and incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration showing no visible bacterial growth, while the MBC was defined as the lowest concentration yielding no growth upon subculture.

HPLC Profiling

Approximately 2 g of Extract was mixed with 20 mL of 1:1 acetonitrile–methanol, agitated for 30 minutes, and the organic phase was transferred to a 25 mL volumetric flask. Analysis was performed on an Agilent 1200 RP-HPLC system using a Hypersil BDS C18 column (250 mm × 4.0 mm i.d.) with a mobile phase of 0.1% formic acid in water (A) and acetonitrile (B), gradient elution, 0.6 mL/min flow rate, 20 µL injection, and 280 nm detection. Compounds were identified by comparing retention times and UV spectra with standards (23).

Generation and Preparation of Compound Library

Twelve (12) phytochemical compounds identified from Polyalthia longifolia by HPLC analysis were selected for *in silico* screening. The corresponding chemical structures of these HPLC-identified compounds, along with that of the reference drug, were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in Structure Data File (SDF) format. All molecules were imported into the Schrödinger workspace (Schrödinger, 2021) and prepared using the LigPrep module to optimize molecular geometry, assign appropriate protonation states at physiological pH, and generate relevant tautomers and stereoisomers for subsequent computational analyses.

Protein Retrieval and Preparation

The X-ray crystallographic structures of the target proteins—*Klebsiella pneumoniae* metallo-β-lactamase-1 (NDM-1, PDB ID: 4EYB) and *Acinetobacter baumannii* DNA gyrase B (PDB ID: 7PQI)—were obtained from the RCSB Protein Data Bank. The selection of reference ligands for *in silico* screening was based on their mechanistic relevance to the chosen protein targets and known resistance pathways rather than phenotypic susceptibility profiles. Accordingly, Ofloxacin and novobiocin were used as standard inhibitors due to their established interactions with DNA gyrase B and ATP-binding enzymes, respectively. Protein preparation and grid generation were performed using the Schrödinger Protein Preparation Wizard with the OPLS4 force field.

Structure-based Virtual Screening

Prepared compounds from *P. longifolia*, along with the standard ligand, were docked against the target proteins using the extra precision (XP) Glide protocol in Maestro (Schrödinger Suite, 2021). XP docking was employed due to its high accuracy in ranking ligand binding affinities, albeit with longer computational time (23).

Binding Energy Calculations

Docked protein-ligand complexes were further refined using the local optimization feature in Prime. The binding free energy (△_bind) of each complex was estimated through MM/GBSA calculations with the OPLS4 force field, providing quantitative insights into the stability and affinity of ligand interactions.

Statistical Analysis

All experimental data were processed using SPSS v22. One-way ANOVA was performed to compare group means, followed by Duncan's New Multiple Range Test for post hoc analysis. Statistical significance was considered at $p \leq 0.05$.

3. Results

Antibacterial Activity of P. longifolia

The antibacterial activity of the different solvent fractions of *P. longifolia* against multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* is presented in Table 1. All fractions demonstrated inhibitory effects, with inhibition zones ranging between 18.33 ± 0.33 mm and 23.00 ± 0.58 mm. The aqueous fraction (F4) produced the highest zones of inhibition against both test organisms (22.33 ± 0.33 mm for *A. baumannii* and 23.00 ± 0.58 mm for *K. pneumoniae*), followed by the n-hexane fraction (F1). The ethyl acetate fraction (F3) exhibited the lowest activity, particularly against *A. baumannii*. The aqueous fraction exhibited the lowest MIC (6.25 mg/mL) against both *A. baumannii* and *K. pneumoniae*, suggesting the greatest potency. The ethyl acetate and n-hexane: ethyl acetate fractions recorded higher MICs of 25 mg/mL against *A. baumannii*. In contrast, all fractions except Fraction 4 required 12.5 mg/mL to inhibit *K. pneumoniae*. The aqueous fraction again demonstrated the lowest MBC against *A. baumannii* (12.5 mg/mL), whereas the ethyl acetate fraction was most effective against *K. pneumoniae* (12.5 mg/mL). Fractions 1 and 2 exhibited higher MBCs (25–50 mg/mL).

Phytochemical Profile by HPLC

The HPLC chromatographic analysis (Table 2, Figure 1) revealed the presence of several bioactive compounds in the *P. longifolia* leaf extracts, notably Quercetin, Apigenin, catechin, and kaempferol.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

Table 1: Antibacterial Activity of P. longifolia Extracts

Test organisms	Zone of Inhibition (mm)				MIC (mg/mL)				MBC (mg/mL)			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
A. baumannii	21.33 ± 0.67	19.33 ± 0.58	18.33± 0.33	22.33± 0.33	12.5	25	25	6.25	50	50	25	12.5
K. pneumoniae	22.67 ±0.67	21.67 ±0.58	21.33± 0.67	23.00± 0.58	12.5	12.5	12.5	6.25	25	25	12.5	25

Legend: F1- n-hexane fraction, F2- n-hexane/ethyl acetate fraction, F3- ethyl acetate fraction, and F4- aqueous fraction.

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/L]	Grp	Name
1.317	VV E	20.25056	1.00097e-1	2.02702		Caffeic Acid
1.567	VV E	28.07921	4.69604e-3	1.31861e-1		Ferulic Acid
2.454	VV E	40.72340	3.20764e-2	1.30626		Maleic Acid
3.227	VV E	36.74120	1.7158e-2	1.13671		Catechin
4.051	VB	1399.65674	6.70379e-3	9.38301		Magiferin
4.598	BB	142.88849	4.25662e-2	6.08223		Naringin
5.845	BB	80.81638	1.42089e-2	1.14831		Myricetin
8.147	VV X	1.19665e-1	7.75186	9.27626e-1		Baicalin
9.273	MM	20.02323	2.00894e-2	4.02254e-1		Quercetin
10.875	VV X	1.47642e-2	19.68298	2.90603e-1		Phloretin
11.063	MM	10.13986	8.18996e-2	8.30450e-1		Apigenin
12.257	MM	20.87885	3.63575e-2	7.59102e-1		Kaempferol

Table 2: HPLC-Identified Compounds of Polyalthia longifolia Leaf Extracts

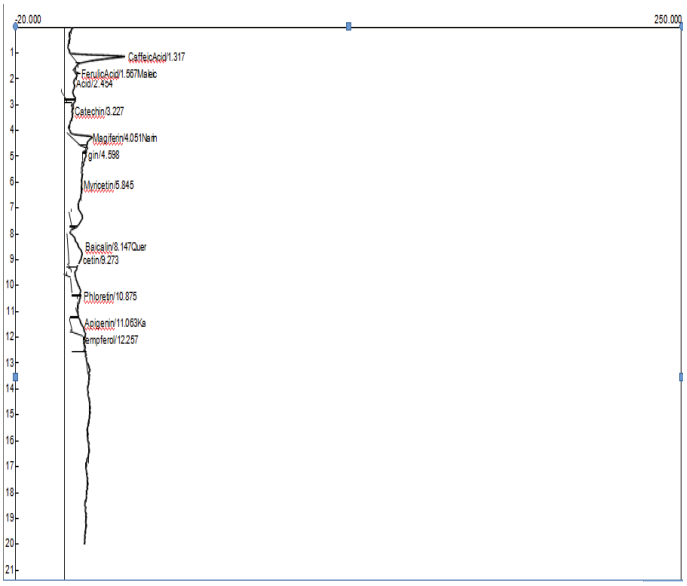


Figure 1: Chromatograph of Polyalthia longifolia Leaf Extract

Molecular Docking Interaction of P. longifolia with K. pneumoniae NDM-1

The binding affinities of the identified phytochemicals toward NDM-1 were compared with standard drugs (Ofloxacin and novobiocin). Among the tested compounds, mangiferin (-9.263 kcal/mol), myricetin (-8.703 kcal/mol), and Baicalin (-8.411 kcal/mol) exhibited stronger binding affinities than Ofloxacin (-6.327 kcal/mol) and oxacillin (-6.286 kcal/mol) (Figure 2). These top-ranking ligands showed multiple hydrogen bonds with key active site residues, including GLN123, ASN220, and ASP124 (Figure 4, Table 3). Hydrophobic interactions were primarily observed with residues such as MET67, LEU65, ILE35, and TRP93, while mangiferin and Baicalin displayed π–π stacking with PHE70. The high binding affinities suggest potential inhibition of the bacterial β-lactamase enzyme comparable to or greater than that of the reference drug.

Molecular Docking Interaction of P. longifolia with A. baumannii DNA Gyrase B

For *A. baumannii*, Quercetin (-7.4 kcal/mol), catechin (-7.262 kcal/mol), and Apigenin (-7.216 kcal/mol) demonstrated the highest binding affinities compared to the standard novobiocin (-6.111 kcal/mol) (Figure 3). These compounds formed multiple hydrogen bonds and hydrophobic interactions with key residues such as ASP87, THR279, and VAL57 (Figure 5, Table 4). Kaempferol and myricetin also interacted favorably, forming π–cation or hydrogen bonds within the catalytic pocket, suggesting competitive inhibition potential against DNA gyrase B.

ADME and Drug-Likeness Properties

The ADME profiles of the top bioactive compounds are presented in Table 5. Most phytochemicals complied with Lipinski's rule of five, indicating good oral bioavailability and pharmacokinetic potential. Compounds such as Quercetin, myricetin, and kaempferol displayed moderate lipophilicity (iLOGP ≈ 1.1–2.0) and acceptable polar surface areas (TPSA ≤ 150 Å²).

High gastrointestinal absorption was predicted for most compounds, particularly Apigenin, kaempferol, and catechin. The compounds

exhibited variable CYP enzyme inhibition, with limited interactions with CYP2C9 and CYP2C19, suggesting low metabolic interference.

Toxicity Prediction

The toxicity profiles (Table 6) revealed that all tested compounds were non-carcinogenic and exhibited low hepatotoxicity and cardiotoxicity risks (low hERG inhibition). None of the lead compounds showed significant Ames mutagenicity or acute oral toxicity, indicating favorable safety margins. Among all, mangiferin, Quercetin, and myricetin presented the most promising safety–efficacy balance, supporting their potential as lead candidates for further development against multidrug-resistant *K. pneumoniae* and *A. baumannii*.

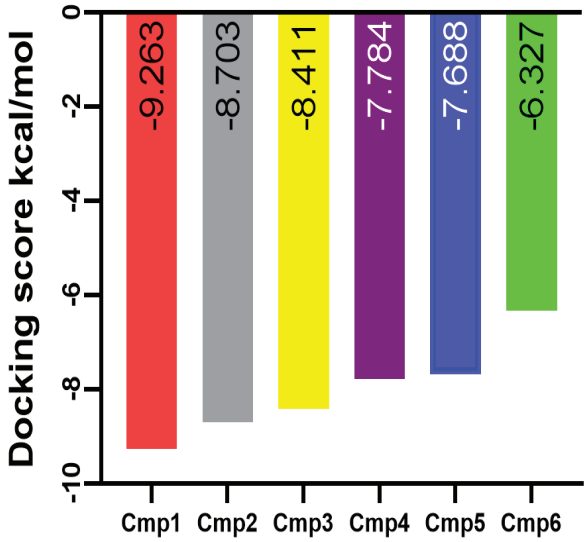


Figure 2: Graphical representation of the binding affinity calculation of the lead compounds from P. longifolia against K. pneumoniae NDM-1

Legend: cmp 1= Mangiferin cmp 2= Myricetin cmp 3= Baicalin cmp 4= Quercetin cmp 5 = Maleic acid cmp 6 = Ofloxacin

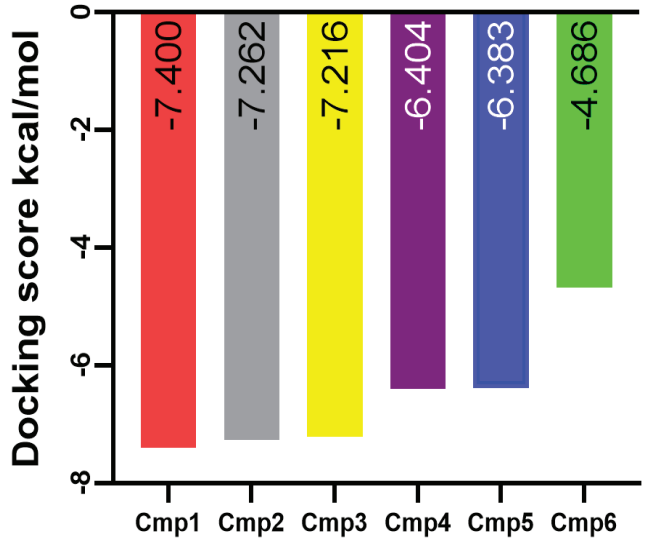
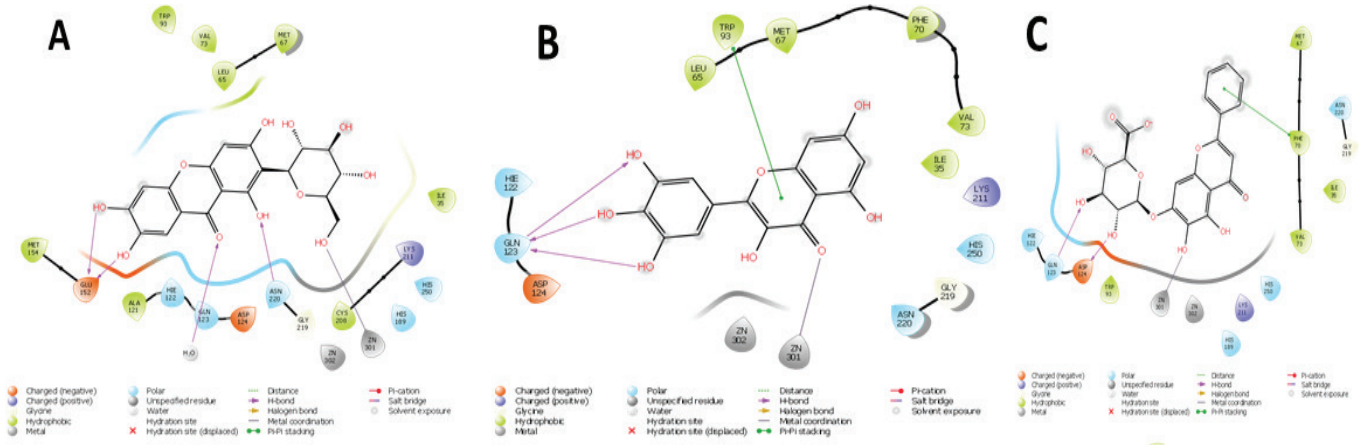


Figure 3: Graphical representation of the binding affinity calculation of the lead compounds from P. longifolia against A. baumannii DNA gyrase B

Legend: cmp 1= Quercetin cmp 2= Catechin cmp 3= Apigenin cmp 4= Kaempferol cmp 5 = Myricetin cmp 6 = Novobiocin



Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

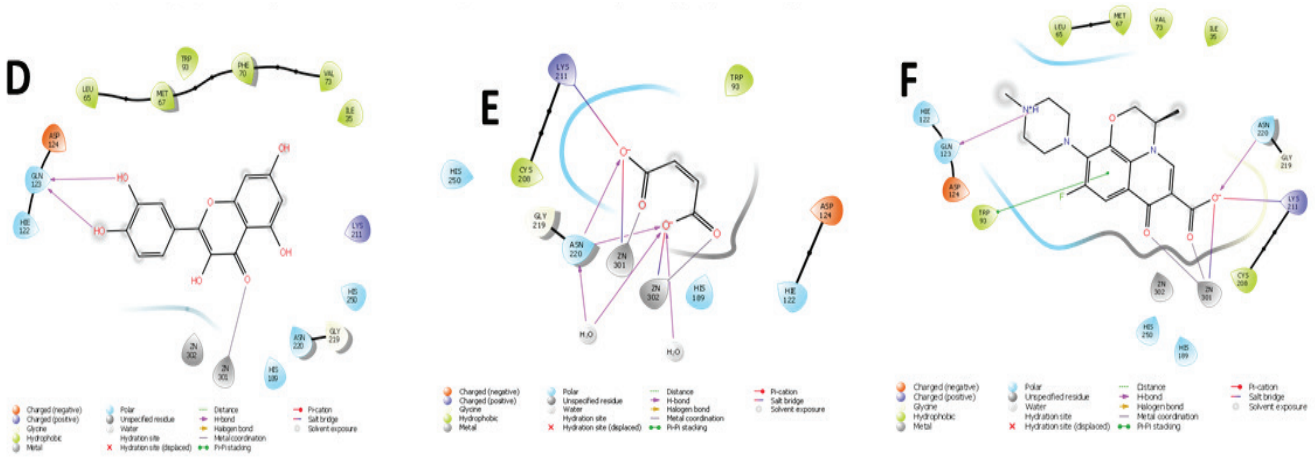


Figure 4: 2D Interaction between P. longifolia lead compounds and Klebsiella pneumoniae protein. Legend : (A) Mangiferin; (B) Myricetin; (C) Baicalin; (D) Quercetin; (E) Maleic acid; (F) Ofloxacin (standard).

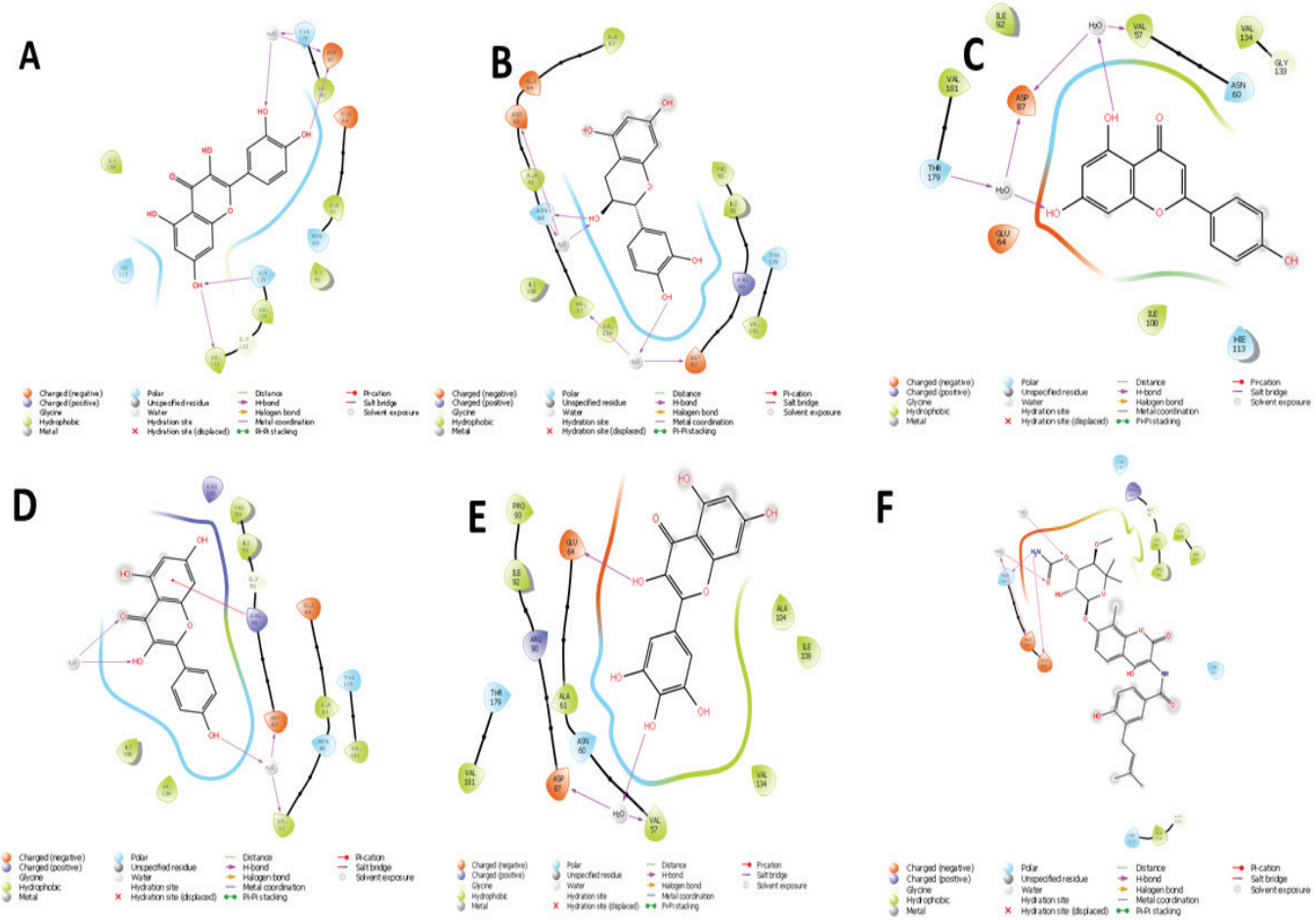


Figure 5: 2D interaction between lead compounds and Acinetobacter baumannii protein
Legend: (A) Quercetin; (B) Catechin; (C) Apigenin; (D) Kaempferol; (E) Myricetin; (F) Novobiocin (standard).

Table 3: Hydrogen Bonds and Hydrophobic Interactions of the Lead Phytochemicals from P. longifolia with K. pneumoniae

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
Mangiferin	GLU152, ASN220	MET154, ALA121, CYS208, ILE35, LEU65, MET67, VAL73, TRP93	NONE
Myricetin	GLN123	TRP93, LEU65, MET67, PHE70, VAL73, ILE35	Pi-Pi stacking: TRP93
Baicalin	GLN123, ASP124	MET67, PHE70, VAL73, ILE35	Pi-Pi stacking: PHE70
Quercetin	GLN123	LEU65, MET67, TRP93, PHE70, VAL73, ILE35	NONE
Maleic acid	ASN220	TRP93, CYS208	Salt bridge: LYS211, ZN302, ZN302
Ofloxacin	GLN123, ASN220	LEU65, MET67, VAL73, ILE35, TRP93, CYS208	Salt bridge: LYS211, ZN301, Pi-Pi stacking: TRP93

Table 4: Hydrogen Bonds and Hydrophobic Interactions of the Lead Phytochemicals from P. longifolia with A. baumannii

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
Quercetin	THR279, ASP87, SER135, VAL132	VAL181, ALA61, ILE92, VAL134, VAL132, ILE108	NONE
Catechin	ASP63, ASN60, ASP87, VAL57	ALA67, PRO93, ILE92, ALA61, ILE108, VAL57, VAL134, VAL181	NONE
Apigenin	ASP87, THR179, VAL57,	VAL57, ILE92, VAL134, VAL181, ILE108	NONE
Kaempferol	ASP87, VAL57,	PRO93, ILE92, ALA61, ILE108, VAL134, VAL57, VAL181	Pi cation: ARG90
Myricetin	GLU64, ASP87, VAL57	ALA61, VAL57, VAL134, ILE108, ALA104, ILE92, PRO93, VAL181	NONE
Novobiocin	ASN60, GLU64	ILE92, ILE108, VAL107, PRO93, ALA114	NONE

Table 5: Drug likeness and ADME profile of the top bioactive compounds of P. longifolia

Compound Name	MW	HBA	HBD	TPSA	iLOGP	ROV	ESOL Log S	GIA	CYP2C19 inhibitor	CYP2C9 inhibitor
Quercetin	302.04	7	5	131.36	1.447712	0	-3.72159	0.133589	0.005855004	0.432249367
Ofloxacin	361.14	7	1	75.01	-0.11273	0	-1.97932	3.46E-06	2.59E-05	5.96E-06
Novobiocin	612.23	13	6	200.01	1.790881	1	-4.47123	0.122134	0.711179674	0.007009505
Myricetin	318.04	8	6	151.59	1.115212	0	-3.44294	0.31806	0.002221007	0.995218813
Mangiferin	422.08	11	8	201.28	0.059778	1	-3.15526	0.751839	1.91E-08	0.000311245
Maleic acid	116.01	4	2	74.6	-0.02442	0	0.404371	0.949129	3.26E-10	5.12E-05
Kaempferol	286.05	6	4	111.13	1.965317	0	-3.64796	0.015143	0.132401496	0.79935813
Catechin	290.08	6	5	110.38	1.172588	0	-2.58137	0.016103	9.82E-06	2.47E-06
Baicalin	446.08	11	6	187.12	1.300846	1	-2.30448	0.008194	6.57E-06	7.80E-07
Apigenin	270.05	5	3	90.9	2.980917	0	-4.21599	0.001684	0.112379447	0.002128024

Legend: MW= molecular weight, HBA= Hydrogen bond acceptor , HBD= Hydrogen bond donor, ROV=, GIA= Gastrointestinal absorption, BA= bioavailability

Table 6: Toxicity profile of the top bioactive compounds of P. longifolia

Compounds	Ames	hERG	Hepatoxicity	Carcinogenecity	LD50(mg/kg)
Quercetin	0.586042	0.052722	0.337382	0.600177	['-']
Ofloxacin	0.920446	0.405136	0.988498	0.76824	[(9, 8, 7, 6, 5, 4, 25, 16, 15, 13, 12, 11)]
Novobiocin	0.748943	0.141221	0.88874	0.34689	['-']
Myricetin	0.657129	0.040223	0.325381	0.501712	['-']

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

Mangiferin	0.845841	0.015823	0.498865	0.282849	['–']
Maleic acid	0.207986	0.036178	0.242399	0.021734	['–']
Kaempferol	0.545969	0.069277	0.386179	0.715984	['–']
Catechin	0.458893	0.1343	0.556749	0.226083	['–']
Baicalin	0.499818	0.009341	0.428817	0.045225	['–']
Apigenin	0.617563	0.099782	0.435063	0.793352	['–']

Legend: Ames = Mutagenecity potential, hERG= Cardiotoxicity, LD50= Acute oral toxicity

- **Abbreviations**
- **ADME** – Absorption, Distribution, Metabolism, and Excretion
- **AMES** – Mutagenicity potential (Ames test)
- **ATP** – Adenosine Triphosphate
- **CYP** – Cytochrome P450 enzymes
- **DMSO** – Dimethyl sulfoxide
- **DNA** – Deoxyribonucleic acid
- **F1–F4** – Extract fractions (F1: n-hexane, F2: n-hexane:ethyl acetate, F3: ethyl acetate, F4: aqueous)
- **HPLC** – High-Performance Liquid Chromatography
- **hERG** – Human Ether-à-go-go-Related Gene (Cardiotoxicity potential)
- **iLOGP** – Lipophilicity prediction index
- **LD50** – Median lethal dose (Acute oral toxicity)
- **MBC** – Minimum Bactericidal Concentration
- **MIC** – Minimum Inhibitory Concentration
- **MDR** – Multidrug-resistant
- **NDM-1** – New Delhi Metallo-β-lactamase-1
- **PDB** – Protein Data Bank
- **TPSA** – Topological Polar Surface Area
- **XP Glide** – Extra Precision Glide docking protocol

β-lactam – Beta-lactam antibiotics

4. DISCUSSION

This study evaluated the antibacterial activity of different solvent fractions of *Polyalthia longifolia* leaves against multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*, two critical-priority nosocomial pathogens (8,24). The rising prevalence of these resistant bacteria underscores the urgent need for alternative therapeutics, particularly natural products with established bioactive efficacy.

Antibacterial screening revealed variable inhibitory potencies among the solvent fractions. The aqueous fraction consistently produced the largest zones of inhibition and the lowest MIC and MBC values against both pathogens, indicating a higher concentration of polar antibacterial constituents. HPLC profiling confirmed the presence of Quercetin, rutin, and other phenolic compounds, which are known to act synergistically to enhance antibacterial activity. These findings align with previous reports demonstrating that polar extracts of *Polyalthia longifolia* exhibit broad-spectrum antibacterial activity largely attributed to flavonoids, phenolics, and glycosides (25–27). Flavonoids such as Quercetin have been reported to disrupt bacterial membrane integrity, inhibit nucleic acid synthesis, and interfere with energy metabolism (27), thereby supporting the observed potency of the aqueous fraction.

In addition to polar phytochemicals, non-polar and moderately polar fractions of *P. longifolia* in the present study also exhibited antibacterial activity, albeit to a lesser extent. The n-hexane fraction showed appreciable inhibitory effects, particularly against *Klebsiella pneumoniae*, suggesting the involvement of lipophilic constituents such as terpenoids and fatty acids with membrane-disrupting and quorum-sensing inhibitory properties (28,29). Notably, clerodane-type diterpenoids previously isolated from *P. longifolia* have been reported to possess significant antibacterial activity**,** supporting the bioactivity observed in non-polar fractions. Faizi et al. (30) demonstrated that clerodane diterpenoids isolated from *P. longifolia* exhibited marked antimicrobial effects against pathogenic bacteria, highlighting the contribution of diterpenoid scaffolds to the plant's antibacterial profile.

The ethyl acetate fraction also demonstrated notable antibacterial activity and recorded the lowest MBC value against *K. pneumoniae*, emphasizing the bactericidal potential of moderately polar metabolites, including terpenoids, phenolic acids, and other semi-polar compounds. Collectively, these results underscore the influence of solvent polarity on phytochemical extraction efficiency and antibacterial efficacy, and further corroborate earlier reports that both polar phenolics and non-

polar diterpenoids contribute to the antimicrobial potential of P. longifolia (31).

Variability in bacterial susceptibility was evident, with *K. pneumoniae* being more sensitive than *A. baumannii*, consistent with the latter's robust efflux pumps and biofilm-mediated resistance (7,32). Fractionation preserved antibacterial efficacy, with the aqueous fraction achieving inhibition zones of 22.3 mm (*A. baumannii*) and 23.0 mm (*K. pneumoniae*), MICs of 6.25 mg/mL, and MBCs as low as 12.5 mg/mL, comparable to previous studies using crude extracts (26,33). The strong performance of the aqueous fraction likely reflects synergistic interactions among diverse metabolites (15).

At the molecular level, resistance in *K. pneumoniae* and *A. baumannii* is largely mediated by β-lactamases and DNA gyrase enzymes (34,35). The metallo-β-lactamase NDM-1 hydrolyzes β-lactam antibiotics (36,37), while Ofloxacin inhibits bacterial replication via stabilization of the DNA–DNA gyrase complex. DNA gyrase B in *A. baumannii* catalyzes ATP-dependent negative supercoiling of DNA, and Novobiocin competes at its ATP-binding site (38-40). Docking of *P. longifolia* phytochemicals against these targets provides mechanistic insight into their observed antibacterial activity.

Docking analyses revealed that Mangiferin, Myricetin, and Baicalin exhibited stronger binding affinities toward NDM-1 than Ofloxacin. Mangiferin (-9.263 kcal/mol) formed key hydrogen bonds with GLU152 and ASN220 and hydrophobic contacts with MET154, ALA121, CYS208, ILE35, LEU65, MET67, VAL73, and TRP93, stabilizing the complex. Myricetin (-8.703 kcal/mol) and Baicalin (-8.411 kcal/mol) showed robust interactions via hydrogen bonds, hydrophobic contacts, and π–π stacking, consistent with previous findings that hydroxyl-rich flavonoids enhance binding to target enzymes (41). Quercetin (-7.784 kcal/mol) and Maleic acid (-7.688 kcal/mol) demonstrated moderate binding, while reference antibiotics Oxacillin (-6.286 kcal/mol) and Ofloxacin (-6.327 kcal/mol) displayed weaker affinities. These findings indicate that natural compounds may demonstrate superior inhibitory interactions compared to standard drugs, in agreement with previous studies (22,42).

For *A. baumannii*, Quercetin exhibited the highest binding affinity (-7.400 kcal/mol), forming multiple hydrogen bonds with THR279, ASP87, SER135, VAL132, and ILE108, complemented by hydrophobic interactions. These results suggest that the natural compounds adopt favorable conformations within the receptor's active site, enabling stable and meaningful interactions, consistent with previous findings (43,44). Catechin (-7.262 kcal/mol) and Apigenin (-7.216 kcal/mol) displayed comparable stabilizing interactions, while Kaempferol (-6.404 kcal/mol) and Myricetin (-6.383 kcal/mol) engaged in hydrogen bonding, hydrophobic contacts, and π–cation interactions with ARG90. By contrast, reference antibiotics Novobiocin (-4.686 kcal/mol) and Ofloxacin (-4.415 kcal/mol) showed lower docking scores, reflecting weaker binding tendencies and highlighting the potential of these natural compounds as effective inhibitors.

The correlation between *in silico* binding affinities and *in vitro* antibacterial outcomes reinforces the predictive accuracy of docking results. The aqueous fraction's superior potency is likely attributable to hydrophilic flavonoids, including Quercetin and Mangiferin, which interact favorably with both NDM-1 and DNA gyrase B, supporting dual inhibitory mechanisms that impair β-lactam hydrolysis and DNA replication.

ADMET profiling confirmed the pharmacological potential of the lead phytochemicals. Most compounds complied with Lipinski's Rule of Five, indicating favorable oral bioavailability and membrane permeability (45). Moderate lipophilicity (iLOGP: -0.024 to 2.98) supported membrane transport while maintaining solubility. Gastrointestinal absorption was high, and limited CYP450 inhibition suggested minimal metabolic interference. Notably, Myricetin and Catechin exhibit significant antioxidant and antibacterial activity with negligible CYP450 inhibition (46,47), consistent with the present study's findings. In contrast, Baicalin and Mangiferin showed restricted bioavailability due to high TPSA and numerous hydrogen bond donors, reflecting pharmacokinetic limitations typical of glycosylated polyphenols (41,48). Toxicity predictions indicated low mutagenic, hepatotoxic, and cardiotoxic risks for the natural compounds, whereas reference antibiotics Ofloxacin and Novobiocin displayed higher hepatotoxicity and Ames values, corroborating clinical reports of dose-related hepatocellular injury (49).

Overall, the combined *in silico* and *in vitro* evidence indicates that *P. longifolia* bioactive compounds exhibit dual antibacterial mechanisms—targeting β-lactamase and DNA gyrase B enzymes—similar to Novobiocin and Ofloxacin, while maintaining superior safety and pharmacokinetic profiles.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

5. Conclusion

Polyalthia longifolia leaf fractions exhibit significant antibacterial activity against multidrug-resistant *A. baumannii* and *K. pneumoniae*, with the aqueous fraction showing the highest efficacy. The n-hexane fraction also demonstrated notable activity, highlighting contributions from both polar and non-polar phytochemicals. HPLC analysis identified flavonoids, phenolic acids, and alkaloids—including Mangiferin, Myricetin, Baicalin, Catechin, and Quercetin—as key bioactive compounds likely acting synergistically.

Molecular docking revealed strong binding affinities of these phytochemicals to clinically relevant bacterial targets (NDM-1 and DNA gyrase B), surpassing or matching standard antibiotics and supporting dual inhibitory potential observed *in vitro*. ADMET profiling confirmed favorable pharmacokinetic and safety profiles, with good oral bioavailability, low CYP450 inhibition, and minimal toxicity, though some polyhydroxylated flavonoids showed limited gastrointestinal absorption.

These findings underscore the therapeutic potential of *P. longifolia* as a source of bioactive compounds with dual antibacterial mechanisms, combining cell wall disruption and DNA replication inhibition. Further *in vitro* and *in vivo* validation, along with optimization for solubility and absorption, is essential to translate these findings into clinically useful antimicrobial therapies.

References

1. World Health Organization. Antimicrobial resistance: Global report on surveillance Report: 2021 [Internet]. www.who.int. 2021. Available from: https://www.who.int/publications/i/item/9789240027336

2. Geng Y, Liu Z, Ma X, Pan T, Chen M, Dang J, et al. Infection prevention and control measures for multidrug-resistant organisms: a systematic review and network meta-analysis. Infection. 2025 Mar 17;53.

3. Abebe AA, Birhanu AG, Tessema TS. Antibiotic Resistance and Virulence Mechanisms in Klebsiella pneumoniae: Understanding for Better Interventions. Bacteria. 2026 Feb 2;5(1):9.

4. Hao SY, Lin HY, Yeh PI, Hsiao HC, Lin CY, Huang WH, et al. Multidrug-Resistant Acinetobacter baumannii Pneumonia in Clinical Therapy: A Systematic Review and Network Meta-analysis on Effectiveness and Safety of Different Antibiotic Combination Regimens. International Journal of Antimicrobial Agents. 2026 Apr;107815.

5. Kulaç Ö, Başkan C, Koşar N, Balcı PÖ, Havuz SG, Sırıken B. Klebsiella pneumoniae clinical isolates: extended spectrum β-lactamase production, biofilm formation, and virulence factors. Biologia. 2024 Aug 6;79(10):3209–17.

6. Mukhopadhyay H, Bairagi A, Mukherjee A, Prasad AK, Roy AD, Nayak A. Multidrug resistant Acinetobacter baumannii: A study on its pathogenesis and therapeutics. Current Research in Microbial Sciences. 2024 Dec 6;8:100331.

7. Oluyele O, Olaoluwa SF, Akanji V, Egunjobi G, Eweola E. Fractionation-Guided Evaluation of Justicia secunda and Kalanchoe pinnata as Inhibitors of DNA Gyrase and Penicillin-Binding Proteins in Multidrug-Resistant Acinetobacter baumannii and Klebsiella pneumoniae. Journal of Natural Products Discovery. 2026;4(4).

8. World Health Organization. WHO Bacterial Priority Pathogens List, 2024 [Internet]. 2024. Available from: https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf

9. Patra M, Gupta A, Kumar D, Kumar B. Antimicrobial Resistance: A Rising Global Threat to Public Health. Infection and Drug Resistance. 2025 Oct;Volume 18:5419–37.

10. Asmare Z, Tamrat E, Erkihun M, Endalamaw K, Alelign D, Getie M, et al. Antimicrobial resistance pattern of Acinetobacter baumannii clinical isolate in Ethiopia. A systematic review and meta-analysis. BMC Infectious Diseases. 2025 Apr 12;25(1).

11. Totaro V, Guido G, Cotugno S, De Vita E, Asaduzzaman M, Patti G, et al. Antimicrobial Resistance in Sub-Saharan Africa: A Comprehensive Landscape Review. The American journal of tropical medicine and hygiene. 2025;tpmd250035.

12. Gargate N, Laws M, Rahman KM. Current economic and regulatory challenges in developing antibiotics for Gram-negative bacteria. npj Antimicrobials and Resistance. 2025 Jun 11;3(1).

13. Oluyele O, Bimboye PT. Harnessing Blighia sapida as a Source of Novel Antimicrobials: Activity of Crude and Fractionated Extracts Against MDR Pathogens. Biology, Medicine, & Natural Product Chemistry. 2026;15(1):221–7.

14. Akorful RAA, Asafo-Adjei K, Kotey FCN, Donkor ES. Effectiveness of African Herbal Preparations against Multidrug-Resistant Klebsiella pneumoniae: A Systematic Review. Scientific African. 2025;28(2).

15. OLUYELE O. HPLC PROFILING AND ANTI-CANDIDAL ACTIVITY OF CRUDE AND FRACTIONATED EXTRACTS OF TITHONIA DIVERSIFOLIA. Quantum Journal of Medical and Health Sciences. 2025 Jun 28;4(3):48–55.

16. Goswami C, Pawase PA, Shams R, Pandey VK, Tripathi A, Rustagi S, et al. A Conceptual Review on Classification, Extraction, Bioactive Potential and Role of Phytochemicals in Human Health. Future foods. 2024 Feb 1;9:100313–3.

17. Dattatray TV, Baburao SP, Shivaji CS. A comprehensive review on Polyalthia longifolia. Traditional Medicine Research. 2021;6(2).

18. Musa N, Ayisa T. Medicinal properties of Polyalthia longifolia (Masquerade tree). International Journal of Agriculture and Earth Science. 2024;10(5):140–9.

19. Ismaila M, Dharmaraj S, Ong E, Jun TG, Sasidharan S. Polyalthia longifolia var. angustifolia thw.: a review of ethnobotany, phytochemistry, pharmacology applications and future prospects as a green anti-aging agent. Advances in Traditional Medicine. 2025 Jun 6;25(4).

20. Firdous SM, Ahmed SN, Hossain SM, Ganguli S, Fayed MAA. Polyalthia longifolia: phytochemistry, ethnomedicinal importance, nutritive value, and pharmacological activities review. Medicinal Chemistry Research. 2022 Jun 10;31(8):1252–64.

21. Oluyele O, Oladunmoye M, Ogundare A, Onifade A, Okunnuga N. Microbial spectrum and susceptibility profile of opportunistic pathogens isolated from cancer patients attending a tertiary healthcare centre in Akure, Nigeria. Microbes, Infection and Chemotherapy. 2023;3:1–10.

22. Oluyele O, Oladunmoye M. Susceptibility Patterns of Staphylococcus aureus Isolated from Wounds Swabs to Extracts of Vernonia amygdalina. Journal of Advances in Medical and Pharmaceutical Sciences. 2017 Jan 10;13(4):1–11.

23. Oluyele O, Omoboyowa DA, Aderogba AE, Osei KA. Piper guineense (Swhan and Thon) Inhibits Lanosterol-14α-demethylase in Multi-Drug Resistant Non-albicans Candida Species: In vitro and In silico Studies. Advances in Medical, Pharmaceutical and Dental Research. 2025 Mar 15;5(1):10.

24. Olaniyan TO, Martínez-Vázquez AV, Escobedo-Bonilla CM, López-Rodríguez C, Huerta-Luévano P, Castrejón-Sánchez O, et al. The Prevalence of ESKAPE Pathogens and Their Drug Resistance Profiles in Aquatic Environments Around the World. Microbiology Research. 2025 Sep 4;16(9):201.

25. Chioma D, Reminus O. Phytochemical Analysis of Polyalthia Longifolia (Fresh Leaves). Direct Research Journal of Chemistry and Material Science. 2023 Sep;11(7):49.

26. Isamotu BA, Dikwa KB, Appah J. Antibacterial activity of aqueous and methanolic extracts of *Polyalthia longifolia* and *Hyptis suaveolens* against clinically relevant isolates from Nigerian Defence Academy Hospital, Kaduna, Nigeria. Science World Journal. 2025 Aug 20;20(2):815–23.

27. Liu Y, Zhu J, Liu Z, Zhi Y, Mei C, Wang H. Flavonoids as Promising Natural Compounds for Combating Bacterial Infections. International Journal of Molecular Sciences. 2025 Mar 10;26(6):2455.

28. Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran TB, et al. Terpenes and Terpenoids as Main Bioactive Compounds of Essential oils, Their Roles in Human Health and Potential Application as Natural Food Preservatives. Food Chemistry: X. 2022 Mar;13(100217):100217.

29. Câmara JS, Perestrelo R, Ferreira R, Berenguer CV, Jorge, Castilho PC. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. Molecules. 2024 Aug 15;29(16):3861–1.

30. Faizi S, Khan RA, Mughal NR, Malik MS, Sajjadi KS, Ahmad A. Antimicrobial activity of various parts of *Polyalthia longifolia* var. *pendula*: isolation of active principles from the leaves and the berries. Phytotherapy Research. 2008 Apr 3;22(7):907–12.

Advances in Medical, Pharmaceutical and Dental Research Journal (AMPDR)- ISSN 2812-4898
http://dx.doi.org/10.21622/AMPDR.2026.06.1.1808

31. Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria. PeerJ. 2019 Oct 9;7:e7857.

32. Abdi SN, Ghotaslou R, Ganbarov K, Mobed A, Tanomand A, Yousefi M, et al. Acinetobacter baumannii Efflux Pumps and Antibiotic Resistance. Infection and Drug Resistance. 2020 Feb 12;13:423–34.

33. Umar FJ, Idris FT, Usman A, Balarabe FT, Adamu A. Antibacterial Activity of Polyalthia longifolia Leaf Extracts against Staphylococcus aureus and Escherichia coli. UMYU Journal of Microbiology Research (UJMR). 2024 Jun 9;9(3):8–12.

34. Li Y, Kumar S, Zhang L. Mechanisms of Antibiotic Resistance and Developments in Therapeutic Strategies to Combat Klebsiella pneumoniae Infection. Infection and Drug Resistance. 2024 Mar;17:1107–19.

35. Ramachandran S, Deepak K, Shrikumaran M, et al. Unveiling meropenem resistance and co-resistance patterns in *Klebsiella pneumoniae and Acinetobacter baumannii*: A global genome analysis using ML/DL and association mining. *BMC Microbiol.* 2025;25:699. https://doi.org/10.1186/s12866-025-04394-6

36. Raghunath D. New metallo β-lactamase NDM-1. The Indian journal of medical research. 2010 Nov;132(5):478–81.

37. King DT, Worrall LJ, Gruninger R, Strynadka NCJ. New Delhi Metallo-β-Lactamase: Structural Insights into β-Lactam Recognition and Inhibition. Journal of the American Chemical Society. 2012 Jul 5;134(28):11362–5.

38. Dighe SN, Collet TA. Recent advances in DNA gyrase-targeted antimicrobial agents. European Journal of Medicinal Chemistry. 2020 Aug;199:112326.

39. Cotman AE, Durcik M, Benedetto Tiz D, Fulgheri F, Secci D, Sterle M, et al. Discovery and Hit-to-Lead Optimization of Benzothiazole Scaffold-Based DNA Gyrase Inhibitors with Potent Activity against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Journal of Medicinal Chemistry. 2023 Jan 12;66(2):1380–425.

40. Rajakumari K, Aravind K, Balamugundhan M, Jagadeesan M, Somasundaram A, Brindha Devi P, et al. Comprehensive review of DNA gyrase as enzymatic target for drug discovery and development. European Journal of Medicinal Chemistry Reports. 2024 Dec;12:100233.

41. Huang T, Liu Y, Zhang C. Pharmacokinetics and Bioavailability Enhancement of Baicalin: A Review. European Journal of Drug Metabolism and Pharmacokinetics. 2019 Apr 1;44(2):159–68.

42. Pancu DF, Scurtu A, Macasoi IG, Marti D, Mioc M, Soica C, et al. Antibiotics: Conventional Therapy and Natural Compounds with Antibacterial Activity—A Pharmaco-Toxicological Screening. Antibiotics. 2021 Apr 7;10(4):401.

43. Ali M, Hassan M, Ansari SA, Alkahtani HM, Al-Rasheed LS, Shoeb Anwar Ansari. Quercetin and Kaempferol as Multi-Targeting Antidiabetic Agents against Mouse Model of Chemically Induced Type 2 Diabetes. Pharmaceuticals. 2024 Jun 8;17(6):757–7.

44. Boo HJ, Yoon D, Choi Y, Kim Y, Cha JS, Yoo J. Quercetin: Molecular Insights into Its Biological Roles. Biomolecules. 2025 Feb 20;15(3):313.

45. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001 Mar;46(1-3):3–26.

46. Almatroodi SA, Rahmani AH. Unlocking the Pharmacological Potential of Myricetin Against Various Pathogenesis. International Journal of Molecular Sciences. 2025 Apr 28;26(9):4188–8.

47. Nadalin P, Kim JK, Park SU. Recent studies on myricetin and its biological and pharmacological activities. EXCLI Journal. 2023 Nov 28;22:1223.

48. Imran M, Arshad MS, Butt MS, Kwon JH, Arshad MU, Sultan MT. Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipids in Health and Disease. 2017 May 2;16(1).

49. Andrade RJ, Tulkens PM. Hepatic safety of antibiotics used in primary care. Journal of Antimicrobial Chemotherapy. 2011 Jul 1;66(7):1431–46.