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Exploring the antimicrobial potential of *Azadirachta indica* (Neem) phytoconstituents against drug-resistant *Acinetobacter baumannii*: A molecular docking and ADMET screening study

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Abstract:

Background: The emergence of multidrug resistant (MDR) *Acinetobacter baumannii* remains at the top of the health care concern around the world which thereby emphasizes the need to discover novel antimicrobials from medicinal plants, such as *Azadirachta indica* (neem) in treatment of infections caused by MDR *A. baumannii*. The current in-silico study was aimed to evaluate inhibitory potential of Neem derived phytoconstituents against essential proteins of *A. baumannii* and their evaluation of pharmacokinetic characteristics.

Methodology: Selected bioactive constituents of *A. indica* extracted from proteomic databases were subjected to systematic molecular docking protocol (using Auto Dock Vina in PyRx interface) against three important *A. baumannii* protein targets; 4Y0A, 5YDB and 4DXV crystallographic structures extracted from PubChem database. The resulting binding affinity values along with detailed insights into the landscape of ligand-protein interactions provided information on the inhibitory potential of each compound. Thereafter, the highest-ranking candidates by their docking values were subjected to ADMET profiling (using ADMETlab 3.0), which entailed evaluation of absorption, distribution, metabolism, excretion, and toxicity (ADMET), *in silico* to assess their pharmaceutical suitability and prospective clinical application.

Results: Through rigorous molecular docking studies, several phytochemicals were extracted from *A. indica* proteomic databases, which exhibited significant high binding affinities against the three key proteins targets of *A. baumannii*. Among these, isonimocinolide, nicotiflorin, and nimbaflavone showed the highest activity with binding energy ranging from -8.7 to -9.9 (kcal/mol), thus suggesting significant potential for inhibition. However, assessments of ADMET profile indicated major limitations of the pharmacokinetic and toxicological nature. Although the metabolic interactions obtained were acceptable for some of the compounds, *in-silico* predictions suggest considerably low oral bioavailability for the three phytochemical compounds. Furthermore, toxicological screening outlined possible hepatotoxic as well as genotoxic effects.

Conclusion: In this *in-silico* study, computational analysis showed that some *A. indica* phyto-constituents have significant inhibitory activity against MDR *A. baumannii* protein targets. However, low oral bioavailability, and large tolerance risk such as liver damage and genotoxicity, indicate the need for improvement in the structures of the phytochemicals. Although these neem derived phytochemicals show promising inhibitory activity, systematic optimization with rigorous *in vitro* validation will be necessary to improve their safety profiles and therapeutic efficacy.

Keywords: *Azadirachta indica*; *Acinetobacter baumannii*; molecular docking; ADMET; AMR; phytochemicals

Received May 6, 2026; Revised Jul 10, 2026; Accepted Jul 12, 2026

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Exploration du potentiel antimicrobien des phytoconstituants d'*Azadirachta indica* (Neem) contre *Acinetobacter baumannii* résistant aux antibiotiques: étude de docking moléculaire et de criblage ADMET

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Résumé:

Contexte: L'émergence d'*Acinetobacter baumannii* multirésistant (MDR) demeure une préoccupation majeure de santé publique à l'échelle mondiale, soulignant ainsi la nécessité de découvrir de nouveaux antimicrobiens issus de plantes médicinales, telles qu'*Azadirachta indica* (Neem), pour le traitement des infections causées par *A. baumannii* MDR. La présente étude *in silico* visait à évaluer le potentiel inhibiteur de phytoconstituants dérivés du neem sur des protéines essentielles d'*A. baumannii*, ainsi que leurs caractéristiques pharmacocinétiques.

Méthodologie: Des constituants bioactifs sélectionnés d'*A. indica*, extraits de bases de données protéomiques, ont été soumis à un protocole de docking moléculaire systématique (à l'aide d'AutoDock Vina dans l'interface PyRx) contre trois cibles protéiques importantes d'*A. baumannii*: les structures cristallographiques 4Y0A, 5YDB et 4DXV, extraites de la base de données PubChem. Les valeurs d'affinité de liaison obtenues, associées à une analyse détaillée des interactions ligand-protéine, ont permis d'évaluer le potentiel inhibiteur de chaque composé. Les candidats les plus prometteurs, selon leurs valeurs d'amarrage moléculaire, ont ensuite fait l'objet d'un profilage ADMET (à l'aide d'ADMETlab 3.0), comprenant l'évaluation *in silico* de l'absorption, de la distribution, du métabolisme, de l'excrétion et de la toxicité (ADMET), afin d'évaluer leur potentiel pharmaceutique et leur application clinique potentielle.

Résultats: Grâce à des études rigoureuses d'amarrage moléculaire, plusieurs composés phytochimiques extraits des bases de données protéomiques d'*A. indica* ont présenté des affinités de liaison élevées et significatives pour les trois protéines cibles clés d'*A. baumannii*. Parmi ceux-ci, l'isonimocinolide, la nicotiflorine et la nimbaflavone ont montré l'activité la plus importante, avec une énergie de liaison comprise entre -8,7 et -9,9 kcal/mol, suggérant ainsi un potentiel d'inhibition significatif. Cependant, l'évaluation du profil ADMET a révélé des limitations majeures d'ordre pharmacocinétique et toxicologique. Bien que les interactions métaboliques obtenues soient acceptables pour certains composés, les prédictions *in silico* suggèrent une biodisponibilité orale considérablement faible pour les trois composés phytochimiques. De plus, le criblage toxicologique a mis en évidence des effets hépatotoxiques et génotoxiques potentiels.

Conclusion: Dans cette étude *in silico*, l'analyse computationnelle a montré que certains constituants phytochimiques d'*A. indica* présentent une activité inhibitrice significative contre les cibles protéiques d'*A. baumannii* multirésistant. Cependant, la faible biodisponibilité orale et le risque élevé d'effets indésirables, tels que des lésions hépatiques et une génotoxicité, indiquent la nécessité d'améliorer la structure de ces composés phytochimiques. Bien que ces composés phytochimiques dérivés du neem présentent une activité inhibitrice prometteuse, une optimisation systématique, associée à une validation *in vitro* rigoureuse, sera nécessaire pour améliorer leur profil de sécurité et leur efficacité thérapeutique.

Mots-clés: *Azadirachta indica*; *Acinetobacter baumannii*; amarrage moléculaire; ADMET; AMR; phytochimiques

Introduction:

Antimicrobial resistance (AMR) has become a pre-eminent global health challenge of the twenty-first century. These are changes in microorganisms due to mutations resulting in resistance to conventional therapeutic agents. The increase in the rates of infection due AMR, together with a lack of new development of new antimicrobial drugs, has led to a critical public health gap (1). The projections suggest that the current toll of 700 000 deaths a year may rise to 10 million by 2050, and this will be accompanied by a staggering rise in healthcare expenditure (2).

While antibiotics are indispensable in clinical practice (instrument), inappropriate and prolonged use have imposed such strong selective pressure leading to the emergence of resistant bacterial phenotypes (3). In response, the World Health Organization (WHO) revised the Bacterial Priority Pathogens List (BPPL) in 2024 and outlines 24 organisms that are high, critical and medium priority in response to the need to guide research and development efforts towards the most important threats (4).

Among the most formidable organisms in the 'critical' category is *Acinetobacter baumannii*, a predominant aetiological agent of nosocomial infections such as ventilator-associated pneumonia and bacteremia. This is enhanced by its ability to form clonal outbreaks, and its frighteningly broad resistance to carbapenem antibiotics (5). Carbapenem-resistance among *A. baumannii* (CRAB) has become a worldwide healthcare burden, associated with prolonged hospital stay, high mortality and significant economic costs (6).

As the need for new antimicrobials re-emerges, interest in medicinal botanicals with known pharmacological content and demonstrated efficacy in the past has been revived. Among these *Azadirachta indica* commonly known as Neem, belonging to Meliaceae family, emerges as well documented source of diverse bioactive constituents (7). Its efficacy against both Gram-positive and Gram-negative, bacterial strains is attributed to its species of phytochemicals such as nimbin, nimbidin, azadirachtin and gedunin with pronounced bacteriostatic and bactericidal effects. Owing to its rich source of phenolic, flavonoid and terpenoid, Neem is a

promising natural source of hits for the rational design of new drug scaffolds to fight multidrug resistant infections (8).

While the antimicrobial potential of *A. indica* is well documented, there are still large knowledge gaps in understanding the interaction between individual neem-derived phytochemicals and drug-resistant mediators in bacteria, most notably in *A. baumannii*. The current study was aimed at determining the potential inhibitory activity of the phytochemical constituents of neem on a set of clinically relevant protein targets of *A. baumannii* (4Y0A, 5YDB and 4DXV), using structure-based molecular docking. The pharmacokinetic compatibility and toxicity risk of the phytochemicals were further evaluated using complimentary assessment of ADMET profiling.

Materials and method:

Study design:

This research is an *in-silico* study to evaluate the antimicrobial efficacy of *A. indica* against MDR *A. baumannii*. The research followed a computational workflow i. e. bioactive Neem ligands and essential bacterial target proteins first extracted from proteomic databases and preprocessed for simulation. Later, molecular docking was performed in order to quantify binding affinities (kcal/mol) and describe structural interactions between selected phytochemicals and the bacterial targets. High affinity candidates were then prioritized for comprehensive ADMET screening to assess the viability of the candidates during clinically relevant pharmacokinetic screening tests as well as safety profiles of the candidate compounds. This combined approach was aimed to identify highly potent lead compounds with the best drug likeness for further experimental settings.

Selection of phytochemical compounds and protein targets:

Structures of bioactive compounds of *A. indica* were obtained from PubChem database (9). Three key targets for protein production in *A. baumannii* were obtained from the RCSB Protein Data Bank (PDB), namely 4Y0A, 5YDB and 4DXV (10).

Data collection and measurements:

Molecular docking simulation was carried out using Auto Dock vina software in PyRx environment version (v 0.8), which was subsequently visualized and scrutinized for the interactions of proteins and ligand compounds using BIOVIA Discovery Studio version 2025. The pharmacokinetic and toxicological characteris-

tics of the best ligands were predicted in ADMETlab web 3.0 server (11). The results were expressed in terms of ranked inhibitory efficacy using binding free energies that were reported in kcal/mol, while toxicity metrics were represented in terms of probability indices ranging from 0.0 to 1.0.

QSAR modeling:

A Quantitative Structure-Activity Relationship (QSAR) structure was built for correlating the molecular descriptors of the *A. indica* constituents (independent variables) with the experimental IC₅₀ values (dependent variable). Using Genetic Function Approximation (GFA) tool within Materials Studio, we successfully generated the GFA (Multiple Linear Regression - GFA (MLR - GFA)) equation; Predicted IC₅₀ = a + b₁x₁ + b₂x₂ + b_nx_n. This model provides a strong validation-based statistical framework tool for predicting neem derivatives inhibitory capacity against *A. baumannii* based on their distinctive molecular characters.

Molecular docking:

The three high resolution protein targets for *A. baumannii* (PDB IDs: 4Y0A, 5YDB, 4DXV) were selected from the RCSB Protein Data Bank based on stringent selection criteria which included the resolution of the X-ray diffraction data (1.5-2.5 Å), the co-crystallization status, and the mutational stability. Protein preparation involved removal of heteroatoms and crystalline waters followed by the addition of polar hydrogens using the Auto Dock Tools. The ligand structures extracted from PubChem were energy minimized and then converted to PDBQT format with Open bolt.

Docking calculations were done using Auto Dock Vina software in the PyRx program, using site specific coordinates of grid boxes adapted to active site of each target (for example, center X = -1.69 Y = 8.19 Z = 14.17 for 4Y0A). The resulting binding poses were ranked according to the force field scoring functions, defining Nimbaflavone, Isonimocinolide and Nicotiflorin as the best ligands to the target 4Y0A, 5YDB and 4DXV respectively. Interaction geometries were defined and visualized by using BIOVIA Discovery Studio 2025.

ADMET screening:

The lead phytochemicals were subjected to a stringent ADMET screening using ADMETlab 3.0 computational platform (11). The screening protocol included Lipinski's Rule of Five, oral bioavailability, gastrointestinal absorption, and critical cytochrome P450 enzymes interactions predictions. Safety assessments in-

cluded predictions of the hepatotoxicity, mutagenicity and systemic clearance rates. Molecules that showed both high affinity of binding and positive numbers for safety and drug memorial properties were selected for further experimental verification.

Results:

Molecular docking results:

The detailed results of docking that include binding energies in kcal/mol and the different forms of ligand-receptor interactions between selected phytochemicals from *A. indica* with the three protein structures of *A. baumannii* (4Y0A, 5YDB and 4DXV) are summarized in Tables 1 to 3 corresponding to the individual targets for bacterial protein.

Docking simulation of *A. indica* phytochemicals against 4Y0A protein of *A. baumannii* yielded binding affinities in the range of -4.8 to -8.7 (kcal/mol). Nimbaflavone had the highest binding inhibitor with binding energy of -8.7 (kcal/mol) while meliantriol and beta-sitosterol-beta-D- glucoside had strong binding energy of -7.7 (kcal/mol) and -7.5 (kcal/mol), respectively.

Moderate binding affinities were observed for deacetylnimbin, 28-deoxonibolide and deacetylsalannin within the range -6.5 to -7.0 (kcal/mol), which suggests a fair degree of biological activity. In contrast, squalene and hexamethyltetracosahexaene derivatives had lower binding energies (i.e. 4.8 to 5.6 kcal/mol), thereby showed reduced inhibitory ability towards this specific target. All of the ligands tested showed a Root Mean Square Deviation (RMSD) of 0.0A, thus confirming the stability of the ligand orientation and the credibility of the docking poses in the active site of 4Y0A (Table 1).

The molecular docking analysis studies performed on the phytochemicals from *A. indica* against *A. baumannii* 5YDB protein target showed the binding affinity with values from -5.0 (kcal/mol) to -9.1 (kcal/mol). Among the phytochemicals, isonimocinolide was the most promising one with binding energy of -9.1 (kcal/mol), therefore indicating the highest interaction and specificity for bound activation site of the target protein. Additional ligands with great inhibitory potential were Meliantriol (-8.5 kcal/mol), Beta-sitosterol-Beta-D-glucoside (-8.2 kcal/mol) and 28-deoxonimbolide (-8.0 kcal/mol) (Table 2).

Table 1: Binding results of *Azadirachta indica* with 4Y0A_ *Acinetobacter baumannii*

S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	4Y0A_Meliantriol_uff_E=997.15	-7.7	0	0
2	4Y0A_Deacetylnimbin_uff_E=982.24	-7.0	0	0
3	4Y0A_2,6,10,15,19,23-Hexamethyltetracosahexaene_uff_E=248.91	-5.6	0	0
4	4Y0A_(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.94	-5.0	0	0
5	4Y0A_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-7.5	0	0
6	4Y0A_Elemene; delta- Elemene_uff_E=218.48	-5.6	0	0
7	4Y0A_gamma- Elemene_uff_E=179.27	-6.1	0	0
8	4Y0A_(1S,4aS,8aR)-1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene_uff_E=173.65	-6.1	0	0
9	4Y0A_deuterio-squalene_uff_E=190.36	-4.8	0	0
10	4Y0A_(14E,18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.57	-5.2	0	0
11	4Y0A_(18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.57	-5.3	0	0
12	4Y0A_Deacetylsalannin_uff_E=1158.52	-6.5	0	0
13	4Y0A_28-Deoxonimbolide_uff_E=1088.15	-6.7	0	0
14	4Y0A_Nimbaflavone_uff_E=311.89	-8.7	0	0

UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

A moderate binding affinities were observed for the molecules; deacetylsalannin, deacetylnimbin, gamma-murolene, and elemene, which ranged from -6.2 thousand calories (kcal) per mole to -7.9 (kcal/mol). On the other hand, the derivatives of deuterio-squalene and hexamethyl-tetracosahexaene had relatively low inhibitory potential, with affinities ranging from -5.0 (kcal/mol) to -5.7 (kcal/mol). The technical robustness of the docking results was also supported by an RMSD value of 0.0 Å for all ligands and provided stable and predictable binding orientation. Accordingly, isonimocinolide piloted series of potent binders were sucked out and prioritized for further ADMET profiling and validation through additional experiments.

Molecular docking of *A. indica* phytochemicals on *A. baumannii* 4DXV protein target showed binding affinity between -4.8 and -9.9 (kcal/mol). Nicotiflorin had the most potent in-

hibitory activity with a binding energy of -9.9 (kcal/mol). Strong interactions were also shown by Nimbaflavone (with -8.5 kcal/mol), beta-sitosterol-beta-beta-glucoside (-7.7 kcal/mol) and 28-deoxonimbolide (-7.6 kcal/mol).

Moderate affinities were recorded for deacetylsalannin (-7.3kcal/mol) and deacetylnimbin (-6.8kcal/mol). On the contrary, hexamethyltetracosahexaene derivatives and squalene-related compounds exhibited weak to moderate interactions with a value range between (-4.8 to -6.2 kcal mol). Technical validation of these results was confirmed by a Root Mean Square Deviation (RMSD) of 0.0 Å within all the ligands which indicates the consistency of the docking orientations and completeness of a reliable binding pose. Overall, nicotiflorin and nimbaflavone were found to be the most promising inhibitors of the 4DXV protein (Table 3).

Table 2: Binding results of *Azadirachta indica* with 5YDB_Acinetobacter baumannii

S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	5YDB_Meliantriol_uff_E=997.15	-8.5	0	0
2	5YDB_Deacetylnimbin_uff_E=982.24	-7.4	0	0
3	5YDB_Super Squalene_uff_E=248.91	-6.3	0	0
4	5YDB_(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.94	-5	0	0
5	5YDB_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-8.2	0	0
6	5YDB_Elemene_uff_E=218.48	-6.2	0	0
7	5YDB_Elemene_uff_E=179.27	-6.6	0	0
8	5YDB_gamma-Murolene_uff_E=173.65	-7.2	0	0
9	5YDB_deuterio-squalene_uff_E=190.36	-5.7	0	0
10	5YDB_(14E,18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.57	-6.1	0	0
11	5YDB_(18E)-2,6,10,15,19,23-hexamethyltetracosahexaene_uff_E=254.57	-5.4	0	0
12	5YDB_Deacetylsalannin_uff_E=1158.52	-7.9	0	0
13	5YDB_28-Deoxonimbolide_uff_E=1088.15	-8	0	0
14	5YDB_Isonimocinolide_uff_E=1018.00	-9.1	0	0

UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

Table 3: Binding results of *Azadirachta indica* with 4DXV_ *Acinetobacter baumannii*

S/N	Ligand	Binding affinity	RMSD/ub	RMSD/lb
1	4DXV_Nicotiflorin_uff_E=736.07	-9.9	0	0
2	4DXV_Deacetylrimbin_uff_E=982.24	-6.8	0	0
3	4DXV_2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene_uff_E=248.91	-5.8	0	0
4	4DXV_(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene_uff_E=254.94	-6.1	0	0
5	4DXV_beta-Sitosterol-beta-D-glucoside_uff_E=869.46	-7.7	0	0
6	4DXV_Elemene_uff_E=218.48	-5.7	0	0
7	4DXV_(-)-gamma Elemene_uff_E=179.27	-6.2	0	0
8	4DXV_gamma-Murolene_uff_E=173.65	-6	0	0
9	4DXV_deuterio-squalene_uff_E=190.36	-5.6	0	0
10	4DXV_(14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene_uff_E=254.57	-5	0	0
11	4DXV_(18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene_uff_E=254.57	-4.8	0	0
12	4DXV_Deacetylsalannin_uff_E=1158.52	-7.3	0	0
13	4DXV_28 Deoxonimbolide_uff_E=1088.15	-7.6	0	0
14	4DXV_Nimbaflavone_uff_E=311.89	-8.5	0	0

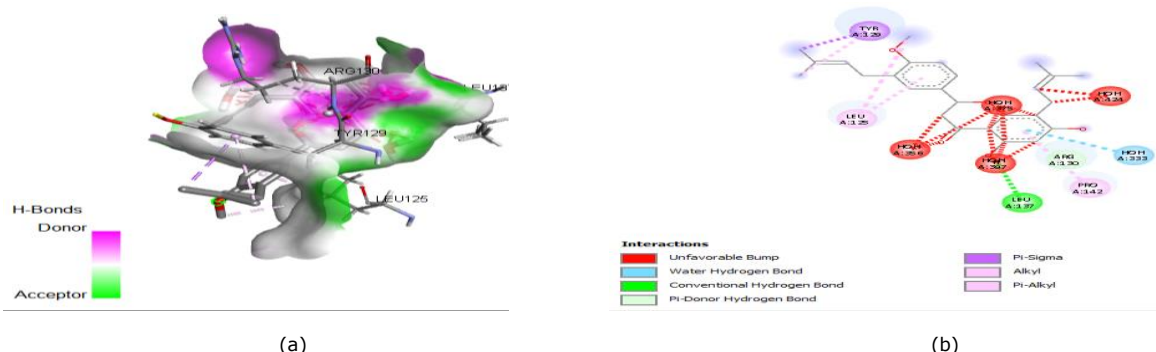
UFF- Universal Force Field, E- Energy (_uff_E- stability of the ligand structure); RMSD = Root mean square deviation

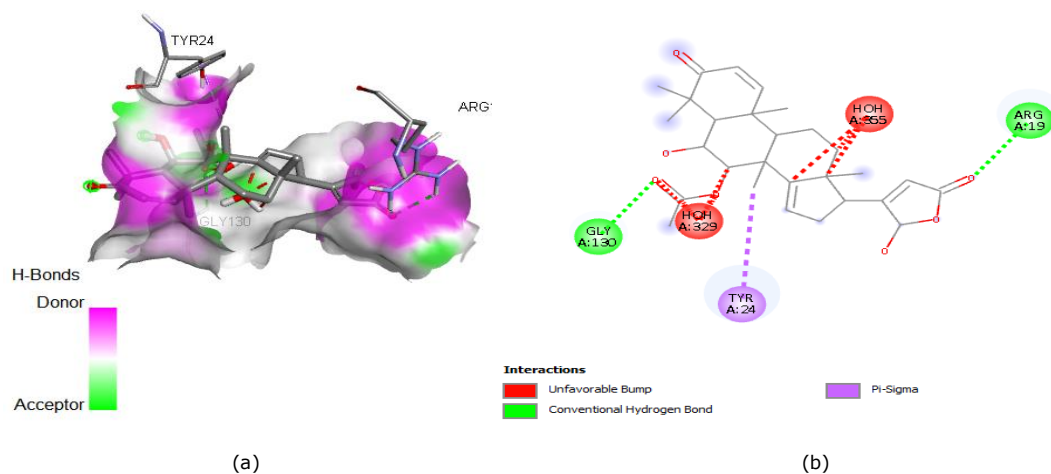
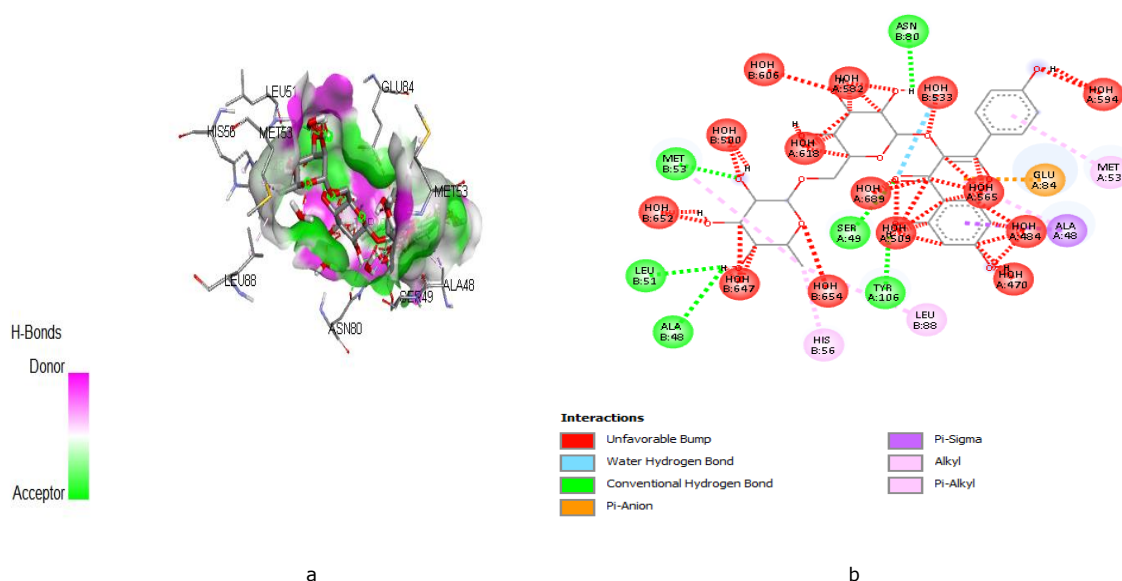
The technical validity of the predicted ligand-protein interactions for all three targets (5YDB, 4DXV and 4Y0A) was confirmed by the Root Mean Square Deviation (RMSD), which showed continuously 0.0 Å at the upper and lower limits. Such results represent very stable docking conformations and reliable binding conformations for all ligands tested.

The comparative analysis identified the strongest binders for each protein target; isonimocinolide had the highest affinity ligand for 5YDB protein with an estimated binding free energy of -9.1 (kcal/mol), which left nicotiflorin the strongest ligand for the 4DXV target with a binding free energy of -9.9 (kcal/mol), while

nimbaflavone was the most effective ligand for the 4Y0A target with a binding free energy of -8.7 (kcal/mol). All three phytochemicals were consistently ranked at the top of the binding hierarchy that supports their high inhibitory potential.

In summary, the results of docking support that nimbaflavone (Fig 1), isonimocinolide (Fig 2) and nicotiflorin (Fig 3) were the most promising bioactive derivatives of *A. indica*. In response to their binding affinities and stably persistent conformations seen for all of the *A. baumannii* proteins assayed, these compounds were prioritized for further pharmacokinetic and toxicity profiling.

Fig 1: (a) 3D and (b) 2D binding interaction of nimbaflavone with 4Y0A of *Acinetobacter baumannii*

Fig 2: (a) 3D and (b) 2D binding interaction of isonimocinolide with 5YDB of *Acinetobacter baumannii*Fig 3: (a) 3D and (b) 2D binding interaction of nicotiflorin with 4DXV of *Acinetobacter baumannii*

Physicochemical properties of the lead phytochemical compounds of *A. indica*:

The pharmacokinetic and toxicity profiles of the top-scoring ligands were carried out to test their drug-like and oral bioavailability seen in Table 4. Isonimocinolide had the best ADMET profile with acceptable molecular weight, lipophilicity and aqueous solubility. These are favorable characteristics for obtaining a promising balance between intestinal absorption and metabolic stability. On the other hand, nimbaflavone, although meeting many drug likeness criteria, is associated with high lipophilicity

and low solubility and this may be associated with poor systemic bioavailability.

Nicotiflorin was found to violate some of Lipinski's Rule of Five criteria, specifically in terms of being high in molecular weight and polar. Such violations would suggest the possibility of membrane permeability problems, as well as sub-optimal oral absorption. Consequently, from the combination of integrated ADMET characteristics, isonimocinolide proves to be the most promising lead compound for further structural optimization and antimicrobial development.

Table 4: Physicochemical properties of the lead phytochemical compounds of *Azadirachta indica*

Compound	M/W	HBA	HBD	TPSA	logP	logS	Structural stability
Nimbaflavone	422.21	5.0	2.0	75.99	6.069	-5.65	Stable
Isonimocinolide	484.25	7.0	2.0	110.13	2.294	-4.514	Moderate/poor
Nicotiflorin	594.16	15.0	9.0	249.2	1.16	-2.557	Stable

ADMET properties of lead phytochemical compounds of *A. indica*:

The ADMET evaluation of the *A. indica* derivatives showed a general low extent of oral bioavailability in the selection of compounds as shown in Table 5. Although the ligands generally showed moderate membrane permeability and intestinal absorption, their predicted interaction as a substrate or inhibitors of P-glycoprotein (P-gp) suggest a significant susceptibility to active efflux which likely will result in reduced systemic absorption.

Of the candidates, nimbaflavone had the most promising pharmacokinetic profile with respect to absorption. Conversely, isonimocinolide and nicotiflorin showed pharmacokinetic determinants related to poor bioavailability to be of concern for their ability to reach therapeutic concentrations using oral administration. These observations reveal the need for the structural improvement or devising of special drug delivery technologies, to generate a clinical viability of these neem-derived antimicrobials.

Table 5: ADMET properties of the lead phytochemical compounds of *Azadirachta indica*

Compound	ADMET properties								
	Absorption								
	CaCO-2 permeability	MDCK permeability	PAMPA permeability	Human intestinal absorption (HIA)	Pgp-inhibitor	Pgp-substrate	Bioavailability		
	Nimbaflavone	-4.813	-4.63	0.049	0.22	0.986	0.014	Poor	
	Isonimocinolide	-5.033	-4.637	0.96	0.059	0.385	0.02	Poor	
Nicotiflorin	-6.532	-5.029	1.0	0.137	0.0	0.924	Poor		
	Distribution								
	Plasma binding protein (PPB)		Fraction unbound	Volume of distribution	Blood brain barrier penetration		Distribution stability		
	Nimbaflavone	96.41%	3.375	0.541L/Kg	0.0		Moderate		
	Isonimocinolide	84.228%	15.366	-0.117 L/Kg	0.168		Moderate/poor		
Nicotiflorin	85.118%	13.389	-0.038 L/Kg	0.0		Poor			
	Metabolism								
	CYP1A2 inhibitor		CYP3A4 substrate		CYP2C9 inhibitor				
	Nimbaflavone	0.008	0.743		1.0				
	Isonimocinolide	0.0	0.24		0.0				
Nicotiflorin	0.0	0.0		0.0					
	Excretion								
	Plasma clearance			Half-life (t1/2)					
	Nimbaflavone	5.687	1.1						
	Isonimocinolide	6.082	0.961						
Nicotiflorin	1.415	4.272							
	Toxicity								
	hERG blockers	Liver toxicity (DILI)	AMES mutagenicity	Acute oral toxicity	Skin sensitization	Genotoxicity	Neurotoxicity	Overall toxicity profile	
	Nimbaflavone	0.071	0.742	0.793	0.888	0.923	0.979	0.599	Poor
	Isonimocinolide	0.019	0.869	0.609	0.889	0.96	0.997	0.175	Poor
Nicotiflorin	0.011	0.915	0.734	0.045	0.984	0.884	0.002	Poor	

The distribution profiles of nimbaflavone, isonimocinolide and nicotiflorin were investigated in order to appraise their systemic disposition and tissue penetration dynamics. As shown in Table 5, nimbaflavone displayed the most favorable pharmacokinetic behavior which could be described as moderate volume of distribution and fairly stable plasma residence. Conversely, both isonimocinolide and nicotiflorin showed poor penetration into tissue and poor metabolic stability which are potentially limiting factors for their efficacy to the deep infection sites. None of the three compounds are predicted to cross the blood brain barrier, which arguably constitutes a limited central nervous system permeability and thus, at least to a degree, reduces the neurotoxicity, however, does not enable its use in central nervous system-related infections. Overall, these results highlight that nimbaflavone has the greatest therapeutic potential, yet all three of these phytochemicals require structural screening for enhanced pharmacokinetic stability to achieve efficacious therapeutic distribution.

The metabolic profiling of main lead compounds revealed large differences in their interactions with the cytochrome P450 (CYP) enzymatic network. As shown in Table 5, nimbaflavone proved to be a potential substrate of CYP3A4 and a strong inhibitor of CYP2C9; proposing it as an increased potential for clinically relevant drug-drug interactions (DDIs) if used in combination with other agents metabolized through these pathways and an inhibitory potential on CYP1A2 was found to be negligible. On the contrary, isonimocinolide and nicotiflorin showed negligible or non-observed interaction with CYP isoforms tested, signaling better metabolic safety margin and consequences of lesser chances of adverse metabolic interactions. Accordingly, the inhibitory profile of nimbaflavone makes it a careful candidate in polypharmacy settings; however, isonimocinolide and nicotiflorin appear to be metabolically more stable and therefore better candidates for possible therapeutic development against *A. baumannii*.

The pharmacokinetic study of nimbaflavone, isonimocinolide and nicotiflorin revealed large differences in their kinetics of clearance and systemic half-life. As shown in Table 5, nimbaflavone and isonimocinolide displayed rapid clearance, leading to rather short elimination half-lives. These kinetic features suggest a rapid hepatic processing and renal elimination of drug from the systemic compartment and can therefore cause a need for more frequent dosing regimens to maintain therapeutic levels of drug concentration. In contrast, nicotiflorin displayed a significantly slow rate of clearance and half-

life, indicating a slow systemic residence time. Such a sustained exposure leads one to believe that nicotiflorin may be endowed with a greater duration of action of the pharmacologic action of the two other compounds. Overall, although the rapid rate at which nimbaflavone and isonimocinolide are eliminated may limit the possibility of drug accumulation, the long half-life of nicotiflorin has unique advantage in terms of dose frequency when targeting *A. baumannii* infections.

A thorough *in silico* toxicological evaluation of nimbaflavone, isonimocinolide and nicotiflorin identified serious safety concerns, despite a relatively low predicted risk of cardiotoxicity and neurotoxicity. As shown in Table 5, all three compounds had high possibility of hepatotoxicity, mutagenicity and genotoxicity. In particular, nicotiflorin had the highest probability of drug-induced liver injury (DILI), while nimbaflavone and isonimocinolide showed high correlation with the genotoxic and mutagenic potential. Concerning acute systemic safety, predictions showed that nimbaflavone and isonimocinolide had high acute oral toxicity profile. Furthermore, each one of the phytochemicals was found to have the ability to cause skin sensitization, which suggests potential allergic or inflammatory response on exposure to the chemical. Whilst the lack of significant cardiac and neurological hazards can be considered a beneficial feature, the overall toxicological profiles are less than ideal. Consequently, these derivatives of *A. indica* should undergo a confidential matter of safety assessment and structure modifications to reduce hepatotoxic and genotoxic risks before further clinical development occurs.

The computational analysis of *A. indica* (Neem) reveals the phytoconstituents have significant antimicrobial activity against MDR pathogen (*A. baumannii*). Through molecular docking several constituents were found to have potent inhibition of the essential bacterial proteins with nimbaflavone showing most binding affinity especially towards the 5YDB target. Pharmacokinetic profiling done through ADMET screening resulted in a complex balance of efficacy and drug-likeness. Nimbaflavone is also shown to have moderate intestinal absorption and a stable systemic distribution whereas Nicotiflorin is linked with an extended elimination half-life and therefore the potential for long term therapeutic exposure. However, the oral bioavailability of these crude phytochemicals still has a considerable limitation. From a metabolic perspective, the compounds do not generally exhibit a particularly unfavorable safety profile, however, the inhibition of CYP2C9 by nimbaflavone raises awareness of the need to be vigilant for the occ-

urrence of possible drug-drug interactions in a polypharmacy setting.

Toxicological evaluation revealed considerable risks for hepatotoxicity and genotoxicity among the lead molecules and these become the major obstacles for clinical development. These results highlight an absolute need for thorough exercise of safety profiling before carrying any of these agents forward to a therapeutic application.

Discussion:

The escalating threat of AMR, particularly among opportunistic pathogens like *A. baumannii* (1), underscores the urgent need for alternative therapeutic strategies. This study aimed to investigate the *in-silico* inhibitory activity of *A. indica* phytochemicals against key proteins of *A. baumannii*. The molecular docking studies performed during this study showed that the chosen neem phytochemicals have good binding affinities ranging from -8.7 to -9.9 (kcal/mol). These values point to highly promising leads for inhibitors. Subsequent research by Mahmoud et al., (12) highlighted the latest progress in the computational drug discovery, especially the combination of molecular docking and ADMET screening, which can rapidly evaluate the pharmacokinetic properties of plant-derived compounds before experimental evaluation at a later stage, which is very costly.

The binding energies that are observed here are comparable to the studies that were published in the literature (12), and constituents of neem, which displayed similar affinities while tested against bacterial enzymes such as DNA gyrase and FtsZ, thereby suggesting that these components can be useful in the disruption of key functions in bacteria such as replication and cell division. Furthermore, the antimicrobial possibilities of neem do not end up in the computerized projections. As reported in a study by Owolabi et al., (13), significant zone of inhibition against different clinical and food-borne pathogens has been demonstrated by experimental investigations conducted with neem leaf extracts. These empirical observations are complimented by the study of Mudenda et al., (8) which confirmed the efficacy of Neem against diverse Gram-positive and Gram-negative bacteria and attributed these broad-spectrum activities to the synergies of the various phytochemicals present in the extract.

Despite the good docking scores indicative of potent inhibition, ADMET screening during this study demonstrated that there are significant pharmacokinetic limitations. Many of the phytochemicals which scored well had low

oral bioavailability as well as potential toxicity. This agrees with the study by Owolabi et al., (11) who reported that many plant-derived compounds have failed to satisfy rigorous drug-likeness criteria in their native form without chemical modification. Therefore, although these phytochemicals demonstrate excellent *in silico* properties, there is still a need for empirical *in vitro* and *in vivo* validation.

This study is limited on the fact that it relies entirely on computational predictions based on no experimental validation, which raises the concern that the reported binding affinity for *A. baumannii* may not express itself as actual biological activity. Moreover, the computational predicted limitations such as the insufficient bioavailability and potential prospective toxicity require full *in vitro* and *in vivo* evaluations before consideration of therapeutic deployment.

Conclusion:

Azadirachta indica shows good *in-silico* potential activity against drug-resistant *A. baumannii* and some effective compounds including isonimocinolide, nicotiflorin and nimbaflavone were identified as high-affinity leads. Despite such promising scaffolds, the limitations of ADMET i. e. poor oral bioavailability and risk of hepatotoxicity/genotoxicity, emphasizes the importance of structural optimization. Further *in vitro* validation of these neem-derived compounds is mandatory to refine the pharmacokinetic profiles and the safety of these compounds in an effort to develop therapeutic material.

Prospective investigations should be focused on isolation and *in-vitro* validation of the identified lead entities in order to validate their inhibitory function against MDR *A. baumannii*. In view of known ADMET drawbacks, it is advised to pay attention to structural optimization and development of new approaches to drug formulation for the sake of higher bioavailability and safety. More so, *in-vivo* safety analyses, as well as studies investigating the synergistic interactions underlying the simplicity between these phytochemicals and standard antibiotics, are imperative to bypass existent resistance mechanisms.

Ethical statement:

This study was conducted entirely *in-silico*, using computational tools, and did not require human subjects, use animal subjects or clinical specimens, and was therefore not subject to the overview of local institutional ethics board scrutiny.

Contributions of authors:

PD supervised the study; IB conceived the study and was the main author in the research; and KP co-supervised the study. All authors read and approved the final manuscript submitted for publication.

Source of funding:

No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

Artificial Intelligence (AI) use disclosure:

Generative AI; ChatGPT and Claude AI, were used to assist in the interpretation and clarification of results. Stealthwriter AI and Humanize AI, were used in this study for paraphrasing and spell check. My bib was used to arrange the references. The authors reviewed, edited and validated all results to ensure accuracy and scientific integrity.

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