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Antibacterial Potential and Molecular Docking Analysis of Anogeissusleiocarpus Against Multi-Drug Resistant Pseudomonas Aeruginosa

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Abstract: Pseudomonas aeruginosa is a form of bacteria that is resistant to numerous medications and can cause infections in persons with compromised immune systems owing to illnesses such as cystic fibrosis. Resistance of P. aeruginosa has posed a serious health concern globally and need to be addressed. Phytochemicals from plants have been used over decades to serve as potential inhibitors for many healths related complications. Anogeissusleiocarpusmethanol and ethanol extracts were analyzed using GC-MS to find the most abundant bioactive compounds. The antibacterial assay was conducted using agar disc diffusion methodsEthanolic extracts shows high zone of inhibition of 12.00 mm at 100mg/dl when compared with the methanolic extracts. The GC-MS analysis revealed the presence of 15 bioactive compounds which were subjected to physicochemical analysis using DataWarrior tool.These Molecules were assayed in docking studies using the AutoDock 4.2 tool to calculate the binding free of each protein-ligand complex.Four compounds CID-9601770, CID-319068771, CID-596590, and CID-596591 were found to have high binding affinity with the protein. CID-319068771had the best binding of -6.13kcal/mol and was analyze to interact with RNA dependent RNA polymerase via a single hydrogen bond with Ile25, (distance = 2.83Å).This is followed by CID-9601770 with binding affinity of -4.77kcal/mol, CID-596590 with -4.66kcal/mol, and CID-596591 with -4.66kcal/mol. These bindings indicate the potential of the compounds as antibacterial agents against multi-drug resistant P. aeruginosa.

Keywords: Antibacterial, A. leiocarpus,docking, multidrug resistant, P. aeruginosa

Introduction

Pseudomonas aeruginosa is a type of bacteria that is resistant to multiple drugs and can cause infections in individuals with weakened immune systems due to conditions such as cystic fibrous(Rossi et al., 2021).One of the methods for this resistance is through the formation of biofilm. Antibiotic resistance is an escalating issue resulting from microorganisms that have developed resistance to routinely employed antibiotics, hence rendering these medications ineffective ((Ventola, 2015). This resistance can arise either spontaneously, or acquired by genetic mutations, or as a result of horizontal gene transfer among microorganisms. In 2019, the United States Centers for Disease control and Prevention (CDC)on their antibiotic resistance concern, identified multi-drug resistance Pseudomonas aeruginosa as a significant and urgent concern that demands immediate and continuous action (Center for Disease Control and Prevention, 2019).

For centuries, plants have served as medicinal source for treating a wide range of ailments, including bacterial infections. Several plant species possess inherent chemicals with antibacterial properties that can effectively hinder or kill microorganisms (Jadimurthy et al., 2023). Plants phytochemicals such as quercetin and kaempferol have been reported to ameliorate disease complications(Ibitoye et al., 2018).

Anogeissusleiocarpus, also known as African Birch, is an indigenous tree species found in the savannah region of particularly West Africa. Ethanolic extracts of Anogeissus leiocarpus has been reported by (Dahiru et al., 2023) to have low heavy metals contents, thereby rendering it good for use in folklore medicine. The study also suggests the utilization of the bioactive compounds in development of good therapeutics due to their pharmacological properties. The increasing prevalence of P. aeruginosa infections and the emergence of drug resistance have made them difficult to treat. Therefore, this study explores the effectiveness of Aleiocarpus againstP. aeruginosa.

Materials and methods

Chemicals/ reagents.

All chemicals/reagents employed in the present study were of analytical grades.

Plant collection and extraction

Parts of fresh leaves, stems and roots samples of Anogeissus leiocarpus were collected from different locations at University of Maiduguri, Borno State, Nigeria. The plant was identified at the Herbarium of the department of Botany (UM/2137). The plant was carefully processed and extracted as follows: samples were dissolved

in two different laboratory solvent (methanol and ethanol), Ethanolic and methanolic extraction of the plant materials were carried out by suspending 100g of pulverized Anogeissus leiocarpus extraction 100 ml of ethanol and 100ml of methanol respectively and left for 24 hours; this was then filtered using Whatman filter paper (No. 1). The ethanolic and the methanolic extract were allowed to evaporate at room temperature. The residues obtained were reconstituted in distilled water and 95% ethanol at stock concentration of 0.5 g/ml and stored in the o refrigerator at 4^0C before use (Faboro et al., 2023).

Phytochemical analysis

A comprehensive phytochemical study was performed on powdered African Birch extract, including its leaves, root, and stem. Various screening procedures were employed to measure the quantities of flavonoids, alkaloids, glycosides, tannins, phenols, volatile oils, saponins, and terpenoids that are present in the extract. The screening tests employed well-established methodologies developed by(Harborne, 1973),(P. Van Buren & B. Robinson, 2002), Evans and Trease, 2009 and (Alhaithloul, 2023).

Gas chromatography-mass spectrometry (GC-MS) analysis

For GC-MS analysis, the entire Anogeissus leiocarpus plant was extracted in methanol. The analysis was performed using the GC (Agilent Technologies 7890B model) and MS (Silent Technologies 5977A MSD model) models.The gas chromatograph is interfaced to a mass spectrometer instrument.The gas chromatograph settings are as follows: 2g of plant extract was dissolved in 4 test tubes with methanol and 5ml of acetone added; the extract was then centrifuged (TDL-50B model) for 5 minutes at a resolution range of 5000r/m. Three tubes of the GC machine were filled with centrifuged supernatant for use in GC-MS; two of the tubes were used for cleaning, while the other tube functioned as a dustbin. The GC model was cleaned using aceton as the solvent, and the entire GC operating time was 40 minutes.

Susceptibility testing

To prepare antimicrobial disc, the discs were saturated with different quantities of African Birch extract, which were estimated based on previous studies or preliminary tests. The susceptibility test using the disk diffusion method was performed in accordance with known recommendations, such as those provided by EUCAST. Uniforminoculums were used to inoculate bacterial suspension onto Mueller-Hinton agar plates. The plants extract discs were placed on already inoculates plates and then incubated at 35-37°C for 16 to 20 hours. The inhibitory concentration was determined by measuring the Zone of inhibition surrounding each

disc and interpreting the data according to established parameters. The experiment was repeated three times to guarantee the reproducibility of the results.

Preparation of crystal of PASW

The crystallographic arrangement of PAsw in complex with metallic ions OHN (N-3-Oxo- Dodecanoxyl-Homo Serine Lactone) was acquired from the Protein Data Bank (PDB; ID: 3IX3) with a resolution of 1.40 Å (Bernstein, 1973). The OHN bonds were detached, the structure was purified, and absent atoms were introduced using energy minimization and protein optimization using Chimera (Pettersen et al., 2004), and SwissPDB Viewer (Johansson et al., 2012).

Molecular Docking

The compounds chosen for docking study were selected based on their distinct physicochemical features. An extension of the Python Molecular Viewer, AutoDock 4.2, was utilized for the docking study.Automated docking was performed using Lamarckian genetic algorithm. During this procedure, the ligands' torsion bonds and side chains were let to rotate without constraint, whereas PAsw remained inflexible. The PAsw structure was modified by adding polar hydrogen atoms, and Gasteiger charges were calculated. The docking procedure consisted of 10 iterations, usi ng a population size of 150, a maximum of 2,500,000 evaluations, and a maximum of 27,000 generations. The grid applied had a spacing of 0.375 Å and dimensions of 60 Å × 60 Å × 60 Å, facilitating a comprehensive examination of the docking space to conduct a detailed investigation of ligand binding to PAsw.

Pharmacokinetic Analysis

After conducting docking tests, compounds with favorable binding energies underwent a secondary screening. The purpose of this screening was to assess the pharmacokinetic features of the compounds, including their absorption, distribution, metabolism, and excretion (ADME), in order to determine their suitability as possible therapeutic candidates. AdmetSAR tool and ADME/TOX program were used to achieve this purpose. Toxicity assessment of the identified compounds was also done using DataWarrior tool along with the AdmetSAR tool and ADME/TOX tools. This extensive evaluation facilitated the identification of compounds that possess both advantageous binding energies and attractive pharmacokinetic profiles, while also maintaining acceptable safety levels.

Results and discussion

Phytochemical analysis of A. leiocarpus

The results of the phytochemical analysis indicates the presence of five groups of bioactive compounds belonging to the groups of flavonoids, Tannins, Phlobatinnin, Caumarins, and Terpenoid as shown in Table 1.

Table 1: Phytochemical screen of A. leiocarpus

Extracts/ Phytochemical Constituents	**Methanol**	**Ethanol**
Flavonoid	+	+
Tannins	+	+
Phlobatinnin	+	+
Caumarins	+	+
Terpenoid	+	+

Key: +Ve = Presence

Antibacterial activity of Methanol and Ethanol extract of A. leiocarpus for Zone of Inhibition

The antimicrobial effectiveness of methanol and ethanol extracts derived from A. leiocarpus at varying concentrations (25, 50, 75 and 100 mg/dl) was evaluated against isolates of MDR P. aeruginosa. Methanolic extract shows lower zone of inhibition even at 75 and 100 mg/dl when compared to the ethanolic extract that shows 10.00 mm and 12.00 mm at same concentrations respectively (Table 2). This is almost similar to the study by(Dahiru et al., 2021) where they found zone of inhibition of ethanolic extract at 15.67 mm. Other studies such as (Edewor et al., 2016) shows a high zone of inhibition from the methanolic extracts. These contradicting differences in results may arise due to the effects of solvents as highlighted by(Ghali et al., 2022).

Table 2: Zone of Inhibition of Methanol and Ethanol Extract ofA. leiocarpus extract

Extracts	**25 mg/dl**	**50 mg/dl**	**75 mg/dl**	**100 mg/dl**
Methanol	0.00	0.00	6.00	6.00
Ethanol	.00	0.00	10.00	12.00

GC-MS Analysis

GC-MS analysis of A. leiocarpus extract yielded a total of 15 components. The chromatogram is shown in Fig. 1 while the chemical components together with their compounds and peaks, molecular weight (MW), retention time (RT), and molecular

formula are shown in Table 3. The compounds were subsequently filtered based on their physiochemical properties according to Christopher A. Lipinski's rule of five or Pfizer's rule of five (Molecular weight (≤500), Number of HBA (≤10), Number of HBD (≤5), MolLogP (≤5)to evaluate Drug likeness or determine the chemical and physical properties of pharmacological agent. This led to the selection of all the 15 compounds (Table 2).The chromatogram of these compounds can be depicted in figure 1 with their peaks clearly viewed. These Molecules were assayed in docking studies using the AutoDock 4.2 tool to calculate the binding free of each protein-ligand complex. Based on the docking results CID-319068771has the best binding of -6.13kcal/mol and was analyze to interact with RNA dependent RNA polymerase via a single hydrogen bond with Ile25, (distance = 2.83Å). Also, CID-319068771 has been reported to have antimicrobial effect (Delmondes et al., 2020). Likewise, CID-9601770possess the binding affinity of -4.77kcal/mol interacting 1hydrogen bonds with Ile25 (distance = 2.71Å). And also,CID-596590has a binding energy of -4.66kcal/mol which interact with RNA dependent RNA polymerase via 2 hydrogen bond with Glu47(distance = 2.68Å) and Glu13 (distance = 2.82Å). Compound with Pubchem I.D of CID-596591, has binding energy of -4.66kcal/mol and was examined to interact with via 2 hydrogen bond with Glu47 (distance=2.69Å) and Glu13 (distance=2.82Å). Moreover, other compounds interact with RNA dependent RNA polymerase via the weak hydrophobic bond, these include; CID-41687which possessed the binding energy of -4.75kcal/mol (Table 4)

Table 3: Analysis of the identified bioactive compounds via GC-MS

S/N	PubChem ID	Compound	Formula	Molecular Weight	Retention Time(min)	Peaks
1	CID-8857	Ethyl Acetate	$C_4H_8O_2$	88	3.694	1
2	CID-8900	Heptane	C_7H_{16}	100	4.815	3
3	CID-5363148	2-Amino-octadec-7-ene-1,3-diol butaneboronate	$C_{22}H_{44}BNO_2$	365	5.154	4
4	CID-21459	Pentane, 1-(ethenyloxy)-	$C_7H_{14}O$	114	5.154	4
5	CID-76029	Propanoic acid, 2-(aminooxy)-	$C_3H_7NO_3$	105	5.495	5
6	CID-537288	9,9-Dimethoxybicyclo[3.3.1]nona-	$C_{11}H_{16}O_4$	212	6.404	6

		2,4-dione				
7	CID-534521	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	$C_7H_9FN_2O_2$	172	6.404	6
8	CID-41687	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	$C_{20}H_{40}$	280	7.065	8
9	CID-8180	Undecanoic acid	$C_{11}H_{22}O_2$	186	7.312	9
10	CID-534521	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	$C_7H_9FN_2O_2$	172	8.074	11
11	CID-9601770	Bicyclo[3.2.0]heptan-3-one, 2-hydroxy-1,4,4-trimethyl-, O-acetyloxime	$C_{12}H_{19}NO_3$	225	18.181	19
12	CID-319068771	3-Cyclohexene-1-methanol, α,4-dimethyl-α-(4-methyl-3-pentenyl)-, [R-(R*,R*)]-	$C_{15}H_{26}O$	222	18.181	19
13	CID-596590	1,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,4-c)triazine	$C_6H_7N_5O$	165	21.001	21
14	CID-5363377	8-Dodecen-1-ol, acetate, (Z)-	$C_{14}H_{26}O_2$	226	21.001	21
15	CID-596590	1,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,4-c)triazine	$C_6H_7N_5O$	165	26.231	24

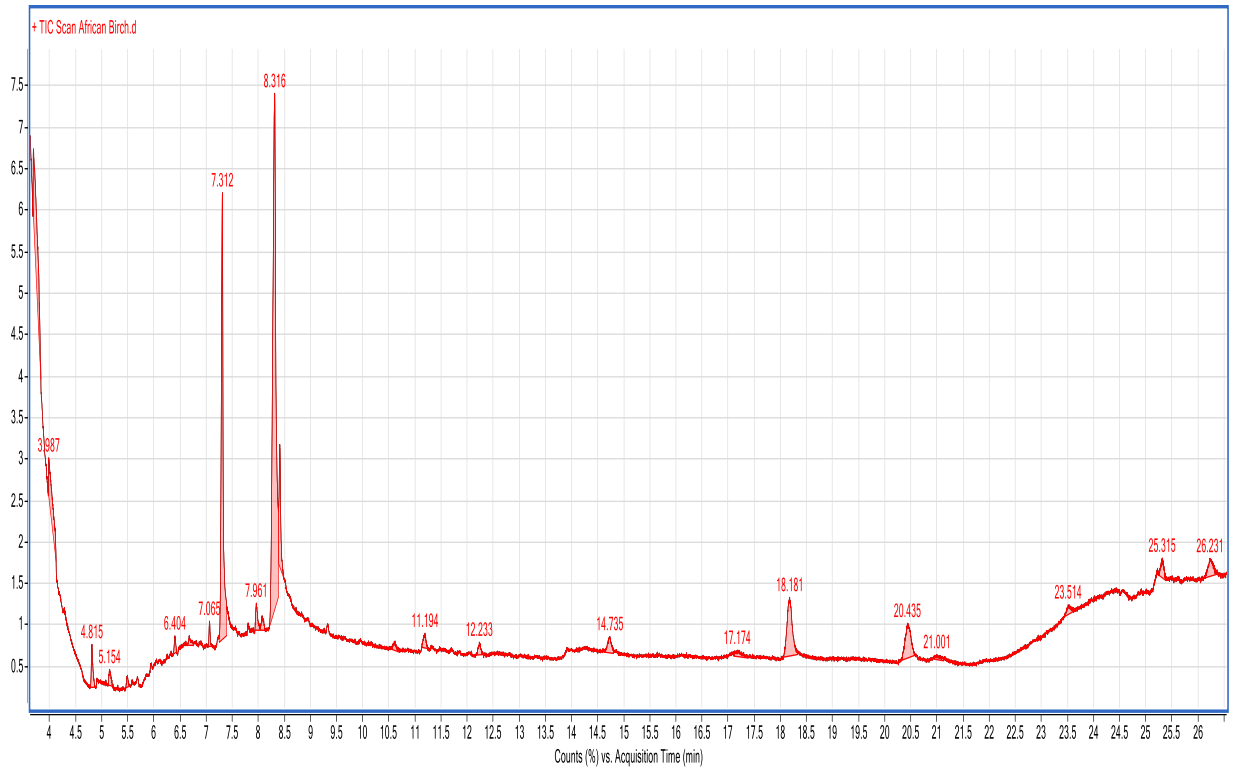

Figure 1: Chromatogram of African Birch as obtained from GC-MS analysis

Table 4: Docking scores and residues involved in H-Bond formation

S/no.	Compound Id	Docking score (kcal/mol)	Residues involve in H-bonds	Distance (Á)
1	CID-8857	-2.27	0	0
2	CID-8900	-2.69	0	0
3	CID-5363148	-2.75	0	0
4	CID-21459	-2.40	0	0
5	CID-76029	-2.75	0	0
6	CID-537288	-3.65	0	0
7	CID-534521	-2.82	0	0
8	CID-41687	-4.75	0	0
9	CID-8180	-3.29	0	0
10	CID-534521	-2.84	0	0
11	CID-9601770	-4.77	Ile25	2.71
12	CID-319068771	-6.13	Ile25	2.83
13	CID-596590	-4.66	Glu47 Glu13	2.68 2.82
14	CID-5363377	-3.56	0	0
15	CID-596591	-4.66	Glu47 Glu13	2.69 2.82

All the compounds obtained from GC_MS analysis were further filtered for physiochemical properties(molecular ≤ 500 Da, number of hydrogen bond acceptor ≤ 10, number of hydrogen bond donors ≤ 5, logP ≤ 5 and logs ≤ 5) using DataWarrior tool (Table 5). Two of the selected compounds (CID-5363148 and CID-41687) failed one of the Lipinski's rule, i.e having a logP>5.

Table 5: physicochemical analysis of the selected compounds

S/N	PubChem ID	Molecular weight (≤500)	Number of HBA (≤10)	Number of HBD (≤5)	MolLogP (≤5)	Drug likeness
1	CID-8857	88.1055	2	0	0.5351	-4.9668
2	CID-8900	100.204	0	0	3.2492	-12.388
3	CID-5363148	365.407	3	1	6.8407	-35.284
4	CID-21459	114.187	1	0	2.0682	-12.135
5	CID-76029	105.093	4	2	-1.3119	-1.4365
6	CID-537288	212.244	4	0	0.8154	-16.496
7	CID-534521	172.158	4	0	0.7781	-1.9363
8	CID-41687	280.538	0	0	7.3351	-3.8753
9	CID-8180	186.294	2	1	3.7905	-25.216
10	CID-534521	172.158	4	0	0.7781	-1.9363
11	CID-9601770	225.287	4	1	2.2086	-3.6962
12	CID-319068771	222.370	1	1	4.4711	-1.4665
13	CID-596590	165.156	6	0	0.4256	5.7268
14	CID-5363377	226.358	2	0	4.8269	-17.916
15	CID-596591	165.156	6	0	0.4256	5.7268

Based on the docking studies, four compounds (CID-9601770, CID-319068771, CID-596590, and CID-596591) shows good binding energy with the protein (Figure 2). The ADMETSAR 2.0 tool was used to identify features such as Human Intestinal Absorption (HIA), Cytochrome P450 (CYP450 2D6) inhibition, and Blood-Brain Barrier (BBB), which are possible drug transporters to the target site (Table 4).

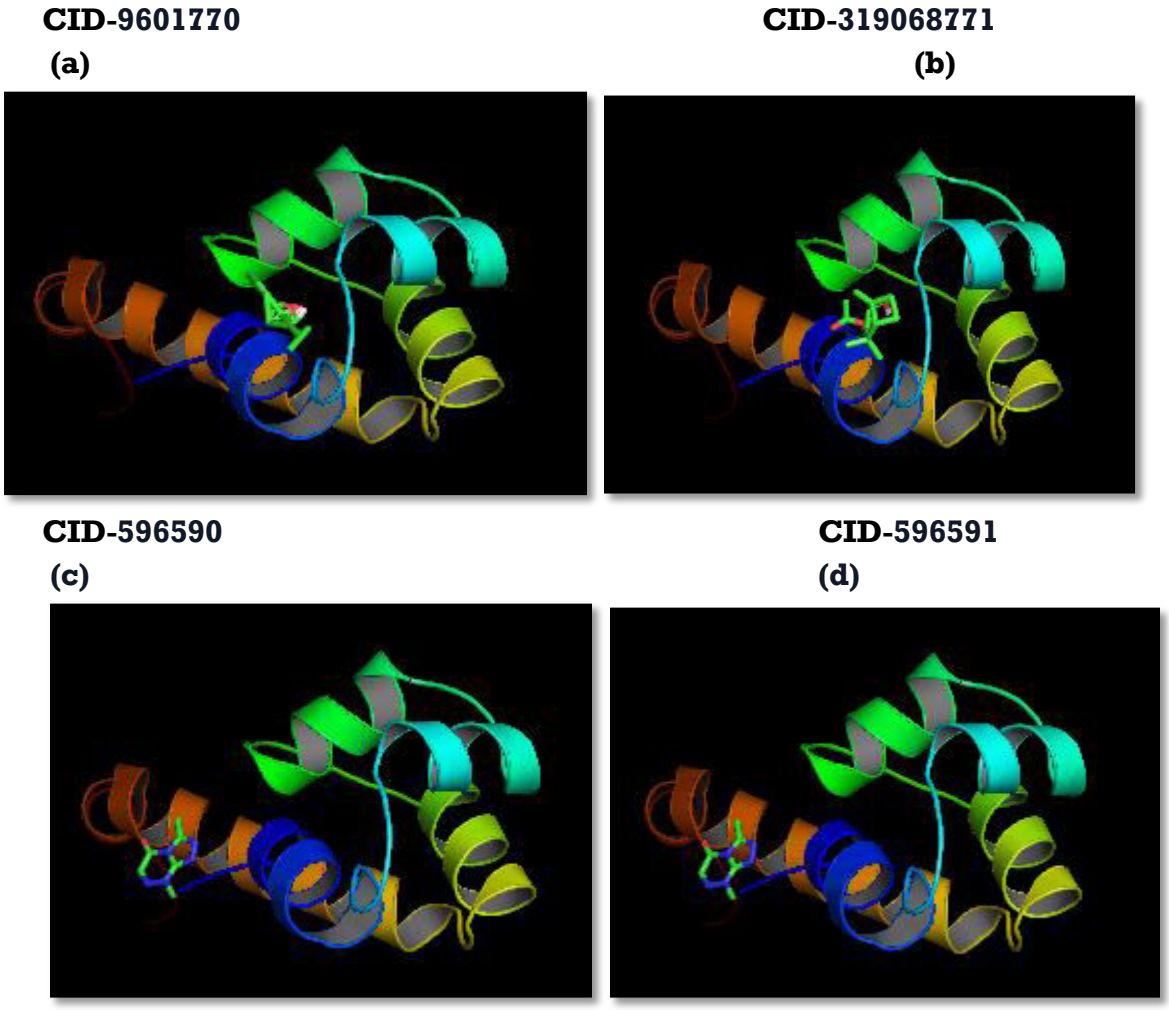

Figure 2 (a-d): docking predictions of the four selected compounds

All compounds showed positive human intestinal absorption, with the exception of CID-2 and CID-8, which showed negative results.The anticipated CYP450 2D6 of the compounds revealed that all compounds were non-inhibitors of Cytochrome, and all compounds were determined to be BBB positive (i.e., they have the ability to transfer drug candidates to specific receptors), with the exception of CID-2 and CID-8, which were negative. The toxicity parameters such as mutagenicity, Tumorigenicity, Reproducibility, and Irritability was accessed using the DataWarrior tool, and four compounds (CID-8857, CID-21459, CID-76029andCID-9601770) were found to be highly mutagenic, and the others are none mutagenic. The two chemical compounds (CID-21459 and CID-9601770) are tumorgenic, while the others are not.In the analysis, three compounds have limited reproducibility (CID-21459, CID-596590, and CID-596590), whereas the remaining compounds are non-reproducible. Finally, five compounds have high irritability (CID-8857, CID-21459, CID-8180, CID-

319068771, and CID-5363377), while three compounds (CID-21459, CID-596590, and CID-596591) have mild irritability; the other compounds are non-irritable.
In general, the study is limited to invitro and insilico studies, furthers study should explored the mechanisms of action of individual compounds in animal-based experiment.

Conclusion

Overall, a total of fifteen (15) compounds with good affinities against PAsw protein were selected and screened for pharmacokinetic properties. Four (4) of these compounds with desirable pharmacokinetic properties were selected. In silicoevidence shows their abilities to bind to specific amino acids of PAsw protein.
Thus, the present compounds are considered as suitable prospective inhibitors of PAsw protein and encourage the use of phytochemicals to treat multi-drug resistant P. aeruginosa. Further experiments are needed to explore the insights into the types of inhibition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in thispaper.

References

1. Alhaithloul, H. A. S. (2023). Phytochemical Screening of Some Medicinal Plants in Al Jouf, KSA. Open Journal of Ecology, 13(02), 61–79.
2. Center for Disease Control and Prevention. (2019). Antibiotic resistance threats in the United States, 2019, Atlanta, GA: U.S. Department of Health and Human Services. In Center for Disease control and Prevention.
3. Dahiru, Adamu, M., OS, K., & MS, I. (2021). Anogeissus leiocarpus: A Potential Inhibitor of Some ExtendedSpectrumβ-Lactamase Bacteria. Acta Scientific Microbiology, 4(11), 75–81.
4. Dahiru, M. M., Badgal, E. B., & Musa, N. (2023). Phytochemical Profiling and Heavy Metals Composition of Aqueous and Ethanol Extracts of Anogeissus Leiocarpus. 47(2), 311–323.
5. Delmondes, G. D. A., Santiago Lemos, I. C., Dias, D. D. Q., Cunha, G. L. Da, Araújo, I. M., Barbosa, R., Coutinho, H. D. M., Felipe, C. F. B., Barbosa-Filho, J. M., Lima, N. T. R. De, De Menezes, I. R. A., & Kerntopf, M. R. (2020). Pharmacological applications of farnesol (C15H26O): a patent review. In Expert Opinion on Therapeutic Patents (Vol. 30, Issue 3).
6. Edewor, T., Ibibia, Akpor, O. B., & Owa, S. O. (2016). Determination of antibacterial activity, total phenolic, flavonoid and saponin contents in leaves of Anogeissus leiocarpus (DC.) Guill and Perr. Journal of Coastal Life

Medicine, 4(4), 310–314.
7. Evans WC, Evans D, Trease GE. Trease and Evans Pharmacognosy. 16th ed. Edinburgh, New York: Saunders/ Elsevier, 2009.
8. Faboro, E. O., Adekunle, D. O., Obisesan, I. A., & Oyinlola, T. A. (2023). Optimization of extraction conditions for phytochemicals from Senna fistula using cheminformatics. SN Applied Sciences, 5(8).
9. Ghali, U. M., Teralı, K., Dalmızrak, Ö., & Özer, N. (2022). Rethinking common solvents in butyrylcholinesterase activity assays. Inorganic Chemistry Communications, 143(May).
10. Harborne, J. B. (1973). Methods of Plant Analysis. Phytochemical Methods, 1–32.
11. Ibitoye, O. B., Olofinsan, K. A., Teralı, K., Ghali, U. M., & Ajiboye, T. . (2018). Bioactivity - guided isolation of antidiabetic principles from the methanolic leaf extract of Bryophyllum pinnatum. June, 1–11.
12. Jadimurthy, R., Jagadish, S., Nayak, S. C., Kumar, S., Mohan, C. D., & Rangappa, K. S. (2023). Phytochemicals as Invaluable Sources of Potent Antimicrobial Agents to Combat Antibiotic Resistance. 1–34.
13. Johansson, M. U., Zoete, V., Michielin, O., & Guex, N. (2012). Defining and searching for structural motifs using DeepView/Swiss-PdbViewer. BMC Bioinformatics, 13(1).
14. P. Van Buren, J., & B. Robinson, W. (2002). Formation of complexes between protein and tannic acid. Journal of Agricultural and Food Chemistry, 17(4), 772–777.
15. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. Journal of Computational Chemistry, 25(13), 1605–1612.
16. Rossi, E., La Rosa, R., Bartell, J. A., Marvig, R. L., Haagensen, J. A. J., Sommer, L. M., Molin, S., & Johansen, H. K. (2021). Pseudomonas aeruginosa adaptation and evolution in patients with cystic fibrosis. In Nature Reviews Microbiology (Vol. 19, Issue 5).
17. Ventola, C. L. (2015). The Antibiotic Resistance Crisis Part 1 : Causes and Threats. 40(4), 277–283.