

Targeting penicillin binding protein 2a in methicillin resistant *Staphylococcus aureus* using selected phytocompounds: an environmental surveillance and in silico molecular docking study

Benson Okorie

okoriebenson09@gmail.com

Federal University of Technology

Blessing Awoyemi

Federal University of Technology

Emeka Okorie

Modibbo Adama University of Technology

Ukamaka Okpor

Delta State University

Tracy Osatohan

Delta State University

Promise Ogungbemi

Delta State University

Akin Oyewumi

University of Benin

Richmore Ibeh

Federal University of Technology

Babatunde Adegboyega

Federal University of Technology

Christopher Olowosoke

RMIT University

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant public health threat owing to its resistance to beta-lactam antibiotics and persistence in diverse environments. Harvested rainwater, widely used for household purposes in Nigerian communities, represents an understudied potential reservoir for antimicrobial-resistant organisms. This study had two linked objectives: to investigate the occurrence of *mecA*-positive *S. aureus* in household harvested rainwater from Oregbeni Community, Ikpoba-Okha Local Government Area, Benin City, Nigeria, and to conduct an in silico molecular docking analysis of selected phytocompounds against penicillin-binding protein 2a (PBP2a) as a hypothesis-generating step toward identifying candidate PBP2a inhibitors. Thirty household rainwater samples were collected from randomly selected zinc-roofed households during the rainy season and analyzed by culture-based, biochemical, and molecular methods. Nineteen presumptive *S. aureus* isolates were recovered and confirmed by PCR amplification of the *nuc* gene; 8 of these 19 isolates (42.1%) harbored the *mecA* gene, indicating the presence of methicillin resistance determinants. Molecular docking of 25 phytocompounds against PBP2a (PDB: 5M18) using AutoDock Vina identified epigallocatechin gallate (−8.1 kcal/mol), luteolin (−7.3 kcal/mol), andrographolide (−7.2 kcal/mol), and apigenin (−7.1 kcal/mol) as priority compounds. These findings are strictly preliminary; experimental validation through molecular dynamics simulations, enzyme inhibition assays, and in vitro antibacterial testing is required before any conclusions on anti-MRSA efficacy can be drawn. The study underscores the need for improved microbiological surveillance of harvested rainwater and provides a structural basis for prioritizing phytocompounds for future experimental investigation.

INTRODUCTION

The threat to the human populations and global public health posed by antimicrobial resistance has enormous and significant consequences. One of the most crucial bacteria responsible for Antimicrobial resistance is *Staphylococcus aureus* because it develops resistance to the drugs commonly used to kill them. MRSA causes much greater public health problems, primarily due to the fact that they are resistant to most beta lactam drugs, are associated with a number of different diseases in the hospital setting and the community (Abebe and Birhanu 2023; Chaturvedi 2022). Until recently, Methicillin-resistant *Staphylococcus aureus* has been almost exclusively identified in healthcare institutions; however, mounting evidence has indicated that MRSA is no longer only restricted to clinical settings but can be recovered from a variety of non-clinical sources including soil, wastewater and surface water, providing possible avenues for dissemination of AMR into the environment and linking it to the One Health paradigm of the relationship between human, animal and environmental health (Kallen 2010). In Nigeria there are limited facilities to provide safe drinking water in majority of homes, which makes households resort to other sources, some of which include harvested rainwater. In urban and peri-urban environments, such as Benin City, harvesting of rainwater often represents a convenient way of supplying water. In the public eye, harvested rainwater is generally seen as clean; however the method of collection system, pollution from the roofing material, air, environmental sources, storage, and handling may compromise the microbial quality. While the contamination of harvested rainwater by

microorganisms has been reported in the past, there has been a dearth of information about AMRs (specifically Methicillin-resistant *Staphylococcus aureus*) in harvested rainwater in Nigeria (Imarhiagbe et al. 2023). Methicillin resistance in *Staphylococcus aureus* is almost invariably caused by the presence of *mecA* gene which encodes a novel penicillin binding protein 2a (PBP2a) which has very low binding affinities for beta lactam antibiotics resulting in resistance (Lade and Kim 2023). Due to its central role in clinical strains, PBP2a remain the prime target in the pursuit for anti-MRSA drug discovery. The current study, thus, has two related components. The first component investigates the environmental surveillance aspect: investigating whether harvested household rainwater from Oregbeni Community, Benin City, could potentially act as reservoir for *mecA* positive *S. aureus* (detected by molecular methods). The second component will focus on in silico study: investigation on whether a series of phytocompounds had favorable binding affinities with PBP2a when tested in a structure-based molecular docking investigation as a hypothesis for future experimental work. The two components are linked since detection of *mecA* positive *S. aureus* from the environment would ascertain the importance of PBP2a as a drug target in public health context; meanwhile, the docking study would indicate how and where certain phytocompounds interact and have the potentials to be useful in future experimental studies. Among the tested phytocompounds, EGCG, luteolin, andrographolide and apigenin have high and computed binding affinities for PBP2a however these findings are still very preliminary and does not imply any antibacterial nor inhibitory activity in vitro or in vivo.

MATERIALS AND METHODS

2.1 Study area and water sampling

The study area was Oregbeni Community in the Ikpoba-Okha Local Government Area, Edo State, Nigeria. Ikpoba-Okha was carved out of the old Oredo Local Government Council and its geographic location lies between latitudes 6°15'N and 6°25'N and longitudes 5°30'E and 5°50'E (Fig. 1). The area has a rainy season which lasts from March to November and a dry season covers the rest of the year. The average annual rainfall in this area ranges from 1200mm to 2200mm. Heavy rainfall and environmental change is observed in the area and its environs, especially Oregbeni, due to industrialization and urbanization in process.

2.2 Sample collection

A total of 30 rainwater samples were harvested from 30 random household in Oregbeni Community, Benin City, Edo State, Nigeria during the rainy season (July-August, 2013). Houses were selected randomly throughout the community. One sample was taken per household so that they were independent samples. All sampled households used zinc-roofed (galvanized iron sheet) roof-catchment systems, with runoff channeled via gutters into storage drums or plastic containers. The sample was collected aseptically removing the lid from the stored water container, using sterilized 500ml sampling bottles. The samples were labeled and transported to the laboratory within 2hrs of the sample being collected packed in cooler with ice.

2.3 Isolation of bacteria

The isolates of bacteria were identified using traditional culture methods. 1ml of each water sample was spread on blood agar (BA), mannitol salt agar (MSA), and Vogel-Johnson agar (VJA). Media were prepared according to manufacturer's specification (Oxoid, UK) and methodology previously described in Agha et al. (2022). Control plates of each type of media, without being inoculated, were incubated at 37 °C for 24h to check sterility of each type of media. The inoculated media were then incubated at 37 °C for 24-48h. Colonies which produced a yellow halo on Mannitol salt agar and a black colony on Vogel-Johnson agar were assumed to be *S. aureus*. More than one colony type was picked per plate where they occurred. This technique revealed 19 presumptive *S. aureus* from the 30 water samples. Pure cultures were obtained from initial isolations. All isolates were then stored in nutrient agar at 4°C until required for the subsequent study, and where the colony types on the above two agar media differed on multiple colonies were initially regarded as distinct samples, however biochemical characterisation confirmed the initial suspicion of *S. aureus* (it should be noted that ATCC strains, e.g. ATCC 25923 and ATCC 33591, were not included as positive controls which should be noted when analyzing results shown in section 4.0).

3.5 2.4 Morphological and biochemical characterization

Morphological characterization of bacterial isolates was performed according to Gram staining characteristics and colonial appearance. Smears were made from pure isolated colonies, air-dried and heat-fixed then Gram stained according to a conventional method by Cheesbrough (2000). Microscopic examination was done to reveal the Gram reaction and morphology of the cell.

Biochemical characterization of bacterial isolates was based on the metabolic activity of each organism by studying its enzymatic activities using various tests. The tests conducted included catalase test, coagulase test, indole test, urease test, citrate test, oxidase test, sugar fermentation tests of lactose, sucrose and glucose. The test were performed following standard procedures (Salwan et al. 2023; Hafezi and Khamar 2024; Pasrija and Kumari 2025). After culturing, purification and biochemical tests on 19 isolates identified as *Staphylococcus aureus* were selected to further confirmation of *Staphylococcus aureus* presence by identifying the nuc gene and screening of the isolates for the *mecA* gene.

3.6 2.5 Molecular confirmation of *Staphylococcus aureus* and detection of the *mecA* gene

2.5.1 Genomic DNA extraction

Genomic DNA was extracted by following the protocol developed by Agbonlahor et al., 2018 and Ajayi, 2022. Bacteria cells were suspended in ZR BashingBead Lysis tubes and 0.5% of b-mercaptoethanol was added and allowed to homogenize, this helps with cell lysis. Centrifugation of lysate was conducted for 1 minute at 10,000 x g and the filtrate was applied to Zymo-Spin IV Spin Filters. Filter was collected and

diluted with Fungal/Bacterial DNA Binding Buffer and then loaded onto Zymo-Spin IIC Column in portions of 800ul, this was carried out until the lysate has been completely loaded onto the columns in order for optimal binding of DNA to the column membrane. The DNA was eluted in DNA Elution Buffer and run on a 1% agarose gel and stained using ethidium bromide and visualized on gel doc system (Agbonlahor et al. 2018).

2.5.2 PCR amplification of the *nuc* and *mecA* gene

The *nuc* and *mecA* genes were amplified by conventional PCR. The *nuc* primers were originally described by Brakstad et al. (1992) and the *mecA* primers were described by Murakami et al. (1991). Primer sequences used were *nuc* F (5'TCAGCAAATGCATCACAAACAG3'), *nuc* R (5'CGTAAATGCACTTGCTTCAGG 3'); *mecA* F (5'-AAAATCGATGGTAAAGGTTGGC-3') and *mecA* R (5'-AGTTCTGCAGTACCGGATTTGC-3'). The reaction mixture included 12.5µL of 2x Master Mix, 1µM of both forward and reverse primers, 5µL of template DNA, and nuclease-free water to adjust the total volume to 25µL. Separate cycling conditions were used for each gene. For the *nuc* gene: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min, yielding an expected amplicon of 225 bp. For the *mecA* gene: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min, yielding an expected amplicon of 532 bp. Each PCR run included a no-template negative control (nuclease-free water substituted for DNA template). No positive control ATCC strain was available; this is acknowledged as a limitation. DNA quality was confirmed by electrophoresis on a 1% agarose gel prior to PCR (Agbonlahor et al. 2018; Igbinosa et al. 2016).

2.5.3 Gel electrophoresis of PCR Products

The PCR products were analyzed by electrophoresis. Gels were run on 1% agarose containing Tris-borate-EDTA buffer, then stained with ethidium bromide. 8 µl of each PCR product, mixed with 2 µl loading dye, was loaded into the wells of each gel. These gels were run at 120 V for 30 minutes then photographed with a gel documentation system (Agbonlahor et al. 2018).

2.6 Protein and ligand retrieval and preparation

Penicillin-binding protein 2a (PBP2a) which confers methicillin resistance in *Staphylococcus aureus* by virtue of being encoded by the *mecA* gene and is thus the primary drug target of this study. The X-ray crystal structure of MRSA PBP2a in complex with cefepime (PDB ID: 5M18; resolution 1.98 Å) was used because it contains the cefepime-bound, open conformation of the PBP2a active-site, which reveals a biologically relevant binding pocket suitable for structure-based virtual screening (Mahasenan et al. 2017). Cefepime is present within the transpeptidase active site in 5M18 and as such, docking was directed to this same pocket. The crystal structure was downloaded from the RCSB Protein Data Bank (<https://www.rcsb.org/>) in PDB format. The PBP2a protein was prepared in UCSF Chimera v1.13.1 and BIOVIA Discovery Studio: crystal water molecules and co-crystallized cefepime ligand were removed, missing hydrogen atoms added and partial charges assigned using the Gasteiger method. Energy

minimization was performed prior to docking to resolve steric clashes (Damilare et al., 2025). No re-docking validation of the docking protocol (re-docking cefepime into 5M18 and calculating RMSD) was carried out, and this is a weakness of this study. The reproducibility of the experimental binding pose would need to be established. Three dimensional structures of the 25 phytocompounds were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format and were converted to PDB/PDBQT format using OpenBabel GUI version 2.4.1 (Awoyemi et al. 2025)

2.7 In silico molecular docking

Structure-based virtual screening was carried out using PyRx (Python Prescription, 0.8) which utilize AutoDock Vina as the docking program. The 25 phytocompounds and cefepime (PubChem CID: 5479537) as the reference compound were minimized in PyRx using a default Universal Force Field (UFF) and subsequently converted into the PDBQT format which used by AutoDock using Open Babel pipeline included in PyRx. The compound was docked against the PBP2a transpeptidase active-site using the AutoDock Vina module in PyRx. Grid box was set to be $14.57 \times 17.65 \times 12.85$ and center at $x = 18.13$, $y = 17.65$ and $z = 51.02$ and enclosing the active-site cavity of transpeptidase; an exhaustiveness of 8 was set in AutoDock Vina. Cefepime was chosen as the reference control because it is the co-crystal ligand of PDB: 5M18 however the binding with only cefepime alone would be expected to have a very limited controls due to the complex binding-site structure and allosteric activation of PBP2a. The best scoring pose (lower binding energy) for each compound was taken into consideration. It should be pointed out that the AutoDock Vina binding scores are merely estimates of binding free energy and cannot be viewed as an accurate indicator of the binding affinity, inhibitory constant, or antibacterial activity (Olowosoke et al. 2026). The conformations and the interactions of the compound with the active-site were visualized using BIOVIA Discovery Studio (Awoyemi et al. 2025; Olowosoke et al. 2026).

3.0 RESULTS

3.1 Distribution of bacterial isolates

All 30 harvested rainwater samples yielded mixed bacterial growth on primary culture. Selective isolation targeting *Staphylococcus aureus* was performed by sub-culturing colonies showing characteristic morphology on mannitol salt agar (yellow zones) and blood agar. Nineteen (19) of the 30 samples yielded presumptive *S. aureus* isolates, representing an isolation rate of 63.3%. The remaining 11 samples showed no colonies consistent with *S. aureus* on selective media or were overgrown by competing flora. The 19 presumptive isolates were taken forward for biochemical and molecular confirmation.

3.2 Molecular identification of *Staphylococcus aureus*

After phenotypic and biochemical characterization, the presumptive *Staphylococcus aureus* isolates were identified using PCR analysis amplifying the *nuc* gene. All 19 isolates yielded the expected 225 bp *nuc*

amplicon, confirming their identity as *Staphylococcus aureus*. All confirmed isolates were also screened for the *mecA* gene which confers methicillin resistance. This is illustrated in Fig. 2.

3.3 Detection of the *mecA* Gene in *Staphylococcus aureus* Isolates

Among the 19 isolates of *S. aureus* tested by PCR, there were 8 positive isolates for the *mecA* gene (42.1%). The other 11 isolates tested were negative for the *mecA* gene (57.9%). The isolates R1 through R10 in the first gel were positive with R1, R7 and R10 showing the band at 532 bp. While R2, R3, R4, R5, R6, R8, R9 tested negative. The second gel with the isolates R11 through R19 showed the isolates R15, R16, R17, R18 and R19 testing positive for the *mecA* gene and the isolates R11, R12, R13 and R14 tested negative for the gene. The no-template control did not give an amplification product. These findings suggest that *mecA* positive *S. aureus* is present and is able to be detected in the harvested rainwater sample from households. No phenotypic testing was performed to confirm these results so these results are classed as *mecA* positive *S. aureus* and not MRSA and interpreted as molecular detection of the genes determining methicillin resistance in the environmental water sample. The agarose gel electrophoresis profile of *mecA* gene amplification can be viewed on Fig. 3.

Table 1 Summary of rainwater sample collection and bacterial isolation outcomes (Oregbeni Community, Benin City; July–August 2023)

Sample ID	Household Selection	Roof Type	Primary Culture Result	<i>S. aureus</i> Isolated	nuc PCR Result	<i>mecA</i> PCR Result
S1–S30	Random selection, Oregbeni Community, Benin City	Zinc (galvanized iron sheet)	Mixed bacterial growth (all 30 samples)	19 of 30 samples (63.3%)	19/19 Positive (225 bp amplicon)	8/19 Positive (532 bp); 11/19 Negative
<i>Note: All 30 samples yielded mixed bacterial growth on primary culture. Pure culture isolation yielded 19 S. aureus isolates across the sample set. Individual sample-to-isolate mapping was not recorded; results are presented as aggregate outcomes. nuc PCR (225 bp) confirmed all 19 isolates as S. aureus; 8/19 (42.1%) were mecA-positive (532 bp). All households used zinc roof catchment. Sampling period: July–August 2023 (rainy season).</i>						

3.4 Molecular docking analysis

The binding affinity of the selected phytocompounds to PBP2a (PDB: 5M18) determined by molecular docking simulations, where more negative values show better binding affinity, vary from higher to lower affinity: EGCG (– 8.1 kcal/mol), luteolin (– 7.3 kcal/mol), andrographolide (– 7.2 kcal/mol), THC (– 7.1 kcal/mol), apigenin (– 7.1 kcal/mol) (Table 1). THC was chosen because studies have previously shown that it has in vitro anti-MRSA activity thus there was a reason for investigating the affinity of interaction between its structure and PBP2a and because it is not an illegal drug which would impact its potential applications, but is also not approved as medicine in all parts of the world. The binding affinities were in

order of descending values: chlorogenic acid (– 7.0 kcal/mol), fisetin (– 6.9 kcal/mol), curcumin (– 6.8 kcal/mol), quercetin (– 6.8 kcal/mol). Ginsenosides (– 4.4 kcal/mol), allicin (– 3.7 kcal/mol), silymarin (– 1.7 kcal/mol) have poor binding affinities. Additionally, the reference drug cefepime gave a binding score of – 5.4 kcal/mol, showing poorer binding than several selected compounds under the same circumstances.

3.5 Protein–Ligand Interaction Profiles

All the top-ranked compounds formed stable interaction with the active site of PBP2a, and these interactions are predominantly hydrogen bonds, hydrophobic contacts and van der Waals force (Fig. 1). The compound THC showed mostly hydrophobic-alkyl and alkyl- interactions with Val, Met and Tyr residues and one conventional H-bond with Arg241. The compound Epigallocatechin gallate generated strong binding through widespread H-bonds (Glu, Ser, Asp) and π -interactions, while Apigenin and luteolin were attached through the hydrogen bonds with Arg241, Ser240, Asn, and Ser and the hydrophobic contact to Val, Pro, Met372 (–sulfur). Chlorogenic acid and Andrographolide contained both H-bonds (Glu, Asn, Thr, Ser, Lys) and the alkyl/ π -alkyl interaction with Val, Arg. The compound Cefepime, the control, generated the mixture interaction with the moderate binding energy according to its binding energy against the highly favorable phytocompound, reflecting the ability of the phytocompounds to bind to PBP2a efficiently.

Table 1
Docking Scores of Selected Phytocompounds and Reference Drug against
PBP2a (PDB: 5M18)

S/N	Name	PubChem ID	Docking score (kcal/mol)
1.	Resveratrol	445154	-6.5
2.	Berberine	2353	-5.8
3.	Curcumin	969516	-6.8
4.	Quercetin	5280343	-6.8
5.	Thymol	6989	-5.1
6.	Eugenol	3314	-5.2
7.	Cinnamaldehyde	637511	-5.0
8.	Epigallocatechin gallate	65064	-8.1
9.	Gingerol	442793	-5.5
10.	Oleuropein	5281544	-6.7
11.	Limonene	22311	-5.0
12.	Ginsenosides	3086007	-4.4
13.	Allicin	65036	-3.7
14.	Caryophyllene	5281515	-6.4
15.	Andrographolide	5318517	-7.2
16.	Rutin	5280805	-5.5
17.	Tetrahydrocannabinol	16078	-7.1
18.	Kaempferol	5280863	-6.6
19.	Luteolin	5280445	-7.3
20.	Fucosterol	5281328	-5.8
21.	Hydroxytyrosol	82755	-5.2
22.	Fisetin	5281614	-6.9
23.	Chlorogenic Acid	1794427	-7.0
24.	Silymarin	5213	-1.7
25.	Apigenin	5280443	-7.1
26.	Cefepime (Control)	5479537	-5.4

4.0 Discussion

The present findings demonstrate that the collected domestic rainwater samples from Oregbeni Community had varying loads of bacteria including *Staphylococcus aureus* and other organisms of public health importance, which is consistent with reported evidence of issues with microbial contamination and presence of pathogens such as harmful bacteria in roof harvested rainwater samples from Nigeria. Ewelike et al. (2019) reported on the poor microbial quality of collected rain water samples and presence of *S. aureus*. The collected rain water from Port Harcourt was found by Aleruchi et al. (2025) to contain numerous bacterial species. In addition, Nwogu et al. (2024) found that the microbial quality of collected rainwater samples are variable and dependent on roof material, season, climate conditions, and storage methods. This implies that the presence of bacterial contamination is a function of rainfall collection, storage and handling in addition to rainfall it-self. The predominance of *S. aureus* in the present study suggests the presence of possible sources contributing to the contamination of collected rainwater samples like debris on the roof, dust, storage containers, and human hand contact. A striking aspect of this investigation is the contrast in number of samples collected versus number of isolates identified. From thirty collected domestic rain water samples nineteen *Staphylococcus aureus* isolates were obtained. Out of this nineteen *S. aureus* isolates, 8 contained the *mecA* gene. Molecular characterization of *Staphylococcus aureus* using the *nuc* gene confirmed the isolates. According to Ibraheim et al. (2023), *nuc* is a species specific gene which has long been employed to reliably differentiate *S. aureus* from other staphylococci. This molecular confirmation complimented the phenotypic and biochemical analysis of these isolates and enhances the reliability of the *S. aureus* isolates used in subsequent *mecA* screening. Of importance is the fact that the 42.1% referred to the percentage *S. aureus* isolates carrying the *mecA* gene rather than percentage of the total rain water samples which could lead to interpretation errors. The molecular evidence of the presence of the *mecA* gene in 8 out of the 19 *S. aureus* isolates indicates the presence of methicillin resistance gene in bacteria sourced from collected domestic rain water. This supports previous findings that bacteria contaminate domestic rainwater and the resistance gene present in these bacteria are confirmed using a molecular approach. Azuma et al. (2022), in their study, confirmed bacteria from collected domestic rain water had the resistance gene of public health importance. The findings of this study also complement previous findings that resistant staphylococci are not restricted to the hospitals environment but may be sustained in environmental sources, thus forming a reservoir for antibiotic resistant bacteria which in turn may lead to human exposure. Alhadrami et al. (2020) confirmed the presence of methicillin-resistant staphylococci in water samples from environments not related to hospitals. The Public health implications of this study is considerable in communities where collected rain water are used for cleaning purposes, food preparation and even sometimes as drinking water in the absence of proper quality checks. However this investigation should be looked at from an environmental health perspective; that collected rain water may be a vehicle for exposure to antibiotic resistant bacteria but not conclusive evidence of infection transmission risk. This investigation had the limitation of not carrying out phenotypic antimicrobial susceptibility testing hence its results should be treated as evidence of *mecA* gene in the bacteria of interest from collected domestic rain water rather than as full characterization of the resistance pattern in them. However,

confirmed *mecA* gene can serve as an initial good sign for potential methicillin resistant strain in the environment of *S. aureus*. Conclusively the present data suggest that untreated collected rain water may serve as a potential reservoir for the environment to which antibiotic resistant bacteria are transmitted. Further studies with proper monitoring protocols and combined molecular identification with phenotypic screening are therefore warranted to assess their public health implications.

Molecular docking analysis In silico has revealed initial structural information about binding between 25 phytocompounds and PBP2a active sites. Computed docking energies were 8.1 kcal/mol for epigallocatechin gallate, followed by luteolin, andrographolide, THC, apigenin and chlorogenic acid respectively (lower binding energy, indicates higher binding) compared to reference standard cefepime using the same protocol for analysis. The compounds bind to PBP2a by hydrogen bonds, hydrophobic and Van Der Waal interactions within the active site pocket of the enzyme forming computationally stable orientations. These findings represent a hypothetical basis; docking scores from AutoDock Vina represents binding free energy and should not be taken as direct indicator of inhibitory potency, anti-bacterial efficiency and utility for clinical purposes. Do not conclude that since some phytocompounds show higher docking energy than cefepime, they are better anti-MRSA agents, as the mechanism of cefepime binding to PBP2a resistance mechanism relies on allosteric binding which cannot be assessed using rigid docking protocols. Also, the docking procedure was not validated by the re-docking of co-crystallized ligand to calculate RMSD. In order to determine if the analyzed compounds possess actual anti-MRSA properties, the subsequent MD simulations, MM-GBSA/MM-PBSA calculations of free binding energies and in vitro tests for antibacterial activity should be performed before conclusions can be made, present finding serves as the initial step towards identification of anti-MRSA drugs for future experimental works (Alhadrami et al. 2020).

5.0 Conclusion

In this study we were able to molecularly detect methicillin resistant *Staphylococcus aureus* (*mecA*-positive) in harvested household rainwater from Oregbeni Community, Benin City, Nigeria. Thirty samples of household rainwater were collected and we recovered 19 presumptive *Staphylococcus aureus* isolates; these were confirmed as being of *Staphylococcus aureus* by PCR to detect the presence of the *nuc* gene and amplification was achieved for all 19 presumptive isolates. We detected *mecA* in 8 of the *Staphylococcus aureus* isolates confirming the molecular presence of methicillin resistant determinants among the environmental isolates. We wish to state here that we did not perform antimicrobial susceptibility testing; therefore, these isolates are merely described as *mecA*-positive *S. Aureus* and not phenotypically *mecA*-positive *S. Aureus* (we just performed molecular detection). Our results suggest that the water, once harvested and untreated, can serve as a source of antibiotic resistance determinants among the community, this study supports further microbiological surveillance of environmental water samples and suggests proper hand washing techniques after use. Concerning the in silico study, from the binding of 25 phytocompounds to PBP2a (PDB: 5M18), four of the highest estimated binding energies were associated with epigallocatechin gallate, luteolin, andrographolide, and apigenin, however these are initial hypothesis generating results only and in no way demonstrate an anti

MRSA effect. Binding of these phytochemicals should be assessed using molecular dynamics simulation and enzyme inhibition and MIC testing before any of these can be claimed as potential anti-MRSA compounds. We must perform phenotypic antimicrobial susceptibility testing of the isolates and test all aspects of the docking procedure, however we have presented our data and it suggests that certain compounds that can potentially block PBP2a activity could be useful in developing an alternative anti-MRSA treatment.

Limitations

Several limitations of this study should be acknowledged. First, phenotypic antimicrobial susceptibility testing (cefoxitin or oxacillin disc diffusion, or MIC determination) was not performed on the *S. aureus* isolates; therefore, methicillin resistance was inferred solely from *mecA* gene detection and the isolates cannot formally be classified as phenotypically *mecA*-positive *S. aureus* (molecular detection only). Future studies should include standardized phenotypic testing per CLSI or EUCAST guidelines. Second, no reference ATCC quality-control strains (e.g., ATCC 25923 for *S. aureus* and ATCC 33591 for *mecA*-positive MRSA) were included as positive controls in PCR assays, which limits assay quality assurance. Third, the molecular docking protocol was not validated by re-docking the co-crystallized cefepime ligand into PDB: 5M18 and computing the RMSD; validation is a standard step to confirm protocol reproducibility and should be included in future work. Fourth, none of the phytochemicals identified in the docking analysis were experimentally tested against the recovered isolates; therefore, all docking findings are preliminary and hypothesis-generating only, with no in vitro or in vivo evidence of anti-MRSA activity. Fifth, pharmacokinetic properties including bioavailability, solubility, cell permeability, and potential toxicity of the candidate phytochemicals were not evaluated, which limits the translational relevance of the docking results. Finally, the relatively small sample size (30 households, one collection period) limits the generalizability of the environmental surveillance findings.

6.0 Recommendations

Regular microbiological examinations of collected rainwater should be recommended for communities using large amounts of collected rainwater for domestic purposes. Health education is necessary to alert the communities of potential dangers associated with the usage of untreated collected rainwater and ways of collecting, storing and treating collected rainwater safely. Future research studies should involve both molecular detection and phenotypic evaluation of antibiotic susceptibilities for a comprehensive profile of resistance pattern of the environmental *S. aureus* strains. Further research should also authenticate these findings with in-vitro antibacterial tests, specific inhibition assays and MD simulations to explore further the anti-MRSA potential of top scoring phytochemicals.

Declarations

Ethical Approval: This study did not involve human or animal subject. Therefore, ethical consideration was not applicable.

Conflict of interest: The authors declare no conflict of interest.

Authors Contributions: Benson Okorie was responsible for the conceptualization, supervision, investigation, writing—original draft preparation, review, methodology, and laboratory analysis. Blessing Awoyemi contributed to the writing—original draft preparation, review, and molecular docking analysis. Emeka Okorie, Ukamaka Okpor, Tracy Osatohan, Promise Ogungbemi, Akin Oyewumi, Richmore Ibeh, and Babatunde Adegboyega were involved in the original draft preparation, writing—review. Christopher Olowosoke handled the conceptualization, supervision, original draft preparation, writing—review, and methodology. All authors have read and approved the final version of the manuscript for submission.

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References

1. Abebe A, Birhanu A (2023) Methicillin resistant *Staphylococcus aureus*: molecular mechanisms underlying drug resistance development and novel strategies to combat. *Infect Drug Resist* 16:7641–7662. <https://doi.org/10.2147/IDR.S428103>
2. Adediji A, Felix I (2012) Risk assessment analysis of accelerated gully erosion in Ikpoba Okha local government area of Edo State, Nigeria. *Environ Nat Resour Res* 3:68. <https://doi.org/10.5539/enrr.v3n1p68>
3. Agbonlahor DE, Ehiaghe JI, Eremwanarue OA, Ehiaghe AF, Oviasogie FE, Iyen RI, Ogbu EC, Ikhiuwu PE, Oseji MO, Abbey S, Tاتفeng MS (2018) A primal incrimination of *Cedecea davisae* with post-prostatectomy urinary tract infection in Nigeria. *Int J Biol Chem Sci* 12:676. <https://doi.org/10.4314/ijbcs.v12i2.6>
4. Agha Y, Najim AH, Talaat RA, Bahjat SA (2022) Detection of atypical motile *Staphylococcus aureus* from rain floods. *Open Access Maced J Med Sci* 10:1373–1377. <https://doi.org/10.3889/oamjms.2022.8686>
5. Aleruchi ON, Godshalom O, Robinson V, Chibuike A (2025) Microbiological assessment of rainwater and air quality of some areas in Port Harcourt. 15
6. Alhadrami HA, Hamed AA, Hassan HM, Belbahri L, Rateb ME, Sayed AM (2020) Flavonoids as potential anti-MRSA agents through modulation of PBP2a: a computational and experimental study. *Antibiotics* 9:562. <https://doi.org/10.3390/antibiotics9090562>
7. Awoyemi BO, Damilare BJ, Aborode AT, Ismail CMKH, Adebayo A, Umar HI, Makinde IA, Bello RO, Fakorede S, Alshehri MM, Alanazi AF (2025) In silico identification of GTPase inhibitors from selected anti-gonorrheal plants against *Neisseria gonorrhoeae*. <https://doi.org/10.1007/s13237-025-00600-y>. *Nucleus*

8. Azuma T, Murakami M, Sonoda Y, Ozaki A, Hayashi T (2022) Occurrence and quantitative microbial risk assessment of methicillin-resistant *Staphylococcus aureus* (MRSA) in a sub-catchment of the Yodo River basin, Japan. *Antibiotics* 11:1355. <https://doi.org/10.3390/antibiotics11101355>
9. Chaturvedi A (2022) *Staphylococcus aureus*: cases representing MRSA with their complications and diagnostic tests. Booksclinic Publishing
10. Cheesbrough M (2000) Microbiological tests. In: Cheesbrough M (ed) *District laboratory practice in tropical countries*. Cambridge University Press, Cambridge
11. Ewelike NC, Ogbonnaya PC, Ekwebelem VC, Onyema JU, Chuku PE, Anoka EO (2019) Microbiological quality of harvested rainwater used for drinking and domestic purposes in Southeastern Nigeria. *Singap J Sci Res* 10:47–51. <https://doi.org/10.3923/sjsres.2020.47.51>
12. Hafezi A, Khamar Z (2024) The method and analysis of some biochemical tests commonly used for microbial identification: a review. *Compr Health Biomed Stud* 3. <https://doi.org/10.5812/chbs-160199>
13. Ibraheim H, Fayez R, Jasim A, Gharban H (2023) Role of nuc gene in *Staphylococcus aureus* to phagocytic activity in different cattle infections. *Open Vet J* 13:1021. <https://doi.org/10.5455/OVJ.2023.v13.i8.8>
14. Igbinosa E, Beshiru A, Akporehe L, Oviasogie F, Igbinosa O (2016) Prevalence of methicillin-resistant *Staphylococcus aureus* and other *Staphylococcus* species in raw meat samples intended for human consumption in Benin City, Nigeria: implications for public health. *Int J Environ Res Public Health* 13:949. <https://doi.org/10.3390/ijerph13100949>
15. Imarhiagbe EE, Onwudiwe CC, Akahomhen M (2023) Water, sanitation, hygiene and health status of Ekosodin community residents, Benin City, Edo State, Nigeria. *J Appl Sci Environ Manag* 27:2611–2618. <https://doi.org/10.4314/jasem.v27i11.33>
16. Kallen AJ (2010) Health care–associated invasive MRSA infections, 2005–2008. *JAMA* 304:641. <https://doi.org/10.1001/jama.2010.1115>
17. Lade H, Kim J-S (2023) Molecular determinants of β -lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA): an updated review. *Antibiotics* 12:1362. <https://doi.org/10.3390/antibiotics12091362>
18. Mahasenani KV, Molina R, Bouley R, Batuecas MT, Fisher JF, Hermoso JA, Chang M, Mobashery S (2017) Conformational dynamics in penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*, allosteric communication network and enablement of catalysis. *J Am Chem Soc* 139:2102–2110. <https://doi.org/10.1021/jacs.6b12565>
19. Nwogu FU, Ubuoh EA, Kanu SC (2024) Chemical characteristics and microbiological loads of harvested rainwater run-off from roof tops in South Eastern Nigeria. *Discov Sustain* 5:4. <https://doi.org/10.1007/s43621-023-00177-z>
20. Olowosoke CB, Nwankwo DO, Izu CS, Chukwuemeka PO (2026) The integration of artificial intelligence and biotechnology in medicine: accelerating novelty in biomarker-targeted discovery

and drug delivery systems. Adv Biomark Sci Technol 8:137–140.

<https://doi.org/10.1016/j.abst.2025.12.002>

21. Pasrija R, Kumari D (2025) Urease assay for *Cryptococcus* sp. Protocols for the study of pathogenic fungi. Springer Nature Singapore, Singapore, pp 189–197. https://doi.org/10.1007/978-981-96-7047-5_16
22. Salwan R, Rana A, Sharma V (2023) Basic experiments in microbial biochemistry. Laboratory methods in microbiology and molecular biology. Elsevier, pp 87–104. <https://doi.org/10.1016/B978-0-323-95078-7.00011-5>

Figures

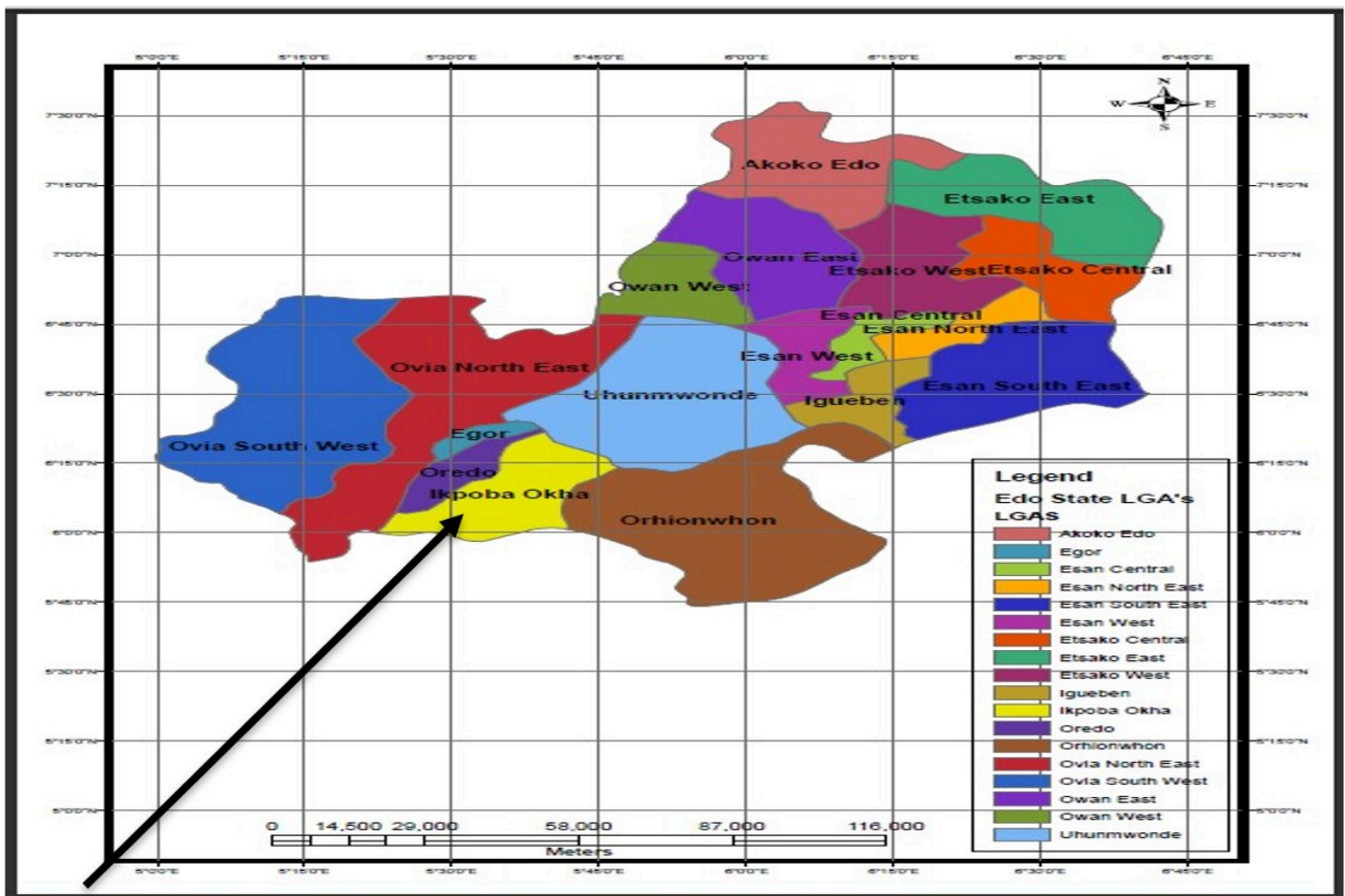


Figure 1

Map of Edo State LGAs and the study area (Adediji and Felix 2012)

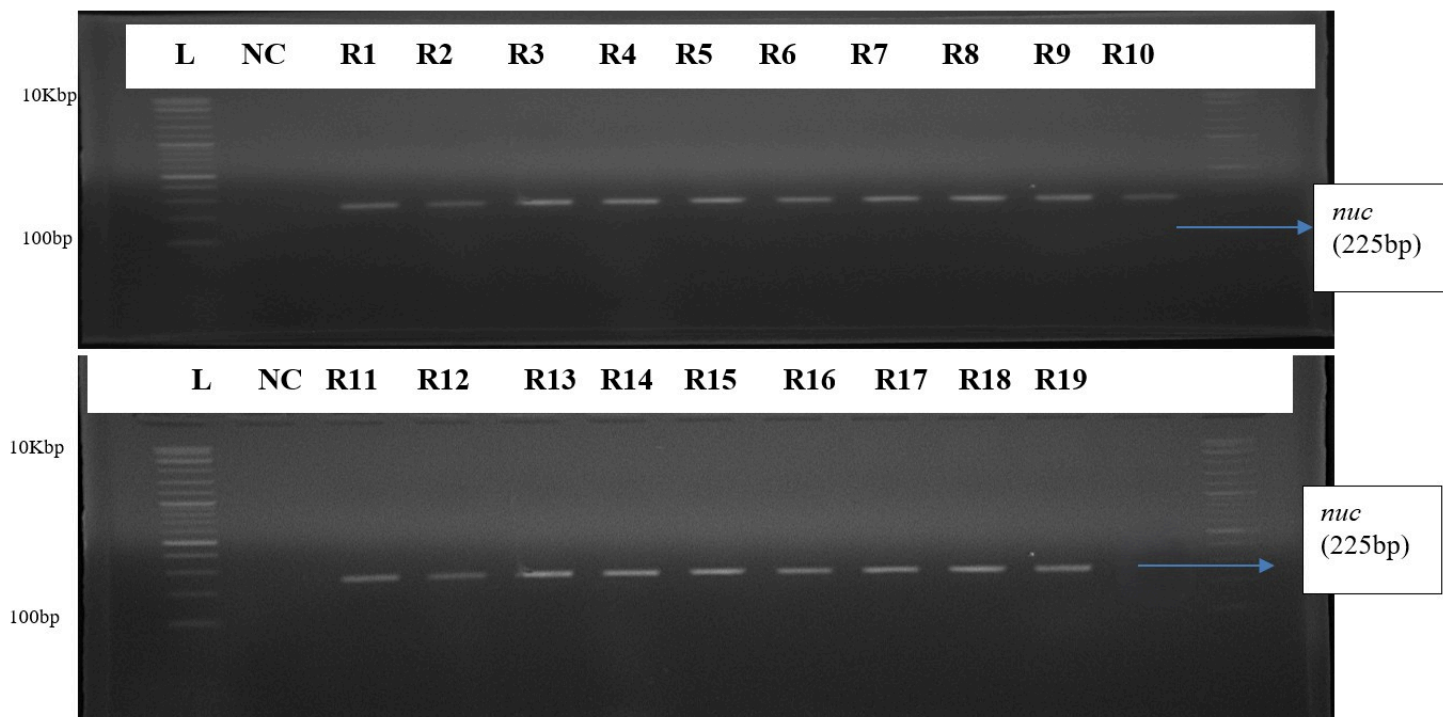


Figure 2

Agarose gel electrophoresis of PCR products showing detection of the *nuc* gene on *Staphylococcus aureus* isolates recovered from harvested household rainwater. (A) Lanes R1–R10; (B) lanes R11–R19. L = 1 kb plus DNA ladder; NC = no-template negative control, No ATCC positive control strain was included (see Section 5.1). Bands at 225 bp indicate *nuc*-positive isolates.

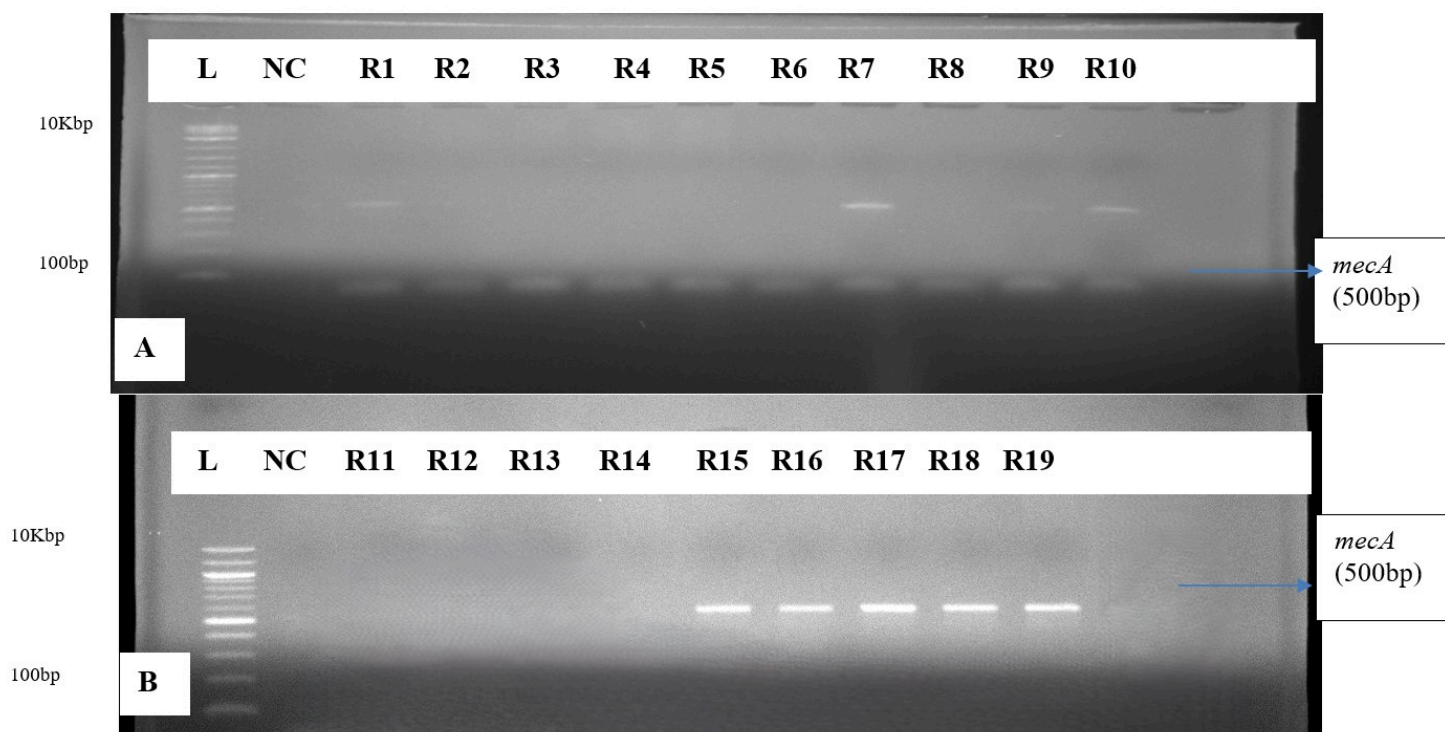


Figure 3

Agarose gel electrophoresis of PCR products showing detection of the *mecA* gene in *Staphylococcus aureus* isolates recovered from harvested household rainwater. (A) Lanes R1–R10; (B) lanes R11–R19. L = 1 kb plus DNA ladder; NC = no-template negative control. Bands at 532 bp indicate *mecA*-positive isolates.

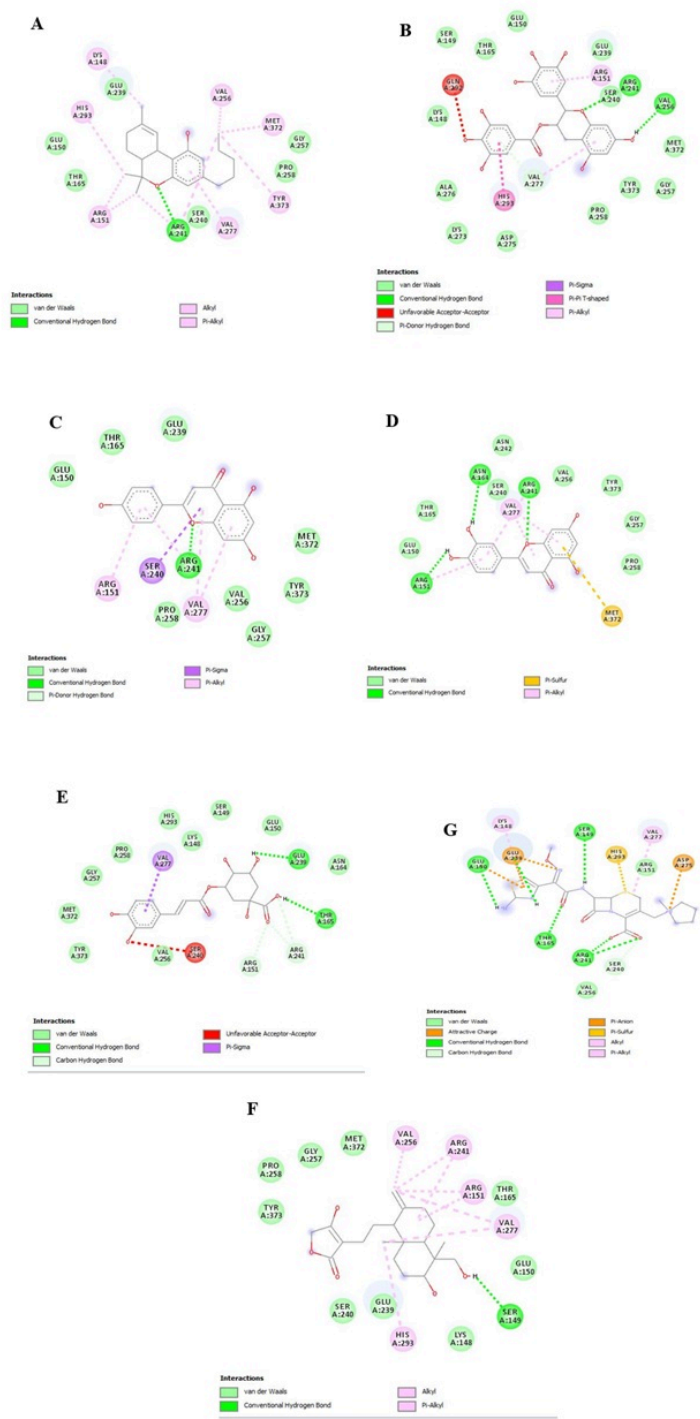


Figure 4

Molecular interaction profiles of selected compound derivatives with PBP2a (PDB: 5M18). (A) Tetrahydrocannabinol, (B) Epigallocatechin gallate, (C) Apigenin, (D) Luteolin, (E) Chlorogenic acid, (F) Andrographolide, and (G) Cefepime (control). Each compound exhibited distinct binding interactions within the active site of the target protein, highlighting variations in hydrogen bonding, hydrophobic interactions, and binding orientation.

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