

Research

In vitro and in silico screening and identification of potential bioactive anthraquinones of *Morinda lucida* benth against pathogenic bacterial target proteins

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Abstract

Background The use of plant extracts as antibiotics is gaining more attention because bacteria are one of the biggest threats to global health and the resistance of bacteria to antibiotics in humans and animals is increasing. Antibacterial susceptibility is used to determine which specific antibiotics a particular bacterium is sensitive to. This research is focused on the phytochemical, in vitro antibacterial susceptibility, and in silico analysis of *Morinda lucida* root extracts against gram-negative *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus*. The root of *M. lucida* was extracted separately with ethanol, chloroform, and ethyl acetate.

Results Crude extracts of *M. lucida* had active antibacterial activity and were a promising natural antibiotic when compared to gentamicin. The in vitro results showed that the extracts of *M. lucida* had good susceptibility properties against the two drug-resistant bacteria while the in silico showed that 2-hydroxy-1-methoxy anthraquinone is predicted to have a better susceptibility with *S. aureus* while alizarin has better susceptibility properties against *P. aeruginosa*. Finally, the MD simulation studies of Alizarin and 9,10-Anthracenedione 2-hydroxy-1-methoxy- define the stability of protein–ligand complexes within a 50 ns time scale.

Article Highlights

The research article studied the way selected compounds from the *Morinda lucida* plant affects selected bacteria. This study was carried out in vitro and in silico so as to identify why anthraquinone poses the best potential for novel drugs. The alizarin compound and the compound 2-hydroxyl-1-methoxyanthraquinone have the best properties.

Keywords *Morinda lucida* · Antibacterial · Antibiotic resistance · Susceptibility · *Staphylococcus aureus* · Extract · Phytochemistry

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1 Introduction

Morinda lucida is a plant species that is widely distributed in the tropics, especially in West Africa. The plant has numerous medicinal properties. It is used traditionally in various communities for the treatment of different ailments [1]. One of the reported applications of *M. lucida* is the treatment of bacterial infections [2, 3]. In recent years, the use of plant extracts for the development of new antibacterial agents has been on the increase, particularly in the face of the increasing prevalence of antibiotic-resistant bacteria [4, 5]. Approximately 80% of people worldwide still rely on plant medicines for relief from illnesses, according to the World Health Organization (WHO). Since they are an essential source of physiologically active compounds, medicinal plants have a big impact on human health. [6–8] The study of *M. lucida* root extracts by in vitro and in silico antibacterial analysis is highly intriguing as it offers important information about the plant's potential for producing new antibacterial chemicals. Previous studies have reported the antibacterial potential of the leaves and stem bark of *M. lucida* against a range of bacterial strains and its use in traditional medicine [2, 5, 9–11]. The *M. lucida* leaf extracts have been found to have the potential to inhibit the growth of *E. coli* and *Salmonella* species, indicating its potential in the treatment of infections involving these bacteria [6]. *Morinda lucida* leaf aqueous extract has strong immunostimulatory and immunorestorative qualities, which may account for why it works so well for treating infections and immuno-inflammatory conditions [7]. The ethanol extract of *M. lucida* leaves possesses antiemetic properties, suggesting its potential for use in the development of new antiemetic drugs [8] and the leaves may have potential applications in anticoccidial therapy [12].

A different study discovered that the *M. lucida* leaf aqueous extract had the greatest inhibitory effect on two enzymes (α -Amylase and α -Glucosidase), and this activity might be because of phytochemicals like tannins, flavonoids, and saponins [13]. Importantly, research indicates that consuming *M. lucida* leaf extract has no negative effects on liver or kidney function, indicating that it might be a safe therapeutic herb [14]. According to Saganuwan et al., the kidney, lung, liver, heart, and spleen may have mild to moderate adverse effects at higher dosages. However, the aqueous extract of *M. lucida* is safe when administered for 28 days at test dosages of 16.26 and 162.65 mg/kg body weight. [15] The *Staphylococcus aureus* (a Gram-positive multidrug resistant [16, 17] facultative anaerobe bacteria typically causing a variety of clinical diseases [18]) and *Pseudomonas aeruginosa* (a ubiquitous facultatively anaerobic, toxin-producing gram-negative bacterium [19] associated with serious infections in immunocompromised hosts [20]). The GC–MS analysis of *M. lucida* was discussed by Adekunle et al., [21] indicating that the root of *M. lucida* is very rich in phytochemicals. The information regarding the phytochemical profile and biological functions of *M. lucida* roots has not yet been fully utilized. As a result, the study sought to qualitatively examine the phytochemical components and assess the antibacterial properties of the chloroform extract of *M. lucida* root on several chosen clinical bacterial pathogens. However, the root extract of *M. lucida* has not been extensively studied for its antibacterial potential, especially against antibiotic-resistant strains.

Therefore, this study investigates the antibacterial activity of *M. lucida* root extracts against selected two bacterial strains using in vitro and in silico assays.

2 Methodology

2.1 Materials and Methods

2.1.1 Bacteria

Selected gram-positive and gram-negative bacterial strains include; *Staphylococcus aureus* and *Pseudomonas aeruginosa* used in the in vitro antibacterial susceptibility study of *M. lucida* root crude extracts on the bacterial species. These isolates were subcultured onto sterile nutrient agar plates and incubated for 24 h at 37°C, and the pure cultures were used for the antibacterial analysis.

2.1.2 Plant Sample

The fresh roots of *M. lucida* were carefully harvested from the Ebulawe Area, Iwo, Osun State, Nigeria in July 2021. Plant specimens were authenticated and a voucher specimen number was given (BUH036). The roots were washed

and air dried for two weeks after cutting into small chunks then homogenized to fine powder which was then stored in airtight containers for further analysis.

2.1.3 Extraction

After macerating 150 g of powdered *M. lucida* root in 1 L of chloroform for 48 h at room temperature with gentle shaking, the material was filtered through filter paper (Whatman, No. 42). The process was repeated three times to ensure adequate extraction. The extracts were combined and concentrated using a rotary evaporator. Phytochemicals were screened using the procedure outlined by Harborne (1984) [22] before being analyzed, the concentrated extracts were sealed and kept in a refrigerator. The yield of the extract was determined and noted.

2.1.4 Antibacterial assay

The agar diffusion method was used to evaluate the in vitro antibacterial activities of the extracts [23] using a modification of Adeleke and Owoseni [24] protocol and the guidelines of the Clinical Laboratory Standards Institute [25] with slight modifications. 10% DMSO served as the negative control and gentamicin as the positive reference standard medication for the test bacterium. The agar plates were inoculated with the bacteria suspension using a sterile swab on the surface of a Mueller–Hinton agar plate. After applying uniformly, they were allowed to dry for a few minutes. Using sterile forceps, the gentamicin-impregnated disks were placed onto the inoculated agar surface. Precautions were taken to ensure that there was sufficient spacing between the disks and a complete contact between the disk and the agar surface. The inoculated agar plates were incubated at 35 °C for a period of 24 h.

The average zone of susceptibility and average deviation for the test substances against the bacteria were recorded during the duplicate experimentation.

2.1.5 Data organization and statistical analysis

With the aid of Microsoft Word 2016 and Microsoft Excel 2016, the data was arranged and tallied. The studies were performed in triplicate, and the mean and standard deviation (MSD) were determined as the average of the clear zone and standard deviations (SD).

2.2 Molecular docking of Compounds from Plant Extracts of *Morinda lucida* against selected Microorganisms.

2.2.1 Preparation of compounds

The compounds used for molecular docking studies were retrieved from a previously published work [21]. The Ligprep module of the Schrodinger suit was used to prepare the compounds. The OPLS4 (optimized potentials for liquid simulations) was utilized for force field and energy minimization [26]. The ionization state was established using the Epik ionization tool in ligprep at a pH range of 7.0 ± 1.0 [26]. The 3D conformer structure of the ligands was downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). To prepare the receptor, crystal structures of the different proteins, *S. aureus* GyrB ATPase, and *Pseudomonas aeruginosa* DNA gyrase B 24 kDa ATPase subdomain complexed with EBL2888, were retrieved from the protein data bank (PDB) [3U2D and 7PTG] (<https://www.rcsb.org>).

A grid box was generated around the centroid of the co-crystallized ligand on the prepared protein using the Glide Receptor Grid Generation module of the Schrodinger suit. The grid center coordinates on the receptor were set at 7PTG ($x = 18.805600541666667$, $y = -17.018923583333333$, $z = -0.7439418333333333$) and 3u2d ($x = 0.2916116222222224$, $y = 2.6178757555555547$, $z = 24.385581244444444$) and the inner box defines the diameter midpoint restrain of each ligand docked was set at $12 \text{ \AA} \times 12 \text{ \AA} \times 12 \text{ \AA}$.

2.3 Molecular docking

The prepared compounds were docked using extra precision (XP) glide docking into the produced receptor grid. The force field of choice in the docking computation was the OPLS4 force field.

2.4 Molecular dynamics simulation

The two protein–ligand complexes that showed the best binding affinity for both strains of bacteria studied were selected for molecular dynamics simulation. 2-hydroxy-1-methoxy anthraquinone for *S. aureus* while alizarin for *P. aeruginosa*. In the molecular dynamics simulation studies, parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and Intermolecular hydrogen bonding were taken into consideration.

3 Results and discussion

3.1 In vitro analysis

Figure 1 shows the antibacterial activities of the three extracts tested against two organisms. These were compared with the antibacterial activity of Gentamicin.

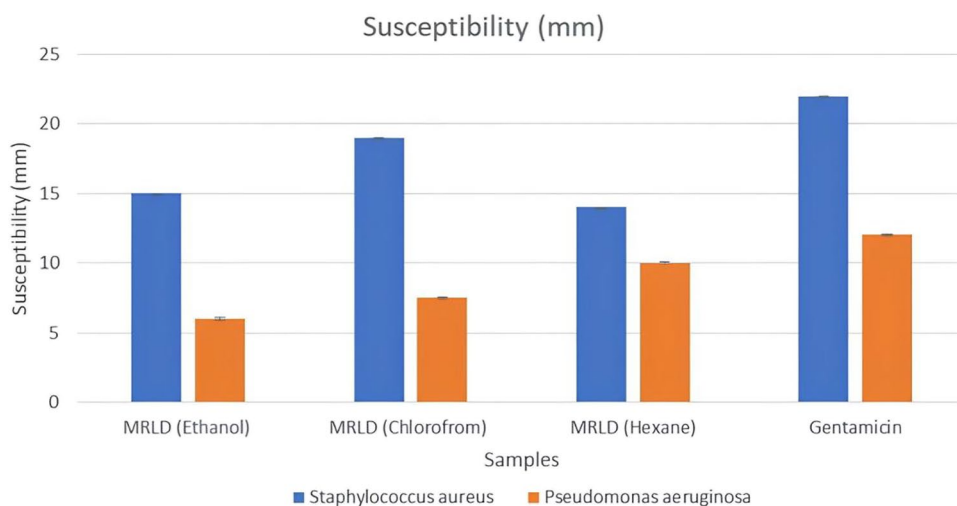
The extract showed different antibacterial activities against *S. aureus* and *P. aeruginosa*. The hexane extract had the weakest activity for *S. aureus* when compared to other extracts while the other extracts showed excellent susceptibility with chloroform extract having the best susceptibility against *S. aureus*. Hexane extract has better activity against *P. aeruginosa* while ethanol extract has a more promising activity against *S. aureus*.

These results are in agreement with the earlier published reports [2], which confirmed the bioactivities of the aqueous and ethanol extracts of *M. lucida* against *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhorum*, and *Escherichia coli*. In addition, several parts of *M. lucida* have proven to possess antibacterial potential. [27]

P. aeruginosa, which is also resistant to different antibiotics [5] had its growth inhibited by ethanol and chloroform extracts of *M. lucida*.

Burns and wound infections are caused by *P. aeruginosa*; these infections frequently occur in diabetic patients and those who have recently undergone surgery. Hospital-acquired wound infections are most frequently brought about by *S. aureus*. [27, 28]

Fig. 1 Susceptibility of bacteria to *M. lucida* root extracts



3.2 In silico

The in silico analysis was used to screen and identify the bioactive compounds with the potential to influence the microbial activity noted in the in vitro analysis. The binding affinity, amino residues, interactions, and bond distances for *Pseudomonas aeruginosa* (7PTG) and *Staphylococcus aureus* (5TW8).

3.2.1 *Pseudomonas aeruginosa* (7PTG)

It is evident from the interaction of the quinones with the 7PTG protein as seen in Table 1 that gentamicin (Fig. 2) had the highest binding affinity with a docking score of -7.79 kcal/mol which indicates that it has the best binding to the receptor. Gentamicin forms multiple hydrogen bonds with the following amino acid residues: Glu44, Asp51, Asn48, Ile98, Val99, Leu100, Ile96, and Gly121. It also shows electrostatic interactions with negatively charged Asp residues, forming salt bridges (Fig. 2). The variety of hydrogen bonds and electrostatic attractions with key binding site residues lead to its tight binding. [28]

From the quinones identified in *M. lucida* root extracts, all the compounds with the hydroxyl and methoxy functional groups had bonding affinity ranging from -5.65 to -3.85 kcal/mol (Figs. 3, 4). 1-Hydroxy-4-methylanthraquinone (-5.47 kcal/mol) (Fig. 5) and 1-Hydroxy-2-methylanthraquinone (-5.59 kcal/mol) (Fig. 6) show affinity with amino acid residues such as Thr167 and other hydrophobic interactions. The interaction of the methyl derivatives 9,10-Anthracenedione, 2-methyl- (Fig. 3) and 9,10-Anthracenedione, 1-methyl- (Fig. 4) interacting with amino acid residues indicates that the presence of the methyl group did not effectively increase the interaction as the hydroxyl groups with amino acid residues of the 7PTG protein.

Others such as 3-Hydroxyl-1-methoxyanthraquinone (Fig. 9) had an affinity of -3.85 kcal/mol, 2-hydroxyl-1-methoxyanthraquinone (Fig. 7) -5.65 kcal/mol, and 9,10-Anthracenedione, 1,5-dimethoxy- (Fig. 8) -4.54 kcal/mol.

The methoxy substituted anthraquinones like 1,5-dimethoxy anthraquinone (-4.54 kcal/mol) and 1,8-dimethoxy anthraquinone (-5.56 kcal/mol) (Figs. 9, 10 and 11 respectively) had an affinity with similar amino acids except for VAL 73. The methyl-substituted anthraquinones have varied affinities which that the position of the methyl group influences that binding affinity.

Naturally occurring alizarin (Fig. 11) shows the highest binding energy among the natural anthraquinone compounds from *M. lucida* (-6.2 kcal/mol) owing to multiple hydrogen bonds, consistent with its antibiotic potential as described by Khan et al. [29]. Thus it can be predicted that alizarin contributes strongly to the susceptibility properties of *M. lucida* against *Pseudomonas aeruginosa*.

3.2.2 *Staphylococcus aureus* (5TW8)

Table 2 presents the results of the docking for *S. aureus* showing the affinities, distance, and natures of bonds. Gentamicin (Fig. 12) shows the highest binding affinity of -7.04 kcal/mol, consistent with its potent antibacterial activity against *S. aureus*. [30]

2-Methyl (Fig. 13) and 1-methyl (Fig. 14) substituted anthraquinones have weaker affinities than Gentamicin with -3.07 and -3.67 kcal/mol respectively due to limited hydrophobic contacts.

Alizarin (Fig. 21), 1-Hydroxy-4-methylanthraquinone (Fig. 15), and 1-hydroxy-2-methylanthraquinone (Fig. 16) showed improved binding affinities (-3.7 , -3.8 , and -4.36 kcal/mol respectively) over the methyl anthraquinones, attributed to additional hydrogen bonds with binding site residues like Tyr291. The hydroxy group enhances their antibacterial potential as noted experimentally. [31]

9,10-Anthracenedione-2-hydroxy-1-methoxy (Fig. 17) also shows a higher binding energy (-5.5 kcal/mol) than the other hydroxyl-methyl derivative of anthraquinone, facilitated by hydrogen bonding interactions. The dimethoxy anthraquinones 1,5-dimethoxyanthraquinone (Fig. 18), 3-Hydroxyl-1-methoxyanthraquinone (Fig. 19), and 1,8-dimethoxyanthraquinone (Fig. 20) have lower affinities than the other anthraquinone derivatives potentially due to steric effects.

Naturally occurring 2-hydroxy-1-methoxyanthraquinone (Fig. 17) shows the highest binding energy among the natural anthraquinone compounds from *M. lucida* (-5.5 kcal/mol) owing to multiple hydrogen bonds with amino acids such as ASN 141, ALA 74, LEU 115, SER 116.

Table 1 Binding affinity, amino residues, interactions, and bond distances for *Pseudomonas aeruginosa* (7PTG)

Ligand	Binding affinity (Kcal/Mol)	Amino acid residue	Distance	Interactions
Gentamicin	− 7.78991	GLU 44	1.81647	Hydrogen bond; electrostatic
		ASP 51	2.05542	Hydrogen bond; electrostatic
		ASP 51	4.38912	Electrostatic
		ASP 47	5.27187	Electrostatic
		ASP 51	4.39319	Electrostatic
		ASP 51	5.27825	Electrostatic
		VAL 122	2.99873	Hydrogen bond
		ASN 48	2.49708	Hydrogen bond
		ILE 98	2.02908	Hydrogen bond
		VAL 99	2.16504	Hydrogen bond
		LEU 100	1.96026	Hydrogen bond
		ILE 96	2.81088	Hydrogen bond
		GLY 121	2.56262	Hydrogen bond
		GLY 121	2.76346	Hydrogen bond
		GLY 121	2.87489	Hydrogen bond
		ASP 51	2.57938	Hydrogen bond
		ASP 51	2.63844	Hydrogen bond
		ASP 51	2.29913	Hydrogen bond
1-Hydroxy-4-methylanthraquinone	− 5.47002	THR 167	3.09343	Hydrogen bond
		ASN 48, SER 49	4.81844	Hydrophobic
		ASN 48, SER 49	4.6486	Hydrophobic
		GLY 79, ILE 80	5.37543	Hydrophobic
		ILE 96	3.71347	Hydrophobic
		ILE 80	4.3234	Hydrophobic
		VAL 122	5.4246	Hydrophobic
		VAL 169	5.49339	Hydrophobic
1-Hydroxy-2-methylanthraquinone	− 5.59469	THR 167	3.06606	Hydrogen bond
		ILE 80	2.50686	Hydrophobic
		ASN 48, SER 49	4.77792	Hydrophobic
		ASN 48, SER 49	4.67811	Hydrophobic
		PRO 81	5.33488	Hydrophobic
		ILE 80	4.37619	Hydrophobic
		VAL 122	5.30302	Hydrophobic
		VAL 169	5.45946	Hydrophobic
9,10-Anthracenedione, 2-hydroxy-1- methoxy-	− 5.64862	GLU 52	2.79628	Hydrogen bond
		ILE 80	2.53863	Hydrophobic
		ASN 48;SER49	4.79358	Hydrophobic
		ASN 48;SER49	4.54147	Hydrophobic
		ARG 78	4.8321	Hydrophobic
		ILE 80	4.82034	Hydrophobic
		ILE 80	4.35204	Hydrophobic
1,5-dimethoxylantraquinone	− 4.54796	ASP 75	2.5043	Hydrogen bond
		ASN 48, SER 49	4.92059	Hydrophobic
		GLY 79, ILE 80	5.62106	Hydrophobic
		PRO 81	4.38698	Hydrophobic
		ILE 80	4.01842	Hydrophobic
		ILE 80	4.57666	Hydrophobic
		ILE 80	4.44715	Hydrophobic
1,8-dimethoxylantraquinone	− 5.55999	THR 167	2.73232	Hydrogen bond

Table 1 (continued)

Ligand	Binding affinity (Kcal/Mol)	Amino acid residue	Distance	Interactions
3-Hydroxy-1-methoxyanthraquinone	− 3.85129	THR 167	2.35472	Hydrogen bond
		GLU 52	2.57359	Hydrogen bond
		THR 167	2.58227	Hydrogen bond
		ASN 48, SER 49	4.79781	Hydrophobic
		ASN 48, SER 49	4.82209	Hydrophobic
		GLY 79, ILE 80	5.50277	Hydrophobic
		ARG 78	4.39307	Hydrophobic
		VAL 73	5.25738	Hydrophobic
		ILE 80	4.37714	Hydrophobic
		ILE 80	4.00422	Hydrophobic
		VAL 45	5.48031	Hydrophobic
		VAL 122	5.05071	Hydrophobic
		VAL 169	5.28631	Hydrophobic
		GLU 52	2.63436	Hydrogen bond
		GLY 79	2.51058	Hydrogen bond
		GLU 52	2.65973	Hydrogen bond
		ILE 80	2.34658	Hydrophobic
		ASN 48, SER 49	4.82606	Hydrophobic
		ASN 48, SER 49	4.57935	Hydrophobic
		GLY 79, ILE 80	5.41592	Hydrophobic
9,10-Anthracenedione, 2-methyl-	− 5.13266	ARG 78	4.25467	Hydrophobic
		ILE 80	4.32306	Hydrophobic
		ILE 80	2.68035	Hydrophobic
		ASN 48, SER 49	4.77139	Hydrophobic
		ASN 48, SER 49	4.7086	Hydrophobic
		VAL 45	4.90315	Hydrophobic
		VAL 73	4.62431	Hydrophobic
		VAL 169	4.50234	Hydrophobic
		ILE 80	4.36528	Hydrophobic
		VAL 169	5.44207	Hydrophobic
9,10-Anthracenedione, 1-methyl-	− 4.57458	ILE 80	2.50097	Hydrophobic
		ASN 48, SER 49	4.87332	Hydrophobic
		ASN 48, SER 49	4.50621	Hydrophobic
		GLY 79, ILE 80	5.53306	Hydrophobic
		ILE 80	4.85373	Hydrophobic
		ILE 80	4.28144	Hydrophobic
Alizarin	− 6.21997	GLU 52	2.19827	Hydrogen bond
		THR 167	2.64967	Hydrogen bond
		ASN 48, SER 49	4.78073	Hydrophobic
		ASN 48, SER 49	4.75924	Hydrophobic
		ILE 80	4.39392	Hydrophobic
		ILE 80	4.20164	Hydrophobic
		VAL 45	5.45861	Hydrophobic
		VAL 122	5.09899	Hydrophobic
		VAL 169	5.30321	Hydrophobic

Fig. 2 Gentamicin interacting with amino acid residues of 7ptg protein

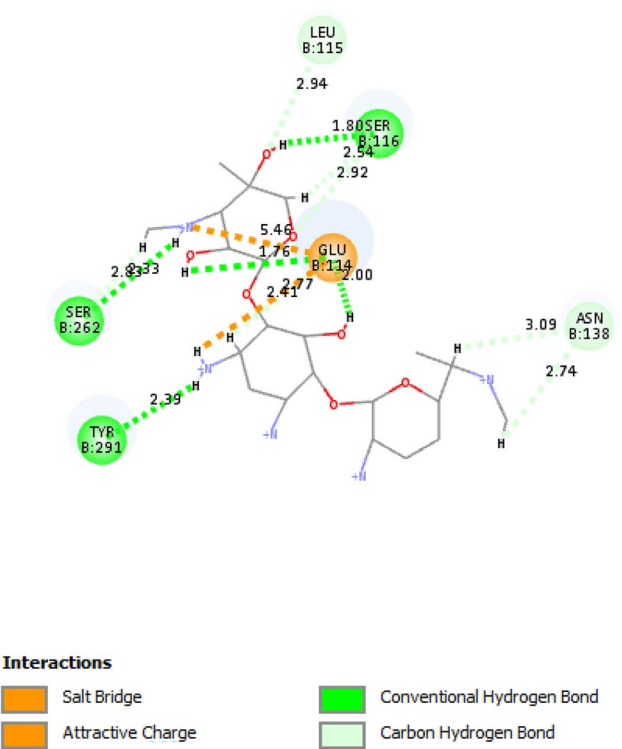


Fig. 3 9,10-anthracenedione, 2-methyl-interacting with amino acid residues of 7ptg protein

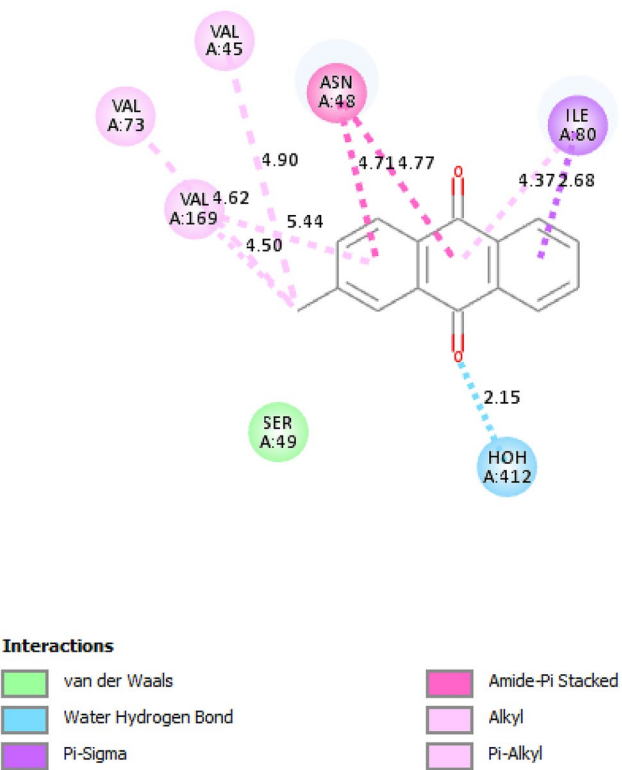


Fig. 4. 9,10-anthracenedione, 1-methyl-interacting with amino acid residues of 7ptg protein

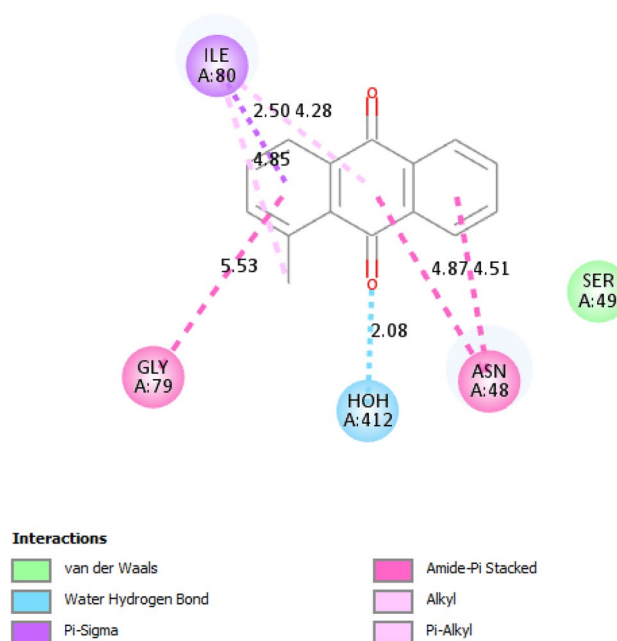
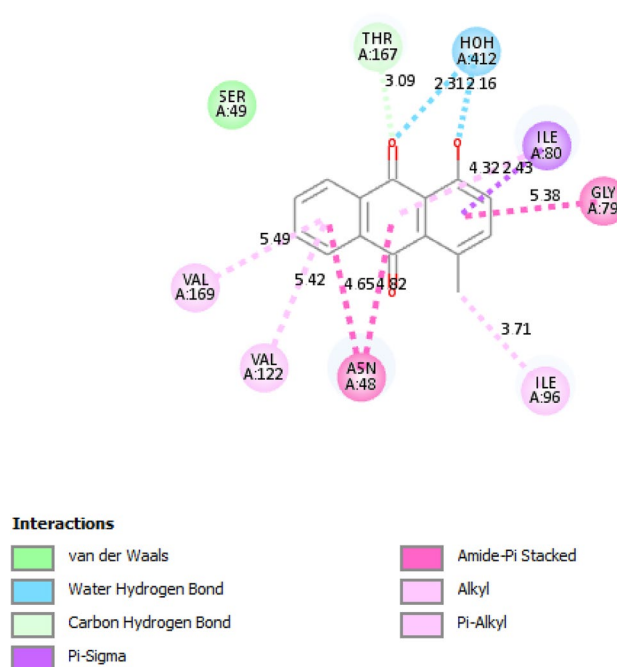


Fig. 5. 1-hydroxy-4-methylantraquinone interacting with amino acid residues of 7ptg protein



2-hydroxyl-1-methoxyanthraquinone isolated from the stem bark of *M. lucida* has been reported to possess antibacterial activities. [32]. Figure 21, shows the binding interactions of alizarin with amino acids in the 5tw8 protein. Alizarin (Fig.21) showed improved binding affinities (– 3.7 kcal/mol) attributed to additional hydrogen bonds.

3.3 Molecular dynamics (MD) simulations

3.3.1 The root mean square deviation (RMSD) of protein–ligand

RMSD of *S. aureus* (5TW8) in complex with 2-hydroxy-1-methoxy anthraquinone and *P. aeruginosa* (7tgp) in complex with alizarin is shown in Figs. 22 and 23. The RMSD of protein–ligand was done for 50 ns. The RMSD value of

Fig. 6. 1-hydroxyl-2-methylanthraquinone interacting with amino acid residues of 7ptg protein

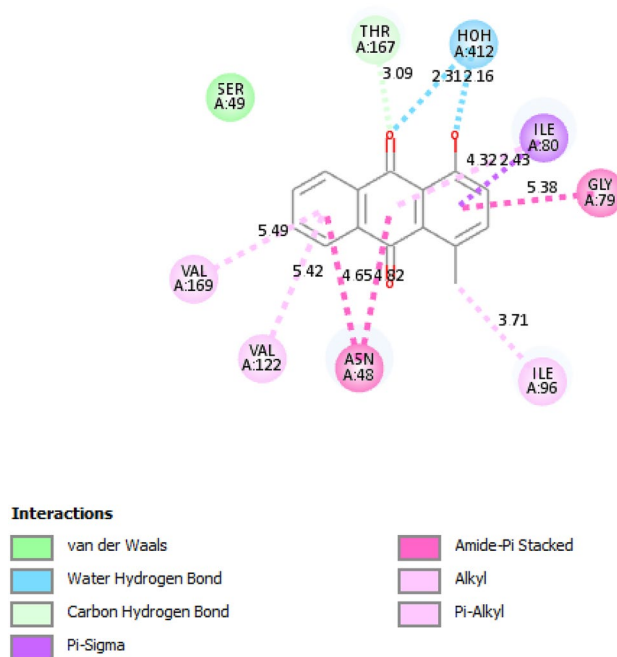
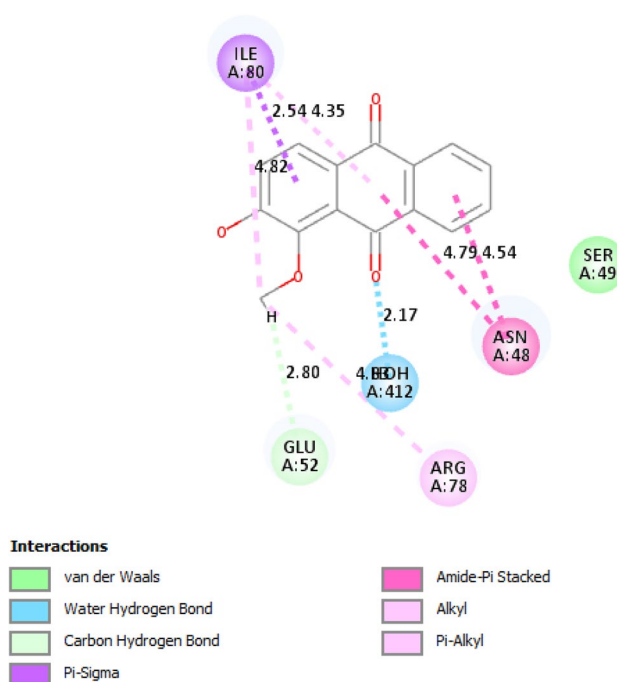


Fig. 7. 2-hydroxyl-1-methoxyanthraquinone interacting with amino acid residues of 7ptg protein



2-hydroxy-1-methoxy anthraquinone with 5TW8 is stabilized at 1.5 Å and 20 ns and remains stabilized for 50 ns (Fig. 22). Similarly, in the case of alizarin complex with 7tgp is stabilized at 5 Å and 5 ns and remains unstable for 50 ns (Fig. 23). The RMSD of the ligand with respect to the alpha carbon (prot_CA) of the protein with the reference frame shows that in Fig. 22 the protein is relatively stable and is not undergoing major conformational changes which could disrupt activities such as catalysis. It is expected that a protein being in the range of 3 Å as expected for non-globular proteins [33]. The RMSD explains how the ligand interacts with the receptor and the stability over time [34]. The alizarin ligand in the binding site of the 7tgp protein is not very stable as seen in Fig. 23.

Fig. 8. 9,10-Anthracenedione, 1,5-dimethoxy-interacting with amino acid residues of 7ptg protein

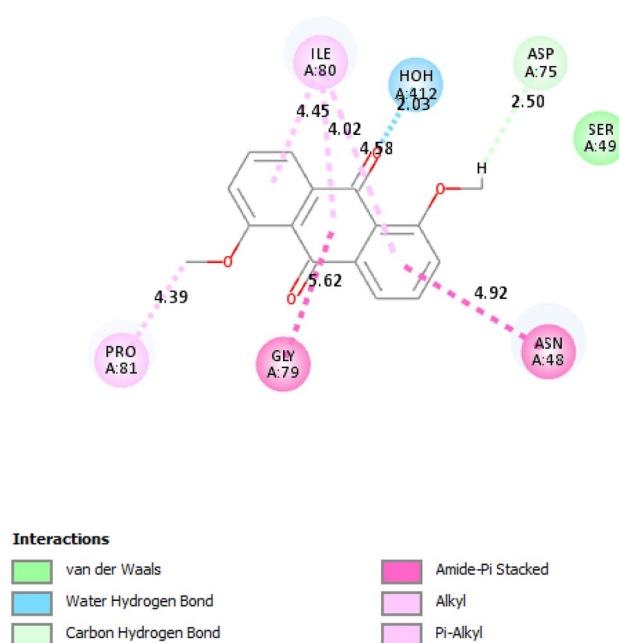
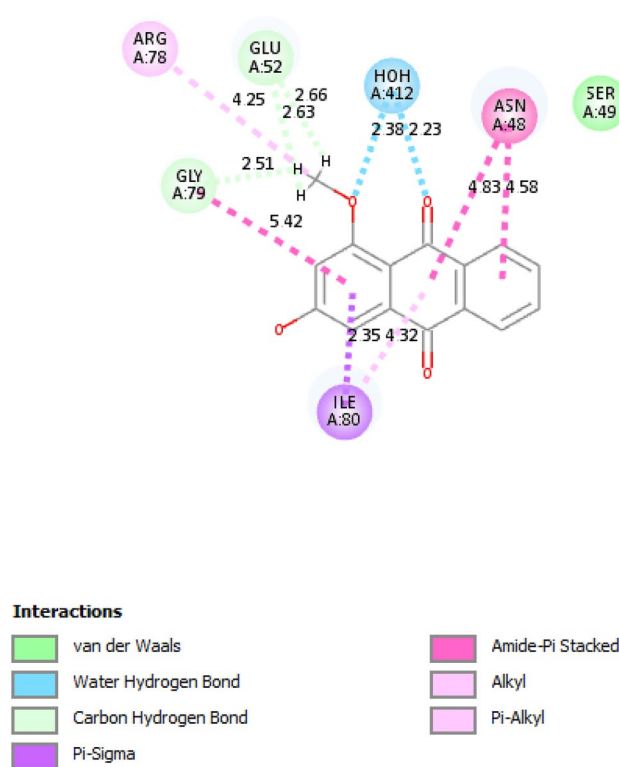


Fig. 9. 3-hydroxyl-1-methoxyanthraquinone interacting with amino acid residues of 7ptg protein



3.3.2 The root mean square fluctuation (RMSF) of protein–ligand

To determine the flexibility of protein structures and determine the deviation of the particles from the original position of macromolecules (3-D structure of the protein) the RMSF is used [35]. As seen in Fig. 24, low fluctuations are observed for 5TW8 in a complex with 2-hydroxy-1-methoxy anthraquinone between 0.5 and 2.0 Å. Similarly, 7ptg in complex with alizarin also showed low fluctuation between 0.5 and 2.5 Å (Fig. 25). Thus, the above results revealed that RMSF plots for the two complexes and ligand RMSF have stability within the binding pocket of the target RMSF. [35]

Fig. 10. 1,8-dimethoxyanthraquinone interacting with amino acid residues of 7ptg protein

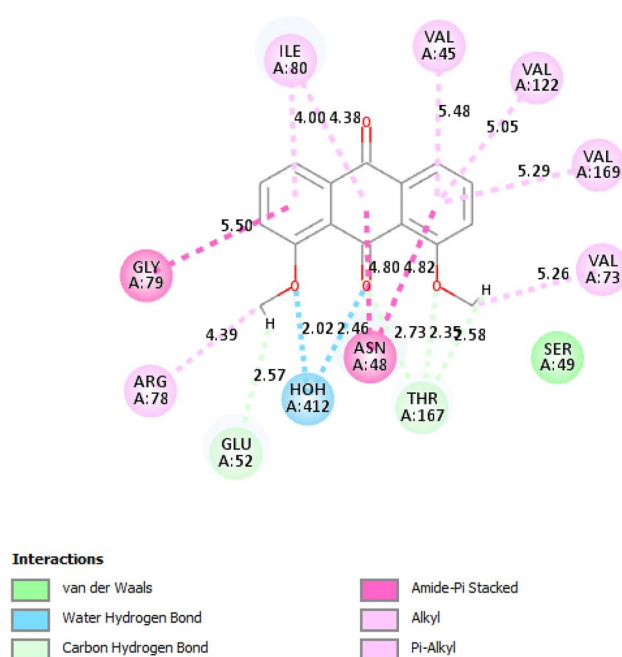
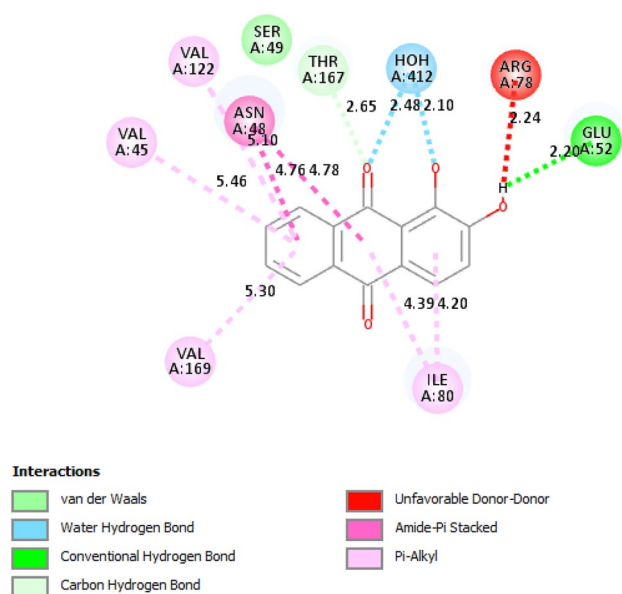


Fig. 11 Alizarin interacting with amino acid residues of 7ptg protein



Figures 26 and 27 further confirm the stability of the proteins by showing interaction fractions of the various residues of the protein by which the amino acids bond to the various ligands. SER 116 had the highest interaction fraction for 5TW8. SER had 87% interaction with the hydroxyl group of 2-hydroxy-1-methoxy anthraquinone (the ligand) while the oxygen on the methoxy group of the ligand had a 60% interaction (Fig. 28). ASP 75 shows the highest interaction fraction for 7PTG with water bridges. The most common type of interaction between alizarin ligand and the 7PTG protein is hydrophobic interactions. Figures 28 and 29 buttresses the nature of the most important bonding. The presence of intramolecular hydrogen bonding as seen in Fig. 29.

Table 2 Binding Affinity, Amino residues, interactions, and bond distances for *Staphylococcus aureus* (5TW8)

Ligand	Binding affinity (Kcal/Mol)	Amino acid residue	Distance	Interactions
Gentamicin	− 7.04272	GLU 114	2.41304	Hydrogen bond; electrostatic
		GLU 114	5.45627	Electrostatic
		GLU 114	1.99993	Hydrogen bond
		TRY 291	2.38736	Hydrogen bond
		GLU 114	1.76273	Hydrogen bond
		SER 262	2.32656	Hydrogen bond
		SER 116	1.79566	Hydrogen bond
		LEU 115	2.93701	Hydrogen bond
		SER 116	2.92156	Hydrogen bond
		ASN 138	3.09098	Hydrogen bond
		ASN 138	2.74461	Hydrogen bond
		GLU 114	2.76657	Hydrogen bond
		SER 116	2.53536	Hydrogen bond
		SER 262	2.82619	Hydrogen bond
9,10-Anthracenedione, 2-methyl-	− 3.66524	SER 75	2.18258	Hydrogen bond
		SER 116	2.70362	Hydrogen bond
		SER 262	1.88854	Hydrogen bond
		SER 75	2.41938	Hydrogen bond
		SER 116	2.7027	Hydrogen bond
		GLY 261	2.65559	Hydrogen bond
		SER 139	3.30955	Hydrogen bond
		PHE 241	4.48781	Hydrophobic
		ALA 74	5.38199	Hydrophobic
		ALA 182	4.95781	Hydrophobic
		SER 75	2.32821	Hydrogen bond
		SER 262	2.16029	Hydrogen bond
		SER 75	2.52997	Hydrogen bond
		SER 116	2.74265	Hydrogen bond
9,10-Anthracenedione, 1-methyl-	− 3.06871	GLY 261	2.95526	Hydrogen bond
		SER 139	3.07973	Hydrogen bond
		ALA 182	5.34628	Hydrophobic
		SER 75	2.58544	Hydrogen bond
		SER 139	1.92545	Hydrogen bond
1-Hydroxy-4-methylanthraquinone	− 3.73278	THR 260	2.10343	Hydrogen bond
		TYR 291	2.9518	Hydrogen bond
		SER 75	2.66179	Hydrogen bond
		SRE 262	2.65995	Hydrogen bond
		TRY 268	5.48611	Hydrophobic
1-Hydroxy-2-methylanthraquinone	− 3.7866	SER 75	2.02955	Hydrogen bond
		SER 116	3.01298	Hydrogen bond
		SER 262	2.27608	Hydrogen bond
		GLU 114	1.8679	Hydrogen bond
		SER 75	2.46781	Hydrogen bond
		SER 116	2.67621	Hydrogen bond
		GLY 261	3.00639	Hydrogen bond
		SER 139	2.82683	Hydrogen bond
		LEU 115	5.03111	Hydrophobic

Table 2 (continued)

Ligand	Binding affinity (Kcal/Mol)	Amino acid residue	Distance	Interactions
9,10-Anthracenedione, 2-hydroxy-1-methoxy-	− 5.53845	SER 116	2.36077	Hydrogen bond
		ASN 141	2.1947	Hydrogen bond
		SER 116	2.59604	Hydrogen bond
		ALA 182	4.78349	Hydrophobic
		ALA 74	5.07036	Hydrophobic
		LEU 115	5.48742	Hydrophobic
1,5-dimethoxylantraquinone	− 3.25953	ALA 182	4.14704	Hydrophobic
		SER 75	2.81528	Hydrogen bond
		SER 262	1.94085	Hydrogen bond
		SER 75	2.46767	Hydrogen bond
		GLY 261	2.55392	Hydrogen bond
		SER 262	3.20836	Hydrogen bond
		PHE 241	5.22656	Hydrophobic
		ALA 74	3.97217	Hydrophobic
		ALA 182	4.18828	Hydrophobic
		PHE 241	4.2908	Hydrophobic
3-Hydroxy-1-methoxyanthraquinone	− 3.60737	SER 75	2.37322	Hydrogen bond
		SER 262	2.17035	Hydrogen bond
		SER 75	2.59169	Hydrogen bond
		SER 116	2.56196	Hydrogen bond
		SER 116	2.88028	Hydrogen bond
		GLY 261	3.00972	Hydrogen bond
		SER 139	3.08354	Hydrogen bond
		PHE 241	4.3286	Hydrophobic
		SER 74	2.15611	Hydrogen bond
1,8-dimethoxylantraquinone	− 3.60437	SER 116	2.92998	Hydrogen bond
		SER 262	1.83574	Hydrogen bond
		SER 75	2.39059	Hydrogen bond
		LEU 115	2.54995	Hydrogen bond
		SER 116	2.31613	Hydrogen bond
		SER 116	2.83065	Hydrogen bond
		GLY 261	2.5845	Hydrogen bond
		GLU 114	2.69112	Hydrogen bond
		SER 139	2.983	Hydrogen bond
		ALA 182	5.2674	Hydrophobic
		SER 75	2.30791	Hydrogen bond
Alizarin	− 4.36655	SER 262	2.11579	Hydrogen bond
		GLU 114	1.9707	Hydrogen bond
		GLU 114	1.90117	Hydrogen bond
		SER 75	2.53096	Hydrogen bond
		SER 116	2.4595	Hydrogen bond
		GLY 261	2.92474	Hydrogen bond
		SER 139	2.9822	Hydrogen bond

Fig. 12 Gentamicin interacting with amino acid residues of 5tw8 protein

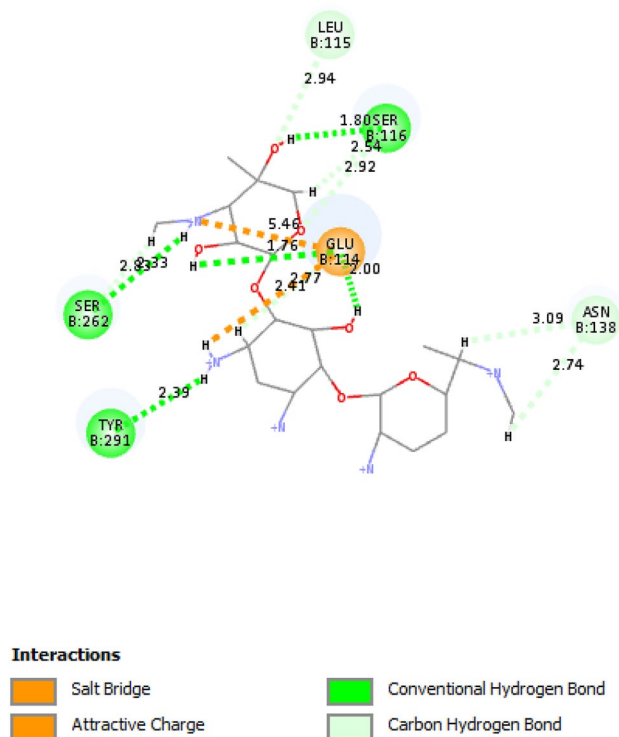


Fig. 13. 9,10-anthracenedione, 2-methyl-interacting with amino acid residues of 5tw8 protein

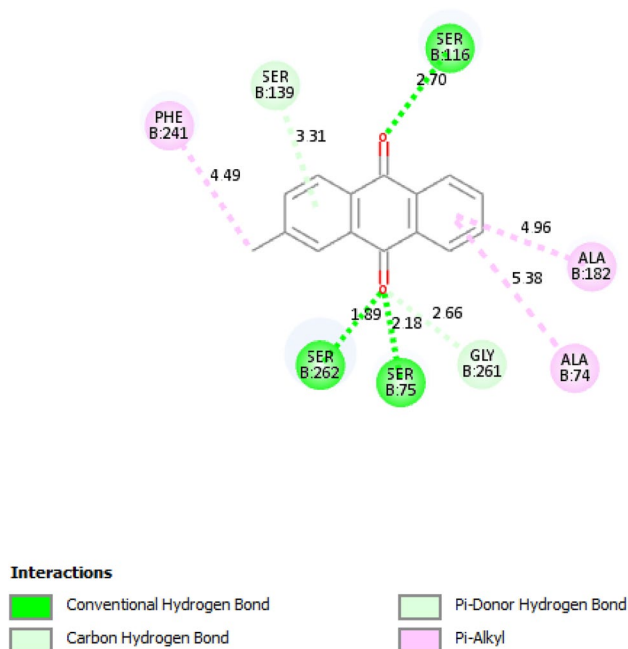


Fig. 14. 9,10-anthracenedione, 1-methyl-interacting with amino acid residues of 5tw8 protein

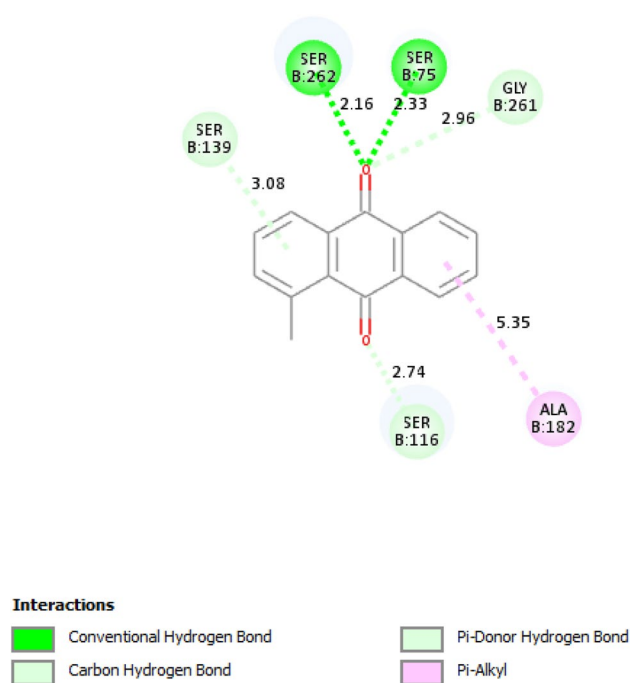


Fig. 15. 1-hydroxy-4-methylantraquinone interacting with amino acid residues of 5tw8 protein

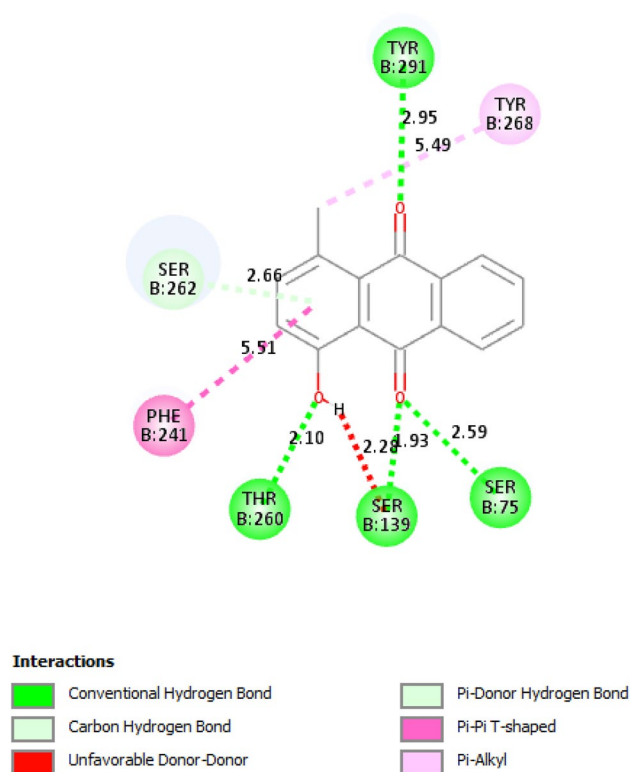


Fig. 16. 1-hydroxyl-2-methylantraquinone interacting with amino acid residues of 5tw8 protein

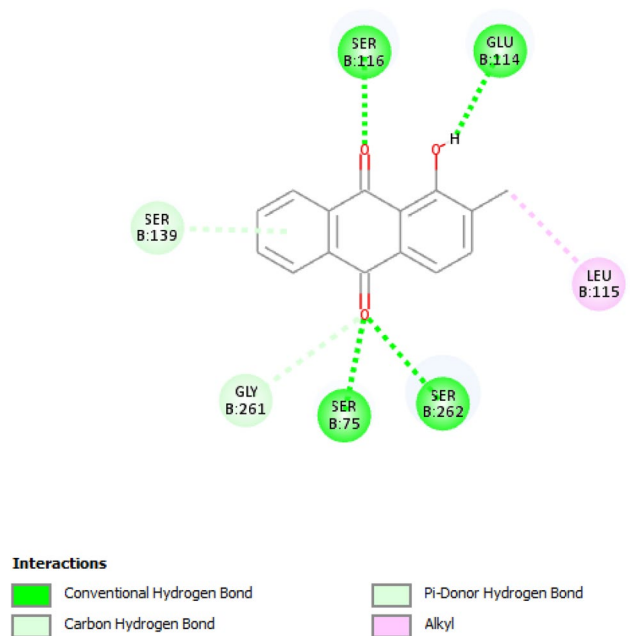


Fig. 17. 2-hydroxyl-1-methoxyanthraquinone interacting with amino acid residues of 5tw8 protein

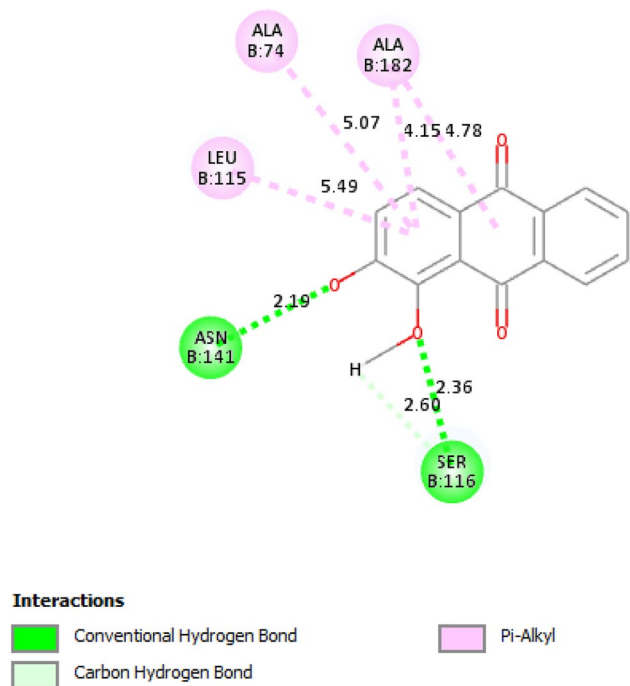


Fig. 18. 9,10-Anthracenedione, 1,5-dimethoxy-interacting with amino acid residues of 5tw8 protein

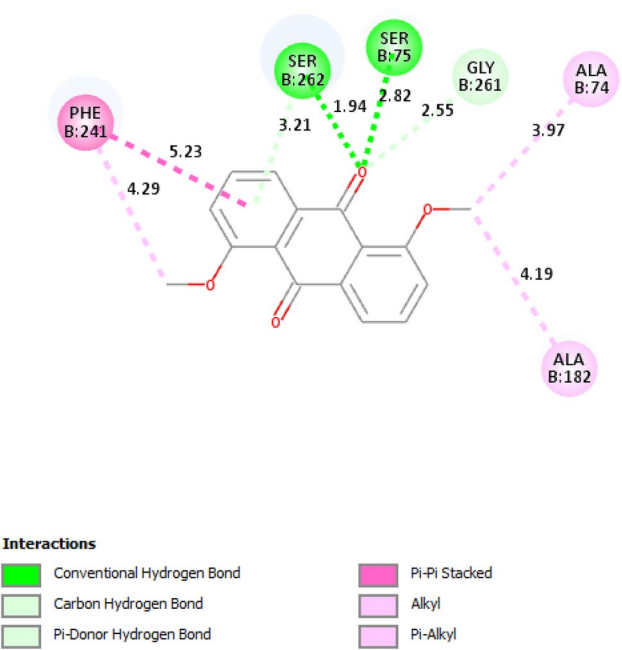


Fig. 19. 3-hydroxyl-1-methoxyanthraquinone interacting with amino acid residues of 5tw8 protein

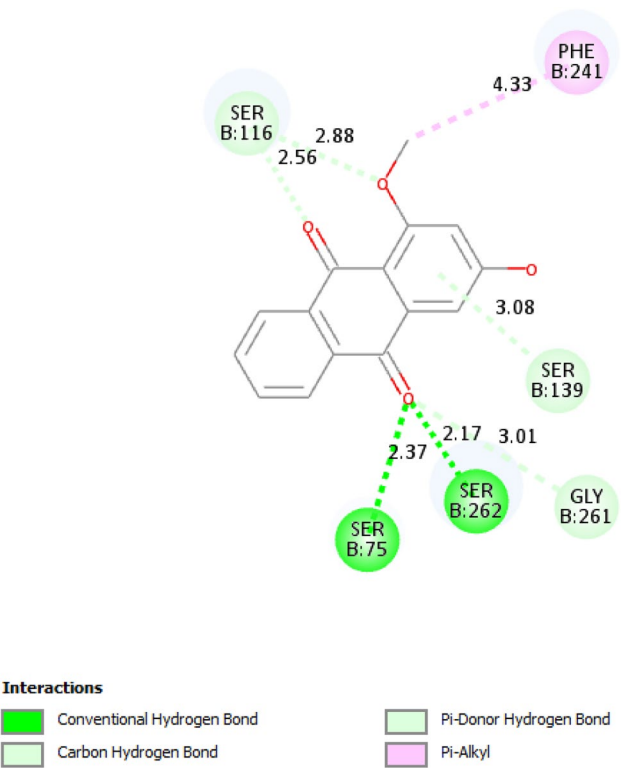


Fig. 20. 1,8-dimethoxyanthraquinone interacting with amino acid residues of 5tw8 protein

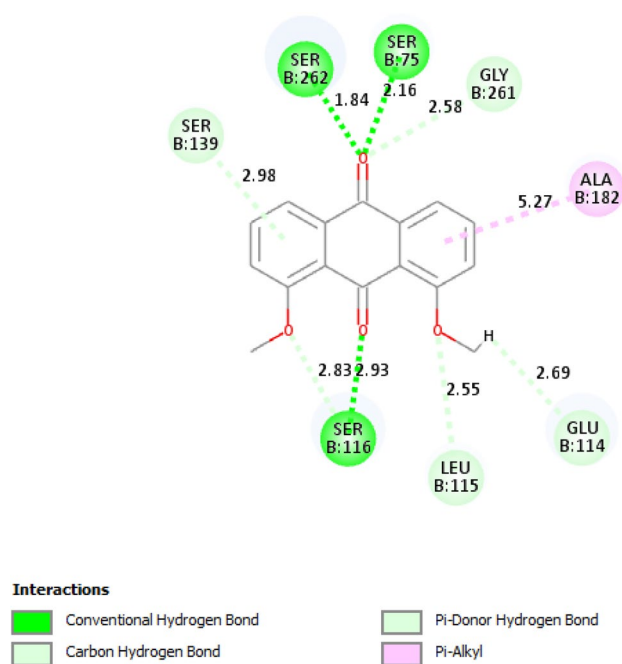


Fig. 21 Alizarin interacting with amino acid residues of 5tw8 protein

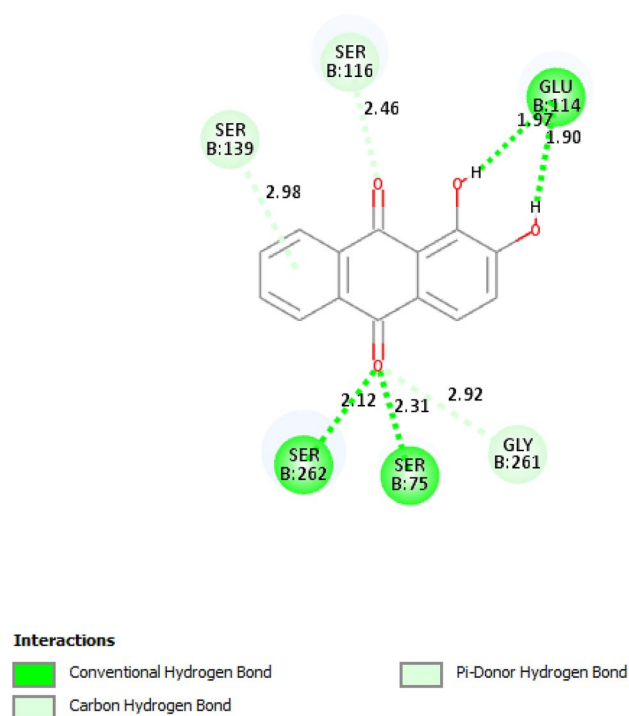


Fig. 22 RMSD graph of *S. aureus* (5TW8) in complex with 2-hydroxy-1-methoxy anthraquinone for 50 ns

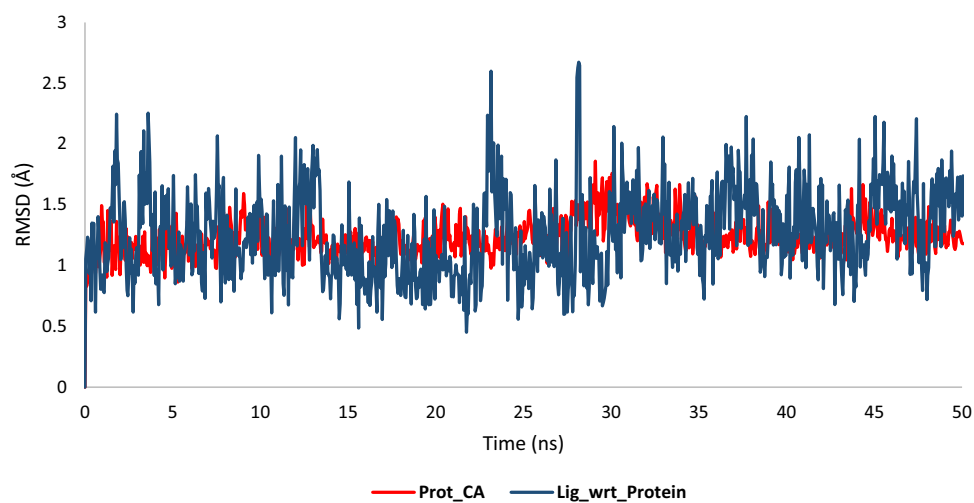


Fig. 23 RMSD graph of *P. aeruginosa* (7PTG) in complex with alizarin for 50 ns

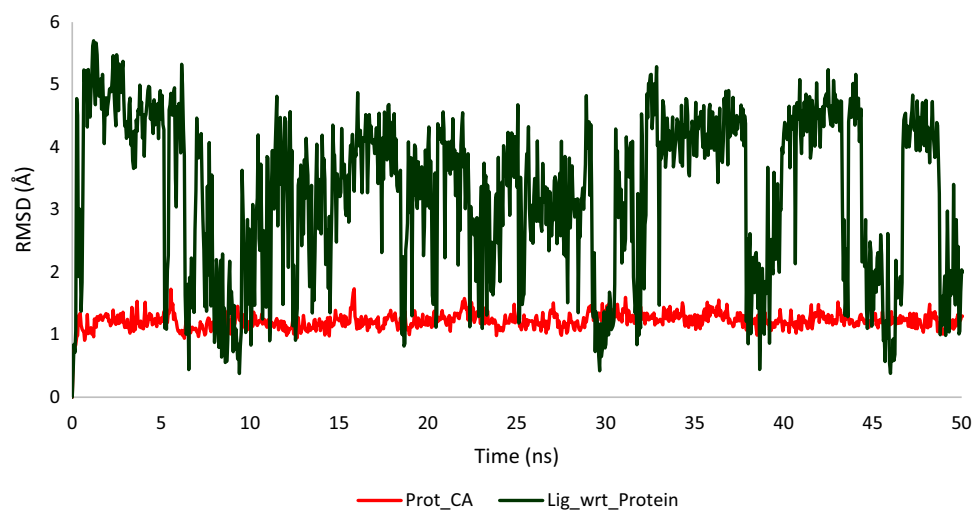


Fig. 24 RMSF graph of 5TW8 in complex with 2-hydroxy-1-methoxy anthraquinone for 50 ns

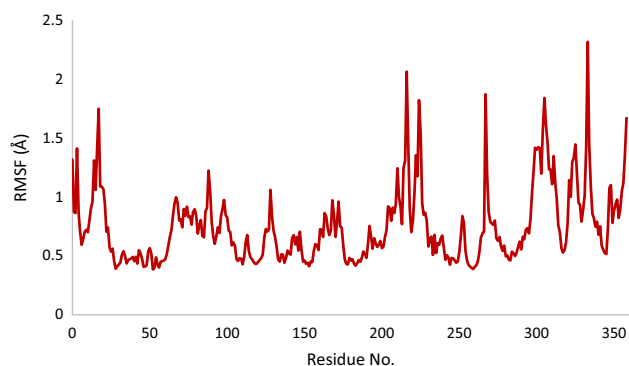


Fig. 25 RMSF graph of *P. aeruginosa* (7PTG) in complex with alizarin for 50 ns

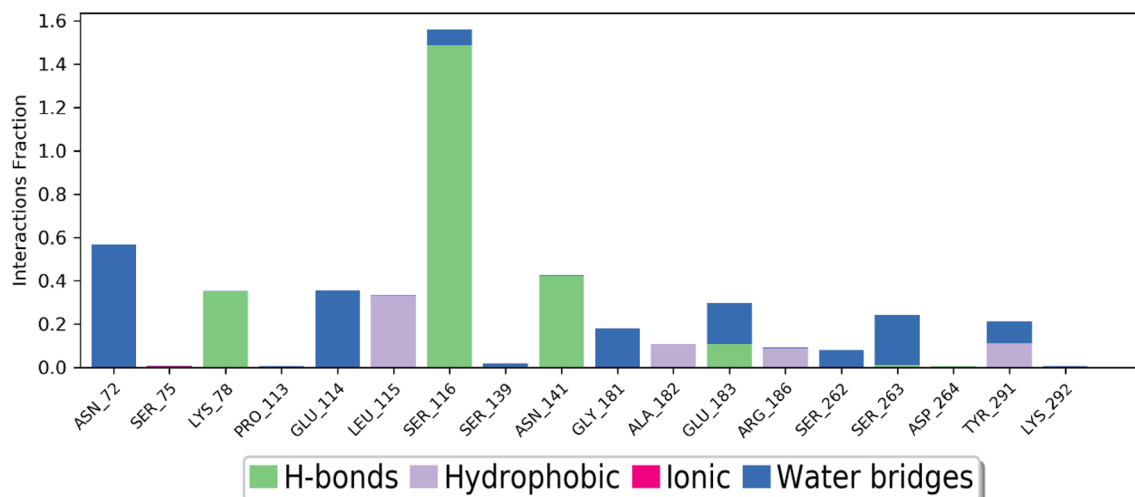
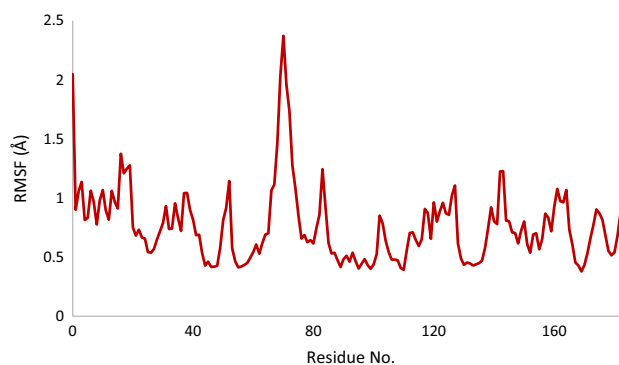


Fig. 26 Contact Histogram of 5TW8 showing the various interaction fractions of the amino acids

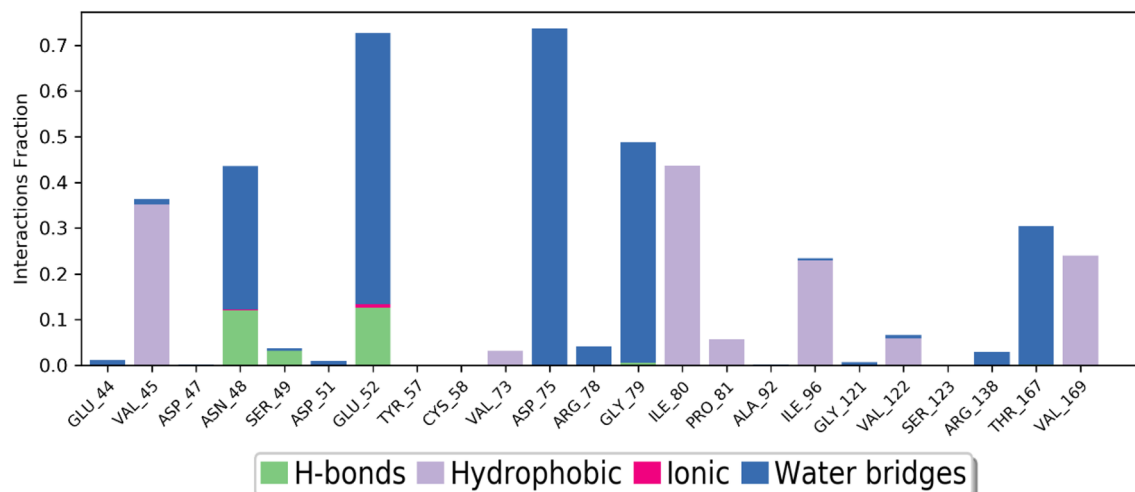
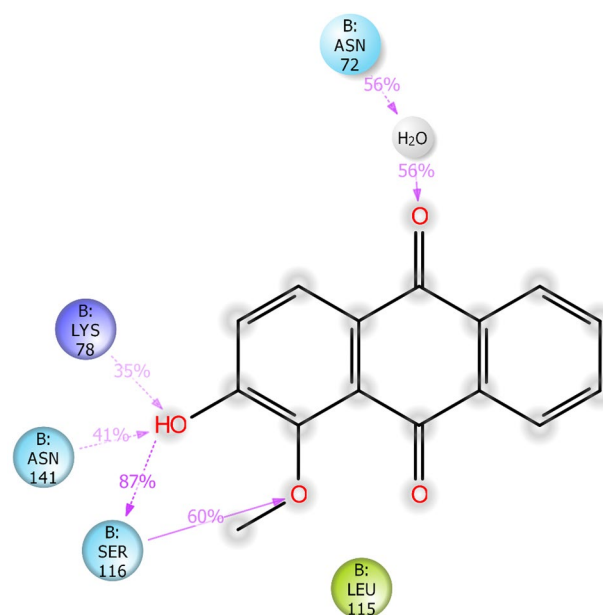
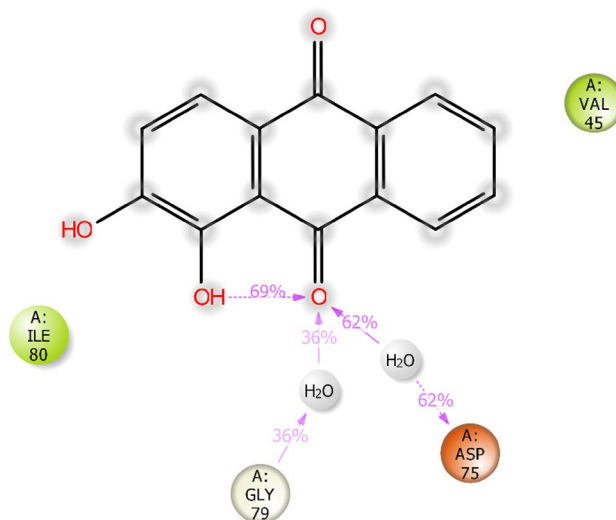


Fig. 27 Contact Histogram of 7PTG showing the various interaction fractions of the amino acids

Fig. 28 Contact summary for 5TW8**Fig. 29** Contact summary for 7PTG

4 Conclusion

Since these bacteria are of public health concern and can produce toxins that are stable under heat [36], utmost care must be taken to properly address them. One of which is the identification of compounds from plants with antibacterial properties. As seen in the in vitro results, the extracts of *M. lucida* had good susceptibility properties against the two drug-resistant bacteria when compared to gentamicin. From the anthraquinones identified in *M. lucida*, 2-hydroxy-1-methoxyanthraquinone is predicted to have a better susceptibility with *S. aureus* while alizarin has better susceptibility properties against *P. aeruginosa*. The MD shows a notable level of stability between the protein and the ligands in this study.

Thus anthraquinone compounds from *M. lucida* show that they have bioactive potentials as antibacterial drugs. This provides a justifiable reason for further identification and characterization of all the compounds that can serve as enzyme inhibitors with potential medicinal applications. Therefore future research would be on the isolation and characterization of secondary metabolites which have been identified in the study to be responsible for the antibacterial properties.

Author contributions AD and AO- carried out the invitro studies AD and OA - worked on the insilico analysis FO and LL- designed the work and worked on the manuscript. AD wrote the manuscript

Funding Therefore future research would be on the isolation and characterization of secondary metabolites which have been identified in the study to be responsible for the antibacterial properties.

Data Availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Declarations

Competing interests The authors declare that they have no competing interests.

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