Saudi Journal of Biological Sciences 30 (2023) 103743

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Assesment of polyphenolic compounds against biofilms produced by clinical *Acinetobacter baumannii strains* using in silico and *in vitro* models

Laraib Mumtaz [a,b], Arshad Farid [a], Suliman Yousef Alomar [c], Naushad Ahmad [d], Asif Nawaz [b], Saadia Andleeb [e], Adnan Amin [b,*]

[a] Gomal Centre of Biochemistry and Biotechnology(GCBB), Gomal University, KPK, 29050 D.I.Khan, Pakistan
[b] Gomal Centre of Pharmaceutical Sciences, Faculty of Pharmacy, Gomal University, D.I.Khan 29050, Pakistan
[c] Doping Research Chair, Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[d] Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[e] Atta Ur Rehman School of Biological Sciences, National University of Science and Technology, Islamabad Pakistan

ARTICLE INFO

Article history:
Received 27 May 2023
Revised 5 July 2023
Accepted 14 July 2023
Available online 20 July 2023

Keywords:
Acinetobacter baumanii
In silico analysis
Polyphenols
Drug resistance
Efflux pump
Metallo-β-lactamase

ABSTRACT

Several types of microbial infections are caused by *Acinetobacter baumanii* that has developed resistance to antimicrobial agents. We therefore investigated the role of plant polyphenols against *A. baumannii* using in silico and *in vitro* models. The clinical strains of *A. baumannii* were investigated for determination of resistance pattern and resistance mechanisms including efflux pump, extended spectrum beta lactamase, phenotype detection of AmpC production, and Metallo-β-lactamase. The polyphenolic compounds were docked against transcription regulator BfmR (PDB ID 6BR7) and antimicrobial, antibiofilm, and antiquorum sensing activities were performed. The antibiogram studies showed that all isolated strains were resistant. Strain A77 was positive in Metallo-β-lactamase production. Similarly, none of strains were producers of AmpC, however, A77, A76, A75 had active efflux pumps. Molecular docking studies confirmed a strong binding affinity of Rutin and Catechin towards transcription regulator 6BR7. A significant antimicrobial activity was recorded in case of quercetin and syringic acid (MIC 3.1 μg/mL) followed by vanillic acid and caffeic acid (MIC 12.5 μg/mL). All tested compounds presented a strong antibiofilm activity against *A. baumanii* strain A77 (65 to 90%). It was concluded that all tested polyphenols samples posess antimicrobial and antibiofilm activities, and hence they may be utilized to treat multidrug resistance *A. baumannii* infections.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) can grow in a wide range of environments (Peleg et al., 2008), and can cause several infections including healthcare-associated pneumonia, skin tissue infections, septicemia, hospital acquired meningitis and respiratory tract infections (Alves et al., 2016; Dijkshoorn and Nemec, 2008). *A. baumannii* infections primarily affect immuno-suppressed patients in ICUs, neonates, children, elderly, and diabetic patients in nursing homes and community hospitals settings (Sengstock et al., 2010). A reported cause for these wide spread infections is "surface adherence" capability of *A. baumannii* and development of layers i.e biofilms on surfaces in hospital settings (McConnell et al., 2013). The bacterial biofilms are non-motile complex layers of bacteria enclosed in a grid of EPSs (extracellular polymeric substances) (Alves et al., 2016; Roy et al., 2018). EPSs are infact dark matter of biofilms since they posess a range of matrix biopolymers (Flemming et al., 2007). EPSs also provide defense from mechanical stress by regulating the easy passage of nutrients, signaling molecules, and toxic substances across cell (Morgan et al., 2014). Quorum sensing is one of regulatory mechanism for biofilm formation in *Acinetobacter* spp. (Bhargava et al., 2010), that includes acyl homoserine lactones (AHL) dependent signaling (Prashanth et al., 2012) that makes such infections resistant to antimicrobial agents. Currently, several antimicrobial agents including sulbactam,

* Corresponding author.
E-mail addresses: director.gcps@gu.edu.pk (L. Mumtaz), syalomar@ksu.edu.sa (S. Yousef Alomar), anaushad@ksu.edu.sa (N. Ahmad), Asifnawaz@gu.edu.pk (A. Nawaz), saadia.andleeb@asab.nust.edu.pk (S. Andleeb), adnan.amin@gu.edu.pk (A. Amin).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103743
1319-562X/

minocycline and polymyxin are commonly used to control *A. baumannii* infections, however growing resistance and toxicity are common limiting factors. (Karageorgopoulos et al., 2011).

Polyphenolic compounds are quiet common in medicinal plants (Daglia et al., 2012; Fraga et al., 2019), and mainly responsible for diverse biological activities alone and in synergism (Manso et al., 2021). Polyphenolic compounds are categorized with at least one aromatic ring and more than one hydroxyl groups. Most of polyphenolics are produced from the oligo or polymerization of simple phenols (Zhang et al., 2020). The SAR investigations on polyphenolic compounds show that considerable increase in antimicrobial activity is observed in the presence of OH groups at 5′ and 7′ positions and 2′ and 4′ or 2′ and 6′ positions of A and B-rings respectively (Babu et al., 2005; Cushnie and Lamb, 2011). Similarly, among phenolic acids, the compounds with higher number of OH and methoxy groups are reported with high bacterial inhibition rates (Li et al., 2020), that is mainly attributed to strong antioxidant activities (Natella et al., 1999). Despite known antimicrobial activity of polyphenolic compounds, a little data is available regarding clinical isolates including *A. baumannii* (Álvarez-Martínez et al., 2020; Rama et al., 2021). Undeniably, natural molecules can affect biofilm formation, influence bacterial adhesion and inhibit communication among cells for biofilm formation Borges et al., 2014. Thus keeping in mind a widespread occurrence of *A. baumannii* infections and limited available treatment options, there exist a great need to look for new drugs or agents that can effectively target biofilm formation, detachment and complete removal (Simões et al., 2010). Aim of this work was to determine antimicrobial, anti Quorum sensing (QS) and antibiofilm activity of natural polyphenolic molecules by using *insilico* and *in vitro* analysis against resistant *A. baumannii* strains.

2. Materials and methods

2.1. Bacterial cultures

Acinetobacter baumannii were obtained from NUST (National University of Science and Technology, Islamabad, Pakistan). *Chromobacterium violaceum* strains (DSM 30191) was purchased from DSMZ Germany, and other quality control strain like *Klebsiella pneumoniae* (ATCC BAA-1705), *Escherichia coli* (ATCC 25922), *Staphylococos aureus* (ATCC 33862) and *Pseudomonas aeruginosa* (ATCC 15442) was provided by NPRL (Natural Products Research Lab, Gomal University, D.I.Khan, Pakistan).

2.2. Antibiotic discs, chemicals, growth media

Antibiotic disc including Piperacillin (100 μg), Ceftazidime (30 μg), Tetracycline (30 μg), Azteronam 30 μg, Cefotaxime (30 μg), Amoxicillin /Clavulanic Acid (30 μg), Imipenum (10 μg), Meropenem (10 μg), Ceftriaxone (30 μg), Ciprofloxacin (5 μg), Amoxicillin (10 μg), Cefoxitin (30 μg), Trimethoprim (5 μg), Gentamicin (10 μg), Sulphamethoxazole (100 μg) and Polymyxin B (300 μg) were purchased from Oxoid (UK). Vegetables and fruit derived polyphenols such as Ferulic acid, Gentistic acid, Gallic acid, Rutin, Cinnamic acid, Quercetin, Caffeic acid, Catechin, Syringic acid, and Vanillic acid were purchased from Sigma (Aldrich, UK). Growth media including Muller Hinton ager, MA (MacConkey Agar), Lauria Bertani broth, and tryptic soya broth were obtained from Hi Media (Mumbai, India).

2.3. Computational analysis

The transcription regulator gene "BfmR" (biofilm regulator protein) (PDB ID: 6BR7) in *A. baumannii* was downloaded from PDB (protein Data Bank) database (Draughn et al.,2018). BfmR domain is comprised of two homodimers i.e chain A and chain B (Lokhande *et al*., 2022) and based on literature review (Farrow et al., 2018, Russo et al., 2016). PDB file of ligands were produced by using Accelrys Discovery Studio visualizer 2.0 (Accelrys Discovery Studio 2012). Molecular docking of targets and ligands was performed using Autodock Vina 6 using Lamarckian Algorithms Trott and Olson, 2010. Docking analysis was performed by Discovery studio visualizer and interaction analysis was performed by using Ligplot plus.

2.4. Determination of resistance pattern

The resistivity pattern of clinical isolates was determined by disc diffusion method (Wayne et al., 2010). The Muller Hinton agar plates were inoculated with bacterial culture (adjusted to 0.5 McFarland) followed by placing discs on Muller Hinton agar plate and incubation at 37 °C for 24 h. Each antibiotic disc's inhibition zone was determined (millimeter) and interpretation was performed in accordance with CLSI criteria.

2.5. Phenotype detection of extended spectrum beta lactamse (ESBLs)

The phenotypic ESLs was determined by using double disc synergy test protocol (Litake et al., 2015). Briefly, five discs of antibiotics including ceftriaxone, amoxicillin-clavulanic acid, aztreonam and cefotaxime. The amoxicillin-clavulanic acid disc was placed in the center, and remaining discs were positioned 1.5 cm apart, trailed by incubation at 3 °C for 24 h. An extension of inhibitory zone towards amoxicillin-clavulanic acid disc after 24 h incubation was considered as ESBLs positive.

2.6. Phenotype detection of Metallo-β-lactamase production (MBL)

A standard methodology with slight modifications was used to analyze the phenotype detection of MBL production (Saha et al., 2010). Concisely, Imipenum and Imipenum + EDTA were positioned on Mueller Hinton agar plate center to center about 20 mm apart. After that plates were incubated at 37 °C for 24 h. An enhancement of Imipenum + EDTA disc's zone of inhibition nearly 7 mm as compare to Imipenum disc was recorded as MBL positive isolate.

2.7. Phenotype detection of AmpC production

The AmpC disc assay was used to identify presence of AmpC-lactamase, with minor modifications (Rafiee et al., 2014). The bacterial strain (matched with 0.5 McFarland) was spread onto the agar plate (Muller Hinton) and allowed to dry for 20 min. Afterwards, cefoxitin disc (30 μg) and AmpC (filter paper discs containing Tris EDTA) disc were positioned on seeded agar plate and incubated at 37 °C for 24 h. Later, blunting of inhibition zones toward Cefoxitin was observed. A positive test was indication of enzymatic inactivation of cefoxitin.

2.8. Efflux pump screening

Ethidium Bromide-agar (EtBr) cartwheel method was used for determination of efflux pump activity (Anbazhagan et al., 2019). Briefly, EtBr diversified concentrations (0–2 mg/mL), were inoculated with 10^6 cells per mL bacterial cell solution on agar plates (Muller-Hinton) and incubated for 24 h in incubator set at 37 °C. Further plates were evaluated for fluorescence after 24 h using a UV light (Cleaver Scientific Ltd). The bacterial strains with active efflux pumps presented fluorescence at minimal EtBr concentration.

2.9. Antibacterial activities (96 microplate)

Antibacterial activity of compounds was performed by modified method (Weseler et al., 2005). An overnight bacterial culture (1.5 x10^7CFU/mL) of about 50 µL was loaded in 96 micro well plates and then added 50 µL of test compounds were added. After 24 h of incubation at 37 °C, an aliquot of 50 µL of resazurin (0.0015%) was applied to each well of 96 micro well plates. After addition of resazurin micro well plates were incubated again for about 1 h at 37 °C and reading were checked by using 96 micro well plate readers (Biosan, Riga, Latvia). For MBC calculation, fresh Muller Hinton agar plates were prepared and 10 µL broth (containing bacterial) from the MIC micro well was loaded and incubated for 24 h at 37 °C. After 24 h on the plates development of bacteria was observed. Ciprofloxacin was used as positive control.

2.10. Anti-biofilm activity

Anti-biofilm activity was performed on the polystyrene 12 well plates with slight changes (Bazargani and Rohloff, 2016). The adjusted bacterial suspension (with 0.5 McFarland) of about 280 µL was inoculated in Tryptic Soya Broth and incubated at 37 °C for 24 h. Afterwards test samples (0.01––4 mg/mL; 100 µL) was added in media and incubated at 37 °C for 24 h. Bacterial growth was monitored at 592 nm. The biofilm was quantified by staining with crystal violet dye. Ethanol (95%) was used for destaining and reading was checked at 592 nm for biofilm assesment. Following formula was used to determine inhibition:

$$\% \text{ inhibition} = [1 - (\text{Absorbance of compound} / \text{Absorbance of control})] \times 100$$

2.11. Anti-quorum sensing studies

The *Chromobacterium violaceum* (*C. violaceum*) overnight culture (1/100) was streaked on petri dishes of LB (Luria Bertani) agar. Over surface of LB media petri dishes planted with *C. violaceum* strain were placed previously prepared sterilized filter paper discs of 6 mm. Test sample (0.02–3 mg/ml;15 µL) were loaded onto a 6 mm disc and kept to become dry for at least 30 min, followed by incubation at 30 °C for 72 h (Koh et al., 2011).

3. Results

3.1. Molecular docking

To ascertain possible interactions between polyphenolic compounds and target site, molecular docking studies were carried out. The resistance of *A. baumannii* is mainly due to biofilm formation ability and based on literature (Draughn et al.,2018), it is regulated by BfmR gene (PDB ID 6BR7). Therefore BfmR gene was used in docking analysis. The BfmR domain was comprised pf of two chains (A and B) and has a sequence length of 133 amino acids. The chain A was selected for further docking and processed for addition of charges and removal of co-crystallized ligands and H atoms. In group A compounds, ferulic acid docking presented maximum number (4) of H-bonding interactions with amino acid residues Gly35, Ile10, Glu50, Lys9 at active site of pose 4 (-5.4 ΔG (kJ mol^{-1}) (Table 1). Other interactions including pi sigma, Pi-Pi and pi Alkyl interactions were seen with Val34, Glu33, Leu11, Pro52 (Fig. 1). Among compounds of Group B, Gallic acid showed best interaction (-4.9 ΔG (kJ mol^{-1}) (Table 1) with Lys107, Asp58, Met60, Asp15 (Pose 3) and neighboring amino acids included Asp16, Glu14 (Fig. 2). The number of hydrogen bonds and amino acid residues that participated in other compound's ligand–protein interaction are shown in (Fig. 1). In Flavonoids (group C), rutin showed presented best fit (-6.6 ΔG (kJ mol^{-1}) in the active pocket of target (pose 1) (Table 1). The H-bond interacting residues included Glu98, Leu97, Ala101, Gly100, Asp102, His78, Arg71.The free binding energies and results of other compounds are presented in Table 1, whereas interaction analysis is presented in Fig. 3.

3.2. Multi drug resistance (MDR) phenotyping

Sixteen antibiotics from various antibiotic classes were used and susceptibility was recorded (Table 2). Azteronam, ciprofloxacin, Imipenem and cefoxitin were active against all clinical isolates. Whereas tetracycline, gentamycin, piperacillin, polymyxin were active against at least two of tested strains (Table 2). Based on these findings the results were compared with National committee for clinical laboratory standard or clinical and laboratory standard institute (NCCLS / CLSI) standard table is categorized as S (sensitive), I (intermediate) and R (Resistant) (Table 3). It was noticed that cefoxitin is sensitive to all tested strains whereras Azteronam, and imipenem are sensitive toward two strains while all other antibiotics are resistant (Table 4).

3.3. Phenotype detection of ESBL

With aim to determine the resistance mechanism, strains were tested for presence of extended spectrum beta lactamase enzyme using double disc synergy method. The plates were showed no ESBL production against FOX (Cefoxitin) and AMC (Amoxicillin Clavulanic Acid), it was therefore confirmed that tested strains were not ESBLs producers (Supplementary data).

3.4. Phenotype detection of MBL (Metallo-β-lactamase) production

Bacterial isolates were screened using the EDTA method for the production of metallo-lactamase. A slight increase in the inhibition zone with EDTA in case of A77 was indication of moderate Metallo-β-lactamase Production. All other tested strains were negative in this case (Table 5).

3.5. Phenotypic screening of isolates for efflux pump activity

EtBr (Ethidium bromide) cartwheel technique was adopted for determination of efflux pump in isolated strains. All *A. baumannii* strains (A77, A76, A75) had active efflux pumps since no fluoresce was observed at minimum concentration of EtBr according to cartwheel method (Supplementary data).

3.6. Phenotype detection of AmpC production

Phenotype Detection of AmpC production was determined by using standard procedure explained earlier (Materials and Methods). Based on observation, since there was no blunting of zone towards neither Cefoxitin disk it was concluded that tested strains were nor producers of AmpC (Supplementary data).

3.7. Antimicrobial activities based assay (MIC and MBC)

The MIC values ranged from 6.3 to 12.5 µg/mL (except Gallic acid and Cinnamic acid) (Table 6), whereas in case of flavonoid it was 3.1 to 12.5 µg/mL (except Rutin and Catechin) (Table 6). In the case of Quercetin, nicer inhibition of A77 was recorded (MIC 3.1 µg /mL), however compare to it lesser inhibition was shown in case of *S. aureus* (MIC 6.3 µg /mL). In the case of A76 strain of *A. baumannii, E.coli, K. pneumonia, S. aureus* and *P. aureginosa* inhibition was recorded (MIC 12.5 µg/mL) in case of Quercetin. In case

Table 1
Results of docking studies of all polyphenols examined with their respective targets 6BR7.

Test sample	ΔG (kJ mol-1)	Pose No	No of H bonds	H- Bond Interacting residues	Other interactions
Caffeic Acid	−4.6	5	3	Asn30, Gly31, Arg119	Leu32, Pro8, Leu122, Arg123
Catechin	−5.6	4	5	Gln23, Glu37,Arg42, Thr38, Glu17	Arg21, Val36,
Cinnamic Acid	−4.7	5	3	Val36, Glu37, Arg42	Thr38, Glu17
Ferulic Acid	−5.4	1	4	Gly35, Ile10, Glu50, Lys9	Val34, Glu33, Leu11, Pro52
Gallic Acid	−4.9	3	4	Lys107, Asp58, Met60, Asp15	Asp16, Glu14,
Gentistic Acid	−4.8	1	3	Arg46, Glu50, Lys9	Leu11, Ile10, Gly35, Pro52
Quercetin	−6.0	2	4	Asn30, Arg119, Thr125, Leu122,	Pro8, Leu7, Glu5, Gly31
Rutin	−6.6	1	7	Glu98, Leu97, Ala101, Gly100, Asp102, His78, Arg71	Met99, Arg74
Syringic Acid	−4.4	1	3	Pro108, Ala106, Arg117	Val113, Val109, Val105
Vanillic Acid	−4.3	1	2	Ile10, Glu33	Lys9, Pro52, Glu50, Gly35, Val34

of *K. pneumoniae* nicer inhibition (MIC 6.3 μg/mL) was recorded in case of Syringic acid, however as compare to this less inhibition (MIC 12.5 μg/mL) was shown in *A. baumannii* (A75, A76 and A77).

3.8. Anti-biofilm activity

All tested compounds were active against A75 strain (65 to 90%). A comparably lower antibiofilm activity was recorded in case of A76 (49 to 81%) whereas a week antibiofilm activity was recorded in case of A77 (15% to 65%) (Table 7).

3.9. Anti-quorum (QS) sensing activity

Polyphenolic compounds were assessed for antiquorum sensing activity and no inhibition was recorded. That clearly shows that the antibiofilm activity of tested compounds could possibly due to certain other mechanism (Supplementary data).

4. Discussion

A. baumannii is an important health challenge as of its great inherent resistance against wide range of antimicrobials. Moreover, an ability of resistant *A. baumannii* strains to sustain for extended periods of time in a hospital setting significantly increases antibiotic resistance, thus making it very difficult to manage infection control (Raorane et al., 2019, Manchanda et al., 2010). Our tested compounds were catargirised as Group A (caffeic acid, ferulic acid, cinnamic acid,), Group B (gallic acid, syringic acid, gentistic acid, and vanillic acid) and flavonoids (Catechin, Rutin and Quercetin) for more clear overview based on structural similarities. In group A compounds, the number of phenolic group (OH) was higher in cinnamic acid (3) followed by ferulic acid (2) and caffeic acid (1). Ferulic acid was associated with a methoxy group and carboxylic acid was common in all compounds. Based on structural features, a diverse interaction mechanism was expected in all compounds with transcriptional regulator 6BR7. The best docking pose for each molecule was further used to study intramolecular correlations. Among Group A compounds, Ferulic acid presented significant interaction with target site, that possibly could be the result of methoxy group present in ferulic acid, since presence of hydroxyl, carboxyl and/or methoxy groups in compounds hydrophilic interactions are possible that results in high docking scores (Stefaniu et al., 2020). Further, presence of hydroxyl, carboxyl and/or methoxy groups also facilitate better absorption and permeation properties (Veber et al., 2002). This comparably better interaction of gallic acid to target with mainly attributed to presence of three OH groups. Further an increase in increasing alkyl chain length may also result in enhanced activity (Takai et al., 2011), since it may affect membrane binding capability (Kahkeshani et al., 2019). The Group B compounds has quiet diversity in structure based on position and number of OH and OCH_3 groups. Gallic has highest number of OH groups (3) followed by Gentistic acid (2). Both Syringic acid and Vanillic acid posess only one OH group with additional OCH_3 groups. Among tested compounds, Among OCH_3 group containing compounds, Syringic acid has highest number (03), however its binding energy (−4.4 ΔG (kJ mol^{-1}) was lesser compared to gallic acid and showed only three H-bonding interactions with Pro108, Ala106, Arg117 (Pose 1). Upon comparison it is obvious gallic acid and syringic acid have OH/OCH_3 replacement at meta position and despite interaction of OCH_3 with Ala 106 and Arg117 interaction lesser binding energy was observed. It has been reported previously that number and position of OCH_3 group may result in diverse interaction (Sato et al., 2014). In compounds of Group C, the OH groups mainly participate in the H bonding interaction (bond length 2.87°A to 3.25A °) and stabilized by hydrophobic interactions with Ala101, Leu97 and His 78.

To identify the resistance mechanism of *A. baumannii* antimicrobial susceptibility test was performed. In this study, some frequently used antibiotics were screened. *A. baumannii* strains were resistant for Ceftazidime, Amoxicillin, Cefotaxime, and Sulphamethoxazole. Likewise resistance of tested strains towards Piperacillin and Polymixin B antibiotics was recorded, that was in accordance with earlier investigation in Egypt (Deaf et al., 2013), where a significant resistance to *A. baumannii* (33.3%) was observed. Further, a corresponding (Abdar et al., 2019) higher resistance to Ceftazidime (93%) and Meropenem (71%) has been reported earlier. The higher resistance levels were attributed to drug abuse in the clinical settings (Zhanel et al., 2013). *A. baumannii* cancels the effects of several different antibiotics, as well as carbapenem due to its β-lactamases hydrolyzing potential that is crucial in the treatment of nosocomial infections are caused by gram-negative pathogens (Irfan et al., 2008). In *A. baumannii*, carbapenem resistance results from a number of interrelated mechanisms, including hydrolysis by β-lactamases, changes to the outer membrane proteins and penicillin-binding proteins, and enhanced action of efflux pumps (Amudhan et al., 2011). Our tested strains had carbapenem resistance might be due to presence of other mechanisms such as alterations in the outer membrane proteins and penicillin-binding proteins and increased activity of efflux pumps.

EDTA disc synergy test revealed that one strain *of A. baumannii* (A 76) was capable of producing MBL. Researchers have shown that MBL-producing strains may be susceptible to monobactam with respect to certain investigations (aztreonam) (Yousefi et al., 2010), we observed that one of our tested strain presented resistance to Azteronam. It was therefore concluded that OXA-type carbapenemase production may have presented carpabenemase positive results rather than metallo β-lactamase synthesis, which is often cited as a key mechanism of resistance in A. *baumannii* isolates. Some Metallo-β-lactamase negative strains have resistance for different types of antibiotics due to presence of over expression or reduce expression of efflux pumps. Multidrug resistance is an outcome of over expression of efflux pumps (Viveiros et al.,

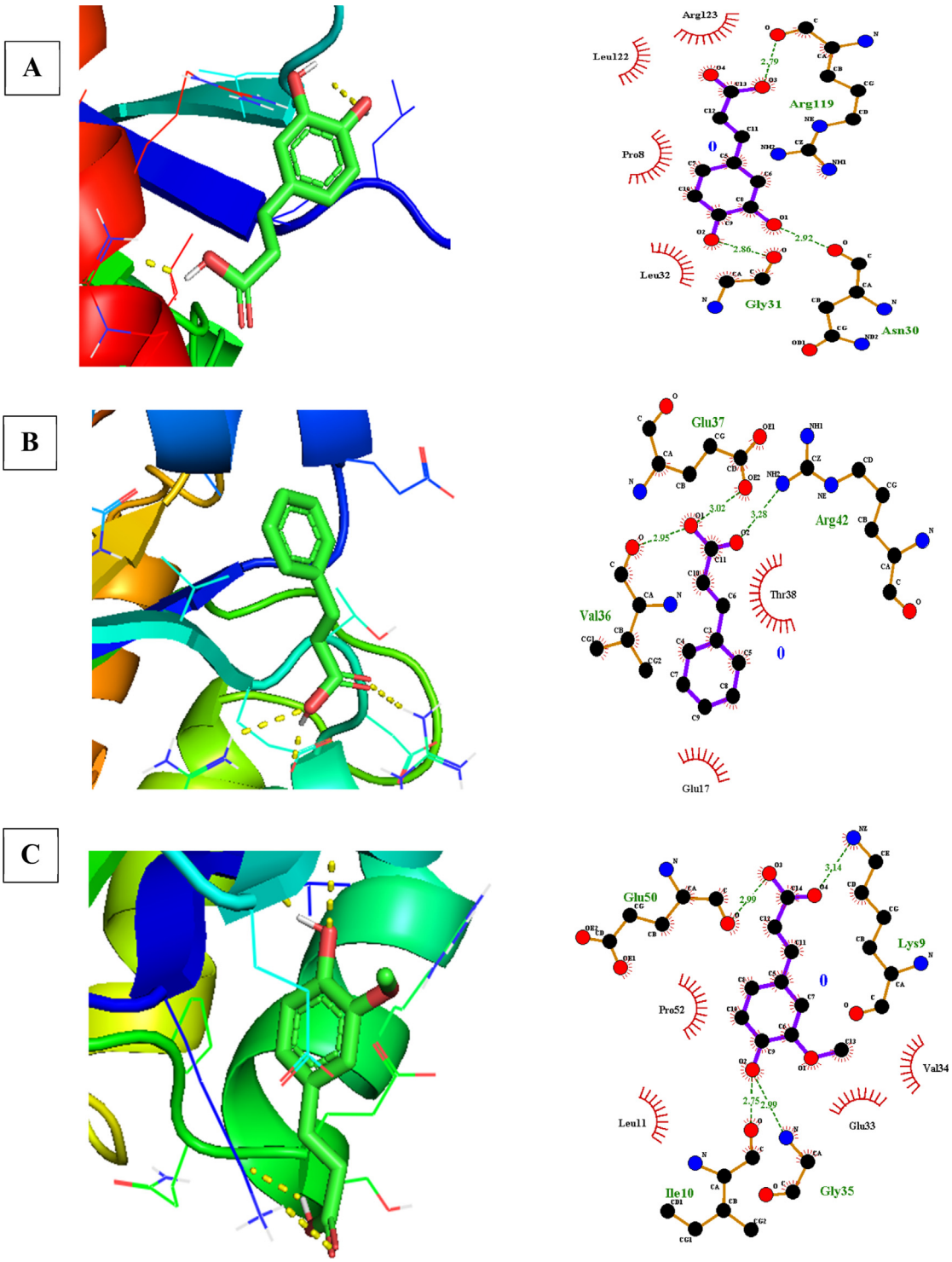

Fig. 1. Three-dimensional interaction and hydrogen, non-hydrogen Bonding interaction of caffeic acid A (Pose 5), Ferulic acid B (Pose 1) and Cinnamic acid C (Pose 7) on target site of 6BR7 with pose rank 5. (Group 1).

2008), that facilitates removal of antibiotics prior to reaching the planned targets. A variety of processes, including enzyme modification, reduced membrane permeability, protective shield development, drug target modification, can also contribute to acquired MDR in bacteria (Martins et al., 2011). In our study our all isolates of *A. baumannii* were resistant that may be ascribed to presence of active efflux pumps. Efflux pumps are membrane proteins expressed in gram-negative bacteria and gram-positive that permit antibiotics for expulsion from the cells. Resistance to antibiotics in bacteria is frequently linked to the presence of efflux pumps.

AmpC positive bacterial infections are typically settled with carbapenem, yet efflux pump activation due to induced mutations in bacteria may limit their usage. Our tested strains had AmpC negative results and represents resistance for carbapenem. Resistance to carbapenem have been ascribed to several factors such as elevated efflux activity, lessened expression of outer membrane pro-

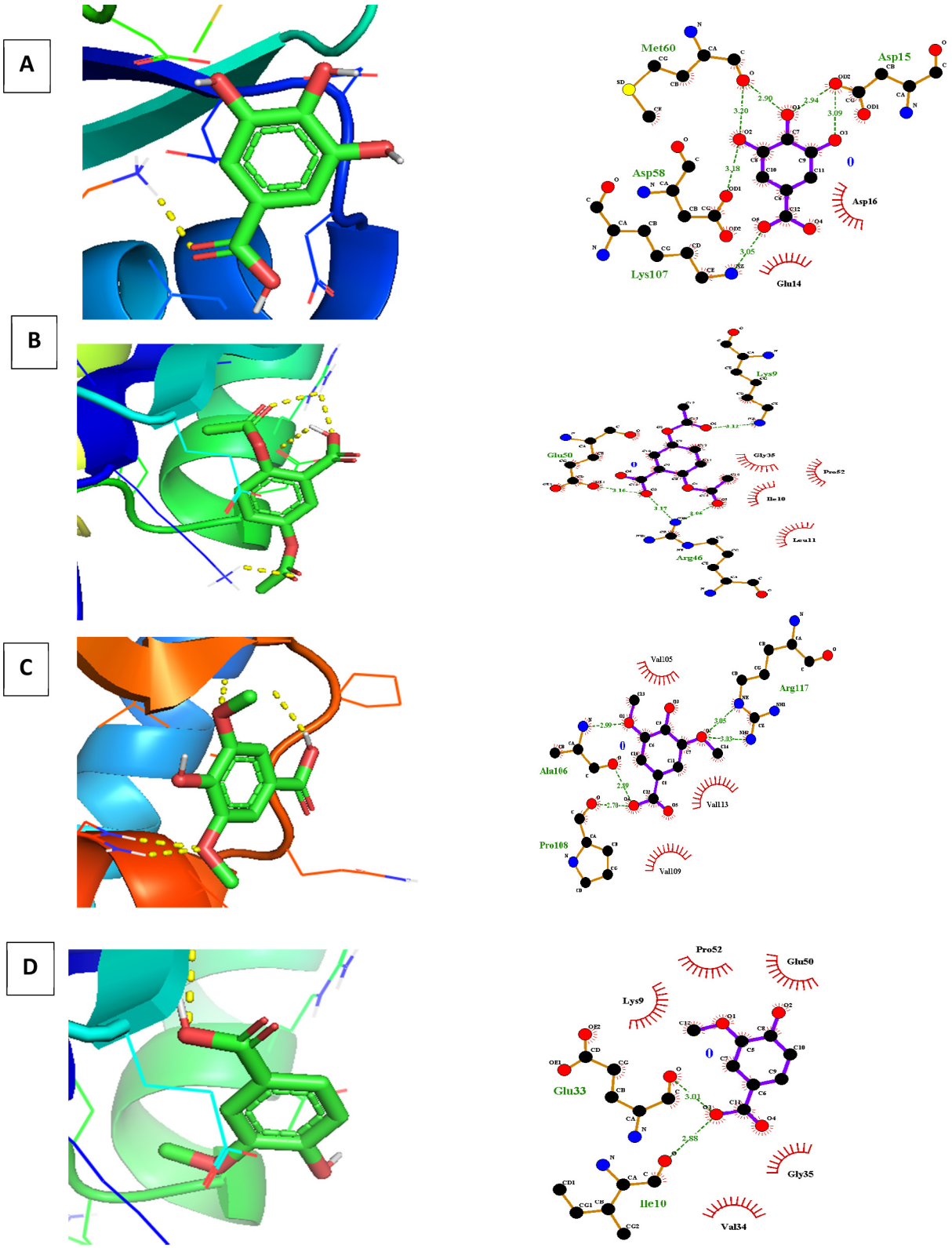

Fig. 2. Three-dimensional interaction and hydrogen, non-hydrogen Bonding interaction of gallic acid A (gallic acid pose 3), B Gentistic acid (Pose 1), Syringic acid C (Pose 1), Vanilic acid D (Pose 1) on target site of 6BR7. (Group 2).

teins and carbapenemase production (Pitout., 2010). However, there are no CLSI guidelines are available for detection of AmpC mediated resistance in Gram–negative strains and thus it frequently reflects misleading results, especially so in phenotypic tests (Handa et al., 2013). In our study, Cefoxitin resistance was recorded but no AmpC reduction was noticed. The reason of negative results can be these selected isolates were obtained from those patients which not have prolonged hospital stay.

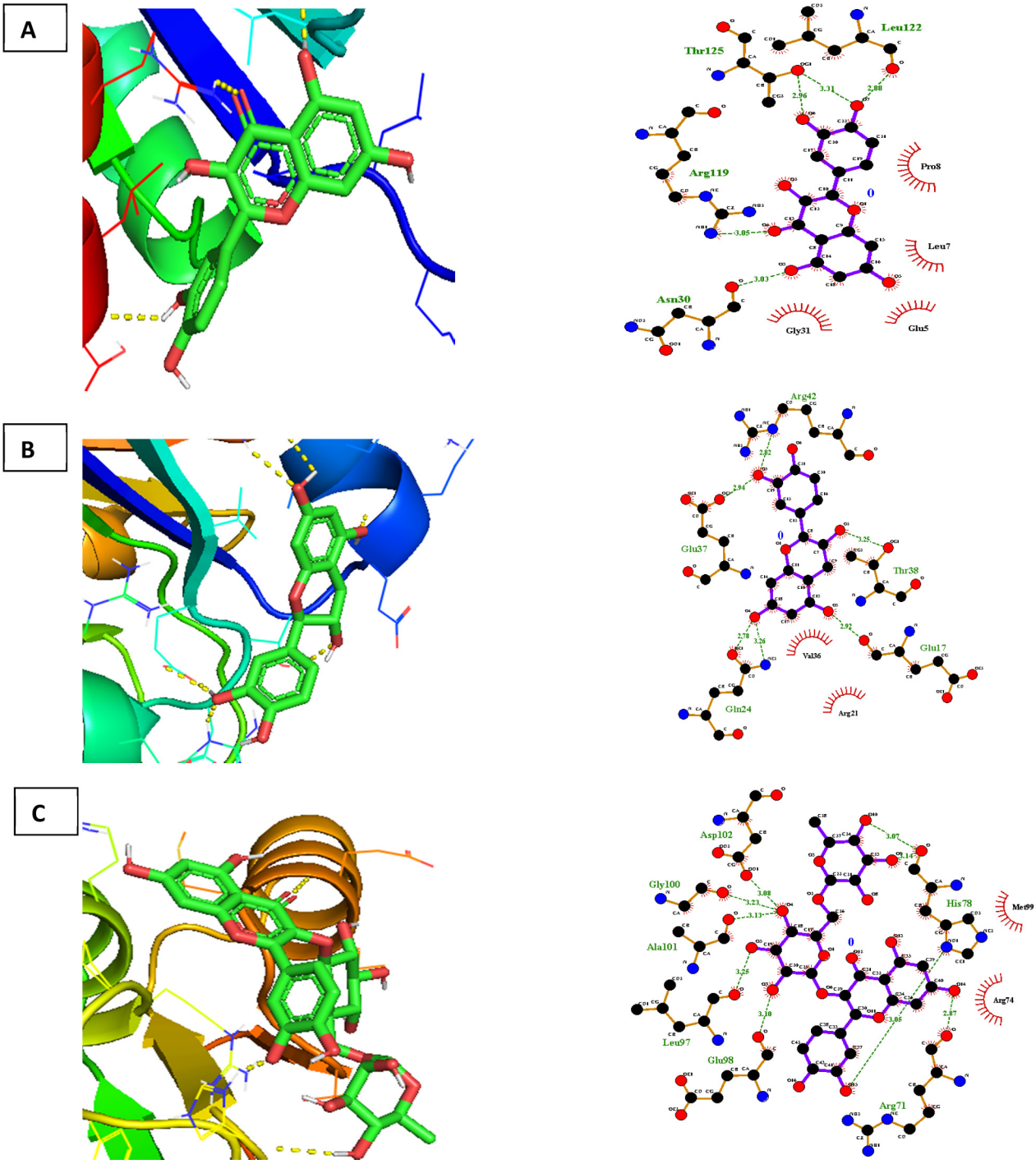

Fig. 3. Three-dimensional interaction and hydrogen, non-hydrogen Bonding interaction of Catechin A (Pose 4), Quercetin B (pose 2), Rutin C (Pose 2) on target site of 6BR7. (Group 3).

Based on initial screening and resistance pattern, it was confirmed that all isolated strains resistant to antibiotics. We therefore screened our polyphenolic compounds against the *A. baumannii*, and reference standard strains including *S. aureus, K. pneumoniae, E. coli,* and *P. aeruginosa*. Our results show that all polyphenols were active against *A. baumannii*, however quercetin and syringic acid had significant inhibition. Plant poly phenols have long been reported to posess antimicrobial activities (Khameneh et al., 2019; Klancnik et al. 2017), due to inhibition of exopolysaccharide activity (Ferreira et al., 2014). The tested compounds were further screened for antibiofilm activity, and it was evident that all tested compounds inhibited biofilm formation in case of A75 and A76, whereas a week inhibition was recorded in case A77, that reflect resistance. Interestingly, no of the tested compounds was able to presented antiquorum sensing activity against tested strains which clearly shows antibiofilm activity is possibly due to other mechanisms (Singh et al., 2017) than inhibition of cell to cell signaling and that may be due to efflux pump inhibition (Richmond et al., 2016) that was positive in our isolated strains(Supplementary data).

Table 2
Antibiotic Susceptibility testing (AST) of *A. baumannii* clinical isolates against some antibiotics.

S.No	Antibiotic Disc	Potency (μg/disc)	Zone of inhibition (mm)		
			A75	A76	A77
1	Ceftazidime	30	0	0	0
2	Aztreonam	30	16	25	18
3	Amoxycillin	10	0	0	0
4	Cefotaxime	30	0	0	0
5	Amoxycillin/Clavulanic Acid	30	8	0	0
6	Imipenem	10	24	29	16
7	Meropenem	10	0	0	0
8	Ceftriaxone	30	3	0	6
9	Ciprofloxacin	5	16	16	22
10	Tetracycline	30	10	8	30
11	Cefoxitin	30	22	30	26
12	Trimethoprim	5	0	0	29
13	Gentamicin	10	10	0	19
14	Sulphamethoxazole	100	0	0	0
15	Piperacillin	100	18	0	15
16	Polymixin B	300	8	15	15

Table 3
CLSI Criteria for determination of antimicrobial susceptibility.

S.no	Antibiotic Disc	Potency (μg/disc)	Resistance Pattern		
			S	I	R
1	Ceftazidime	30	≥18	15–17	≤14
2	Aztreonam	30	≥18	15–17	≤14
3	Amoxycillin	10	–	–	–
4	Cefotaxime	30	≥23	15–22	≤14
5	Amoxycillin/Clavulanic Acid	30	≥15	11–14	≤11
6	Imipenem	10	≥22	19–21	≤18
7	Meropenem	10	≥18	15–17	≤14
8	Ceftriaxone	30	≥21	14–20	≤13
9	Ciprofloxacin	5	≥21	16–20	≤15
10	Tetracycline	30	≥15	12–14	≤11
11	Cefoxitin	30	≥18	15–17	≤14
12	Trimethoprim	5	≥16	11–15	≤10
13	Gentamicin	10	≥15	13–14	≤12
14	Sulphamethoxazole	100	≥16	11–15	≤10
15	Piperacillin	100	≥21	18–20	≤17
16	Polymixin B	300	–	–	–

Table 4
Antibiotic Susceptibility test of *A. baumannii clinical* isolates against some antibiotics.

S.no	Antibiotic disk	Potency (μg/disc)	Resistance Pattern		
			A75	A76	A77
1	Ceftazidime	30	R	R	R
2	Aztreonam	30	I	S	S
3	Amoxycillin	10	R	R	R
4	Cefotaxime	30	R	R	R
5	Amoxycillin/Clavulanic Acid	30	R	R	R
6	Imipenem	10	S	S	R
7	Meropenem	10	R	R	R
8	Ceftriaxone	30	R	R	R
9	Ciprofloxacin	5	I	I	S
10	Tetracycline	30	R	R	S
11	Cefoxitin	30	S	S	S
12	Trimethoprim	5	R	R	S
13	Gentamicin	10	R	R	S
14	Sulphamethoxazole	100	R	R	R
15	Piperacillin	100	R	R	R
16	Polymixin B	300	R	R	R

Table 5
Phenotype detection of MBL production by Imipenum and IPM/EDTA disc positional test of *A. baumannii* strains A77, A76, and A75.

Bacterial Strains	Inhibition Zone (mm)			Inference
	IPM	IPM/EDTA	(IPM/EDTA) – IPM	
A77	16	18	2	Positive
A76	29	29	_	Negative
A75	24	24	_	Negative

Table 6
Determination of MIC of given polyphenols against *A. baumannii*, *E. coli*, *K. pneumoniae*, *S. aureus* and *Pseudomonas aureginosa* strains.

Samples	*A. baumannii*			*E.coli*	*K. pneumoniae*	*S. aureus*	*P. aureginosa*
	A75	A76	A77				
	MIC(μg/mL)						
Gentistic Acid	12.5	25	12.5	12.5	12.5	12.5	12.5
Ferulic Acid	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Gallic Acid	25	12.5	12.5	12.5	12.5	12.5	12.5
Rutin	12.5	25	12.5	12.5	12.5	12.5	12.5
Cinnamic Acid	25	12.5	12.5	25	12.5	12.5	12.5
Catechin	25	12.5	12.5	12.5	12.5	12.5	12.5
Quercetin	25	12.5	3.1	12.5	12.5	6.3	12.5
Caffeic Acid	12.5	12.5	12.5	12.5	In active	12.5	12.5
Syringic Acid	12.5	12.5	12.5	12.5	6.3	12.5	12.5
Vanillic Acid	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Ciprofloxacin	1.6	6.3	6.3	0.4	1.6	0.2	0.8

Table 7
Antibiofilm assay of polyphenols.

Samples	A75	A76	A77
Ciprofloxacin	74%	64%	21%
Syringic Acid	90%	68%	56%
Vanillic Acid	83%	81%	54%
Quercetin	87%	57%	50%
Caffeic Acid	83%	73%	57%
Gallic Acid	72%	72%	15%
Ferulic Acid	69%	49%	65%
Rutin	72%	55%	64%
Cinnamic Acid	71%	63%	55%
Catechin	73%	72%	47%
Gentistic Acid	65%	79%	63%

5. Conclusion

A. baumanii infections are of great health concern in clinical settings as they are involved in healthcare-associated pneumonia, skin and soft tissue infections and nosocomial meningitis. Especially, *A. baumannii* primarily affects immuno-suppressed patients in hospital intensive care units. We used plant polyphenols that are secondary metabolites found in all higher plants including fruits and vegetable against resistant *A. baumannii*. Our findings revealed a strong interaction of tested compounds with target site in in silico analysis. further, it was observed that key resistance mechanism involved in these strains was efflux pump. All tested compounds were able to present significant inhibition of bacteria and biofilm formation that was attributed to efflux pump, since no antiquorum sensing activity was reported. It was therefore concluded that our tested compounds can be employed for treatment of *A. baumanii* infections.

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 this paper.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs; Research Chair of Doping. Authors wish to thanks researchers supporting project Number (PNURSP2022R30) at King Saud University Riyadh Saudi Arabia.

Authors' contributions

A.A. designed and supervised the project, whereas methodology was written by A.F. Formal analysis L.M., AN and S.A and validation of results was conducted by A.A., S.Y.A and N.A Software analysis was performed by A.N. and L.M. Original draft preparation was written by L.M. and A.A. whereas final review and editing was performed by A.A and A.F. All authors contributed to the article and approved the submitted version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103743.

References

Abdar, M.H., Taheri-Kalani, M., Taheri, K., Emadi, B., Hasanzadeh, A., Sedighi, A., Sedighi, M., 2019. Prevalence of extended-spectrum beta-lactamase genes in *Acinetobacter baumannii* strains isolated from nosocomial infections in Tehran, Iran. GMS Hyg. Infec Control. 14.

Accelrys Discovery Studio (2012) Inc, A. S. Discovery studio modeling environment, release 3.5. Accelrys Software Inc, San Diego 2012.

Álvarez-Martínez, F.J., Barrajón-Catalán, E., Encinar, J.A., Rodríguez-Díaz, J.C., Micol, V., 2020. Antimicrobial Capacity of Plant Polyphenols against Gram-positive Bacteria: A Comprehensive Review. Curr. Med. Chem. 27, 2576–2606.

Alves, S., Duarte, A., Sousa, S., Domingues, F.C., 2016. Study of the major essential oil compounds of *Coriandrum sativum* against *Acinetobacter baumannii* and the effect of linalool on adhesion, biofilms and quorum sensing. Biofouling. 32 (2), 155–165.

Amudhan, S.M., Sekar, U., Arunagiri, K., Sekar, B., 2011. OXA beta-lactamase mediated carbapenem resistance in *Acinetobacter baumannii*. Ind. J. Med. Microbiol. 29 (3), 269–274.

Anbazhagan, P.V., Thavitiki, P.R., Varra, M., Annamalai, L., Putturu, R., Lakkineni, V.R., Pesingi, P.K., 2019. Evaluation of efflux pump activity of multidrug-resistant Salmonella Typhimurium isolated from poultry wet markets in India. Infect. Drug. Resist. 6 (12), 1081–1088.

Babu, K.S., Babu, T.H., Srinivas, P.V., Sastry, B.S., Kishore, K.H., Murty, U.S.N., 2005. Synthesis and in vitro study of novel 7-O-acyl derivatives of oroxylin A as antibacterial agents. Bioorg. Med. Chem. Lett. 15, 3953–3956.

Bazargani, M.M., Rohloff, J., 2016. Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food. Control. 61, 156–164.

Bhargava, N., Sharma, P., Capalash, N., 2010. Quorum sensing in Acinetobacter: an emerging pathogen. Critic. Rev. Microbiol. 36 (4), 349–360.

Borges, A., Serra, S., Cristina Abreu, A., Saavedra, M.J., Salgado, A., Simões, M., 2014. Evaluation of the effects of selected phytochemicals on quorum sensing inhibition and in vitro cytotoxicity. Biofouling 30 (2), 183–195.

Cushnie, T.P.T., Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrob. Agents. 38 (2), 99–107.

Daglia, M., 2012. Polyphenols as antimicrobial agents. Curr. Opin. Biotechnol. 23 (2), 174–181.

Dijkshoorn, L., Nemec, A., 2008. The diversity of the genus Acinetobacter. In: Gerischer, U. (Ed.), Acinetobacter Molecular Biology. 1st ed. Caister Academic Press, Norwich, UK, pp. 1–34.

Draughn, G.L., Milton, M.E., Feldmann, E.A., Bobay, B.G., Roth, B.M., Olson, A.L., Thompson, R.J., Actis, L.A., Davies, C., Cavanagh, J., 2018. The structure of the

biofilm-controlling response regulator BfmR from Acinetobacter baumannii reveals details of its DNA-binding mechanism. J. Mol. Boil. 430 (6), 806–821.
Farrow III, J.M., Wells, G., Pesci, E.C., 2018. Desiccation tolerance in *Acinetobacter baumannii* is mediated by the two-component response regulator BfmR. PloS One. 13, (10) e0205638.
Ferreira, S., Silva, F., Queiroz, J.A., Oleastro, M., Domingues, F.C., 2014. Resveratrol against Arcobacter butzleri and Arcobacter cryaerophilus: activity and effect on cellular functions. Int. J. Food. Microbiol. 180, 62–68.
Flemming, H.C., Neu, T.R., Wozniak, D.J., 2007. The EPS matrix: the "house of biofilm cells". J. Bacteriol. 189 (22), 7945–7947.
Fraga, C.G., Croft, K.D., Kennedy, D.O., Tomás-Barberán, F.A., 2019. The effects of polyphenols and other bioactives on human health. Food Funct. 10 (2), 514–528.
Handa, D., Pandey, A., Asthana, A.K., Rawat, A., Handa, S., Thakuria, B., 2013. Evaluation of phenotypic tests for the detection of AmpC beta-lactamase in clinical isolates of *Escherichia coli*. Ind. J. Pathol. Microbiol. 56 (2), 135–138.
Irfan, S., Zafar, A., Guhar, D., Ahsan, T., Hasan, R., 2008. Metallo-β-lactamaseproducing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital. Ind. J. Med. Microbiol. 26 (3), 243–245.
Kahkeshani, N., Farzaei, F., Fotouhi, M., Alavi, S.S., Bahramsoltani, R., Naseri, R., Bishayee, A., 2019. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. Iran. J. Basic. Med. Sci. 22 (3), 225.
Khameneh, B., Iranshahy, M., Soheili, V., Bazzaz, F.B.S., 2019. Review on plant antimicrobials: a mechanistic vie wpoint. Antimicrob. Resist Infect Control. 8, 118.
Klancnik, A., Sikic Pogacar, M., Trost, K., Tusek Znidaric, M., Mozetic Vodopivec, B., Smole, M.S., 2017. Anti-campylobacter activity of resveratrol and an extract from waste pinot noir grape skins and seeds, and resistance of camp. Jejuni planktonic and biofilm cells, mediated via the CmeABC efflux pump. J. Appl. Microbiol. 122 (1), 65–77.
Koh, K.H., Tham, F.Y., 2011. Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. Journal of Microbiology, Immun. Infect. 44 (2), 144–148.
Litake, G.M., Ghole, V.S., Niphadkar, K.B., Joshi, S.G., 2015. Phenotypicb ESBL detection in Acinetobacter baumannii: a real challenge. Americ. J. Infect. Dis. 11 (3), 48.
Liu, J., Du, C., Beaman, H.T., Monroe, M.B.B., 2020. Characterization of Phenolic Acid Antimicrobial and Antioxidant Structure-Property Relationships. Pharmaceutics. 12 (5), 419.
Manchanda, V., Sanchaita, S., Singh, N.P., 2010. Multidrug resistant *acinetobacter*. J. Global Infect. Dis. 2 (3), 291.
Manso, T., Lores, M., de Miguel, T., 2021. Antimicrobial Activity of Polyphenols and Natural Polyphenolic Extracts on Clinical Isolates. Antibiotics (Basel) 11 (1), 46.
Martins, A., Machado, L., Costa, S., Cerca, P., Spengler, G., Viveiros, M., Amaral, L., 2011. Role of calcium in the efflux system of *Escherichia coli*. Int. J. Antimicrob. Agent. 37 (5), 410–414.
McConnell, M.J., Actis, L., Pachón, J., 2013. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. FEMS microbiology reviews. 37 (2), 130–155.
Morgan, J.L., McNamara, J.T., Zimmer, J., 2014. Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP. Nat. Struct. Mol. Biol. 21 (5), 489–496.
Natella, F., Nardini, M., Di Felice, M., Scaccini, C., 1999. Benzoic and cinnamic acid derivatives as antioxidants: Structure-activity relation. J. Agric. Food Chem. 47, 1453–1459.
Peleg, A.Y., Seifert, H., Paterson, D.L., 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21 (3), 538–582.
Prashanth, K., Vasanth, T., Saranathan, R., Makki, A.R., Pagal, S., 2012. Antibiotic resistance, biofilms and quorum sensing in *Acinetobacter* species. Antibiotic resistant bacteria: a continuous challenge in the new millennium., 179–212
Rafiee, R., Eftekhar, F., Tabatabaei, S.A., Tehrani, D.M., 2014. Prevalence of extended-spectrum and metallo β-lactamase production in AmpC β- lactamase producing Pseudomonas aeruginosa isolates from burns. Jundishapur J. Microbiol. 7 (9), e16436.
Rama, J.L.R., Mallo, N., Biddau, M., Fernandes, F., de Miguel, T., Sheiner, L., Choupina, A., Lores, M., 2021. Exploring the powerful phytoarsenal of white grape marc against bacteria and parasites causing significant diseases. Environ. Sci. Pollut. Res. Int. 28, 24270–24278.
Raorane, C.J., Lee, J.H., Kim, Y.G., Rajasekharan, S.K., García-Contreras, R., Lee, J., 2019. Antibiofilm and antivirulence efficacies of flavonoids and curcumin against *Acinetobacter baumannii*. Front. Microbiol 10, 990.
Richmond, G.E., Evans, L.P., Anderson, M.J., Wand, M.E., Bonney, L.C., Ivens, A., Chua, K.L., Webber, M.A., Sutton, J.M., Peterson, M.L., Piddock, L.J., 2016. The *Acinetobacter baumannii* two-component system AdeRS regulates genes required for multidrug efflux, biofilm formation, and virulence in a strain-specific manner. MBio. 7 (2), e00430–e516.
Roy, R., Tiwari, M., Donelli, G., Tiwari, V., 2018. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence 9 (1), 522–554.
Russo, T.A., Manohar, A., Beanan, J.M., Olson, R., MacDonald, U., Graham, J., Umland, T.C., 2016. The response regulator BfmR is a potential drug target for Acinetobacter baumannii. MSphere 1 (3), e00082–e116.
Saha, R., Jain, S., Kaur, I.R., 2010. Metallo beta-lactamase producing pseudomonas species–a major cause of concern among hospital associated urinary tract infection. J. Ind. Med. Assoc. 108 (6), 344–348.
Sato, H., Chuang, V.T.G., Yamasaki, K., Yamaotsu, N., Watanabe, H., Nagumo, K., Maruyama, T., 2014. Differential effects of methoxy group on the interaction of curcuminoids with two major ligand binding sites of human serum albumin. PLoS One 9, (2) e87919.
Sengstock, D.M., Thyagarajan, R., Apalara, J., Mira, A., Chopra, T., Kaye, K.S., 2010. Multidrug-resistant *Acinetobacter baumannii*: an emerging pathogen among older adults in community hospitals and nursing homes. Clin. Infect. Dis. 50 (12), 1611–1616.
Simões, M., Simões, L.C., Vieira, M.J., 2010. A review of current and emergent biofilm control strategies. LWT-Food. Sci. Technol. 43 (4), 573–583.
Singh, S., Singh, S.K., Chowdhury, I., Singh, R., 2017. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. Open Microbiol. J. 28 (11), 53–62. https://doi.org/10.2174/1874285801711010053.
Stefaniu, A., Pirvu, L., Albu, B., Pintilie, L., 2020. Molecular docking study on several benzoic acid derivatives against SARS-CoV-2. Molecules 25 (24), 5828.
Takai, K.K., Kibe, T., Donigian, J.R., Frescas, D., De Lange, T., 2011. Telomere protection by TPP1/POT1 requires tethering to TIN2. Mol. Cell 44 (4), 647–659.
Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput Chem. 31 (2), 455–461.
Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45 (12), 2615–2623.
Viveiros, M., Martins, A., Paixão, L., Rodrigues, L., Martins, M., Couto, I., Fähnrich, E., Kern, W.V., Amaral, L., 2008. Demonstration of intrinsic efflux activity of Escherichia coli K-12 AG100 by an automated ethidium bromide method. Int. J. Antimicrob. Agent. 31 (5), 458–462.
Wayne, P.A., 2010. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document, M100–M120.
Weseler, A.H.K.R., Geiss, H.K., Saller, R., Reichling, J.J.D.P., 2005. A novel colorimetric broth microdilution method to determine the minimum inhibitory concentration (MIC) of antibiotics and essential oils against Helicobacter pylori. Pharmazie 60 (7), 498–502.
Yousefi, S., Farajnia, S., Nahaei, M.R., Akhi, M.T., Ghotaslou, R., Soroush, M.H., Jazani, N.H., 2010. Detection of metallo-β-lactamase–encoding genes among clinical isolates of *Pseudomonas aeruginosa* in northwest of Iran. Diagnostic microbiology and infectious disease 68 (3), 322–325.
Zhanel, G.G., Lawson, C.D., Adam, H., Schweizer, F., Zelenitsky, S., Lagacé-Wiens, P.R. S., Denisuik, A., Rubinstein, E., Gin, A.S., Hoban, D.J., Lynch, J.P., Karlowsky, J.A., 2013. Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination. Drug. 73 (2), 159–177.
Zhang, N., Liu, W., Qian, K., 2020. In-Vitro antibacterial effect of tea polyphenols combined with common antibiotics on multidrug-resistant Klebsiella pneumoniae. Minerva Med. 111, 536–543.

Further Reading

Borges, A., Serra, S., Cristina Abreu, A., Saavedra, M.J., Salgado, A., Simões, M., 2014. Evaluation of the effects of selected phytochemicals on quorum sensing inhibition and in vitro cytotoxicity. Biofouling. 30 (2), 183–195.
Dijkshoorn, L., Nemec, A. 2010. The diversity of the genus Acinetobacter. Acinetobacter molecular microbiology 2008, 1-34. de Benedictis, F. M., Sengstock, D. M., Thyagarajan, R., Apalara, J., Mira, A., Chopra, T., Kaye, K. S. Multidrug-resistant *Acinetobacter baumannii*: an emerging pathogen among older adults in community hospitals and nursing homes. Clin. Infect. Dis. 50 (12), 1611-1616.
Duarte, A.F., Ferreira, S., Oliveira, R., Domingues, F.C., 2013. Effect of coriander oil (Coriandrum sativum) on planktonic and biofilm cells of Acinetobacter baumannii. Nat. Prod. Commun. 8, (5) 1934578X1300800532.
Karageorgopoulos, D.E., Falagas, M.E., 2008. Current control and treatment of multidrug-resistant *Acinetobacter baumannii* infections. The Lancet. Inf. Dis 8 (12), 751–762.
Khatoon, A., Rizvi, M., Sultan, A., Khan, F., Sharma, M., Shukla, I., Khan, H.M., 2015. Chronic suppurative otitis media: a clinico-microbiological menace. Int. J. Res. Med. Sci. 3 (8), 1932–1936.
Lokhande, K.B., Pawar, S.V., Madkaiker, S., Nawani, N., Venkateswara, S.K., Ghosh, P., 2023. High throughput virtual screening and molecular dynamics simulation analysis of phytomolecules against BfmR of Acinetobacter baumannii: anti-virulent drug development campaign. J. Biomol. Struc. Dynamic. 41 (7), 2698–2712.
Pitout, J.D., 2010. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. Drugs 70 (3), 313–333.
Suresh Babu, K., Hari Babu, T., Srinivas, P.V., Sastry, B.S., Hara Kishore, K., Murty, U.S. N., Madhusudana Rao, J., 2005. Synthesis and in vitro study of novel 7-O-acyl derivatives of oroxylin A as antibacterial agents. Bioorg. Med. Chem. Lett. 15 (17), 3953–3956.
Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput Chem. 31 (2), 455–461.
Vila, J., Pachón, J., 2008. Therapeutic options for *Acinetobacter baumannii* infections. Expert. Opinion. Pharmacother. 9 (4), 587–599.