ORIGINAL ARTICLE

Molecular and insilico Screening of RND-Type Efflux Pump *oprJ* Gene in Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Burn Patients

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Key words:
Efflux pumps inhibitor, OPRJ gene, Pseudomonas aeruginosa, Molecular docking

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ABSTRACT

Background: *The oprJ gene in Pseudomonas aeruginosa encodes a component of the MexCD-OprJ efflux pump, contributing to multidrug resistance.* ***Objective:*** *This study aimed to perform molecular and in silico analyses of oprJ, investigate mutations in antibiotic-resistant strains, examine the relationship between amino acid changes and resistance, determine the three-dimensional efflux pump structure, and identify potential inhibitors.* ***Methodology:*** *A total of 140 burn patient swabs were collected, identified, and tested for antimicrobial susceptibility using the VITEK 2 system. DNA from isolates was extracted, amplified by PCR, sequenced by Macrogen (Korea), translated into proteins, and analyzed via bioinformatics to model 3D structures and identify potential efflux pump inhibitors.* ***Results:*** *Out 140 specimens, 118 (84.3%) were culture-positive, including 60 (50.8%) P. aeruginosa, while 22 (15.7%) showed no growth. Isolates were susceptible to colistin (78%). The oprJ gene was detected in 30 isolates (50%), showing 98% sequence and 99% protein similarity to global strains. Structural analysis revealed leucine-to-methionine mutation at position 317. Molecular docking identified natural and synthetic compounds with strong efflux pump binding, evaluated by Lipinski's Rule of Five. Statistical analysis indicated significant correlations between mutations and antibiotic resistance ($P \leq 0.05$, $P \leq 0.01$).* ***Conclusion:*** *P. aeruginosa was the predominant pathogen, with Marked resistance to antibiotics, Mutations included transversion and transition substitutions, causing amino acid changes (Tyrosine-to-Phenylalanine at P415, P520; Leucine-to-Methionine at 467; Leucine-to-Glutamine at 535; Tyrosine-to-Cysteine at 441). Conserved and variable residues were observed, and molecular docking suggested that curcumin, palmatine, berberine, and PAβN could serve as potential efflux pump inhibitors.*

INTRODUCTION

Pseudomonas aeruginosa is among the most frequently isolated pathogens from burn patients worldwide[1]. As an opportunistic bacterium, it is commonly linked to healthcare-associated infections, including those in intensive care units (ICUs), ventilator-associated pneumonia (VAP), surgical site infections, and burn wounds[2]. *P. aeruginosa* exhibits resistance to a wide range of antibiotics through multiple mechanisms, including intrinsic, acquired, and adaptive strategies. Its intrinsic resistance is largely attributed to multidrug efflux pumps, reduced outer membrane permeability, and the production of antibiotic-inactivating enzymes[3,4].

Active efflux pumps are a key resistance mechanism in *P. aeruginosa*, which expelling antibiotics before they reach their targets. In Gram-negative bacteria, these multidrug pumps span both inner and outer membranes[5].The OprJ protein forms part of the MexCD-OprJ efflux pump, a Resistance-Nodulation-Division (RND) system that extrudes antimicrobial agents and contributes to resistance. MexCD-OprJ is one of four major RND pumps in *P. aeruginosa*, along with MexAB-OprM, MexXY, and MexEF-OprN[6].

MexCD-OprJ is mainly associated with resistance to fluoroquinolones such as levofloxacin and ciprofloxacin[7,8], but can also extrude other antimicrobial agents such as macrolides, novobiocin, zwitterionic cephalosporins, such as cefepime and cefpirome[9], tetracyclines, chloramphenicol[10], β-lactams Cefepime and other later-generation β-lactams[11]. and the biocide chlorhexidine[12]. However, strains that overexpress MexCD-OprJ commonly show greater susceptibility to aminoglycosides and some β-lactams[13].

Therefore, it is crucial to the role vital of efflux pump inhibitors (EPIs) for restoring antibiotic efficacy by increasing intracellular drug concentration. Inhibition can occur through various mechanisms, including

disruption of regulatory pathways, chemical modification of antibiotics to evade recognition, prevention of pump assembly, competitive or non-competitive inhibition of antibiotic binding, blockage of outer membrane channels, and interference with the pump's energy supply[14,15]. The present study aimed to perform molecular and in silico analyses of *oprJ*, investigate mutations in antibiotic-resistant strains, examine the relationship between amino acid changes and resistance, determine the three-dimensional efflux pump structure, and identify potential inhibitors.

METHODOLOGY

Study design:

The present study was conducted in the laboratory of Imam Al-Sadiq Hospital in Babylon, Iraq. and DNA laboratory at the University of Babylon's College of Sciences' Biology Department, Babylon, Iraq. A total of 140 samples were collected from two different hospitals The burn unit at Imam Al-Sadiq Hospital in Babylon, and the burn Center at Al-Hussein Teaching Hospital in Karbala during the period from November 2023 to March 2024, the clinical samples were collected from infected wound sit and labelled with patients' information and transported to the laboratory immediately and cultured on blood, MacConkey, and Cetrimide agars incubate 24hours at 37C°.The isolates *P. aeruginosa* were subjected to antibiotic susceptibility testing using the Vitek2 Compact System with the AST-GN222 card examined against 16 antibiotics agents related to 7 antibiotics classes[27].

DNA extraction and PCR technique:

DNA from pure colonies of *P. aeruginosa* were extracted according to the protocol of the manufacturing company (Favorgen, Biotech, Taiwan.) PCR was performed on all *P. aeruginosa* (60 isolates) by using specific primers as illustrated table 1. All isolates were successfully sequenced by DNA Macrogen, Korea. And then it was aligned with a number of other samples from different countries of the world through the Gene Bank. by using the program BLAST Nucleotide in site National Center Biotechnology Information (NCBI).

Bioinformatics Programs and Websites:

Programs and websites used in the current study are shown in table 2.

Table 1: Primers used in present study.

Gene	Primer	Product Size (bp)	Annealing Temperature (◦C)
OprJ	**F** CTGCCGCCTCGATGTACC **R** GTATCGGCGCTGCTGATCG	**1412**	**55**

Table 2: Programs and websites with purpose of usage.

Program or website	Purpose of use
Nucleotides BLAST	Determine sequence similarity and phylogenetic distances
BLAST X	Translate polynucleotides to polypeptide
MEGA 11	Alignment of sample sequence
Phyre 2	Build 3D structure form protein
UCSF Chimera	Make protein picture with some actions
Molecular docking	Shows the intensity binding of the inhibitor to the protein.

Statistical analysis: was conducted with SPSS v21 using chi-square tests to assess correlations between amino acid sequence mutations and antibiotic resistance, with significance set at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

One hundred eighteen (84.28%) showed positive culture results. Sixten (50.84%) was identified as suspected *P. aeruginosa* the remaining isolates included various Gram-positive and Gram-negative bacteria, while 22 samples (15.71%) showed no growth. As presented in Table3.

The most prevalent bacteria were *P. aeruginosa* (50.84%), followed by *Klebsiella* spp. (6.77%), *Acinetobacter* spp (5.93%), *E. coli*. (2.54%), and *Proteus* spp. (1.69%) figure 1.

Antibiotic susceptibility testing (AST):

All of *P. aeruginosa* were subjected to antibiotic susceptibility testing using the Vitek2 Compact System with the AST-GN222 card. All isolates were examined against 16 antibiotics agents related to 7 antibiotics classes table 4.

Table 3: Number and percentage of positive and negative bacterial growth.

Sample source	Total number	Bacterial growth		
		No growth	Gram- positive and negative isolates	*P. aeruginosa*
Burns	**140**	**22(15.71%)**	**118 (84.28%)**	**60 (50.84%)**

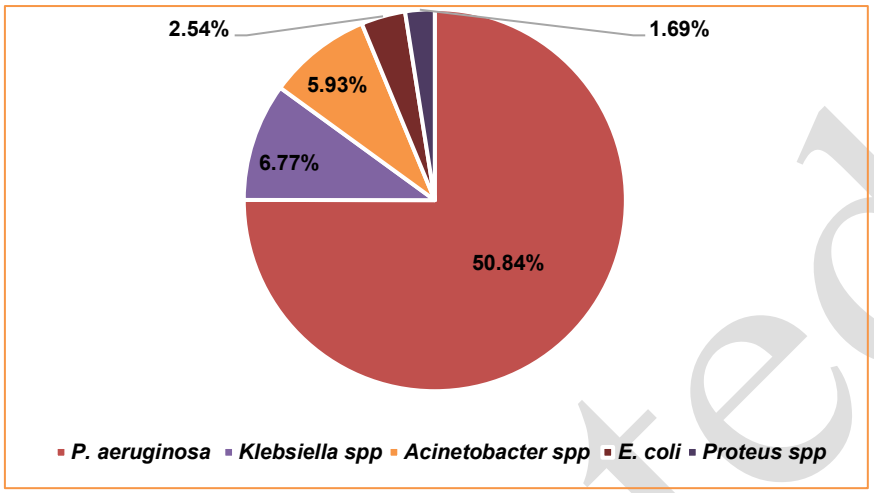

Fig. 1: The percentage of Gram-negative bacterial isolates from burned patients.

Table 4: Antibiotics susceptibility test of *P. aeruginosa* isolates.

Antibiotics classes	Antibiotics	Susceptible (S) NO. of Isolates (%)	Intermediate (I) NO. of Isolates (%)	Resistant (R) NO. of Isolates (%)
Penicillins	Piperacillin PIP	14(23)	0	46(76)
	Ticarcillin TIC	16(26)	0	44(73)
	Ticarcillin/Clavulanic Acid TCC	18(30)	0	42(70)
	Piperacillin/Tazobactam TZP	19(31)	0	41(68)
	Amoxicillin \ Clavulanic acid AUG	0	0	60(100)
Cephalosporin	Cefepime FEP	19(31)	3(5)	38(63)
	Cefoxitin CX	0	0	60(100)
	Ceftazidime CAZ	19(31)	2(3)	39(65)
Carbapenem	Imipenem IPM	17(28)	0	43(71)
	Meropenem MEM	16(26)	2(3)	42(70)
monobactam	Aztreonam ATM	15(25)	2(3)	43(71)
Aminoglycoside	Gentamicin GM	20(33)	2(3)	38(63)
	Amikacin AN	22(36)	0	39(65)
	Tobramycin TM	21(35%)	0	39(65)
Quinolone	Ciprofloxacin CIP	19(31)	0	41(68)
Polypeptides	Colistin CS	47(78)	2(3)	11(18)

Amplification of *OprJ* gene from *P. aeruginosa*:

PCR was performed to *P. aeruginosa* isolates using specific primers. The study detected the *OprJ* gene in 30 isolates (50%) from burn patients. The PCR results were compared with a molecular weight marker (DNA ladder) using electrophoresis figure 2.

DNA sequencing analysis:

In the present study, DNA sequencing was performed on 30 *OprJ* gene isolates. Comparative analysis revealed that some isolates have 98% similarity with global reference strains, with variations arising from single-nucleotide substitutions: adenine replacing thymine at one site; adenine to guanine at three sites; adenine to cytosine at one site; thymine to guanine at one site; and cytosine to guanine at one site figure 3. While Polypeptide alignment showed 99% amino acid identity with global strains, with a single substitution of alanine (Ala) by serine (Ser) figure 4.

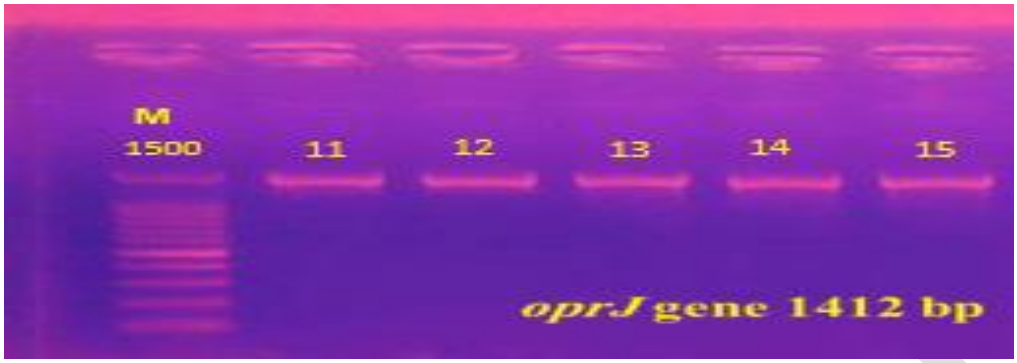

Fig. 2: PCR amplification products stained with dye of ethidium bromide for *oprJ* gene of *P. aeruginosa* isolates. the size of the PCR product is 1412 bp, the gel was 1.5%, V: 90, Time: 45 minutes, M: DNA ladder only isolates.

```
>Pseudomonas aeruginosa strain 2021CK-01315 chromosome, complete genome
Sequence ID: CP124667.1 Length: 6596513
Range 1: 5482008 to 5482821

Score:1421 bits(769), Expect:0.0,
Identities:800/815(98%),  Gaps:1/815(0%), Strand: Plus/Plus

Query  109      GCGTGCCTAGGCCAGCTTCAGGGCTTCGTTGCTGCTGTTCGCCAGGGCGCGCAGGGCCTT  168
                ||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482008  GCGTGCCTTGGCCAGCTTCAGGGCTTCGTTGCTGCTGTTCGCCAGGGCGCGCAGGGCCTT  5482067

Query  169      CTCTTCGCGACGCAGGGTATCGCTGGCCGCCAGGGCGTCGGCGACCTCGCGGAAAGCGGT  228
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482068  CTCTTCGCGACGCAGGGTATCGCTGGCCGCCAGGGCGTCGGCGACCTCGCGGAAAGCGGT  5482127

Query  229      CTGGATGGTCCCCTCGTAGGCGGCCACCGCCGAATCCTTGCGCGCCTCGGCCAGGCTCAG  288
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482128  CTGGATGGTCCCCTCGTAGGCGGCCACCGCCGAATCCTTGCGCGCCTCGGCCAGGCTCAG  5482187

Query  289      GTTGGCACGGTTGCGCCCGCCATCGAAGATCGGCAGCGTCAGCGTCGGCAGGAAGCTCCA  348
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482188  GTTGGCACGGTTGCGCCCGCCATCGAAGATCGGCAGCGTCAGCGTCGGCAGGAAGCTCCA  5482247

Query  349      GGAGCGCGAGCCGCCATCGAACAGGCCGGACATTTCCGCGCTGGAGGTGCCGAAGCTGCC  408
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482248  GGAGCGCGAGCCGCCATCGAACAGGCCGGACATTTCCGCGCTGGAGGTGCCGAAGCTGCC  5482307

Query  409      GGTCAGGCTGATGCGCGGGAAGAAcgccgcgcgcgccgcgccgatatccgcgttgcgcgc  468
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482308  GGTCAGGCTGATGCGCGGGAAGAACGCCGCGCGCGCCGCGCCGATATCCGCGTTGCGCGC  5482367

Query  649      CAGGTTGCGCTCCTGCTCGGCGCGCGCCTGCTCCACCAGGCCAAGGGCTTCCTGGTAGTC  708
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482548  CAGGTTGCGCTCCTGCTCGGCGCGCGCCTGCTCCACCAGGCCAAGGGCTTCCTGGTAGTC  5482607

Query  709      CAGCGCGGTGGCGGCGCCAGACGCGCGGCGCTGGTCGATCAGGGAGAAGGAATACTCACG  768
                |||||||||||||||| | |||||||||||||||||||| |||||||||| || ||||||
Sbjct  5482608  CAGCGCGGTGGCGGCGCCGGCCGCGCGGCGCTGGTCGATCAGGGCGAAGGAATACTCGCG  5482667

Query  769      GCTGACCATGGTCTGACCAGATCAGCGCCAGGCGCCGCAGGGCGCCGTCATAGCTCAGGT  828
                |||||||| ||||||||| | ||||||||||||||||||||||||||||||||||||||
Sbjct  5482668  GCTGACCAGGGTCTGACCGG-TCAGCGCCAGGCGCCGCAGGGCGCCGTCATAGCTCAGGT  5482726

Query  829      AGGCCTGGCTGACCTCGGCGACCAGGGCGATCCGTGCCGCGCGCGCTGCCTCCTCGCTAA  888
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5482727  AGGCCTGGCTGACCTCGGCGACCAGGGCGATCCGTGCCGCGCGCGCTGCCTCCTCGCTGG  5482786

Query  889      ACATGTACTGTTGCAGGGCTGCGTCGGTCACGCTC  923
                 || |||||||||||||||||||||||| |||||
Sbjct  5482787  CCAGGTACTGTTGCAGGGCTGCGTCGGTCAGGCTC  5482821
```

Fig. 3: Alignment of nucleotides *OprJ* gene (Query) for *P. aeruginosa* by nucleotide BLAST Which shows 98 % identities with global isolates (Subject).

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>multidrug efflux transporter outer membrane subunit OprJ [Pseudomonas aeruginosa]
Sequence ID: HCL3709806.1 Length: 167
Range 1: 68 to 155

Score:172 bits(435), Expect:6e-49,
Method:Compositional matrix adjust.,
Identities:87/88(99%), Positives:88/88(100%), Gaps:0/88(0%)

Query  88   GVALDNNRSLRQTLLDIEAARAQYRIQRADRVPGLNAAATGNRQRQPADLSAGNRSEVAS  267
            GVALDNNRSLRQTLLDIEAARAQYRIQRADRVPGLNAAATGNRQRQPADLSAGNRSEVAS
Sbjct  68   GVALDNNRSLRQTLLDIEAARAQYRIQRADRVPGLNAAATGNRQRQPADLSAGNRSEVAS  127

Query  268  SYQVGLALPEYELDLFGRVKSLTDASLQ  351
            SYQVGLALPEYELDLFGRVKSLTDA+LQ
Sbjct  128  SYQVGLALPEYELDLFGRVKSLTDAALQ  155
```

Fig. 4: Polypeptide sequence alignment for *Oprj* gene (Query) Shows 99% match with global isolates. (Sbjct).

Multiple sequence alignment of *OprJ* gene:

The current study showed multiple alignment of DNA for *oprJ* gene Between local isolate reveal high number of mutation Fig 5. The number of mutation present were affected in changing coding protein such as GCT\GTT (Alanine to valine) While changing GCC\TGC (Alanine to Cysteine) another location showed change CAG\CTG (Glutamine to Leucine) CTC\GCC (Leucine to Alanine) last location CTG\AGG (Leucine to Arginine).

The present study shows multiple alignment of *oprJ* gene when compare local isolates with global isolates reveal high number of mutations in many of isolate while other showed high similar with global isolate for *P. aeruginosa* Fig 6. All mutations that appeared in the current study are mutations (Transversion or Transition) nitrogenous base with another base. mutations leading to changes in the genetic code, and then a change in the amino acids at the translation level. such as GCT\GTT (Alanine to valine) While changing CGC\CTG (Arginine to Leucine) another location showed change GAC\ G TT (Aspartic acid to Vaine) CCT\CGC (Proline to Arginine) last location CCT\CAG (Proline to Glutamine).

Multiple alignment of amino acid sequences to *OprJ* gene in current study showed the presence of a mutation between local isolates in sample (6,11,18) at position 467 that led to a change in amino acid (leucine to methionine), while the sample (6,11,12,18) showed mutations in another position 520 that led to a change in the amino acid (Tyrosine to phenylalanine). last position 535 in sample (11,16,18) change in amino acid (Leucine to Glutamine) Figure 7.

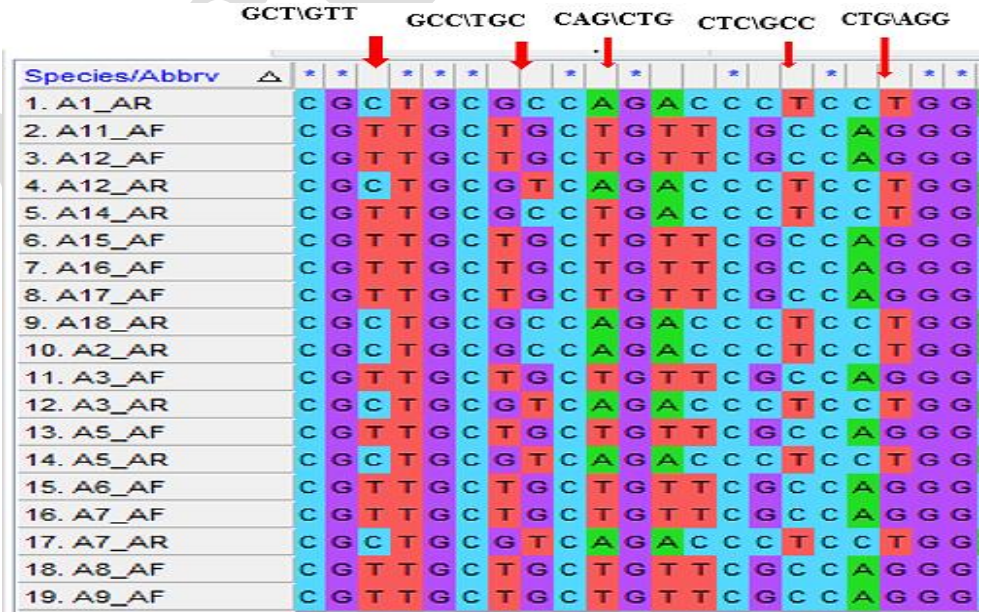

Fig. 5: Alignment of *OprJ* gene sequences of local isolates

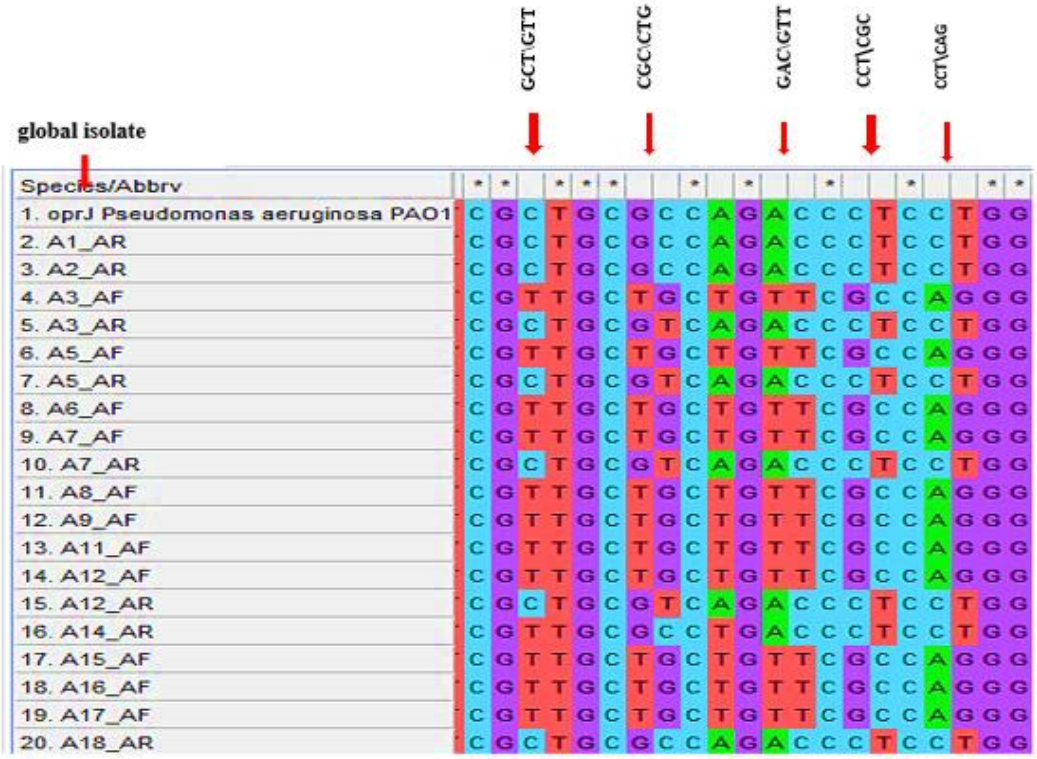

Fig. 6: Alignment of *OprJ* gene sequences of local isolates comparison with sequences of global isolates.

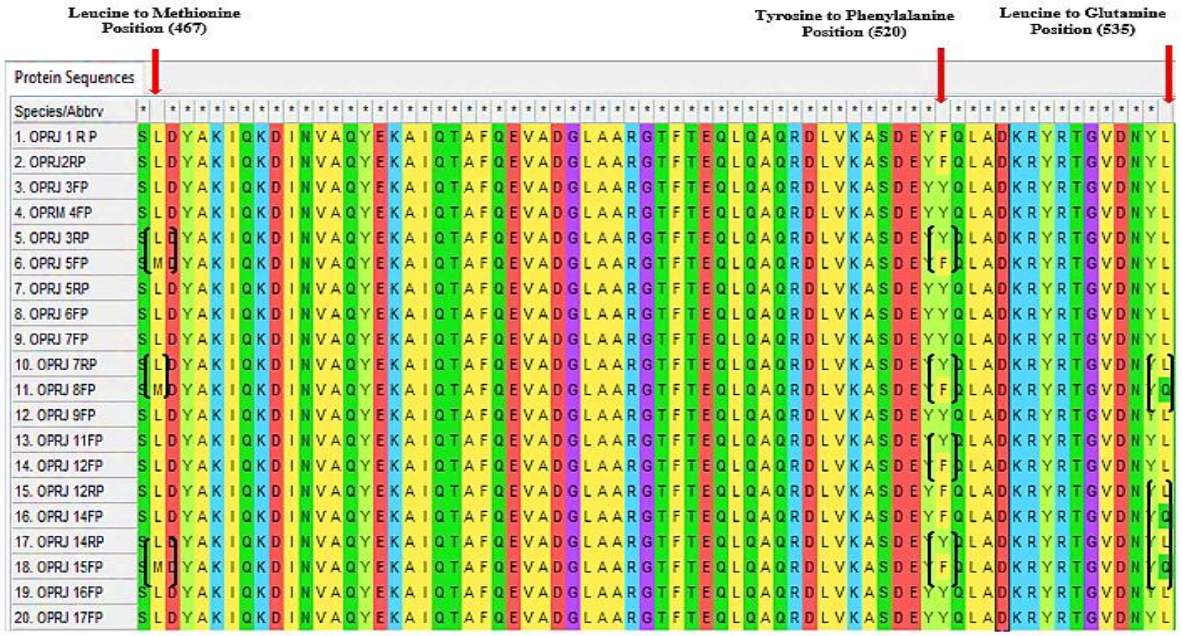

Fig. 7: multiple alignment of amino acid sequences of the *oprJ* gene for local isolates

The of local isolates results when comparing with global isolates showed the presence of mutations at position 451 for sample (2,3,7,12,15,16,17,19) led to a change in amino acid (Tyrosine to phenylalanine), another position 430 for sample (12,17,19) change in amino acid (leucine to glutamine), last position 441 for sample (14) change in amino acid (threonine to cysteine) Figure 8.

The current study showed relationship between antibiotic resistances and change amino acid in protein by used Statistical analysis significant association at ($p \leq 0.05$) and ($p \leq 0.01$) table 5.

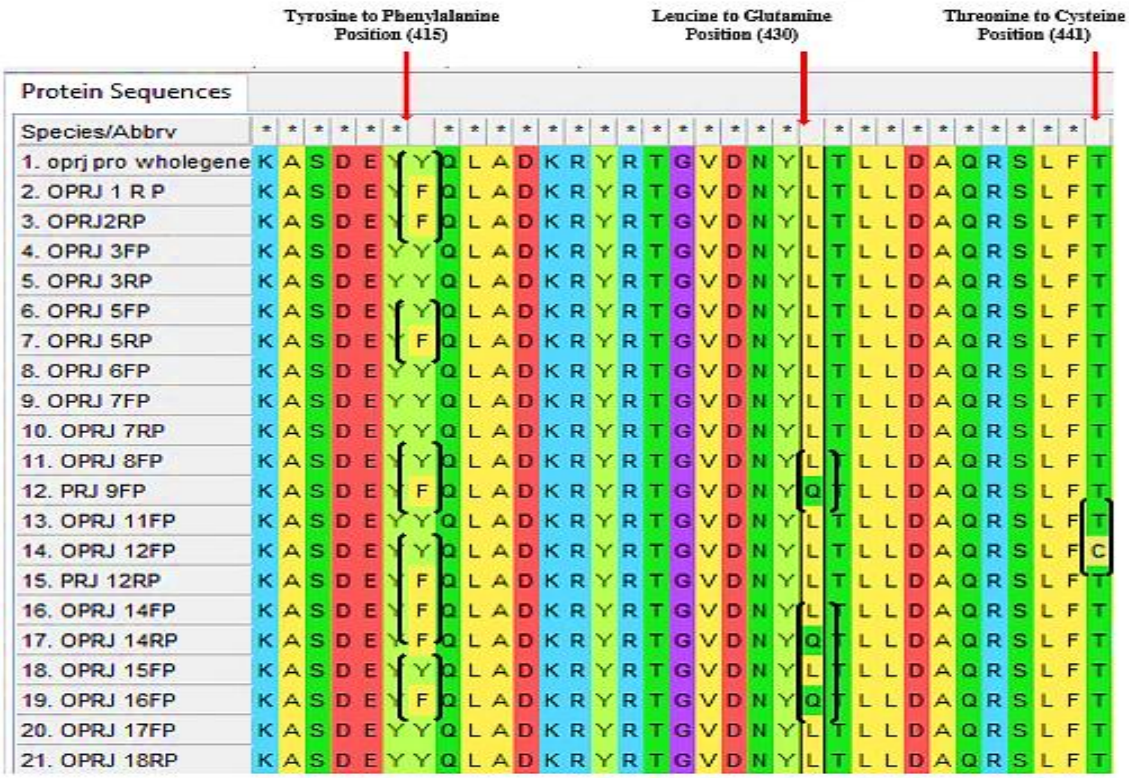

Fig.8: multiple alignment of amino acid sequences of the *oprJ* gene for local isolates comparing with global isolates.

Table 5: showed Statistical analysis between change amino acid and antibiotic resistance

CS	CIP	TM	GM	AN	ATM	IPM	MEM	CX	FEP	CAZ	AUG	TCC	TZP	TIC	PIP	Mutation & location	
0.538	1.167	1.167	1.167	1.167	0.737	0.737	0.946	39.093	0.946	0.946	39.093	0.737	0.032	0.538	0.538	χ^2	Tyrosine to Phenylalanine 415
0.463	0.280	0.280	0.280	0.280	0.391	0.391	0.623	0.0001**	0.623	0.623	0.0001**	0.391	0.857	0.463	0.463	p-valne	
0.444	0.350	0.350	0.350	0.350	0.221	0.221	0.284	39.093	0.284	0.284	39.093	0.221	0.350	0.162	0.162	χ^2	Leucine to Methionine (467)
0.801	0.554	0.554	0.554	0.554	0.638	0.638	0.868	0.0001**	0.868	0.868	0.0001**	0.638	0.554	0.688	0.688	p-valne	
1.988	0.538	0.538	0.538	0.538	0.340	0.340	0.437	39.093	0.437	0.437	39.093	0.340	0.538	0.249	0.249	χ^2	Tyrosine to Phenylalanine 520
0.370	0.463	0.463	0.463	0.463	0.560	0.560	0.804	0.0001**	0.804	0.804	0.0001**	0.560	0.463	0.618	0.618	p-valne	
1.292	0.350	0.350	0.221	0.350	0.221	0.221	0.284	39.093	0.284	0.284	39.093	0.221	0.350	0.162	0.162	χ^2	Leucine to Glutamine (535)
0.524	0.554	0.554	0.638	0.554	0.638	0.638	0.868	0.0001**	0.868	0.868	0.0001**	0.638	0.554	0.688	0.688	p-valne	
2.126	0.737	0.737	0.737	0.737	0.465	0.465	0.597	39.093	0.597	0.597	39.093	0.465	0.737	0.340	0.340	χ^2	Leucine to Glutamine (430)
0.346	0.391	0.391	0.391	0.391	0.495	0.495	0.742	0.0001**	0.742	0.742	0.0001**	0.495	0.391	0.560	0.560	p-valne	
2.285	0.171	0.171	0.171	0.171	0.108	0.108	0.138	39.093	0.138	0.138	39.093	0.108	0.171	0.079	0.079	χ^2	Threonine to Cysteine (441)
0.319	0.679	0.679	0.679	0.679	0.743	0.743	0.933	0.0001**	0.933	0.933	0.0001**	0.743	0.679	0.779	0.779	p-valne	

**** refer to significant association at (p≤0.01).**
*** Refer to significant association at (p≤0.05)**
Piperacillin **PIP**, Ticarcillin **TIC,** Piperacillin/Tazobactam **TZP**, Ticarcillin/Clavulanic Acid **TCC**, Amoxicillin \ Clavulanic acid **AUG**, Cefepime **FEP**, Cefoxitin **CX**, Ceftazidime **CAZ,** Imipenem **IPM,** Meropenem **MEM,** Aztreonam **ATM**, Gentamicin **GM**, Amikacin **AN**, Tobramycin **TM**, Ciprofloxacin **CIP**, Colistin **CS.**

The result according to table 5 showed that all significant associations ($p < 0.01$) were linked to Cefoxitin (CX), Amoxicillin-clavulanate (AUG)) This strongly supports the *OprJ* mutations in burn isolates that enhance resistance to β-lactam antibiotics, there is no statistically significant association with resistance to Colistin (CS), Carbapenems (IPM) and Aminoglycosides (GM, AN, TM). Change Tyrosine to Phenylalanine Mutations Locations: P415, P520, Significant Resistance to Cefoxitin (CX) and Aztreonam-clavulanate (AUC) Extremely high values (39.093, $p \leq 0.01$) strong resistance. these mutations may disrupt β-lactam binding sites, leading to resistance to cephalosporins (CX) and monobactams (AUC). Other mutation in current study changes amino acid Leucine to Methionine (Location 467) Significant Resistance to Cefoxitin (CX) and Aztreonam-clavulanate (AUC)with values (0.0001, $p \leq 0.01$). because Hydrophobic-to-hydrophobic substitutions (Leu→Met) in efflux pump components may enhance resistance to certain β-lactams.

Change Leucine to Glutamine (Location 535) Significant Resistance to Meropenem (MEM) and AUC (0.0001, $p \leq 0.01$). this indicates the Polar Glutamine introduction may alter membrane permeability or carbapenemase activity, explaining meropenem resistance. Last mutation changes Tyrosine to Cysteine (Location 441) Significant Resistance for MEM and AUC (0.0001, $p \leq 0.01$). because Cysteine's thiol group could disrupt disulfide bonds in porins (e.g., OprD), reducing carbapenem uptake.

Three-dimensional structure of *oprJ* gene:

The three-dimensional structure of *oprJ* gene was determined comparing local isolates with global isolate, the results showed both similarities and differences in amino acid sequence. change amino acid Leucine in global isolate change to Methionine in local isolate at position 317 figure 9.

Molecular docking of *OprJ* protein to inhibit of efflux pump in *P. aeruginosa*:

In current study, the best efflux pump inhibitor (EPI) was selected by preparing 3D structures of efflux pump proteins by using the UCSF Chimera program figure 10 shows the efflux pump in *p. aeruginosa* contain drug target and amino acid sequencing in outer membrane (RQRQPADLSAGNRSEVASSYQ) at start with amino acid argnine91, glutamine 92, arginine 93, glutamine 94, proline 95, alanine 96, aspartic acid 97, leucine 98, serine 99, alanine 100, glycine 101, asparagine 102, argnine103, serine 104, glutamic acid 105, valine106, alanine 107, serein 108,109, tyrosine 110, and end with glutamine 111. Based on the molecular docking score (delta G), the inhibitors in current study were arrangement according to their docking score and binding to the outer part of the efflux pump *Oprj* gene fore *P. aeruginosa* table 6.

The result show according to the Table 6the two compounds (Cinnamtannin B1, Dieckol,) exhibited the highest binding affinity to the efflux pump Docking score (-7.4, -7.3 Kcal / Mol). Figure11.

Then, by using the tool Property calculator available on the platform Mcule in Molecular docking to determine the **physical** and **chemical** properties of important compounds for drug pharmacokinetics within the human body and subjecting them to **Lipinski's Rule of Five** (RO5) is a set of guidelines used to evaluate the drug-likeness of a compound based on its pharmacokinetic properties, table 7.

According to table 7 Several compounds including, Conessine, curcumin, palmatine, phenyl-arginine-beta-naphthylamide (PABN), kaempferol, daidzein, and berberine—demonstrated effectiveness (total score 6) and were non-toxic for burn patients, also meeting the criteria of Lipinski's Rule of Five. molecular docking and chemical structure of these compound from the following figures 12 and 13.

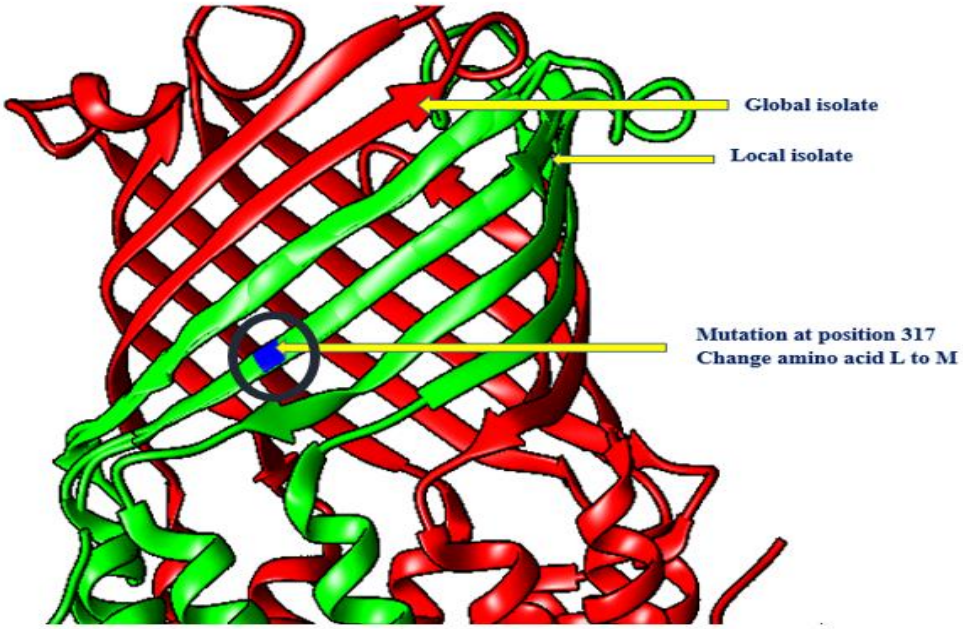

Fig.9: showed 3D structure of *OprJ* gene for local isolate (green color) and match with global isolate (red color) by used UCSF chimera program the differential between local and global isolate at position (317) blue color represent change amino acid Leucine in global isolate change to Methionine in local isolate at position 317.

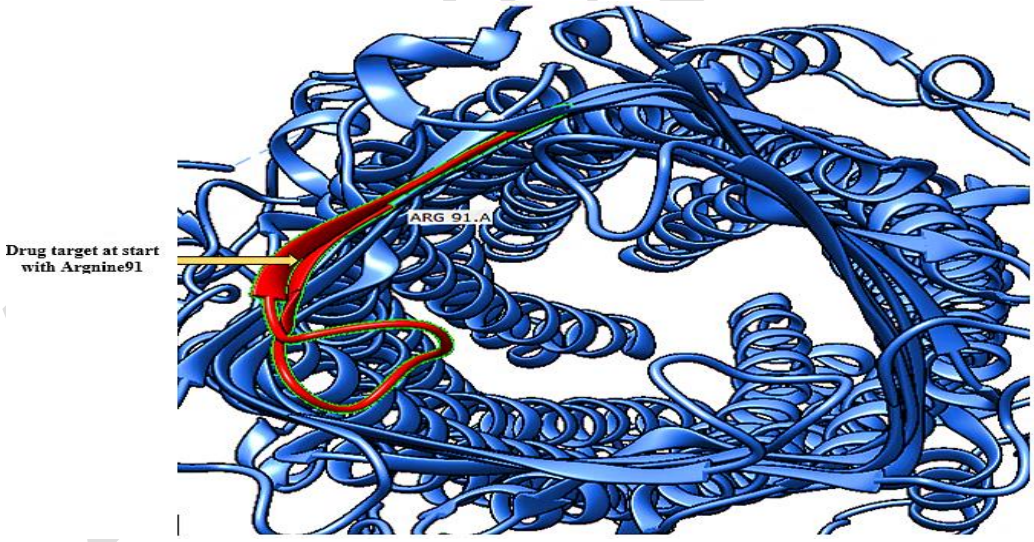

Fig.10: showed efflux pump in outer membrane (blue color) for *Oprj* gene contain drug target (red color) contained amino acid sequencing (RQRQPADLSAGNRSEVASSYQ) at start with amino acid argnine91, and end with glutamine 111.

Table 6: Inhibitors targeting *OprJ* efflux pump and their binding affinities.

NO.	Inhibitor	International Chemical Identifier (InChIKey)	Docking score Kcal/Mol
1	Cinnamtannin B1	BYSRPHRKESMCPO-LQNPQWRQSA-N	-7.4
2	Dieckol	DRZQFGYIIYNNEC-UHFFFAOYSA-N	-7.3
3	Tetrandrine	WVTKBKWTSCPRNU-KYJUHHDHSA-N	-7.1
4	Celastrol	KQJSQWZMSAGSHN-JJWQIEBTSA-N	-6.5
5	Epigallocatechin-3-gallate (EGCG)	WMBWREPUVVBILR-WIYYLYMNSA-N	-6.5
6	Uvaol	XUARCIYIVXVTAE-ZAPOICBTSA-N	-6.5
7	Tiliroside	DVGGLGXQSFURLP-VWMSDXGPSA-N	-6.3
8	Valinomycin	FCFNRCROJUBPLU-DNDCDFAISA-N	-6.3
9	α, amyrin	FSLPMRQHCOLESF-SFMCKYFRSA-N	-6.3
10	β-Amyrin	JFSHUTJDVKUMTJ-QHPUVITPSA-N	-6.3
11	Urs-12-en-28-oic acid, 3-hydroxy-, (3beta)-	WCGUUGGRBIKTOS-UHFFFAOYSA-N	-6.3
12	7-O-B-D-Glucopyranoside	WKUHPOMCLBLCOV-UHFFFAOYSA-N	-6.3
13	Pimozide	YVUQSNJEYSNKRX-UHFFFAOYSA-N	-6.2
14	Balsaminol F	WKIUWXJRQZRPNJ-HCBDOCHMSA-N	-6.1
15	Balsaminol A	AVFNYXHRDYAHNF-HEVJPHJFSA-N	-6
16	Oreganol	MFOVLHFTNQGRLH-BFMVXSJESA-N	-6
17	Olaanolic acid	MIJYXULNPSFWEK-GTOFXWBISA-N	-6
18	5′-Methoxyhydnocarpin (5′-MHC)	NIOAGUZTTGFPGK-ILBGXUMGSA-N	-6
19	Carnosol	PCMDDYKLZIZJHI-SULUTDIPSA-N	-6
20	Karavilagenin D	CUJVAMKVNDHSSR-DLGSBZDHSA-N	-5.9
21	Chlorogenic Acid	CWVRJTMFETXNAD-JUHZACGLSA-N	-5.9
22	Balsaminoside A	SIFXZLRBHSSMMW-CUSGLKLLSA-N	-5.8
23	Berberine	YBHILYKTIRIUTE-UHFFFAOYSA-N	-5.8
24	Ginsenoside 20(S)-Rh2	CKUVNOCSBYYHIS-IRFFNABBSA-N	-5.7
25	Reserpine	QEVHRUUCFGRFIF-MDEJGZGSSA-N	-5.7
26	Mpc-3100	CVBWTNHDKVVFMI-LBPRGKRZSA-N	-5.6
27	(+)-Epicatechin	PFTAWBLQPZVEMU-ZFWWWQNUSA-N	-5.6
28	Ferruginol	QXNWVJOHUAQHLM-AZUAARDMSA-N	-5.6
29	D13-9001	RVJNIKFVEAWLQC-HXUWFJFHSA-N	-5.6
30	Slupp-417	CXKARUGMFISLBH-LFYBBSHMSA-N	-5.5
31	Baicalein	FXNFHKRTJBSTCS-UHFFFAOYSA-N	-5.5
32	Orobol	IOYHCQBYQJQBSK-UHFFFAOYSA-N	-5.5
33	Amitriptyline	KRMDCWKBEZIMAB-UHFFFAOYSA-N	-5.5
34	Phenyl-arginine-beta-naphthylamide	ZLDXMOCDBCFTAJ-FQEVSTJZSA-N	-5.5
35	Galbanic acid	CVWWNYPTZYQCSE-YFBXQHAESA-N	-5.4
36	Carnosic acid	QRYRORQUOLYVBU-VBKZILBWSA-N	-5.4
37	Quercetin	REFJWTPEDVJJIY-UHFFFAOYSA-N	-5.4
38	Ellagic Acid	AFSDNFLWKVMVRB-UHFFFAOYSA-N	-5.3
39	Kaempferol	IYRMWMYZSQPJKC-UHFFFAOYSA-N	-5.3
40	Curcumin	VFLDPWHFBUODDF-FCXRPNKRSA-N	-5.3
41	Sertraline	VGKDLMBJGBXTGI-SJCJKPOMSA-N	-5.3
42	daidzein	ZQSIJRDFPHDXIC-UHFFFAOYSA-N	-5.3
43	1-Caffeoylquinic acid	GWTUHAXUUFROTF-AVXJPILUSA-N	-5.2
44	Luteolin	IQPNAANSBPBGFQ-UHFFFAOYSA-N	-5.2
45	Timcodar	MQSMWZHHUGSULF-QNGWXLTQSA-N	-5.2
46	palmatine	QUCQEUCGKKTEBI-UHFFFAOYSA-N	-5.2
47	Apigenin	KZNIFHPLKGYRTM-UHFFFAOYSA-N	-5.1
48	Cholecalciferol	QYSXJUFSXHHAJI-YRZJJWOYSA-N	-5.1
49	Resveratrol	LUKBXSAWLPMMSZ-OWOJBTEDSA-N	-5
50	Piperine	MXXWOMGUGJBKIW-YPCIICBESA-N	-5

*Docking score(Kcal / Mol) refer to the binding affinity of the inhibitory compound to the protein. (Efflux pump).

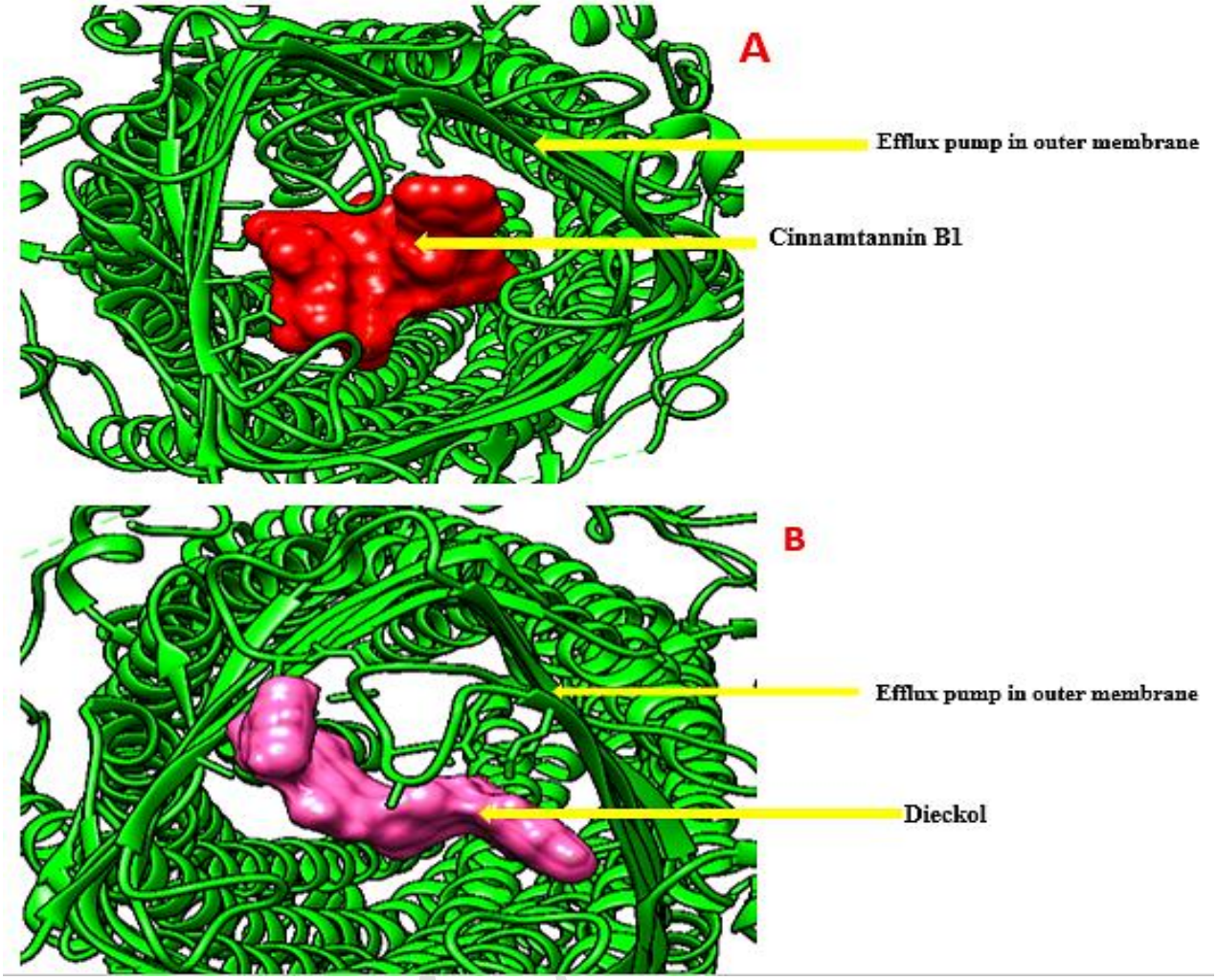

Fig.11: A\Compound Cinnamtannin B1 binding to the efflux pump protein by *Oprj* gene and Docking score (-7.4 Kcal / Mol). proteins retrieve 3D structures by used program UCSF chimera. B\ Compound Dieckol binding to the efflux pump protein by *OprJ* gene and Docking score (-7.3Kcal / Mol).

Table 7: Compounds with more likely to be oral drug.

NO.	Inhibitor	Toxicity	NO. of Rotatable bonds	Logp	M.W	H-bond Acceptor	H-bond donor	Total Score
1	Curcumin	1	1	1	1	1	1	6
2	palmatine	1	1	1	1	1	1	6
3	daidzein	1	1	1	1	1	1	6
4	Kaempferol	1	1	1	1	1	1	6
5	Berberine	1	1	1	1	1	1	6
6	Phenyl-arginine-beta-naphthylamide PABN	1	1	1	1	1	1	6
7	Ferruginol	1	1	0	1	1	1	5
8	Uvaol	1	1	0	1	1	1	5
9	α, amyrin	1	1	0	1	1	1	5
10	β-Amyrin	1	1	0	1	1	1	5
11	Urs-12-en-28-oic acid, 3-hydroxy-, (3beta)-	1	1	0	1	1	1	5
12	Pimozide	1	1	0	1	1	1	5
13	Olaanolic acid	1	1	0	1	1	1	5
14	5′-Methoxyhydnocarpin (5′-MHC)	0	1	1	1	1	1	5
15	Carnosol	0	1	1	1	1	1	5
16	Epicatechin	0	1	1	1	1	1	5
17	Slupp-417	0	1	1	1	1	1	5
18	Baicalein	0	1	1	1	1	1	5
19	Orobol	0	1	1	1	1	1	5
20	Amitriptyline	0	1	1	1	1	1	5
21	Carnosic acid	0	1	1	1	1	1	5
22	Quercetin	0	1	1	1	1	1	5
23	Ellagic Acid	0	1	1	1	1	1	5
24	Sertraline	1	1	0	1	1	1	5
25	Luteolin	0	1	1	1	1	1	5
26	Apigenin	0	1	1	1	1	1	5
27	Resveratrol	0	1	1	1	1	1	5
28	Piperine	0	1	1	1	1	1	5
29	Celastrol	1	1	0	1	1	1	5
30	Balsaminol F	0	1	0	1	1	1	4
31	Balsaminol A	0	1	0	1	1	1	4
32	Karavilagenin D	0	1	0	1	1	1	4
33	Balsaminoside A	0	1	1	0	1	1	4
34	Mpc-3100	0	1	1	0	1	1	4
35	Galbanic acid	0	1	0	1	1	1	4
36	Cholecalciferol	0	1	0	1	1	1	4
37	Chlorogenic Acid	0	1	1	1	1	0	4
38	1-Caffeoylquinic acid	0	1	1	1	1	0	4
39	Tetrandrine	0	1	0	0	1	1	3
40	Reserpine	0	1	1	0	0	1	3
41	Epigallocatechin-3-gallate (EGCG)	0	1	1	1	0	0	3
42	7-O-B-D-Glucopyranoside	0	1	1	1	0	0	3
43	Oreganol	0	1	1	1	0	0	3
44	Ginsenoside 20(S)-Rh2	0	1	1	0	1	0	3
45	D13-9001	0	0	1	0	0	1	2
46	Timcodar	0	0	0	0	1	1	2
47	Cinnamtannin B1	0	1	1	0	0	0	2
48	Tiliroside	0	1	1	0	0	0	2
49	Valinomycin	0	1	1	0	0	0	2
50	Dieckol	0	1	0	0	0	0	1

*(1)Refers to a compound that meets the criteria according to Lipinski's Rule of Five for drugs.
* (0)Refers to a compound that does not meet the criteria according to Lipinski's Rule of Five for drugs.

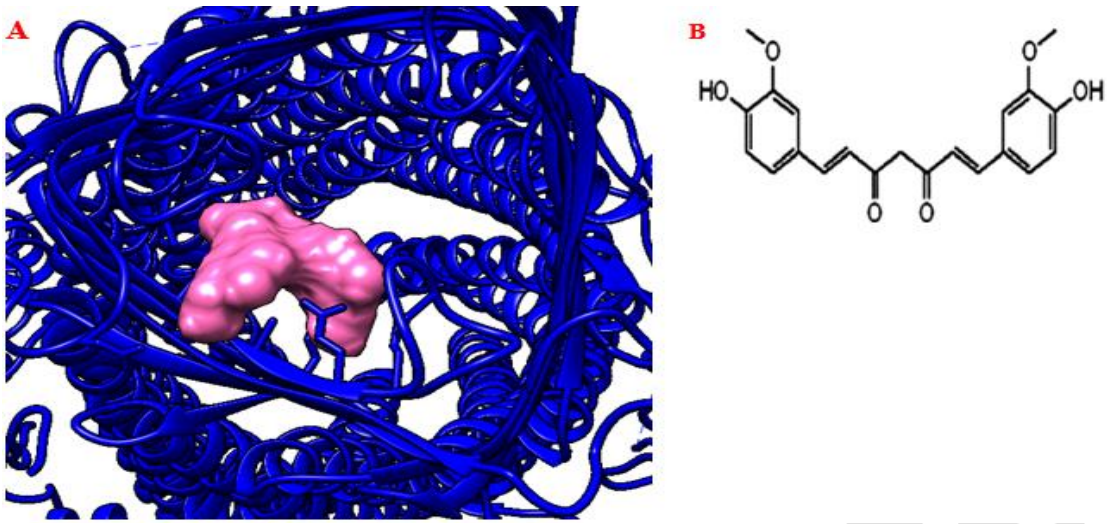

Fig. 12: A- Molecular docking of Curcumin compounds (pink color) and efflux pump of *Oprj* (blue color) with (-5.3 Kcal / Mol) and total score 6 by used program 1-click docking and UCSF chimera.
B- chemical structure of Curcumin.

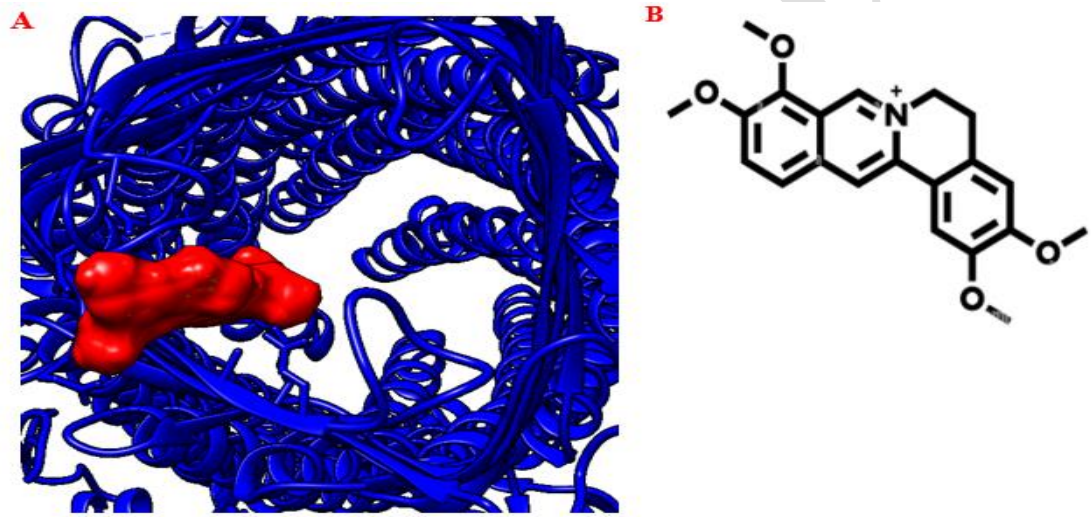

Fig. 13: A- Molecular docking of Palmatine compounds (red color) and efflux pump of *Oprj* (blue color) with (-5.2 Kcal / Mol) and total score 6 by used program 1-click docking and UCSF chimera.
B- chemical structure of Palmatine.

DISCUSSION

Pseudomonas aeruginosa is a leading cause of burn wound infections, largely due to its high virulence, multidrug resistance, and capacity to induce severe complications. The frequent occurrence of *P. aeruginosa* in burn units has been attributed to prolonged hospital stays and the extensive use of antibiotics[17].The predominance of this pathogen is further influenced by its resistance to both antibiotics and antiseptics, as well as the transformation of burn wounds into a favorable environment for bacterial growth, resulting from the impaired defense of damaged skin tissues. Additionally, the widespread presence of *P. aeruginosa* in the surrounding hospital environment, on medical equipment, and on the hands of healthcare personnel, combined with periods of overcrowding in burn units, contributes significantly to its high prevalence[18].

Moreover, the present data showed the high resistance of *P. aeruginosa* to Carbapenems represented by 71% Imipenem ,70 % Meropenem. *Pseudomonads* may develop resistance to carbapenems through combined mechanisms such as target inaccessibility,

stable derepression of AmpC β-lactamase, overexpression of efflux systems and production of Metallo-β-lactamases (MBLs)[19].

The results showed *P. aeruginosa* isolates were sensitive to Polypeptides (Colistin) was78 because the mechanism of action Colistin interacts with the lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria, disrupting membrane integrity, leading to leakage of cellular contents and bacterial cell death. This interaction is primarily electrostatic, as colistin is cationic and binds to the anionic LPS[20,21].

Molecular docking has identified several promising compounds with relatively low toxicity profiles. One such compound is curcumin, known for its antioxidant, anti-inflammatory, antibacterial, and wound-healing properties[22]. In its nano-formulation, curcumin functions as both an efflux pump inhibitor and an antibiotic potentiator against *Pseudomonas aeruginosa* isolated from burn wounds. Its mechanism involves direct inhibition of efflux activity, as well as the ability to disrupt bacterial membranes, biofilms, and virulence systems[23,24]. Another notable compound is palmatine, an isoquinoline alkaloid structurally related to berberine and naturally occurring in medicinal plants such as *Coptis chinensis*, *Phellodendron amurense*, *Tinospora* species, and *Stephania* species[25]. Palmatine demonstrates a wide spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, anticancer, and neuroprotective effects[26,27].

CONCLUSION

P. aeruginosa was the predominant pathogen, with Marked resistance to antibiotics, Mutations included transversion and transition substitutions, causing amino acid changes (Tyrosine-to-Phenylalanine at P415, P520; Leucine-to-Methionine at 467; Leucine-to-Glutamine at 535; Tyrosine-to-Cysteine at 441). Conserved and variable residues were observed, and molecular docking suggested that curcumin, palmatine, berberine, and PAβN could serve as potential efflux pump inhibitors.

Ethical Approval

A valid consent was achieved from hospital administration and from each (patients) before subjecting them to the study. Prior to samples collection, procedures were informed. For every patient or their followers, making absolutely sure that they understood the procedure that were to be carried out. patients had the right to reject giving sample and to be involved in the present study. Ethical Committee is 6/4265-2023/11/22

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Contribution of the author: Each author made an equal contribution to the research.

Conflict of interest: non

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