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Investigation of Bacterial Outer Membrane Proteins and Suggesting Antiadhesive Agents from Guava Plant in Skin Infections

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Abstract

Klebsiella pneumoniae is an opportunistic pathogenic bacterium belonging to *Enterobacteriaceae* family that owns outer membrane proteins called *omp35* and *omp36* which are responsible for colonization and adhesion to host tissues such as skin. Twenty pre-diagnosed bacterial isolates of *K. pneumoniae* of wounds and burn swabs were selected in this study, and the diagnosis was confirmed by VITEK-2 automated system. Whole DNA was isolated then amplified by PCR then gel electrophoresis was performed to detect the prevalence of the genes that encode both of *omp35* and *omp36* in bacterial isolates. Phylogenetic tree of both studied genes was conducted to investigate the precursor of tested isolates in relation to NCBI data. Virtually, the affinity of two medicinal components of fruit Guava *(Psidium guajava L.)*, which are Quercetin and L-arabinopyranoside, were tested as antiadhesive materials against omp35 and omp35 proteins by molecular docking tools. Results showed that 15 out of 20 skin-infecting *K. pneumoniae* isolates owned both *omp35* and *omp36* genes as detected by gel electrophoresis and the phylogenetic trees of both genes illustrated that they were related to surgical and nosocomial swab infection precursors in their origin in NCBI. High affinity of Quercetin was recorded towards *omp35* protein and *omp36* protein which were (-8.1 and -7.8) respectively, in the same time the affinity of L-arabinopyranoside was (-5.2 and -5.3) towards *omp35* and *omp36* proteins; resulting in suggesting medicinal ingredients of Guava fruit to prevent adhesion and colonization of *K. pneumoniae* and other *Enterobacteriaceae* members which might invade wounded skin by bacterial outer membrane proteins.

1. Introduction

Klebsiella pneumoniae is one of the coliforms bacteria members. It is Gram- negative bacteria which cause many nosocomial and community-acquired infections in many organs and after surgeries that are considered an important opportunistic pathogen that has multidrug resistance (MDR) worldwide as a public health challenge [1]. One of its resistance mechanisms is mediated by the permeability of the outer membrane proteins of *K. pneumoniae*

which are called OMPs like *Omp35* and *Omp36*, which are specific to *K. pneumoniae* and crucial in antibiotic penetration into the bacterial cell [2]. In ordinary cases, *K. pneumoniae* harbor *Omp35*, *Omp36* or both genes which have a very important role in bacterial pathogenicity and drug influx into *K. pneumoniae* cells [2]. Nowadays, antibiotic resistance results in the need to investigating the influence of some medicinal plants on OMPs after a deep study of the nature of Omps genes which are responsible for MDR and *K. pneumoniae* virulence factors [3].

Guava (*Psidium guajava*) is considered a seasonal plant of *Myrtaceae* family whose fruits, leaves and bark are full of beneficial antimicrobial agents, made researchers tested the influence of various extracts of guava parts against clinical isolates of bacteria, especially MDR *Enterobacteriaceae* [4]. The expression of virulence genes leads to a high tendency of pathogenic bacteria to colonize host tissues with a potent virulence pattern of MDR clinical isolates, especially wounds and burns in human beings [5]. So this study aims to:

1) Isolation and identification of *K. pneumoniae* from different clinical samples and confirming *K. pneumoniae* diagnosis by VITEK-2 system.
2) Detection of antibiotic resistance profile genes that encode *Omp35* and *Omp36* by PCR technique and performing their sequence, and phylogenetic tree to detect the ancestors of clinical isolates.
3) Investigation of medicinal components of Guava (Quercetin and L-arabinopyranoside) that can bind to the studied virulence proteins virtually by *In silico* tools.

2. Experimental Procedure

2.1. Ethical Approval

Twenty pre-diagnosed skin swabs from wounds and burns, identified as *Klebsiella pneumoniae* isolates, were collected from patients at the Teaching Hospital of Baghdad Medical City, following their verbal consent.

2.2. Materials and Methods

1) Samples collection and diagnosis: Pre-diagnosed 20 skin swabs of (wounds and burns) isolates of *K. pneumoniae* were collected from patients who attended the teaching hospital of Baghdad Medical City. Isolated and pre-diagnosed *K. pneumonia* strains were confirmed in their diagnosis by using differential and culture media; then were further diagnosed by using VITEK-2 compact system. Both bacterial diagnosis and drug susceptibility tests were done simultaneously, all VITEK-2 cards of diagnosis and antibiotic susceptibility tests were supplied by Biomereux Company (France).

2) Genetic study: It was performed by detection of OMPs gene is a technique which is based on the amplification of a part of bacterial DNA that was pre-extracted by Wizard DNA extraction kit (Promega/ USA); followed by the use of Polymerase Chain Reaction (PCR) by Green Master Mix kit (Promega) and by two primers (forward and reverse primers for each gene) which were designed in this study by using Genius software program (ver. 2019) for 40 cycles of PCR (each cycle included the PCR stages: Denaturation, Annealing and Extension). Gel electrophoresis was applied for analyzing the products of PCR and for evaluation of positive OMPs results (for both genes) in bacterial isolates Agarose (Promega) of 1% suspended in 1X TAE buffer (Promega) was used for this purpose. Ethidium bromide dye (Promega) was used as 5 microliter for each 100 ml of melted agarose for visualizing DNA bands under UV-illuminator, in comparison with the size of DNA Ladder (1500 bp) with loading dye (6X) (Promega). The optimal electrophoresis conditions and primer sequence of the studied genes are listed in Table (1). After that, the sequencing of amplified fragments of detected *Omp35* and *Omp36* genes of *K. pneumoniae* were sent to South Korea (Macrogen/ Korea) by applying of the Sanger method using ABI3730XL, automated DNA sequences. The advantage of this technique is that it is a highly discriminatory method [6]. The results of *Omp35* and *Omp36* sequencing were then analyzed by using Genius software ver. 2019. Pairwise comparison was performed between each studied gene with the same gene of a standard strain of *K. pneumoniae* with a specific NCBI codes to analyze each pair of compared genes and identify the phylogenetic tree for Omps genes.

Table (1): Optimal gel electrophoresis conditions and the primers sequence of target genes in current study.

Gene symbol	Forward primer sequence (5′…3′)	Reverse primer sequence (5′…3′)	No. of PCR cycles	Gel electrophoresis period at 100Volt of 1% agarose	Gene size (bp)
Omp35	TGAGTTTTACCAGCGAAGTG	AAACGGCAACAAACTGGA	40	75 minutes	780
Omp36	TCAACATATTTCAGCAGGTCC	ACCGTAACTCTGATTTCTTCG	40	90 minutes	1317

3) Molecular docking: Guava fruit medicinal components, Quercetin and L-arabinopyranoside, were virtually used in the docking process with both Omp35 and Omp36 as protein receptors of the outer membrane in *Klebsiella pneumoniae* which are both responsible for attachment to host cells and together give a higher virulence of pathogenicity with MDR. *In silico* approach was used aided by the PubChem website (www.pubchem.com) and Protein bank database (PBD) databases to get information about each one of the Guava fruit ingredients which is supposed to be a medicinal compound, and its strength to bind with the omp35 or omp36 were tested by using the software programs such as **PyRx** [7] for molecular docking; and **Discovery Studio version 0.8** for visualization of docked compounds [8].

2.3. Preparation of Outer Membrane Protein (Omps) Receptors

By using the Protein Bank Database (PDB) website, all information and chemical formulas related to the omp35 and omp36 proteins were obtained and saved. The structure of OMP genes was initially visualized by **Discovery Studio version 0.8** [8] software and only chain A was kept from all structural configurations of this protein. Mainly, water molecules and non-essential residues were all deleted. The new configuration shape of the OMP proteins was saved to be docked later with each ligand, elsewhere Quercetin or L-arabinopyranoside [3].

2.4. Preparation of Medicinal Components as Ligands

The chemical formulas of Quercetin or L-arabinopyranoside were all downloaded as SDF formulas by using www.pubchem.com website. To perform the virtual binding, the ligand should be prepared as a special form to make it suitable to occupy the active site of the OMP proteins. Quercetin or L-arabinopyranoside formulas were modified by the option of "convert to ligand in **PyRx** software" then were saved after some chemical configurational changes to bind with the modified OMPs receptor in the earlier step above [9]. The chemical formulas of Quercetin and L-arabinopyranoside are clarified in Table (2).

Table (2): Studied ligands and their chemical formula.

No.	Guava components	Chemical Formula
1	Quercetin	$C_{15}H_{10}O_7$
2	L-arabinopyranoside	$C_5H_{10}O_5$

2.5. Docking Process

The programs of molecular docking were downloaded by the links available on the network for free in order to perform the virtual binding of the medicinal components of guava with outer membrane proteins omp35 and omp36. The visualization of 2D and 3D formulas, chemical bonds and scored affinity of binding are all obtained by **Discovery Studio version 0.8 (2021)** and **PyRx (2021) programs** [7, 8].

3. Results and Discussion

3.1. Detection of OMPs Genes

After being previously diagnosed and confirmed by VITEK-2 system in hospital, the target genes (*Omp35* and *Omp36*) of *K. pneumoniae* pre-isolated DNA were detected by binding to their complimentary designed forward and reverse primers for all swab bacterial strains. Twenty-five microliters of PCR mixture for each isolate were divided for genetic the study: five microliters were used in gel electrophoresis detection of target genes, while the

rest 20 microliters were used for the Sanger method to get the nucleotide sequencing and phylogenetic tree in comparison with NCBI database [6]. It was obvious that *Omp35* gene was expressed in 18 out of 20 swab isolates. On the other hand, *Omp36* gene was present in 16 out of 20 isolates of swabs, and 15 swabs of 20 (75%) of the total isolates owned both *Omp35* and *Omp36* genes (Figure 1).

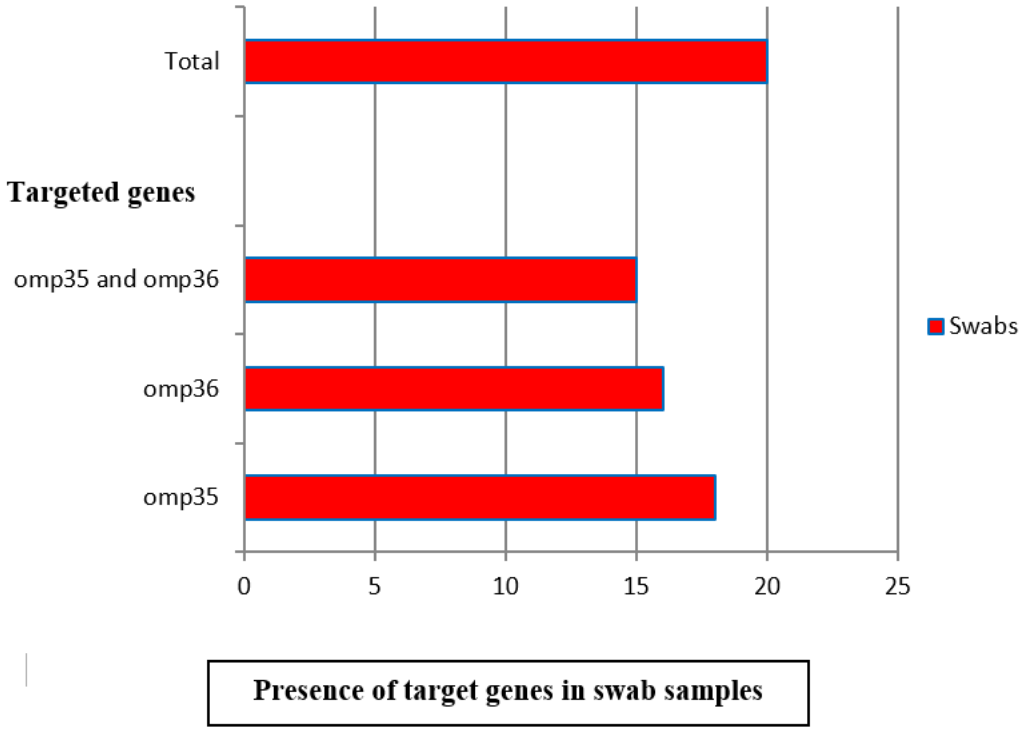
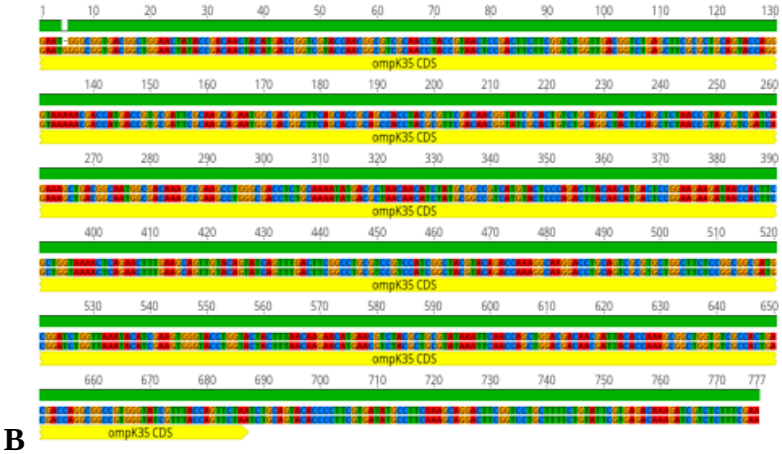


Figure (1): Prevalence of outer membrane porins genes in tested swab isolates.

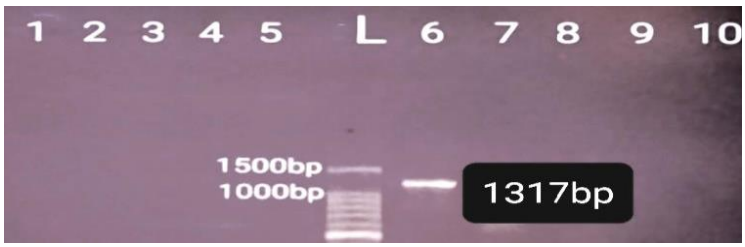
The positive results of both target genes which had a glitter appearance under the UV-illuminator apparatus after accomplishing the electrophoresis approach of PCR-positive amplicons, are clarified in Figure (2 A & B) and Figure (3 A & B) by comparison of *OmpK-35* and *OmpK-36* genes with the same genes of standard strains of NCBI, cp047336 and cp04719, respectively. Many researchers said that OMPs proteins are e crucial for the penetration of antibiotics into the cells, and they resemble a potent virulence-aid for bacteria to survive and increase MDR towards carbapenems, cephalosporins, quinolones and β-lactams antibiotics, especially in infected wounds and burns [10, 11].

A

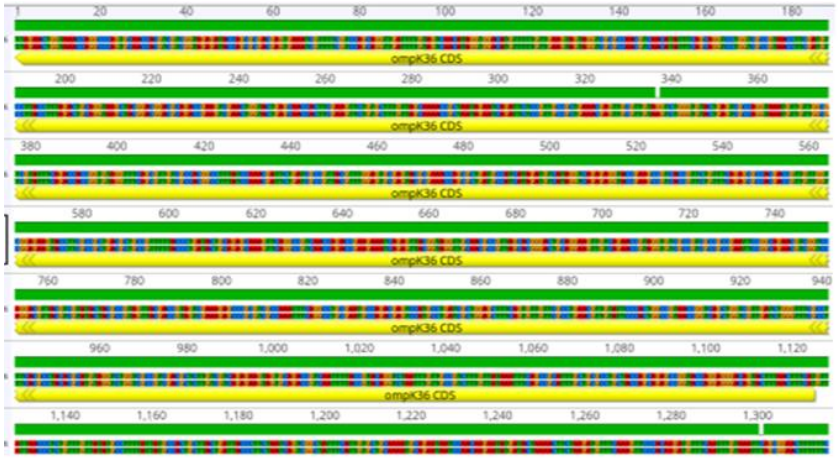


B

Figure (2): A: Gel electrophoresis of PCR product of *OmpK-35* gene (780bp amplicon) of *K. pneumoniae* by using 1% agarose gel/100ml at 100 Volt for 75 min. lane L represents 100-1500bp of DNA ladder, all ten lanes give a positive result for *OmpK-35* gene. B: Comparison between *K. pneumoniae OmpK-35* gene with the *OmpK-35* gene of NCBI strain (cp047336).



A



B

Figure (3): A: Gel electrophoresis of PCR product of *OmpK-36* gene (1370bp amplicon) of *K. pneumoniae* by using 1% agarose gel/100ml at 100 Volt for 75 min. lane L represents 100-1500bp of DNA ladder, all ten lanes give a positive result for *OmpK-36* gene. B: Comparison between *K. pneumoniae OmpK-36* gene with the *OmpK-36* gene of NCBI strain (cp047192).

OMPs are the main principal outer membrane proteins of Gram negative bacteria and they are highly conserved among the Enterobacteriaceae, also important for preventing the bacteria from being sensitized to the concentrations of drugs, or preventing accumulation of drug peptides via bacterial cell membrane [5]. *K. pneumoniae* resistance towards antimicrobials increases due to random use of antibiotics; this means that there is either insertion elements (IS) or nucleotide insertion that make these strains much more virulent with complex numerous drug resistance [12].

3.2. Phylogenetic Tree Using *Omp35* and *Omp36* Sequencing

Molecular data of DNA sequencing for *Omp35* and *Omp36* genes were analyzed by using Genius software (ver. 2019) and then were aligned by nucleotide blast of similar strains saved in NCBI to obtain the homologous sequence for each gene; then the phylogenetic tree was conducted and analyzed [6]. The phylogenetic tree refers to the relatedness of the studied strain (query) with the close strains of the same bacterial genus; for example the hospital-isolates of skin swab source might have a tight relationship with other nosocomial ancestors of infected surgical wounds and burns, which were distributed among the same country or other countries, related with patients infected with opportunistic *K. pneumoniae* [12]. In other words, this relationship refers to that the nosocomial strains are widely prevalent worldwide due to misuse, over use and bacterial resistance of antibiotics which results in multidrug resistance in skin-infecting *Enterobacteriaceae* locally or globally [13]. Phylogenetic tree of *Omp35* (Figure 4) showed that the query (current studied *Omp35* gene of the ID: 62165) was not very close in their origin to the standard sequences; except for NDM-71 *K. pneumoniae* strain (Gene bank ID: CP082936.1) which is belongs to nosocomial infection of human being by *Enterobacteriaceae* and locates at the same branch of the phylogenetic tree and originated from the same node; while other similar sequences of *K. pneumoniae* for Omp35 gene were gathered at another different branches of the tree. Otherwise the Iraqi isolation was unique and this result is also obtained by a previous study in Iraq [14].

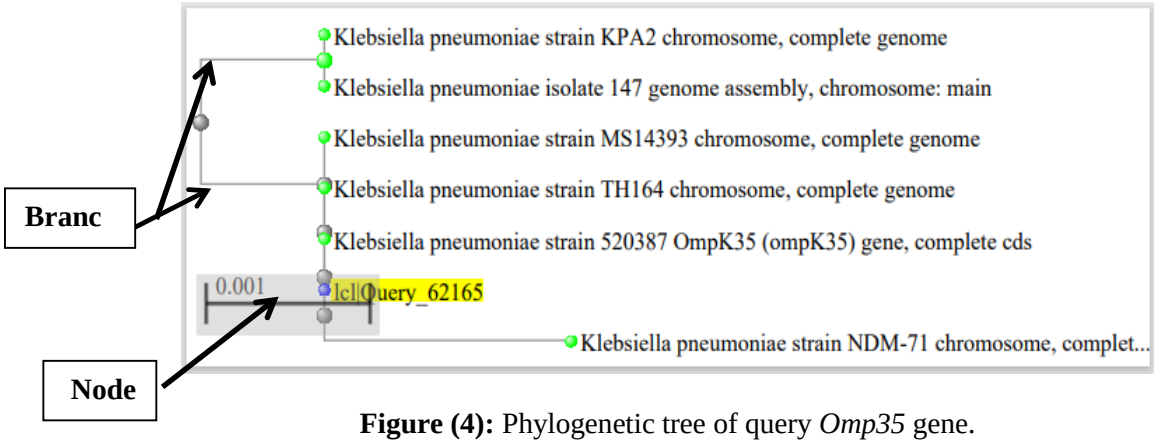


Figure (4): Phylogenetic tree of query *Omp35* gene.

The Phylogenetic tree of Omp36 in relation to other *K .pneumoniae* clinical isolates in NCBI (Figure 5) demonstrated that there was not any relatedness of query (ID: 44903) with other NCBI isolates regarding Omp36 gene sequence; even the similar *K. pneumoniae* strains of the other branch were of human being source. The other branch is further divided into two branches and all strains were obtained from infected surgical or after-operation isolates (for example the strain MIN-109 of Gene Bank ID: CP086128.1). The heterogeneous gene sequence results in being in a distinct location in the tree, refers to the enhancement of bacterial attachment to host endothelial tissue by Omp-mutant isolates of *Enterobacteriacea*, particularly *K. pneumoniae* [15].

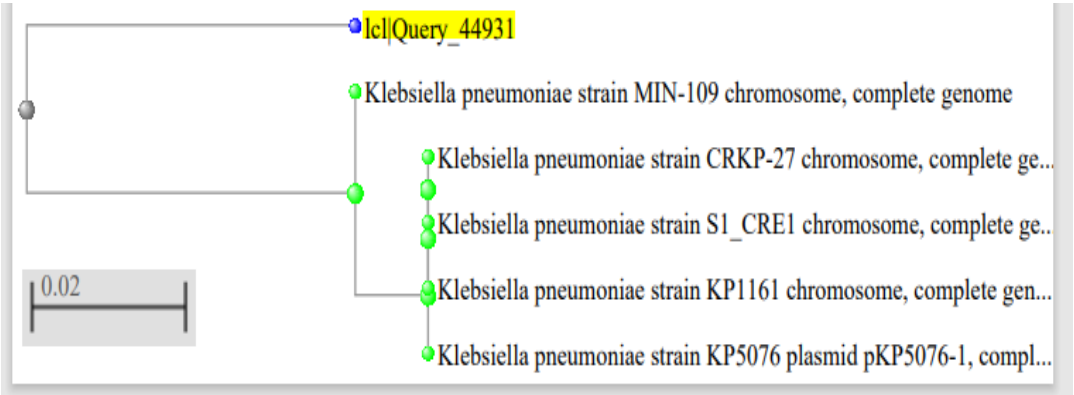


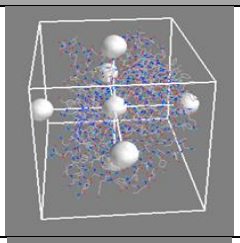
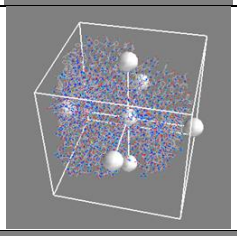
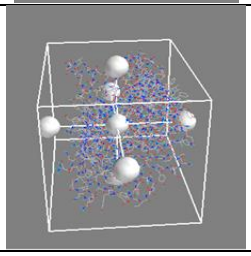
Figure (5): Phylogenetic tree of *Omp36* gene.

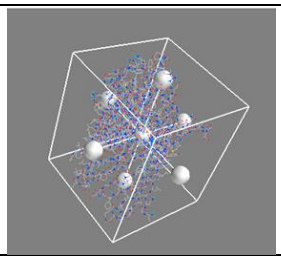
As concluded by the constructed phylogenetic tree of *Omp35* and *Omp36* above, *K. pneumonia* isolates displayed recurrent and frequent mutation in either *Omp35* or *Omp36* or both genes in numerous locations which results in aggressive resistance mode towards antibiotics, even the novel drugs, and these multiple alterations in Omps genes are strongly associated with increased MDR resistance and bacterial high virulence and invasive pathogenicity [12]. The dramatic biodiversity besides the evolutionary bacterial MDR of *K. pneumoniae* results in the need to find new and safe medications against resistant pathogenic *Enterobacteriaceae* members, as they all share the horizontal gene transfer mechanisms of antibiotic resistance [13].

3.3. Guava Medicinal Materials Docking with Bacterial Receptors Omp35 and Omp36

Virtual binding of *Omp35* and *Omp36* with medicinal components Quercetin and L-arabinopyranoside of Guava fruit was performed virtually, and *In silico* approach was used aided by PubChem website (www.pubchem.com) and Protein Bank Database (PBD) databases giving the necessary information about each one of the Guava ingredients which is supposed to be a medicinal compound, and its strength to bind with the *Omp35* or *Omp36* were tested by using the software programs such as **PyRx** for molecular docking; and **Discovery Studio version 0.8** for visualization of docked compounds. After preparing and saving all suitable forma of ligands and receptors that will be docked together, **PyRx** tool was used. VINA box is a representative box that gave all angles and rotation capacity of the binding process for each ligand with the same OMP proteins respectively and virtually [3]. Table (3) illustrates the configurational characteristics of VINA box search space of each studied ligand (Quercetin or L-arabinopyranoside) in the docking process with both *Omp35* and *Omp36* proteins.

Table (3): The configurational characteristics of VINA box search space of each studied ligand in the docking process with OMP proteins.

No.	Tested Ligand	VINA Box Dimensions (Angstrom)			VINA box Shape Of Docking
		X	Y	Z	
1	Quercetin and *Omp35*	46.3627	63.9584	44.9863	
2	Quercetin and *Omp36*	74.8624	68.1646	73.3141	
3	L-arabinopyranoside and *Omp35*	21.5819	34.3800	22.9825	

4	L-arabinopyranoside and *Omp36*	46.9900	47.3500	53.6823	

After accomplishing molecular docking of Quercetin and L-arabinopyranoside with OMP proteins, the affinity was calculated automatically and computationally by using python language by the same program, then the results of the binding strength were all written down for a comparison of the affinity of binding, measured by minus values as the bigger one in an inversive relationship [9]. The compounds that resulted from docking were all saved and then displayed by **discovery visualizer 0.8** to show the binding sites among amino acids of both combined molecules; in addition to the bonds that binds them together [7].

3.4. Visualizing Amino Acids and Chemical Bonds

Each docked compound, which resulted from the molecular docking of OMP proteins with each of the mentioned ligands, was then displayed in the **Discovery visualizer 0.8** program to get more information about the bound amino acids and the bonds among them [8]. As recorded below the affinity, which refers to the energy of the binding by minus, was listed in Table (4) from the highest to the lowest values, besides the type of the chemical bonds which bind each of the ligand to the Omp35 and Omp36 [3, 9].

Table (4): The strength and chemical bonds of Quercetin and L-arabinopyranoside with Omp proteins.

Docking molecules	Affinity (kcal/ mol)	Chemical bond/s
Quercetin and *Omp35*	-8.1	Pi-Pi, Vander Waals Pi-Alkyl, Pi-Anion Hydrogen bond, Unfavorable binding
Quercetin and *Omp36*	-7.8	Vander Waals, Pi-Alkyl Hydrogen bond, Unfavorable binding
L-arabinopyranoside and *Omp35*	-5.3	Vander Waals, Pi-Alkyl Carbon-Hydrogen bond, Unfavorable binding
L-arabinopyranoside and *Omp36*	-5.2	Vander Waals, Hydrogen bond Unfavorable binding

Quercetin and L-arabinopyranoside belong to a group of plant pigments called flavonoids, which give color to the guava fruit. They are also present in guava leaves and their extracts have been tested in previous studies as safe, plant-origin pharmaceuticals with low cost and many benefits as anticancer, antifungal antimicrobial and antidiarrheal agents [4, 9].

As antiadhesive materials, that prevent bacterial adhesion to host tissues and colonization or invasion, both Quercetin and L-arabinopyranoside extracts were very successful in urinary tract infections and respiratory complications [16], besides bacterial strains in particular and pathogens in general cannot develop resistance against them like the dramatic mode of MDR [17]. Traditionally, phytochemical pigments of guava are considered “Generally Recognized As Safe” (GRAS) with high efficacy, and they enhance the antibiotic activity in a synergistic effect [3, 18].

The 2 Dimensions (to the right) and 3 Dimensions (to the left) of molecular docking of guava components with the Omps receptors are clarified via *In Silico* tools below (Figures 6, 7, 8, & 9).

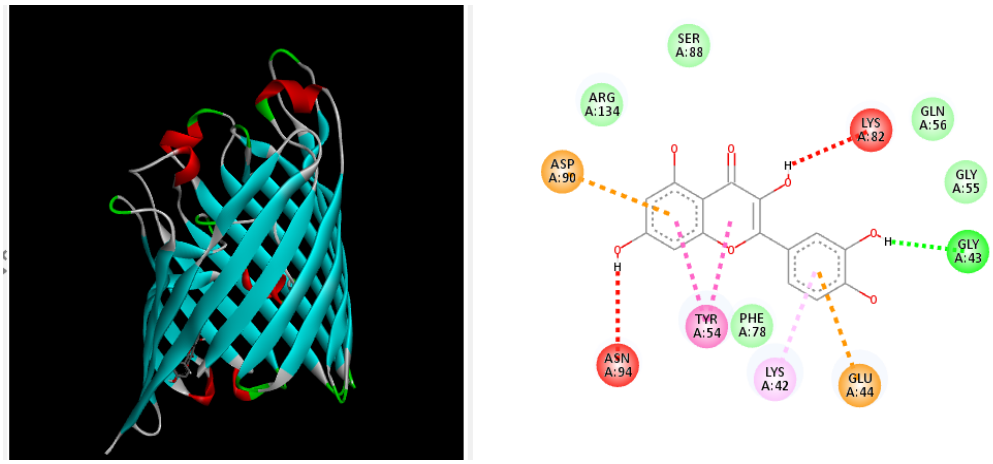


Figure (6): Molecular docking of Quercetin and *Omp35*.

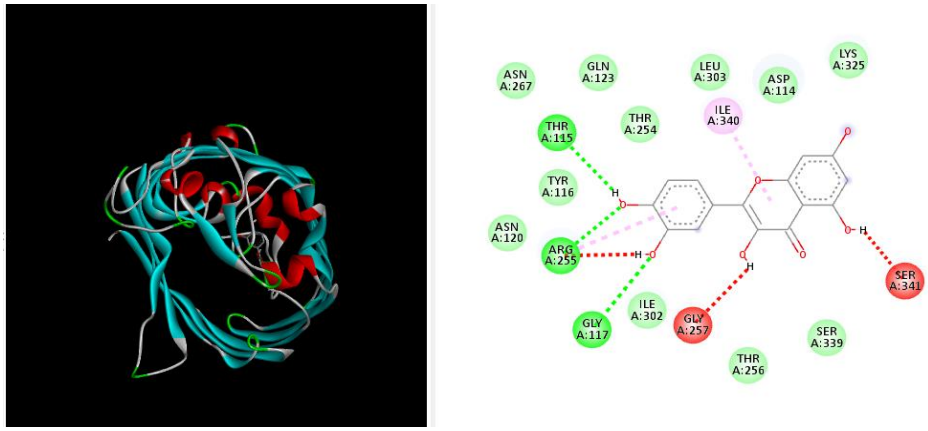


Figure (7): Molecular docking of Quercetin and *Omp36*.

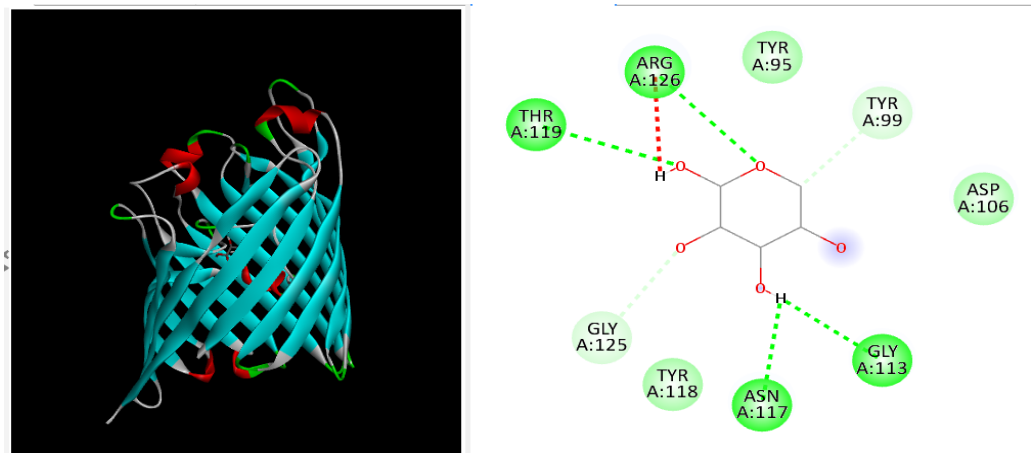


Figure (8): Molecular docking of L-arabinopyranoside and *Omp35*.

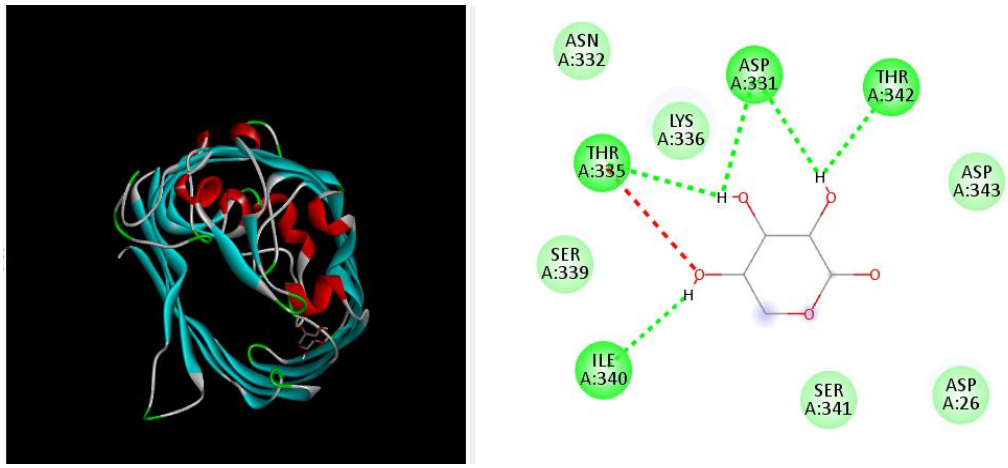


Figure (9): Molecular docking of L-arabinopyranoside and *Omp36*.

The configuration and dimensions of both flavonoids, Quercetin and L-arabinopyranoside, displayed a high affinity of binding towards bacterial OMPs which are portentous in nature. They occupied them by binding to the amino acids in Omp35 and Omp36 by many strong chemical bonds like Carbon-Hydrogen bond, even though there were weak bonds (like Vander Waals) and some unfavorable binding; because it is a virtual binding aided with computed tools as a technique used for identifying the presence of certain functional sites and groups to enable ligands to interact with their specific receptors [3]. The available information can be used to design potent formulas of plant-origin drugs regarding quantitative structure and activity relationship, from guava or any other medicinal plants by bioinformatics tools [16, 19].

In short, there is a great chance for plant-origin antibacterials to be used as the antibiotic adjuvant and some molecules like Quercetin already have been shown as adhesion inhibitors of the pathogenic bacterial attachment to injured host skin considering the interfaces, regarding the communication between naturally originated products, human and external environments to figure out with main fundamentals of the importance of Antibiotic Resistance Genes (ARGs) in the "One Health" dimension [20].

4. Conclusions

Quercetin recorded a high affinity towards *omp35* and *omp36* protein, while the affinity of L-arabinopyranoside slightly lower towards them; suggesting medicinal ingredients of Guava fruit as antiadhesive agents and antibacterial products against *K. pneumoniae* and other *Enterobacteriaceae* in skin infections. The current study is the first one that performed in both *In vitro* and *In silico* approaches.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

References

[1] S. Karolina, A. L. Adrianna, D. and E. Laskowska, "Antibiotic Heteroresistance in *Klebsiella pneumoniae*," *Int. J. Mol. Scien.*, vol. 23, no. 449, 2022.

[2] E. Sugawara, S. Kojima, and H. Nikaido, "*Klebsiella pneumoniae* Major Porins *OmpK35* and *OmpK36* Allow More Efficient Diffusion of -Lactams than Their *Escherichia coli* Homologs *OmpF* and *OmpC*," *J. Bacteriology*, vol. 198, no. 23, 2016.

[3] E. Ugbogu *et al.*, "The ethnobotanical, phytochemistry and pharmacological activities of *Psidium guajava L.*," *Arab. J. Chem.*, no. 103759, 2022.

[4] A. Garode and S. Waghode, "Antibacterial activity of *Psidium guajava linn* (guava) leaves extracts on bacterial pathogens," *Int. J. Bioassays*, vol. 3, no. 2, pp. 1794-1796, 2014.

[5] P. Ashwath *et al.*, "Biofilm formation and associated gene expression in multidrug resistant *Klebsiella pneumoniae* isolated from clinical samples," *Curr. Microbiol.*, vol. 79, no. 73, 2022.

[6] S. Alles *et al*., "Development of Resistance during Antimicrobial Therapy Caused by Insertion Sequence Interruption of Porin Genes," *Antimicrob. Agents Chemother*., vol. 43, no. 4, pp. 937–939, 2017.
[7] PyRx program for site specific docking which include AutoDock 4 and AutoDock Vina, 2021. Online. Available: https://pyrx.sourceforge.io/.
[8] Biovia Discovery Studio Visualizer program, 2021. Online. Available: https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php.
[9] S. K. Sharma, E. Sharma, and Y. Sharma, "A review: Recent computational approaches in medicinal chemistry: Computer aided drug designing and delivery," *The Pharma Innovation*, vol. 6, no. 5, p. 5, 2017.
[10] M. Chanoine *et al*., "Interplay between membrane permeability and enzymatic barrier leads to antibiotic-dependent resistance in *Klebsiella pneumoniae*," *Front. Microbiol*., vol. 9, no. 1422, 2017.
[11] J. Pages *et al*., "Role of the Outer Membrane and Porins in Susceptibility of Beta-Lactamase Producing *Enterobacteriaceae* to Ceftazidime-Avibactam," *Antimicrob. Agents Chemother*., vol. 60, no. 3, pp. 1349-1359, 2017.
[12] D. Jinrong Yan *et al*., "Multidrug Resistance Mechanisms of Carbapenem Resistant *Klebsiella pneumoniae* Strains Isolated in Chongqing, China," *Ann. Lab. Med*., vol. 37, no. 5, p. 398, 2017.
[13] E. A. Muhsin, L. A. Said, and S. S. Al-jubori, "Correlation of Type 1 and Type 3 Fimbrial Genes with the Type of Specimen and the Antibiotic Resistance Profile of Clinically Isolated *Klebsiella Pneumoniae* in Baghdad," *Al-Mustansiriyah J. Sci*., vol. 33, no. 3, pp. 1–11, 2022.
[14] R. Aziz and S. Al-Jubori, "Genetic elements responsible for extreme drug resistance (XDR) in *Klebsiella pnumoniae var pnumoniae* isolated from clinical samples of Iraqi patients," *World J. Pharm. Res.*, vol. 5, no. 5, 2016, DOI: 10.20959/wjpr20165-6020.
[15] R. Nisa *et al*., "Genetic relatedness of local *Cronobacter sakazakii* based on invasion gene *ompA*," *IOP Conf. Ser.: Earth Environ. Sci*., Preprint issue, 2020, art. no. 443012034.
[16] A. Yahaya *et al*., "Extracts on *Staphylococcus aureus* Isolated from Patients with Urinary Tract Infections Attending a Tertiary-Care Hospital," *Sci. World J*., vol. 14, no. 1, pp. 47-51, 2019.
[17] A. Sule, A. Olalemi, and A. Ogundare, "Efficacy of leaf extracts of *Psidium guajava (L.)* on enteric bacterial isolates from faecally impacted groundwater," *Water Pract. Technol.*, vol. 17, no. 1, p. 167, 2021, DOI: 10.2166/wpt.2021.113.
[18] Y. Fumi *et al*., "Antibacterial Effects of Guava Tannins and Related Polyphenols on *Vibrio* and *Aeromonas* Species," *Nat. Prod. Commun*., vol. 3, no. 5, pp. 711-720, 2008.
[19] D. Manika and G. Subhagata, "Antifungal and Antibacterial Property of Guava (*Psidium guajava*) Leaf Extract: Role of Phytochemicals," *Int. J. Health Sci. Res*., vol. 2, no. 19, pp. 39-45, 2019.
[20] P. Singh and M. Sillanpa, Degradation of antibiotics and antibiotic-resistant bacteria from various sources: A book, 1st ed. United Kingdom: Elsevier, 2023.