ORIGINAL PAPER

ANTIBACTERIAL PROPERTY AND MOLECULAR DOCKING STUDIES OF *TAMARIX RAMOSISSIMA* PLANT EXTRACT

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Abstract. *Researchers try to develop new broad-spectrum antibiotics against bacteria, because the extended use of antibiotics has led to drug resistance. It directed the researchers to find new medical plants which are a rich source of many antibacterial compounds such as polyphenols. The current research aimed to investigate the antibacterial activity shown by Tamarix ramosissima tincture against five human pathogenic bacteria: Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Klebsiella pneumoniae. Using molecular docking technique we explained the antibacterial effect of plant extract.*

Keywords: *molecular docking, Tamarix ramosissima, antibacterial plant effect.*

1. INTRODUCTION

Antibacterial activity is the most widely studied aspect of plant extracts. Antibiotics extensively produced and consumed in large quantities, have proved to be problematic due to various types of adverse effects. The development of bacterial resistance to currently available antibiotics needed the search for new antibacterial agents [1]. One of the alternative strategies for fighting antibiotic - resistant bacteria is the use of natural antimicrobial substances such as plant extracts [2]. Polyphenols are a group of highly hydroxylated phenolic compounds which exist in the extractive fraction of several plant components. Polyphenols are proved to have bactericide activities against a huge number of pathogenic bacteria [3]. Polyphenols in plants include flavanols, flavonols, flavanones, flavones, anthocyanins, proanthocyanidins (tannins), hydroxystilbenes, and aurones [4-11]. It was observed that regulate consumption of diets rich in plant polyphenols have positive antioxidant effects on the human body, reducing the development risk of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [12].

Tamarix ramosissima (Tamaricaceae) is a small tree that grows spontaneously in Europe and Asia. This species is considered an invasive species in geographically hot climates (south of the US and the desert of California) [13]. In Romania, it grows along the rivers, (i.e., Siret, Danube), forming phytocoenoses that can advance to the sub-zone of the oak. Using HPLC method recent studies shows that *Tamarix ramosissima folium et flos* (TRFF) tincture contains quercetol 250-300 [µg/mL], and chlorogenic acid, kaempferol, apigenin-7-glucoside, apigenin and isoquercitrin in traces [14]. *Tamarix ramosissima* has anti-inflammatory, analgesic and antioxidant effects. The plant extract (extracted with acetone-

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water and ethylacetic acid) showed antibacterial, antifungal and DNA-damaging effect in *in vitro* studies [15].

It was tested the antimicrobial activity of *Tamarix ramosissima* extracts against five bacterial species: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginos* and *Klebsiella pneumoniae.* Protein-ligand docking is the process of computationally predicting the placement and binding affinity of a small organic molecule in the binding pocket of a protein, usually for the purposes of drug discovery. Lot of techniques, starting from simple point-matching algorithms to explicit physical simulation methods has been developed to solve this problem [13]. Docking is widely used for the study of biomolecular interactions and mechanisms, and is applied to structure based drug design [14] and as well as to elucidate fundamental biochemical processes [16].

2. MATERIALS AND METHODS

2.1. SAMPLES PREPARATION

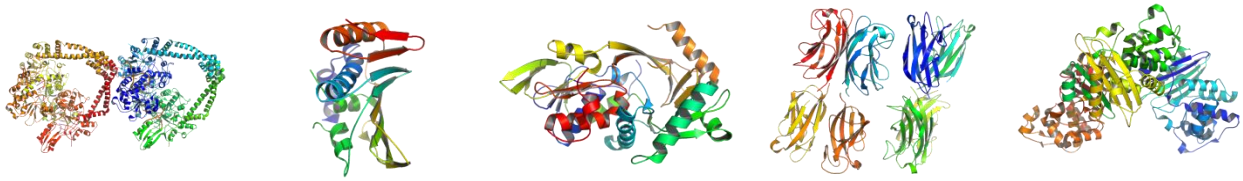
The vegetal products were used as tinctures, obtained by simple percolation, in 1:5 ratio of vegetal product / solvent (ethanol 70°) (the preparation method complies with the Romanian Pharmacopoeia, 10th Edition - F.R.X) [17].

2.2. ANTIBACTERIAL POTENTIAL TEST

There was poured nutrient agar (Mueller-Hinton) in Petri plates with a diameter of 100 mm, in a 4 mm uniform layer. Inoculum preparation was performed by 2-3 standard colony suspending in physiological saline, turbidity of the suspension is controlled by nephelometry. The culture medium must have a pH of 7.2-7.4 and a suitable composition that is proper for development of the tested bacterial species. Seeding was carried out by flooding the nutrient medium with the bacterial suspension, followed by removal of the excess. Drying is achieved by keeping the inoculated plates for 10 min at room temperature (22 °C) prior to the samples insertion in the oven. The microorganisms to be tested coming from standard reference strain were purchased from the Cantacuzino Institute, being sensitive to the antibiotics of choice. In order to test the antibacterial effect of our vegetal extracts we used the diffusion method of the nutrient agar (Kirby Bauer) according to F.R.X. Sterile filter paper tablet (Ø = 6 mm), were impregnated with a volume of 25 µL of plant extract to be tested, and then were maintained in oven for 24 h, at 20°C, so that alcohol evaporates. Sample discs are prepared in the same conditions. The disks were impregnated with the proper antibiotic (control +), according to the sensitivity of bacterial species. Deposition of the impregnated slices with test samples was carried out after drying of the paper, about 15 min after sowing, using an ophthalmic forceps, applying each sample to be analyzed on the surface of the culture medium. Test discs were placed at 1.5 cm distance from the edge of the Petri dish and 3 cm distance from each other. Incubation was carried out for 18 h at 37°C, in the inverted position of the Petri plate. The results were read by visual inspection, using a graduated ruler, and the average diameter of the inhibition zone (DZI), in millimeters, induced by the test samples was recorded. Results were expressed as average values obtained by calculating the arithmetic mean of diameters for the three tests. Very small colonies were not considered, neither subsequent invasion of the inhibition zone or discrete increments within the zone of inhibition [18].

2.3. MOLECULAR DOCKING TECHNIQUE

Quercetol and antibiotics were optimized using the Gaussian 09 software (Gauss View 16 interface) by the DFT / B3LYP / 6-31G method. The X-ray crystal structure of the bacterial species was taken from the Protein Data Bank (2XCT code for *Staphylococcus aureus*, 1KZN code for *Escherichia coli*, 5I39 code for *Proteus vulgaris*, 4LKD code for *Pseudomonas aeruginosa*, 2OV5 code for *Klebsiella pneumoniae*).



Staphylococcus aureus | *Escherichia coli* | *Klebsiella pneumoniae* | *Pseudomonas aeruginosa* | *Proteus vulgaris*

Figure 1. Bacterial targets.

The molecular docking analysis was performed using the Autodock 4.2.6 software together with the AutoDockTools molecular viewer. For the molecular protein docking method we added all the polar hydrogen atoms and we selected the Gasteiger charge. In the grid stage we set the 100X100X100 grid box at a distance of 0.75 angstroms from the center of the protein. In the docking stage was chosed the Lamarckian Genetic Algorithm (LGA) with a number of 30 runs. The images of the protein-ligand complexes were visualized using the PyMol (Schrodinger) [19] and Discovery Studio Visualization (Biovia) [20].

3. RESULTS AND DISCUSSION

Staphylococcus aureus is a pathogenic bacteria that can cause suppurative infections or septicemia. The manifestations of these infections are varied: impetigo, pyoderma, stafiloderma, staphylococcal pneumonia, Lyell's syndrome [21]. *E. coli* is part of the *Enterobacteria* group, living as epiphyte in the digestive tract. In some cases of intestinal microflora imbalance, these bacteria are massively multiplied, resulting in the emergence of toxicogenic strains [22]. *Proteus vulgaris* is isolated from patients with physical disabilities, hospitalized for a long time and underlying illness or low immunity. Patients with recurrent infections with structural urinary tract abnormalities, those with urethral instruments and those whose infections were acquired in the hospital are at high risk of contamination with *Proteus vulgaris*.

Table 1. Media average diameters [mm] of inhibition of bacterial growth after testing tinctures.

Test Product	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
TRFF	5*	19,5***	4*	14.7**	22.6***
amoxicillin	31,3***	nt	nt	nt	nt
levofloxacin	nt	34,2***	nt	nt	nt
amikacin	nt	nt	33,6***	nt	nt
ceftazidime	nt	nt	nt	28,6***	nt
cefotaxime	nt	nt	nt	nt	35,8***

** resistant ** intermediate *** sensitive, nt-untested.*

Proteus and *Pseudomonas* are the most frequently responsible microorganisms for gram-negative bacteraemia and sepsis [23]. *TRFF* extract show antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*, but is inactive against *Staphylococcus aureus* and *Proteus vulgaris.* The plant extract has a lower antibacterial activity comparative with the antibiotic of choice. Because *TRFF* contains quercetol like a principal component we realized the docking technique ligand-target using it.

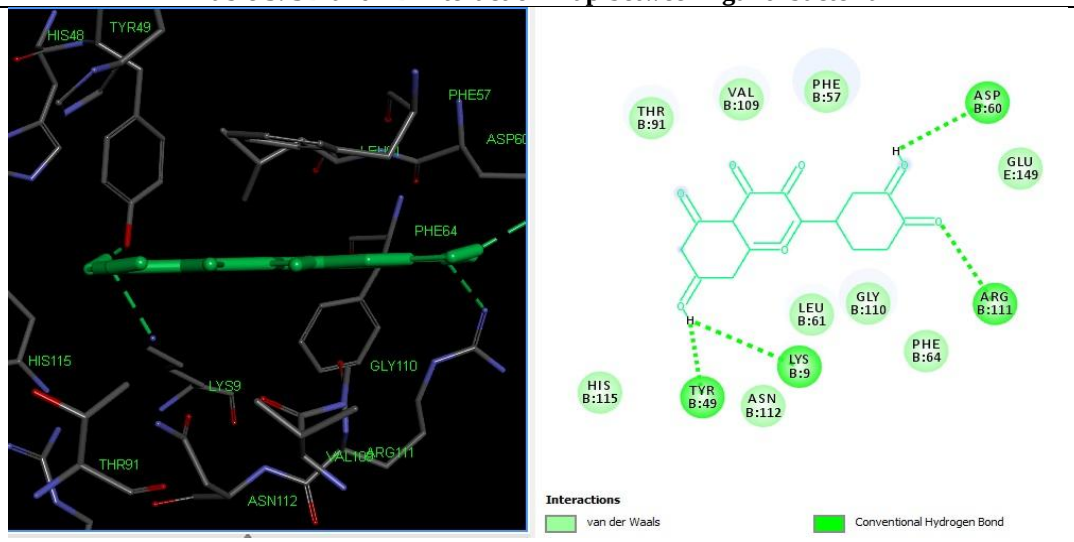
The docking technique allows visualization of interaction between the ligand and the biological receptor and predicts the optimized conformation of the stable complex formed by interaction.

Table 2. Binding energy ligand (quercetol/antibiotic/ ethyl alcohol –bacteria, [kcal/mol]).

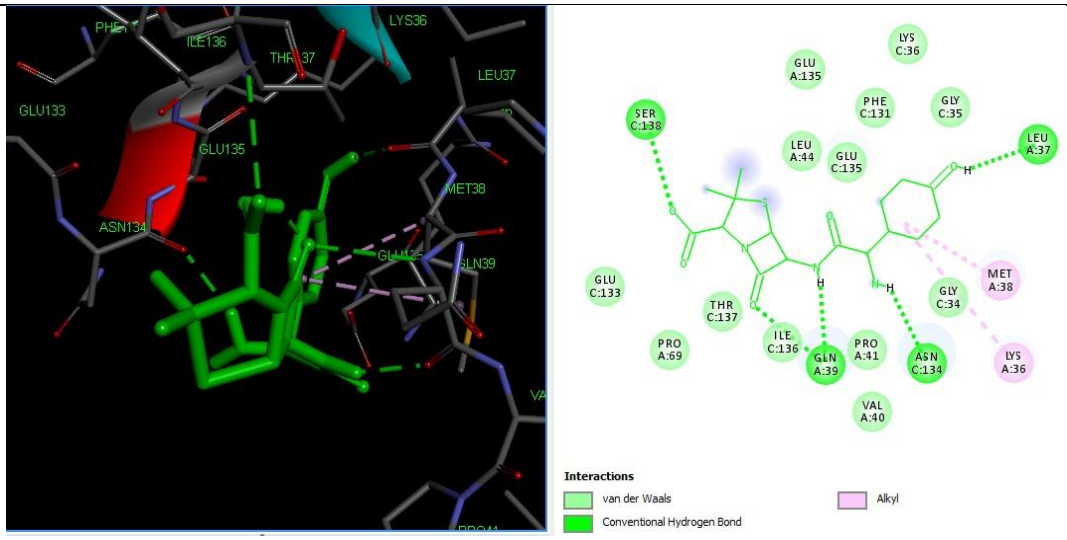
Test Product	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
TRFF(quercetol)	-1.18	-4.25	-0.14	-1.17	-3.25
amoxicillin	-4.43	nt	nt	nt	nt
levofloxacin	nt	-6.07	nt	nt	nt
amikacin	nt	nt	-2.03	nt	nt
ceftazidime	nt	nt	nt	-3.03	nt
cefotaxime	nt	nt	nt	nt	-3.34

nt-untested.

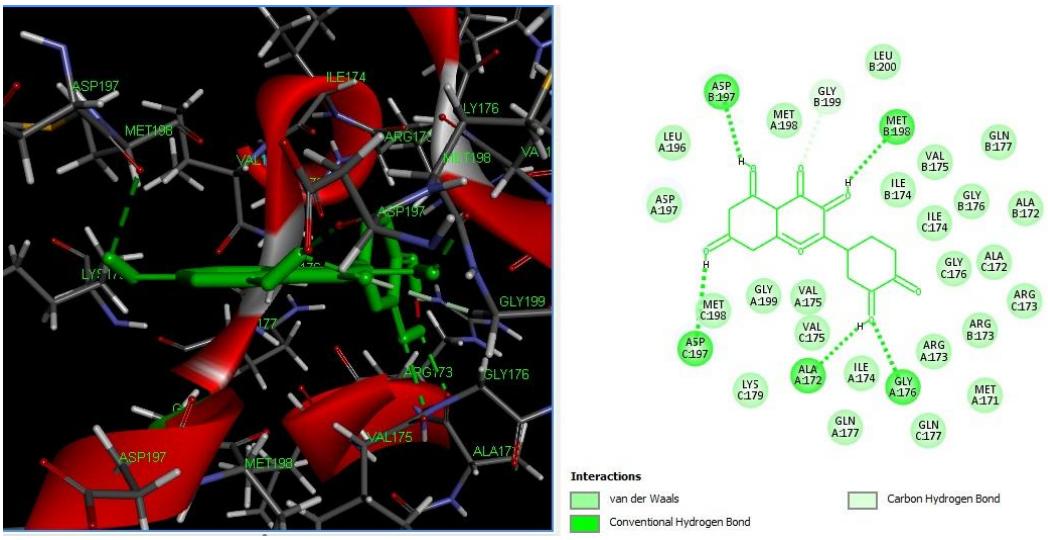
Table 3. 3D and 2D interaction map between ligand-bacteria



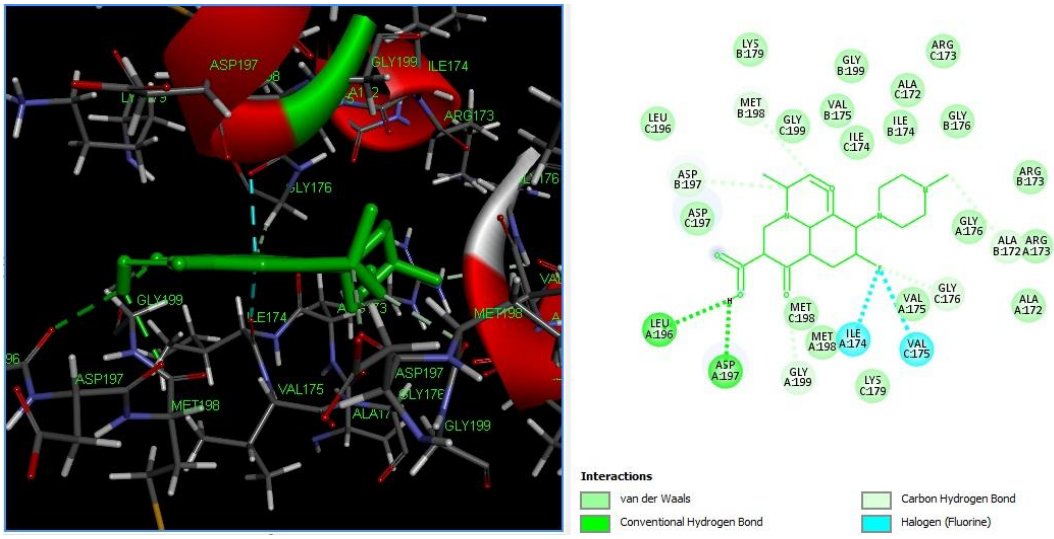
3D (left) and 2D(right) interaction maps between quercetol- *Staphylococcus aureus*



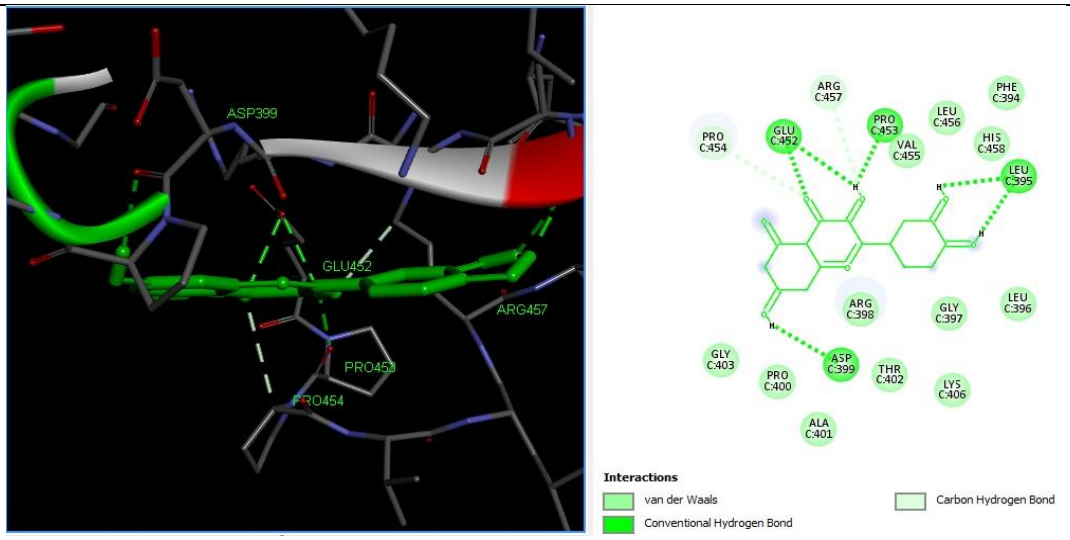
3D (left) and 2D(right) interaction maps between amoxicillin - *Staphylococcus aureus*



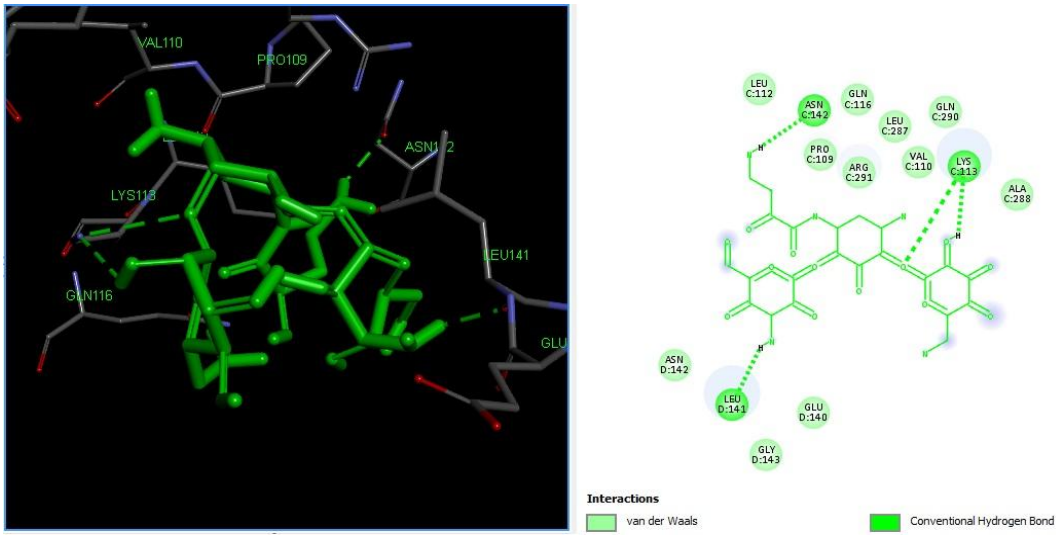
3D (left) and 2D(right) interaction maps between quercetol- *Escherichia coli*



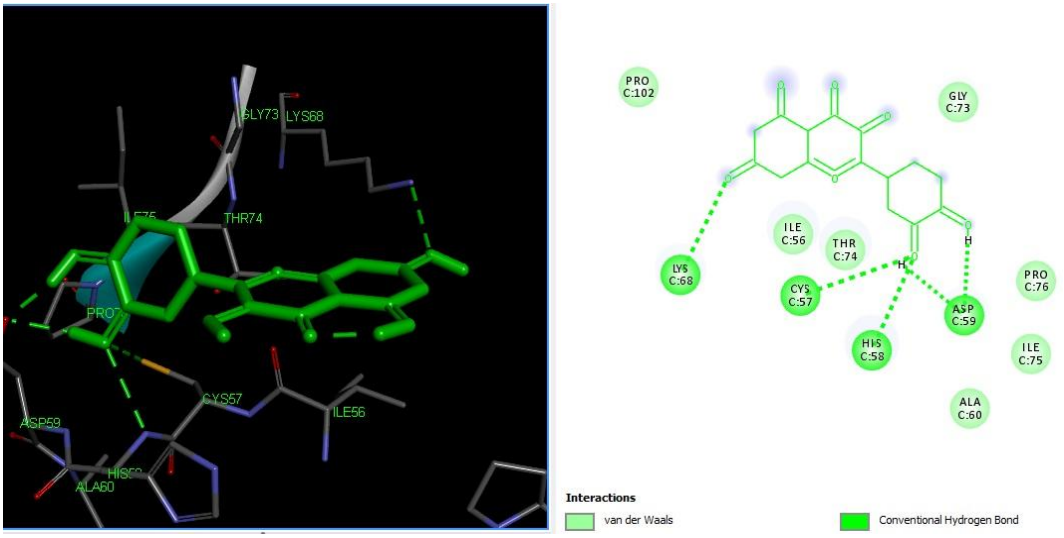
3D (left) and 2D(right) interaction maps between levofloxacin - *Escherichia coli*



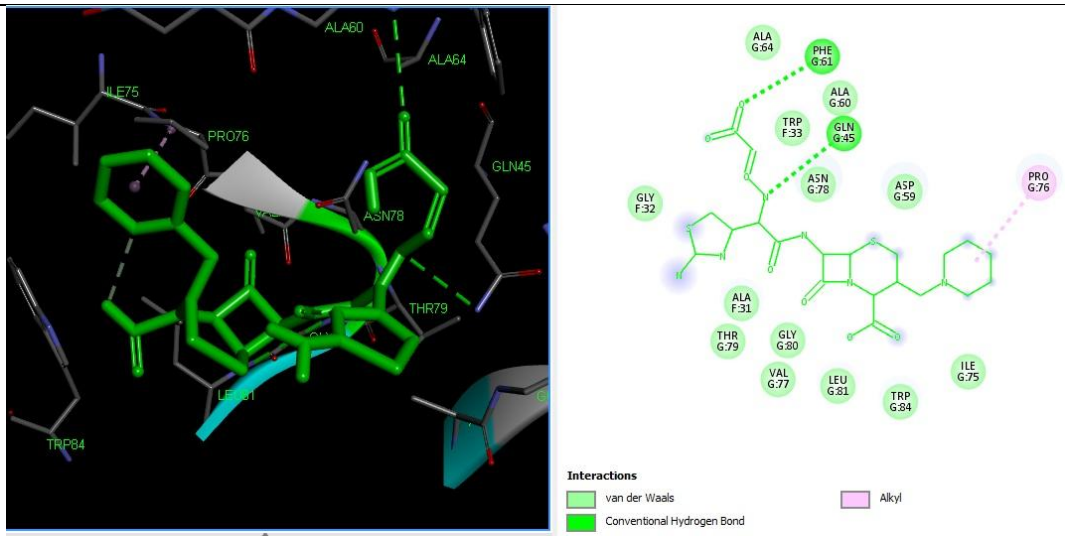
3D (left) and 2D(right) interaction maps between quercetol- *Proteus vulgaris*



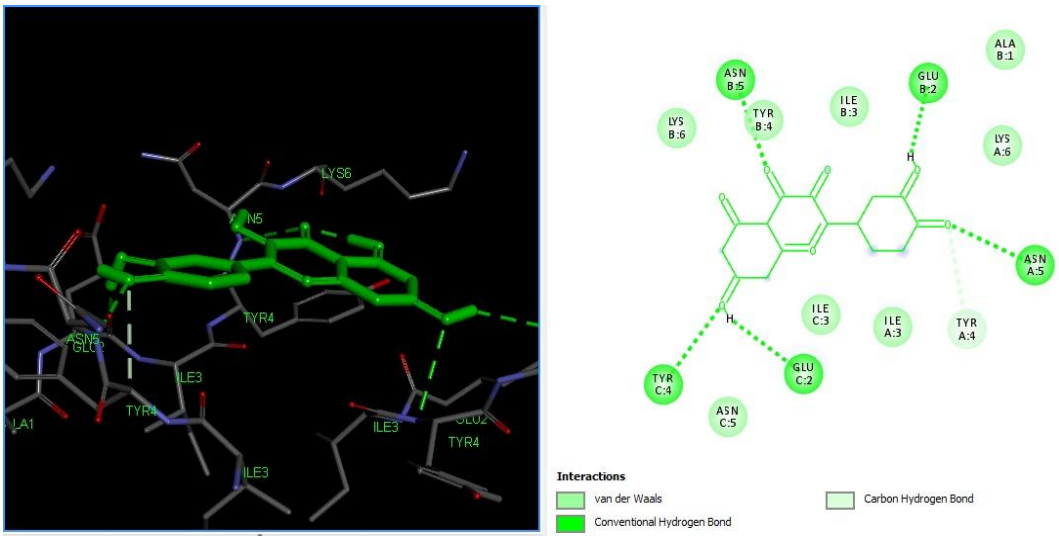
3D (left) and 2D(right) interaction maps between amikacin - *Proteus vulgaris*



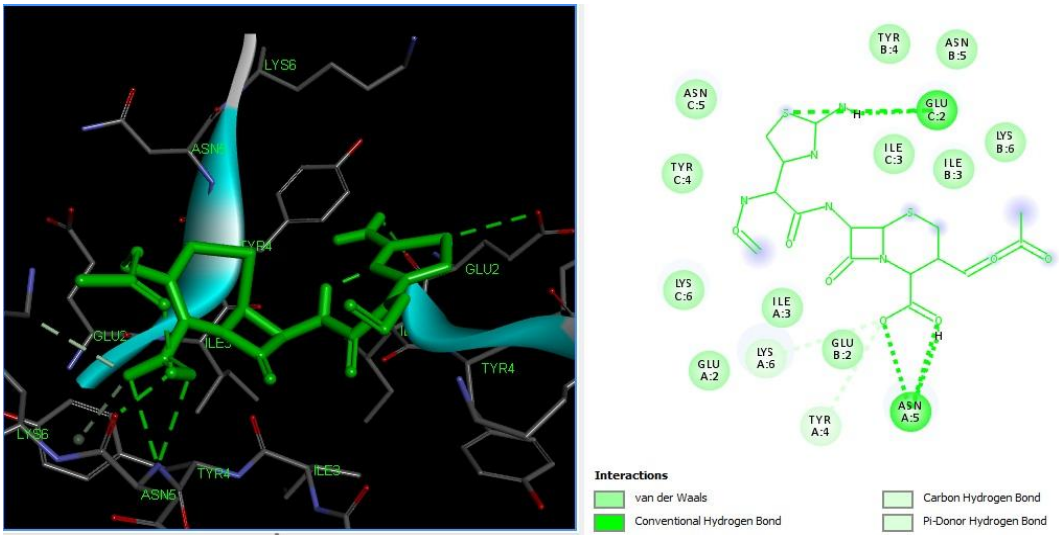
3D (left) and 2D(right) interaction maps between quercetol- *Pseudomonas aeruginosa*



3D (left) and 2D(right) interaction maps between ceftazidime - *Pseudomonas aeruginosa*



3D (left) and 2D(right) interaction maps between quercetol - *Klebsiella pneumonia*



3D (left) and 2D(right) interaction maps between cefotaxime - *Klebsiella pneumonia*

From the docking procedure results we can see that quercetol is an active antibacterial agent against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, making ligand-bacteria complexes with a low binding energy values. From 2D and 3D map we observed that quercetol have the same active situs with levofloxacin against *Escherichia coli* and with cefotaxime against *Klebsiella pneumonia.* Quercetol have common aminoacids with ceftazidime in the *Pseudomonas aeruginosa* active pocket (PRO76, ILE75, ALA60).

4. CONCLUSION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. For a long period of time, plants have been a valuable source of natural products for maintaining human health with more intensive studies for natural therapies. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. This study confirms that *TRFF* plant extract shows *in vitro* antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* and no activity against *Staphylococcus aureus* and *Proteus vulgaris.* Molecular docking technique support the experimental results.

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