ORIGINAL PAPER

ANTIBACTERIAL ACTIVITY OF FRESH ONION AND GARLIC JUICES

LUCIANA TEODORA ROTARU[1*], MARIUS NOVAC[2], OANA NICOLAESCU[3]

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Abstract. *Herbal extracts with antimicrobial potential represent an important research directive, in the current medical world, aiming to isolate active components, to develop new chemotherapeutic agents with applicability in the treatment or use as adjuvant therapy in infectious states. Antimicrobial properties of plants are conferred by their ability to synthesize certain secondary metabolites with relatively complex structures. The last century has been marked by sustained efforts to search for new natural compounds with antibacterial therapeutic properties, due to the gradual reduction in the number of effective allopathic antibiotics and the toxic effects of antibiotic residues. Numerous in vitro studies have shown that plants have antibacterial efficacy, discovering the importance of little-studied natural resources in this regard, as being effective in fighting against bacterial resistance and destroying bacterial agents. In this study, the antibacterial effect of the fresh onion and garlic juice was compared to the antibiotics of choice, using the diffusimetric agar method. Both plant products tested have antibacterial effect, the bacterial species being classified as sensitive to their action. The molecular docking method helps us to see the type of interaction between ligands and targets, allicin having no common binding site with antibiotics of choice.*

Keywords: *allicin; molecular docking; antibiogram.*

1. INTRODUCTION

Antibiotic resistance is considered to be one of the most pressing public health issues in the world, the vast majority of bacteria becoming less sensitive to antibiotic treatment. The bacteria genetic ability to acquire multiple resistance to the chemotherapeutic agents used, leads to unfavorable evolution of the infections and to therapeutic failure. The development of bacterial resistance to classical antibiotics has led to the development of research in this field, to search new effective antibacterial agents [1].

Herbal medicine has been effective in treating many diseases. Since ancient times, people have used whole plants or their vegetable parts rich in active, fresh or processed principles (in the form of infusion, decoction, tincture, aqueous macerate) for their healing properties [2-7]. Unlike allopathic therapy, phytotherapeutic treatment shows potentiating synergism of the components and fewer adverse reactions. Scientists use theoretical physicochemical data to explain experimental results [3, 8, 9]. Based on many findings in the

[1] University of Medicine and Pharmacy Craiova, Emergency First Aid & Prehospital Care Department, 200349 Craiova, Romania.
[2] University of Medicine and Pharmacy Craiova, Anestesiology and Intensive Care Department, 200349 Craiova, Romania
[3] University of Medicine and Pharmacy Craiova, Faculty of Pharmacy, Department of Pharmaceutical Technology, 200349 Craiova, Romania.
[*] *Corresponding author*: lucianarotaru@yahoo.com.

pharmaceutical field, research involving quantum chemical calculations is the starting point [10-14].

Onion (*Allium cepa, Liliaceae)* is a biannual plant being also the most used vegetable in the world. It contains cycloalliin, methylalliin, propyl cycloalliin, flavonoids (quercetol and camferol glycosides), saponozides, amines, enzymes [15, 16]. It has antibacterial, antifungal properties and hypoglycemic effect similar to glibenclamide and insulin and hypolipemic effect [17]. It is used as a condiment in the kitchen. In medicine, together with garlic, helps restore and normalize blood circulation. The onion has an antimicrobial action, regulates the metabolism of the lipids, stimulates the immune system, helps to combat the cold [18].

Garlic (*Allium sativum, Liliaceae*) is an edible plant, used as food and spice. In folk medicine, it is considered, along with onions, a real medicine, due to its rich content of vitamins and mineral substances. The chemical composition of garlic comprises: sulfur derivatives, flavonozides, vitamins (A, B1, B2, C), phytosterol, glycerides of palmitic, stearic, oleic, linoleic, myristic, allicin, sterol (erubozide) derivatives [19].

A study has shown that garlic extract supplementation inhibits vascular calcification in human patients with high blood cholesterol [20]. Garlic has antiplatelet effect, so it is recommended to reduce the dose of anticoagulant when consuming garlic [21]. There are studies conducted on cohorts where the antitumor effect of garlic in different types of gastric cancers is highlighted [22, 23].

The present study aimed to investigate the antimicrobial potential of some plant extracts from the *Alliaceae* family: *Allium sativum* and *Allium cepa*, taking as reference the recognized antibacterial effect of antibiotics of choice (control +) on the reference strains from: *Staphylococcus aureus* (ATCC 25)), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603).

2. MATERIALS AND METHODS

2.1. SAMPLE PREPARATION

The vegetable products were used in the form of fresh juice, obtained by mechanical pressing of the fresh bulbs.

2.2. ANTIBIOGRAM PREPARATION

In order to test the antibacterial activity for the two fresh juices to be analyzed, the diffusimetric method on nutritional agar (Kirby-Bauer) was used, in accordance with the provisions of F.R. X. This is a very simple and fast method, which allows to determine the sensitivity spectrum of the microorganism. Fresh juice was impregnated on the rounds filter paper and deposited on the surface of the agarized medium sown "in cloth" with the standardized inoculum (obtained from the reference strain) [24]. The procedure is based on the radial diffusion in agar gel of the antimicrobial substances from the test vegetable juices. The active principles will diffuse into the environment, achieving a concentration gradient inversely proportional to the diameter of the diffusion zone, so with the distance to the disk. If the strain is sensitive to a particular antibiotic / plant juice, the microbial growth will be inhibited on a certain surface around the disk impregnated with the respective sample, a

surface called a bacterial growth inhibition zone. How the antibiogram was performed has been previously described in other studies [25, 26].

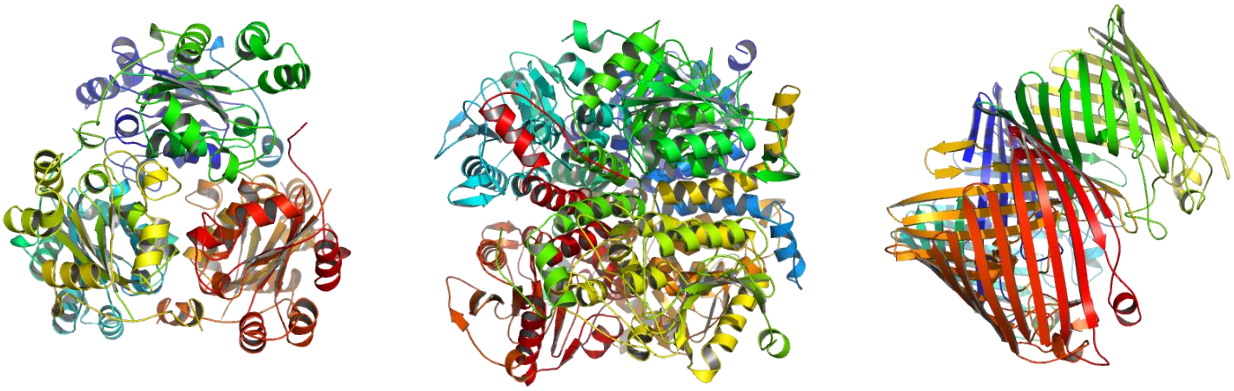
In practice, is used the technique of discs impregnated with antibiotic (as control +), standardized, recommended by NCCLS (National Committee for Clinical Laboratory Standardization).

Table 1. Sensitivity of tested germs.

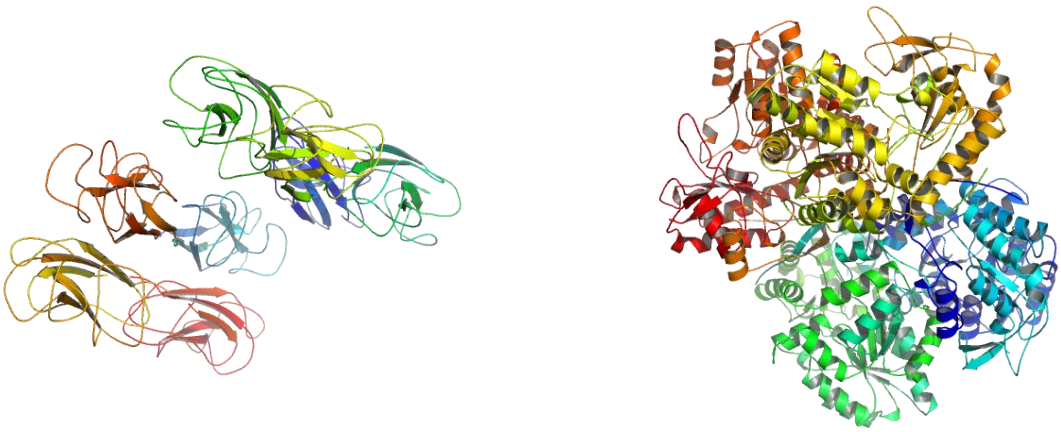
Microorganism test	Chemotherapeutic agent (control +)	R	IS	S
Staphylococcus aureus	amoxicillin	≤15	16-19	≥20
Escherichia coli	levofloxacin	≤13	14-16	≥17
Proteus vulgaris	amikacin	≤14	15-16	≥17
Pseudomonas aeruginosa	ceftazidime	≤14	15-17	≥18
Klebsiella pneumoniae	cefotaxime	≤14	15-22	≥23

2.3. THE THEORETICAL STUDY OF THE ANTIBACTERIAL EFFECT USING AUTODOCK 4.2

Gaussian software (DFT / B3LYP / 6-31G) is used in the first stage to optimize antibiotics and polyphenolic compound (allicin).



Staphylococcus aureus *Escherichia coli* *Klebsiella pneumoniae*



Pseudomonas aeruginosa *Proteus vulgaris*

Figure 1. Bacterial targets used in the docking technique.

The X-ray crystalline structure of the bacterial species was taken from the Protein Data Bank (3Q8U code (Fig. 1) for *Staphylococcus aureus*, 3T88 code for *Escherichia coli*,

5o79 code for *Klebsiella pneumoniae*, 4lKD code for *Pseudomonas aeruginosa*, 1AX4 code for *Proteus vulgaris*).

Molecular docking analysis was performed using Autodock 4.2.6 software along with the AutoDockTools molecular viewer. Autodock can calculate and visualize the most stable ligand-receptor conformation and can estimate the minimum binding energy. Preparation of the receptor molecules involved the addition of polar hydrogen, calculating the Gasteiger charge; Autogrid 80 × 80 × 95 Å in the x, y and z directions with a distance of 1 Å from target center. All calculations were performed in vacuum. For the docking process was chosen the Lamarckian genetic algorithm, with a population of 150 and a number of 30 rounds [27].

The Gaussian 09 application and the graphical interface GaussView 6.0.16 was used in order to provide the optimized molecules using the extension * zmt. In the next step were drawn the molecules with the descriptor assignment program and note the obtained values. The calculation of the molecular descriptors was done using MOPAC 2016, in the files out we could find the physico-chemical information of the substances studied [28].

3. RESULTS AND DISCUSSION

Fresh juices of both onion and garlic have antibacterial effects, possessing a wide range of action. All bacterial tested species are considered to be sensitive to Allium cepa and Allium sativum, respectively but the diameter of the bacterial growth inhibition zone was smaller compared to the reference antibiotics (Table 2).

Table 2. Average mean diameters [mm] of bacterial growth inhibition.

Sample	***Staphylococcus aureus***	***Escherichia coli***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***
Allium cepa	25.5***	27.4***	28.3***	19.7***	26.7***
Allium sativum	27.3***	28.2***	29.1***	21.9***	27.7***
amoxicilin	32.6***	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 - not tested*

The dipole moment is an important molecular parameter, which shows the partial separation of the electrical charge in the molecule, providing information about the physicochemical behavior of the molecules (Table 3).

Table 3. Molecular descriptors.

Compound	μ [D]	ΔE	ΔHf [kcal/mol]	TotE [eV]	MSA [A^2]	CMV [A^3]
allicin	3.121	7.987	-0.182	-1519.394	187.51	200.22
amoxicillin	5.639	8.468	-148.094	-4433.118	340.41	421.46
levofloxacin	8.604	7.666	-157.624	-4686.033	342.82	398.89
amikacin	7.049	10.492	-592.785	-8053.688	481.60	645.44
ceftazidime	17.24	6.695	-49.446	-6231.421	440.79	552.34
cefotaxime	5.492	7.926	-160.093	-5544.037	417.14	479.84

Allicin has the lowest dipole moment value, which anticipates lower antibacterial activity compared to antibiotics of choice (Table 3). Receptor-ligand interaction is favorable if there is an interaction of the boundary orbitals: HOMO (the highest energy level in the

electron-occupied molecule) and LUMO (the lowest electron-occupied molecular level) that expresses how a molecule will interact with a biological receptor through electron transfer processes. The energy difference between HOMO and LUMO levels ($\Delta E = E_{LUMO} - E_{HOMO}$) is a chemically important molecular descriptor, which explains the stability of the molecule, a low value indicating that the molecule is highly reactive (Table 3).

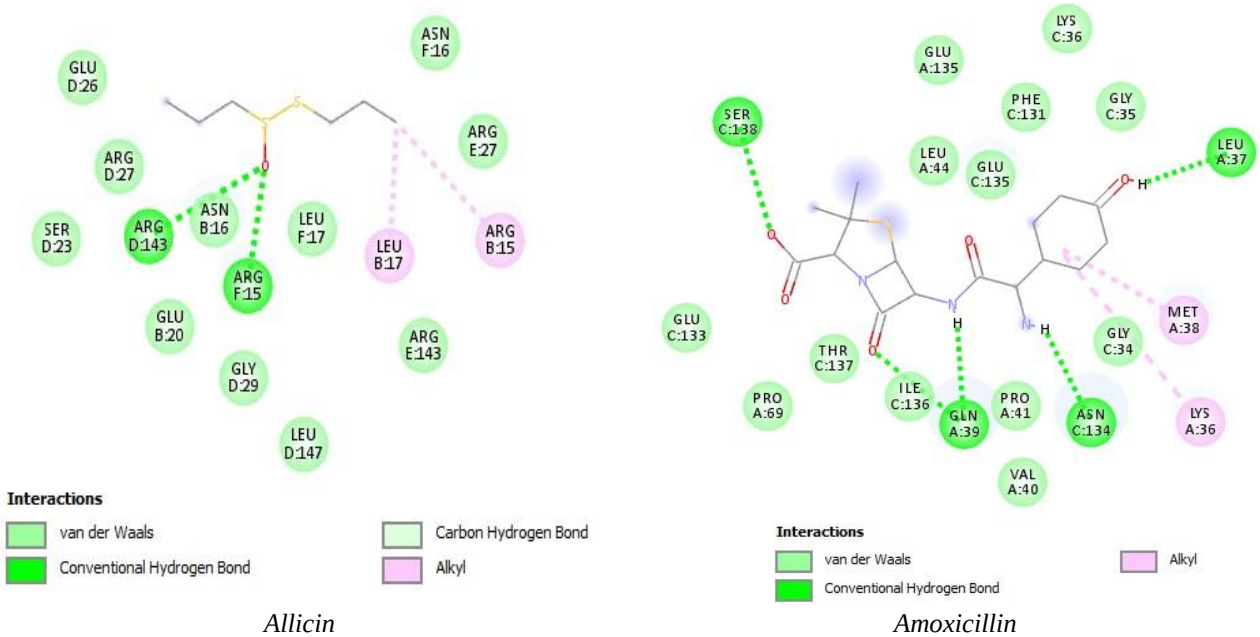
All antibiotic molecules are more reactive than allicin, which explains their higher antibacterial activity. The total energy of a molecule is the expression of stability, a lower value meaning high stability. Allicin is a less stable compound compared to the reference antibiotics. Molecular surface area (MSA) is a geometric descriptor (Table 3) that characterizes the molecular compound and play an important role in the target-ligand interaction [29].

The COSMO (Mopac) calculation process generates a polygonal surface around the molecules, at van der Waals distance, being accessible to the polar groups of the solvent and the stationary phase. The lowest value of the geometric descriptors belongs to allicin, correlating with the lower antibacterial potency. A high value of the molecular surface area correlated with a high polarity of the molecule that means a high interaction with the target. The enthalpy of formation represents the amount of heat released or absorbed during the formation of the chemical species, a small value of which reflects the stability of the substance [30]. Allicin has the highest value of the enthalpy of formation, having lower stability compared to the reference antibiotics (Tables 3 and 4).

Table 4. Binding energy value [kcal/mol].

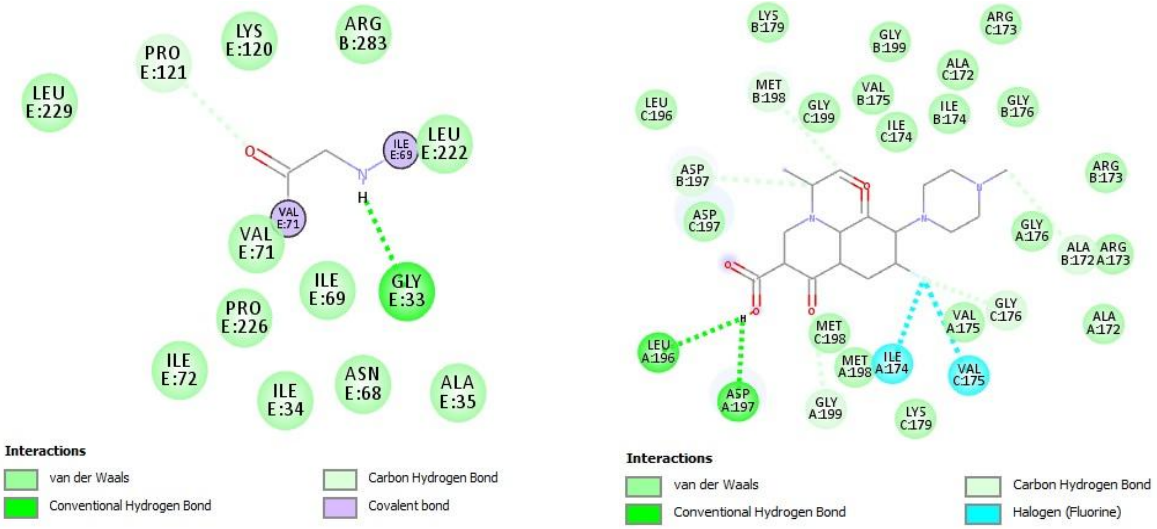
Sample	***S. aureus***	***E. coli***	***P. vulgaris***	***P. aeruginosa***	***K. pneumoniae***
allicin	-3.75	-5.20	-4.21	-4.12	-3.43
amoxicillin	-4.43	-	-	-	-
levofloxacin	-	-6.07	-	-	-
amikacin	-	-	-2.03	-	-
ceftazidime	-	-	-	-3.03	-
cefotaxime	-	-	-	-	-3.34

The docking studies (Figs 2 - 6) were performed with allicin, because it is the major organosulfur compounds in garlic and onion being considered to be biologically active [31].



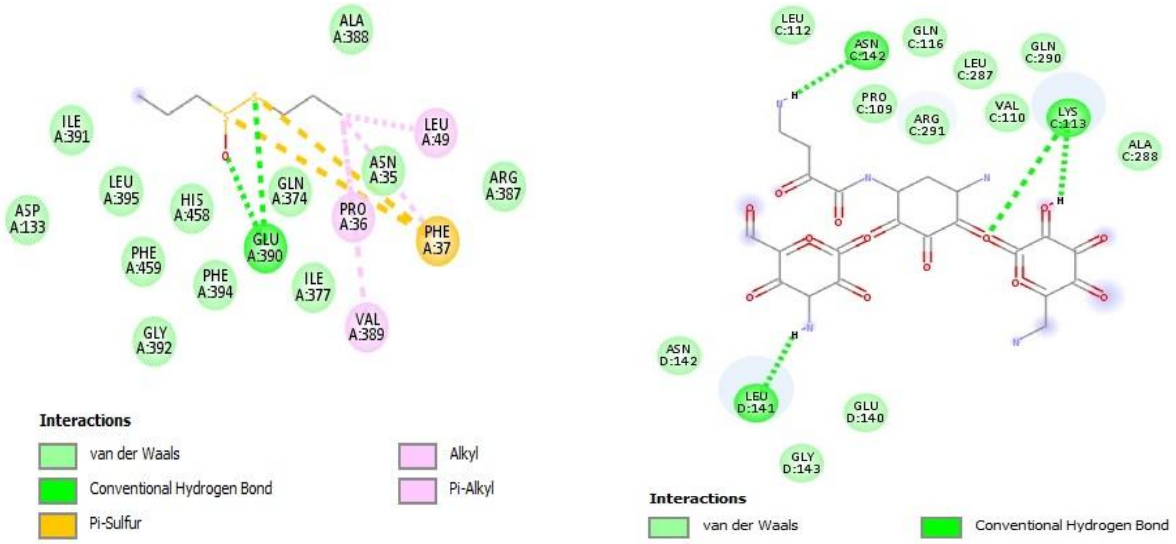
Allicin *Amoxicillin*

Figure 2. 2D interaction maps between ligands - *Staphylococcus aureus*



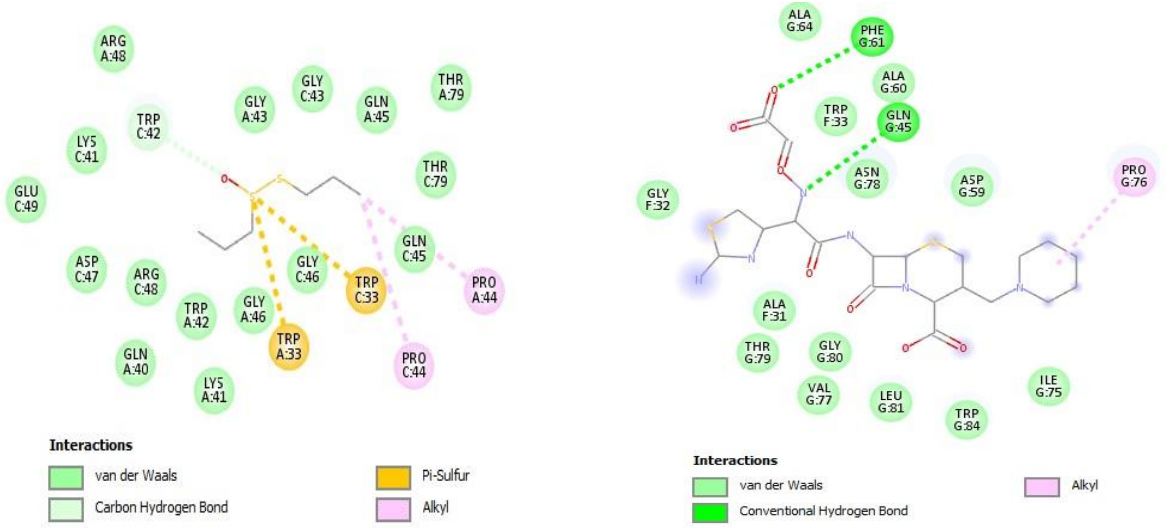
Allicin *Levofloxacin*

Figure 3. 2D interaction maps between ligands - *Escherichia coli.*



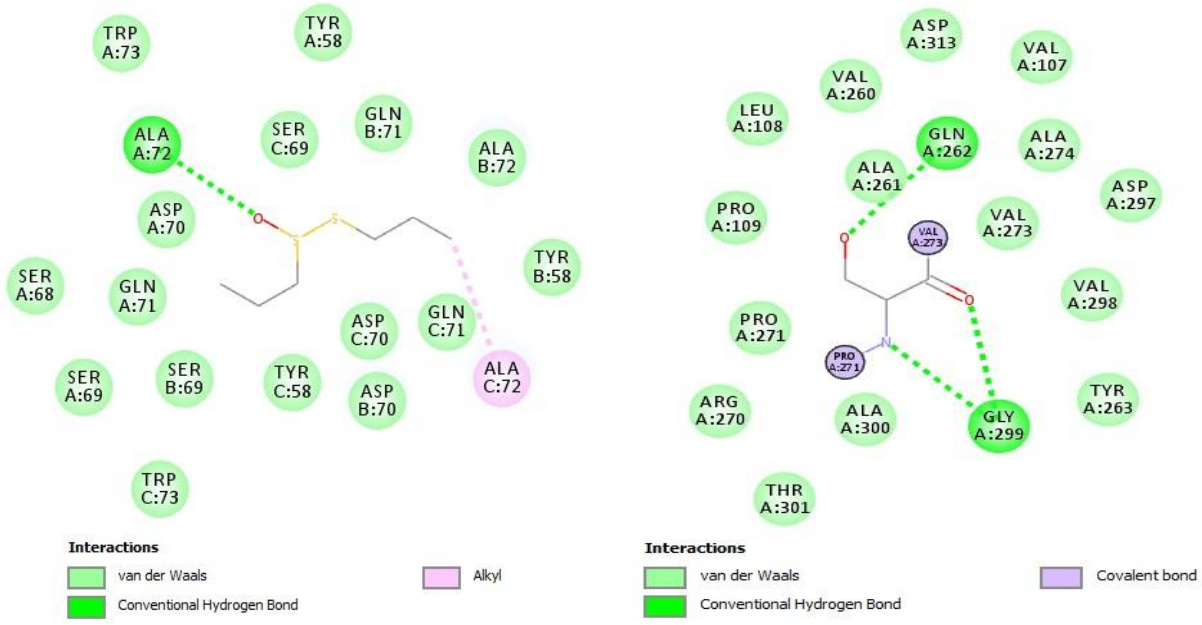
Allicin *Amikacin*

Figure 4. 2D interaction maps between ligands - *Proteus vulgaris.*



Allicin *Ceftazidime*

Figure 5. 2D interaction maps between ligands- *Pseudomonas aeruginosa.*



Allicin *Cefotaxime*

Figure 6. 2D interaction maps between ligands - *Klebsiella pneumoniae.*

From the 2D interaction maps, it can be observed that allicin and the reference antibiotics did not have a common site of action, allicin binding to the common site of polyphenolcarboxylic acids.

4. CONCLUSIONS

Physico-chemical descriptors represent valuable information about chemicals, being the result of quantum chemical calculations performed computationally with various software. The pharmaceutical industry benefits from such advances in quantum chemistry and molecular modeling, stimulated by the impressive vanguard of computing techniques. The driving force in drug research is the acquisition of new pharmacological agents, which help to characterize, at the molecular level, fundamental aspects of physiology and pharmacology.

Herbal extracts with antimicrobial potential represent an important research directive in the current medical world, with the aim of isolating the active components, in order to develop new chemotherapeutic agents with applicability in the treatment, biomarkers [32], or use as adjuvant therapy in infectious conditions. Both plant products tested have antibacterial effect, although they bind to different site of the bacterial targets, the bacterial species being classified as sensitive to their action.

REFERENCES

[1] Rotaru, L.T., Istratoaie, O., Udrescu, L., *et al*., *Rev. Chim. (Bucharest)*, **69**, 1939, 2018.
[2] Varut, R.M., Gird, C.E., Rotaru, L.T., et al., *Pharm. Chem. J*., **51**, 1088, 2018.
[3] Radulescu, C., Stihi, C., Ilie, M., Lazurca, D., Gruia, R., Olaru, O., Bute, O., Dulama, I. D., Știrbescu, R.M., Teodorescu, S., Florescu, M., *Analytical Letters*, **50**(17), 2839, 2017.
[4] Radulescu, C., Olteanu, R.L., Stihi, C., Florescu, M., Lazurca, D., Dulama, I.D., Stirbescu, R.M., Teodorescu, S., *Analytical Letters*, **52**(15), 2393, 2019.

[5] Buruleanu, L., Radulescu, C., Georgescu, A.A., Nicolescu, M.C., Olteanu, R.L., Dulama, I.D., Stanescu, G.S., *Analytical Letters*, **52**(8), 1195, 2019.
[6] Buruleanu, L., Radulescu, C., Georgescu, A.A., Danet, A.F., Nicolescu, M.C., Olteanu, R.L., Dulama, I.D., *Analytical Letters*, **51**(7), 1039, 2018.
[7] Radulescu, C., Olteanu, R.L., Stihi, C., Florescu, M., Stirbescu, R.M., Stanescu, S.G., Nicolescu, C.M., Bumbac, M., *Journal of Chemometrics*, **34**(6), e3234, 2020.
[8] Alecu (Holban), G.-C., Olteanu, R.L., Radulescu, C., Stirbescu, R.M., Necula, C., Boboaca-Mihaescu D.-N., *Journal of Science and Arts*, **2**(51), 475, 2020.
[9] Radulescu, C, Stihi, C., Barbes, L., Chilian, A., Chelarescu, E.D., *Rev. Chim. (Bucharest)*, **64**(7), 754, 2013.
[10] Rotaru, L.T., Varut, R.M., *JOSA*, **1**(50), 135, 2020.
[11] Telehoiu Bordei, A.T., Nuță, D.C., Căproiu, M.T., Dumitrascu, F., Zarafu, I., Ioniță, P., Bădiceanu, C.D., Avram, S., Chifiriuc, M.C., Bleotu, C., Limban, C., *Molecules*, **25**(2), article no. 266, 2020.
[12] Limban, C., Nuta, D.C., Missir, A.V., Roman, R., Caproiu, M.T., Dumitrascu, F., Pintilie, L., Stefaniu, A., Chifiriuc, M.C., Popa, M., Zarafu, I., Arsene, A.L., Pirvu Dinu, C.E., Udeanu, D.I., Papacocea, I.R., *Processes*, **8**(5), article no. 503, 2020.
[13] Radulescu, C, *Rev. Chim. (Bucharest)*, **56**(2), 151, 2005.
[14] Radulescu, C, Hossu, A.M., *Rev. Chim. (Bucharest)*, **56**(7), 742, 2005.
[15] Gazuwa, S.Y., Makanjuola, E.R., Jaryum, K.H., *et al*, *Asian J. Exp. Biol. Sci*., **4**(3), 406, 2013.
[16] Slimestad, R., Fossen, T., Vagen, I.M., *J. Agric. Food Chem*, **55**(25), 10067, 2007.
[17] Bnouham, M., Ziyyat, A., Mekhfi, H., *et al*, *Int J Diabetes Metab.*, **14**, 1, 2006.
[18] Lee, E.J., Rezenom, Y.H., Russell, D.H., et al., *Fochd*., **131**(3), 852, 2012.
[19] Martínez, M.C., Corzo, N., *Trends Food Sci Tech*., **18**(12), 609, 2007.
[20] Durak, I, Kavutcu, M., Aytaç, B., et al., *J. Nutr. Biochem*. **15**(6): 373, 2004.
[21] Rohner, A., Ried, K.S., Igor, A., Bucher, H.C., et al., *Am. J. Hypertens*, **28**(3), 414, 2015.
[22] Zhou, Y., Zhuang, W., Hu, W.L., et al., *Gastroenterology*. **141** (1), 80, 2011.
[23] David, M., Serban, A., Radulescu, C., Danet, A.F., Florescu, M., *Bioelectrochemistry*, **129**, 124, 2019.
[24] Woods, G.L., Washington J.A., Antibacterial susceptibility tests: dilution and disk diffusion methods, *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, DC, 1995, 1327.
[25] Varut, R.M. Rotaru, L.T., *Rev. Chim. (Bucharest)*, **68**, 228, 2017.
[26] Varut, R.M., Rotaru, L.T., *Rev. Chim. (Bucharest)*, **68**, 1419, 2017.
[27] Florescu, C., Rotaru, L.T., Varut, R.M., et al. *Rev. Chim. (Bucharest)*, **69**, 837, 2018.
[28] Rotaru, L.T., Nedelea, P., Varut, R.M., et al, *Rev. Chim. (Bucharest)*, **70**, 814, 2019.
[29] Varut, R.M., Rotaru, L.T., Varut, M.C., *Rev. Chim. (Bucharest)*, **68**, 1776, 2017.
[30] Istratoaie, O., Rotaru, L.T., Varut, R.M. et al., *Rev. Chim. (Bucharest)*, **69**, 459, 2018.
[31] Rahman, M.S., *Int. J. Food Prop*., **10**, 245, 2007.
[32] Dinescu, V.C, Puiu, I, Dinescu, S.N, et al. *Rev. Chim. (Bucharest)*, **70**, 63**,** 2019.