Disciplinarum Scientia. Série: Naturais e Tecnológicas, Santa Maria, v. 23, n. 3, p. 79-102, 2022.
Recebido em: 10.11.2022. Aprovado em: 19.12.2022.

ISSN 2176-462X

DOI: 10.37779/nt.v23i3.4391

IN SILICO EVALUATION OF PHARMACOKINETIC PARAMETERS OF NATURAL BIOACTIVES IN THE OIL OF *Olea europaea L.* FOR POSSIBLE APPLICATIONS IN SKIN INFECTIONS[1]

AVALIAÇÃO IN SILICO DE PARÂMETROS FARMACOCINÉTICOS DE BIOATIVOS NATURAIS PRESENTES NO ÓLEO DE Olea europaea L. PARA POSSÍVEIS APLICAÇÕES EM INFECÇÕES CUTÂNEAS[1]

Aline Rossato[2], Walter Paixão de Souza Filho, Mirkos Martins[3], Liana da Silva Fernandes[3] e Michele Rorato Sagrillo[3]

ABSTRACT

Olive oil (*Olea europaea L.*) contains many useful substances that positively affect human health; they are called bioactive compounds and include phenolic compounds with high antioxidant potential, a-tocopherols (vitamin E), triglycerides, sterols and essential fatty acids such as oleic and linoleic acids. Its molecules give the oil several beneficial health effects due to its antihypertensive, antidiabetic, anticancer, antiatherosclerotic and anti-inflammatory activities. The discovery of new medicines, such as those from medicinal plants, is a more complex problem than it was in the past. The problem is the complexity of the molecules of medicinal plants. In this context, in silico studies, which use computer systems that store, manipulate and display chemical structures and the information associated with them, have become an important tool and increasingly used in research. Based on this, this work sought to evaluate the biological and toxicological parameters of the main natural bioactives of olive oil (oleic and linoleic acid) through the study of in silico predictions, with computational tools (Pass online, Molinspiration , pkCSM and ProTox-II) and discover the best points of interaction between the compounds with proteins that form bacterial biofilms, through molecular docking. Although this is a preliminary study, which requires further clinical trials and evidence, the results obtained in the study showed that the major fatty acids present in olive oil can be strong candidates to be used in biological applications because in prediction studies computational and molecular docking, proved to be biologically safe, with good pharmacological properties and with good ligand-protein interactions.

Keywords: Olive oil, Bioactive Compounds, Computational Analysis.

RESUMO

O óleo de oliva (Olea europaea L.) contém muitas substâncias úteis que afetam positivamente a saúde humana; eles são chamados de compostos bioativos e incluem compostos fenólicos com alto potencial antioxidante, a-tocoferóis (vitamina E), triglicerídeos, esteróis e ácidos graxos essenciais, como ácidos oleico e linoleico.As suas moléculas conferem ao óleo vários efeitos benéficos à saúde devido às suas atividades anti-hipertensiva, antidiabética, anticancerígena, anti-aterosclerótica e anti-inflamatória. A descoberta de novos medicamentos, como os de plantas medicinais, é um problema mais complexo do que era no

1 Study performed at Nanosciences Postgraduate Program - Fransciscan University (UFN).

2 PhD Students of the Nanosciences Postgraduate Program - Fransciscan University (UFN). E-mail: aline.rossato@ufn.edu.br, walter.paixao@ufn.edu.br

3 Professors of the Graduate Program in Nanosciences - Franciscan University (UFN). E-mail: mirkos@ufn.edu.br, liana@ufn.edu.br, sagrillomr@ufn.edu.br

passado. O problema é a complexidade das moléculas das plantas medicinais. Nesse contexto, os estudos in sílico, que utilizam sistemas computacionais que armazenam, manipulam e mostram estruturas químicas e as informações a elas associadas, têm se tornado uma ferramenta importante e cada vez mais utilizada em pesquisas. Com base nisso, este trabalho buscou avaliar os parâmetros biológicos e toxicológicos dos principais bioativos naturais do óleo de oliva (ácido oleico e linoleico) por meio do estudo de predições in sílico, com ferramentas computacionais (Pass online, Molinspiration , pkCSM e ProTox-II) e descobrir os melhores pontos de interação entre os compostos com proteínas formadoras de biofilme bacteriano, através do docking molecular. Embora este seja um estudo preliminar, o qual requer a realização de mais ensaios clínicos e comprovações, os resultados obtidos no estudo demonstraram que os ácidos graxos majoritários presentes no óleo de oliva podem ser fortes candidatos para serem utilizados em aplicações biológicas pois nos estudos de predições computacionais e docking molecular, mostraram-se biologicamente seguros, com boas propriedades farmacológicas e com boas interações ligante-proteínas.

Palavras-chave: *Óleo de oliva, Compostos Bioativos, Análises Computacionais.*

INTRODUCTION

As an essential bond between the body and the external climate, our skin acts as the main line of defense against the entry of pathogens. *Staphylococcus aureus*, *Streptococcus pyogenes, Pseudomonas aeruginosa*, *Escherichia coli*, and fungus, such as *Candida albicans*, have an adverse effect on the skin, can all contribute to skin infections. (DIAS *et al*., 2022). Microbiological control of pathogenic microorganisms is necessary to prevent infections, reduce human-to-human transmission and prevent the emergence of resistant strains of bacteria (RODRÍGUEZ-BAÑO *et al*., 2015).

With the advent of multidrug-resistant pathogens, there is a growing interest in natural products, including essential oils and plant extracts, in bacterial and fungal skin infections (SOUNOUVOU *et al*., 2021). *Olea europaea L*. oil, popularly known as olive oil, contains many suitable substances that positively affect human health; they are called bioactive compounds and include phenolic compounds with high antioxidant potential, a-tocopherols (vitamin E), triglycerides, sterols and essential fatty acids such as oleic and linoleic acids, phytosterols and squalene (SÖNMEZ *et al*., 2020).

In addition to its sensory attributes, olive oil stands out among other vegetable oils thanks to its chemical composition. The presence of oleic and linoleic acid and, above all, minor constituents give olive oil the status of a product that benefits human health. These benefits are related to cardiovascular diseases and cancer, anti-inflammatory actions, and LDL cholesterol transporter reduction levels (TRIPOLI *et al*., 2005; COVAS *et al*., 2006). In addition, oils have exceptional properties for skin care, such as improving skin hydration and elasticity and exerting a protective, emollient and regenerating action (SANCHEZ-RODRIGUEZ *et al*., 2019).

he discovery of new medicines, such as those from medicinal plants, is a more complex problem now than it was in the past. The problem is the complexity of the molecules of medicinal plants. In this context, *in silico* studies, which use computer systems that store, manipulate and display

chemical structures and the information associated with them, have become an important tool and are increasingly used in research. Computing shows promise for allowing the early detection of problematic molecules and for guiding research toward molecules with great potential (OLIVEIRA *et al*., 2018).

Based on this, this work sought to evaluate the biological, toxicological parameters and main pharmacological activities of the natural bioactive of Olea europaea L. oil through the study of in silico predictions with computational tools (Pass online, Molinspiration, pkCSM, and ProTox- II), and molecular docking, to highlight the main pharmacological activities and theoretical oral bioavailability, as well as possible antimicrobial activities, for potential application in cutaneous infections.

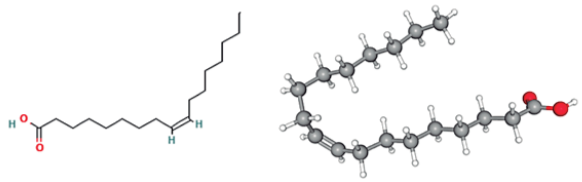
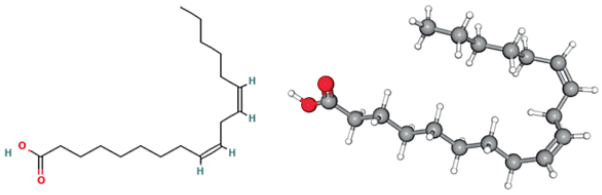
MATERIALS AND METHODS

IN SILICO BIOLOGICAL ACTIVITY ASSAYS

Test substances

The test substances used in the study were the major constituents of olive oil (oleic acid and linoleic acid). All chemical information for the test substances was obtained from the NIH (National Institutes of Health) Pubchem® website (https://pubchem.ncbi.nlm.nih.gov) for chemical information (canonical SMILES form) of the molecules as per shown in table 1. These codes were later copied into computational analysis tools (software).

Table 1 - 2D and 3D structure and SMILES codes of major compounds in olive oils. Data obtained from the National Center for Biotechnology Information (NCBI) (2021).

COMPOST	OLEIC ACID
Chemical Structure (2D;3D)	
IUPAC name	(Z)-octadec-9-enoic acid
SMILES code	CCCCCCCCC=CCCCCCCCC(=O)O
COMPOST	LINOLEIC ACID
Chemical Structure (2D;3D)	
IUPAC name	(9Z,12Z)-octadeca-9,12-dienoic acid
SMILES code	CCCCCC=CCC=CCCCCCCCC(=O)O

Source: Author's construction.

COMPUTATIONAL PREDICTIONS

Prior to the simulations, open-source platforms were used for computational prediction of properties about the chemical structures used in the research. The information obtained from the data made it possible to analyze the safety profile and bioactive characteristics of the compounds. For this purpose, the following platforms were used: Pro-Tox-II and pkCSM for toxicity assessment; Molinspiration and PASS online® for bioactivity assessment, in addition to other properties.

To analyze the theoretical oral bioavailability of the product, the Molinspiration program (http://www.molinspiration.com/cgi-bin/properties) was used. Molinspiration calculates the physicochemical properties relevant to the solubility and permeability of a given compound, such as the octanol-water partition coefficient (Kow), the topological polar surface area (TPSA), the number of atoms (nAtom), the number of number of acceptors (nON) or hydrogen bond donor (nOHNH), the number of bond rotations (nRot), volume and molecular mass (MM) (LIPINSKI *et al.,* 1997), which are associated with Lipinski's Rule , also known as Lipinski's Rule of Fives (LIPINSKI, 2004).

For predictions of ADMET properties (adsorption, distribution, metabolism, elimination, toxicity) the pkCSM platform (http://biosig.unimelb.edu.au/pkcsm/) was used. It proposes graph-based properties that encode patterns of distance between atoms, representing small molecules and thus testing predictive models. The interpretation of the values in the table is obtained by the column "values/reference units", while the results yes and no suggest activity or not of the respective forecast.

For the analysis of the pharmacological properties of the constituents of olive oil, the PASS online® software (http://way2drug.com/passonline/) was used. The activity spectrum prediction for substances - PASS online®, is a software that aims to evaluate the biological potential of an organic molecule when in contact with the human body. With this, it is possible to have simultaneous predictions of various types of biological activities based on the structure of organic compounds, in addition to allowing the estimation of the activity potential of a substance, which can be classified as Pa indices ("probability of being active") and Pi ("probability of being inactive").

Finally, for the analysis of hepatotoxicity, carcinogenicity, mutagenicity and cytotoxicity, a free ProTox-II software was used, using the address: https://tox-new.charite.de/protox_II. The free ProTox-II software results page shows us the predicted median lethal dose (LD50) in mg/kg body weight, toxicity class and prediction accuracy, as well as the mean similarity along with three most similar toxic compounds from the set of Rodent data known oral toxicity value.

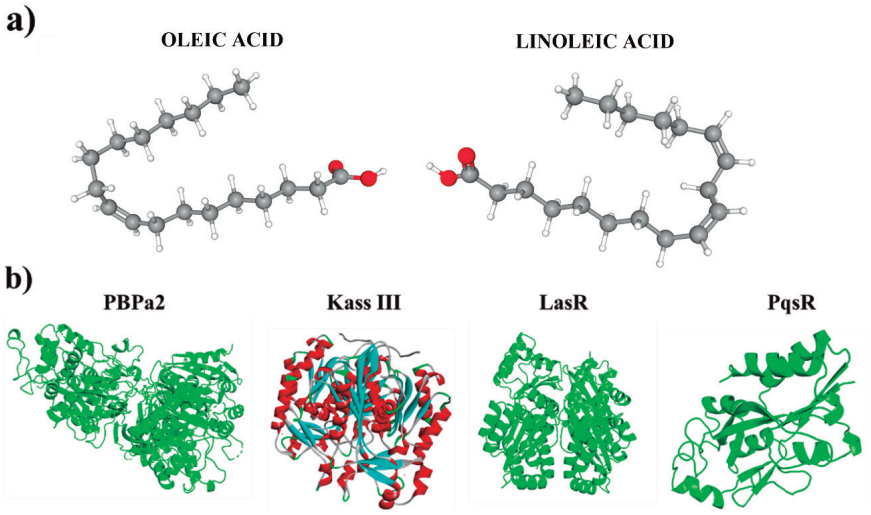
It is worth mentioning that these computational prediction platforms have been used in pharmacokinetic studies aiming at the discovery of new drugs, since evaluating the cytotoxicity and bioactivity profile in an economical way in terms of time, financial and material resources is important in the current scenario (AMMAR; AMMAR, 2017; PATHAK *et al*., 2017; AZMINAH *et al.,* 2019; CRUZ *et al.,* 2019; BALMEH *et al.,* 2020; BITEW *et al.,* 2021; DORCHEH; BALMEH; SANJARI, 2022; FERRARI, 2021).

Molecular Docking

The molecular docking study was carried out using the AutoDock Vina® program, which consists of a set of open source software that allows the modeling of structures, more specifically, provides the 3D geometric and energetic adjustment between a macromolecule (protein) and a small molecule (ligand) (TROTT and OLSON, 2009). Thus, the evaluation consisted of the interaction between the major fatty acids of olive oil with four proteins present in the formation of the biofilm of *Pseudomonas aeruginos*a bacteria (LasR and PqsR proteins), methicillin-resistant *Staphylococcus aureus* (PBP2a protein), and *Propionibacterium acnes* (protein KASS III) in order to verify the affinity and mode of binding between these systems.

The ligands (Figure 1a) were obtained from the Pubchem® website (https://pubchem.ncbi.nlm.nih.gov) of the NIH (National Institutes of Health) and their structures were converted to the PDBQT format using the AutoDockTools4 software. The 3D structures of inflammatory proteins, LasR - PDB ID: 2UV0; PqsR - PDB ID: 4JVD; PBP2a - PDB ID: 1VQQ and KASS III - PDB ID: 6A9N (Figure 1b) were acquired from the PDB (Protein Data Bank) database, and optimized in PyMol® and AutoDockTools format.

Figure 1 - (a) Molecular structures of the ligands and (b) 3D structures of the bacterial proteins used.



Source: Author's construction.

Subsequently, the search space for the protein by the ligand was delimited by the parameterization of the docking box. The center of the box, which refers to the active sites of the proteins, was predicted by the Deepsite® software (freely available at https://www.playmolecule.org) (JIMÉNEZ *et al*., 2017). The dimensions of the docking box were automatically calculated using a script (available free of charge at https://www.brylinski.org/eboxsize), according to the size of each of the binders (FEINSTEIN; BRYLINSKI, 2015).

After the optimization of the structures, the Autodock Vina® was run to perform the molecular coupling, setting the parameter exhaustivity to 8, the default value used in docking experiments (FORLI *et al.,* 2016). To model the complexes formed between proteins and ligands, the free energy of binding (FEB) was calculated based on the Gibbs free energy function (ΔG). This is obtained by adding the individual molecular mechanical terms of the chemical potentials, according to equation 1:

$$FEB \approx \Delta G(kcal/mol) = \Delta G_{vdW} + \Delta G_H + \Delta G_{electrost} + \Delta G_{int} \quad (1)$$

where ΔG_{vdw} are the van der Waals interactions, ΔG_H the hydrogen bonds, $\Delta G_{eletrost}$ the electrostatic interactions and ΔG_{int} the intramolecular ligand interactions.

ΔG values are commonly expressed in kcal.mol-1, where negative values indicate affinity between the structures of the receptor-ligand complex, while positive values and greater than zero indicate absence of affinity and, therefore, an unfavorable docking (ASLANLI *et al*., 2020). Furthermore, the more negative the value of ΔG, the greater the spontaneous interaction of the receptor-ligand system (DU *et al*., 2016). Together with ΔG, another important metric for docking is the root mean square deviation or RMSD (Root Mean Square Deviation), represented by equation 2 and whose value is associated with a favorable docking when RMSD < 2 Å and different from 0 Å , serving as a form of validation for each interaction (SCHWEIKER; LEVONIS, 2020). Only favorable interactions were selected for the final analysis of docking results (RMSD < 2 Å and RMSD ≠ 0 Å).

$$RMSD\ (pose_{i_{lig}}, pose_{i_{prot}})\ (\text{Å}) = \sqrt{\frac{\Sigma_n (atom_{(i_{lig})} - atom_{(i_{prot})})^2}{n}} \quad (2)$$

where RMSD represents the average distance between atoms in a system, n the average position of all atoms in the system, and and the average position of atoms in the system. ligand and protein, respectively.

Finally, the analysis of the 3D result of the simulations was performed using the software Pymol™ 1.7.x and the non-covalent intermolecular interactions of the macromolecule-ligand complex were analyzed using 2D diagrams automatically plotted by the software LigPlot v.4.5.3 (LASKOWSKI; SWINDELLS, 2011).

RESULTS AND DISCUSSION

ASSESSMENT OF ORAL BIOAVAILABILITY IN SILICO

In the analysis of pharmacological parameters, the theoretical oral bioavailability of the product was evaluated according to Lipinski's "Rule of Five". The 2 major compounds in *Olea europaea L.* oil

violated only one rule. The prediction results obtained through the Molinspiration website (http://www.molinspiration.com/cgi-bin/properties) for the compounds studied are presented in Table 2.

Table 2 - Analysis of the theoretical in silico oral bioavailability of the compounds. Results obtained through the Molinspiration website (http://www.molinspiration.com/cgi-bin/properties).

Bioavailability Tests - Molinspiration								
Compost	PubChem CID	Nº of violations	MiLogP	TPSA	nON	nOHNH	MM	nRot
Oleic acid	445639	1	7.08	37.3 Å	2	1	268,44	14
Linoleic acid	5280450	1	5.85	37.3 Å	2	1	252,40	12

miLogP (octanol/water partition coefficient) = ≤5; TPSA (topological polar surface area) = ≤140 Å; nON (number of hydrogen bond acceptor groups) = ≤10; nOHNH (number of hydrogen bond donor groups) = ≤5; MM (molar mass) = ≤ 500 g.mol-1; nRot (number of rotating bands).

The results obtained (Table 2) were compared with Lipinski's Rule of Fives (LIPINSKI *et al*., 1997). The value of miLogP should not exceed five, indicating low hydrophilicities that lead to high values of miLogP, resulting in poor absorption or permeation (TEIXEIRA *et al*., 2013). Based on the results found in this Molinspiration prediction, the values of oleic acid and linoleic acid were above the value recommended by Teixeira *et al*., (2013) indicating a low hydrophilicity of the compounds.

According to Lipinski's Rule of Five, a bioactive molecule to be absorbed by passive diffusion must have a miLogP less than 5, MM must not exceed 500 daltons (Da), polar surface area (TPSA) less than or equal to 140 Å2 or the sum of the number of acceptors and donors of hydrogen bonds less than 12; and must not have more than 5 nOHNH functional groups and 10 nON groups. Such a rule was formulated to assess similarity or determine whether a chemical compound with a given biological or pharmacological activity has properties that make it a likely drug for oral use in humans (LIPINSKI, 2004). Thus, the molecule may have only one violation of one of these parameters to be a drug candidate.

In addition to these parameters analyzed, there is also the parameter number of rotating bands (nrotb), which is related to the flexibility of the molecule to predict bioavailability, because the greater its flexibility, the easier the interaction with the enzyme. The flexibility of the molecule is associated with the number of rotating bonds, which corresponds to the number of single bonds, outside a ring, attached to a non-terminal atom. The simpler the bonds the molecule has, the greater the interaction with the enzyme, facilitating the transposition of the barrier, that is, the greater the bioavailability of the drug (OLIVEIRA *et al*., 2018). It is observed in Table 3 that the two compounds had only one of the rules violated, and the miLogP was greater than 5. Bioavailability problems can be solved by applying micro and nanoencapsulation technologies.

ADMET PROPERTIES (ADSORPTION, DISTRIBUTION, METABOLISM, ELIMINATION, TOXICITY)

The pkCSM software was used to analyze the pharmacokinetic and toxicological results of the properties, the results of which are described in Table 3. Oleic and linoleic acids showed high permeability in Caco-2 gastrointestinal cells, with good intestinal and skin permeability, but not permeating the blood-brain barrier, which may be related to its bioavailability.

Table 3 - Analysis of the adsorption, distribution, metabolism, elimination and toxicity properties of the compounds. Results obtained using pkCSM software (http://biosig.unimelb.edu.au/pkcsm/).

Parameters		Compostos majoritários	
		Ác.oleico	**Ác.linoleico**
Absorption	Caco2 permeability	High	High
	Intestinal absorption (human)	High	High
	skin permeability	Good	Good
	P-glycoprotein substrate	No	No
Distribution	Volume of distribution	Low	Low
	BBB permeability	Does not pass 100%	Does not pass 100%
	CNS permeability	Yes	Yes
Metabolism	CYP1A2 inhibitor	No	No
	CYP3A4 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2D6 inhibitor	No	No
Excretion	Total settlement	1,871 mL/min/Kg	1,871 mL/min/Kg
Toxicity	Mutagenic	No	No
	Hepatotoxicity	No	No
	Skin sensitization	Yes	Yes

Source: Author's construction.

ADMET is an acronym used to denote the words absorption, distribution, metabolism, excretion and toxicity. It provides information about the physicochemical properties, drug similarity and physicochemical properties of a compound. The concept of ADMET is based on the fact that for a compound to be orally effective, it must be bioavailable, delivered to selective target areas, properly metabolized, excreted after doing its work, while not causing adverse effects to the body's cells, tissues and organs with which it came into contact. Predicting the ADMET properties of a natural compound early on in the drug discovery process is an important step in increasing the overall chances of success during clinical trials (EGBUNA *et al*., 2021).

Regarding the absorption parameter, we found that both compounds have high permeability in Caco-2 gastrointestinal cells, with good intestinal and skin permeability. In the distribution results, we found that the compounds are permeable through the blood-brain barrier, but do not fully permeate due to their bioavailability, and can easily reach the central nervous system.

For metabolism, the results demonstrate that the compounds are not likely to be metabolized by cytochromes. Human cytochromes P450 (CYPs), a heme-containing superfamily of enzymes with approximately 57 isoforms, catalyze the metabolism of a variety of endogenous and xenobiotic compounds (CHENG *et al.,* 2011). The CYP enzymes of greater clinical significance, 1A2, 2C9, 2C19, 2D6 and 3A4, are responsible for about 90% of oxidative metabolic reactions. Among them, the most relevant is CYP3A4, which acts in the synthesis of lipids, such as cholesterol (WILKINSON, 2005).

Furthermore, from the toxicity test, we found that the analyzed fatty acids can cause skin sensitization, but are neither hepatotoxic nor genotoxic.

IN SILICO TOXICITY ASSESSMENT

Predicting the toxicity of compounds is an important part of the drug development process. Computational estimates of toxicity are not only faster than determining toxic doses in animals, but can also help reduce the amount of animal experimentation (BENERJEE *et al.,* 2018).

ProTox-II incorporates molecular similarity, fragment propensity, more frequent features, and machine learning (CLUSTER cross-validation based on fragment similarity), based on a total of 33 models to predict various toxicity endpoints such as dose median lethal (LD50), class toxicity, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. The results found are shown in table 4.

Table 4 - Results of in silico cytotoxicity tests of compounds using the ProTox-II online platform (https://tox-new.charite.de/protox_II/).

Compost	Expected toxicity class	LD50	Target	Prediction	Probability of being inactive (%)
Oleic acid	II	48 mg/kg	Hepatotoxicity	Inactive	0,55
			Carcinogenicity	Inactive	0,64
			Immunotoxicity	Inactive	0,99
			Mutagenicity	Inactive	1,0
			Cytotoxicity	Inactive	0,71
Linoleic acid	VI	10.000 mg/Kg	Hepatotoxicity	Inactive	0,59
			Carcinogenicity	Inactive	0,65
			Immunotoxicity	Inactive	0,97
			Mutagenicity	Inactive	0,93
			Cytotoxicity	Inactive	0,70

Source: Author's construction.

The prediction of cytotoxicity is important for the screening of compounds that can cause unwanted and desired cellular damage (as in the case of tumor cells) (ZHANG *et al*., 2014).

A significant cause of acute liver failure, drug-induced hepatotoxicity is a major reason for drug withdrawal from the market. Drug-induced liver injury (DILI) is a chronic process or a rare

event. However, the prediction of DILI is important and one of the safety concerns for drug developers, regulators and clinicians (SIRAMSHETTY *et al.,* 2015).

Chemicals can damage cells and result in certain diseases such as cancer. Chemicals that can induce tumors or increase the incidence of tumors are called carcinogens, and chemicals that cause abnormal genetic mutations, such as changes in the DNA of a cell, are called mutagens (AMES *et al.,* 1973). Furthermore, the adverse effect of xenobiotics on the immune system is called immunotoxicity (SCHREY *et al*., 2017).

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose, meaning the dose at which 50% of test subjects die after exposure to a compound (PRO-TOX II). From the results obtained, we verified that the LD50 of the linoleic acid compound was 10,000 mg/Kg, as well as showing toxicity class VI. Oleic acid, on the other hand, had a lower LD50 of 48mg/kg, as well as toxicity class II. Furthermore, the results demonstrated that both compounds were not shown to be hepatotoxic, carcinogenic, immunotoxicogenic, mutagenic and cytotoxic.

In silico pharmacological predictions

In the pharmacological analysis, the major compounds showed several biological effects of therapeutic importance, and for all values of Pa (probability of being active) they were higher than those of Pi (probability of being inactive). The potential biological effects of the main compounds in *Olea europaea L.* oil are shown in table 5.

Table 5: *In silico* predictions of potential biological effects, using the PASS online® tool, of the main compounds.

Activity	Probability	Compounds	
		Oleic acid	Linoleic acid
CYP2J2 substrate	Pa	0,973	0,749
	Pi	0,001	0,022
Antieczematic	Pa	0,947	0,388
	Pi	0,001	0,207
Mucomembranous protector	Pa	0,958	0,949
	Pi	0,003	0,003
CYP2J substrate	Pa	0,974	0,749
	Pi	0,001	0,022
CYP4A11 substrate	Pa	0,902	0,870
	Pi	0,002	0,002
CYP4A2 substrate	Pa	0,869	0,712
	Pi	0,001	0,001
Antimutagenic	Pa	0,852	0,792
	Pi	0,003	0,004
Anti-hypercholesterolemic	Pa	0,744	-
	Pi	0,006	-
Vasoprotectant	Pa	0,872	0,852
	Pi	0,003	0,004
Angiogenesis stimulant	Pa	0,773	0,832
	Pi	0,003	0,002

Anti-inflammatory	Pa	0,614	0,730
	Pi	0,029	0,012
CYP3A1 substrate	Pa	0,213	0,538
	Pi	0,001	0,035
Antiseborrhoeic	Pa	0,803	0,771
	Pi	0,019	0,024
Antithrombotic	Pa	0,707	0,732
	Pi	0,007	0,006
Peptoglycan glycosyltransferase inhibitor	Pa	0,743	0,720
	Pi	0,003	0,003
Cytoprotective	Pa	0,737	0,721
	Pi	0,004	0,004
CYP2C9 inducer	Pa	0,701	0,426
	Pi	0,004	0,058
Antipruritic, antiallergic	Pa	0,649	0,642
	Pi	0,006	0,007
Antiulcer	Pa	0,635	0,658
	Pi	0,008	0,007
Antiviral (herpes)	Pa	0,433	0,526
	Pi	0,023	0,013
Membrane Permeability Enhancer	Pa	0,579	-
	Pi	0,004	-
Anti-infective	Pa	0,665	0,607
	Pi	0,009	0,013
Antifungal	Pa	0,498	0,500
	Pi	0,031	0,030
Antimycobacterial	Pa	0,401	0,440
	Pi	0,039	0,030
CDP-glycerol glycerophosphotransferase inhibitor	Pa	0,428	-
	Pi	0,169	-
Anti-bacterial	Pa	0,332	0,335
	Pi	0,048	0,047
Inhibition of bacterial wall biosynthesis	Pa	0,325	-
	Pi	0,042	-

Source: Author's construction.

Data from the scientific literature indicate that essential fatty acids (oleic and linoleic acids) have several pharmacological activities, such as anti-inflammatory, antimutagenic, cytoprotective, antihypercholesterolemic, antibacterial, among others (DILIKA *et al*., 2000; CARRILLO *et al*., 2012; SALES-CAMPOS *et al*., 2013; PEGORARO *et al*., 2021.) .

In line with what has been said, in this study, following the in silico research model, it is possible to notice that many of these pharmacological activities, previously listed by other researchers, are present in our results after analysis by the PASS online® software, which evaluates the ability of the molecule to have a pharmacological effect based on predictions.

Among the various biological possibilities for the analyzed compounds, strong anti-inflammatory, anti-hypercholesterolemic, antibacterial, antiviral, antimutagenic activities and metabolic

influence on cytochrome P450 enzymatic complexes were indicated, either acting as a substrate or as an enzyme inducer. In addition to these biological effects, the results in silico as a potentiator of membrane permeability were also analyzed. the ac. oleic acid have the potential for this effect (Pa>Pi). The increase in cell permeability facilitates access to medicines (KOHANSKI *et al*., 2010).

Different tests have been used to evaluate the pharmacological properties of substances. In this context, tests that use in silico models stand out (expression used with the meaning of "run on the computer"), which are fast, reproducible and generally based on human bioregulators, thus ensuring safety for the use of the natural product as a future medicine (CRUZ *et al*., 2019).

Pass online® software provides simultaneous predictions of various types of biological activities based on the structure of organic compounds. Therefore, it can be used to estimate biological activity profiles, in relation to virtual molecules, before their chemical synthesis and biological tests. Pa (probability of being active) and Pi (probability of being inactive) assess the categorization of potential compounds to belong to the subclass of active or inactive compounds, respectively (KHURANA *et al.,* 2011). In this study, the fatty acid oleic and linoleic acids, among many pharmacological aspects that they have, have activities that, if explored, can act well as agents, mainly anti-inflammatory and antibacterial, being able to act in the treatment of skin infections.

MOLECULAR DOCKING

Tables 6 and 7 show the grid box4 parameters used. The evaluation consisted of the interaction between the major fatty acids of *Olea europaea L.* oil, with four proteins present in the biofilm formation of the bacteria *Pseudomonas aeruginosa* (LasR and PqsR proteins), methicillin-resistant *Staphylococcus aureus* (PBP2a protein), and *Propionibacterium acnes* (KASS III protein). The central coordinate values for the box determine the active site of each protein.

Table 6 - Center of the docking box for the proteins studied.

Proteins	**Coordinates (x, y, z) - units in Å**
LasR	[23.6; 16.1; 79.5]
PqsR	[-33.2; 57.6; 8.6]
PBP2a	[20.5; 32.1; 39.1]
Kass III	[8.6; 16.8; 181.4]

Source: Author's construction.

Table 7 - Dimensions of the grid box used during docking (calculated according to the binder).

Binders	**Coordinates (x, y, z) - units in Å**
Oleic acid	[19.236; 19.236; 19.236]
Linoleic acid	[19.000; 19.000; 19.000]

Source: Author's construction.

Protein-ligand docking allows an analysis of favorable biochemical interactions to be made, and thus, to predict whether the ligand substance will potentially bind to the receptor (protein) in vitro or in vivo. This study was carried out in AutoDockTools4 software and the results of the best complexation configurations are presented in table 8. For the analysis of the obtained data, it was taken into account that the favorable docking occurs only if the FEB values are negative and the values of RMSD are < 2 Å and different from 0.

Table 8 - Affinity and RMSD values for the best fitting configurations for each studied system.

Binders	Proteins	FEB (kcal/mol)	RMSD (Å)	Mode
Linoleic acid	LasR	-7.3	1.278	4
	PqsR	-6.0	1.802	2
	PBP2a	-4.8	1.705	2
	Kass III	-7.0	1.061	2
Oleic acid	LasR	-7.4	1.034	4
	PqsR	-6.2	1.651	2
	PBP2a	-4.9	1.985	2
	Kass III	-6.9	1.002	3

Source: Author's construction.

The RMSD is an important variable to determine the accuracy of molecular docking, and values below 2 Å are considered successful, indicating the validity of the process (COLE *et al*., 2005). In this study, all RMSD values were below 2 Å.

Furthermore, according to the results found, there is affinity between all ligands and proteins. The FEB values of the linoleic acid - LasR and the oleic acid - LasR complexes had the most negative interaction energy (-7.3 kcal/mol and -7.4 kcal/mol, respectively), being the strongest interaction when compared to the other systems that showed molecular affinity. The other couplings all showed negative energies close (between -7.0 and -4.9 kcal/mol) to the complexes with the best interaction energy. These results, with better interactions, are probably due to the stronger interaction of the majority compounds of the studied olive oil with Gram-negative bacteria.

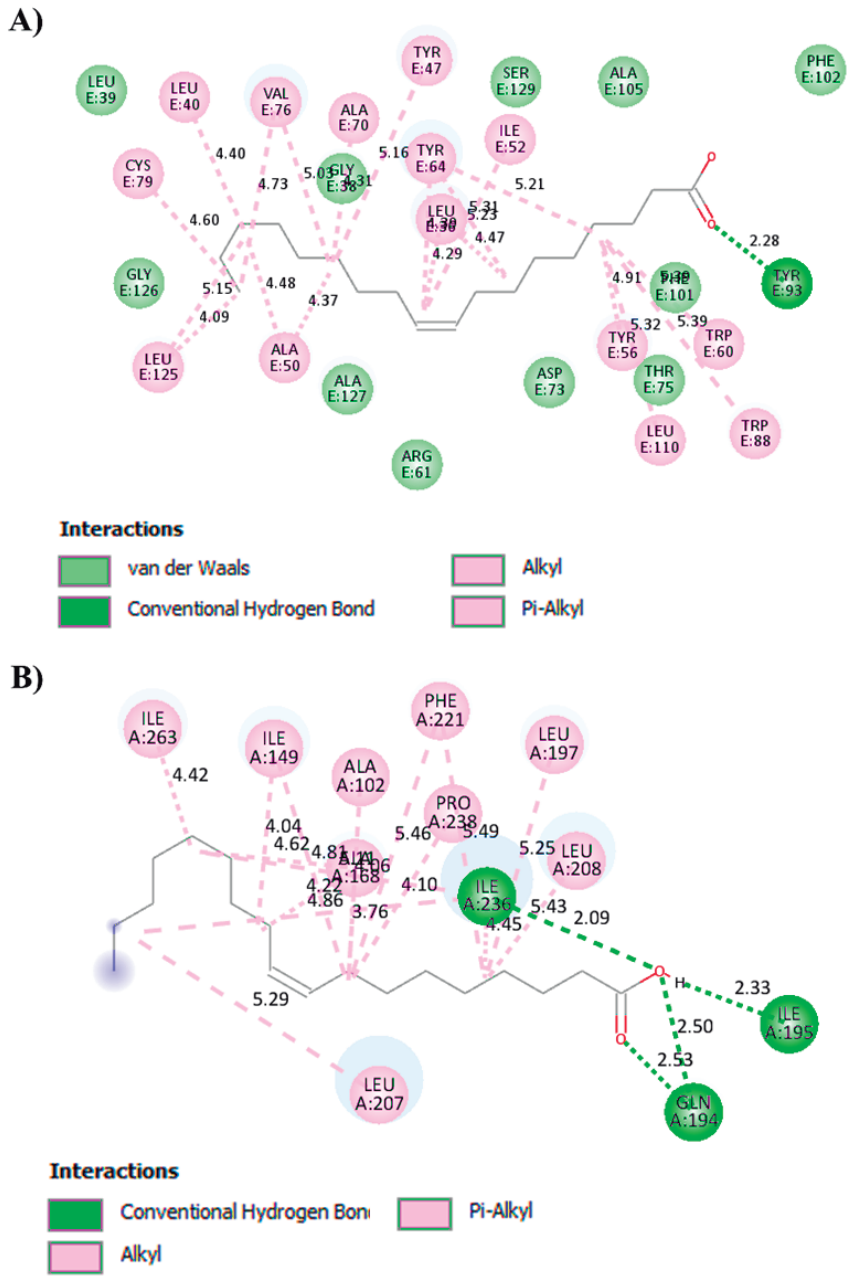
The lowest energy complexes, but still with favorable docking, were the complexes of all fatty acids, with methicillin-resistant Staphylococcus aureus protein PBP2a, probably because it is a multi-drug-resistant microorganism, presenting greater difficulty in interacting with drugs.

The interaction between a protein and a given ligand results in the formation of a protein-ligand complex, since the ligand has high binding affinity and specificity with the protein. The affinity and specificity between the receptor and the ligand are given by intermolecular interactions, among which are Van der Waals forces, hydrophobic interactions, π-π, ionic or electrostatic interactions, hydrogen bonds and covalent bonds (GURYANOV *et al*., 2016). Hydrogen bonds are very important interactions that occur in biological systems and are responsible for maintaining the structure of proteins, and covalent bonds in biological systems are of high energy, and are rarely broken, drugs

that interact through covalent bonds inactivate the receptor site or completely inhibit the action of the enzymes (BARREIRO and FRAGA, 2015).

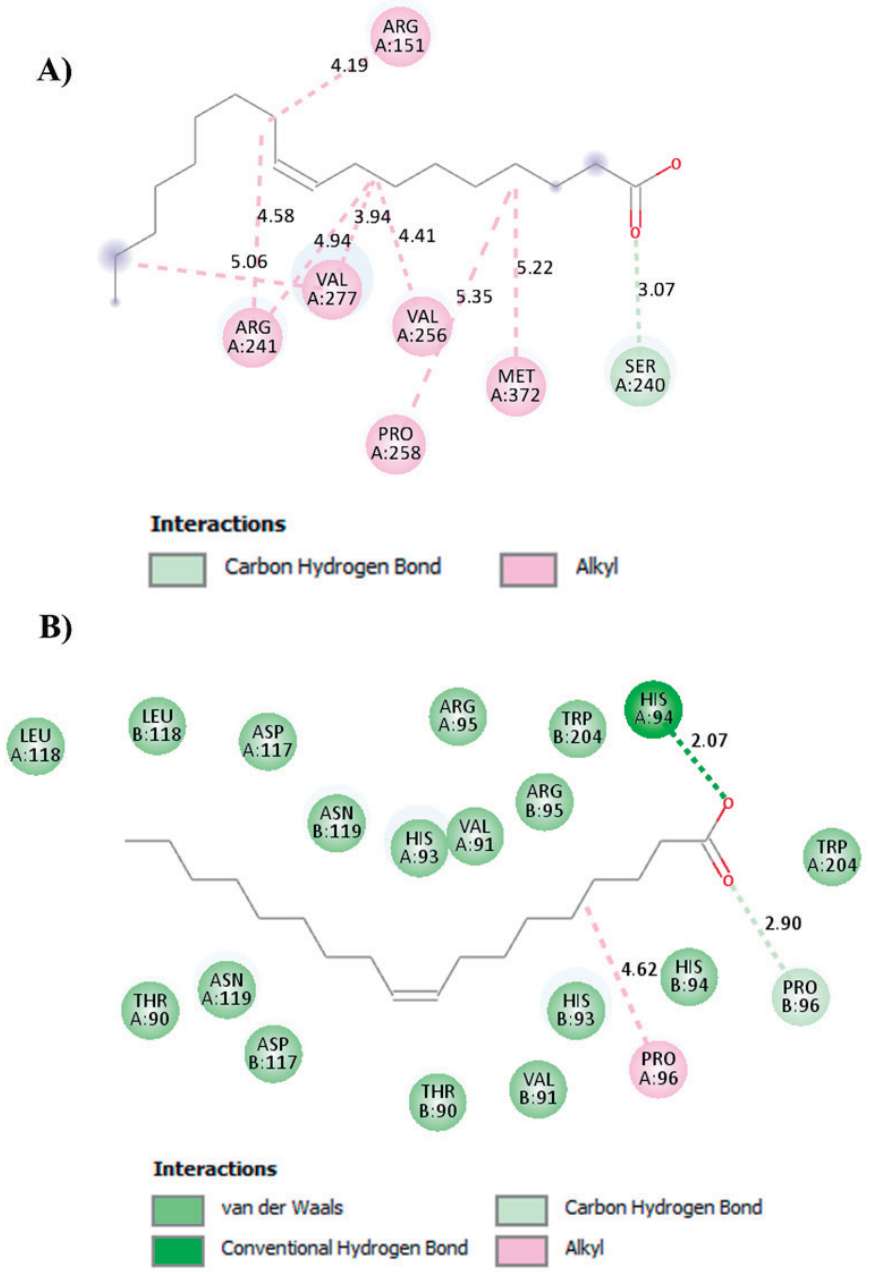
The 2D diagrams that show the amino acid residues that participate in the link between the fatty acid-protein couplings are shown below, in figures 2, 3, 4 and 5 respectively.

Figure 2 - Possible binding sites between the oleic acid ligand and proteins A) LasR and B) PqsR.



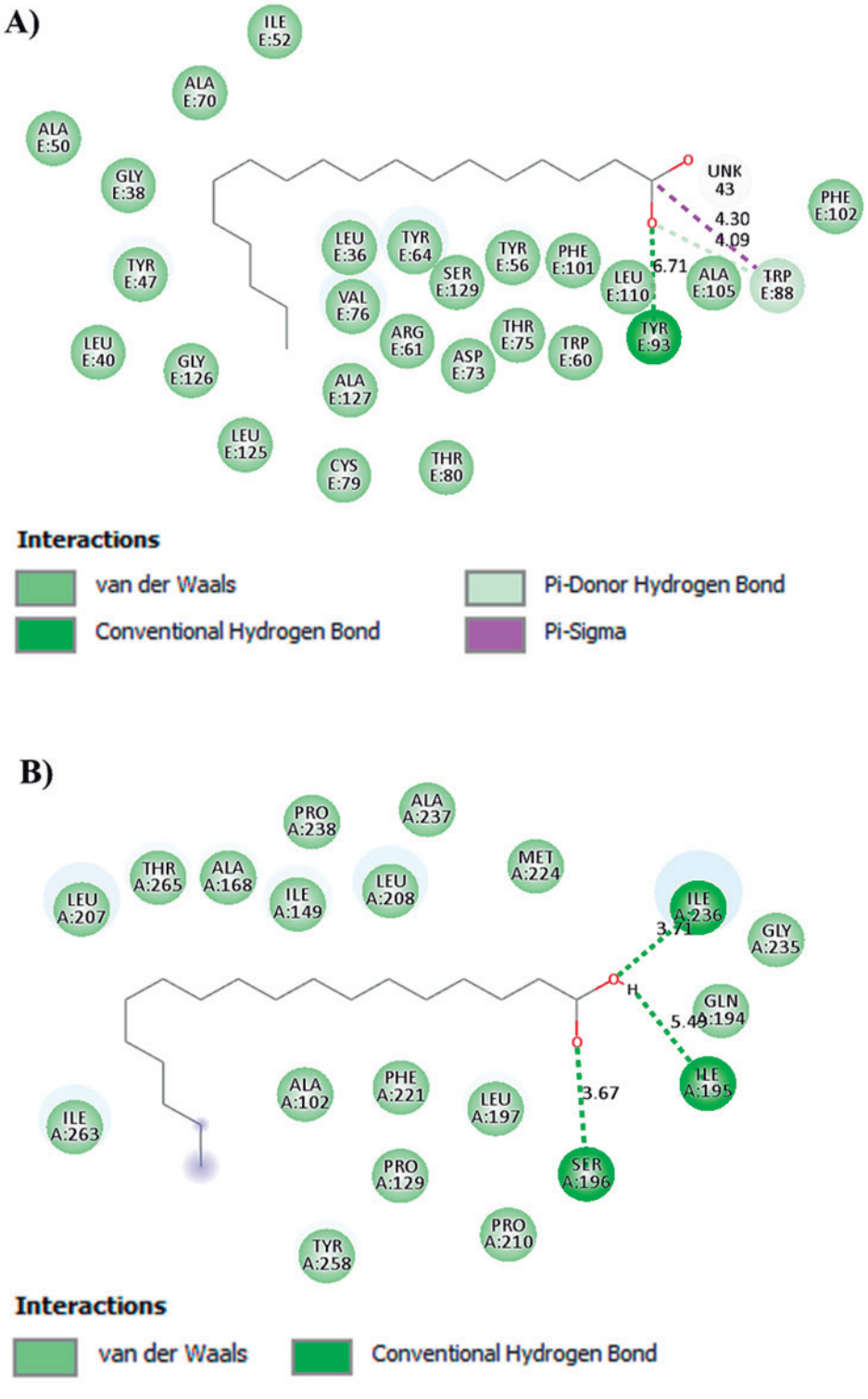
Distances (Å -Angstrom): A) Leucine-LEU125 (4.09; 5.15); Cysteine-CYS79 (4.6); Leucine-LEU40 (4.40); Alanine-ALA50 (4.48; 4.37); Valine-VAL76 (4.73; 5.0); Alanine-ALA70 (4.31); Tyrosine-TYR47 (5.16); Tyrosine-TYR64 (4.47; 4.38; 5.21); Leucine-LEU35 (4.30; 4.47); Isoleucine-ILE52 (5.31); Tyrosine-TYR56 (4.91); Leucine-LEU110 (5.32); Tryptophan-TRP88 (5.39); Tryptophan-TRP60 (5.39); Tyrosine-TYR93 (2.28); **B)** Leucine-LEU207 (5.29); Isoleucine-ILE263 (4.42); Isoleucine-ILE149 (4.04; 4.62); Alanine-ALA102 (4.06); Phenylalanine-PHE221 (5.46; 5.49); Proline-PRO238 (4.10; 5.46); Isoleucine-ILE236 (5.43; 4.45; 3.76); Isoleucine-ILE195 (2.09; 2.33); Glutamine-GLN194 (2.53; 2.50);

Figure 3 - Possible binding sites between the oleic acid ligand and proteins A) PBP2a and B) KASS III.



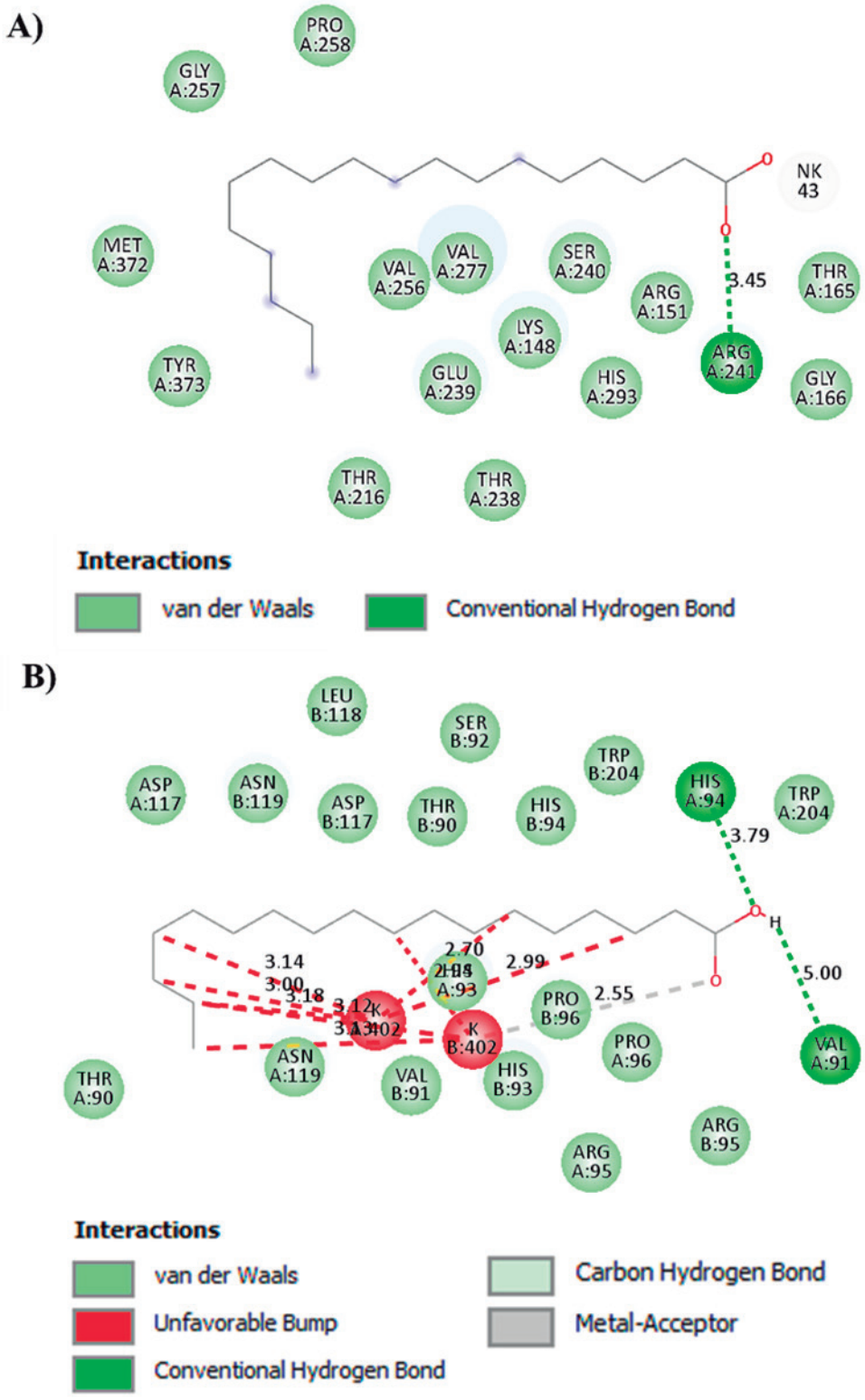
Distances (Å -Angstrom): A) Arginine-ARG241 (4.58; 4.94); Arginine-ARG151 (4.19); Valine-VAL277 (5.06; 3.94); Valine-VAL256 (4.41); Proline-PRO258 (5.35); Methionine-MET372 (5.22); Serine-SER240 (3.07); **B)** Proline-PRO96 (4.62); Proline-PRO96 (2,90); Histidine-HIS94 (2.07).

Figure 4 - 2D images of possible binding sites between the linoleic acid ligand and proteins A) LasR and B) PqsR.



Distances (Å -Angstrom): A) Tyrosine-TYR93 (6.71); Tryptophan-TRP88 (4.09; 4.30).
B) Isoleucina-ILE236 (3.71); Isoleucine-ILE195 (5.49); Serine-SER196 (3.67).

Figure 5 - 2D images of possible binding sites between the linoleic acid ligand and proteins A) PBP2a and B) KASS III.



Distances (Å -Angstrom): A) Arginine-ARG241 (3.35).
B) Histidine-HIS94 (3.79); Valine-VAL91 (5.00); Proline-PRO96 (2.55)

All complexes showed hydrophobic contact interactions, which, although considered relatively weak interactions when compared to the others, are of great relevance in the complexation process with a protein, and hydrogen bonds (more intense intermolecular force) between the carboxyl group of the acids fatty acids and different amino acid residues.

Other important interactions such as alkyl interactions and Pi-alkyl interactions have also been reported. The high affinity of the compounds was also associated with the presence of Van der Waal forces created in the structure of the amide substituents with the respective amino acids, which undoubtedly created a strong cohesive environment, thus stabilizing the formed complex. The interatomic distance was <7 Å, considered relevant for the coupling process between systems in molecular docking (DURRUTHY *et al.,* 2017).

CONCLUSION

Although this is a preliminary study, which requires further clinical trials and evidence, the results presented here allow us to infer that the major active compounds present in the oil of *Olea europaea L*. may be strong candidates for applications in the treatment of skin infections, since in the studies of computational predictions, they were shown to be biologically safe and with good antimicrobial, anti-inflammatory, antifungal, antiviral, mucomembranous protective properties, among others.

The two ligands of the study (oleic and linoleic acids) obey the rules for proposed drugs, obeying the parameters proposed by Lipinski. As for the physicochemical properties related to the pharmacokinetic profile, it was observed that the two ligands have a high probability of permeation in Caco-2 cells, and showed a good percentage of absorption through the intestine. As they present high lipophilicity values, it is indicated that the compounds have a moderate to high probability of crossing the BBB, and penetrating the CNS. The results also indicate that the ligands are not cytotoxic.

In view of the results found, it can be concluded that molecular docking indicated that all substances showed good binding affinity with the proteins present in the formation of the biofilm of bacteria *Pseudomonas aeruginosa* (LasR and PqsR proteins), methicillin-resistant *Staphylococcus aureus* (PBP2a protein), and *Propionibacterium acnes* (KASS III protein), suggesting that the ligands have potential to act as antibacterial agents.

As future perspectives, it is necessary to carry out in vitro and in vivo tests, to better evaluate the pharmacokinetic and antimicrobial efficacy of the binding compounds, since the results represent that the use of olive oil can be a promising strategy for the development of new (nano)materials for application in the treatment of skin infections.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENT

This paper was carried out with the support of the Coordination for the Improvement of Higher Education Personnel-Brazil (CAPES)-Financing Code 001, TELEMEDICINA1690389P.

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