



Molecular Modeling of the Major Compounds of Sesquiterpenes Class in Copaiba Oil-resin

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Authors' contributions

This investigation was performed in collaboration with all authors. Authors NSRS, CFS and CBRS designed the study, wrote the protocol, involved in writing the first draft, participated in data collection.

Authors LKSG, FSB, CSL and DSBB managed the literature search, analyses of the study and manuscript preparation. Authors JRA, CHTPS, LISHM and CBRS performed data interpretation and were actively involved in reading the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the Structure-Activity Relationship (SAR) and pharmacokinetic and toxicological properties (ADME/Tox) of the major compounds of sesquiterpenes class oil-resin extracted from the copaiba, using quantum chemistry methods B3LYP/6-31G*, employing the resulting information as a guide to obtain more stable compounds, less reactive and toxic with better absorption, distribution, metabolism and excretion.

Place and Duration of Study: Laboratory of Modeling and Computational Chemistry (LMCC) at Federal University of Amapá (UNIFAP), Macapá, Brazil, between March 2014 and February 2015.

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Methodology: Major compounds were selected from the literature, totaling 12 compounds, and modeled with the GaussView 5.0 program. The optimization was performed using the DFT method and B3LYP/6-31G* base set implemented in the Gaussian 03 program. Maps of molecular electrostatic potential (MEP's) were generated from the atomic charges, and the construction of the MEP's and frontier orbital's (HOMO and LUMO) were visualized with the aid of Molekel program. Pharmacophore pattern was determined from the online server PharmaGist, and the ADME/Tox properties of the compounds studied were calculated using the online server PreADMET and the results were compared with those of DEREK software.

Results: MEP's in the compounds studied have shown no uniformity in their region of electrostatic potential. HOMO is localized to at the double bonds (C=C), and LUMO orbitals were on the carbon atoms of the double bonds. Pharmacophore pattern evidenced 11 regions hydrophobic. Pharmacokinetic property of human intestinal absorption (HIA) was 100% for all compounds. Cell permeability P_{CaCO_2} was classified as medium, and the values ranged from 56.3475 nm/sec (compound 2) to 23.405 nm/sec (compound 10). Cell permeability P_{MDCK} was classified as high, because presented higher values than 25 nm/sec, ranging from 218.885 nm/sec (compound 2) to 40.0711 nm/sec (compounds 7 and 11). The compounds showed strong plasma protein binding with values ranging from 90.849656% (compound 2) to 100% for the other compounds. Penetration of the blood brain barrier was classified as active ($C_{Brain}/C_{Blood}>1$), and the values ranged from 3.75249 (compound 2) to 15.0642 (compound 10). Therefore, these compounds studied evidenced toxic effects on the CNS. In toxicity tests, the compounds 1-2, 4-6 and 8-10 show mutagenic predictions, and only the compounds 3, 7, 11 and 12 presented non-mutagenic predictions. Carcinogenicity was significant in mouse. However, the predictions in rats the compounds 1-4 and 6-12 presented positive predictions (non-carcinogenic), and only the compound 5 showed a negative prediction (evidence of carcinogenicity), when analyzed in DEREK software the compounds showed no toxiphoric alerts generated by epoxides groups.

Conclusion: The B3LYP/6-31G* method was adequate to optimize the major compounds of copaiba oil-resin. Compounds studied are viable for administration in different pathways, and may have their improved pharmacokinetic properties according to the formulation to enhance the efficacy and safety.

Keywords: *Copaiba oil-resin; B3LYP/6-31G*; MEP's; pharmacokinetic and toxicological properties.*

1. INTRODUCTION

The genus *Copaifera* tree of *Fabaceae* family and sub-family *Caesalpinioideae*, covers more than 30 native species in Latin America, distributed between Honduras and southern Brazil [1]. In Brazil it is widely found in the Amazon region, and its species are known as "*copaiba stands*" or "*pau d'oil*". The incision in the tree trunks provides copaiba oil in the form of an oil-resin yellow to brown coloration, which is used industrially in varnishes, in the restoration of old paintings, such as odor fixative in fragrances, and as a flavor in foods [2,3]. In popular medicine, especially in the Brazilian Amazon, the copaiba oil is used as cicatrizing, anti-inflammatory, antiseptic, anti-tumor, and as an agent to treat bronchitis, and skin diseases [4]. Its chemical composition is predominantly from terpenes. Many drugs are formulated as solutions or added to the liquid in the form of powder or solution. Therefore, there are great chances of sesquiterpene, diterpenes and triterpenes compounds are related with

abovementioned medicinal effects. Thereby, it is necessary to group these in terms of solubility as soluble, insoluble and amphipathic having aqueous solution as reference [5].

With regard to chemical composition of oil-resin from the species of *Copaifera*, it is observed predominance of sesquiterpenes, as the α -humulene, α and β -selinene, β -bisabolene and β -caryophyllene [6,7]. The sesquiterpene compounds are divided into oxygenated sesquiterpenes (alcohols) and sesquiterpene hydrocarbons, and have greater anti-inflammatory activity when compared with compounds of the diterpene class [8,9]. The β -bisabolene has antiinflammatory and analgesic properties and β -caryophyllene is described in the literature as anti-endemic, anti-inflammatory, antibacterial and insect-repelling [10].

The copaiba oil-resin has several ethnopharmacological indications, such as: anti-inflammatory, healing, antimicrobial, leishmanicide, larvicide, antineoplastic and

antinociceptive activities. Such activities were confirmed with *in vivo* pharmacological studies [11]. The substitution of copaiba oil for new drugs confirms their therapeutic efficacy. In addition, the fact that copaiba oil is a natural compound of popular acceptance, especially by the Brazilian Amazon population has no access to pharmaceuticals and public health services. However, this justifies an effort that allows enable its use as herbal medicine, of a form safe, effective and with quality [12]. Therefore it is necessary to know which of the major compounds of oil-resin exhibit molecular stability, chemical reactivity, pharmacophoric region, pharmacokinetic and toxicological properties to enable its use with biological action [13].

In this work, we propose to study the structure-activity relationship and pharmacokinetic and toxicological properties of 12 major compounds of sesquiterpenes class extracted from copaiba oil-resin, using quantum chemistry methods and B3LYP/6-31G* basis set, maps of electrostatic potential (MEP's), frontier orbital's (HOMO and LUMO), derivation of the pharmacophore pattern, determination of pharmacokinetic properties: Human intestinal absorption (AIH), Cell permeability (P_{CaCO_2}), Cell permeability of Maden Darby Canine Kidney (P_{MDCK}), Skin Permeability (P_{Skin}), Plasma protein binding (PPB) and Penetration of the blood brain barrier (C_{Brain}/C_{Blood}), and Toxicological: mutagenicity and carcinogenicity, employing the resulting information as a guide to obtain more stable compounds, less reactive and toxic and with better absorption, distribution, metabolism and excretion.

2. METHODOLOGY

The study was performed in Laboratory of Modeling and Computational Chemistry at Federal University of Amapá, Macapá, Brazil, between March 2014 and February 2015.

2.1 Selection and Modeling of Compounds

Major compounds were selected from the literature [11]. The major compounds of the sesquiterpene class found in copaiba oil-resin used for modeling were: (1) β -caryophyllene, (2) caryophyllene oxide, (3) α -humulene, (4) δ -cadinene, (5) α -cadinol, (6) β -elemene, (7) α -copaene, (8) α -selinene, (9) β -selinene, (10) β -bisabolene, (11) α -cubebene and (12) trans- α -bergamotene, see Fig. 1. Major compounds were

modeled with GaussView 5.0 program [14], following the strategy described: initially the structure of β -caryophyllene, compound 1, Fig. 1, was built and completely optimized with the DFT method and B3LYP/6-31G* basis set implemented in the Gaussian 03 program [15], and all the other structures (see Fig. 1) were built and optimized with the same method and basis set. The frequencies calculations were also calculated using B3LYP/6-31G*, and observed that there were no negative frequencies, thereby ensuring that local minimum energy structures.

2.2 Maps of Molecular Electrostatic Potential (MEP) and Frontier Orbital's (HOMO AND LUMO)

MEP's for β -caryophyllene and 11 derivatives were generated from the atomic charge in the DFT method and B3LYP/6-31G* basis set calculated in the Gaussian 03 program. The construction of the frontier orbital's (HOMO and LUMO), and of the MEP's were visualized with the aid of Molekel program [16].

2.3 Derivation of the Pharmacophore Pattern

Pharmacophore pattern was determined from the online server PharmaGist (<http://bioinfo3d.cs.tau.ac.il/PharmaGist/>) for β -caryophyllene and 11 derivatives. From the derivation, the highest score was chosen by annealing of all the compounds studied [17-19].

2.4 Prediction of Pharmacokinetic and Toxicological Properties (ADME/Tox)

The ADME/Tox properties (Absorption, Distribution, Metabolism, Excretion/Toxicology) of the major compounds were calculated using the online server PreADMET (<http://preadmet.bmdrc.org/>). This server calculates pharmacokinetic properties as: Human Intestinal Absorption (HIA), cell permeability Caco-2 *in vitro* (P_{CaCO_2}), cell permeability of Maden Darby Canine Kidney (P_{MDCK}), skin permeability (P_{Skin}), Plasma Protein Binding (PPB) and the penetration of the blood brain barrier (C_{Brain}/C_{Blood}), and toxicological properties such as: mutagenicity and carcinogenicity [20].

The DEREK software was also used in comparison to the results achieved using the online server PreADMET. The DEREK knowledge-based expert system collected alerts, specific toxic endpoints, including

carcinogenicity, genotoxicity, hepatotoxicity, reproductive toxicity, chromosome mutagenicity, teratogenicity, respiratory damage, neurotoxicity, irritancy, sensitization, thyroid toxicity and skin sensitization, which may each provide toxicity predictions made by the system [21,22].

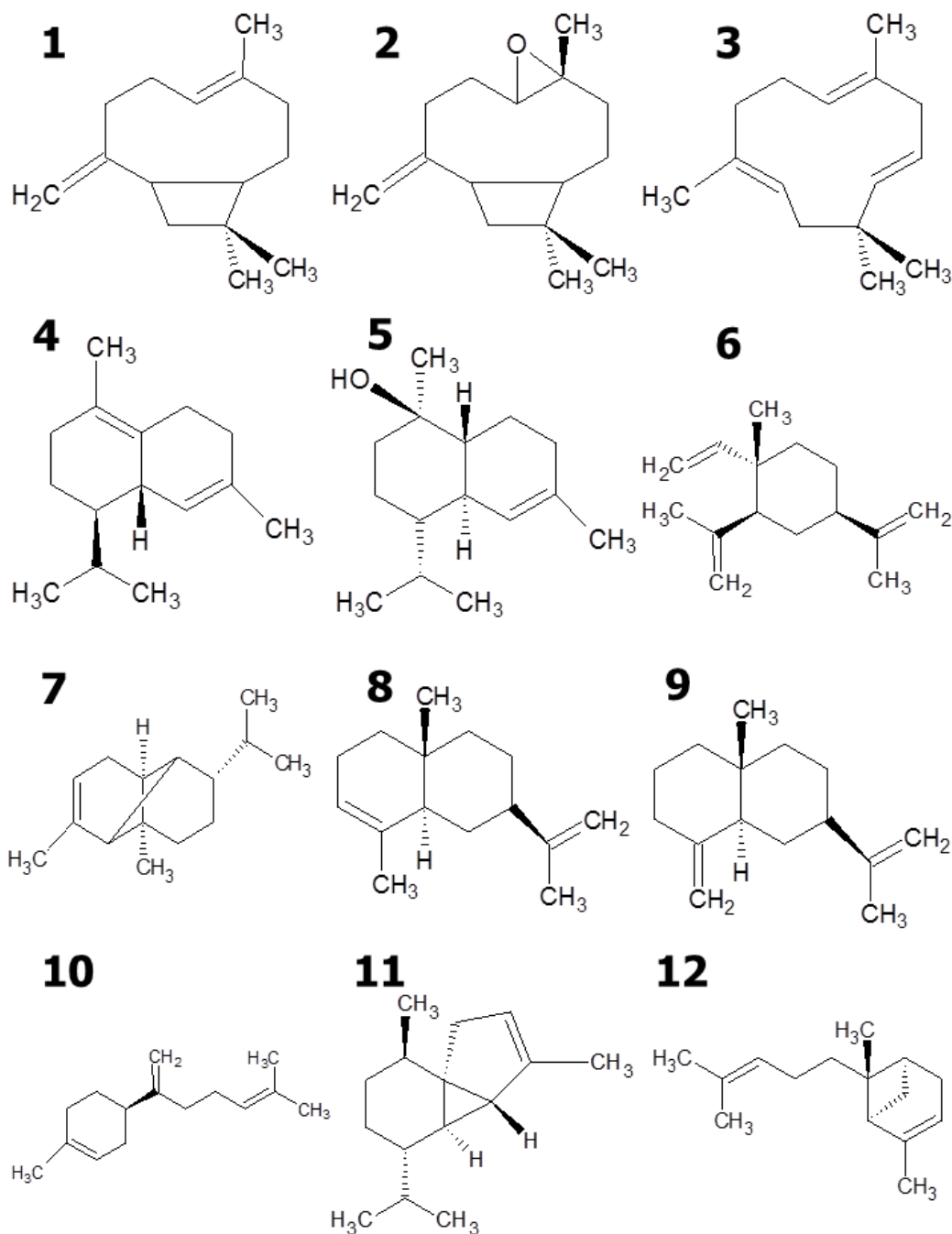


Fig. 1. Structure of β -caryophyllene (1) and its derivatives (2-12) of the sesquiterpene class oil-resin extracted from the copaiba

3. RESULTS AND DISCUSSION

3.1 Maps of Molecular Electrostatic Potential (MEP's)

MEP's allows the use of a qualitative analysis to find reactive sites in a molecule, and determine the roles played by both electronic and steric effects (size/shape) on its power, and it is important that visualization of MEP provide qualitative information on the molecules, such as the behavior of the interaction between a ligand and receptor, ie, is an important tool for process analysis of recognition by another molecule, as drug-receptor interactions and types of enzyme-substrate, due to the potential that a species see another in a biological recognition process [23,24].

MEP's are shown in Fig. 2, and regions highlighted in different colors may vary from green to red and represent the negative regions (indicating excess negative charges which can cause attraction of positively charged molecules), the highlight of these regions in the molecule indicates the region with higher probability of perform nucleophilic attack, and bluish regions represent the positive regions (indicating excess of positive charges which can cause repulsion of positively charged molecules), the evidence of these regions characterizes the portion having a higher probability to suffer nucleophilic attack [25].

The MEP of compound 1 (β -caryophyllene) has a negative potential region (red region) in the double bond ($\text{CH}_2=\text{C}$) in the bicyclo (meta position), having a maximum positive electrostatic potential of 0.04109u.a. (blue region) and minimum negative electrostatic potential of -0.04876u.a. (red region). In compound 2 (caryophyllene oxide) the negative region is on the oxygen atom, its maximum positive electrostatic potential (blue region) was of 0.04558u.a, and minimum negative potential (red region) of -0.09429u.a. In the compound 3 (α -humulene) the negative potential region is located bicyclo inside and close to the double bond at para position, and its maximum positive electrostatic potential was of 0.03804u.a, and minimum negative potential of -0.04587u.a. It was observed that the negative electrostatic potential (orange color) in the compound 4 (δ -cadinene) is located mainly in the aromatic ring of the double bond region, and its maximum positive potential (blue region) was of

0.04153u.a, and the minimum negative potential (red region) was of -0.04947u.a.

The potentially negative region (red region) of compound 5 (α -cadinol) is located on hydroxyl (-OH), and its maximum positive electrostatic potential was of 0.12327u.a, and the minimum negative potential -0.09787u.a. In the compounds 6 (β -elemene) and 7 (α -copaene) the negative region located at methyl group (- CH_3) in the meta position, being the maximum positive electrostatic potential of β -elemene of 0.05351u.a, and the minimum negative potential -0.04665u.a, and the maximum positive electrostatic potential of α -copaene was of 0.03849u.a, and minimum negative potential of -0.03352u.a. The negative regions of the compounds 8 (α -selinene) and 9 (β -selineno) are located predominantly on the double bond ($=\text{CH}_2$), but especially the α -selinene has a negative region in the hydrogen atom (H) of carbon (C) in the (α) alpha position, and in the β -selineno the negative region in the hydrogen atom (H) is located on the carbon (C) in the (β) beta position, being this feature which distinguishes the compounds. The maximum positive electrostatic potential for the compound 8 (α -selinene) is 0.04516u.a, and the minimum negative potential is -0.04711u.a, and for compound 9 (β -selineno) is 0.03643u.a and -0.04779u.a, respectively. Being that the potential difference between the maximum and minimum values of compounds 8 and 9 are $\pm 8.73 \cdot 10^{-3}$ u.a and $\pm 6.8 \cdot 10^{-4}$ u.a, respectively.

In the compound 10 (β -bisabolene) the negative regions are concentrated in the double bonds, being its maximum positive electrostatic potential of 0.02839u.a. (blue region) and minimum negative potential of -0.04564u.a. (red region). The predominant negative regions in the compounds 11 (α -cubebene) and 12 (trans- α -bergamotene) are close to the double bond, and the maximum positive electrostatic potential for the compound 11 (α -cubebene) is 0.02984u.a, and for compound 12 (trans- α -bergamotene) is 0.02349u.a (blue region), the minimum negative electrostatic potential for the compound 11 (α -cubebene) is -0.03839u.a, and for compound 12 (trans- α -bergamotene) is -0.04127u.a (red region). The negative regions have a higher probability of nucleophilic attack, being these regions highlighted in the major compounds of sesquiterpene class of copaiba oil-resin that are possibly binding targets, as shown in Fig. 2.

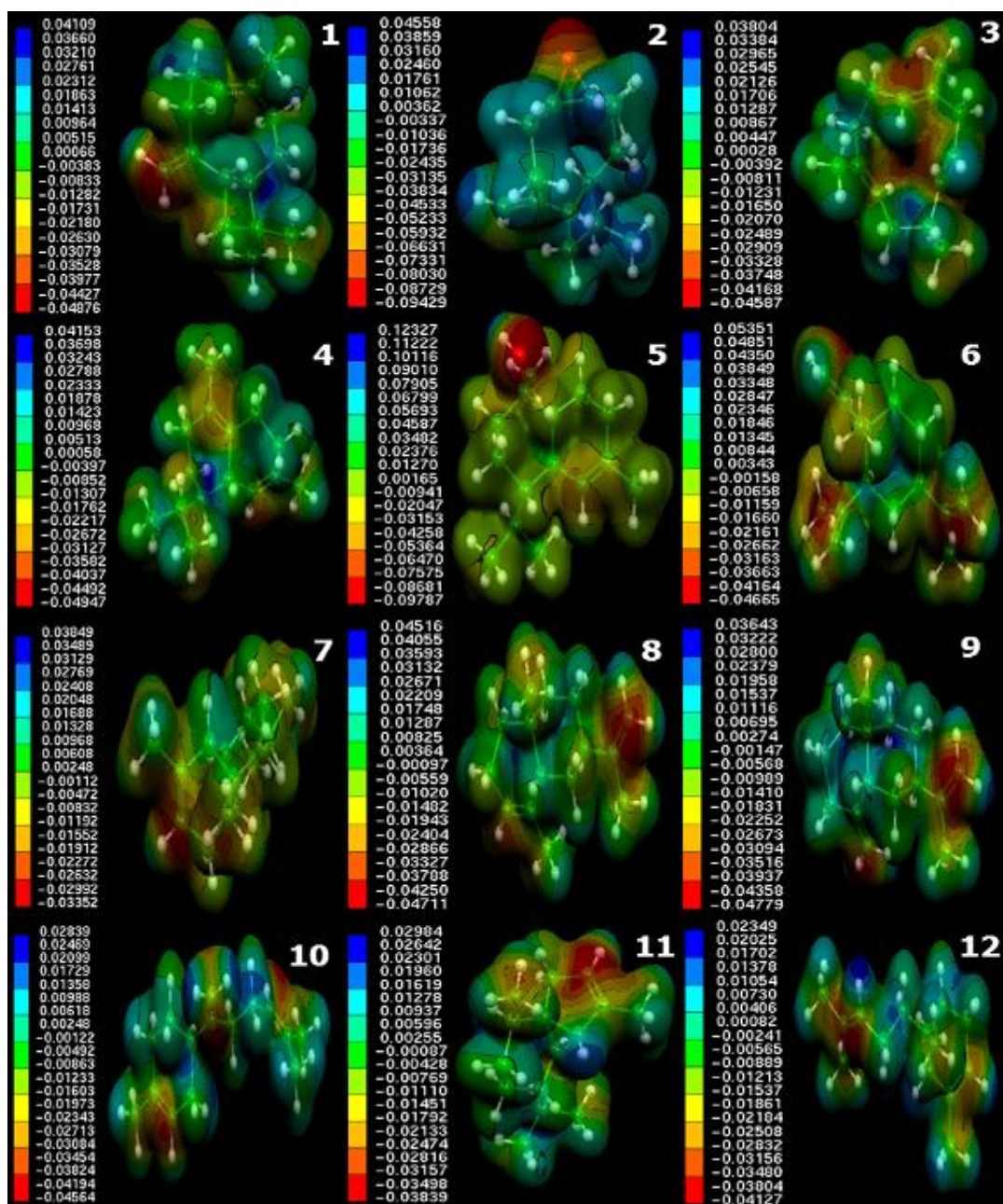


Fig. 2. Potential electrostatic maps of the major compounds of sesquiterpene class of copaiba oil-resin

3.2 Frontier Orbital's (HOMO and LUMO)

The energy of the frontier orbital's is one of the descriptors used in the chemical-quantum studies, being used the highest occupied molecular orbital (HOMO) energies, and the lowest unoccupied molecular orbital (LUMO) energies. This happens because the properties

provide information related to the character electron-donor and electron-acceptor of the compounds and therefore the formation of a charge transfer complex (CTC) [26]. The HOMO energy is related to the electron-donor character, to the ionization potential and features the ability of the molecule to perform nucleophilic attack [27,28].

Fig. 3 represents HOMO orbitals for the compounds studied and their respective values, presenting minimum and maximum value -6.3663eV and -5.6640eV for compounds 2 and 4, respectively. Thus, it is noteworthy that the HOMO values are directly related to the greater tendency to perform nucleophilic attacks (electron-donor trend), ie, higher HOMO value, the greater its ability to perform such an attack. The sesquiterpene compounds 3 (HOMO= -5.7742eV), 4 (HOMO= -5.6640eV), 7 (HOMO=-

5.8942eV) and 11 (HOMO= -5.7190eV) were those with the highest values, therefore higher tendency to perform nucleophilic attacks. The β -caryophyllene (1) compound is found in greater percentage (51%) in the copaiba oil-resin, and volatile portion is one of the major components accounting for 70% of this portion, also being one of those responsible for anti-inflammatory biological activity [11,29], and presented a HOMO value of -6.0031 eV.

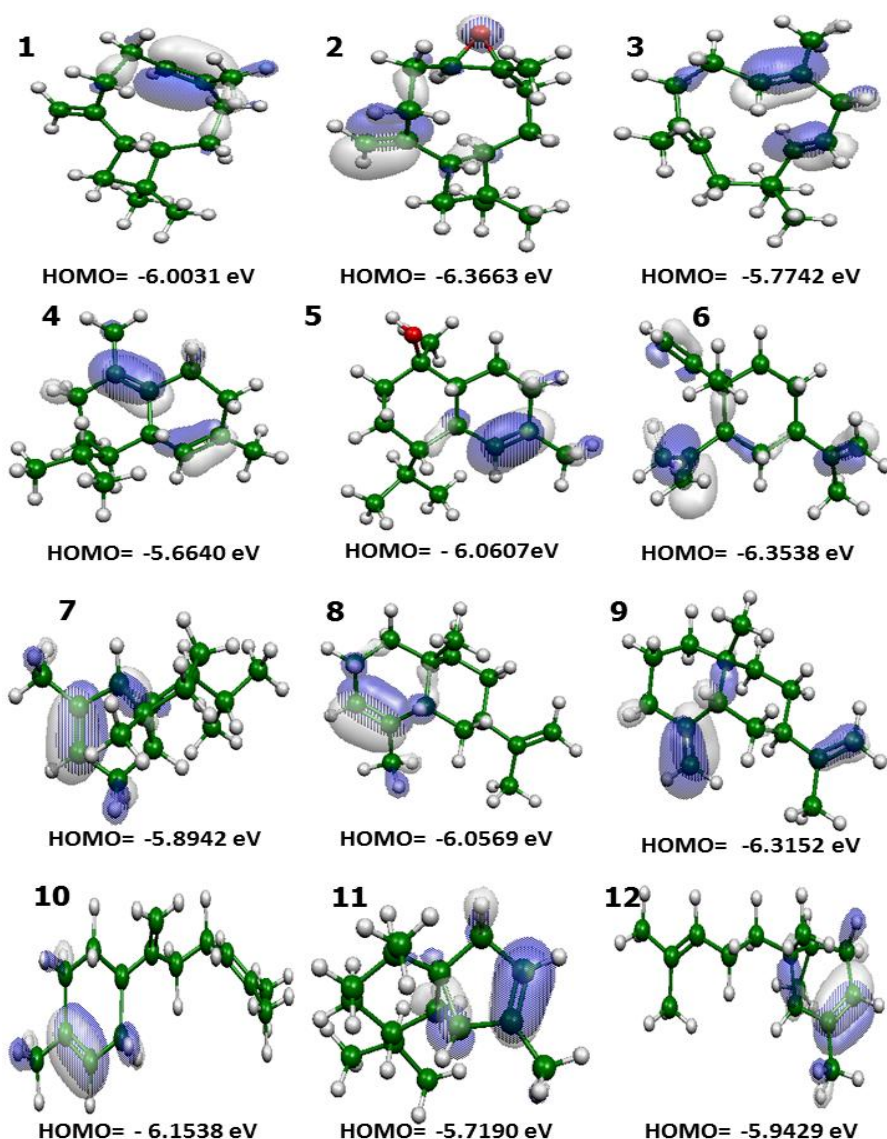


Fig. 3. Frontier orbital (HOMO) of the major compounds of sesquiterpene class of copaiba oil-resin

When analyzing the HOMO orbitals in Fig. 3, it is observed that the globules are double bonds predominantly in the cycles and/or in their ramifications, being also evident in the hydrogens atoms (H) of all compounds studied. In compound 1 (β -caryophyllene) the globules are located on the double bond and single bonds between carbon of the cycle, and on carbon of their ramification. In compounds 2, 6 and 9 that have the smaller HOMO values, the globules are presented in the double bonds located in the ramifications of the cycle, and the compound 2 is shown in double bond between carbons in ramification, in single bond between carbons in the bicyclo, in hydroxyl (-OH) and on the hydrogens atoms (-H); in compound 6, the globules are located specifically between carbons of the cycle in binding (-C=CH₂) in the meta position, on the carbons of binding (-C=CH₂) in the ortho position, in the carbons and hydrogens in ramification of the cycle in the meta position. In the compound 9 these globules are located in bond between carbons of the cycle, in double bond (-C=CH₂) of its ramification in the para position, in the hydrogen bonded directly to the carbon of the cycle, and in double bond (-C=CH₂) in the extreme of the compound.

When comparing the HOMO values and the location of globules with compound 1 (β -caryophyllene) of the compounds studied, note that their values are close and that the location of their globules are similar. Thereby, sharing the characteristic of having tendency to perform nucleophilic attack, favoring the attack with a minimum amount of energy to react with electrons in regions. Unlike of HOMO energy, LUMO energy is related to the affinity by electrons, and consequently the susceptibility of the compounds with respect to nucleophilic attack, presenting electron-acceptor characteristics. The LUMO energy measures the electron-acceptor character of the compounds. However, the smaller energy, the lower it will be this strength to accept electrons [30].

Fig. 4 represents the LUMO orbitals for the compounds studied and values, presenting the minimum and maximum value of 0.2843eV and 0.8822 eV for compounds 2 and 5, respectively. The major compound β -caryophyllene (1) presented LUMO value of 0.4713 eV. When analyzing the representation of LUMO orbitals, Note that the globules are located on carbons that perform double bonds and about hydrogens

(H), differing of HOMO orbitals (occupied orbitals), such globules located on the double bonds. In compound 1 (β -caryophyllene), the globules were located on carbons that perform the double bond in the ramification of the bicyclo. In the compound with minimum value (compound 2) the globules are also located on carbons, and in the compound with maximum value of LUMO (compound 5), the globules follow the pattern of other compounds, located on the carbon atoms that perform the double bond.

Thus, it is noted that there is a similarity between the compound 2 and 5 when compared to compound 1 (β -caryophyllene) which is found in greater proportion in oils of copaiba. Hence, such compounds share of the characteristic in relation to the affinity by electrons in regions where globules are evident, then these regions are more susceptible to nucleophilic attack, and specifically the compound that had the lowest energy value (compound 2) is the one with the least resistance to accept electrons.

3.3 Prediction of Pharmacophore

A pharmacophore is the three-dimensional arrangement (3D) of characteristics that are essential for molecule ligand to interact with a specific mode receptor. The pharmacophore hypothesis is given by the alignment of compounds, identifying these regions share. The identification of a pharmacophore can serve as an important model in the rational drug development, since it can aid in the discovery of novel compounds that can bind to a target receptor [18].

The derivation of the pharmacophore of main compounds of the sesquiterpene class of the copaiba oil-resin was given by the alignment of compound 12 with a score of 12.781, see Fig. 5. The aligned compounds share 11 spatial characteristics, being 11 hydrophobic groups. According to Lipinski Rule proposes that a compound to be well absorbed orally administered needs to adapt the following physical-chemical parameters: molecular weight of less than 500Da., logP (lipophilicity) of less than 5 (five); maximum of five (5) hydrogen donor groups and a maximum of ten (10) groups acceptor [31]. Therefore, the compounds of sesquiterpene class of the copaiba oil-resin did not suit this rule, evidencing that are not viable candidates for this administration route.

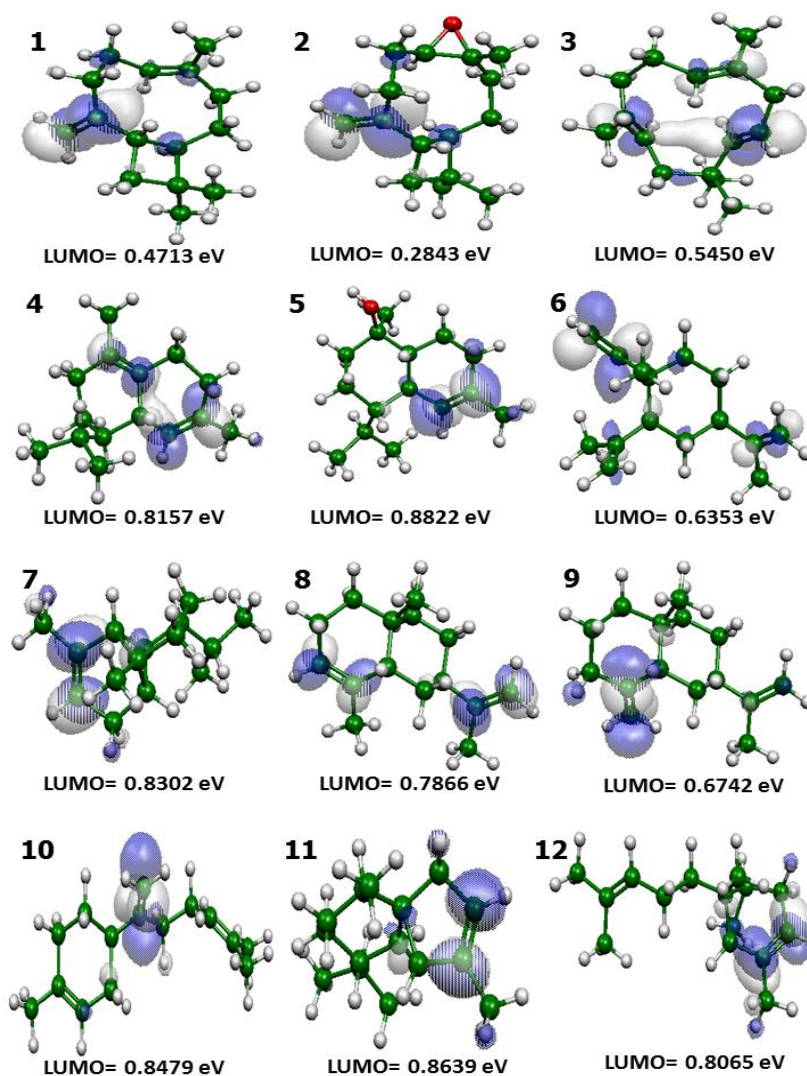


Fig. 4. Frontier orbital (LUMO) of the major compounds of sesquiterpene class of copaiba oil-resin

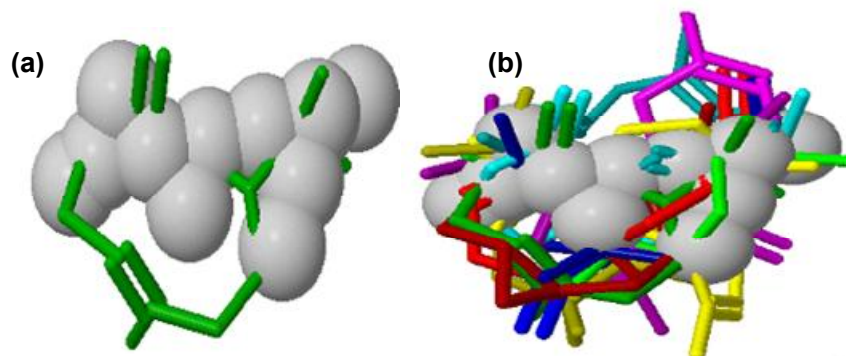


Fig. 5. Pharmacophore group of β -caryophyllene compound (a) and the alignment of the major compounds of sesquiterpene class of copaiba oil-resin (b)

3.4 Prediction of Pharmacokinetic and Toxicological Properties

Currently the discovery of new drugs is not based only by its pharmacodynamics, evaluating the activation or inhibition of biological receptors, also includes the pharmacokinetic process, investigating the influence of the biological system in the effects produced by drug [32]. The prediction of intestinal absorption is the main objective of the discovery and selection of new drugs in order to be administered orally [33]. The absorption processes are related to the permeation of compounds through biological membranes under the influence of the physicochemical characteristics [34].

The results of ADME test (absorption, distribution, metabolism and excretion) can be observe the human intestinal absorption values (HIA), permeability in special cell lineage (P_{Caco2} and P_{MDCK}), skin permeability (P_{skin}), plasma protein binding (PPB) and permeability of the blood-brain barrier (C_{Brain}/C_{Blood}) are shown in Tables 1 and 2. Toxicological tests were evaluated the mutagenicity and carcinogenicity of major compounds, shown in Table 3. The HIA results were 100% for all major compounds classified as good human intestinal absorption, see Table 1.

The Caco-2 permeability (P_{Caco-2}), cell line of human colon adenocarcinoma, enables the evaluation of the potential of intestinal absorption and passive transport of drugs, can be used to predict the dissolution-absorption relationship in

drug discovery using data from dissolved and absorbed fractions in time function [33-35]. Table 1 shows the P_{Caco-2} values, and according with the values obtained may be classified as medium permeability (value between 4 and 70 nm/sec) and high permeability (>70nm/sec). So, it is noted that all the compound showed a medium permeability, and the values ranged from of 56.3475 nm/sec (compound 2) to 23.4050 nm/sec (compound 10), and β -caryophyllene (compound 1) presented a permeability value of 23.6315 nm/sec.

In prediction of MDCK permeability (P_{MDCK}), is used canine kidney cells, possessing similarities with Caco-2 cells. This prediction method is used as a rapid screening tool of permeability [36-38]. The values shown in Table 1 for predicting PMDCK are greater than 25 nm/sec, and classified as compounds which possess high permeability, ranging from of 218.8850 nm/sec (compound 2) to 40.0711 nm/sec (compounds 7 and 11).

The skin permeability (P_{skin}) predicts the possibility of a drug to be transdermally administered [39]. Since this is the parameter used to evaluate of possible risk case the substance accidentally please contact with skin [40]. The sesquiterpenes compounds showed negative values, showing that this administration route (transdermally) would not be the first choice for the possible formulation and administration of the drug, but only compound 6 showed the best value of $P_{skin}=-0.5475$.

Table 1. Absorption properties of the compounds of sesquiterpene class of copaiba oil-resin

Compounds	Absorption			
	HIA(%) ^[a]	P_{Caco2} (nm/sec) ^[b]	P_{MDCK} (nm/sec) ^[c]	P_{skin} ^[d]
1	100	23.6315	56.2164	- 0.6764
2	100	56.3475	218.8850	-1.4101
3	100	23.6330	60.6852	-0.5677
4	100	23.6391	59.3726	-0.7971
5	100	55.4076	169.7890	-1.0828
6	100	23.4917	56.8713	-0.5475
7	100	23.6323	40.0711	-1.0337
8	100	23.6320	56.2164	-0.6767
9	100	23.4924	57.2175	-0.6424
10	100	23.4050	63.6779	-0.5857
11	100	23.6324	40.0711	-1.0316
12	100	23.4054	55.1386	-0.7204

^[a]percentage of human intestinal absorption; ^[b]cell permeability (Caco-2 in nm/s); ^[c]cell permeability Maden Darby Canine Kidney in nm/s; ^[d]skin permeability

However, this does not impede its utilization transdermally according to a study conducted by Silva [41], that used topical microemulsion containing copaiba oil for anti-inflammatory activity and mutagenic, and showed to be effective in relation to their mutagenicity, confirming the anti-inflammatory activity of copaiba oil and the viability of using in topical formulation.

The drug is present in two forms in the blood, in its bound form and its fraction unbound to the plasma proteins. These bonds between other factors determine plasma half-life of drugs, because when the greater its bond with these, higher half-life in the body of individual, because of these bonds transforming themselves into deposits of slow drug release [42,43]. Would otherwise, only drug in its unbound fraction can overcome the membranes for that binds with the target in which it is intended and display the pharmacological effects [44].

Table 2. Distribution properties in percentages of PPB and penetration of the blood brain barrier of the compounds of sesquiterpene class of copaiba oil-resin

Compounds	Distribution	
	PPB (%)	$C_{\text{Brain}}/C_{\text{Blood}}$
1	100	13.3193
2	90.8496	3.7524
3	100	14.2219
4	100	13.9265
5	100	9.2187
6	100	13.4359
7	100	11.1471
8	100	13.3193
9	100	13.4992
10	100	15.0642
11	100	11.1471
12	100	13.1358

According to Table 2, it is verified that all the compounds bind strongly to plasma proteins with values ranging from of 90.8496% (compound 2) to 100% for the other compounds. Such values evidence that the compounds of copaiba oil-resin by present high binding values the display of their pharmacological effects are slow, because will only be evident when they turn off of plasma proteins. The compounds will present a longer plasma half-life, influencing also in its renal excretion, because are only available for glomerular filtration the fraction unbound and its renal excretion is decreased, due to bound portion acts as a reservoir where the drug is

slowly released, so that it will remain in equilibrium with unbound fraction that will be metabolized and excreted [45,46].

The Blood-Brain Barrier (BBB) preventing entry of toxic drugs in the Central Nervous System (CNS), but allows the circulation of adequate amounts of arterial blood through the brain tissue, regulating the exchange of substances between the blood and CNS. However, drugs that act on the CNS need to overcome this barrier to act in its molecular target and produce pharmacological effects. For drugs with peripheral target is important that the drug has little or no penetration, in order to avoid undesired effects [47]. Study performed by Silva et al. [41], used computational methods to assess physical-chemical, pharmacokinetic and toxicological in proposed modifications of antimalarials inhibitors (deoxyhypusine synthase), evidencing satisfactory results in the prediction of these properties using the PreADMET server, mainly in relation the penetration in blood-brain barrier [46]. Thus, it has an importance in pharmacology since the compounds are classified as: inactive (if not overcome the barrier) and active (if overcome barriers), ie, the inactive compounds do not cause collateral effects [48].

Table 2 shows the penetration values and classified according with Ma et al. [49], compounds that have values greater than 1 ($C_{\text{Brain}}/C_{\text{Blood}} > 1$) are considered active in the CNS can cause collateral effects, and compounds having values less than 1 ($C_{\text{Brain}}/C_{\text{Blood}} < 1$) are classified as inactive in the CNS. Therefore, all compounds of copaiba oil-resin presented higher values than 1 ($C_{\text{Brain}}/C_{\text{Blood}} > 1$), being considered active and may cause collateral effects. The values ranged from of 3.75249 (compound 2) to 15.0642 (compound 10), and the major compound β -caryophyllene (compound 1) showed a value of 13.3193, these values can evidence the toxic effect of this oil in the CNS.

The computational methods using experimental data, and this new approach can contribute to the reduction of animal testing, facilitating the process of evaluation and applicability for toxicological study of chemically identified individual components, mixtures or chemical products of natural origin, which have not been submitted to *in vivo* assays. With this, can improve the drug discovery studies and reduce costs [50].

Table 3. Toxicological properties of mutagenicity (Ames test) of the compounds of sesquiterpene class of copaiba oil-resin

Compounds	Ames test				Mutagenicity
	TA100_10RLI (+S9)	TA100_NA (-S9)	TA1535_10RLI (+S9)	TA1535_NA (-S9)	
1	Negative	Negative	Negative	Negative	Mutagenic
2	Negative	Negative	Positive	Negative	Mutagenic
3	Negative	Negative	Negative	Negative	Non-mutagenic
4	Negative	Negative	Negative	Negative	Mutagenic
5	Negative	Negative	Positive	Negative	Mutagenic
6	Negative	Negative	Negative	Negative	Mutagenic
7	Negative	Negative	Negative	Positive	Non-mutagenic
8	Negative	Negative	Negative	Negative	Mutagenic
9	Negative	Negative	Negative	Negative	Mutagenic
10	Negative	Negative	Negative	Positive	Mutagenic
11	Negative	Negative	Negative	Negative	Non-mutagenic
12	Negative	Negative	Negative	Negative	Non-mutagenic

The Ames test evaluates mutagenicity of compounds, and this assay utilizes *Salmonella typhimurium*, TA100_10RLI (+S9), TA100_NA (-S9), TA1535_10RLI (+S9), TA1535_NA (-S9), strains with modifications in the genes responsible for histidine synthesis. Thus, the test evaluates the ability of mutagenic agent to cause growth inhibition in environment without histidine [49]. According to Table 3 which details the results of the simulation of mutagenicity tests in different strains of *S. typhimurium* in the presence (+S9) and absence (-S9) of S9 mixture, a post-mitochondrial supernatant of rat liver treated with phenobarbital/ β -naphthoflavone mixture, in order to induce hepatic microsomal enzymes [51,52]. Table 3 shows the compounds of copaiba oil-resin submitted to this test, these compounds 1-2, 4-6 and 8-10 showed predictions mutagenic, and only the compounds 3, 7, 11 and 12 showed predictions non-mutagenic.

Table 3 shows the results for compound 1 (β -caryophyllene) to TA100 strain in the absence (TA100_NA) and presence (TA100_ORLI) of S9 mixture were negative, corroborating with studies made by Di Sotto et al. (2008) that investigated the mutagenic and antimutagenic effects of terpenes including β -caryophyllene, being evaluated by bacterial reverse mutation assay in *S. typhimurium* TA 98 and TA 100, and in *Escherichia coli* WP2uvrA strains. The β -caryophyllene showed mutagenicity in colonies revertant in WP2uvrA, both with and without S9 mixture. However, the β -caryophyllene showed strong antimutagenic activity on 2NF in highest concentration tested (6.40 mg/plate), the number of revertant colonies induced by 2NF was

reduced by 83.9%. Suggesting that the antimutagenic activity of β -caryophyllene observed presents possible chemopreventive properties [53].

In another study carried out by Di Giacomo et al. [52] aimed evaluate the ability of natural sesquiterpenes β -caryophyllene and β -caryophyllene oxide in inhibit the mutagenicity and the genotoxic potential of methanol extract from cigarette butts commonly discharged using the *S. typhimurium* strains TA100 and TA98, being studied as a possible prevention strategy. The antimutagenicity was strong in all experimental conditions for both sesquiterpenes.

In this way, Ames test via preADMET online server confirmed the negative results of sesquiterpene compounds tested for mutagenicity of TA100 strain with and without S9 mixture (Table 3), including the β -caryophyllene (compound 1) and caryophyllene oxide (compound 2) as observed in the studies cited previously, evidenced no mutagenicity of the compounds for this strain. In the TA1535 strain showed negative results in the presence and absence of S9 mixture for compounds (1, 3, 4, 6, 8, 9, 11 and 12), the compounds (2 and 5) gave positive for strain with the presence of S9 mixture and the compounds (7 and 10) gave positive for TA1535 strain with absence of S9 mixture. However, the foul of other conclusive studies on mutagenicity of sesquiterpene compounds, made it impossible to explain the fact that the results it has shown negative for all strains with or without the presence of S9 mixture, and even then, they are classified as mutagenic (only the compounds 1, 4, 6, 8 and 9).

The complexity and the difficulty of interpreting these results can be explained by the fact, even if all four strains appear to be capable of detecting all mutagenic agents, however, It is not fully comprehensive. The mutagenicity test is not desirable as the unique test, because it is less sensitive than a specific back mutation test (because of the many spontaneous mutations of various kinds) and may, in special cases, not work at all [54].

Carcinogenicity tests possess the objective of identifying the tumorigenic potential in animals and risk evaluation in humans, and this requires much time study (> 2 year) and the preADMET online server predicts the results from data NTP (National Toxicology Program) and EUA/FDA, because they are data made *in vivo* in rats and mouse for 2 years [55].

Based on the results obtained from the DEREK software for these compounds, compound 2 showed the only toxicophoric alerts, generated by its epoxide group that can lead to carcinogenicity, irritation of the eye and skin and skin sensitization. The epoxide ring is highly strained and therefore reactive. It is thought to act by ring opening to form a reactive ion which alkylates the DNA, which may result in carcinogenicity [56]. Low molecular weight (MWt <200) epoxides tend to be the most irritating. The presence of two epoxide groups increases irritancy compared with one. High molecular weight (>300) di-epoxides of low aqueous solubility are frequently only slightly irritating to skin and eyes. A structural alert for irritancy indicates some potential for this effect. Additionally, except for highly reactive corrosive substances, the skin and eye irritation potential of a chemical is very dependent on physicochemical properties which influences the concentrations at and exposure to component tissues [57].

The Custom Prediction section of the results for all twelve compounds displayed by DEREK showed predictions based on knowledge added to the knowledge-based by the user; none predictions has been added here and the display states simply that there is "Nothing to report". Thus, the presence of the toxicophoric group (epoxide) within the compound 2 is not an assurance that the toxic effects described will be demonstrated.

In Table 4, it is noted that the compounds 1 and 5-12 presented negative predictions showing

great evidence of carcinogenicity in mouse, and the compounds 2-4 showed positive predictions, ie, non-carcinogenic in mouse. The predictions in rats, the compounds 1-4 and 6-12 showed positive predictions (non-carcinogenic), and only compound 5 showed a negative prediction (evidence of carcinogenicity).

Table 4. Toxicological properties carcinogenicity (mouse and rat) of the compounds of sesquiterpene class of copaiba oil-resin

Compounds	Carcinogenicity	
	Mouse	Rat
1	Negative	Positive
2	Positive	Positive
3	Positive	Positive
4	Positive	Positive
5	Negative	Negative
6	Negative	Positive
7	Negative	Positive
8	Negative	Positive
9	Negative	Positive
10	Negative	Positive
11	Negative	Positive
12	Negative	Positive

4. CONCLUSION

The DFT method and B3LYP/6-31G* basis set proved adequate to optimize the major compounds of copaiba oil-resin for further study. Maps of electrostatic potential (MEP's) for the compounds studied showed no uniformity in their region of electrostatic potentials, however, the compounds 2 and 5 have their negative regions located in the oxygen, and the other compounds such regions were located in the double bonds, suggesting that this have a reactive site, and these being possible targets of binding of these molecules because negative regions have the highest probability of suffer nucleophilic attack.

In the HOMO of the compounds, it was observed that globules were located predominantly in the double bonds of the cycles and/or in its ramifications, thus favoring, the nucleophilic attack with a minimum amount of energy to react with electrons in regions. In the LUMO of the compounds, note that the globules are located on carbons that perform double bonds and on the hydrogens atoms (H), differing thus of the HOMO (occupied orbitals).

The alignment of the pharmacophore group showed that the compounds share 11

hydrophobic groups. Therefore, the compounds did not fit in the Lipinski rule, evidencing that the compounds are not viable to be administered orally because they must adapt some specific parameters.

The investigated compounds showed value of human intestinal absorption HIA>90% for all major compounds studied, and permeability values P_{CaCo2} were classified as medium, and for the P_{MDCK} were higher than 25 nm/sec. However, compound 5 obtained results of $P_{CaCo2}=55.4076$ nm/sec and $P_{MDCK}=169.7890$ nm/sec, highlighting among other.

In evaluating of distribution properties, the compounds bind strongly to plasma proteins (PPB). In relation to the penetration of the blood-brain barrier, the values of all compounds were greater than 1 ($C_{Brain}/C_{Blood}>1$), can cause serious collateral effects. However, compound 2 showed PPB=90.8496% and $C_{Brain}/C_{Blood}=3.7524$, ie, this compound will be with greater availability to bind with the target receptor, showing pharmacological effect for possessing a lower penetration of the blood-brain barrier, causing less collateral effects when compared to other compounds. Regarding the toxicity tests, the compounds (3, 7, 11 and 12) were predicted as non-mutagenic, and of these only the compound 3 showed no carcinogenic in mouse; and in rats the carcinogenicity was only evident in the compound 5. When the compounds were tested in DEREK software that emits alerts toxicophoric indicating carcinogenicity generated by epoxy groups neither compound gave alert, even in the compound 2 that features this group in its structure. Thus, the copaiba oil compounds are viable for administration in different pathways and can have their pharmacokinetic properties optimized in accordance with its formulation to enhance the efficacy and safety.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dwyer JD. The Central American, West Indian and South American Species of *Copaifera* (Caesalpinaceae). *Brittonia*. 1951;7(3):143-172.
2. Rose A, Rose E, Turner FM. The Condensed Chemical Dictionary, 6. ed, New York: Reinhold Publishing; 1961.
3. Opdyke DLJ. Balsam copaiba. *Food Cosmet. Toxicology*. 1976;14:687.
4. Lewis WH, Elvin-Lewis PF. *Medical Botany – Plants affecting Man's health*, New York: John Willey & Sons; 1977.
5. Florence AT, Attwood D. *Princípios físico-químicos em farmácia*. 2th ed. São Paulo: Pharmabooks; 2011.
6. Langenheim JH, Feibert EB. Leaf resin variation in *Copaifera langsdorffii*: Relation to irradiance and herbivory, *Phytochemistry*. Oxford. 1988; 27(8):2527-2532.
7. Pinto AC, Braga WF, Rezende CM, Garrido FMS, Veiga Jr VF, Bergter L, Patitucci M L, Antunes OAC. Separation of acid diterpenes of *Copaifera cearensis* Huber ex Ducke by flash chromatography using potassium hydroxide impregnated silica gel. *J. Braz. Chem. Soe*. 2000;11(4): 355-360.
8. Cascon V, Gilbert B. Characterization of the chemical composition of oleoresins of *Copaifera guianensis* Desf., *Copaifera duckei* Dwyer and *Copaifera multijuga* Hayne. *Phytochemistry*. 2000;55(7):773-8.
9. Maciel MAM, Pinto AC, Veiga Jr VF. Plantas medicinais: A necessidade de estudos multidisciplinares. *Quim. Nova*. 2002;25(3):429-38.
10. Veiga Jr VF, Pinto AC. O Gênero *Copaifera*, *Quím. Nova*. 2002;25:273-286.
11. Leandro LM, Vargas FS, Barbosa PCS, Neves JKO, Silva JA, Veiga Jr VF. Chemistry and Biological Activities of Terpenoids from Copaiba (*Copaifera* spp.) Oleoresins. *Review in Molecules*. 2012;17: 3866-3889.
12. Pieri FA, Mussi MCM, Moreira MAS. Óleo de copaiba (*Copaifera* sp.): Histórico, extração, aplicações industriais e propriedades medicinais. *Rev. Bras. Plantas Med*. 2009;11:465-472.

13. Sousa RB, Silva RAO, Marques LGA, Morais LS, Santos MRM, Pessoa CÓ. Aplicações do óleo resina de Copaifera L. na medicina popular: Uma prospecção tecnológica. *Revista GEINTEC – ISSN: 2237-0722*. 2012;3(1):144-155.
14. Dennington R, Keith T, Millam J. *GaussView, Version 5*, Semichem Inc., Shawnee Mission KS; 2009.
15. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR et al. *Gaussian 03, Revision C.02*, Gaussian, Inc. Wallingford, CT; 2004.
16. Plukiger P, Lutthi HP, Portmann S, Webber J. *MOLEKEL 4.1*, Swiss Center for Scientific Computing, Switzerland; 2001.
17. Inbar Y, Schneidman-Duhovny D, Dror O, Nussinov R, Wolfson JH. Deterministic Pharmacophore Detection via Multiple Flexible Alignment of Drug-Like Molecules. *RECOMB, LNBI 4453*. 2007;412-429.
18. Schneidman-Duhovny D, Dror O, Inbar Y, Nussinov R, Wolfson HJ. *PharmaGist: a webserver for ligand-based pharmacophore detection*. *Nucl. Acids. Res.* 2008;36:223-228.
19. Dror O, Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. Novel approach for efficient pharmacophore-based virtual screening: method and applications. *J. Chem. Inform. and Model.* 2009;49(10): 2333-43.
20. Yamashita S, Furubayashi T, Kataoka M, Sakane T, Sezaki H, Tokuda H. Optimized Conditions for Prediction of Intestinal Drug Permeability Using Caco-2 Cells. *Eur. J. Pharmacol.* 2000;10:195-204. Available:[http://dx.doi.org/10.1016/S0928-0987\(00\)00076-2](http://dx.doi.org/10.1016/S0928-0987(00)00076-2)
21. *Derek for Windows, version 10.0.2*, Lhasa Ltd., UK, Leeds; 2007.
22. Ridings JE, Barratt MD, Cary R, Earnshaw CG, Eggington CE, Ellis MK, Judson PN, Langowski JJ, Marchant CA. Computer prediction of possible toxic action from chemical structure: An update on the DEREK system. *Toxicology.* 1996;106: 267-79.
23. Santos CBR, Lobato CC, Sousa MAC, Macêdo WJC, Carvalho JCT. *Molecular Modeling: Origin, Fundamental Concepts and Applications Using Structure-Activity Relationship and Quantitative Structure-Activity Relationship*. *Rev. in Theor. Sci.* 2014;2:91-115.
24. Santos CBR, Lobato CC, Braga FS, Morais SSS, Santos CF, Fernandes CP, Brasil DSB, Hage-Melim LIS, Macêdo WJC, Carvalho JCT. Application of Hartree-Fock method for modeling of bioactive molecules using SAR and QSPR. *Comput. Mol. Biosci.* 2014;4:1-24.
25. Mendes APS, Schalcher TR, Barros TG, Almeida ED, Maia CSF, Barros CAL, Monteiro MC, Borges RS. A Geometric and Electronic Study of Dapsone. *Journal of J. Comput. Theor. Nanosci.* 2011;8:1-4.
26. Honório KM, Da Silva ABF. An AM1 study on the electron-donating and electron-accepting character of biomolecules. *Int. J. Quant. Chem.* 2003;95:126.
27. Contreras R, Domingo LR, Andrés J, Pérez P, Tapia O. Nonlocal (Pair Site) Reactivity from Second-Order Static Density Response Function: Gas- and Solution-Phase Reactivity of the Acetaldehyde Enolate as a Test Case. *J. Phys. Chem.* 1999;103:1367.
28. Heaton CA, Miller AK, Powell RL. Predicting the reactivity of uorinated compounds with copper using semi-empirical calculations. *J. Fluorine Chem.* 2001;107:1-3.
29. RAMOS MFS. Desenvolvimento de microcápsulas contendo a fração volátil de copaíba por spray-drying: um estudo de estabilidade e atividade farmacológica. 2006. 118f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto; 2006.
30. Arroio A, Honório KM, Silva ABF. Propriedades Químico-Quânticas Empregadas em Estudos das Relações Estrutura-atividade. *Quim. Nova.* 2010; 33(3):694-699.
31. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 2001;23:3-26.
32. Avdeef A, Testa B. Physicochemical profiling in drug research: A brief survey. *Cell Mol Life Sci.* 2002;59:1681-1689.
33. Zhao YH, Le J, Abraham MH, Hersey A, Eddershaw PJ, Luscombe CN, Butina D, Beck G, Sherborne B, Cooper I, Platts JA. Evaluation of human intestinal absorption data and subsequent derivation of a

- quantitative structure-activity relationship (QSAR) with the Abraham descriptors. *J. Pharm. Sci.* 2001;90:749.
34. Balimane PV, Chong S, Morrison RA. Current methodologies used for evaluation of intestinal permeability and absorption. *J. Pharmacol. Toxicol. Methods.* 2000;44: 301-312.
 35. Chong S, Dando S, Morrison R. Evaluation of biocoat intestinal epithelium differentiation environment (3-Day Cultured Caco-2 Cells) as an absorption screening model with improved productivity. *Pharm Res.* 1997;14(12): 1835-1837.
 36. Horie K, Tang F, Borchardt RT. Isolation and characterization of Caco-2 subclones expressing high levels of multidrug resistance protein efflux transporter. *Pharm. Res.* 2003;20:161-167.
 37. Ginski JM, Taneja R, Polli JE. Prediction of dissolution-absorption relationships from a continuous dissolution/Caco-2 system. *AAPS Pharm. Sci.* 1999;1(3):1-12.
 38. Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, Grove JR. MDCK (Madin-Darby canine kidney) cells: A tool for membrane permeability screening. *J. Pharm. Sci.* 1999;88:28-33.
 39. Verster JC, Volkerts ER. Antihistamines and driving ability: evidence from on-the-road driving studies during normal traffic. *Ann. Allergy Asthma Immunol.* 2004;92: 294-304.
 40. Singh S, Singh J. Transdermal drug delivery by passive diffusion and iontophoresis: A review. *Med. Res. Rev.* 1993;13:569-621.
 41. Silva DTC. Avaliação da atividade anti-inflamatória e mutagênica de microemulsão tópica contendo óleo de copaíba. 2014. 30f. Trabalho de Conclusão de Curso (Graduação em Farmácia)- Universidade Estadual da Paraíba, Campina Grande; 2014.
 42. Godin DV. Pharmacokinetics: Disposition and Metabolism of Drugs. In: Munson PL, editor, *Principles of Pharmacology.* New York: Chapman & Hall; 1995.
 43. Pratt WB, Taylor P. Principles of drug action: The basis of pharmacology. 3rd ed, New York: Churchill Livingstone; 1990.
 44. Smith DA, Van de Waterbeemd H, Walker DK. *Pharmacokinetics and Metabolism in Drug Design.* Weinheim: Wiley-VCH; 2001.
 45. Brunton LL Goodman & Gilman: *As Bases Farmacológicas da Terapêutica.* 12th ed, Rio de Janeiro: McGraw-Hill; 2012.
 46. Silva, NSR, Gonçalves LKS, Duarte JL, Silva JS, Santos CF, Braga FS, Silva RC, Costa JS, Hage-Melim LIS, Santos CBR. Computational analysis of physicochemical, pharmacokinetic and toxicological properties of deoxyhyposine synthase inhibitors with antimalarial activity. *Comput Mol Biosci.* 2014;4:47-57.
 47. Banks WA. Blood-Brain Barrier as a Regulatory Interface. *Forum of Nutrition.* 2010;63:102-110.
 48. Bemis GW, Murcko MA. Designing Libraries with CNS Activity. *J. Med. Chem.* 1999;42:4942-4951.
 49. Ma X, Chen C, Yang J. Predictive model of blood-brain barrier penetration of organic compounds. *Acta Pharmacol. Sin.* 2005; 26:500-512.
 50. Rijju A, Sithara K, Suja SN, Eapen SJ. Prediction of toxicity and pharmacological potential of selected spice compounds. In: *Anais do Simpósio Internacional de Bioinformática.* ACM. 2010;31.
 51. Ames BN, Gurney EG, Miller JA, Bartsch H. Carcinogens as Frameshift Mutagens: Metabolites and Derivatives of 2-Acetylaminofluorene and Other Aromatic Amine Carcinogens. *Proc Natl Acad Sci USA.* 1972;69:3128-3132.
 52. Di Giacomo S, Mazzanti G, Di Sotto A. Mutagenicity of cigarette butt waste in the bacterial reverse mutation assay: The protective effects of β -caryophyllene and β -caryophyllene oxide. *Environ. Toxicol.* 2015;30:2:1-10.
 53. Di Sotto A, Evandri, MG, Mazzanti G. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. *Mutat Res-Gen Tox En.* 2008;653(1):130-133.
 54. Ames BN, Lee FD, Durston WE. An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens (Salmonella typhimurium/lipopolysaccharide/frameshift mutations). *Proc. Nat. Acad. Sci. USA.* 1973;3:782-786.
 55. Woo YT. Mechanisms of action of chemical carcinogens, and their role in

- structure-activity relationships (SAR) analysis and risk assessment. In: Benigni R, ed., Quantitative Structure-Activity Relationship (QSAR) Models of Mutagens and Carcinogens, Boca Raton: CRC Press; 2003.
56. Hine C, Rowe VK, White ER, Darmer KI, Youngblood GT. In: Clayton GD, Clayton FE, editors. Epoxy compounds. Patty's Industrial Hygiene and Toxicology, 3th ed. New York: John Wiley; 1981.
57. Gardiner TH, Waechter JM, Stevenson DE. In: Clayton GD, Clayton FE, editors. Epoxy compounds. Patty's Industrial Hygiene and Toxicology, 4th ed. New York: John Wiley; 1993.

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