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RESEARCH ARTICLE

Molecular Modeling of Substances Isolated from the Essential Oil of the Species *Drimys angustifolia* and *Drimys brasiliensis*

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Abstract: ***Background*:** Carry out an *in silico* study of chemical substances isolated from the species: *Drimys angustifolia* and *Drimys brasiliensis*.

***Methods*:** A theoretical study of global reactivity, QSAR descriptors, MEP construction and molecular docking was performed to analyze the interaction of substances with acetylcholinesterase of *Drosophila melanogaster* and the prediction of skin permeation and toxicological properties of the substances.

***Results*:** Chemical reactivity and molecular stability investigation suggest that the substance which presents stability values similar to the standard substance D-limonene, was Terpinen-4-ol. The MEPs of the investigated substances were evenly distributed along the hydrogens and oxygens. The molecular docking studies suggest interesting and promising results for the substance Myristicin. Regarding skin permeability, the results suggests low skin absorption for all substances. Regarding toxicological properties, bicyclogermacrene indicated non-carcinogenic and mutagenic activity.

***Conclusion*:** Our results suggest that the substance, bicyclogermacrene, is a potential candidate for usage as a repellent.

Keywords: *Drimys angustifolia*, *Drimys brasiliensis*, molecular modeling, natural insecticides, predictions *in silico*.

1. INTRODUCTION

Diseases transmitted by insects affect many people, mostly in tropical and subtropical regions. Mosquitoes, in addition to being vectors of infectious diseases, can cause discomfort through their bites, especially in people with hypersensitivity. The bites of some species of mosquitoes can cause local irritation, pruritus, papules, vesicles, scrofulous, local secondary infection, pain, discomfort, among others. Research is of interest to prevent insect bites using natural or artificial repellents, with the aim of avoiding diseases and uncomfortable bites. Repellents are substances that when applied to the skin, prevents the approach of insects. The use of these substances reduces the risk of

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transmission of infectious diseases and immunoallergic reactions resultant from the bites [1].

Acetylcholinesterase (AChE) is the enzyme which catalyzes the hydrolysis process of acetylcholine (ACh) neurotransmitter resulting in choline and acetic acid in order to restore activated cholinergic neurons. AChE has gained widespread attention due to its association with neurodegenerative diseases and is the primary target of inhibition regarding organophosphorus compounds such as nerve agents and pesticides [2].

Synthetic repellents are often used. However, their usage is often inappropriate and does not ensure adequate protection. In addition, some repellents suffer liver metabolism, which may cause toxicity in some tissues. There is also the development of resistance of some species of mosquitoes to repellents. Consequently, it is necessary to investigate new repellents which are safe and effective against mosquitoes [3]. Research involving plants with repellent action has evolved in recent years. The use of plant extracts can reduce the cost of the product, in addition to reducing the risk of intoxication that synthetic repellents may cause.

Essential oils that several species of plants produce occupy a prominent place in the industry of repellents, mainly in the production of agricultural defenses, since they have activity against diverse insects that can cause diseases. They present advantages when compared to synthetic products, since they are obtained from renewable resources and are rapidly volatized. However, the development of repellents of natural origin requires more research in order to define greater selectivity against insects as well as low human toxicity and economic benefits for a viable large-scale production. It was identified that leaves of the species *D. angustifolia* and *D. brasiliensis* present repellent action attributed to their high content of essential oils [3, 4]. Based on this information, this research pursued the investigation of the physicochemical, pharmacokinetic and toxicological properties of isolated substances from *D. brasiliensis* and *D. angustifolia.* Molecular modeling methods [5-11] were used to identify their repellent action and analyze their interaction with the enzyme acetylcholinesterase of *Drosophila melanogaster* (DmAchE).

2. MATERIALS AND METHODS

2.1. Compounds Studied

In the study developed by Gomes *et al*. it was identified that the essential oils of the species *D. angustifolia* and *D. brasiliensis* indicated potent repellent action [4].

Based on this information, the substances contained in these essential oils have been selected, such as the monoterpenes Sabinene and Terpinen-4-ol; the sesquiterpenes Bicyclogermacrene and Cyclocolorenone; and the phenylpropanoid Myristicin. The 2D molecular structures of the substances were downloaded from the PubChem database [12]. For the purpose of comparison, we used the substance D-limonene, which has already proven to be repellent against various species of insects (Fig. **1**).

2.2. Molecular Modeling of Substances Studied and Descriptor Calculations

The substances studied were constructed as follows. Initially, the structures of D-limonene, Bycyclogermacrene, Cyclocolorenone, Myristicin, Sabinene and Terpinen-4-ol were modeled using the GaussView 3.0 software [13] and optimized using the DFT method and B3LYP/6-31G∗ basis sets implemented in the Gaussian 03 software [14]. After the structures were determined in three dimensions, various descriptors for each molecule were calculated. They represent different sources of chemical information (features) regarding the molecules and include geometric, electronic, quantum-chemical, physical-chemical and topological descriptors, among others [15, 16].

2.2.1. Quantum Chemical Descriptors

In this study, with the aid of the GaussView 5.0 software [17] and using the basis set B3LYP/6-31G** implemented in the Gaussian 03 software and the method of Density Functional Theory (DFT) reactivity descriptors were calculated, *i.e.* Total Energy (TE), mulliken electronegativity (χ), molecular hardness (η), molecular softness ($1/\eta$), chemical potential (μ), global electrophilicity index (ω), Highest Occupied Molecular Orbital (HOMO), one level below highest occupied molecular orbital (HOMO-1), lowest unoccupied molecular orbital (LUMO), one level above lowest

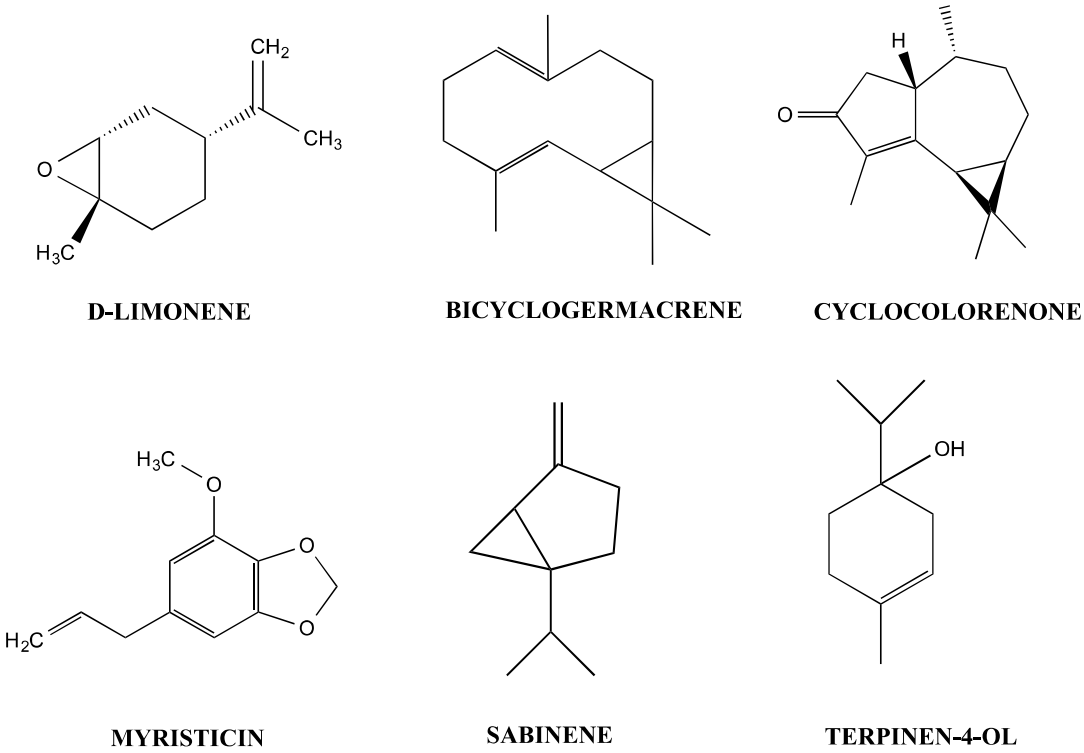

Fig. (1). 2D structure of the substance with repellent activity (D-limonene) and compounds present in the species *D. angustifolia* and *D. brasiliensis*.

unoccupied molecular orbital (LUMO+1), difference between HOMO and LUMO (GAP). These descriptors are indicators of stability and chemical reactivity [18, 19].

2.2.2. Quantitative Structure-activity Relationship (QSAR) Descriptors

QSAR descriptors were calculated using the HyperChem 6.02 software [20] for the natural substances. These descriptors are Total Surface Area (TSA), Molecular Volume (MV), Molecular Refractivity (MR), Molecular Polarizability (MP), Coefficient of Lipophilicity (logP), Molecular Mass (MM) and Hydration Energy (HE). The selected molecular descriptors provided important information regarding the electronic, steric, hydrophilic and hydrophobic influence of the selected substances.

2.3. Statistical Analysis

When the measurements are made on a number of objects, the results are usually organized in a data matrix. The measurements provided for this study (natural substances) were arranged in rows, and the objects (molecular descriptors) arranged in columns. Pearson correlation was performed with the Piroutte 3.10 [21] and Statistica 6.1 [22] softwares in order to evaluate the descriptors that have better correlations. The correlation of descriptors was performed with the Hydration Energy (HE) in order to evaluate molecular stability and chemical reactivity.

2.4. Maps of Molecular Eletrostatic Potential (MEP)

An important concept explored in this study is the structure-activity relationships of the studied substances *via* the characteristics of the respective electrostatic potential maps (MEPs). These maps allow the use of a qualitative analysis to locate reactive sites in a molecule, and to determine the roles played by both electronic and steric effects (size/shape) on potency. The visualization of MEPs can provide qualitative information such as the behavior of the interaction between a ligand and receptor. The MEPs were generated from the atomic charges at the DFT B3LYP/6-31G** level, calculated using the Gaussian 03 sofware, and the results were visualized with the Molekel software [23].

2.5. Molecular Docking

Molecular docking allows investigation of the possible orientations that a given molecule takes within the binding site of a receptor. It involves energy functions containing electrostatic parameters, van der Waals, hydrophobic and hydrogen bonds, which generate mathematical models that predict the best orientations of the ligands, according to a list of energy scores [24, 25].

For the docking simulations, hydrogen atoms were added to the structure of the enzyme acetylcholinesterase. Subsequently, the AutoDock Vina 1.5.6 software was used [26] to simulate the interaction of each inhibitor with the enzyme. AutoDock Vina 1.5.6 uses an automatic procedure to predict the interaction of ligands with the biomolecular target. Docking validation was performed by calculating the Root-Mean-Square-Deviation (RMSD) between the three-dimensional coordinates (x, y, and z) that the inhibitor has in the crystallographic structure and the coordinates in the best pose generated from docking. The Grid map was centered on residues Tyr71, Trp83, Met153, Tyr370 [27], with the following values: x = 34.212, y = 72.536 and z = 10.913.

2.6. Prediction of Skin Permeability and Toxicological Properties

To evaluate the skin absorption and toxicological properties of the substances, the PreADMET webserver [28] was used to predict ADME/Tox properties. PreADMET predicts the result from it model, which is built from the data of NTP (National Toxiciology Program) and US FDA which are the results of the *in vivo* carcinogenicity tets of mice and rats for 2 years. The results of the skin absorption prediction is given by a mathematical expression, where:

$$Kp=Km \times D/h$$

Km is the coefficient of distribution between the stratum corneum and the vehicle, D is the mean diffusion coefficient (cm^2/h), and h represents the skin thickness in cm.

To perform the prediction of mutagenicity, the server uses the Ames test, which is a simple method to test the mutagenicity of a substance. It uses several strains of the bacteria *Salmonella typhimurium* with mutations in genes involved in histidine synthesis. Thus, it can estimate the mutagenic activity of a substance [28].

3. RESULTS AND DISCUSSION

3.1. Chemical Reactivity and Molecular Stability of Studied Substances

The correlation was performed between the physicochemical properties and the values of Hydration Energy (HE). The correlation was also performed between properties (chemical-quantum descriptors) and Total Energy (TE). The Pearson correlation coefficient method was used, whereas for a total positive correlation, the values must be close to or equal to 1. When no correlation is found, the values are equal to 0. It is possible to identify which descriptors are essentials for biological activity of the molecule in the receptor site, The molecular descriptors are chemical characteristics that predict physical-chemical and stereoelectronic properties that allow the study of the structure-activity relationship of a series of substances [9].

The molecular polarizability (α') e hardness (η) are generally correlated with lipophilicity, molar volume and stereo impediments of the molecules. The studies of these descriptors helps interpretations of the mechanisms of interaction between the molecule and its biological target [29]. It was thus possible to identify that the substance Sabinene presented lower value of polarizability (17.38 $Å^3$) whereas Myristicin indicated the highest value (26.55 $Å^3$). When compared to D-limonene (18.02 $Å^3$), Myristicin presented a higher polarizability value. Pearson's correlation of polarizability with hydration energy was -0.826. In relation to hardness, the molecule that presented the lowest value was D-limonene (-0.107 eV) when compared with the substance Myristicin that presented higher value (0.205 eV). However, it was possible to identify that the substance Terpine-4-ol (-0.098 eV) indicated the value of hardness closest to that of D-limonene. Pearson correlation of hardness with hydration energy was -0.849.

The Molecular Mass (MM) is a parameter that is indirectly related to the volume, not being able to express the three-dimensional profile of a molecule [30]. Thus, Sabinene showed a lower molecu-

Table 1. Molecular descriptors of chemical reactivity and molecular stability of compounds.

Compounds	α' (Å³)	η (eV)	MM	MR (Å³)	MV (cm^3)	X (eV)	1/η (eV)	Log P	HE (eV)
D-limonene	18.02	-0.107	152.2	45.24	55.83	0.107	0.133	1.84	0.11
Biciclogermacreno	26.37	-0.086	204.3	68.52	705.1	0.086	0.111	4.27	-2.93
Cyclocolorenone	25.87	0.010	218.3	66.19	707.7	-0.108	0.047	3.51	-2.1
Myristicin	26.55	0.205	192.2	56.45	614.1	-0.205	0.402	-4.6	-6.65
Sabinene	17.38	-0.096	136.2	43.65	532.2	0.096	0.124	2.84	1.89
Terpinen-4-ol	18.79	-0.098	154.2	48.31	558.6	0.098	0.129	2.16	-1.3
α'	1.000	0.655	0.948	0.913	0.882	-0.502	0.295	-0.441	-0.826
H	-	1.000	0.488	0.304	0.262	-0.342	0.811	-0.926	-0.849
MM	-	-	1.000	0.960	0.963	-0.681	0.024	-0.202	-0.677
MR	-	-	-	1.000	0.991	-0.548	-0.104	-0.039	-0.572
VM	-	-	-		1.000	-0.616	-0.182	0.025	-0.506
X	-	-	-	-	-	1.000	0.260	-0.025	0.203
1/η	-	-	-	-	-	-	1.000	-0.971	-0.712
Log P	-	-	-	-	-	-	-	1.000	0.794
HE	-	-	-	-	-	-	-	-	1.000

lar mass value (136.2) when compared to the Cyclocolorenone that had a higher value (218.3). However, the molecule with a molar mass closest to that of D-limonene (152.2) was Terpine-4-ol (154.2). For this molecule the Pearson correlation of molecular mass with the hydration energy was -0.677.

Molar Refractivity (MR) is a property that depends on the structure of the bioactive substance and expresses the lipophilic and electronic character of substituent groups present in the molecule [31]. The lowest Molar Refractivity (MR) value was 43.65 Å³ for Sabinene. This substance indicated MR value closest to that of D-limonene (45.24 Å³). The highest MR value was found for bicyclogermacrene (68.52 Å³). Pearson correlation of molar refractivity with hydration energy was -0.572 for Terpinen-4-ol.

The Molecular Volume (MV) is defined as the impenetrable space of another molecule, obeying the law of mass action, however, it is known that the three-dimensional coordinates that define a certain region of the molecule can be altered in function of the physical state [32]. After the analysis of the results, it was possible to identify that the molecule with the lowest volume was D-limonene (55.83 cm^3) and the highest value was Cyclocolorenone (707.7 cm^3). The Pearson correlation value of the molecular volume with the hydration energy was -0.506 for Terpinen-4-ol.

Electronegativity (x) is a property that can be used to estimate the capacity of one molecule to attract electrons to another when there is an interaction between two molecules [33]. In Table **1**, it can be observed that the molecule with the lowest electronegativity value was Myristicin (-0.205 eV) and the one with the highest value was D-limonene (0.107 eV). In addition, it is possible to observe that the molecule which obtained the electronegativity value closest to D-limonene was Terpinen-4-ol (0.098 eV). The Pearson correlation value for electronegativity with hydration energy was 0.203 for Terpinen-4-ol.

Softness (1/η) represents the facility with which a molecule deforms itself. Thus, the lower the hardness or higher the molecular softness, the

lower the amount of energy needed for the transition of an electron from the HOMO to the LUMO [34]. In relation to softness, Cyclocolorenone indicated the lowest value (0.047 eV) whereas Myristicin presented the highest value (0.402 eV). The substance that indicated a softness value closest to that of D-limonene (0.133 eV) was Terpinen-4-ol (0.129 eV). The Pearson correlation with the hydration energy was -0.712 for Terpinen-4-ol.

The partition coefficient (logP) of a chemical entity is defined by the concentration of a substance in the organic phase and the ratio of the substance in the aqueous phase. Thus, it can be said that positive logP values indicate that a substance is more soluble in the organic phase and when the logP values are negative it indicates high solubility of a molecule in aqueous environment [35]. Based on what was described, Myristicin showed logP equal to -4.6, thus indicating solubility in water, whereas Bicyclogermacrene had the highest logP value (4.27) indicating affinity to the organic phase. The substance that indicated closest logP values to that of D-limonene (1.89) was Terpinen-4-ol (2.16) for which the Pearson correlation of logP with hydration energy was 0.794.

HOMO and LUMO are chemical-quantum descriptors that play important roles in chemical reactions and formation of several charge transfer complexes. HOMO energy is related to the ionization potential of a molecule and is characterized by the ability of a molecule to perform nucleophilic attacks. The energy of LUMO is directly related to the electronic affinity whereas the characteristic is the susceptibility of the molecule to undergo nucleophilic attacks [36].

Regarding the HOMO results, it was verified that Sabinene had the lowest value (-0.221 eV). This value is close to that of the standard D-limonene (-0.241 eV). Cyclocolorenone indicated the highest HOMO value (-0.379 eV).

In the qualitative analysis of the molecules, it was possible to identify that globules were present in the double bonds between the carbons (-C=C-) of the molecules D-limonene, Bicyclogermacrene, Cyclocolorenone, Sabinene and Terpinen-4-ol. In addition, it was possible to observe globules in the ketone group (-C = O) of Cyclocolorenone and in single bonds (-C-C-) of aromatic rings of Myristicin (Fig. **2**).

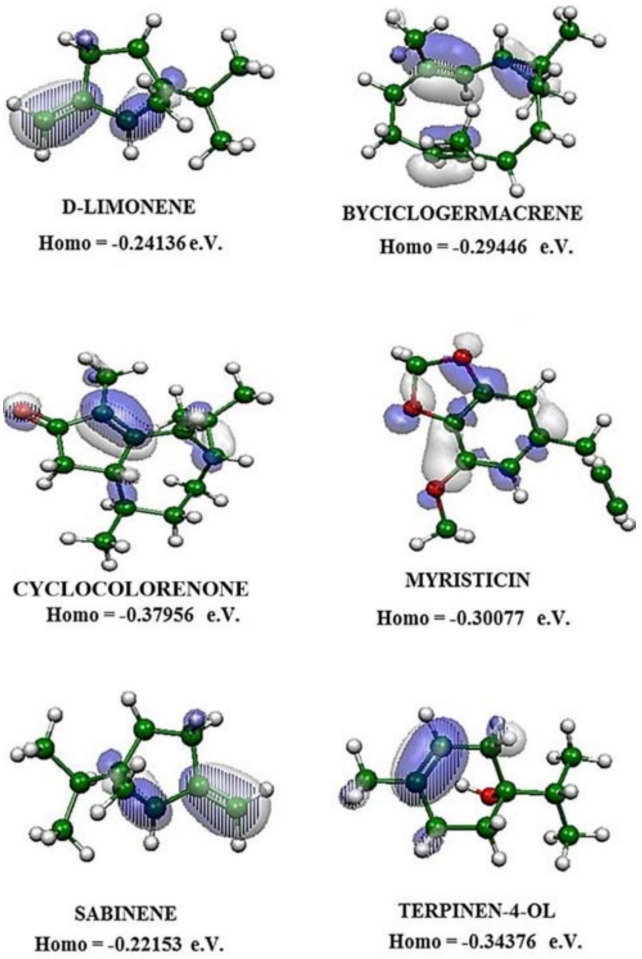

Fig. (2). Representation of HOMO of the studied compounds. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In Table **2**, LUMO values are given and it is possible to observe that Sabinene has the lowest value (0.027 eV), which is the closest LUMO value to that of standard D-limonene (0.029 eV). Bicyclogermacrene indicated the highest value of LUMO (0.189 eV).

Analyzing LUMO of the substances, it was found that D-limonene have globules in the alkynyl group near the methyl group. In Bicyclogermacrene the globules were distributed in the methyl groups (-CH3-). in Sabinene, It was verified in Sabinene that the globules were located in the alkynyl group (C=C). For Terpinen-4-ol, the globules were in the alkyl group. In Myristicin, globules were present in the carbons of double bonds whereas Cyclocolorenone showed globules

Table 2. Calculation of E_{HOMO}, E_{HOMO-1}, E_{LUMO}, E_{LUMO+1} and GAP with energy level B3LYP/6-31G * and Pearson's correlation matrix.

Compounds	E_{Homo} (eV)	E_{Homo-1} (eV)	E_{Lumo} (eV)	E_{Lumo+1} (eV)	*Gap* (eV)	ET (a.u)
D-limonene	-0.241	-0.239	0.029	0.034	-0.267	-465.9
Byciclogermacrene	-0.294	-0.327	0.189	0.195	-0.223	-586.0
Cyclocolorenone	-0.379	-0.379	0.119	0.232	-0.095	-660.0
Myristicin	-0.300	-0.321	0.150	0.158	-0.804	-652.0
Sabinene	-0.221	-0.268	0.027	0.083	-0.249	-390.6
Terpinen-4-ol	-0.343	-0.399	0.187	0.245	-0.259	-467.1
HOMO	1.00	0.922	-0.686	-0.902	-0.149	0.667
HOMO -1	-	1.000	-0.791	-0.981	-0.137	0.449
LUMO	-	-	1.000	0.856	-0.172	-0.539
LUMO +1	-	-	-	1.000	0.131	-0.523
GAP	-	-	-	-	1.000	0.302
Total energy		-	-	-	-	1.000

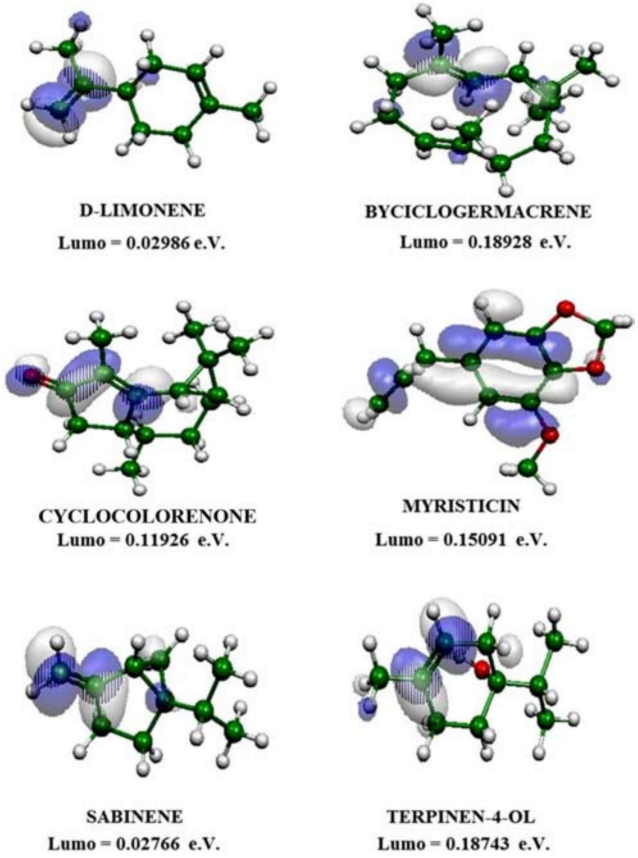

Fig. (3). Representation of LUMO of the studied compounds. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

in the alkenyl (C=C), alkyl (C-C), and oxygen (O) groups. These regions are likely to perform nucleophilic attacks with minimal amount of energy required for the reaction (Fig. **3**).

Molecular stability can be described by the GAP values. A large GAP value suggests high molecular stability (low reactivity in chemical reactions) whereas smaller GAP values generally indicate more reactive molecules [37]. Based on the above, it was possible to identify that Cyclocolorenone had a smaller GAP value (0.095 eV), and Myristicin had a larger GAP value (0.804 eV). We note that Terpinen-4-ol indicaed GAP value (0.259 eV) which is close to that of the standard D-limonene (0.267 eV).

3.2. Map of Molecular Electrostatic Potential

The identification of MEP is an approach that tries to elucidate the electrostatic contribution of atoms in a given molecule. The MEP is one of the most used descriptors in QSAR studies and is important to identify the total molecular size and location of potential electrostatic potentials. The color of the MEP can vary from blue to red whereas electronegative regions are represented by red, characterized as areas that can perform nucleo-

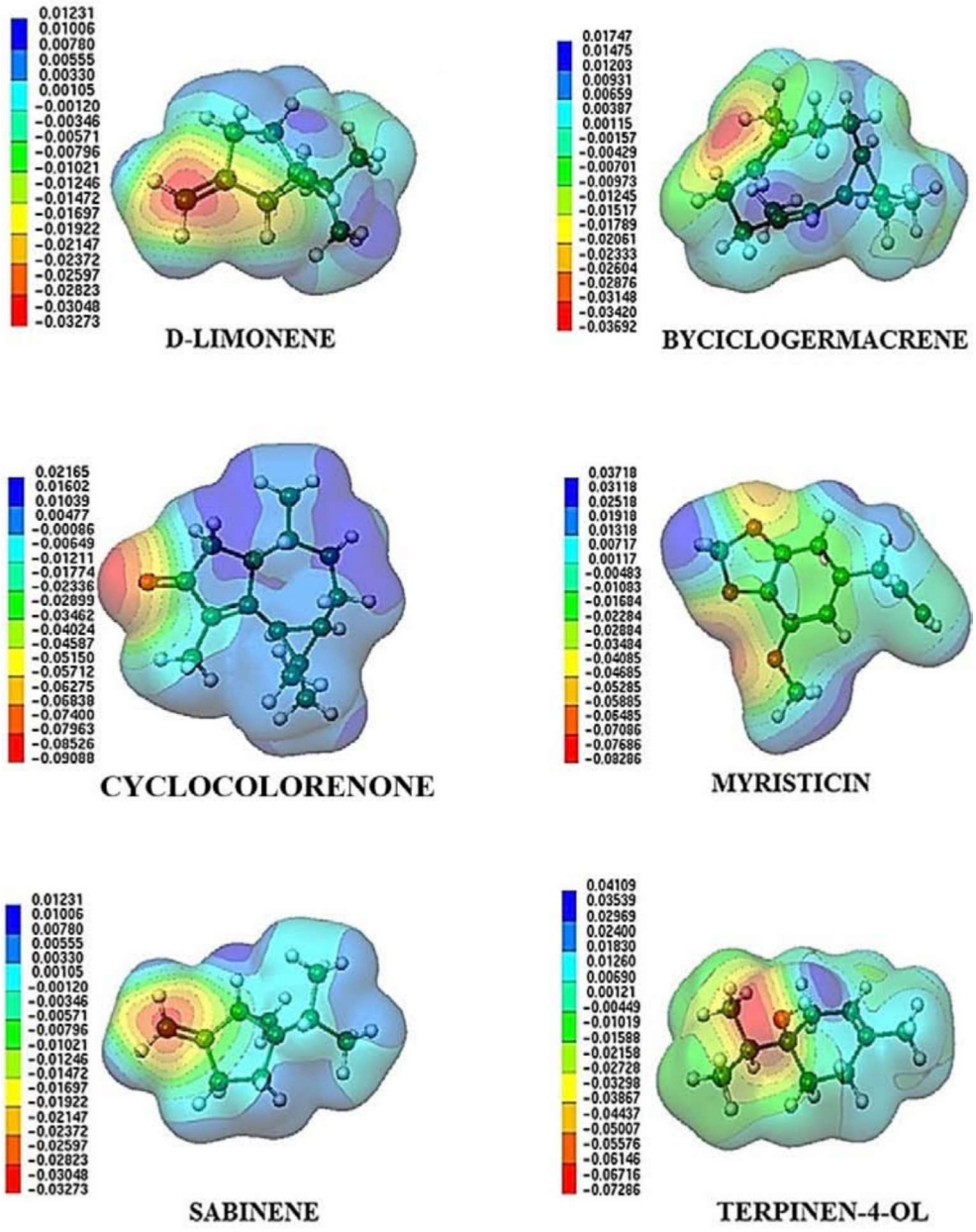

Fig. (4). Representation of the Electrostatic Potential Map of the substances. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

philic attack when interacting with other molecules. Positive regions are represented by blue, indicating regions that are more likely to undergo nucleophilic attack [37, 38]. In Fig. (**4**) we show the MEPs of the substances investigated.

In a qualitative analysis, it is possible to observe that the negative electrostatic potential in D-limonene is distributed in double bonds (-C = C-) with positive electrostatic potentials present in methyl (-CH_3). The maximum negative electrostatic potential is -0.3273 a.u, whereas the maximum positive electrostatic potential is 0.01231 a.u.

In Bicyclogermacrene, the negative molecular electrostatic potential lies in the carbonyl group (O = CH_2-) of the molecule whereas the maximum negative electrostatic potential is -0.03420 a.u. The positive potential is present in the single bonds (-CH_2-CH_2-) and the maximum positive potential is 0.01747 au.

For Cyclocolorenone, it was observed that the negative molecular electrostatic potential lies in the carbonyl group (O = CH_2-) present in the molecule and the positive molecular electrostatic potential is found in the hydrogen of the methyl

group (-CH_3-). The maximum negative electrostatic potential is 0.09088 a.u, whereas the maximum positive potential is 0.02165 a.u.

Myristicin indicated negative electrostatic potential at the oxygen present in the pentane cycle and in the ether group (-O-CH_3-) as well as positive molecular potential in the hydrogens of the methyl group (-CH_3-). The maximum negative potential is -0.08286 a.u and the maximum positive potential is 0.03718 a.u.

Sabinene indicated negative electrostatic potential in the methyl group (-CH_3-) as well as positive electrostatic potential on the hydrogens of the molecule. The maximum negative potential was -0.03273 a.u and the maximum positive potential was 0.03273 a.u.

Terpine-4-ol indicated negative electrostatic potential at the oxygen in the molecule as well as positive molecular potential at hydrogens of the methyl (-CH_3-) group. The maximum negative potential was -0.07286 a.u and the maximum positive potential was 0.04109 a.u.

3.3. Molecular Docking

In order to start the molecular docking studies, the enzyme acetylcholinesterase of *D. melanogaster* was obtained from Protein Data Bank (PDB) deposited in complex with tacrine derivative 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine under the code PDB ID 1DX4, at 2.7 Å resolution whereas water molecules and the receptor complexed to the inhibitor were withdrawn. Subsequently, polar hydrogen atoms as well as Gasteiger-Marsili partial charges were added. After the enzyme treatment, the tacrine derivative 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine ligand was prepared.

In the docking simulation, the validation of the result was performed by calculating RMSD between the experimental ligand and the conformation of the ligand with highest score after docking. Best docking simulation should have RSMD lower than 2Å [39, 40]. In this study, the RMSD calculation of docking simulation between the crystallographic orientation of the tacrine derivative 9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine inhibitor and the docking result with acetylcholinesterase was 0.6358 Å.

In the docking analysis, it was possible to verify that hydrophobic interactions were prevalent among residues Tyr70, Trp83 and Tyr370 and the substances D-limonene, Bicyclogermacrene, Cyclocolorenone and Myristicin. Sabinene indicated hydrophobic interactions with the amino acids Tyr83 and Tyr370 and the compounds D-limonene and Terpinen-4-ol showed interaction *via* hydrogen bonds with the amino acid Tyr370 (Fig. **5**).

The affinity and specificity in the ligand-receptor interaction are determined by several factors, including hydrophobic and hydrogen interactions. The hydrophobic interactions are relatively weak and occur due the interaction in apolar chains or subunits. These chains are present at both the receptor and the ligand sites and are solvated by layers of water molecules. In view of the large number of hydrophobic subunits present in molecules, these interactions may be considered important for the recognition of the ligand by the biomacromolecule. On the other hand, the hydrogen interactions are also considered important in biological systems, responsible for the maintenance of bioactive conformations of macromolecules.

These interactions are formed between electronegative heteroatoms such as oxygen, nitrogen, sulfur and the hydrogen atom as a result of their marked polarizations [41]. After analyzing the docking simulation, it was possible to identify that Myristicin indicated similar docking results and receptor affinity levels close to results of the standard D-limonene. However, the substance Myristicin had no interaction of hydrogen with the amino acid Tyr370. In Table **3**, it is possible to identify the affinity level of each molecule and the types of interactions between the aminoacids and the active site of DmAchE and the studied substances.

3.4. Skin Permeability and Toxicological Properties of the Studied Substances

The prediction of skin permeability properties and toxicological properties *in silico* is an approach that has been widely used in the initial study for planning and development of substances of pharmacological interest. This methodology aims to reduce the unnecessary expense in biological assays of substances that may present high

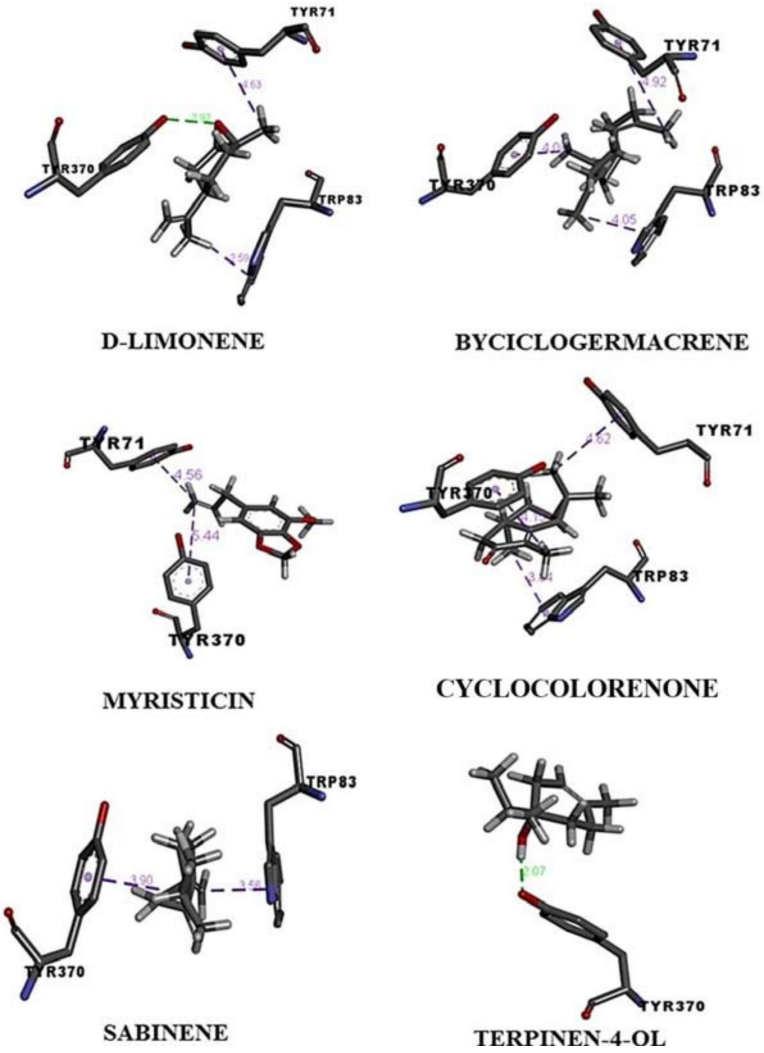

Fig. (5). Docking results of the studied compounds and their interactions with DmAchE binding site. The simulation was performed in AutoDock Vina1.5.6. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 3. Result of docking between studied compounds and the amino acids of the active site of *D. melanogaster* Acetylcholinesterase.

Compounds	Amino Acids	Type of Interaction	Distance (Å)	Affinity (Kcal/mol)
D-Limonene	Tyr71	Hidrofobic	4.63	-6.4
	Trp83	Hidrofobic	2.58	
	Tyr370	Hidrogen bond	2.92	
Bicyclogermagrene	Tyr71	Hidrofobic	4.92	-8.2
	Trp83	Hidrofobic	4.05	
	Tyr370	Hidrofobic	4.08	
Cyclocolorenone	Tyr71	Hidrofobic	4.62	-8.0
	Trp83	Hidrofobic	3.84	
	Tyr370	Hidrofobic	4.15	

(Table 3) contd…

Compounds	Amino Acids	Type of Interaction	Distance (Å)	Affinity (Kcal/mol)
Myristicin	Trp83	Hidrofobic	4.56	-6.9
	Tyr370	Hidrofobic	5.44	
Sabinene	Trp83	Hidrofobic	3.56	-6.3
	Tyr370	Hidrofobic	3.90	
Terpinen-4-OL	Tyr370	Hidrogen bond	2.07	-6.6

Table 4. Skin absorption property of the compounds.

Compounds	Absorption
	PSkin (cm/h)
D-limonene	-187.099
Bicyclogermacrene	-0.715
Cyclocolorenone	-170.612
Myristicin	-242.497
Sabinene	-136.792
Terpinen-4-ol	-130.527

probability of pharmacokinetic and toxic issues, saving time and investment, while allowing promising substances to be selected in the future with a greater probability of not being discarded during the clinical phase [42]. In the study, it was analyzed the skin absorption of the substances since they will be future topical repellents. In addition, the toxicological properties were analyzed to identify the changes that these can cause in the organism. The ability of a molecule to pass through membranes is called permeability. For a substance to have permeability in the skin it is necessary that its physicochemical properties be compatible with the biological properties of the skin. Based on this content, the skin permeability profile of the studied compounds was analyzed by PreADMET, which evaluates the diffusion and distribution of the substance in the stratum corneum. Molecules that show positive skin permeation values indicate that they are highly absorbed by the stratum corneum, but molecules that show negative results should have little absorption by the stratum corneum [30, 32].

Therefore, each substance of the study was analyzed whereas it was possible to verify that all the molecules indicated negative results, indicating low skin permeability. However, it was possible to identify that the substance Cyclocolorenone (-170,612 cm/hour) indicated skin permeability close to the value of D-limonene (-187.099 cm/h) (Table **4**).

The Ames test evaluates mutagenicity of the substances. This assay uses *Salmonella typhimurium* strains that may reveal the ability of a substance to cause genetic mutations responsible for histidine synthesis. Thus, the assay evaluates the ability of a mutagen to cause growth inhibition in histidine-free medium [43].

In Table **5** the substances submitted to the Ames test are shown. It is possible to verify that only Bicyclogermacrene did not present the mutagenicity, which becomes a positive point since the substance D-limonene that is used as repellent showed a positive result for mutagenicity. However, the mutagenicity test is not desirable as a single test because it is less sensitive than a specific mutation test (due to many spontaneous mutations of various types) and may, in special cases, not work at all [44]. There is complexity and difficulty in interpreting these results which can be explained by the fact that even if all four strains of the test seem capable of detecting all mutagenic agents, they may not be fully comprehensive [45]. The lack of other conclusive studies on the mutagenicity of the D-limonene substance makes it difficult to conclude that the results shown are in fact mutagenic or non-mutagenic *via in silico* analysis.

Carcinogenicity is the ability of a substance to induce changes that lead to cancer. Carcinogenicity tests require a long time (usually more than two years). The main methodologies are *in vivo* assays, using mice or rats, exposing them to chemicals, where the observed variable is the existence of

Table 5. Toxicological properties of mutagenicity and carcinogenicity of the studies compounds.

Compounds	Mutagenicity	Carcinogenicity	
		Rats	Mouse
D-limonene	Mutagenic	Positive	Positive
Bicyclogermacrene	Non-Mutagenic	Positive	Positive
Cyclocolorenone	Mutagenic	Positive	Negative
Myristicin	Mutagenic	Positive	Negative
Sabinene	Mutagenic	Negative	Positive
Terpinen-4-ol	Mutagenic	Positive	Negative

functional cell alterations [42]. For the prediction of carcinogenicity in rats, D-limonene, Bicyclogermacrene, Cyclocolorenone, Myristicin and Terpinen-4-ol showed positive prediction, which suggests that they do not present carcinogenic activity. For the prediction of carcinogenicity in mice, D-limonene, Bicyclogermacrene and Sabinene indicated positive results, suggesting no carcinogenicity in this model (Table **5**).

CONCLUSION

In this study we investigated the development of new repellents of natural origin. It was shown that the substance Bicyclogermacrene provided suitable results, in accord with this *in silico* study, since the results of reactivity, stability, docking and toxicity, were superior to the molecule D-limonene, which is a substance already lauched on the market as a repellent. Therefore, it is recommended that further studies be done in order to evaluate their toxicological properties, the concentration at which these substances could present toxicity to human organisms. It is also of interest to investigate the potential inhibitory action on the enzyme AChE of several insects from *in vivo* studies or studies of structural modification, in order to reduce the toxic effects demonstrated in this manuscript.

CURRENT & FUTURE DEVELOPMENTS

This research is the basis for further insecticide studies from the Pharmaceutical and Medicinal Chemistry (PharMedChem) laboratory.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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