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In silico (Eco)toxicology and molecular docking of halogenated and oxygenated rotenoid derivatives

(Eco)toxicologia *in silico* e acoplamento molecular de derivados de rotenóides halogenados e oxigenados

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Resumo
Os rotenóides são compostos amplamente difundidos nos setores agroindustrial e químico-farmacológico devido à sua constituição estrutural e versatilidade em atividades biológicas, porém, apresentam atividade tóxica para peixes. Assim, o objetivo deste estudo é uma investigação *in silico* (eco)toxicológica de rotenoides sintéticos halogenados e oxigenados com o uso de bioindicadores aquáticos e docking molecular visando compreender o comportamento bioquímico e a dinâmica ambiental desses compostos. Portanto, infere-se que os rotenoides sintéticos denotaram resposta toxicológica em nível crônico possibilitada por dados físico-químicos e correlações entre dinâmica ambiental, não obstante, os dados de acoplamento molecular corroborados com ensaio ecotoxicológico, permitindo que os rotenoides apresentem tendências bioconcentradoras ao longo

da cadeia trófica, causando efeitos deletérios em organismos mais desenvolvidos. Assim, o estudo está em um nível inicial, o que possibilita novas abordagens em modelos *in vitro* e *in vivo* para aprimoramento e desenvolvimento deste estudo.
Palavras-chave: Ecotoxicologia. Rotenóides. Acoplamento molecular. *In silico*.

Abstract
The rotenoids are compounds widely disseminated in the agro-industrial and chemical-pharmacological sectors due to their structural constitution and versatility in biological activities, however, these present toxic activity for fish. Thus, the objective of this study is an *in silico* (eco)toxicological investigation of synthetic halogenated and oxygenated rotenoids with the use of aquatic bioindicators and molecular docking aiming to understand the biochemical behavior and environmental dynamics of these compounds. Therefore, it is inferred that the synthetic rotenoids denoted toxicological response at the chronic level made possible through physicochemical data and correlations between environmental dynamics, notwithstanding, the molecular coupling data corroborated with ecotoxicological assay enabling the rotenoids present bio-concentrative tendencies along the trophic chain causing deleterious effects in more developed organisms. Thus, the study is at an initial level, which enables new approaches *in vitro* and *in vivo* models for improvement and development of this study.
Keywords: Ecotoxicology. Rotenoids. Molecular docking. *In silico*.

1. Introduction

Brazil in a climatic, historical, and economic framework that provided the plurality of huge phytophagous arthropods and invasive flora in the fields of monocultures and ecosystems, a fact exemplified by the production and distribution of fruits, cereals, and other products of plant origin for the world market (De Moura *et al*., 2007).

The control of these phytopathogens occurs in the face of the development and application of synthetic chemicals (insecticides) with various groups, such as organophosphates, carbamates, pyrethroids, and as well as natural compounds isolated from nicotinic and veratrine alkaloids. Because of this, the interest in research for new non-polluting insecticides, and reductions in environmental and public health impacts are intertwined with modern society. (De Moura *et al*., 2007).

Thus, rotenoids are alternative compounds from this perspective, consisting of a core of four linked tetrahydrochromene [3,4b] chromene rings in which each core can be substituted (R') for various purposes. These compounds are isolated in legumes and found in tropical countries (Liangsupree & Dangprasert, 2013). In addition, from an ecotoxicological point of view, rotenoids have toxic activity for fish, and some classes of insects, and have broad effect against animal parasitoses, such as sheep scabies (Inácio, 2007).

Nevertheless, these compounds were applied as pesticides (De Oliveira *et al*., 2020; Lucio *et al*., 2019; Reges *et al*., 2019), but due to their structural constitution, they are being investigated for medical bioactivities, such as antibacterial (Silva, 2011), antifungal (David *et al*., 2018) and cytotoxic (Inácio, 2007).

Thus, given the potential of rotenoids in agroindustry and chemical-pharmaceuticals, the aim of this study is an *in silico* (eco)toxicological evaluation of synthetic halogenated and oxygenated rotenoids with the use of aquatic bioindicators and molecular coupling aiming at correlations under biochemical behavior and bioconcentration in face of the environmental dynamics of these compounds given their toxic potential to aquatic organisms.

2. Material and methods

2.1 In Silico (Eco)toxicology

Initially, the halogenated and oxygenated synthetic rotenoids used for this study and the methodological context were described through the studies of Liangsupree and Dangprasert (2013), describing the synthesis process and biological activity in an in vivo model. The structures of these synthetic rotenoids can be visualized in Figure 1.

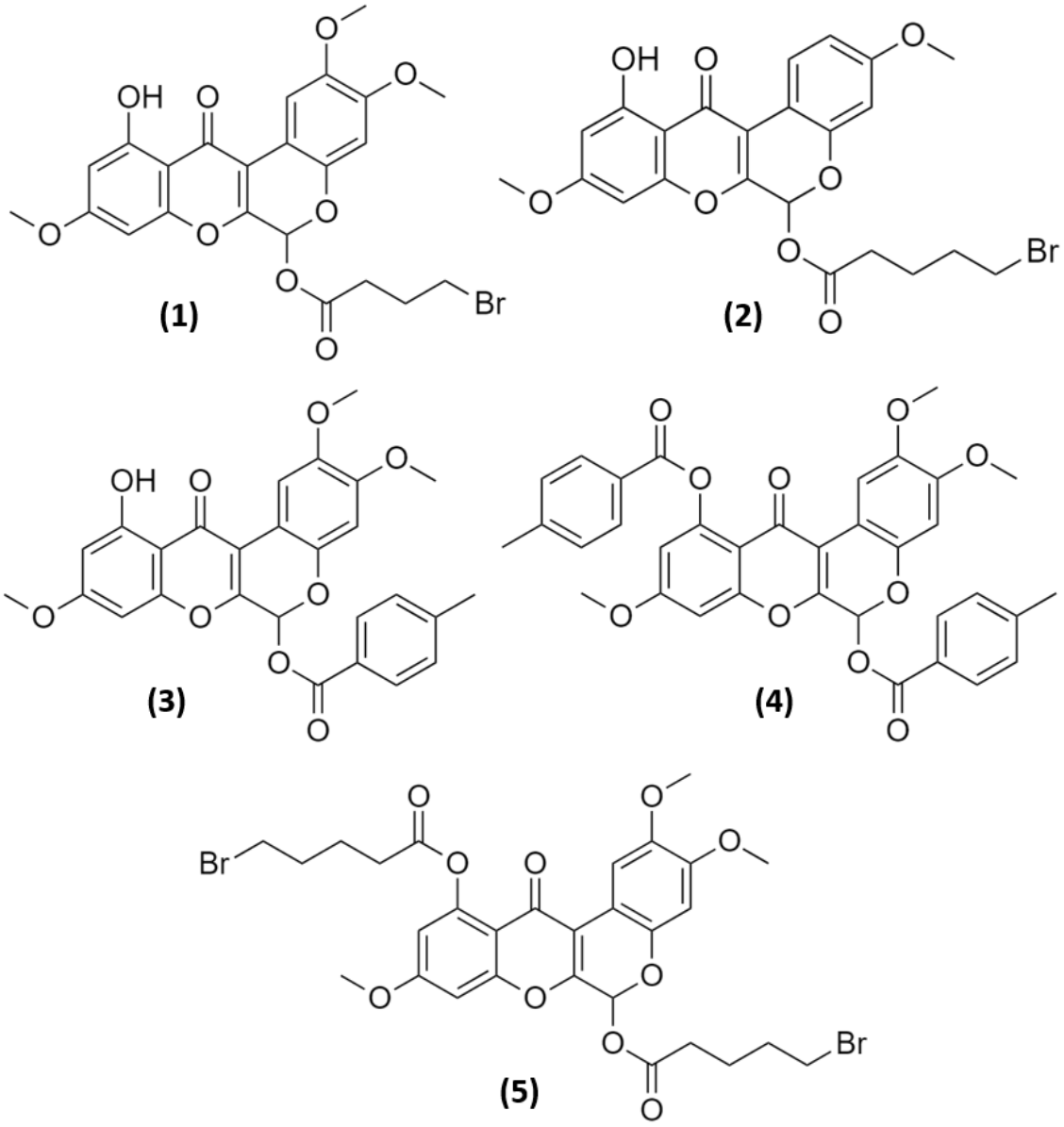

Figure 1 - Two-dimensional structures of halogenated and oxygenated rotenoids.
Notes: (1) pR001; (2) pR002; (3) pT01; (4) pV02 and (5) pR003.

Thus, in silico assays aim to quantify and prove the toxicological levels of a particular chemical compound based on the QSAR (Quantitative Structure-Activity Relationship) method, which enables the rationalization of the use of animals, consequently providing cost reduction and optimization of research time (Victal *et al*., 2014).

Given this, the ECOSAR® (Ecological Structure Activity Relationships) software is a predictive system that evaluates the aquatic toxicity (De Haas *et al*., 2011; Melnikov *et al*., 2016).

$$y = mx + b \quad \textbf{(1)}$$

$$ChV = \log\left(\frac{LOEC \times NOEC}{2}\right) \quad \textbf{(2)}$$

(y): toxic effect concentration (LC_{50} in mmol/L or mg/L); (x): log kow used for compounds that were not tested (Sanderson *et al*., 2003).

The program estimates acute toxicity (short-term exposure) represented by Equation 1 (high: <1 mg/L; moderate: between 1 and 100 mg/L and low: >100 mg/L) and chronic toxicity (long-term exposure) (high: <0.1 mg/L; moderate: between 0.1 and 10 mg/L and low: >10 mg/L) indicated by Equation 2, of a chemical for aquatic organisms such as fish, aquatic invertebrates and aquatic plants, given through the geometric mean of the unobserved effect concentration (NOEC) and the lowest observed effect concentration (ChV) (Austin & Eadsforth, 2014; Claeys *et al*., 2013).

Thus, GraphPadPrism® 8.0.2 software was used to perform the non-parametric one-way ANOVA analysis and application of the Tukey test with significant dissimilarity at $p < 0.05$ to infer significant differences under the mean concentrations of the organisms established in ECOSAR®.

2.2 Molecular Docking

Molecular docking covers the characterization of the behavior of small molecules at the binding site of target proteins, as well as elucidating molecular interactions (Piccirillo & Amaral, 2018).

In this context, the two-dimensional structures of the compounds (STEM control compound), pR001, pR002, pR003, pT01, and pV02) were obtained through the software MarvinSketch®, next, structural and energy optimization calculations were performed aiming to acquire the most stable structure for each compound analyzed by the software Avogadro® (Halgren, 1996; Hanwell *et al*., 2012). Nevertheless, the three-dimensional protein structure was acquired by the database (PDB - Protein Data Bank), with PDB ID code: 3LJE (structure of zebrafish RNase5 (zf- RNAse5)) and adjusted through the use of Chimera® software (Pettersen *et al*., 2004).

Because of this, the enzyme (zf - RNASE5) presents a region where the active site of the protein is located, which is composed of the following amino acids (Lys12.A, His17.A, His117.A, Gln16.A, Arg15.A, Glu120.A, and Thr49.A) (Pizzo *et al*, 2011) in which their inhibition can trigger toxicological biochemical responses that tend to interfere with the reproductive dynamics of the zebrafish organism (Monti *et al*., 2009; Pizzo *et al*., 2006).

It is noteworthy that the gridbox aimed to understand the entire region of the protein aiming to increase the possibilities of ligand interaction, thus the grid box value were: center_x = 83.639, center_y = 52.657, center_z = 22.354, size_x = 116, size_y = 100, size_z = 100, spacing = 0.375 and completeness = 8.

All simulations were incorporated through the AutoDockVina® computer software for each ligand with the analyzed protein 100 simulations were run where each simulation presents 20 possibilities for the formation of the complex (Morris *et al*., 2009; Gaillard, 2018). Because of this,

the selection of each simulation was given through the parameters of RMSD (Root Mean Square Deviation) with values ≤ 2.0Å and Binding Energy (ΔG) ≤ -6.0kcal/mol (Shityakov & Förster, 2014).

For graphical visualization, the programs used were PLIP® for preview (Salentin *et al*., 2015), DiscoveryStudio® for the formation of the three-dimensional complex image (Biovia *et al*., 2016) and their respective interactions with the amino acids, and Chimera® for the image acquisition of the complexes (Pettersen *et al*., 2004).

3. Results and discussion

3.1 (Eco)toxicology Prediction

The development of new drugs has caused major impacts on the environment and there are few studies related to these effects on aquatic organisms, due to the large number of compounds synthesized and in the process of legislation of use. Because of this, it was necessary to investigate the biosafety of these synthetic derivatives in an aquatic environment, since the following compounds will be targeted in this scope, considering the biomarkers (Fish, *Daphnia magna*, Green algae, and *mysid*) in determined duration and concentrations, as expressed in Table 1.

Table 1 - Acute and chronic toxicity testing on aquatic organisms.

			Neutral (organic)/ mg/L							
			Acute				**ChV**			
Compounds	**MW**	**log kow**	**Fish/ 96h**	***D. magna*/ 48h**	**G. algae/ 96h**	***Mysid*/ 96h**	**Fish**	***D. magna***	**G. algae**	***Mysid***
1	521.32	4.948	0.963*	0.718*	1.649*	0.386*	0.130*	0.149*	0.792*	0.070*
2	505.32	5.875	0.137*	0.112*	0.365*	0.640*	0.020	0.029*	0.212*	0.127*
3	490.47	5.625	0.224*	0.177*	0.528*	0.101*	0.032*	0.044*	0.291*	0.005*
4	698.36	6.556	0.046*	0.040*	0.170*	0.024*	0.007*	0.013*	0.114*	0.000
5	608.61	6.927	0.019*	0.017*	0.082	0.011*	0.003*	0.006*	0.059*	0.000

Notes: molecular weight (MW), Octanol/Water Partition Coefficient (log kow), **Asterisk (*)**: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Considering the ECOSAR® software methodology, in acute exposure, initially, it is observed that the compounds derived from stemonal show acute effect in all organisms studied at concentrations ≤ 1mg/L, and correlations with log kow, as this denotes log kow values ≤ 5 (fish, *D. magna* and *mysid*) and log ≤ 6.4 (G. algae) that validate the effect in these organisms.

In contrast, it is observed that synthetic stems have a high lipophilic potential, which in environmental dynamics infers that these compounds are poorly bioavailable in the aqueous medium, and adsorb mostly in organic soil/sediment colloids). Thus, the effect level exceeds the water solubility by 10X, normally no saturation implications (NES) are reported, as observed in all organisms and concentrations studied. However, due to the high lipophilic potential synthetic stems may show bioconcentrative incidences by exposing ≥ 3 kow values.

On chronic exposure, it is inferred that all synthetic stemonals tend to exhibit chronic deleterious effects at extremely low concentration values, i.e., concentrations less than ≤ 0.1mg/L, a fact exemplified by the duration in which these compounds diffused into the environment.

Aiming at accuracy in ecotoxicological analysis, statistical testing was proposed from the mean concentrations of the acute and chronic values for all organisms studied. Given through the one-way ANOVA non-parametric test (***$p < 0.05$, confidence interval).

Due to the mean acute exposure values shown in Figure 2A, there was no significant difference with $r^2 = 0.11$, which corroborates with the acute concentrations, as the synthetic stems will be adsorbed in the soil/sediment, expressing an excess concentration in G. Algae; however, the other organisms presented very similar averages.

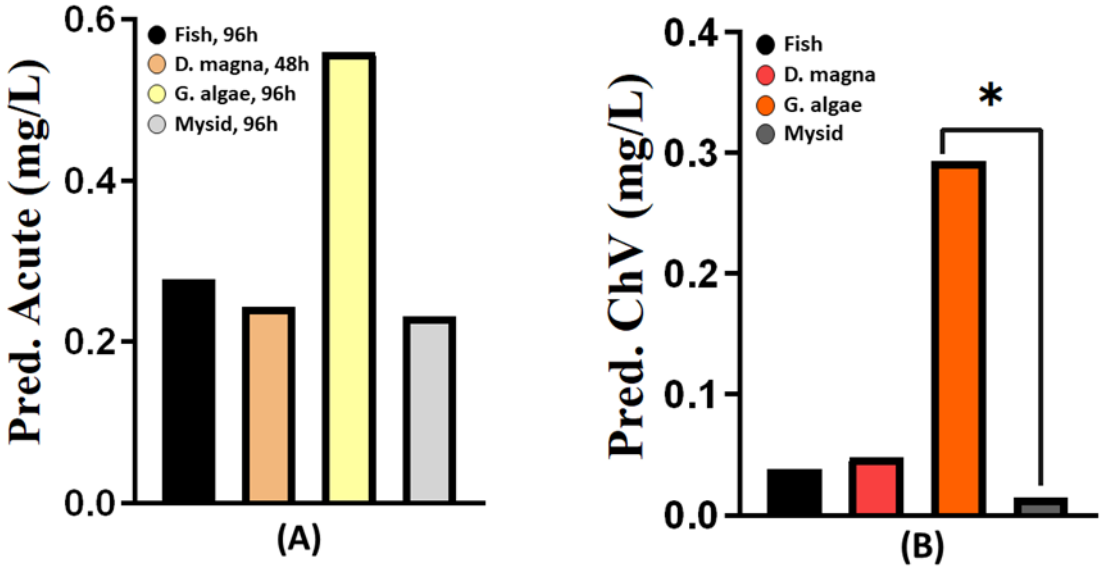

Figure 2 - Tukey's test applied to aquatic organisms

However, in Figure 2B the mean concentrations in chronic exposure denote a significant difference with $r^2 = 0.40$ between G. Algae and *Mysid*, thus, it is inferred that in chronic exposure the synthetic stemonals caused a deleterious effect on *Mysid* due to their low concentrations. Considering the environmental dynamics, the synthetic stemonais presents a bioabsorbent character along the trophic chain that can cause ecological disturbances in developed organisms.

3.1 Molecular Docking

Given the bio-concentrative potential of synthetic halogenated and oxygenated rotenoids, therefore, a molecular docking assay was promoted to describe the behavior of the rotenoids in developed organisms.

Through the computational simulations, the output files were provided for each ligand in complex with the protein analyzed, in Figure 3 all hydrogen bonds and intermolecular interactions that each ligand makes with the amino acids present in the protein are exposed, aiming at possible interactions with amino acids of interest.

In Figure 3 it is remarkable that all ligands (STEM, pR001, pR002, pR003, pT01, and pV02) showed similarity about the interacted region of the protein, but distinct interactions were observed, that are related to the specificity of each simulated ligand.

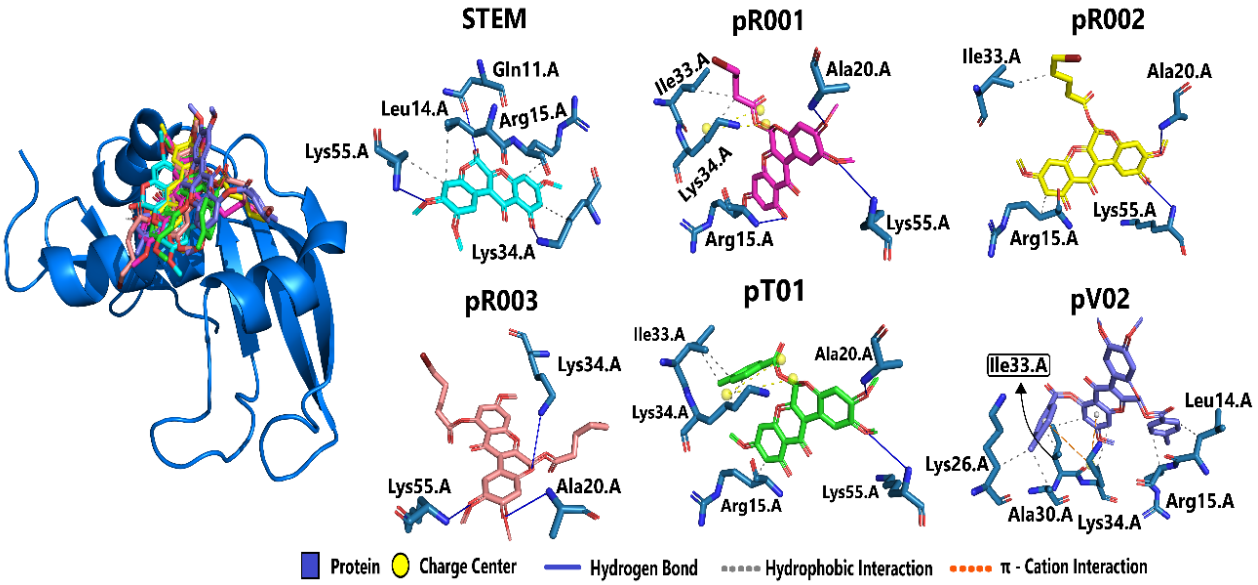

Figure 3 - Formation of complexes with the ligands (STEM, pR001, pR002, pR003, pT01 and pV02), with the RNase5 protein.

The ligand STEM (cyan), exerted interactions with five amino acids present in the protein distributed in hydrophobic bonds and hydrogen bonds; the amino acids (Leu14.A, Arg15.A, Lys34.A, and Lys55.A and Gln11.A), of all interactions are notable only one interaction that is an amino acid inserted in the active site of the protein highlighting the residue (Arg15.A).

The compound pR001 (pink) in Figure 3 presented chemical bonds with five amino acids (Lys55.A, Arg15.A, Lys34.A, Ile33.A, and Ala20.A); referring to the types of hydrophobic bonds exposed, hydrogen bonds and saline bridges; referring to all interactions the ligand showed only one binding to the active site of the protein through the amino acid Arg15.A.

Also present in Figure 3 is the ligand pR002 (yellow), which in its complex formation interacted with four amino acids, (Arg15.A, Ile33.A, Ala20.A, and Lys55.A) with two types of bonds, being hydrophobic bonds and hydrogen, being the amino acid Arg15. The only residue that is present in the catalytic site of the enzyme.

Figure 3 shows the formed pR003 ligand (salmon) simulation complex against the study target protein and found three interactions with amino acids (Lys34.A, Lys55.A, and Ala20.A) all chemical interactions are hydrogen bonds. Regarding the catalytic region, the compound pR003 did not present interactions with the amino acids present in the active site of the protein.

Still referring to Figure 3, chemical bonds are demonstrated from the interaction of the pT01 ligand (green), with the enzyme, where interactions occur with five residues of the protein analyzed (Ile33.A, Lys34.A, Ala20.A, Lys55.A, and Arg15.A) The hydrophobic interaction with the Arg15. A residue is highlighted, characterized in hydrogen bonds, hydrophobic bridges, and saline, when dealing with interactions with reference residues.

Following the other ligands, the compound pV02 (Lilac) also showed interactions with the amino acids present in the analyzed protein, the ligand exerted bonds with six amino acids present in the protein site, being the residues (Lys26.A, Ile33. A, Ala30.A, Lys34.A, Arg15.A, and Leu14.A), hydrophobic interactions and Pi-cation bonds; regarding the region of the active site of the protein is explained the interaction with Arg15.A, through hydrophobic bonds, being the only residue present in the active site.

Table 2 shows the ligands presenting all the interactions exerted with the amino acids present in the protein, providing information regarding the types of interaction and the values for the binding energy, and the corresponding RMSD of each complex formed.

Table 2 - Complexes with distance values from the target ligands for study.

Compounds	Energy/ kcal/mol	RMSD/Å	Interactions	Bond type	Distance/Å
STEM	-6.9	1.683	Leu14.A	Hidrophobic	3.75
			Arg15.A	Hidrophobic	3.84
			Lys34.A	Hidrophobic	3.85
			Lys55.A	Hidrophobic	3.93
			Gln11.A	H-Bond	2.30
			Lys34.A	H-Bond	2.01
			Lys34.A	H-Bond	2.52
			Lys55.A	H-Bond	2.91
pR001	-7.3	1.608	Ile33.A	Hidrophobic	3.63
			Lys34.A	Hidrophobic	3.93
			Arg15.A	H-Bond	3.47
			Ala20. A	H-Bond	2.26
			Lys55.A	H-Bond	3.34
			Lys34.A	Salt Bridges	4.73
			Lys34.A	Salt Bridges	4.65
pR002	-7.5	1.687	Agr15.A	Hidrophobic	3.51
			Ile33.A	Hidrophobic	3.48
			Ala20.A	H-Bond	3.06
			Lys55.A	H-Bond	2.19
pR003	-6.9	1.951	Ala20.A	H-Bond	2.76
			Lys34.A	H-Bond	3.33
			Lys55.A	H-Bond	2.80
pT01	-8.0	1.957	Arg15.A	Hidrophobic	3.54
			Ile33.A	Hidrophobic	3.75
			Ile33.A	Hidrophobic	3.55
			Lys34.A	Hidrophobic	3.57
			Ala20.A	H-Bond	2.10

			Lys55.A	H-Bond	3.27
			Lys34.A	Salt Bridges	5.15
			Lys34.A	Salt Bridges	5.07
			Leu14.A	Hidrophobic	3.58
			Arg15.A	Hidrophobic	3.56
			Lys26.A	Hidrophobic	3.32
			Ala30.A	Hidrophobic	3.35
pV02	-8.2	1.801	Ile33.A	Hidrophobic	3.58
			Ile33.A	Hidrophobic	3.58
			Ile34.A	Hidrophobic	3.86
			Lys34.A	Pi-Cation	4.05
			Lys34.A	Pi-Cation	5.36

All ligands that were performed in the simulations, showed values for interaction energy and RMSD within the established parameter, but among all, the best value for RMSD was the complex formed with the ligand pR001, with a result of 1.608 Å, and regarding the energy the best value was the ligand pV02 with the result equal to -8.2kcal/mol.

The compound STEM (majority control), showed interactions with hydrophobic character with the amino acids (Leu14.A, Arg15.A, Lys34.A, and Lys55.A), presenting the respective distances of 3.75 Å, 3.84 Å, 3.85 Å and 3.93. hydrogen bonds have two bonds with the amino acid Lys34.A with two distances of 2.01 Å and 2.52 Å, and two more interactions with two more protein residues (Gln11.A and Lys55.A), showing distances of 2.30 Å and 2.91 Å, respectively.

In Table 2, the compounds shown are in complex formation with Rnase5 protein, the ligand pR001, referring to the interactions the ligand exerted three types of bonds (Hydrophobic, H-Bond, and Salt Bridges); the amino acids, performed hydrophobic character bonds with their distances of 3.63 Å (Ile33.A) and 3.93 Å (Lys34.A); hydrogen bonds Arg15.A, Ala20. A, Lys55.A, with the best distance of 2.26 Å, through interaction and Salt Bridges with the amino acid (Lys34.A) with distances of 3.34 Å and 4.73 Å.

The studies on complex pointed out that the ligand pR002, exhibited two hydrogen bonds with the residues (Ala20.A and Lys55.A), with their distances of 2.19 Å and 2.76 Å and two chemical bonds of hydrophobic characteristics with the amino acids (Agr15.A and Ile33.A), having their distances equal to 3.51 Å and 3.48 Å.

Table 2 shows the possible interactions performed by the ligand pR003 with the protein residues of RNase5 enzyme, and showed three hydrogen bonds with the residues (Ala20.A, Lys34.A, and Lys55.A), presenting the shortest distance with the residue 2.76 Å with the residue Ala20.A; the other amino acids with hydrogen bonds are Lys34.A and Lys55.A with distances of 3.33 Å and 2.80 Å, respectively.

Still in Table 2, are the interactions that the compound (pT01). The ligand showed three types of interactions, being the hydrophobic ones with residues (Arg15.A, Ile33.A, and Lys34.A), where, the smallest distance is equal to 3.54 Å with Arg15.A; when dealing with hydrogen bonds, they are arranged on two residues (Ala20. A and Lys55.A), with respective distances of 3.08 Å and 3.27 Å;

and finally, two Salt Bridges with only the residue (Lys34.A), with two distances of 5.15 Å and 5.07 Å.

Regarding compound (pV02), entered in Table 2, the ligand exhibited two types of majority interactions, seven hydrophobic interactions with six amino acids (Leu14.A, Arg15.A, Lys26.A, Ala30.A, Ile33.A, and Lys34. A), showing the best distances with residues (Ala30.A, Lys26.A, and Arg15.A), with their respective distances of 3.35 Å, 3.32 Å, and 3.56 Å; regarding Pi-cation bonds two interactions with residue Lys34.A is displayed.

Among the ligands (STEM, pR001, pR002, pR003, pT01, and pV02), it is notable that all the ligands showed similar behavior when interacting with the studied protein, remaining in the same protein region; of all the simulations analyzed and complexes formed seen in Table 2 that only the ligands (STEM, pR001, pR002, pT01, and pV02), are interacting directly with at least one amino acid inserted in the active site of the protein, the residue in question is the Arg15. A, whereas the compound pR003 did not show links with the residues inserted in the active site of the protein.

4. Conclusion

The rotenoids present great biological diversity that enables their wide diffusion in the chemical-pharmacological industry, however, there is incipiency to the impacts of the introduction of these prodrugs in the environmental dynamics.

From the *in silico* (Eco)toxicology model, it was pointed out that the stemonal derivatives, initially, may present some acute effect. However, by evaluating log kow it is established that this toxicity depends on the increase of this which may influence the desorption of these compounds in soil/sediment which will not influence response at an acute level.

When investigating the chronic effect, the stemonal derivatives tend to show deleterious responses due to the bioaccumulation/bioconcentration process along the trophic chain, a fact exemplified by the physicochemical properties, log kow - toxicity ratio, and statistical tests.

The complexes formed between the ligands (STEM, pR001, pR002, pR003, pT01, and pV02), showed that the interactions are determinant when it comes to inhibition potential of the four ligands only the compounds (STEM, pR001, pR002, pT01, and pV02), exerted interaction with Arg15. A, an amino acid present in the active site of protein RNase5; the ligand pR003 showed similarity in the interaction region of the protein but did not exert effective binding with any amino acid present in the site, thus, we can point out that the compound (pR003), has no potential for inhibition of protein RNase5; while, the compounds (STEM, pR001, pR002, pT01, and pV02), formed bonds with the amino acid Arg15. A, residue present in the site, thus possessing potential for inhibition of protein RNase5, that is, corroborating with ecotoxicological analyses and literary support.

Nevertheless, the study is at its initial level, which enables new approaches in in vitro and in vivo models, for improvement and development of this study.

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