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Chemistry

In silico evaluation of erythrinic alkaloids from Erythrina verna in human crystallographic model GABAA R-β3 (4COF) and GABAA R-α1-β2-γ2 (6X3X)

Avaliação *in silico* de alcalóides eritrínicos de Erythrina verna em modelos cristalográficos humanos R-β3 (4COF) e R-α1-β2-γ2 (6X3X)

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ABSTRACT

Erythrina verna, known as mulungu, is a medicinal plant recognized for anxiolytic effects attributed to erythrinic alkaloids. This research aims to enhance our understanding of the mechanism of these alkaloids through *in silico* studies and explore their potential interaction with GABAA sub-receptors. Molecular modeling and docking simulations were performed to evaluate the conformational structure and interaction energies of the molecules as well as toxicity prediction. Multivariate statistical analyses were utilized to highlight the influence of binding affinity. The results indicate strong interactions of the erythrinic alkaloids with key amino acid residues from the GABAA receptor, while multivariate analysis revealed significant interaction patterns for erythradine and erysortrine. Mutagenic, tumorigenic, irritant, or reproductive risks were not observed in these molecules. So, erythrinic alkaloids exposure demonstrates safety and efficacy as a natural anxiolytic. It also contributes to our understanding of erythrinic alkaloids' pharmacological effects and supports the further development of natural anxiolytics for therapeutic applications.

Keywords: Mulungu; Erythrina; *In silico*; Anxiolytic effect; Multivariate analysis

RESUMO

Erythrina verna, conhecida como mulungu, é uma planta medicinal reconhecida pelos efeitos ansiolíticos atribuídos aos alcaloides eritrínicos. Esta pesquisa visa aumentar nossa compreensão do mecanismo desses alcaloides por meio de estudos *in silico* e explorar seu potencial interação com sub-receptores GABAA. Modelagem molecular e simulações de encaixe foram realizadas para avaliar a estrutura

conformacional e as energias de interação das moléculas, bem como a previsão de toxicidade. Análises estatísticas multivariadas foram utilizadas para destacar a influência da afinidade de ligação. Os resultados indicam fortes interações dos alcaloides eritrínicos com resíduos de aminoácidos-chave do receptor GABAA, enquanto a análise multivariada revelou padrões de interação significativos para eritradina e erisortina. Riscos mutagênicos, cancerígenos, irritantes ou reprodutivos não foram observados nessas moléculas. Portanto, a exposição aos alcalóides eritrínicos demonstrou segurança e eficácia como um ansiolítico natural. Também contribui para a nossa compreensão dos efeitos farmacológicos dos alcaloides eritrínicos e apoia o desenvolvimento posterior de ansiolíticos naturais para aplicações terapêuticas.

Palavras-chave: Mulungu; Eritrina; *In silico*; Efeito ansiolítico; Análise multivariada

1 INTRODUCTION

Erythrina verna, taxonomic synonym of Erythrina mulungu, popularly known as mulungu, is a medium-sized tree present in the National List of Medicinal Plants of Interest to the Unified Health System of Brazil (ReniSUS), its popular use is due to the preparation of a tea from the bark of the tree which has anxiolytic and hypnotic properties (Schleier et al., 2016).

The anxiolytic effects of mulungu are associated with its fraction of alkaloids called erythrinic alkaloids. In preclinical studies using different extracts and alkaloid fractions of the inflorescences and stem bark of E. verna and E. velutina, anxiolytic effects and depression of the central nervous system of Wistar rats and Swiss mice were observed, like the action of diazepam (Flausino et al., 2007; Onusic et al., 2002, 2003; Ribeiro et al., 2006; Vasconcelos et al., 2007).

Erythrinic alkaloids is proposed to act on several brain signaling pathways, such as GABAergic and cholinergic. The GABAergic molecular mechanism is not yet fully understood and may be involved in the interaction with subtypes of receptors involved in the processes involved in the development of anxiety (Carvalho et al., 2009; Iturriaga-Vásquez et al., 2010).

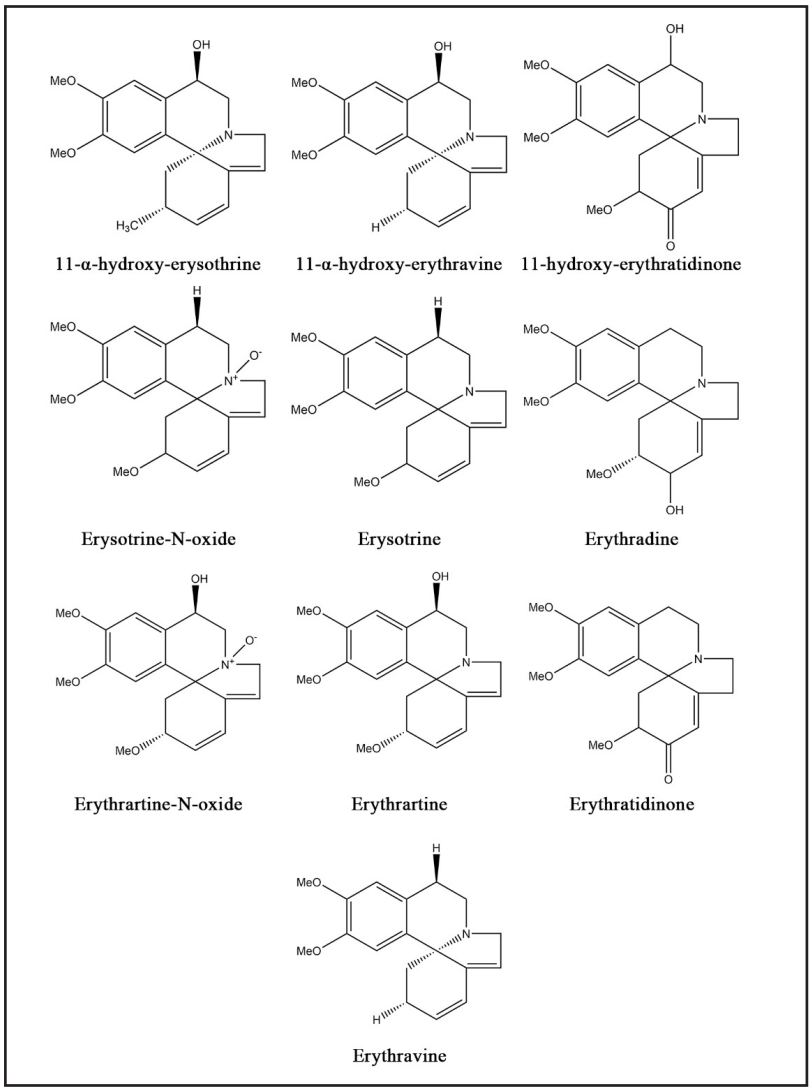
Considering the need for studies that contribute to a better understanding and safety of its use, this study aims to evaluate by in silico methods as molecular modeling and docking to analysis the interactions of a group of erythrinic alkaloids and

diazepam, a reference molecule for GABA interaction, in crystallographic models of GABAA receptor R-β3 (PDB: 4COF) and R-α1-β2-γ2 (PDB: 6X3X), in order to obtain data that corroborate the understanding of a mechanism of action of these compounds in gabaergic system, mainly responsible for the anxiolytic-hypnotic action.

2 MATERIALS AND METHODS

2.1 Compounds

Figure 1 – 2D structures of erythrinic alkaloids



Source: the authors (2025)

The most prevalent erythrinic alkaloid compounds in E. mulungu, 11-α-OH-erysorthrine, 11-α-OH-erythravine, 11-OH-erythratidinone, erysortrine-N-oxide, erysortrine, erythradine, erythrartine-N-oxide, erythrartine, erythratidinone and erythravine were used in this work. Chemical structures of these alkaloids were subjected to molecular modeling and docking studies with the objective of determining information that supports the understanding of the hypnotic and anxiolytic action. All chemical structures are showed in Figure 1.

2.2 In silico Methodology

2.2.1 Molecular Modeling and Prediction of Toxicity of Alkaloid Structures

In molecular modeling studies, the Spartan'08™ for Windows software and the quantum chemistry methodologies included were used to optimize the geometry of chemical structures. The computational calculation protocol used in the geometry optimization and conformational analysis (AC) involved AM1 followed by DFT B3LYP / with 6-311G* data base in gas phase. AC was performed using systematic search with defined torsion angle increment of 30o in the range 0-360o. The lowest energy conformer for chemical structure was saved in PDB file format prior to its use in molecular docking studies. OSIRIS Property Explorer™ was used to predict toxicological risks as mutagenic, tumorigenic, irritant, and reproductive effective parameters of the evaluated molecules (Sander, 2001).

2.2.2 Receptor Models and Treatment

The structures of the GABAergic receptor (GABAA R-beta3) complexed with the compound benzamide, code PDB ID: 4COF and Human GABAA R-α1-β2-γ2 subtype in complex with GABA complexed with diazepam, code PDB ID: 6X3X, were selected from the Protein Data Bank (PDB), to perform the docking studies (Miller and Aricescu, 2014; Kim et al., 2020). The three-dimensional structure was first prepared by identifying and

removing the co-crystallized ligand and water molecules, followed by the addition of polar hydrogens (Clent et al., 2021).

2.2.3 Molecular Docking

Molecular docking studies were performed using iGemDOCK™ 2.1 (Yang and Chen, 2004) slow calculation mode, in which ten individual conformational poses of the same compound were submitted to docking in the protein structure. The chemical structure, benzamide, it is a central molecular site of interaction called cavity BEN, were used in the docking studies in the 4COF model and the chemical structure diazepam central molecular site of interaction called cavity BDZ, were used in the docking studies in the 6X3X model, where the protein active site alternative was used as an option through the application of the dimensional radius box in a range of 5 to 15 angstroms.

Standard iGemDOCK™ docking calculations were performed in Docking Accuracy Setting with genetic algorithm (GA) parameters set to population size, generation, and number of solutions as 200, 70 and 10 respectively, and the IGemDOCK™ scoring function of hydrophobic and electrostatic (1:1 preference). The iGemDOCK™ software was used to infer the main postures that show pharmacological interactions binding energy, such as hydrogen, Van der Waals (VDW), and Electrostatic interaction, between the biological receptor and the studied compounds. In addition, the software was used to generate a dendrogram from the consensus of the results obtained after docking. These interactions were observed in 3D using the BIOVIA Discovery Studio™ (BIOVIA, 2020). The same parameters were used for diazepam docking, PDB ID: DZP, used as control.

To improve the molecular docking analysis conducted with iGemDOCK™, a multivariate approach was applied to the docking data using Past 4.17 software. Initially, principal component analysis (PCA) was performed on the binding energy data of erythrinic alkaloids and diazepam to reduce dimensionality and identify significant interaction patterns. The PCA utilized the correlation matrix, allowing for

the visualization of the main variables contributing to data variability. Furthermore, hierarchical cluster analysis (HCA) was conducted using the Euclidean distance metric and the complete linkage method to assess the similarities between the compounds based on their interactions with GABAA receptor amino acid residues.

3 RESULTS AND DISCUSSION

3.1 Toxicity Prediction

The structures of 11-α-hydroxy-erysorthrine, 11-α-hydroxy-erythravine, 11- hydroxy-erythratidinone, erysotrine-N-oxide, erysotrine, erythradine, erythrartine-N-oxide, erythrartine, erythratidinone and erythravine were individually drawn in OSIRIS software for predicting mutagenic, tumorigenic, irritant, and reproductive effective risks. No risk potential was observed in any of the structures considering these computational tools. These data suggest that these compounds have theoretical biological safety and can be subjected to pharmacological application studies. However, it is recommended additional computational evaluations as well as experimental data in certain conditions to confirm the toxicological security for these erythrinic alkaloids compounds.

3.1.1 Molecular Docking Analysis

The chemical structure of the minimum energy conformer generated in computational calculations was used to insert the bioactive compound in molecular docking studies. All the results of the main interactions between the compounds, the 4COF and 6X3X models (Table 1-2, respectively). Among the compounds, the Erythrartine and Erythradine showed lower energy of interaction between the pose and cavity of GABAA studied (-101.21¬ and -90.83 Kcal mol^{-1}, respectively) on comparison to other erythrinic alkaloids.

Table 1 – Interaction energy (Kcal mol^{-1}) of erythrinic alkaloids and diazepam in 4COF model

Compounds	Total Energy	Van der Waals Interaction	Hydrogen Interactions	Electrostatic
Erythrartine	-101.21	-85.79	-15.43	0.00
Erythradine	-90.83	-86.56	-4.27	0.00
Erythrartine-N-oxide	-88.53	-75.02	-12.41	-1.10
11-α-hydroxy-erythravine	-85.87	-75.99	-9.87	0.00
Diazepam	-83.78	-83.78	0.00	0.00
Erysortrine-N-oxide	-83.53	-72.45	-10.18	-0.90
Erysorthrine	-83.34	-76.98	-6.36	0.00
11-hydroxy-erythratidinone	-81.91	-67.16	-14.75	0.00
Erytravine	-80.70	-74.73	-5.97	0.00
Erythratidinone	-76.76	-67.90	-8.86	0.00
11-α-hydroxy-erysorthrine	-72.48	-64.43	-8.06	0.00

Source: the authors (2025)

Van der Waals (VDW), Hydrogen and Electrostatic Interactions of the compounds and the amino acid residues involved in the active site of the receptor (PDB 4COF) using the iGemDOCK™ (v.2.1)

Van der Waals (VDW), Hydrogen and Electrostatic Interactions of the compounds and the amino acid residues involved in the active site of the receptor (PDB 6X3X) using the iGemDOCK™ (v.2.1)

After docking, the results obtained from the interactions with amino-acid residues from active site were analyzed by consensus within the software itself, where it was possible to observe the main interactions of the compounds with residues ASP 43, TYR 62, TIR 97, TIR 157, PHE 200, THR 202 and TYR 205 (Table 3). Residues TIR 97, PHE 200 and TYR 205 are the important points of local interactions of the active site, cavity BEN, of 4COF model reported in literature (Sahila et al., 2015). Among the

compounds, the Erythradine showed lower energy of interaction between the pose and cavity of GABAA studied (-94.49 Kcal mol^{-1}) on comparison to other alkaloids.

Table 2 – Interaction energy (Kcal mol^{-1}) of erythrinic alkaloids and diazepam in 6X3X model

Compounds	**Total Energy**	**Van der Waals Interaction**	**Hydrogen interactions**	**Electrostatic**
Erythradine	-944.918	-859.918	-8.50	0
11-hydroxy-erythratidinone	-906.669	-860.678	-45.991	0
Diazepam	-902.571	-902.571	0	0
Erythrartine	-879.478	-879.478	0	0
Erythrartine-N-oxide	-860.232	-84.696	-132.717	0
11-α-hydroxy-erysothrine	-856.443	-771.443	-8.50	0
Erythravine	-836.633	-77.669	-599.426	0
Erysortrine-N-oxide	-82.574	-820.681	-0.505941	0
11-α-hydroxy-erythravine	-819.957	-712.603	-107.354	0
Erythratidinone	-817.517	-817.517	0	0
Erysorthrine	-814.455	-805.367	-0.908827	0

Source: the authors (2025)

Main Van Der Waals (VDW) and hydrogen bonding (HI) interactions of compounds and amino acid residues involved in the active site of the $GABA_A$ receptor (PDB 4COF) and application of Consensus Residue Analysis using the iGemDOCK™ program (v.2.1)

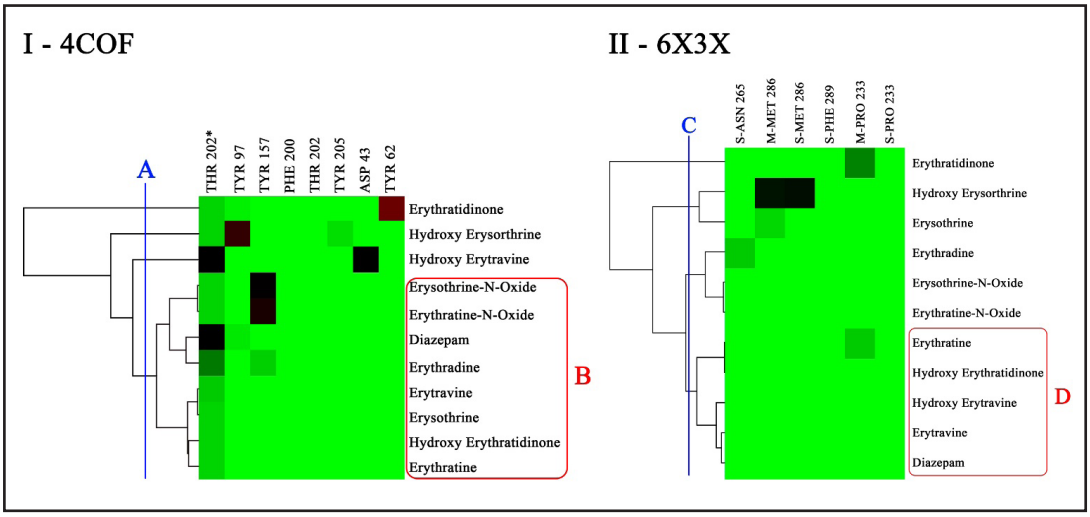
From the consensus of the interactions of erythrinic alkaloids and diazepam with the amino acid residues of the active site of the 4COF and 6X3X models, a dendrogram (Figure 2) generated by iGemDOCK was obtained, which correlates the compounds with the amino acid residues, and the similarity between values of VDW interactions and hydrogen interactions obtained.

Table 3 – Energy of the Van Der Waals (VDW) and Hydrogen Interactions (HI) (Kcal mol-1) of the erythrinic alkaloid and diazepam with 4COF model amino acid residues by consensus

Compounds / Interactions	**S-TYR 97**	**S-TYR 157**	**S-PHE 200**	**S-THR 202**	**S-TYR 205**	**S-ASP 43**	**S-TYR 62**
Diazepam							
VDW	-2.8	-3.9	-18.3	-3.9	-6.9	-8.8	-21.5
HI	0.0	0.0	–	0.0	–	0.0	0.0
Erythrartine							
VDW	-4.10	-8.18	-23.29	-4,69	-10.35	-4.49	-14.60
HI	-3.5	-2.5	–	-2.5	–	-2.5	0.0
Erythrartine-N-Oxide							
VDW	-3.18	0.31	-18.08	-4.50	-12.95	-5.23	-15.46
HI	0.0	-0.5	–	-2.5	–	-2.4	0.0
Erythradine							
VDW	-3.25	-2.44	-21.18	-5.55	-7.78	-3.03	-20.57
HI	0.0	0.0	–	-1.4	–	0.7	-0.5
Erysortrine-N-Oxide							
VDW	-3.27	0.10	-17.36	-4.42	-13.78	-4.50	-14.05
HI	0.0	-0.7	–	-2.5	–	0.0	0.0
11-hydroxy-Erythratidinone							
VDW	-3.97	-7.80	-16.82	-5.55	-7.88	-4.46	-7.69
HI	-3.5	-1.8	–	-2.5	–	0.0	0.0
Erysothrine							
VDW	-3.69	-3.86	-20.71	-6.23	-9.10	-5.41	-9.89
HI	-2.4	0.0	–	-2.5	–	0.0	0.0
11-α-hydroxy-Erythravine							
VDW	-6.0.9	-8.45	-16.81	-3.63	-7.91	1.63	-27.28
HI	-1.3	0.0	–	0.0	–	-3.9	-2.5
Erythravine							
VDW	-0.4	-5.4	-7.8	-4.2	-3.7	-8.6	-0.1
HI	0.0	0.0	–	0.0	–	0.0	0.0
11-α-hydroxy-Erysothrine							
VDW	-4.4	-8.0	-6.6	-7.8	0.0	0.0	-6.9
HI	-2.4	-4.9	–	0.0	–	0.0	0.0
Erythratidinone							
VDW	-1.4	-3.6	4.4	-7.6	-2.9	-14.5	-0.7
HI	-2.0	0.0	–	-1.2	–	-3.5	-0.1

Source: the authors (2025)

Figure 2 – Interaction consensus dendrogram: Interaction strength similarity dendrogram resulting from molecular docking of erythrinic alkaloids and diazepam control in cavity BEN of 4COF and BDZ of 6X3X models



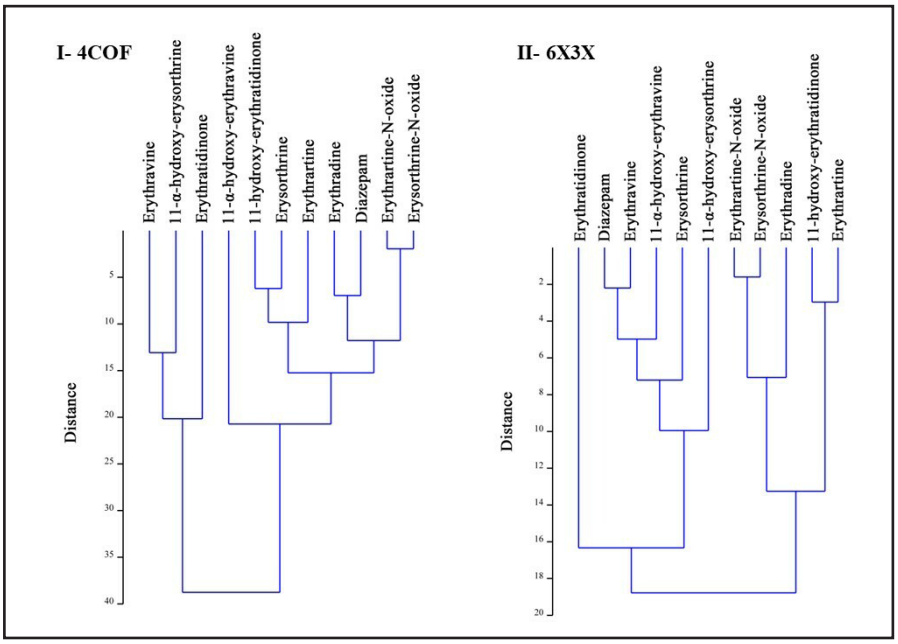
Source: the authors (2025)

The cut line “A” and “C” defines the separation of clusters of compounds, with emphasis on cluster “B” and “D”, which relates the interactions of most erythrinic alkaloids with diazepam. In the 4COF model, the alkaloids erythratidinone, hydroxy-erysorthrine and hydroxy-erythravine, while in the 6X3X model the alkaloids erythratidinone, hydroxy-erysorthrine, erysorthrine, erythradine, erysorthrine-N-oxide and erythrartine-N-oxide were less like diazepam interactions, however, these alkaloids also showed important interactions at the active site in both receptor models.

3.1.2 Hierarchical Cluster Analysis

The hierarchical cluster analysis (HCA) dendrogram corroborated the interaction similarity, showing close clusters between diazepam and alkaloids such as erythradine and erysortrine in the 4COF model, and erythravine and hydroxy erythravine in the 6X3X model (Figure 3) suggesting that these natural compounds have affinity comparable to diazepam for the GABAA receptors (Sigel and Ernst, 2018). Previous studies highlighted the importance of amino acid residues, such as THR 202 and S-ASN 266, for the binding of positive allosteric modulators to GABAA receptors (Sieghart and Savic, 2018).

Figure 3 – Hierarchical Cluster Analysis (HCA) Dendrogram Using Euclidean Distance and Complete Linkage Method for Erythrinic Alkaloids and Diazepam with the I - GABAA R-β3 Receptor (4COF Model) and II - GABAA R-α1-β2-γ2 Receptor (6X3X Model)

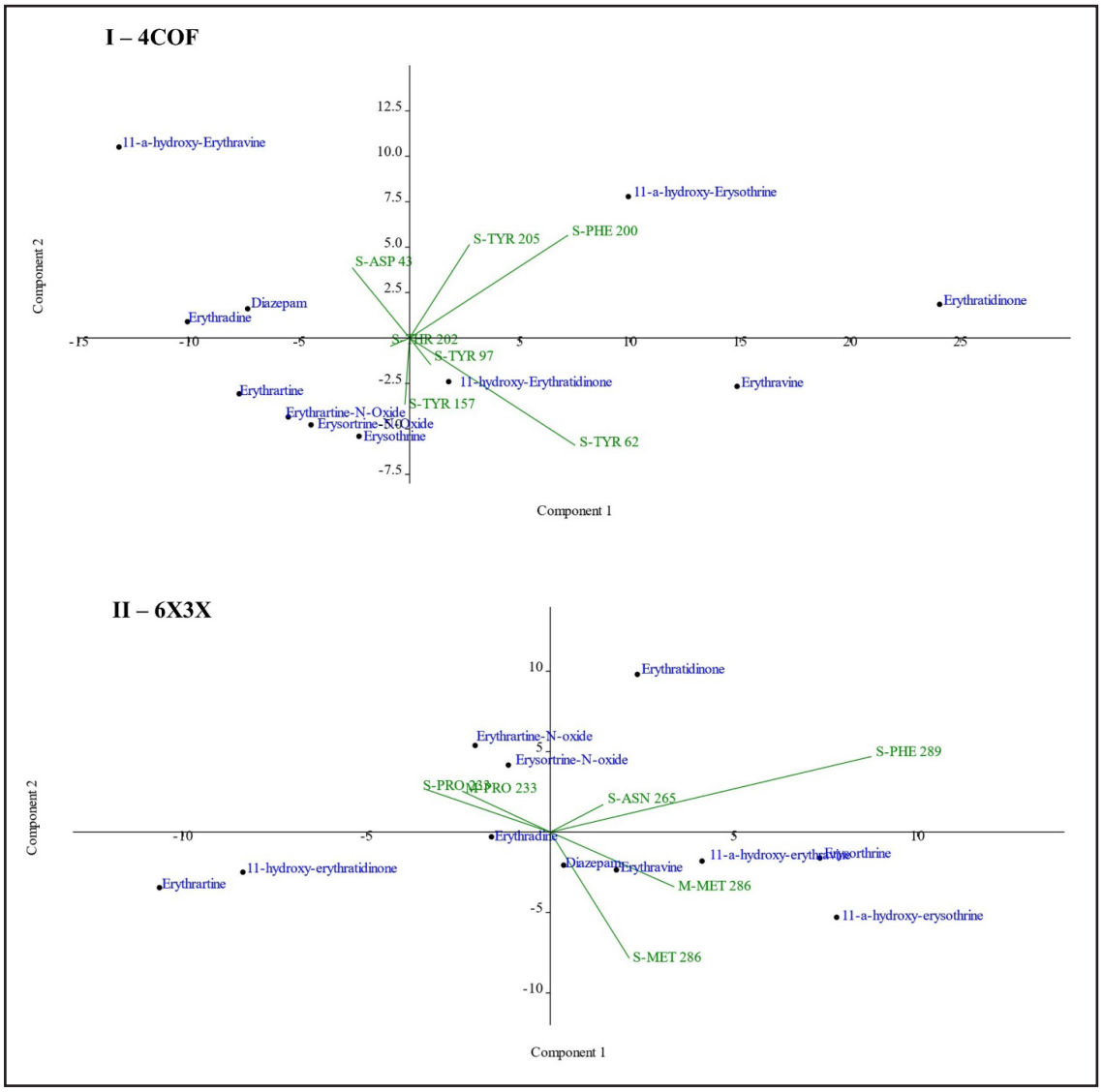


Source: the authors (2025)

3.1.3 Principal Component Analysis

Principal component analysis (PCA) analysis also emphasized that different structural modifications of erythrinic alkaloids, such as the presence of hydroxyl groups, can significantly influence van der Waals interactions and hydrogen bonds, altering binding affinity, consistent with previous findings on the importance of structural chemistry for biological activity (Tretter et al., 1997). PCA reinforced these findings, demonstrating that alkaloids clustering close to diazepam, such as erythradine and erysortrine in the 4COF model, and erythravine and hydroxy erythravine in the 6X3X model (Figure 4), possess interaction profiles that may mimic the anxiolytic effects of the positive control (Olsen, 2018).

Figure 4 – Principal Component Analysis (PCA) Biplot of Component 1 and Component 2 Showing the Interaction Profiles of Erythrinic Alkaloids and Diazepam with the I - GABAA R-β3 Receptor (4COF Model), and II - GABAA R-α1-β2-γ2 Receptor (6X3X Model)



Source: the authors (2025)

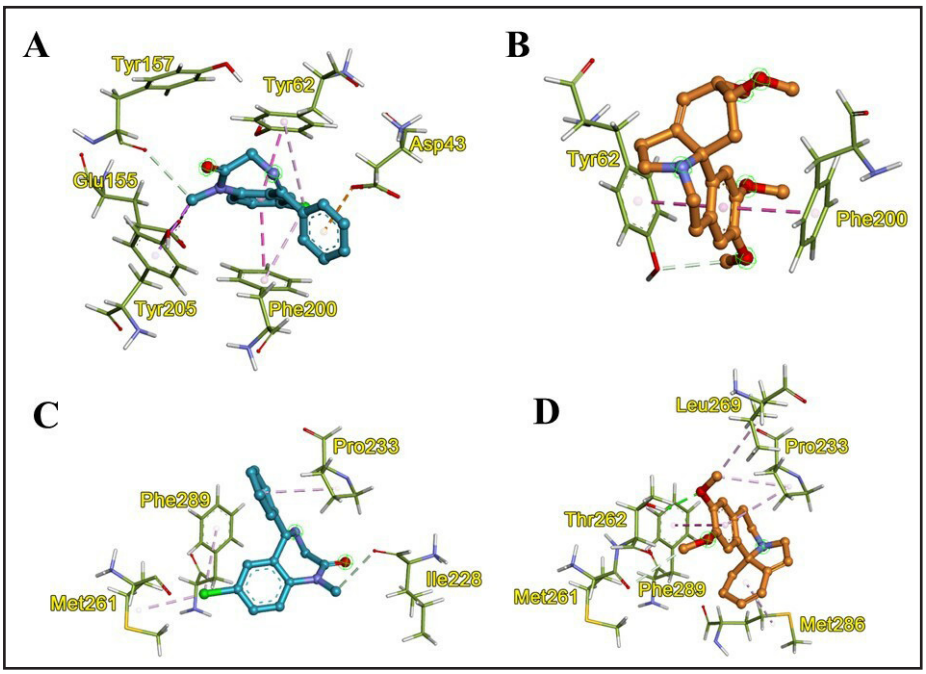
Considering the erythradine also showed low values of pose-cavity of GABAA interaction, the PCA an HCA results indicated that this compound may be better compound candidate to bound with receptor on camparison to others studied. For the receptor 6X3X, the first principal component explained 49.89% of the variance, and the second principal component explained 30.71%, indicating that 80.60% of the total variance is accounted for by these two components. Similarly, for the receptor

4COF, the first principal component accounted for 70.34% of the variance, with the second principal component explaining an additional 14.15%, resulting in a combined total of 84.49% of the variance explained by the first two principal components (Cemin et al., 2024).

3.1.3 Biding Site Interactions

Evaluating the molecules 11-α-hydroxy-erysorthrine, 11-α-hydroxy-erythravine, 11-hydroxy-erythratidinone, erysortrine-N-oxide, erysotrine, erythradine, erythrartine-N-oxide, erythrartine, erythratidinone and erythravine by docking represented in 3D model (Figure 5), it was possible to verify important interactions in amino-acid residues ASP 43, TYR 62, GEN 64, GLU 155, TYR 157, PHE 200, THR 202 and TYR205, with emphasis on TYR 62, GLU 155, PHE 200 and TYR 205 interactions of diazepam and erythravine at the active site of the 4COF model, the consensus of these interactions (Table 3).

Figure 5 – Docked molecules represantation in BEN cavity binding site of 4COF and BDZ cavity biding site of 6X3X models, a – Diazepam, b – erythradine, interactions in the biding site of 4COF model and c – Diazepam, d – erythravine, interactions in the biding site of 6X3X model



Source: the authors (2025)

Table 4 – Energy of the Van Der Waals (VDW) and hydrogen interactions (HI) (Kcal mol^{-1}) of the erythrinic alkaloid and diazepam with 6X3X amino acid residues by consensus

Compounds / Interactions	S-ASN 265	M-MET 286	S-MET 286	S-PHE 289	M-PRO 233	S-PRO 233
Erythradine						
VDW	-2.40	-5.67	-8.38	-20.18	-5.91	-8.96
HI	-6.00	0.0	–	–	0.0	–
11-hydroxy-erythratidinone						
VDW	-12.01	-7.20	-7.20	-24.29	-3.64	-4.91
HI	-1.10	-3.50	–	–	0.0	–
Diazepam						
VDW	-8.40	-3.74	-6.87	-17.89	-7.38	-7.81
HI	0.0	0.0	–	–	0.0	–
Erythrartine						
VDW	-11.39	-7.30	-6.40	-26.60	-2.40	-3.99
HI	0.0	0.0	–	–	0.0	–
Erythrartine-N-oxide						
VDW	-5.03	-6.75	-12.56	-17.05	-4.23	-5.54
HI	-0.77	0.0	–	–	0.0	–
11-α-hydroxy-erysothrine						
VDW	-9.07	-0.31	-0.25	-11.71	-5.54	-9.02
HI	-3.50	0.0	–	–	-2.50	–
Erythravine						
VDW	-7.42	-3.57	-6.29	-17.07	-7.04	-9.46
HI	-3.50	0.0	–	–	0.0	–
Erysortrine-N-oxide						
VDW	-5.19	-5.88	-11.52	-16.86	-4.32	-6.35
HI	0.0	0.0	–	–	0.0	–
11-α-hydroxy-erythravine						
VDW	-7.72	-3.36	-5.05	-14.24	-5.41	-9.72
HI	-3.50	0.0	–	–	-3.50	–
Erythratidinone						
VDW	-9.87	-7.92	-13.05	-9.14	-1.55	-4.35
HI	0.0	0.0	–	–	0.0	–
Erysorthrine						
VDW	-7.38	-2.54	-6.19	-12.16	-9.52	-11.22
HI	0.0	0.0	–	–	0.0	–

Source: the authors (2025)

Main Van Der Waals (VDW) and hydrogen bonding (HI) interactions of compounds and amino acid residues involved in the active site of the $GABA_A$ receptor (PDB 6X3X) and application of Consensus Residue Analysis using the iGemDOCK™ program (v.2.1)

In the 6X3X model the amino-acid residues ILE 228, PRO 233, MET 261, THR 262, LEU 269, MET 286 and PHE 289, with emphasis on PRO 233, MET 261 and PHE 289, there is a consensus of these interactions (Table 4), corroborating the effects of mulungu extracts as like benzodiazepines (Flausino et al., 2007; Sahila et al., 2015).

The main force involved in this process is VDW and, at lower energy, hydrogen bonding stands out. Evaluating the interactions in 4COF model, erythrartine was the compound that obtained the highest binding energy, -101.21 Kcal mol^{-1}, with VDW and hydrogen binding interactions with values of -85.79 and -15.43 Kcal mol^{-1} respectively. It can be considered a good interaction energy when compared to the energy obtained in diazepam docking that presented only VDW interaction, -83.78 Kcal mol^{-1}, in the active site of the 4COF receptor. Furthermore, the erythradine was the compoud with interactions most similar to diazepam (Figure 2) with -90.83 Kcal mol^{-1} biding energy, being -86.56 Kcal mol^{-1} VDW and -4.27 Kcal mol^{-1} hydrogen biding.

Evaluating the interactions in 6X3X model, the compound erythradine have the highest binding energy, -94.49 Kcal mol^{-1}, with VDW and hydrogen binding interactions with values of -85.99 and -8.5 Kcal mol^{-1} respectively. The compound most like diazepam, binding energy -90.26 Kcal mol^{-1}, exclusively VDW interaction, was the erythravine (Figure 3) with binding energy of -83.66 Kcal mol-1, being -77.67 and -5.99 Kcal mol^{-1}, VDW and hydrogen binding interactions respectively.

3.1.4 Erythrinic Alkaloids Action

Evidence that phytochemicals act in the central nervous system indicates that they have an inhibitory action on the GABAergic system due to the reduction in GABA levels and the similarity of anxiety-related responses sensitive to low doses of benzodiazepines in mice in tests such as elevated plus maze, elevated T-maze and light-dark transition (Onusic et al., 2002, Onusic et al., 2003; Flausino et al., 2007; Chu et al., 2019).

The results of the *in silico* study indicate the connection of the molecules present in mulungu extracts to the GABAA receptor (4COF and 6X3X models). However, Flausino

(2006; 2007) suggests other possible pathway, as in high doses, benzodiazepines impair locomotor activity, an event that does not occur with high doses of erythrinic alkaloids. This may be due by an action in a different subunit of GABAA, like evaluated in 4COF and 6X3X, or a possible interaction with 5-HT receptors, as observed by Rogers and collaborators (2001), where the E. vespertilio extract proved to be effective in inhibiting the release of 5-HT in vitro, thus promoting the anxiolytic effects without causing changes in locomotor activities.

Another possibility is a non-selective and dose-dependent action, where in low doses of alkaloids the action occurs mediated by GABA receptors sensitive to low doses of benzodiazepines, and in high doses of alkaloids it occurs on 5-HT receptors, requiring future evaluations.

Considering that all the molecules studied showed a strong correlation among the interactions, it is possible that the anxiolytic, hypnotic, and sedative actions are presented more effectively by the complete set of molecules. Flausino (2007) observed that the pure alkaloids did not alter escape latencies in the elevated T-maze. This possible synergistic action of the alkaloid molecules can be visualized by analyzing the dendrograms and HCA (Figure 2-3), where it is possible to see a major part of the molecules interacting in a very similar way with diazepam in the residues of the active sites, sugesting that sinergic interaction of erithrinic alkaloids group.

The predictions made in the OSIRIS™ software did not indicate potential risks associated with the structures of erythrinic alkaloids in the mutagenic, tumorigenic, irritant, and reproductive effective risks parameters, indicating good safety in the consumption of these alkaloids' substances. Evidence indicates that the extracts are highly safe compared to doses of 2000 and 5000 mg/kg when administered orally, with a minimum risk of acute poisoning (Craveiro et al., 2008; Pitchaiah et al., 2008).

The results of the *in silico* study indicate a great interaction of the molecules present in the mulungu tea to two different GABAA subunits receptor models, being similar to those observed in diazepam, as proposed in previous works, where the

anxiolytic action is presented, in a safe way. By the consensus of docking, is possible observe that erythrinic alkaloids have important interactions in amino acids residues involved in diazepam action.

4 CONCLUSIONS

In silico results demonstrated an important interaction of erythrinic alkaloids present in Erythrina verna plant with active site of the models (PDB: 4COF; 6X3X), similar to diazepam (positive control). In the multivariate analysis, PCA and HCA further supported these findings. Specifically, important interactions were observed with residues THR 202 and S-ASN 266, which are critical for the binding of positive allosteric modulators to GABAA receptors. Moreover, erythrinic alkaloids proved to be safe for consumption based in tested toxicological prediction. These findings provide a solid basis for future studies on the structural optimization and development of new natural anxiolytics, aligning with the literature advocating the search for natural alternatives to synthetic anxiolytic drugs.

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