DISCIPLINARUM SCIENTIA *Disciplinarum Scientia.* **Série: Naturais e Tecnológicas**
Santa Maria, v. 26, n. 3, p. 21-40, 2025
Recebido em: 05.08.2025. Aprovado em: 05.09.2025.
ISSN 2176-462X | DOI: doi.org/10.37779/nt.v26i3.5518

Limonene against GABAA and 5-HT2A receptors: psychopharmacological potential evaluated by docking and ADMET

Limoneno frente aos receptores GABAA e 5-HT2A: potencial psicofarmacológico avaliado por docking e ADMET

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ABSTRACT

Natural products, especially metabolites from essential oils, have been receiving increasing attention in pharmacology due to their therapeutic potential and lower risk of adverse effects. Limonene, a monoterpene found in species of the Rutaceae family, has been associated with anxiolytic and antidepressant effects. This study evaluated, through molecular docking analyses, the interactions of limonene with $GABA_A$ receptor subunits (α1, α2, α3, α5) and with the 5-HT2A receptor. SwissADME, pkCSM, ADMETlab 3.0, and ProTox 3.0 were employed for pharmacokinetic and toxicogenomic predictions, while HEX 8.0.0 was used for molecular docking, with diazepam (DZP) and fluoxetine (FLU) as reference controls. The interactions were further inspected using PyMOL 1.4.7 and Discovery Studio 2021. The results indicated a favorable pharmacokinetic profile, good bioavailability, absence of significant toxicity, and binding affinity comparable to the reference drugs. Interactions with functional receptor residues suggest potential anxiolytic and antidepressant

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modulatory effects. Overall, the findings highlight limonene as a promising candidate for the development of novel phytopharmaceuticals, warranting further in vitro and in vivo validation.

Keywords: Natural Products; Phytochemicals; Terpenes; Molecular docking; ADMET.

RESUMO

Produtos naturais, especialmente metabólitos de óleos essenciais, vêm recebendo crescente atenção na farmacologia por seu potencial terapêutico e menor risco de efeitos adversos. O limoneno, monoterpeno presente em espécies da família Rutaceae, tem sido associado a efeitos ansiolíticos e antidepressivos. Este estudo avaliou, por meio de análises de docking molecular, as interações do limoneno com subunidades do receptor $GABA_A$ (α1, α2, α3, α5) e com o receptor $5\text{-}HT_{2A}$. Foram utilizados os softwares SwissADME, pkCSM, ADMETlab 3.0 e ProTox 3.0 para predição farmacocinética e toxicogenômica, além do HEX 8.0.0 para docking molecular, tendo diazepam (DZP) e fluoxetina (FLU) como controles. As interações foram inspecionadas com PyMOL 1.4.7 e Discovery Studio 2021. Os resultados indicaram perfil farmacocinético favorável, boa biodisponibilidade, ausência de toxicidade relevante e afinidade de ligação comparável aos fármacos de referência. As interações com resíduos funcionais sugerem efeito modulador ansiolítico e antidepressivo. Em conjunto, os achados reforçam o limoneno como candidato promissor para o desenvolvimento de novos fitofármacos, recomendando-se validação experimental in vitro e in vivo.

Palavras-chave: *Produtos Naturais; Fitoquímicos; Terpenos; Ancoragem Molecular; ADMET.*

1 INTRODUCTION

Mental health disorders, especially those related to anxiety, are among the leading causes of global disease burden (Morin and Jarrin, 2022). Anxiety is characterized as a diffuse and unpleasant emotional state, usually accompanied by physical symptoms such as headache, sweating, palpitations, tachycardia and gastrointestinal discomfort (Islam *et al.*, 2022). In addition to affecting psychological well-being, anxiety is associated with various chronic conditions, such as cardiovascular disease, diabetes and hypertension (Khan and Khan, 2020).

Several factors are implicated in the development of anxiety, including genetic, neurobiological, environmental and social aspects (Chowdhury *et al.*, 2024). Alterations in the levels of neurotransmitters such as norepinephrine (NE), dopamine (DA) and serotonin (5-HT) have also been associated with anxiety and depression (Ghallab and Elassal, 2024).

Among the neurochemical targets most studied for the treatment of these disorders are the GABA, AMPA, NMDA and dopamine receptors (Wang *et al.*, 2021; Zhou *et al.*, 2023). The $GABA_A$ receptor, in particular, stands out for its wide brain distribution and inhibitory role in neurotransmission (Teleanu *et al.*, 2022a). This receptor is made up of several subunits, of which α1 is associated with the sedative effects of benzodiazepines (BZDs), while the α2 and α3 subunits are related to anxiolytic effects (Bhuia *et al.*, 2023b). The α5 subunit, on the other hand, seems to be more involved in cognitive functions, such as memory processing (Behlke *et al.*, 2016).

BZDs remain one of the main pharmacological options for anxiety disorders (Edinoff *et al.*, 2021). Other drugs such as pregabalin, buspirone and opipramol are also used, although with limitations in terms of efficacy or side effects (Islam *et al.*, 2020). Prolonged use of these drugs can lead to adverse effects such as excessive sedation, fatigue, memory deficits and motor coordination (Koren *et al.*, 2024).

As a result, there is growing interest in natural compounds with therapeutic potential, such as terpenes, due to their wide bioavailability and lower risk of adverse effects (Bhuia *et al.*, 2023b; Ferdous *et al.*, 2024). Limonene, a monocyclic monoterpene present in essential oils from various plant species, has demonstrated anxiolytic and antidepressant properties, possibly by acting on pathways related to dopamine, serotonin, GABA and glutamate (Masyita *et al.*, 2022; Silveira *et al.*, 2022).

Despite the promising results, the molecular mechanisms involved have yet to be fully elucidated. In this context, computational pharmacology tools such as molecular docking and molecular dynamics simulations offer effective and economical methods for predicting interactions between bioactive molecules and pharmacological targets (Erdogan, 2021; Sahu *et al.*, 2024). Such approaches are especially useful in investigating the therapeutic potential of natural compounds on anxiety-related receptors such as $GABA_A$ and $5\text{-}HT_{2A}$.

Previously, our group demonstrated the potential of limonene to interact with the $GABA_A$ receptor as a whole, suggesting anxiolytic activity. Based on these findings, this study aimed to deepen the investigation by evaluating the interaction with specific $GABA_A$ subunits and with the $5\text{-}HT_{2A}$ serotonin receptor, broadening the understanding of limonene's pharmacological profile.

2. METHODOLOGY

2.1. *IN SILICO* APPROACH

2.1.1 Preparation of ligands

The three-dimensional (3D) conformers of the compound Limonene (PubChem ID: 22311), the standard drug Diazepam - DZP (PubChem ID: 3016), flumazenil - FLU (PubChem ID: 3373) and Fluoxetine (PubChem ID: 3386) were retrieved in SDF format from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al.*, 2021), accessed on April 8, 2025.

2.1.2. Pharmacokinetic properties, drug similarity and toxicity prediction

Drug similarity is a qualitative assessment widely used in the research and development of new drugs, with the aim of estimating the behavior of chemical compounds in parameters related to bioavailability, metabolism and toxicity (ADMET). The physicochemical and pharmacokinetic

properties of Limonene were predicted using the online tools SwissADME (Daina *et al.*, 2017), pkCSM (Pires *et al.*, 2015) and ADMETlab 3.0 (Fu *et al.*, 2024). In addition, the compound's toxicity profile was assessed using the ProTox 3.0 online server, which allows the prediction of parameters such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. The SMILES (Simplified Molecular Input Line-Entry System) notations obtained from PubChem were used as input in the SwissADME, pkCSM, ADMETlab 3.0 and ProTox 3.0 tools for the aforementioned analyses. Results from the different servers were compared and integrated, with emphasis on convergent parameters such as oral bioavailability, gastrointestinal absorption, blood-brain barrier penetration, cytochrome P450 inhibition, hepatotoxicity, mutagenicity, and LD50 values, ensuring consistency of pharmacokinetic and toxicological predictions.

2.1.3. Protein selection and preparation

The α1, α2, α3 and α5 subunits of the human $GABA_A$ receptor were selected as targets for molecular docking studies, based on data from the literature (Bhuia *et al.*, 2023b; Liao *et al.*, 2022; Luscher *et al.*, 2023; Rudolph and Knoflach, 2011; Vollenweider *et al.*, 2011). Also included was the $5\text{-}HT_{2A}$ receptor, which is widely related to the pathophysiology of depression (Savitz *et al.*, 2009). The three-dimensional (3D) structures of the $5\text{-}HT_{2A}$ receptor (PDB ID: 7WC4) and the $GABA_A$ subunits (α1: PDB ID 6HUJ / UniProt ID: P14867; α2: 6HUG / P47869; α3: 6HUJ / P34903; α5: 7QNE / P31644) were obtained from the UniProt and PDB databases (McGarvey *et al.*, 2019; Rudolph and Knoflach, 2011), using the NCBI BLAST tool for sequence alignment (Boratyn *et al.*, 2012). Before docking, all protein structures were pre-processed by removing crystallographic water molecules and native ligands.

2.1.4. Docking protocol and non-bond interactions

Molecular docking between Limonene and the reference ligands with the $GABA_A$ (α1, α2, α3, α5) and $5\text{-}HT_{2A}$ receptors was carried out using the software HEX (version 8.0.0, 64 bits) (Chowdhury *et al.*, 2024), PyMOL (version 1.4.7) (Daina *et al.*, 2017) and Biovia Discovery Studio® 2021. These programs were used to analyze molecular interactions and visualize bonds. All the software used is freely available for academic use.

3. RESULTS

3.1. PHARMACOKINETIC PROFILE

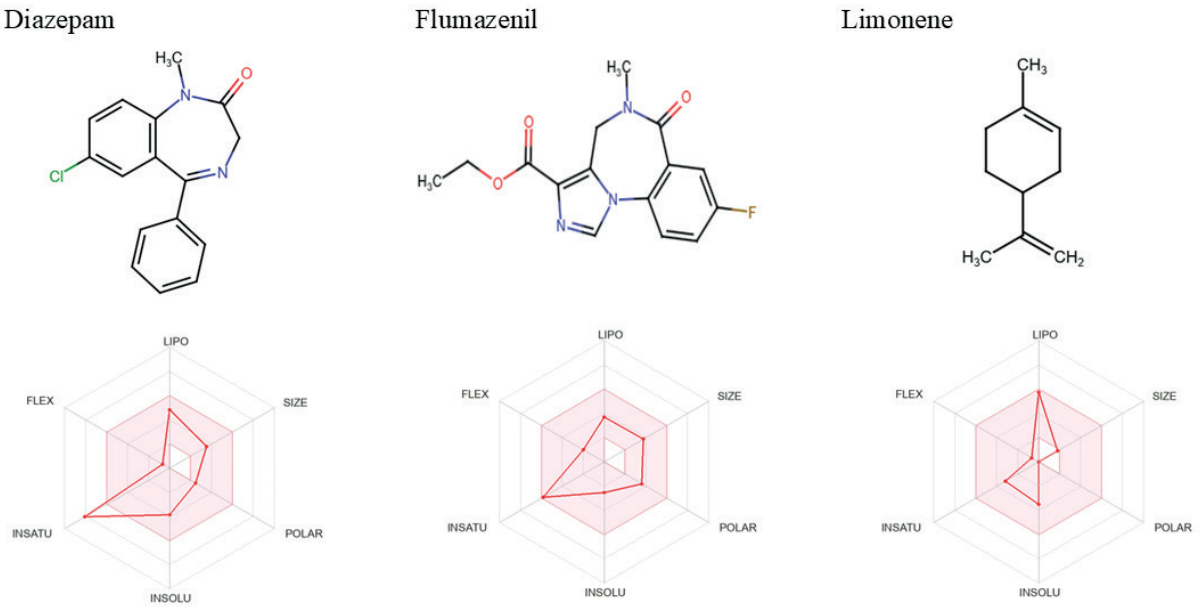
The pharmacokinetic profile of the limonene compound was evaluated using the Molinspiration platform, revealing characteristics favorable to its oral bioavailability. Limonene had a molecular weight of 136.24 g/mol, a topological polar surface area (TPSA) of 0.00 Å² and a molecular volume of 157.30 Å³. The analysis indicated the absence of hydrogen (nOHNH = 0) and oxygen or nitrogen (nON = 0) donor atoms, suggesting low polarity and high lipophilicity, also evidenced by the millogP value of 3.62. The compound showed no violations of Lipinski's rules, which reinforces its potential as a candidate drug for oral administration. With only one rotatable bond (nRotb = 1) and a relatively rigid structure, limonene shows structural characteristics compatible with good permeability in biological membranes (Table 1).

Table 1 - Physicochemical properties of the limonene compound by Molinspiration.

Compound	MW	TPSA	N-atoms	Volume	nON	nOHNH	Nviolations	Nrotb	milogP
Limoneno	136.24	0.00	10	157.30	0	0	0	1	3.62

Source: Construction of the Authors.

Figure 1 - Bioavailability radar related to the physicochemical properties of Limonene. Lipophilicity (LIPO); Polarity (POLAR); Insolubility (INSOLU); Unsaturation (INSATU); Flexibility (FLEX).

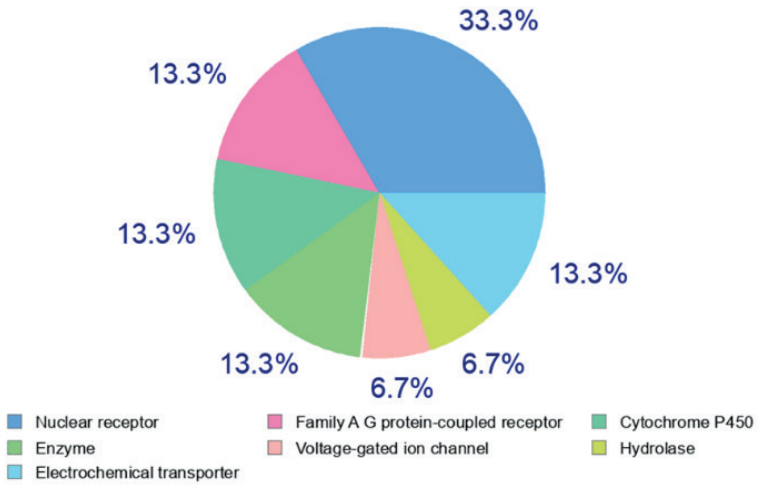


Source: Construction of the Authors.

3.2. PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITY

The prediction of limonene's molecular targets revealed a wide variety of possible biological interactions. The main class of targets corresponds to nuclear receptors, which accounted for 33.3% of the predicted interactions, indicating potential modulation of intracellular regulatory processes, such as gene expression. Other relevant categories included enzymes (13.3%), electrochemical transporters (13.3%), cytochrome P450 (13.3%) and family A G protein-coupled receptors (13.3%), suggesting that limonene may act on different metabolic pathways and cell signaling. Additional targets included voltage-dependent ion channels (6.7%) and hydrolases (6.7%), reinforcing the functional diversity of potential interactions (Figure 2). These results indicate that limonene has a multifaceted biological activity profile, with the possibility of interacting with different classes of biomolecules involved in relevant physiological and pharmacological processes.

Figure 2 - Pie chart of the virtual sorting of the target class prediction.



Source: Construction of the Authors.

3.3. ADME PROFILE AND TOXICITY

Predictive pharmacokinetic analysis of the compounds diazepam (DZP), flumazenil (FLU) and limonene revealed distinct absorption, distribution, metabolism, excretion and toxicity profiles. With regard to human intestinal absorption, limonene showed a high value (95.898%), close to that of diazepam (97.42%) and higher than that of flumazenil (93.567%), indicating good oral bioavailability (Table 2).

With regard to distribution, limonene had a higher volume of distribution (VDss = 0.396 log L/kg) compared to diazepam (0.365) and flumazenil (0.101), suggesting wide tissue dispersion. Permeability through the blood-brain barrier (BBB) was also higher for limonene (log BB = 0.732), demonstrating its good ability to cross this barrier, an essential factor for compounds acting

on the central nervous system. However, its permeability to the CNS (log PS = -2.37) was lower than that of diazepam (-1.397), which may indicate less direct penetration into neural tissues (Table 2).

As far as metabolism is concerned, none of the three compounds was identified as a significant substrate or inhibitor of the main cytochrome P450 isoforms (CYP2D6, CYP3A4, CYP2C19, CYP2C9, CYP1A2), except diazepam, which showed inhibitory action on CYP2C19, which could result in drug interactions in combination therapies. Limonene showed low metabolic interaction potential, which characterizes it as a promising molecule in terms of pharmacological safety (Table 2).

In terms of excretion, limonene had a clearance value of 0.213 log ml/min/kg, lower than flumazenil (0.758) and similar to diazepam (0.294), suggesting slower elimination from the body (Table 2).

Table 2 - ADME and toxicity using the pkCSM tool.

		DZP	Limonene	FLU	Unit
Absorption					
Intestinal absorption (human)		97.42	95.898	93.567	(%Absorbed)
Distribution					
VDss (human)		0.365	0.396	0.101	(log L/kg)
Permeability BBB		0.331	0.732	-0.205	(log BB)
Permeability CNS		-1.397	-2.37	-3.015	(log PS)
Metabolism					
	2D6 substrate	No	No	No	Categorical (Yes/No)
	3A4 substrate	Yes	No	No	Categorical (Yes/No)
	2C19 inhibitor	Yes	No	No	Categorical (Yes/No)
CYP	2C9 inhibitor	Yes	No	No	Categorical (Yes/No)
	2D6 inhibitor	No	No	No	Categorical (Yes/No)
	3A4 inhibitor	No	No	No	Categorical (Yes/No)
	1A2 inhibitor	Yes	No	Yes	Categorical (Yes/No)
Excretion					
Debugging		0.294	0.213	0.758	Numeric (log ml/min/kg)

Source: Construction of the Authors.

Toxicity, the predictive data indicate that limonene has a favorable profile. Its LD_{50} value was 4400 mg/kg, significantly higher than that of flumazenil (1300 mg/kg) and diazepam (48 mg/kg), indicating low acute toxicity. The three compounds were inactive for hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cardiotoxicity (hERG I and II), skin sensitization, drug-induced liver toxicity (DILI), AMES toxicity and acute oral toxicity in

rats. However, diazepam showed cytotoxic activity and was classified as toxicological class 2, while limonene was classified as class 5, reflecting a lower toxicological risk (Table 3).

These findings reinforce the therapeutic potential of limonene as a natural alternative with lower toxicity for disorders related to GABAergic neurotransmission, especially in anxiety disorders.

Table 3 - Toxicity profiling of the test sample Limonene and reference drugs (diazepam and flumazenil).

Toxicity	Parameters	Test sample and reference drugs		
		DZP	FLU	Limonene
	LD_{50}	48 mg/kg	1300 mg/kg	4400 mg/kg
	Toxicity Class	2	4	5
	Hepatotoxicity	Inactive	Inactive	Inactive
	Carcinogenicity	Inactive	Inactive	Inactive
	Immunotoxicity	Inactive	Inactive	Inactive
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	Active	Inactive	Inactive
	hERG I inhibitor	Inactive	Inactive	Inactive
	hERG II inhibitor	Inactive	Inactive	Inactive
	Skin sensitization	Inactive	Inactive	Inactive
	DÍLI	Inactive	Inactive	Inactive
	toxicity AMES	Inactive	Inactive	Inactive
	Acute oral toxicity in rats	Inactive	Inactive	Inactive

LD_{50}: Median lethal dose.

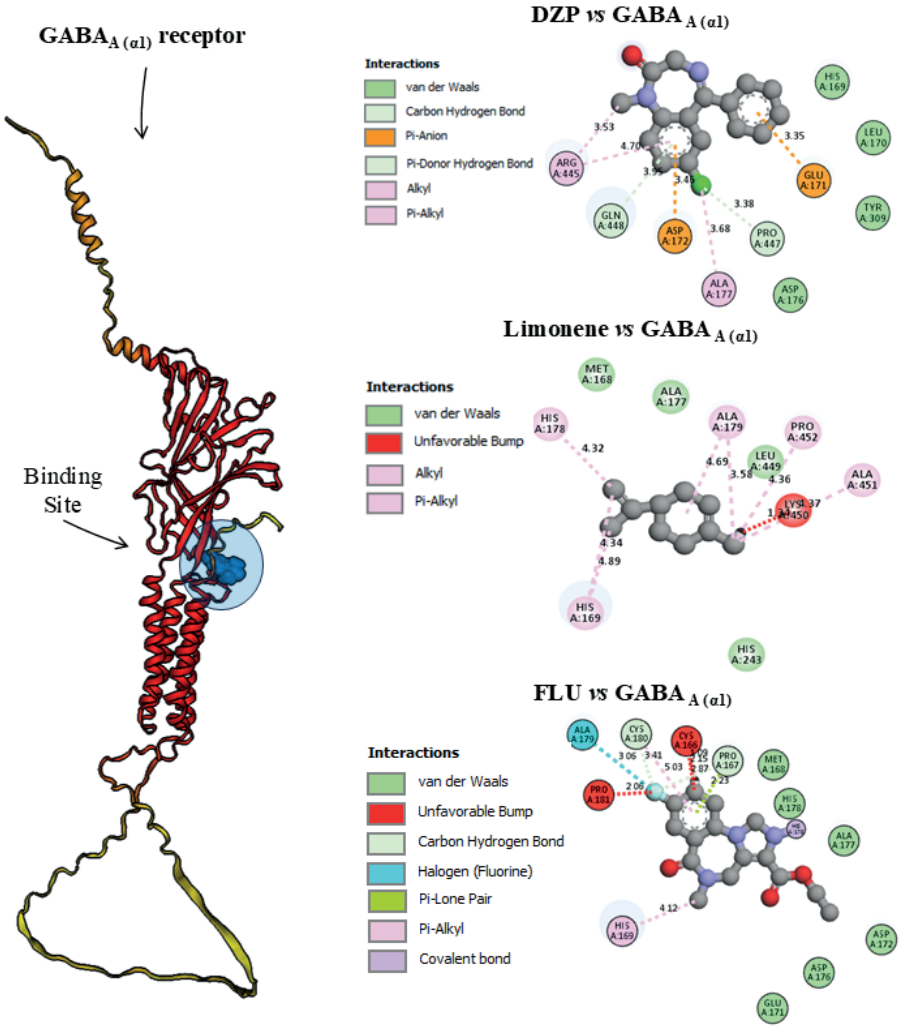
Source: Construction of the Authors.

3.3.1. In silico findings

3.3.1.1 Limonene, DZP and FLU with $GABA_{A\ (\alpha 1)}$ receptor interactions

Molecular docking analysis revealed that DZP showed a binding affinity ranging from -264.89 to -210.08 kcal/mol, and Limonene ranged from -158.62 to -147.95 kcal/mol with the $GABA_{A\ (\alpha 1)}$ receptor. DZP formed van der Waals bonds, carbon hydrogen bond, pi-anion, pi-donor hydrogen bond, alkyl-pi-alkyl with (Asp A: 176, Tyr A: 309, Leu A: 170, His A: 169, Asp A: 172, Glu A: 171, Gln A: 448, Pro A: 447, Arg A: 445, Ala A: 177). Limonene formed van der Waals, unfavorable bump, alkyl, pi-alkyl bonds with (Met A: 168, Ala A: 177, Leu A: 449, Lys A: 450, His A: 178, His A: 169, Ala A: 451, Pro A: 452, Ala A: 179). FLU formed van der Waals bonds, unfavorable bump, carbon hydrogen bond, halogen (fluorine), pi-lone pair, pi-alkyl, covalent bond with (Glu A: 171, Asp A: 176, Asp A: 172, Ala A: 177, His A: 178, Met A: 168, Pro A: 181, Cys A: 166, Cys A: 180, Pro A: 167, Ala A: 179, His A: 169) (Figure 3).

Figure 3 - 2D and 3D structure of the molecular docking interactions of limonene, diazepam and Flumazenil with the α1 subunits of the $GABA_A$ receptor.

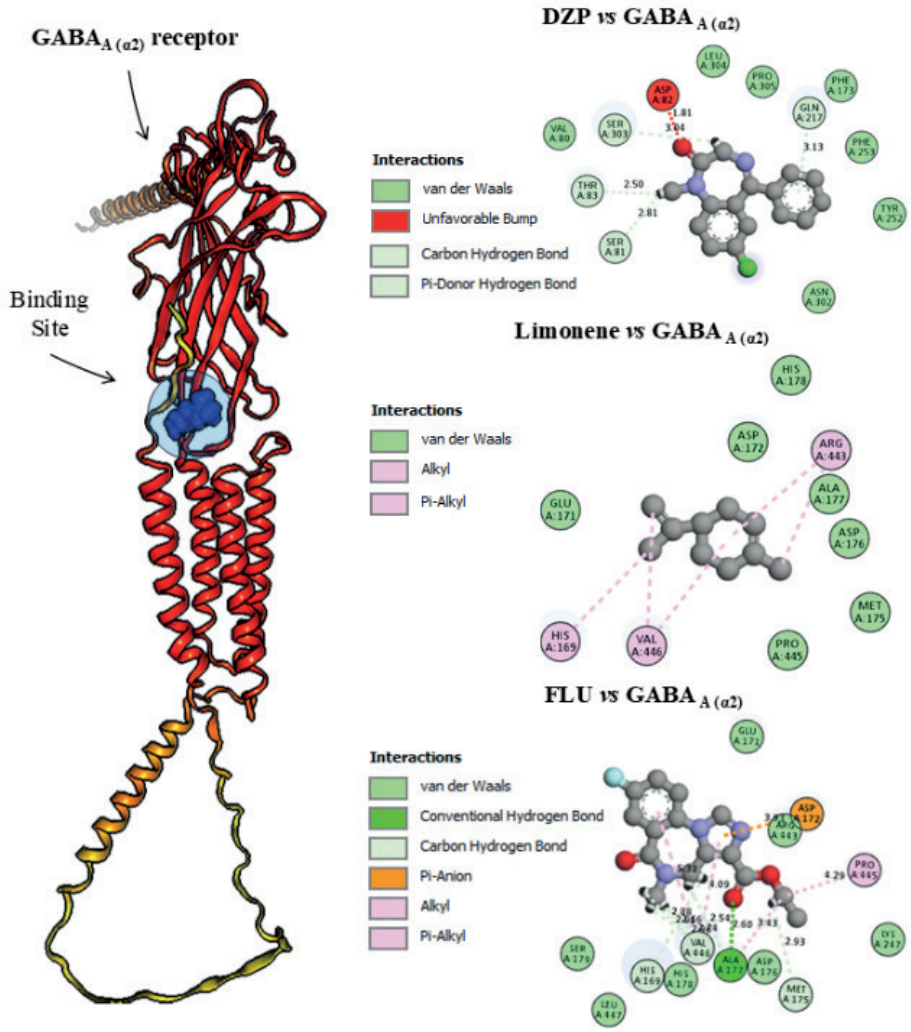


Source: Construction of the Authors.

3.3.1.2. Limonene, DZP and FLU with $GABA_{A\ (\alpha 2)}$ receptor interactions

Molecular docking analysis revealed that DZP showed a binding affinity ranging from -217.69 to -201.21 kcal/mol, and Limonene ranged from -178.41 to -151.73 kcal/mol with the GABA A (α2) receptor. DZP formed van der Waals, unfavorable bump, carbon hydrogen bond and pi-donor hydrogen bonds with (Val A: 80, Leu A: 3004, Pro A: 305, Phe A: 173, Ape A: 173, Phe A: 253, Thr A: 252, Asn A: 302, Ser A: 81, Thr A: 83, Ser A: 303, Gln A: 217). Limonene formed van der Waals, alkyl and pi-alkyl bonds with (Glu A: 171, Asp A: 172, His A: 178, Ala A: 177, Asp A: 176, Met A: 175, Pro A: 445, His A: 169, Val A: 446, Arg A: 443). FLU formed van der Waals, conventional hydrogen bond, carbon hydrogen bond, pi-anion, alkyl, pi-alkyl bonds with (Ser A: 179, Leu A: 447, His A: 178, Asp A: 176, Lys A: 247, Arg A: 443, Glu A: 171, His A: 169, Val A: 446, Met A: 175, Ala A: 177, Asp A: 172 and Pro A: 445) (Figure 4).

Figure 4 - 2D and 3D structure of the molecular docking interactions of limonene, diazepam and Flumazenil with the α2 subunits of the $GABA_A$ receptor.

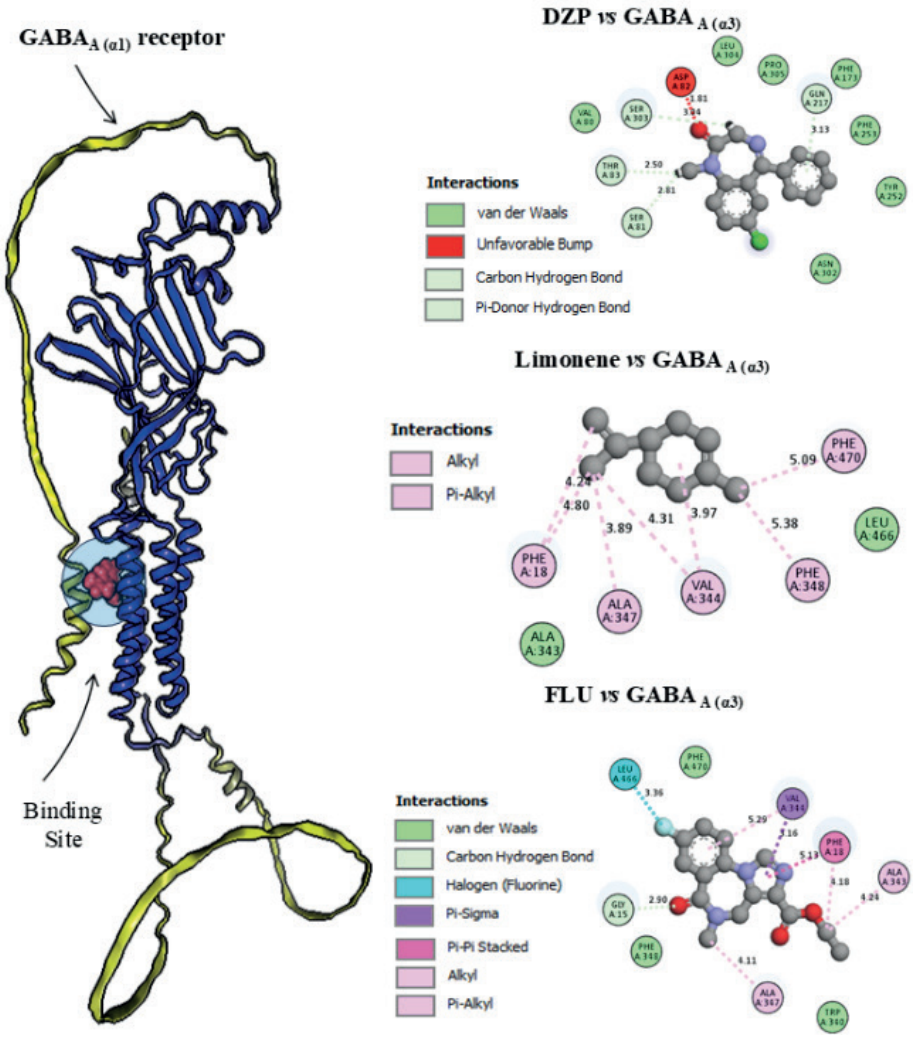


Source: Construction of the Authors.

3.3.1.3 Limonene, DZP and FLU with $GABA_{A\ (α3)}$ receptor interactions

Molecular docking analysis revealed that DZP showed a binding affinity ranging from -229.24 to -211.70 kcal/mol, and Limonene ranged from -165.68 to -145.43 kcal/mol with the $GABA_{A\ (α3)}$ receptor. DZP formed van der Waals bonds, unfavorable bump, carbon hydrogen bond, pi-donor hydrogen bond with (Val A: 80, Leu A: 304, Pro A: 305, Phe A: 173, Phe A: 253, Thr A: 252, Asn A: 302, Asp A: 82, Ser A: 81, Thr A: 83, Ser A: 303, Gln A: 217). Limonene formed alkyl, pi-alkyl bonds with (Ala A: 343, Leu A: 466, Phe A: 18, Ala A: 347, Val A: 344, Phe A: 348, Phe A: 470). FLU formed van der Waals bonds, carbon hydrogen bond, halogen (fluorine), pi-sigma, pi-pi stacked, alkyl, pi-alkyl with (Trp A: 340, Ohe A: 348, Ile A: 469, Phe A: 470, Gly A: 15, Leu A: 466, Val A: 344, Phe A: 18, Ala A: 343, Ala A: 347) (Figure 5).

Figure 5 - 2D and 3D structure of the molecular docking interactions of limonene, diazepam and Flumazenil with the α3 subunits of the $GABA_A$ receptor.

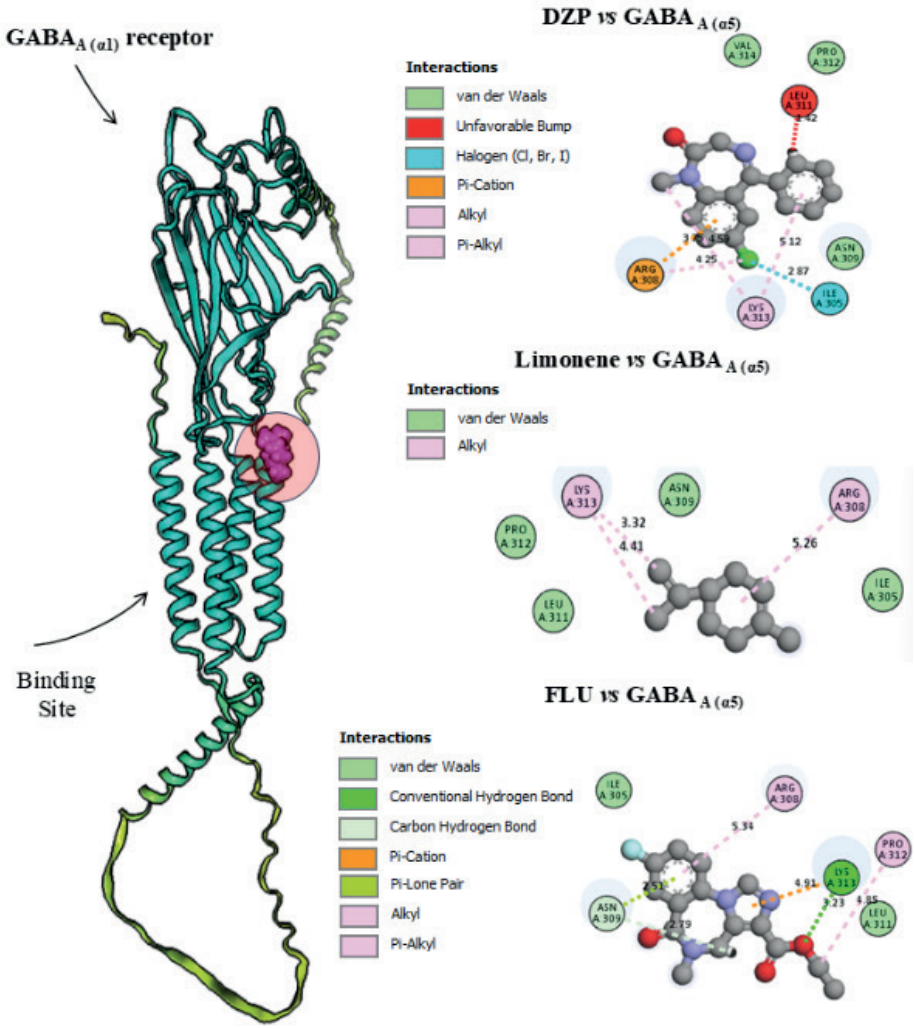


Source: Construction of the Authors.

3.3.1.4 Limonene, DZP and FLU with GABA$_A$ (α5) receptor interactions

Molecular docking analysis revealed that DZP showed a binding affinity ranging from -253.32 to -205.89 kcal/mol, and Limonene ranged from -150.19 to -140.20 kcal/mol with the GABA $_{A\ (\alpha5)}$ receptor. DZP formed van der Waals, unfavorable bump, halogen (Cl, Br, I), pi-cation, alkyl and pi-alkyl bonds with (Val A: 314, Pro A: 312, Asn A: 309, Leu A: 311, Arg A: 308, Ile A: 305, Lys A: 313). Limonene formed van der Waals and alkyl bonds with (Leu A: 311, Pro A: 312, Asn A: 309, Ile A: 305, Lys A: 313, Arg A: 308). FLU formed van der Waals, conventional hydrogen bond, carbon hydrogen bond, pi-cation, pi-lone pair, alkyl and pi-alkyl bonds with (Asn A: 309, Ile A: 305, Leu A: 311, Lys A: 313, Arg A: 308, Pro A: 312) (Figure 6).

Figure 6 - 2D and 3D structure of the molecular docking interactions of limonene, diazepam and Flumazenil with the α3 subunits of the GABA$_A$ receptor.

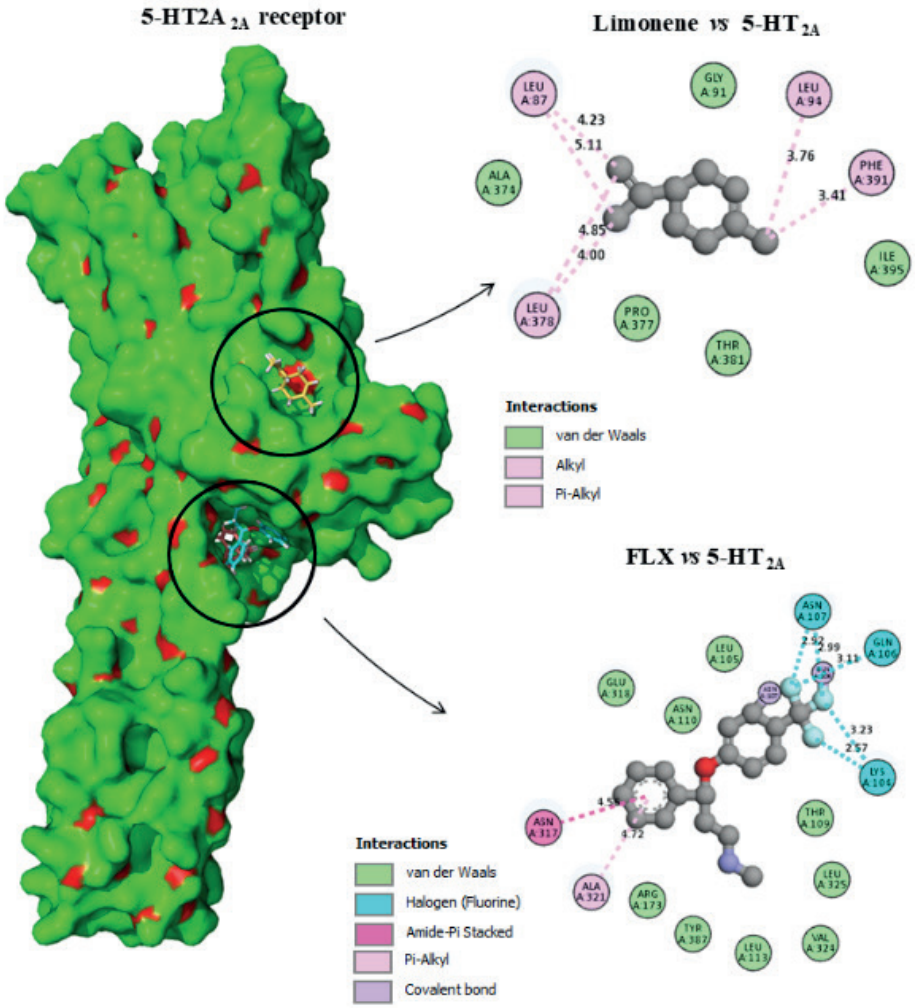


Source: Construction of the Authors.

3.3.1.5. Limonene and FLX with 5HT $_{2A}$ receptor interactions

In the molecular docking analysis with the 5HT $_{2A}$ receptor, FLX exhibited binding affinity ranging from -304.34 to -248.18 kcal/mol and Limonene ranged from -179.71 to -160.70 kcal/mol with the 5HT$_{2A}$ receptor. FLUX interacted with the receptor through multiple interactions, including van der Waals, halogen (fluorine), amide-pi stacked, pi-alkyl and covalent bond interactions with (Thr A: 109, Leu A: 325, Val A: 324, Leu A: 113, Tyr A: 387, Arg A: 173, Glu A: 318, Asn A: 110, Leu A: 102, Lys A: 104, Gln A: 106, Asn A: 107, Ala A: 321, Asn A: 317, Asn A: 107, Gln A: 106). Limonene interacted with the receptor through multiple interactions, including van der Waals, alkyl and pi-alkyl interactions with (Ala A: 374, Pro A: 377, Thr A: 381, Ile A: 395, Gly A: 91, Leu A: 87, Leu A: 378, Phe A: 391, Leu A: 94) (Figure 7).

Figure 7 - 2D and 3D structure of the molecular docking interactions of limonene and fluoxetine with the 5-HT$_{2A}$ receptor.



Source: Construction of the Authors.

4. DISCUSSION

The $GABA_A$ receptor represents one of the main pharmacological targets in the treatment of conditions such as anxiety, seizures and sleep disorders, due to its inhibitory function in the central nervous system (CNS). This ion channel, activated by the neurotransmitter gamma-aminobutyric acid (GABA), contributes to the modulation of neuronal excitability and preservation of synaptic homeostasis(Kalueff and Nutt, 2007; Schür *et al.*, 2016) Benzodiazepines, such as diazepam, act as positive allosteric modulators of this receptor, amplifying the action of GABA and resulting in effects such as sedation, reduced anxiety and muscle relaxation (Bappi *et al.*, 2024). This effect is mediated by the increased entry of chloride ions into neurons, promoting hyperpolarization of the membrane and decreasing electrical activity, especially in brain regions related to behaviour and emotions, such as the cortex and limbic system (Cherubini *et al.*, 2022; Ghit *et al.*, 2021). Despite their efficacy, prolonged use of benzodiazepines can lead to significant adverse effects, such as excessive sedation, the development of tolerance and the risk of addiction (Bhuia *et al.*, 2023a).

Our *in silico* analysis demonstrated that limonene interacts with multiple subunits of the GABAA receptor (α1, α2, α3, and α5), exhibiting significant binding affinities (Figure 1-6). For comparative purposes, DZP was employed as a positive control, while flumazenil (FLU), a competitive antagonist of the BDZ binding site, served as a negative control (González Gómez *et al.*, 2023). The action of DZP is well established on subunits such as α1 and α2 of $GABA_A$, associated with sedative and anxiolytic effects, respectively (Fritschy and Panzanelli, 2014; Liao *et al.*, 2022; Luscher *et al.*, 2023; Ochoa-de la Paz *et al.*, 2021; Vollenweider *et al.*, 2011). FLU, on the other hand, acts as a competitive antagonist with an affinity for these same sites and is widely used to reverse the effects of benzodiazepines (Husain *et al.*, 2024).

In the α1 subunit, DZP showed interactions with critical residues such as Asp176, Tyr309 and His169, corroborating its pharmacological action. FLU showed interactions typical of antagonism, with Glu171 and Cys180. Limonene, on the other hand, interacted mainly through hydrophobic bonds (Van der Waals, pi-alkyl), with residues such as Met168 and Leu449, suggesting possible modulatory activity, although with a different mechanism to the classic drugs (Figure 3).

In the α2 subunit - especially associated with anxiolytic action - limonene showed good binding affinity and interactions with residues such as Met175 and Arg443, similar to those observed for DZP, which reinforces its potential anxiolytic effect (Figure 4).

With regard to the α3 subunit, limonene interacted with residues such as Leu466 and Phe348, suggesting a potentially lower anxiolytic effect. In the α5 subunit, involved in cognitive modulation and sedation, limonene showed relevant interactions, although less complex than DZP and FLU, indicating a milder action. Residues such as His169, Tyr309 and Phe253 are known to be important for binding benzodiazepines and positive allosteric modulators to $GABA_A$, according to structural

and functional studies (Husain *et al.*, 2024), which validates the interaction observed with limonene (Figure 5).

In addition to the docking analyses, limonene showed an excellent pharmacokinetic and safety profile. According to the Lipinski Rule criteria, the compound showed no violations, exhibiting adequate molecular weight, low polarity and good lipophilicity, suggesting high permeability by biological membranes (Bhuia *et al.*, 2023b; Mukty *et al.*, 2024).

Drug similarity is put forward to provide insightful recommendations in the early stages of drug development, thus increasing the likelihood that a chemical can meet the criteria and be approved for clinical trials (Teleanu *et al.*, 2022a). The main reasons for rejecting drug candidates are inadequate pharmacokinetic characteristics or inadequate toxicity. It has become a significant criterion in selecting molecules with optimal bioavailability throughout the early stages of drug development (Glassman and Balthasar, 2019).

According to Lipinski's five rules, a drug candidate must have a PM of 500 g/mol or less, a lipophilicity (LogP o/w) of five or less, no more than 5 HBD and no more than 10 HBA (Lipinski, 2004). Assessing the toxicity of new chemical substances is important to the drug development process, as it helps to select and prioritize molecules with the greatest promise of safe and effective use in humans. Preclinical toxicity tests on various biological systems provide the harmful effects of organisms of specific species and experimental dose content (Amorim *et al.*, 2024). In humans, prolonged chemical exposure often causes many types of organ poisoning, including organ toxicity, immunotoxicity, neurotoxicity, carcinogenicity and genotoxicity. In addition, established reproductive toxicity has been found to produce neurotoxicity mediated by reactive oxygen species, which is favorably associated with Alzheimer's disease (Di; Kerns, 2016).

Predictive toxicity assessment using platforms such as SwissADME, pkCSM, ADMETlab 3.0 and ProTox 3.0 are web-based programs that we used to estimate the drug-like characteristics and ADMET qualities of Limonene. All the parameters estimated for Limonene (drug-like properties and ADMET profile), as shown in Table 2, remained within the permissible limits. It indicated that limonene has high tolerance (LD50 of 4400 mg/kg, Class 5), with no evidence of hepatotoxicity, mutagenicity, immunotoxicity or carcinogenicity. In comparison, FLU has intermediate toxicity (LD50 of 1300 mg/kg, Class 4), while DZP showed greater acute toxicity (LD50 of 48 mg/kg, Class 2), as well as potential cytotoxic effects at high doses or prolonged use (Table 2).

Therefore, the *in silico* data obtained suggests that limonene is a safe and promising candidate with potential anxiolytic and antidepressant activity, possibly mediated by interaction with subunits of the $GABA_A$ receptor and also, in complementary studies, with the serotoninergic $5HT_{2A}$ receptor. These findings justify future *in vitro* and *in vivo* investigations, with the aim of validating its behavioral effects and elucidating its mechanism of action.

5. CONCLUSION

This study has shown, through molecular modeling and in silico predictions, that limonene has pharmacological and toxicological properties compatible with a potential therapeutic agent in the treatment of anxiety and depression disorders. Its ability to interact with specific subunits of the $GABA_A$ receptor and the $5\text{-}HT_{2A}$ receptor, together with its safe pharmacokinetic and toxicological profile, indicates that limonene can act as a positive allosteric modulator with a lower risk of side effects compared to classic drugs such as benzodiazepines. Although promising, this computational evidence requires experimental validation *in vitro* and *in vivo* models to confirm efficacy and fully elucidate the mechanisms of action. Taken together, the findings highlight limonene as a promising phytopharmaceutical candidate for the development of safer and more effective therapeutic strategies for anxiety and depression.

REFERÊNCIAS

AMORIM, A. M. B. *et al.* Advancing drug safety in drug development: bridging computational predictions for enhanced toxicity prediction. **Chemical Research in Toxicology**, v. 37, p. 827-849, 2024. DOI: https://doi.org/10.1021/acs.chemrestox.3c00352.

BAPPI, M. H. *et al.* Quercetin increases the antidepressant-like effects of sclareol and antagonizes diazepam in thiopental sodium-induced sleeping mice: a possible GABAergic transmission intervention. **Phytotherapy Research**, v. 38, p. 2198-2214, 2024. DOI: https://doi.org/10.1002/ptr.8139.

BEHLKE, L. M. *et al.* A pharmacogenetic 'restriction-of-function' approach reveals evidence for anxiolytic-like actions mediated by α5-containing $GABA_A$ receptors in mice. **Neuropsychopharmacology**, v. 41, p. 2492-2501, 2016. DOI: https://doi.org/10.1038/npp.2016.49.

BHUIA, Md. S. *et al.* Anxiolytic-like effects by trans-ferulic acid possibly occur through GABAergic interaction pathways. **Pharmaceuticals**, v. 16, p. 1271, 2023a. DOI: https://doi.org/10.3390/ph16091271.

BHUIA, Md. S. *et al.* Hirsutine, an emerging natural product with promising therapeutic benefits: a systematic review. **Molecules**, v. 28, p. 6141, 2023b. DOI: https://doi.org/10.3390/molecules28166141.

BORATYN, G. M. *et al.* Domain enhanced lookup time accelerated BLAST**. Biology**

Direct, v. 7, p. 12, 2012. DOI: https://doi.org/10.1186/1745-6150-7-12.

CHERUBINI, E. *et al.* Dysregulation of GABAergic signaling in neurodevelopmental disorders: targeting cation-chloride co-transporters to re-establish a proper E/I balance. **Frontiers in Cellular Neuroscience**, v. 15, 2022. DOI: https://doi.org/10.3389/fncel.2021.813441.

CHOWDHURY, R. *et al.* An insight into the anticancer potentials of lignan arctiin: a comprehensive review of molecular mechanisms. **Heliyon**, v. 10, e32899, 2024. DOI: https://doi.org/10.1016/j.heliyon.2024.e32899.

DAINA, A. *et al.* SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules**. Scientific Reports**, v. 7, 2017. DOI: https://doi.org/10.1038/srep42717.

DI GIOVANNI, G. *et al.* Role of serotonin in central dopamine dysfunction. **CNS Neuroscience & Therapeutics**, v. 16, p. 179-194, 2010. DOI: https://doi.org/10.1111/j.1755-5949.2010.00135.x.

EDINOFF, A. N. *et al.* Selective serotonin reuptake inhibitors and adverse effects: a narrative review. **Neurology International**, v. 13, p. 387-401, 2021. DOI: https://doi.org/10.3390/neurolint13030038.

ERDOGAN, T. DFT, molecular docking and molecular dynamics simulation studies on some newly introduced natural products for their potential use against SARS-CoV-2. **Journal of Molecular Structure**, v. 1242, p. 130733, 2021. DOI: https://doi.org/10.1016/j.molstruc.2021.130733.

FERDOUS, J. *et al.* Pharmacological activities of plant-derived fraxin with molecular mechanisms: a comprehensive review. **Chemical Biodiversity**, v. 21, 2024. DOI: https://doi.org/10.1002/cbdv.202301615.

FRITSCHY, J.; PANZANELLI, P. GABA a receptors and plasticity of inhibitory neurotransmission in the central nervous system. **European Journal of Neuroscience**, v. 39, p. 1845-1865, 2014. DOI: https://doi.org/10.1111/ejn.12534.

FU, L. *et al.* ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. **Nucleic Acids Research**, v. 52, p. W422-W431, 2024. DOI: https://doi.org/10.1093/nar/gkae236.

GHALLAB, Y. K.; ELASSAL, O. S. Biochemical and neuropharmacology of psychiatric disorders. p. 25-47, 2024. DOI: https://doi.org/10.1007/978-981-97-2681-3_2.

GHIT, A. *et al.* GABAA receptors: structure, function, pharmacology, and related disorders. **Journal of Genetic Engineering and Biotechnology**, v. 19, p. 123, 2021. DOI: https://doi.org/10.1186/s43141-021-00224-0.

GLASSMAN, P. M.; BALTHASAR, J. P. Physiologically-based modeling of monoclonal antibody pharmacokinetics in drug discovery and development. **Drug Metabolism and Pharmacokinetics**, v. 34, p. 3-13, 2019. DOI: https://doi.org/10.1016/j.dmpk.2018.11.002.

GONZÁLEZ GÓMEZ, L. C. *et al.* Diazepam-induced down-regulation of the GABAA receptor α1 subunit, as mediated by the activation of L-type voltage-gated calcium channel/Ca2+/protein kinase A signaling cascade. **Neuroscience Letters**, v. 810, p. 137358, 2023. DOI: https://doi.org/10.1016/j.neulet.2023.137358.

HUSAIN, Z. *et al.* Tangeretin exerts and modulates the anxiolytic effects of the GABAkine drugs diazepam and flumazenil in mice: molecular interventions through animal behavior and molecular dynamic simulations. **Food Bioscience**, v. 62, p. 105469, 2024. DOI: https://doi.org/10.1016/j.fbio.2024.105469.

ISLAM, Md. S. *et al.* Anxiolytic-like effect of quercetin possibly through GABA receptor interaction pathway: in vivo and in silico studies. **Molecules**, v. 27, p. 7149, 2022. DOI: https://doi.org/10.3390/molecules27217149.

ISLAM, M. T. *et al.* Anxiolytic-like effects of Moringa oleifera in Swiss mice. **Cellular and Molecular Biology**, v. 66, p. 73-77, 2020.

KALUEFF, A. V.; NUTT, D. J. Role of GABA in anxiety and depression. **Depression and Anxiety**, v. 24, p. 495-517, 2007. DOI: https://doi.org/10.1002/da.20262.

KHAN, Md. M. A.; KHAN, Md. N. Effects of psychosocial and socio-environmental factors on anxiety disorder among adolescents in Bangladesh**. Brain and Behavior**, v. 10, 2020. DOI: https://doi.org/10.1002/brb3.1899.

KIM, S. *et al.* PubChem in 2021: new data content and improved web interfaces**. Nucleic Acids Research**, v. 49, p. D1388-D1395, 2021. DOI: https://doi.org/10.1093/nar/gkaa971.

KOREN, A.; KOREN, L.; MARCEC, R.; MARCINKO, D.; LIKIC, R. 2024. Evolving Trends and Economic Burden of Benzodiazepine Use: Insights From a 10-Year Predictive Model. **Value Health Reg Issues** 40, 70-73. DOI: https://doi.org/10.1016/j.vhri.2023.10.005

LIAO, J. *et al.* The mRNA expression levels of GABA A receptor α1 and α2 subunits in patients with major depressive disorder during onset and remission. **International Journal of Neuroscience**, v. 132, p. 606-612, 2022. DOI: https://doi.org/10.1080/00207454.2020.1829618.

LIPINSKI, C.A., 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol 1, 337-341. DOI: https://doi.org/10.1016/j.ddtec.2004.11.007

LUSCHER, B. *et al.* GABAA receptors as targets for treating affective and cognitive symptoms of depression. **Trends in Pharmacological Sciences**, v. 44, p. 586-600, 2023. DOI: https://doi.org/10.1016/j.tips.2023.06.009.

MASYITA, A. *et al.* Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. **Food Chemistry***: X*, v. 13, p. 100217, 2022. DOI: https://doi.org/10.1016/j.fochx.2022.100217.

MCGARVEY, P. B. *et al.* UniProt genomic mapping for deciphering functional effects of missense variants. **Human Mutation,** v. 40, p. 694-705, 2019. https://doi.org/10.1002/humu.23738. MORIN, C. M.; JARRIN, D. C. Epidemiology of insomnia. **Sleep Medicine Clinics**, v. 17, p. 173-191, 2022. DOI: https://doi.org/10.1016/j.jsmc.2022.03.003.

MUKTY, S. A. *et al.* Assessment of sedative activity of fraxin: in vivo approach along with receptor binding affinity and molecular interaction with GABAergic system. **Drug Development Research,** v. 85, 2024. DOI: https://doi.org/10.1002/ddr.22250.

OCHOA-DE LA PAZ, L. D. *et al.* The role of GABA neurotransmitter in the human central nervous system, physiology, and pathophysiology. **Revista Mexicana de Neurociencia**, v. 22, 2021. DOI: https://doi.org/10.24875/RMN.20000050.

PIRES, D. E. V. *et al.* pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. **Journal of Medicinal Chemistry**, v. 58, p. 4066-4072, 2015. DOI: https://doi.org/10.1021/acs.jmedchem.5b00104.

RUDOLPH, U.; KNOFLACH, F. Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. **Nature Reviews Drug Discovery**, v. 10, p. 685-697, 2011. DOI: https://doi.org/10.1038/nrd3502.

SAHU, D. *et al.* A review on molecular docking as an interpretative tool for molecular targets in disease management. **Assay and Drug Development Technologies,** v. 22, p. 40-50, 2024. https://doi.org/10.1089/adt.2023.060.

SAVITZ, J.; LUCKI, I.; DREVETS, W. C. 5-HT1A receptor function in major depressive disorder. **Progress in Neurobiology**, v. 88, p. 17-31, 2009.

SILVEIRA, V. Anxiolytic effect of Anthemis nobilis L. (roman chamomile) and Citrus reticulata Blanco (tangerine) essential oils using the light-dark test in zebrafish (Danio rerio). **Journal of Ethnopharmacology**, v. 298, p. 115580, 2022. DOI: https://doi.org/10.1016/j.jep.2022.115580.

TELLES-CORREIA, D. *et al.* Mental disorder-the need for an accurate definition. **Frontiers in Psychiatry**, v. 9, 2018 DOI:. https://doi.org/10.3389/fpsyt.2018.00064.

Teleanu, R.I. *et al.* 2022a. Neurotransmitters-Key Factors in Neurological and Neurodegenerative Disorders of the Central Nervous System. **Int J Mol Sci 23, 5954**. DOI: https://doi.org/10.3390/ijms23115954

TILLER, J. W. G. Depression and anxiety. **Medical Journal of Australia,** v. 199, 2013. DOI: https://doi.org/10.5694/mja12.10628.

VOLLENWEIDER, I. *et al.* Antidepressant-like properties of α2-containing GABAA receptors. **Behavioural Brain Research**, v. 217, p. 77-80, 2011. https://doi.org/10.1016/j.bbr.2010.10.009.

WANG, H.-Q. *et al.* The receptor hypothesis and the pathogenesis of depression: genetic bases and biological correlates. **Pharmacological Research**, v. 167, p. 105542, 2021. DOI: https://doi.org/10.1016/j.phrs.2021.105542.

ZHOU, J. *et al.* Recent advances in the study of anesthesia-and analgesia-related mechanisms of S-ketamine. **Frontiers in Pharmacology**, v. 14, 2023. DOI: https://doi.org/10.3389/fphar.2023.1228895.