BIOINFORMATICS APPROACH TO ANXIETY MANAGEMENT BASED ON NATURAL COMPOUNDS

CĂTĂLINA MAREŞ, ANDRA MARIA PĂUN**#, ELIZA-MARIA SATMARU*, SPERANŢA AVRAM*, EMILIA RADU***, MARIA MERNEA**

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*Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, 91–95 Splaiul Independenţei, Bucharest, 050095, Romania
**Doctoral School of Biology, Faculty of Biology, University of Bucharest, 91–95 Splaiul Independenţei, Bucharest, 050095, Romania, #paun.andra-maria22@s.bio.unibuc.ro
***Department of Taxonomy, Ecology and Nature Conservation, Institute of Biology Bucharest, Romanian Academy, 296 Splaiul Independenţei, Bucharest, 060031, Romania

Abstract. Anxiety disorders are a growing public health concern, often underestimated in both diagnosis and treatment. Current pharmacological therapies, although effective, are frequently limited by adverse effects and toxicity, highlighting the need for safer and more efficient alternatives. In this study, we investigated the potential of natural compounds as modulators of the $GABA_A$ receptor, a key target in anxiolytic therapy. Using a bioinformatics approach, we performed molecular docking and pharmacokinetic analyses to evaluate the interactions of fifteen plant-derived compounds with the benzodiazepine binding site of the receptor. Several compounds displayed favorable binding affinities and physicochemical properties consistent with drug-likeness criteria. Distribution predictions indicated good blood-brain barrier permeability for most compounds. Notably, myricetin demonstrated both strong predicted binding affinity and stable interactions within the binding site. These results suggest that the analyzed natural compounds may represent promising candidates for the development of new, safer anxiolytic therapies, warranting further experimental validation.

Key words: natural compounds, molecular docking, $GABA_A$ receptor, anxiolytic effects.

INTRODUCTION

Mental health disorders and cognitive impairments have become prominent global public health challenges. Conditions such as depression, anxiety, and environmentally or genetically influenced cognitive dysfunction are increasingly prevalent, with higher incidence reported in women. Current therapeutic strategies remain largely symptom-based [19]. Anxiety is characterized by emotional

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symptoms such as persistent tension; physiological symptoms including muscle tightness, sweating, rapid breathing, and chest constriction; and cognitive symptoms such as impaired concentration and disordered thinking [6]. Despite advances in neuroscience, the development of novel pharmacotherapeutic options for anxiety disorders remains a significant unmet medical need [42]. Current treatments provide relief for many patients but are often limited by issues of efficacy, safety, and long-term tolerability, underscoring the importance of identifying new therapeutic strategies [1, 34].

Lifestyle and diet have been consistently linked to mental health, suggesting a potential role for bioactive compounds in prevention and management [9]. Building on this evidence, the present study examines plant-derived molecules with antioxidant, anti-inflammatory, anticholinesterase, and antiapoptotic properties that support the protection and functional integrity of the nervous system [10]. Plant-derived polyphenols and flavonoids, widely distributed across numerous species, have demonstrated notable neuroprotective effects due to their antioxidant, anti-inflammatory, and neuromodulator activities [20, 28].

Ionotropic membrane proteins such as the GABA receptor have been extensively investigated in the context of anxiety disorders [2]. GABA receptors, present at 20–50 % of synapses in the brain, respond within milliseconds to GABA binding by opening chloride-permeable channels that inhibit neuronal activity [43]. Dysfunction of these receptors contributes to anxiety, epilepsy, and neurodevelopmental disorders, including autism. Their clinical relevance is reflected in the wide range of drugs targeting distinct binding sites within the receptor's structure, which contains approximately ten known ligand-binding sites [45].

Benzodiazepines such as alprazolam, chlordiazepoxide, clonazepam, diazepam, lorazepam, and oxazepam exert their effects through modulation of $GABA_A$ receptors [29]. They play an important role in the management of anxiety disorders, but are generally reserved for second-line or adjunctive therapy due to tolerance and abuse potential [46]. Although initially considered non-addictive, substantial evidence now indicates a risk of dependence, cognitive impairment, and contribution to opioid-related mortality. Current strategies for benzodiazepine discontinuation primarily rely on gradual tapering, as effective pharmacological treatments for dependence remain limited [12, 13].

Other pharmacological options for the symptomatic treatment of anxiety include tricyclic antidepressants (e.g., amitriptyline, clomipramine, imipramine) and antipsychotic agents (e.g., thioridazine, chlorpromazine, haloperidol); however, their use is often limited by significant side effects [3].

Numerous studies have demonstrated that natural compounds with neuroprotective properties interact with molecular targets in the central nervous system. Extensive preclinical research indicates that plant-derived chemicals such as alkaloids, terpenes, flavonoids, phenolic acids, cinnamic lignans, and saponins exhibit significant anxiolytic potential [5]. These effects have been reported in studies investigating *Lippia graveolens, Lavandula officinalis, Trigonella foenum-graecum* seeds, *Tanacetum oarthenium, Elettaria cardamomum, Caesalpinia digyna*

roots, and *Cocos nucifera* roots [17]. Moreover, several recent clinical evaluations have supported the anxiolytic efficacy of plant extracts [33].

In this study, we apply a bioinformatics-driven strategy to evaluate the anxiolytic potential of selected natural compounds by examining their predicted interactions with the $GABA_A$ receptor. Using molecular docking analyses, we assess binding affinities and characterize key interaction profiles that may underlie their modulatory effects. Last, we evaluated their drug-likeness profiles and their ability to reach the central nervous system (CNS). These computational insights provide a foundation for identifying promising candidates and may support the development of alternative therapeutic approaches for anxiety management.

MATERIALS AND METHODS

NATURAL COMPOUNDS WITH POTENTIAL ANXIOLYTIC EFFECTS

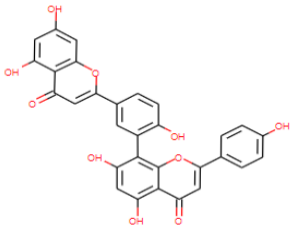
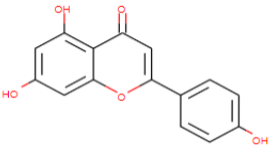
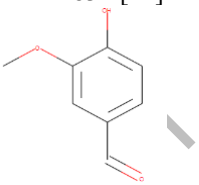
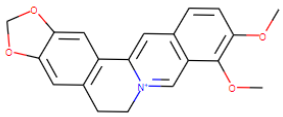
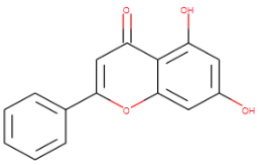
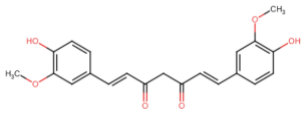
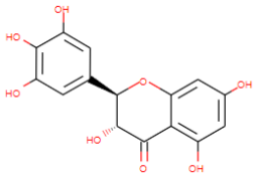
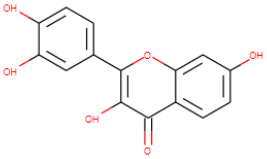
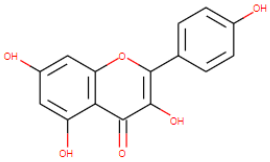
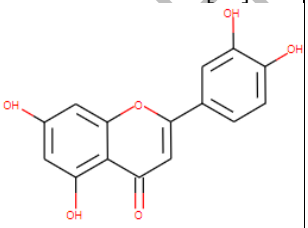
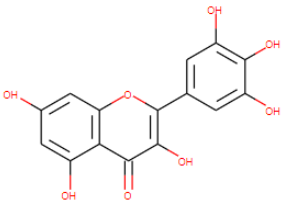
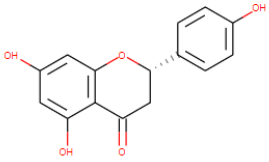
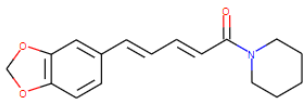
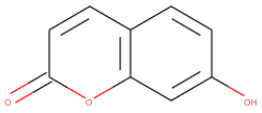
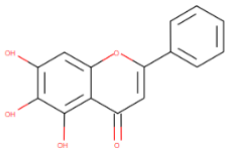
The identification of natural compounds with potential anxiolytic effects was carried out through a literature-based search using the keywords “anxiety” and “natural compounds”. From the results, we selected the fifteen natural compounds with diverse chemical structures (flavones: luteolin, baicalein, chrysin and apigenin; flavonols: myricetin, fisetin, kaempferol, dihydromyricetin; biflavonoid: amentoflavone; flavanone: naringenin; phenolic acids / coumarins / simple phenols: curcumin, umbelliferone, vanillin; alkaloids: piperine, berberine) based on existing evidence linking them to anxiolytic activities. These compounds are presented in Table 1.

MOLECULAR DOCKING

The docking protocol that we applied follows the methodology used in [26]. The three-dimensional structures of the ligands were downloaded from PubChem database [22]. Ligand preparation was carried out in Discovery Studio (v16.1.0.15350, BIOVIA Dassault Systemes, San Diego, CA, USA). Preparation steps included adding hydrogen atoms, protonation at physiological pH (7.4), and energy minimization.

Table 1

Anxiolytic compounds chosen based on prior research papers, their PubChem compound IDs (CID), the bibliographic references for the anxiolytic effect and their 2D structures

Compound name / PubChem ID / Reference/ 2D structure		
Amentoflavone / CID 5281600 / [15] 	Apigenin / CID 5280443 / [27, 30] 	Vanillin / CID 1183 / [11] 
Berberine / CID 2353 / [24] 	Chrysin / CID 5281607 / [35] 	Curcumin / CID 969516 / [14] 
Dihydromyricetin / CID 161557 / [18, 39] 	Fisetin / CID 5281614 / [7] 	Kaempferol / CID 5280863 / [38] 
Luteolin / CID 5280445 / [44] 	Myricetin / CID 5281672/ [41] 	Naringenin / CID 439246 / [36] 
Piperine / CID 638024 / [16] 	Umbelliferone / CID 5281426 / [23] 	Baicalein / CID 64982 / [37] 

The structure of $GABA_A$ receptor was retrieved from Protein Data Bank (PDB ID 6X3X) [21]. This structure contains diazepam molecules bound in both the extracellular and transmembrane regions, with the extracellular ligand defining the benzodiazepine binding site selected for docking the natural compounds. The choice of 6X3X was motivated by the presence of diazepam co-crystallized in this pocket, which provides an experimentally validated ligand-bound conformation. Using a structure with a reference ligand enables a more accurate definition of the docking cavity and allows direct comparison between the binding modes of the natural compounds and a clinically used anxiolytic agent. Prior to docking, the receptor was preprocessed in Discovery Studio by removing water molecules and co-crystallized ligands, adding hydrogen atoms, adjusting protonation states at pH 7.4, and performing energy minimization to optimize the structure.

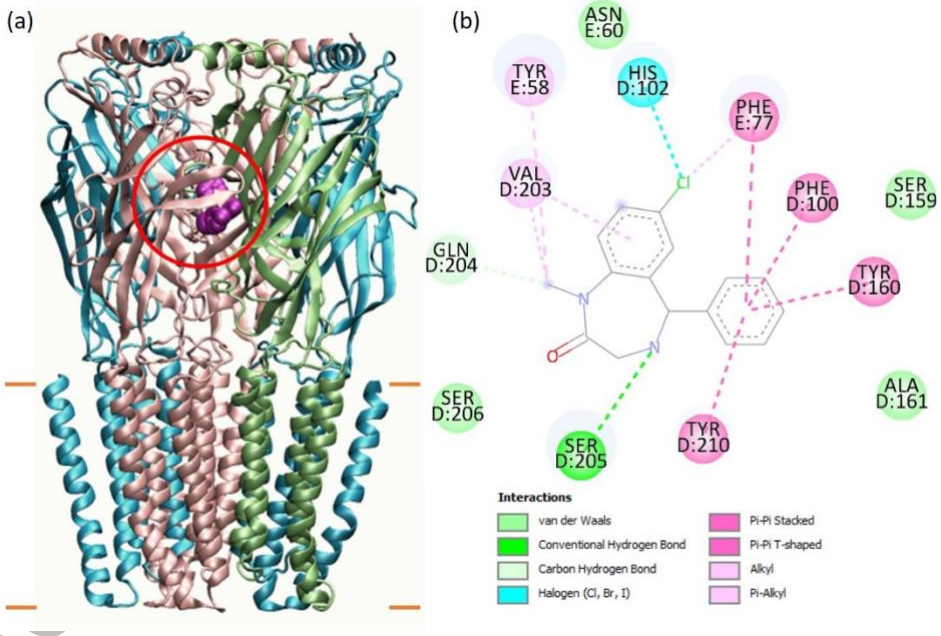


Fig. 1. (a) The structure of $GABA_A$ receptor with the stoichiometry 2β2 (in blue) – 2α1 (in pink) – 1γ2 (in lime) according to 6X3X structure [21]. The diazepam molecule bound in the extracellular region (benzodiazepines binding site) is represented in yellow and its binding site is circled in red. (b) 2D interaction map between diazepam and $GABA_A$ receptor. The residues interacting with diazepam are labeled on the dots and the dots are colored according to the interaction type. Residue labelling involves the amino acid type, the chain belonging to (chain D: α1 subunit, chain E: γ2 subunit) and the numbering from the sequence.

The receptor potential binding cavities were automatically identified, and the extracellular diazepam binding site was selected as the active region of interest (Fig. 1). All selected compounds were docked into benzodiazepines binding site, allowing comparison of their binding affinities and interaction profiles relative to the reference ligand, diazepam. Docking simulations were performed using the CDOCKER algorithm, which positions each ligand into the binding cavity and optimizes its orientation based on interaction energy.

The docking procedure, performed using the CDOCKER protocol, generates ten possible binding poses for each ligand, ranked according to their predicted binding affinity. This affinity is estimated using two scoring functions: CDOCKER ENERGY, which represents the total docking energy (combining pose energy and ligand strain), and CDOCKER INTERACTION ENERGY, which reflects the direct interaction energy between the ligand and the receptor. In Discovery Studio, these scores are reported as negative values, with more negative scores indicating more favorable predicted binding. For clarity of interpretation, in Results section we report absolute values of the scores obtained for the top ranked poses.

PREDICTION OF COMPOUNDS' DRUG-LIKENESS

The drug-likeness profiles of the compounds were assessed using the SwissADME platform [8]. We evaluated the compliance of their physicochemical properties with the acceptable ranges defined by Lipinski's rule of five, which predicts poor absorption when a molecule contains more than five hydrogen-bond donors, more than ten hydrogen-bond acceptors, has a molecular weight above 500 Da, or a Log*P* value greater than 5 [25]. Additionally, SwissADME was used to calculate the Abbott bioavailability score, which estimates the probability that a compound achieves at least 10 % oral bioavailability in rats or exhibits measurable permeability in Caco-2 assays [8].

PREDICTION OF COMPOUNDS' DISTRIBUTION TO THE CNS

The pkCSM [31] platform was used to predict the ability of compounds to reach the CNS. The blood-brain barrier (BBB) permeability is critical for CNS-active compounds, relying on a combination of passive diffusion and active transport mechanisms. CNS permeability is further restricted by tight junctions, which necessitate predominantly transcellular transport [40]. Therefore, we predicted two parameters represented by the BBB permeability, estimated as log*BBB* and CNS permeability, estimated as log*PS*. An effective distribution to the CNS is generally associated with log*BBB* values > –1 and log*PS* values above approximately –3.

RESULTS

MOLECULAR DOCKING RESULTS

The results of docking the selected natural compounds into the benzodiazepine binding site of the $GABA_A$ receptor are presented in Table 2. The molecular docking analysis allowed us to rank ligands based on both predicted binding affinity (-CDOCKER interaction energy) and pose stability (-CDOCKER energy). Amentoflavone exhibited the highest absolute interaction energy (59.11), indicating a strong predicted binding. However, its relatively low absolute total docking energy (37.13) suggests a strained binding pose, which may limit its practical stability within the receptor site.

Table 2

CDOCKER Energy and CDOCKER Interaction Energy scores corresponding to the top-ranked binding poses of the natural compounds docked into the benzodiazepine binding site of the $GABA_A$ receptor

COMPOUND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY
Amentoflavone	37.13	59.11
Myricetin	43.63	50.33
Curcumin	38.88	47.10
Fisetin	38.51	44.14
Baicalein	43.70	43.54
Berberine	–8.20	42.78
Chrysin	40.89	42.57
Kaempferol	33.23	42.18
Naringenin	34.22	41.43
Dihydromyricetin	36.15	40.35
Luteolin	44.64	38.97
Piperin	7.57	37.85
Apigenin	38.66	36.69
Vanillin	24.09	31.64
Umbelliferone	30.18	29.28

In contrast, several compounds combine high predicted affinity with more favorable docking energies, indicating both strong receptor interactions and stable binding poses. This group includes myricetin (absolute interaction energy 50.33, absolute total docking energy 43.63), curcumin (47.10, 38.88), fisetin (44.14, 38.51), baicalein (43.54, 43.7), chrysin (42.57, 40.89), luteolin (38.97, 44.64), kaempferol

(42.18, 33.23), and naringenin (41.43, 34.22). All these compounds are predicted to be potential $GABA_A$ modulators.

Smaller or structurally simpler compounds, including umbelliferone (29.28, 30.18), vanillin (31.64, 24.09), and piperine (37.85, 7.57), exhibited weaker predicted interactions or lower pose stability, indicating less favorable binding. Berberine, despite a relatively high absolute interaction energy (42.78), showed a negative absolute docking energy (−8.2), suggesting strain in its predicted pose.

From the group of ligands that present both favorable interaction energy and docking stability, we selected three top-scoring compounds from different chemical classes, namely flavonols (myricetin), flavones (baicalein) and phenolic acids (curcumin), for a detailed analysis of their interactions with the receptor and comparison with the binding of diazepam.

THE INTERACTION OF TOP SCORING LIGANDS WITH $GABA_A$ RECEPTOR

The Figures 2a and 3 show the 2D interaction maps of myricetin, baicalein and curcumin with the $GABA_A$ receptor. Because several residues are commonly involved in ligand interactions, we chose to present only the surface of residues interacting with myricetin, colored according to hydrogen-bond donor and acceptor potential, hydrophobicity, and electrostatic charge (Fig. 2b–d). The presence of residues capable of forming hydrogen bonds is particularly important for favorable ligand binding, as all three compounds contain multiple polar atoms that can engage in these interactions. In addition, the distribution of hydrophobic and charged residues within the binding site contributes to the overall stability and specificity of the ligand–receptor complex.

Myricetin interacts with His102, Tyr160, Ser205, Thr207, and Tyr210 in the α1 subunit, and with Phe77, Ala79, and Glu189 in the γ2 subunit. The compound forms three hydrogen bonds (Thr207 in α1 and Glu189 in γ2 – two hydrogen bonds), as well as carbon–hydrogen bonds, π–π interactions, π–alkyl interactions, and π-mediated hydrogen bond donor interactions (Fig. 2a).

Baicalein interacts with Ser159, Tyr160, Ala161, Ser205, and Tyr210 in the α1 subunit, and with Phe77 and Ala79 in the γ2 subunit. It forms two hydrogen bonds (Ser159 and Ala161 in α1), along with carbon–hydrogen bonds and aromatic-mediated interactions, including π–anion, π–π, π–alkyl, and π-mediated hydrogen bond donor interactions (Fig. 3a).

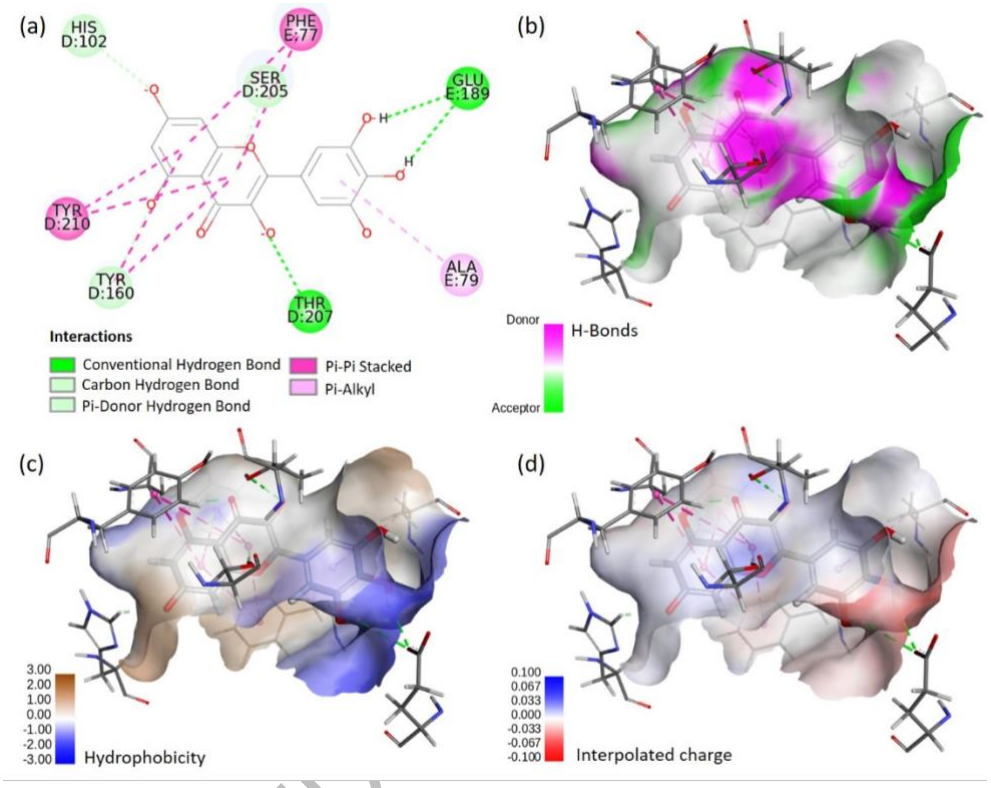


Fig. 2. (a) The 2D interaction map of myricetin with $GABA_A$ receptor. Residue labels follow the conventions presented in the legend of Fig. 1. (b–d) Surface representation of the binding site colored according to hydrogen-bonding potential (b), hydrophobicity (c), and electrostatic charge distribution (d).

Curcumin interacts with Ser159, Tyr160, Ala161, Glu204, Ser206, and Tyr210 in the α1 subunit, and with Tyr58, Phe77, and Met130 in the γ2 subunit. The compound forms three hydrogen bonds (Ser159, Ala161, and Ser206 in α1), in addition to carbon–hydrogen bonds, π–sulfur interactions, π–π interactions, and π–alkyl interactions (Fig. 3b).

To compare the binding of the natural compounds with that of diazepam at the benzodiazepine site, based on the 3D structure 6X3X, Figure 1b shows the 2D interaction map of the diazepam–receptor complex. The residues involved in diazepam binding include Ser159, Tyr160, Ala161, His102, Val203, Gln204, Ser205, Ser206, and Tyr210 in the α1 subunit, and Tyr58, Asn60, Phe77, and Phe100 in the γ2 subunit. A partial overlap is observed between the diazepam binding site and the binding sites of the natural compounds, with Tyr160 and Tyr210 in α1 and Phe77 in γ2 being common to both sets of ligands.

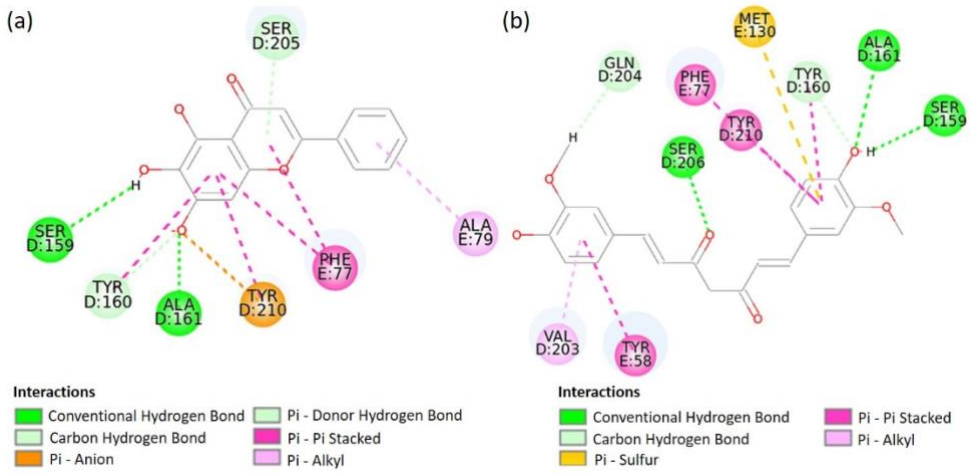


Fig. 3. The 2D interaction maps of baicalein (a) and curcumin (b) with the benzodiazepines binding site in $GABA_A$ receptor. Residue labels follow the conventions presented in the legend of Fig. 1.

DRUG-LIKENESS PROFILE OF NATURAL COMPOUNDS

Most investigated compounds complied with Lipinski's rule of five, indicating generally favorable oral drug-likeness. Only amentoflavone and baicalein exhibited two rule violations each. Consistently, these two compounds also displayed the lowest predicted bioavailability scores, namely 0.17 for amentoflavone and 0.11 for baicalein, whereas all other compounds obtained a score of 0.55.

THE CNS-DISTRIBUTION PROFILE OF COMPOUNDS

To further evaluate the pharmacokinetic suitability of the selected compounds for anxiolytic activity, we examined their predicted ability to penetrate the central nervous system (CNS) using the pkCSM descriptors log*BBB* and log*PS* (Table 3).

BBB permeability predictions varied widely across the dataset. Berberine, chrysin, piperine, and apigenin showed the least negative or even positive log*BBB* values, suggesting a comparatively higher ability to cross the BBB. Other compounds, including myricetin, fisetin, kaempferol, and naringenin, showed moderate predicted permeability. Amentoflavone, baicalein, dihydromyricetin, and myricetin displayed more negative log*BBB* values, indicating limited BBB diffusion.

Table 3

Prediction of drug-likeness properties of compounds based on the compliance with Lipinki's rule [8] and their distribution to the central nervous system as BBB permeability and CNS permeability [31].

Compound	Lipinski rule	Bioavailability score	BBB permeability (log*BBB*)	CNS permeability (log*PS*)
Amentoflavone	No; 2 violations	0.17	–1.653	–3.2
Myricetin	Yes	0.55	–1.493	–3.709
Curcumin	Yes	0.55	–0.562	–2.99
Fisetin	Yes	0.55	–1.039	–2.282
Baicalein	No; 2 violations	0.11	–1.331	–3.811
Berberine	Yes	0.55	0.198	–1.543
Chrysin	Yes	0.55	0.047	–1.912
Kaempferol	Yes	0.55	–0.939	–2.228
Naringenin	Yes	0.55	–0.578	–2.215
Dihydromyricetin	Yes	0.55	–1.167	–3.586
Luteolin	Yes	0.55	–0.907	–2.251
Piperin	Yes	0.55	–0.102	–1.879
Apigenin	Yes	0.55	–0.734	–2.061
Vanillin	Yes	0.55	–0.243	–2.236
Umbelliferone	Yes	0.55	–0.278	–2.741

Predicted CNS permeability (log*PS*) followed the same general pattern, with berberine, piperine, chrysin, and fisetin showing the highest CNS penetration potential, while baicalein, myricetin, amentoflavone, and dihydromyricetin were predicted to penetrate the CNS poorly.

DISCUSSION

Natural products exhibit a wide range of pharmacological and biological activities, making them promising candidates for the treatment of neurological and psychiatric disorders [32]. Numerous studies have identified bioactive plant-derived compounds with neuroprotective and anxiolytic effects mediated through multiple mechanisms, including modulation of neurotransmitter systems, antioxidant activity, and regulation of intracellular signaling pathways [28, 42]. In particular, several natural compounds have been shown to interact with $GABA_A$ receptor [4], a key pharmacological target in anxiety regulation [2].

In the present study, a bioinformatics approach was used to investigate the interaction of selected natural compounds reported to present anxiolytic activity with the benzodiazepine binding site of the $GABA_A$ receptor. Molecular docking analysis revealed that most of these compounds present a high affinity for the receptor: amentoflavone, myricetin, curcumin, fisetin, baicalein, chrysin, luteolin, kaempferol, and naringenin. These compounds present both strong receptor interactions and stable binding poses, except for amentoflavone that, in spite of exhibiting the highest interaction energy, presents a strained binding pose.

By analyzing the binding of myricetin, curcumin, and fisetin in detail, we found that these three compounds share several key contact residues within the benzodiazepine binding site, particularly Ser159, Tyr160, Ala161, Ser205/206, and Tyr210 in the α1 subunit, and Phe77 in the γ2 subunit. All three compounds form multiple hydrogen bonds with the α1 subunit and additional stabilizing interactions, including carbon–hydrogen and aromatic-mediated contacts (π–π, π–alkyl, and π-mediated hydrogen bond donor interactions). These common interaction patterns suggest a similar binding mode, with a combination of polar and hydrophobic contacts contributing to high predicted affinity and pose stability.

Comparison with the binding of diazepam (3D structure 6X3X) shows partial overlap in the binding site. Diazepam interacts with several residues also engaged by the natural compounds, including Tyr160 and Tyr210 in α1 and Phe77 in γ2. Among the natural ligands, curcumin appears to bind most similarly to diazepam, engaging many of the same residues. However, diazepam also forms additional van der Waals contacts and halogen interactions via its chlorine atom, which are absent in the natural compounds. This suggests that while the natural ligands may mimic aspects of diazepam binding, differences in chemical structure influence the precise interaction pattern and potentially the binding strength.

The application of drug-likeness filter showed that most molecules complied with Lipinski's criteria and showed acceptable predicted bioavailability. In what concerns the CNS distribution, berberine, piperine, and chrysin showed the most favorable BBB profiles, while flavonoids such as myricetin, fisetin, kaempferol, luteolin, and naringenin presented intermediate permeability values, still compatible with CNS activity. Amentoflavone and baicalein did not comply with Lipinski rule, presented low bioavailability scores and poor distribution to the CNS.

Overall, integrating drug-likeness criteria with BBB predictions indicates that CNS penetration may limit the in vivo relevance of some strong docked binders (e.g., amentoflavone, myricetin), whereas compounds with moderate affinity but better pharmacokinetic profiles (e.g., berberine, piperine, chrysin) may represent more promising CNS-active candidates.

Collectively, these results highlight the therapeutic promise of plant-derived flavonoids as natural modulators of the GABAergic system. By combining computational modeling with pharmacokinetic predictions, this study provides valuable insights into their molecular mechanisms of action and supports their consideration as potential alternatives or adjuncts to conventional anxiolytic therapies. Future *in vitro* and *in vivo* studies are warranted to validate these

computational findings and further explore the pharmacological efficacy of these natural compounds in anxiety management.

CONCLUSION

This study employed a combined molecular docking and pharmacokinetic-based screening approach to evaluate fifteen natural compounds as potential modulators of the benzodiazepine binding site of the $GABA_A$ receptor. Several molecules, particularly myricetin, curcumin, fisetin, baicalein, chrysin, and luteolin, demonstrated strong and stable predicted interactions with key receptor residues, partially overlapping with the binding profile of diazepam. However, integration of drug-likeness and CNS distribution predictions revealed that high docking affinity does not necessarily translate into optimal pharmacokinetic suitability. While compounds such as amentoflavone showed strong receptor binding, their limited BBB permeability and low bioavailability may restrict *in vivo* efficacy. In contrast, berberine, piperine, and chrysin combined acceptable docking performance with more favorable CNS accessibility, suggesting higher potential to reach effective central concentrations.

Overall, the results highlight several natural compounds with promising $GABA_A$-modulatory properties, while also underscoring the importance of pharmacokinetic constraints in prioritizing candidates for further study. These findings provide a rational basis for selecting natural molecules for experimental validation and support the continued exploration of plant-derived scaffolds in the search for safer anxiolytic agents.

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