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In silico evaluation of *Centella asiatica* compounds as potential antidepressant agents

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ABSTRACT

Major depressive disorder remains a major public health challenge, and plant-derived compounds may provide promising lead structures for antidepressant drug discovery. This study evaluated the predicted interactions of asiatic acid, madecassic acid, and madasiatic acid from *Centella asiatica* (*C. asiatica*) with serotonin 1A, dopamine D2, dopamine D3, and mu-opioid receptors (MORs). Molecular docking protocols were validated by redocking co-crystallized ligands, while drug-likeness, oral bioavailability, pharmacokinetic properties, and toxicity were assessed using established in silico tools. Madasiatic acid showed the most favorable predicted binding to serotonin 1A and dopamine D2 receptors, whereas asiatic acid showed the strongest predicted binding to dopamine D3 receptors and moderate binding to MORs. The three *C. asiatica*-derived compounds generally met common drug-likeness criteria and showed moderate predicted intestinal absorption, possible blood-brain barrier penetration, and no predicted human ether-à-go-go-related gene II inhibition or hepatotoxicity. These findings support further molecular dynamics, receptor-binding, functional, pharmacokinetic, and in vivo validation.

Keywords: *Centella asiatica*, molecular docking, major depressive disorder, medicinal plants, mental health, ethnopharmacology

INTRODUCTION

Major depressive disorder (MDD) is a prevalent psychiatric disorder affecting over 280 million people and is a leading cause of global disability [1]. MDD is marked by persistently low mood, anhedonia, cognitive dysfunction, sleep disturbances, and impaired daily functioning, increasing suicide risk. Despite advances in mental health interventions, depression remains multifactorial, driven by disturbances in monoaminergic neurotransmission, neuroinflammatory signaling, oxidative stress, hypothalamic-pituitary-adrenal axis dysregulation, and neuroplasticity deficits [2, 3]. Current treatments, including selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, tricyclic, and atypical antidepressants, mainly target monoaminergic neurotransmission [4]. Although widely prescribed, their efficacy is limited by delayed onset, incomplete remission, and high variability in responses [5]. Long-term antidepressant use may be associated with adverse effects such as sexual dysfunction, weight gain, gastrointestinal disturbances, insomnia, and emotional blunting, which may contribute to poor adherence and discontinuation [6, 7]. These limitations highlight the need for safer, more effective, and diverse antidepressants.

Recently, attention has been focused on plant-derived natural compounds as alternative or complementary treatments for neuropsychiatric disorders like depression [8]. Natural products may exhibit multitarget pharmacological activity and provide structurally diverse scaffolds for drug discovery, although their efficacy and safety require compound-specific evaluation. *Centella asiatica* (*C. asiatica*) (L.) Urb., or “Bua Bok” in Thailand, is traditionally used in Thai and Ayurvedic medicine for cognitive enhancement, anxiety reduction, wound healing, and inflammation alleviation [9]. Evidence supports *C. asiatica*’s neuroprotective and psychotropic potential, especially on the central nervous system (CNS). Phytochemical analyses have identified triterpenoids as major bioactive constituents, including asiatic, madecassic, and madasiatic acids. Preclinical studies have reported antidepressant-like effects of *C. asiatica*-derived interventions in rodent models, including asiaticoside and standardized *C. asiatica* extract [10, 11]. In the asiaticoside study, these effects were accompanied by reduced inflammatory markers and modulation of the protein kinase A (PKA)/pCREB/BDNF signaling pathway [11].

Depression is linked to alterations in neurotransmitter systems involving serotonin 1A (5-HT1A), dopamine D2 (D2R), dopamine D3 (D3R), and mu-opioid receptor (MOR) signaling,

which are relevant to mood regulation, reward processing, and motivation [2, 3, 12]. These receptors represent biologically relevant targets for exploring potential multitarget approaches to antidepressant drug discovery. Yet, molecular interactions between *C. asiatica*-derived triterpenoids and depression-related protein targets are not well understood. In silico approaches can support early-stage drug discovery by enabling virtual screening of ligand-receptor interactions and prediction of pharmacokinetic properties [13-15]. These computational methods are valuable for natural product research, where experimental screening is time-consuming and resource-intensive.

Therefore, this study used an integrated in silico approach to investigate the antidepressant potential of *C. asiatica*-derived natural compounds—asiatic, madecassic, and madasiatic acids—via molecular docking against four depression-related targets (5-HT1A, D2R, D3R, and MOR) and evaluation of their pharmacokinetic and drug-likeness properties using established ADME prediction tools to prioritize compounds for further experimental antidepressant-related investigation.

MATERIALS AND METHODS

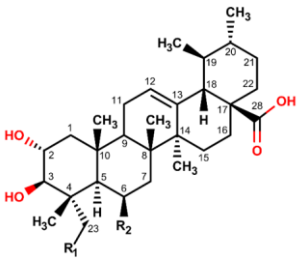
The study protocol was approved by the Walailak University Ethics Committee (WUEC-25-167-02). The present study reports only the *in silico* phase and did not involve human participants or identifiable human data; therefore, informed consent was not applicable.

Antidepressant-Related Protein Selection, Preparation, and Validation

Four targets implicated in MDD pathophysiology (see Introduction) were selected: 5-HT1A, for its central role in anxiety and MDD [12]; the dopamine D2-like receptors D2R and D3R, for their contribution to antidepressant efficacy [2]; and MOR, for its role in the neurochemical and functional deficits observed in MDD [3].

Experimentally determined three-dimensional structures of selected receptors were retrieved from the RCSB Protein Data Bank (PDB; https://www.rcsb.org; accessed on January 5, 2026). These comprised human 5-HT1A resolved by cryo-electron microscopy at 3.00 Å resolution (PDB ID: 7E2Y) [16], human D2R in complex with risperidone determined by X-ray diffraction at 2.87 Å resolution (PDB ID: 6CM4) [17], human D3R bound to eticlopride solved by X-ray diffraction at 2.89 Å resolution (PDB ID: 3PBL) [18], and active MOR bound to the agonist BU72 resolved by X-ray diffraction at 2.07 Å resolution (PDB ID: 5C1M) [19]. The proteins were prepared using the PDB2PQR web server (https://server.poissonboltzmann.org/pdb2pqr; accessed January 5, 2026) to assign atomic charges and radii according to the AMBER force field [20-22]. The protonation states of ionizable residues were predicted using PROPKA3 at physiological pH (7.4) [20, 23]; structural refinement, including hydrogen atom addition and correction of steric clashes, was conducted using the MolProbity web service (http://molprobity.biochem.duke.edu/; accessed on January 5, 2026) [24].

Structural validation was carried out using the UCLA-DOE LAB-SAVES v 6.1 web service (https://saves.mbi.ucla.edu/; accessed on January 5, 2026). Ramachandran plot analysis was employed to evaluate backbone dihedral angles (ϕ and ψ) and residue conformational distributions using PROCHECK [25], while ERRAT was used to assess overall model quality [26]. All prepared protein structures were subsequently used for downstream molecular docking studies.



	R_1	R_2
Asiatic acid	OH	H
Madecassic acid	OH	OH
Madasiatic acid	CH_3	OH

Figure 1. Chemical scaffold and substituent patterns of the *C. asiatica*-derived triterpenoids evaluated in this study (Asiatic acid: R_1 = OH and R_2 = H; madecassic acid: R_1 = OH and R_2 = OH; madasiatic acid: R_1 = CH_3 and R_2 = OH) (the authors' own elaboration)

C. asiatica-Derived Ligand Preparation

The selected compounds comprised asiatic acid, madecassic acid, and madasiatic acid, three structurally related *C. asiatica*-derived triterpenoids. Asiatic acid has demonstrated neuroprotective effects in experimental cerebral ischemia [27], whereas madecassic acid has demonstrated anti-inflammatory activity in LPS-stimulated macrophages [28]. Madasiatic acid was included as a structurally related compound for comparative *in silico* evaluation. Their chemical structures are shown in **Figure 1**. All three compounds were subsequently evaluated as ligands in the molecular docking analyses.

The three natural compounds and receptor-specific comparator ligands were prepared for molecular docking. Serotonin and vortioxetine were used as comparators for the serotonin 1A receptor; risperidone and eticlopride for the dopamine D2 receptor; eticlopride and risperidone for the dopamine D3 receptor; and BU72 and morphine for the MOR. All ligands were subjected to energy minimization using the Merck molecular force field [29] and subsequently converted to PDBQT format using Open Babel version 2.4.1 [30].

Molecular Docking Simulation

The three natural compounds were docked against all four receptor targets, whereas the comparator ligands were docked only against their designated receptor targets using AutoDock Tools v1.5.6 (The Scripps Research Institute, San Diego, CA, USA) [31], which was used to assign Kollman and Gasteiger charges to proteins and ligands, respectively, save all structures in PDBQT format, and define grid boxes around each protein's ligand-binding site using receptor-specific dimensions and center coordinates provided in **Table A1** in **Appendix A** [20]. Docking was performed using AutoDock Vina version 1.1.2 with default parameters [15, 32]. The docking protocol was validated by extracting and redocking the co-crystallized ligands SRO, 8NU, ETQ, and VF1 from the serotonin 1A, dopamine D2, dopamine D3, and MOR structures, respectively. The redocked conformations were compared with their experimentally determined poses, and a root-mean-square deviation (RMSD) below 2.0 Å was considered evidence of acceptable protocol reproducibility [33, 34]. After validation, the same docking parameters were applied to the natural compounds and receptor-specific comparator ligands.

Table 1. Summary of the strongest predicted docking interactions of *C. asiatica*-derived compounds across antidepressant-related targets

Target	Best *C. asiatica*-derived compound	Docking score, kcal/mol	Comparator ligand(s)	Comparator docking score, kcal/mol
5-HT1A receptor (PDB ID: 7E2Y)	Madasiatic acid	-8.5	Vortioxetine; serotonin	-8.0; -6.1
D2 receptor (PDB ID: 6CM4)	Madasiatic acid	-9.6	Risperidone; eticlopride	-12.7; -8.1
D3 receptor (PDB ID: 3PBL)	Asiatic acid	-7.6	Eticlopride; risperidone	-8.3; -10.8
Mu-opioid receptor (PDB ID: 5C1M)	Asiatic acid	-6.7	BU72; morphine	-12.0; -8.5

Note. More negative docking scores indicate stronger predicted binding; Full residue-level interaction profiles, including hydrogen bonds, hydrophobic interactions, π–π stacking interactions, and salt bridges, are provided in **Table A3** in **Appendix A**.

Protein-Ligand Interaction Analysis

Each docked protein-ligand complex was analyzed using the protein-ligand interaction profiler web service (accessed January 6, 2026) to evaluate the intermolecular interactions between the ligands and their corresponding protein targets. Analyses were performed using default parameters to identify hydrogen bonds, hydrophobic interactions, π-π stacking interactions, and salt bridges [35, 36]. All protein-ligand interaction figures were generated and visualized using PyMOL version 2.5.2 (Schrödinger, LLC, New York, NY, USA).

Drug-Likeness, Pharmacokinetic, and Toxicity Prediction

The simplified molecular-input line-entry system (SMILES) representations of asiatic acid, madecassic acid, madasiatic acid, vortioxetine, risperidone, and morphine were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/; accessed January 6, 2026) and are provided in **Table A2** in **Appendix A**. Vortioxetine, risperidone, and morphine were included as clinically relevant comparator drugs for the drug-likeness, pharmacokinetic, and toxicity analyses. Serotonin, eticlopride, and BU72 were used only as receptor-specific docking comparators and were not included in these analyses. Drug-likeness and oral bioavailability parameters were predicted using SwissADME (accessed January 6, 2026) [13]. Pharmacokinetic properties and toxicity profiles were predicted using the pkCSM web server (accessed January 6, 2026) [14]. Acute oral toxicity of the three *C. asiatica*-derived compounds and comparator drugs was additionally predicted using the STopTox web server (accessed January 6, 2026), which applies quantitative structure-activity relationship and machine-learning models to classify toxicity based on molecular structural features [37].

RESULTS

Validation of Antidepressant-Related Protein Structures

The four antidepressant-related protein structures (5-HT1A, D2R, D3R, and MOR) were validated using the UCLA-DOE LAB-SAVES v 6.1 web server prior to molecular docking. Ramachandran plot analysis showed that all four models had more than 90% of residues in favored regions (5-HT1A, 94.0%; D2R, 92.5%; D3R, 90.1%; MOR, 93.8%), with the remainder in additionally allowed regions and none in disallowed regions, confirming good stereochemical quality (**Figure A1** in **Appendix A**). ERRAT analysis further supported high overall model quality, with scores of 98.47%, 99.02%, 96.83%, and 91.95% for 5-HT1A, D2R, D3R, and MOR, respectively—all above the 90% acceptability threshold. Together, these results confirmed that the protein models were structurally reliable and suitable for subsequent docking studies of *C. asiatica*-derived compounds with the selected targets.

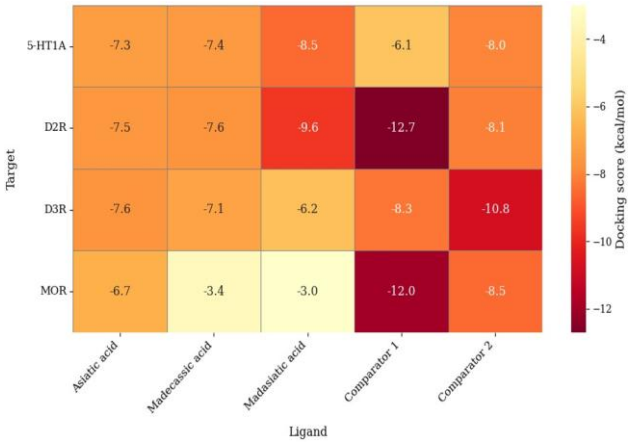


Figure 2. Docking scores of *C. asiatica*-derived compounds and comparator ligands across antidepressant-related targets (Scores [kcal/mol; more negative = stronger predicted binding] for asiatic acid, madecassic acid, madasiatic acid, and comparator ligands against 5-HT1A, D2R, D3R, and MOR & comparator 1 and comparator 2 represent serotonin and vortioxetine for 5-HT1A, risperidone and eticlopride for D2R, eticlopride and risperidone for D3R, and BU72 and morphine for MOR, respectively) (the authors' own elaboration)

Validation of Molecular Docking Studies

The redocked ligands SRO, 8NU, ETQ, and VF1, corresponding to 5-HT1A, D2R, D3R, and MOR, superimposed closely onto their co-crystallized conformations, with RMSD values of 0.920, 1.641, 1.180, and 0.815 Å, respectively (parts a, b, c, and d in **Figure A2** in **Appendix A**), all below the 2.0 Å threshold. These results confirmed the docking protocol's reliability, supporting its use for predicting the binding conformations of *C. asiatica*-derived compounds.

Binding Interactions With Antidepressant-Related Target Proteins

Comparator ligands were included to contextualize the docking scores of the natural compounds. The strongest predicted binding interactions are summarized in **Table 1** and **Figure 2**; full residue-level interaction profiles (hydrogen bonds, hydrophobic interactions, π-π stacking, and salt bridges) are provided in **Table A3** in **Appendix A**.

Among the evaluated *C. asiatica*-derived compounds, madasiatic acid showed the strongest predicted binding to 5-HT1A, with a docking score of -8.5 kcal/mol, which was more favorable than serotonin (-6.1 kcal/mol) and comparable to vortioxetine (-8.0 kcal/mol). Madasiatic acid also showed the strongest predicted binding to D2R among the natural compounds, with a docking score of -9.6 kcal/mol. This score was less favorable than that of risperidone (-12.7 kcal/mol) but more favorable than that of eticlopride (-8.1 kcal/mol).

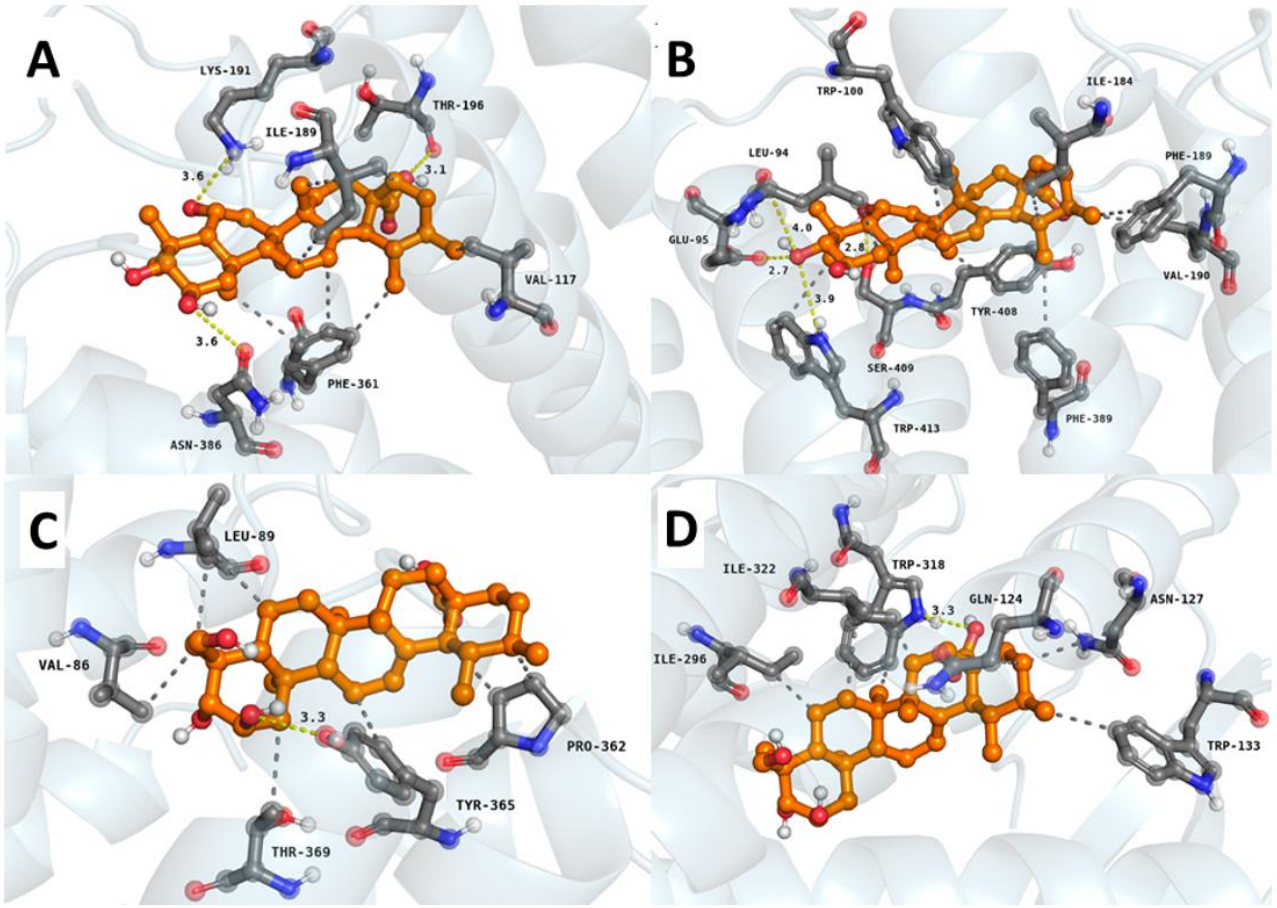


Figure 3. Representative protein-ligand interactions of selected *C. asiatica*-derived compounds (Docking poses for madasiatic acid with the serotonin 1A receptor [A] and dopamine D2 receptor [B], and asiatic acid with the dopamine D3 receptor [C], and MOR [D]) (the authors' own elaboration)

Table 2. Summary of drug-likeness, pharmacokinetic, and toxicity predictions of *C. asiatica*-derived compounds and comparator ligands

Compound	RO5 status	Intestinal absorption (%)	BBB permeability, logBB	CYP3A4 substrate	hERG II inhibitor	Hepatotoxicity
Asiatic acid	No violation	62.855	-0.646	Yes	No	No
Madecassic acid	1 violation: MW > 500	57.113	-0.737	Yes	No	No
Madasiatic acid	No violation	68.106	-0.578	Yes	No	No
Vortioxetine	No violation	92.935	0.901	Yes	Yes	No
Risperidone	No violation	94.226	0.387	Yes	Yes	Yes
Morphine	No violation	73.691	-0.100	Yes	Yes	Yes

Note. RO5: Lipinski's rule of five; BBB permeability is expressed as logBB & detailed physicochemical, permeability, transporter, metabolism, excretion, and toxicity predictions are provided in **Table A4** in **Appendix A**

For D3R, asiatic acid showed the strongest predicted binding among the natural compounds, with a docking score of -7.6 kcal/mol, although this was weaker than eticlopride (-8.3 kcal/mol) and risperidone (-10.8 kcal/mol). For MOR, asiatic acid showed moderate predicted binding, with a docking score of -6.7 kcal/mol, but this was weaker than BU72 (-12.0 kcal/mol) and morphine (-8.5 kcal/mol). Representative docking poses are shown in **Figure 3**, including madasiatic acid with 5-HT1A, madasiatic acid with D2R, asiatic acid with D3R, and asiatic acid with MOR.

Drug-Likeness, Pharmacokinetic, and Toxicity Profiles

Drug-likeness, pharmacokinetic, and toxicity predictions are summarized in **Table 2**, with the full prediction profile provided in **Table A4** in **Appendix A**. Asiatic acid and madasiatic acid showed no Lipinski's Rule of Five violations, whereas madecassic acid showed one violation because of molecular weight (MW) greater than 500 g/mol. The three *C. asiatica*-derived compounds showed moderate predicted intestinal absorption, ranging from 57.113% to 68.106%, and all had logBB values above -1, suggesting potential blood-brain barrier (BBB) penetration.

All three natural compounds were predicted to be cytochrome P450 3A4 substrates. However, none was predicted to inhibit human ether-à-go-go-related gene (hERG) II or to be hepatotoxic. In contrast, the comparator ligands showed higher predicted intestinal absorption but also had less favorable predicted toxicity profiles in some models, particularly hERG II inhibition and hepatotoxicity for risperidone and morphine. Bioavailability radar plots and acute oral toxicity fragment contribution maps are provided in **Figure A3** and **Figure A4**, respectively in **Appendix A**.

DISCUSSION

Beyond its traditional use for CNS-related conditions, *C. asiatica* and its triterpenoids have shown antidepressant-like effects in preclinical models. Asiaticoside reduced inflammatory markers and modulated signaling involving cyclic adenosine monophosphate (cAMP), PKA, cAMP response element-binding protein (CREB), and BDNF in a chronic-stress mouse model [11]. A standardized *C. asiatica* extract also demonstrated antidepressant-like effects in an olfactory-bulbectomy rat model [10]. More recently, a multi-herb formulation containing *C. asiatica* improved depression-like behaviors and CREB/BDNF-related outcomes, although these effects cannot be attributed specifically to *C. asiatica* [38].

Consistent with these findings, previous computational studies have reported strong 5-HT1A binding for structurally diverse phytochemicals, indicating that natural product chemotypes can achieve receptor-compatible interaction profiles [39]. Taken together, these observations support the concept that *C. asiatica* triterpenoids may interact with the serotonin 1A receptor; however, functional modulation cannot be inferred from docking alone. Dopaminergic dysfunction may contribute to motivational anhedonia and reduced motivation in MDD. A recent living systematic review and network meta-analysis found that pro-dopaminergic interventions produced a small reduction in anhedonia, supporting a contributory, although not exclusive, role of dopaminergic pathways in this symptom [39]. Positron emission tomography findings regarding D2/D3 receptor availability are less consistent. It was reported that there was increased striatal D2/D3 receptor availability in patients with MDD and an association with motivational anhedonia [40], whereas it was found no significant differences in D2/D3 receptor availability or dopamine release compared with healthy controls [41]. Nevertheless, the involvement of dopaminergic reward pathways provides a biological rationale for including D2R and D3R as exploratory docking targets.

The endogenous opioid system, particularly the MOR, is implicated in mood regulation and depression [3]. In the present docking analysis, BU72 and morphine showed stronger predicted binding to the MOR than the evaluated triterpenoids and were predicted to form a salt bridge with Asp147. In contrast, the *C. asiatica*-derived triterpenoids showed weaker predicted binding, with docking scores ranging from -3.0 to -6.7 kcal/mol and did not interact with Asp147 under the applied docking conditions. This difference suggests a predicted interaction pattern distinct from those of the opioid comparators; however, it does not establish receptor activation, therapeutic benefit, or a reduced risk of opioid-like adverse effects. Asiatic acid showed the strongest predicted MOR binding among the natural compounds. Molecular dynamics simulations, receptor-binding assays, and functional studies are required to determine the biological relevance of these predicted interactions.

Pharmacokinetic and toxicity predictions were included because inadequate systemic exposure and toxicity-related liabilities are important causes of candidate attrition during drug development. The three *C. asiatica*-derived triterpenoids showed moderate predicted intestinal absorption, ranging from 57.113% to 68.106%, which was lower than that of the comparator drugs and may indicate limited oral exposure without formulation optimization. All three compounds had predicted logBB values above -1, suggesting possible BBB penetration. This finding is broadly consistent with in vitro evidence that asiatic acid can cross a porcine brain endothelial barrier model without compromising barrier integrity [42]. None of the three compounds was predicted to inhibit hERG II or to be hepatotoxic in the applied computational models. Their predicted toxicity profiles were broadly consistent with the reported tolerability of standardized *C. asiatica* extract ECa 233 in healthy volunteers [43]. However, clinical findings for a standardized extract cannot be directly extrapolated to isolated triterpenoids, higher doses, or prolonged systemic exposure. Therefore, these results should be interpreted as preliminary computational predictions rather than evidence of clinical safety. The condensed docking and absorption, distribution, metabolism, excretion, and toxicity (ADMET) summaries suggest that *C. asiatica*-derived triterpenoids may serve as preliminary lead scaffolds for further antidepressant-related investigation (see conclusions for the compound-by-target summary). This approach aligns with the view that antidepressant efficacy may arise from coordinated modulation of multiple neurobiological pathways rather than single-receptor agonism or antagonism [5].

Nevertheless, this study has limitations inherent to its in silico design. Although the docking protocol was internally validated using redocking procedures (RMSD < 2.0 Å), no external validation such as molecular dynamics simulations or experimental assays was performed. Docking scores approximate binding affinity but do not predict functional efficacy, receptor activation, biased signaling, or in vivo pharmacodynamics. In addition, G protein-coupled receptor conformational flexibility and membrane environment effects are not fully captured, and ADMET predictions may not accurately reflect real-world pharmacokinetics, particularly for natural compounds with complex metabolism. Furthermore, the evaluation of a limited number of targets cannot fully represent the multifactorial pathophysiology of depression [2]. Further validation through molecular dynamics simulations, in vitro receptor-binding and functional assays, and in vivo behavioral, toxicological, and pharmacokinetic studies is required to confirm the biological relevance of these computational findings. If adequate preclinical efficacy and safety are demonstrated, clinical development should proceed sequentially. An initial Phase I, randomized, double-blind, placebo-controlled, single-ascending-dose and multiple-ascending-dose study in healthy adult volunteers should evaluate safety, tolerability, dose-limiting adverse events, and pharmacokinetic parameters and establish an appropriate dose range for subsequent studies. This should be followed, if supported by the phase I findings, by a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial in adults with MDD. Participants should be randomly assigned to receive the selected C. *asiatica*-derived compound at one or more predefined doses or matched placebo for an appropriate treatment period, with change in a validated depression severity measure, such as the Montgomery-Åsberg depression rating scale or Hamilton depression rating scale, specified as the primary efficacy endpoint. Secondary outcomes should include response and remission rates, anhedonia-related symptoms, quality of life, safety, tolerability, and pharmacokinetic measures. Such rigorously controlled clinical studies will be necessary to determine whether the predicted receptor interactions identified in the present study translate into clinically meaningful antidepressant efficacy and acceptable safety in patients with MDD.

CONCLUSIONS

This in silico study evaluated the predicted interactions of three *C. asiatica*-derived triterpenoids with depression-related protein targets and assessed their drug-likeness, pharmacokinetic, and toxicity profiles. Madasiatic acid showed the most favorable predicted binding to 5-HT1A and D2R, whereas asiatic acid showed the strongest predicted binding to D3R among the natural compounds and moderate predicted binding to MOR. The evaluated compounds generally showed acceptable predicted drug-likeness, with no predicted hERG II inhibition or hepatotoxicity in the applied models, although their predicted intestinal absorption was lower than that of the comparator ligands. These findings provide preliminary computational evidence that *C. asiatica*-derived triterpenoids may be useful lead scaffolds for antidepressant-related drug discovery. Further validation using molecular dynamics simulations, receptor-binding assays, functional studies, pharmacokinetic assessment, and in vivo depression models is required before therapeutic relevance can be inferred.

Author contributions: AK: conceptualization, data curation, formal analysis, investigation, methodology, resources, software, validation, visualization, writing – original draft, writing – review & editing; **AM:** conceptualization, formal analysis, methodology, writing – original draft, writing – review & editing; **PK:** conceptualization, formal analysis, investigation, methodology, validation, writing – review & editing; **LG:** supervision, writing – review & editing; **WT:** conceptualization, formal analysis, funding acquisition, methodology, project administration, writing – review & editing. All authors have agreed with the results and conclusions.

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Ethical statement: This study was initially approved by the Walailak University Ethics Committee on 20 May 2025 under approval number WUEC-25-167-01. The ethical approval was subsequently renewed under approval number WUEC-25-167-02, valid from 20 May 2026 to 19 May 2027. The present study reports only the in silico phase and did not involve human participants or identifiable personal data; therefore, informed consent and procedures for protecting sensitive or confidential personal data were not applicable.

AI statement: No generative AI or AI-based tools were used in the preparation of this manuscript.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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APPENDIX A

Table A1. Grid box sizes and center coordinates are used for molecular docking

Protein-ligand crystal structure	Grid box size (x, y, z)	Center coordinates			References
		x	y	z	
5-HT1A-SRO (PDB ID: 7E2Y)	16 × 16 × 16	99.279	114.944	109.390	[16]
D2R-8NU (PDB ID: 6CM4)	20 × 20 × 20	9.177	5.470	-8.665	[17]
D3R-ETQ (PDB ID: 3PBL)	20 × 20 × 20	0.369	-14.318	11.611	[18]
MOR-VF1 (PDB ID: 5C1M)	20 × 20 × 20	2.026	15.538	-58.785	[19]

Note. SRO: Serotonin; 8NU: Risperidone; ETQ: Eticlopride; VF1: 2R,3S,3aR,5aR,6R,11bR,11cS-3a-methoxy-3,14-dimethyl-2-phenyl-2,3,3a,6,7,11c-hexahydro-1H-6,11b-(epiminoethano)-3,5a-methanonaphtho[2,1-g/indol-10-ol or BU72]; The grid box for D3R-ETQ was generated to encompass the amino acid residues interacting with the co-crystallized ligand eticlopride

Table A2. Molecular formulas, PubChem compound identifiers, and SMILES representations of the investigated compounds

Compound	Molecular formula	PubChem CID	SMILES
Asiatic acid	$C_{30}H_{48}O_5$	119034	C[C@@H]1CC[C@@]2(CC[C@@]3(C(=CC[C@H]4[C@]3(CC[C@@H]5[C@@]4(C[C@H]([C@@H]([C@@]5(C)CO)O)O)C)C)[C@@H]2[C@H]1C)C)C(=O)O
Madecassic acid	$C_{30}H_{48}O_6$	73412	C[C@@H]1CC[C@@]2(CC[C@@]3(C(=CC[C@H]4[C@]3(C[C@H]([C@@H]5[C@@]4(C[C@H]([C@@H]([C@@]5(C)CO)O)O)C)O)C)[C@@H]2[C@H]1C)C)C(=O)O
Madasiatic acid	$C_{30}H_{48}O_5$	23132225	CC1CCC2(CCC3(C(=CCC4C3(CC(C5C4(CC(C(C5(C)C)O)O)C)O)C)C2C1C)C)C(=O)O
Vortioxetine	$C_{18}H_{22}N_2S$	9966051	CC1=CC(=C(C=C1)SC2=CC=CC=C2N3CCNCC3)C
Risperidone	$C_{23}H_{27}FN_4O_2$	5073	CC1=C(C(=O)N2CCCCC2=N1)CCN3CCC(CC3)C4=NOC5=C4C=CC(=C5)F
Morphine	$C_{17}H_{19}NO_3$	5288826	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(=C(C=C5)O)O[C@H]3[C@H](C=C4)O

Table A3. Full docking interaction profiles of *C. asiatica*-derived compounds and comparator ligands across depression-related targets

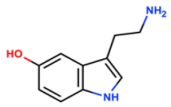
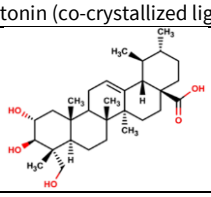
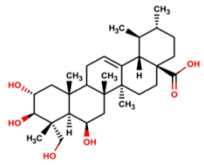
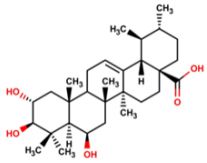
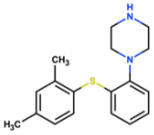
Target	Compounds	Binding energy (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	π-π stacking interactions	Salt bridge interactions
			Amino acid residues	Amino acid residues	Amino acid residues	Amino acid residues
5-HT1A (PDB ID: 7E2Y)	 Serotonin (co-crystallized ligand)	-6.1	VAL117, THR121, TYR390	VAL117, ILE189, PHE362[a]	PHE361	-
	 Asiatic acid	-7.3	THR200	ILE189, TYR195, PHE361[a], VAL364	-	LYS191
	 Madecassic acid	-7.4	THR200	ILE189[a], PHE361, ASN386	-	LYS191
	 Madasiatic acid	-8.5	LYS191, THR196, ASN386	VAL117, ILE189[b], PHE361[b]	-	-
	 Vortioxetine (antidepressant drug)	-8.0	THR121	PHE361[a], ALA365, TYR390	PHE361	-

Table A3 (Continued).

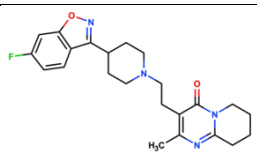
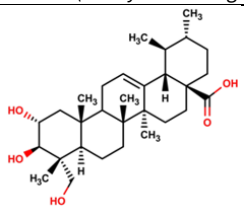
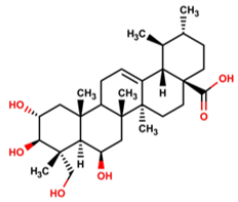
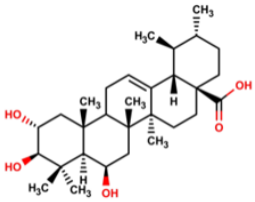
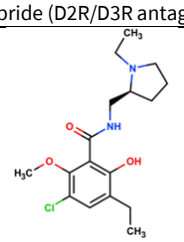
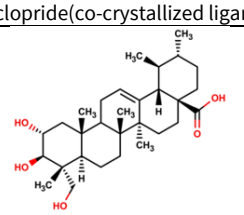
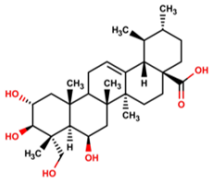
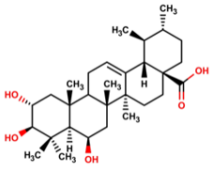
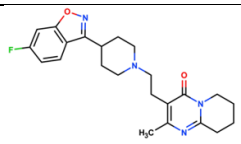
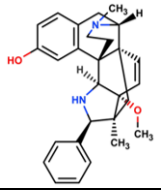
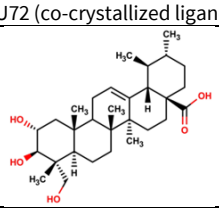
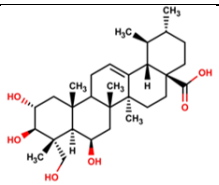
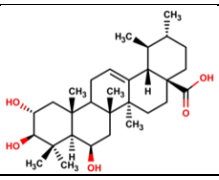
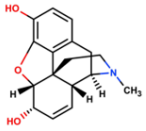
Target	Compounds	Binding energy (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	π-π stacking interactions	Salt bridge interactions
			Amino acid residues	Amino acid residues	Amino acid residues	Amino acid residues
D2R (PDB ID: 6CM4)	 Risperidone (co-crystallized ligand)	-12.7	TYR416 TRP100, TYR408[a], SER409	VAL91, LEU94, ILE184, TRP386, PHE390, THR412 PHE189[a],VAL190, TYR408[a], THR412	TRP100, TRP386[a]	ASP114
	 Asiatic acid	-7.5	TYR416	VAL91, LEU94, ILE184, TRP386, PHE390, THR412	-	-
	 Madecassic acid	-7.6	TYR408[a], TRP413	TRP100, PHE189, VAL190, TYR408[a], THR412	-	-
D2R (PDB ID: 6CM4)	 Madasiatic acid	-9.6	LEU94, GLU95, SER409, TRP413	LEU94, TRP100, ILE184, PHE189, VAL190, PHE389, TYR408, TRP413	-	-
	 Eticlopride (D2R/D3R antagonist)	-8.1	-	VAL91, LEU94, TRP100, PHE110, VAL115, TRP386[a], PHE389, TYR416	-	ASP114
D3R (PDB ID: 3PBL)	 Eticlopride(co-crystallized ligand)	-8.3	TYR373	ILE183, TRP342, PHE345[a], PHE346[a], THR369	PHE345	ASP110
	 Asiatic acid	-7.6	TYR365	VAL86, LEU89[a], PRO362[a], TYR365, THR369	-	-

Table A3 (Continued).

Target	Compounds	Binding energy (kcal/mol)	Hydrogen bonds	Hydrophobic interactions	π-π stacking interactions	Salt bridge interactions
			Amino acid residues	Amino acid residues	Amino acid residues	Amino acid residues
MOR (PDB ID: 5C1M)	 Madecassic acid	-7.1	TYR365, ASP110	VAL86, LEU89, PHE106, PRO362[a], TYR365[a]	-	-
	 Madasiatic acid	-6.2	-	LEU89, ILE183[a], TYR365, THR369	-	-
	 Risperidone (antipsychotic drug)	-10.8	-	PHE106, VAL107, ILE183[a], PHE345, PHE346, TYR365, THR369, TYR373	PHE345[a]	ASP110
	 BU72 (co-crystallized ligand)	-12.0	TYR326	GLN124, VAL143, ILE144, ASP147, TRP293, ILE296[a], VAL300, TRP318, ILE322[a]	-	ASP147
	 Asiatic acid	-6.7	TRP318	GLN124, ASN127, TRP133, ILE296, TRP318, ILE322[a]	-	-
	 Madecassic acid	-3.4	SER55, GLN124, ASN127	GLN124, TYR148, LYS233, VAL236[a], ILE296, VAL300[a], ILE322	-	-
	 Madasiatic acid	-3.0	SER55, ASN127, TYR148, TRP318, TYR326	LYS233, VAL236, ILE296, VAL300[a], TRP318, ILE322	-	-
	 Morphine (opioid analgesic drug)	-8.5	TYR148, TYR326	TYR148, VAL236, TRP293	-	ASP147

Note. Binding energies are reported in kcal/mol, with more negative values indicating stronger predicted binding; Amino acid residues involved in hydrogen bonds, hydrophobic interactions, π-π stacking interactions, and salt bridge interactions are listed for each ligand; Superscript letters (a, b) indicate multiple interactions involving the same amino acid residue within the same interaction category; 5-HT1A, serotonin 1A receptor; D2R, dopamine D2 receptor; D3R, dopamine D3 receptor; MOR, mu-opioid receptor

Table A4. Full drug-likeness, pharmacokinetic, and toxicity predictions of *C. asiatica*-derived compounds and comparator ligands

Compound	RO5 status	MW (g/mol)	HBD/ HBA	CLogP	TPSA (Å^2)	Caco-2 permeability	Intestinal absorption (%)	BBB permeability	CYP3A4 substrate	hERG II I	H	Overall ADMET interpretation
Asiatic acid	Yes; no violations	488.70	4/5	4.45	97.99	0.479	62.855	-0.646	Yes	No	No	Favorable drug-likeness and predicted safety; moderate intestinal absorption and BBB permeability
Madecassic acid	Yes; 1 violation: MW >500	504.70	5/6	3.56	118.22	0.455	57.113	-0.737	Yes	No	No	Acceptable predicted safety; limited permeability and one RO5 violation due to molecular weight
Madasiatic acid	Yes; no violations	488.70	4/5	4.27	97.99	0.609	68.106	-0.578	Yes	No	No	Favorable drug-likeness and predicted safety; best intestinal absorption among the natural compounds
Vortioxetine	Yes; no violations	298.45	1/1	3.74	40.57	1.569	92.935	0.901	Yes	Yes	No	Favorable oral drug-likeness and permeability; predicted hERG II inhibition
Risperidone	Yes; no violations	410.48	0/6	3.62	64.16	1.254	94.226	0.387	Yes	Yes	Yes	High predicted absorption and permeability; predicted hERG II inhibition and hepatotoxicity
Morphine	Yes; no violations	285.34	2/4	1.44	52.93	1.112	73.691	-0.100	Yes	Yes	Yes	Favorable drug-likeness; predicted hERG II inhibition and hepatotoxicity

Note. Caco-2 permeability is expressed as log Papp in 10^{-6} cm/s; BBB permeability is expressed as logBB; CLogP: Calculated 1-octanol/water partition coefficient; CYP: Cytochrome P450; HBA: Hydrogen-bond acceptor; HBD: Hydrogen-bond donor; TPSA: Topological polar surface area; I: Inhibitor; & H: Hepatotoxicity

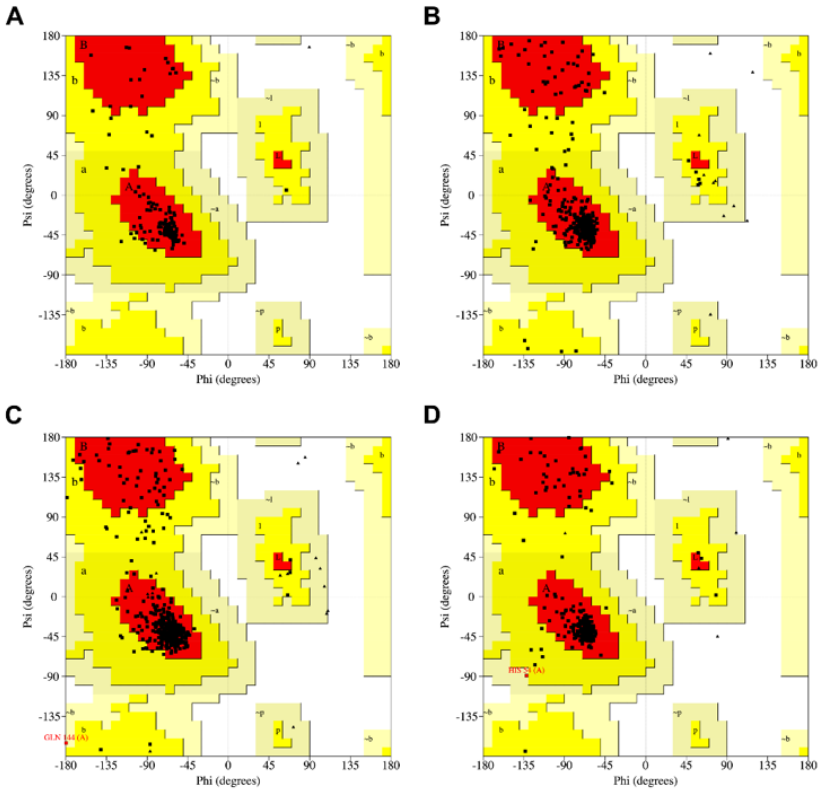


Figure A1. Ramachandran plots of prepared antidepressant-related protein structures (Ramachandran plots showing the distribution of amino acid residues across favored, additionally allowed, generously allowed, and disallowed regions for 5-HT1A [PDB ID: 7E2Y] [A], D2R [PDB ID: 6CM4] [B], D3R [PDB ID: 3PBL] [C], and MOR [PDB ID: 5C1M] [D] & Black dots indicate individual amino acid residues) (the authors' own elaboration)

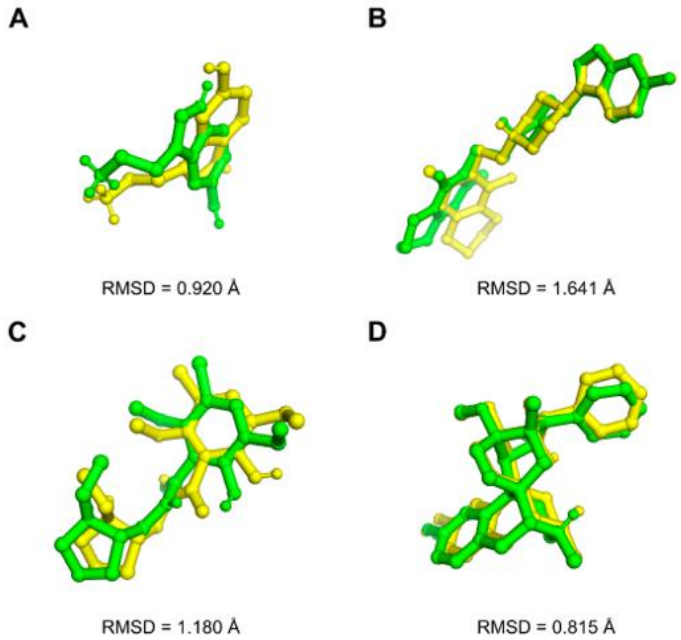


Figure A2. Validation of molecular docking by redocking of co-crystallized ligands (RMSD values between redocked ligands and co-crystallized ligands for serotonin [SRO] with 5-HT1A [A], risperidone [8NU] with D2R [B], eticlopride [ETQ] with D3R [C], and BU72 [VF1] with MOR [D]; Green structures represent redocked ligands, whereas yellow structures represent co-crystallized ligands) (the authors' own elaboration)

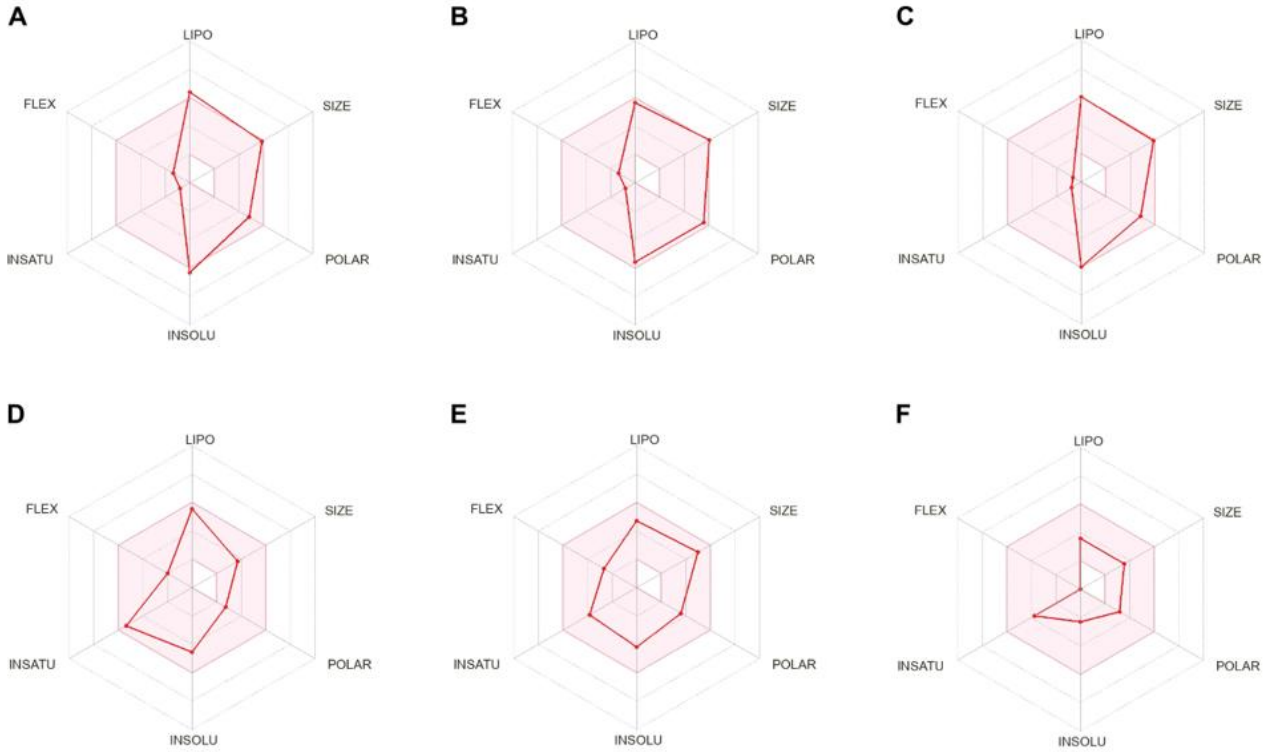


Figure A3. Bioavailability radar plots of the investigated compounds (Bioavailability radar plots generated using SwissADME for asiatic acid [A], madecassic acid [B], madasiatic acid [C], vortioxetine [D], risperidone [E], and morphine [F]; The radar plots display six physicochemical descriptors: lipophilicity, molecular size, polarity, insolubility, insaturation, and flexibility; & The red lines represent the properties of each compound, whereas the pink areas indicate the optimal ranges associated with favorable oral bioavailability) (the authors' own elaboration)

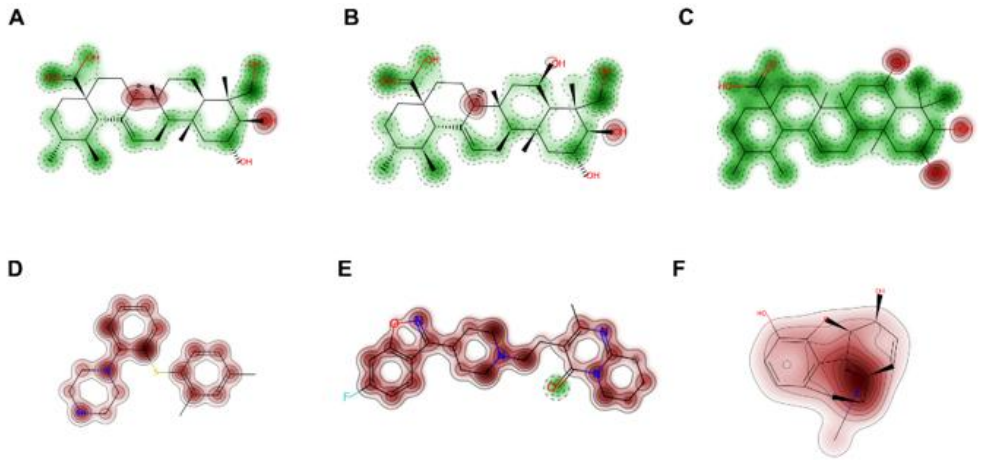


Figure A4. Fragment contribution maps for acute oral toxicity prediction (Fragment contribution maps for acute oral toxicity prediction generated for asiatic acid [A], madecassic acid [B], madasiatic acid [C], vortioxetine [D], risperidone [E], and morphine [F] & Red regions with continuous outlines indicate fragments predicted to increase toxicity, whereas green regions indicate fragments predicted to decrease toxicity) (the authors' own elaboration)