

CENTELLA ASIATICA AS A MULTI-TARGET ANTISTRESS AGENT : AN IN SILICO STUDY

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ABSTRAK

Stres kronis yang berlebihan dapat mengganggu regulasi sumbu hipotalamus–pituitari–adrenal (HPA axis) dan berkontribusi terhadap berbagai gangguan kesehatan. Pendekatan terapi yang menargetkan satu protein seringkali kurang efektif karena kompleksitas sistem ini. *Centella asiatica* diketahui memiliki efek menenangkan dan mengandung senyawa bioaktif yang berpotensi sebagai agen multitarget. Penelitian ini bertujuan untuk mengevaluasi potensi asiatic acid dan madecassic acid sebagai agen antistres multitarget melalui interaksi dengan CRHR1 dan glukokortikoid reseptor (GR). Studi dilakukan secara in silico meliputi analisis ADME menggunakan SwissADME, prediksi aktivitas biologis dengan PASS, serta molecular docking menggunakan AutoDock Vina. Visualisasi interaksi dilakukan dengan Discovery Studio. Hasil docking menunjukkan bahwa kedua senyawa memiliki afinitas sedang terhadap CRHR1 ($-7,7$ kcal/mol), dengan asiatic acid menunjukkan interaksi yang lebih stabil dibandingkan madecassic acid. Pada GR, kedua senyawa menunjukkan afinitas yang lebih rendah ($-4,5$ dan $-2,2$ kcal/mol), meskipun asiatic acid masih mempertahankan interaksi dengan residu penting. Analisis PASS juga menunjukkan potensi aktivitas antiinflamasi dan modulasi faktor transkripsi. Asiatic acid memiliki potensi lebih tinggi sebagai agen antistres multitarget dibandingkan madecassic acid, terutama melalui interaksi dengan CRHR1 dan kemungkinan modulasi GR, meskipun diperlukan validasi eksperimental lebih lanjut.

Kata kunci : asiatic acid, CRHR1, glucocorticoid receptor, madecassic acid, molecular docking

ABSTRACT

Chronic stress can disrupt the regulation of the hypothalamic–pituitary–adrenal (HPA) axis and contribute to various health disorders. Single-target therapeutic approaches are often insufficient due to the complexity of this system. *Centella asiatica* is known for its calming effects and contains bioactive compounds with potential multi-target activity. This study aimed to evaluate the potential of asiatic acid and madecassic acid as multi-target antistress agents through their interaction with CRHR1 and the glucocorticoid receptor (GR). An in silico approach was conducted, including ADME analysis using SwissADME, biological activity prediction using PASS, and molecular docking using AutoDock Vina. Interaction visualization was performed using Discovery Studio. Docking results showed that both compounds exhibited moderate binding affinity toward CRHR1 (-7.7 kcal/mol), with asiatic acid demonstrating a more stable interaction profile compared to madecassic acid. In contrast, both compounds showed lower binding affinity toward GR (-4.5 and -2.2 kcal/mol), although asiatic acid maintained interactions with key residues. PASS prediction indicated potential anti-inflammatory and transcription factor modulation activities. Asiatic acid demonstrated greater potential as a multi-target antistress agent than madecassic acid, particularly through its interaction with CRHR1 and possible modulatory effects on GR, although further experimental validation is required.

Keywords : asiatic acid, CRHR1, glucocorticoid receptor, madecassic acid, molecular docking

INTRODUCTION

The human stress response is an important mechanism for maintaining homeostatic conditions during unfavorable conditions, involving a network of neural and hormonal systems. These mechanisms ensure that various organ systems function properly to maintain the organism's survival (Kivimäki et al., 2023). These mechanisms are also followed by temporal

immune enhancement and immune surveillance and also increased metabolism (hypermetabolism) during acute stress conditions (Alotiby, 2024; Bobba-Alves et al., 2022). However, when stress becomes chronic or recurrently excessive, the involved biological systems may shift from adaptive to maladaptive regulation. Persistent activation of stress responses is associated with increased oxidative burden and neuroinflammatory signaling, which can lead to impaired cognitive function (Hassamal, 2023; Juszczak et al., 2021), and a higher risk of cardiometabolic diseases (Ortiz et al., 2022). Consequently, chronic stress significantly contributes to vulnerability to stress-related neuropsychiatric and systemic disorders. Therefore, understanding the molecular mechanisms that regulate both the initiation and termination of stress signaling pathways is essential for developing therapeutic interventions that restore normal stress-axis function rather than merely suppressing stress responses.

The Hypothalamic–Pituitary–Adrenal (HPA) axis is the primary neuroendocrine system that regulates the physiological response to stress in humans. This axis comprises several interconnected components, with the hypothalamus as the upstream initiator of the stress response, which then signals the pituitary, which in turn signals the adrenal glands to produce cortisol (Sheng et al., 2021). When the stressor is present, whether physical or psychological, is processed in brain regions involved in emotion and regulation, including the amygdala, prefrontal cortex, and hippocampus. These signals activate the paraventricular nucleus (PVN) of the hypothalamus, leading to corticotropin-releasing hormone (CRH) release (Herman et al., 2020). CRH was released and then transported through the hypophyseal portal system, which connects directly to the anterior pituitary. CRH can bind to CRHR1, expressed in corticotroph cells of the pituitary gland, thereby activating downstream signaling that increases transcription of proopiomelanocortin (POMC). POMC is important as a precursor to adrenocorticotropic hormone (ACTH), which is then released into the bloodstream, targeting the adrenal glands to produce cortisol (Caruso et al., 2022; Rasiah et al., 2023). Given the importance of CRHR1 in linking stress signaling to adrenal cortisol production, antagonizing this receptor could be useful for developing an antistress agent that prevents the progression of various diseases (Kim et al., 2023; Pagán-Busigó et al., 2022).

In addition to the activation mechanism of cortisol production, a negative feedback mechanism also regulates it, with its role mediated by the glucocorticoid receptor (GR) (Gjerstad et al., 2018). Cortisol negative feedback initiation is primarily mediated by cortisol binding to GRs in the PVN, anterior pituitary, and hippocampus (Jimeno & Zimmer, 2022). The binding of cortisol to GR in the PVN suppresses CRH neuron activity and CRH gene expression, thereby decreasing CRH release (Su et al., 2025). The binding of cortisol and GR in the anterior pituitary leads to suppression of POMC and ACTH gene expression and a decrease in ACTH secretion (Regazzo et al., 2022). Aside from the PVN and the pituitary gland, the hippocampus also plays a crucial role in indirectly modulating the negative feedback mechanism of the HPA axis. The hippocampus can detect circulating cortisol and binds it to GR within the hippocampus, thereby inhibiting PVN activity via GABAergic relay circuits. This process reduces CRH secretion and constrains prolonged cortisol production (Herman et al., 2020).

However, GR-mediated negative feedback that limits HPA axis activity occurs primarily at the hypothalamic and anterior pituitary levels. Glucocorticoid signaling through GR suppresses CRH and ACTH release, thereby preventing prolonged HPA axis activation. Nevertheless, GR is also present in peripheral tissues, and adrenal signaling may contribute to local regulation of adrenal responsiveness and glucocorticoid production (Paust et al., 2006; Su et al., 2025). The key role of GR in regulating HPA-axis activity and cortisol production through negative feedback may be further leveraged to address chronic stress-related diseases by developing selective GR modulators or partial agonists (De Kloet & Meijer, 2024).

Both CRHR1 inhibitors and GR modulators or agonists have been developed and investigated as therapeutic strategies for stress-related disorders. For example, antalarmin is among the most widely used CRHR1 inhibitors, particularly in experimental settings, and has been shown to reduce HPA-axis activation, anxiety-like behavior, and cortisol/ACTH responses in primate and rodent models of stress (French et al., 2007; Ma et al., 2025). Pexacerfont, a CRHR1 inhibitor that has been clinically tested, also shows promising results in preclinical studies, although the findings are mixed (Kwako et al., 2015). Among GR agonists, dexamethasone is the most prominent, with well-established pharmacological activators of GR signaling that are commonly used as reference compounds in experimental settings and are increasingly used in clinical contexts (Battaglia et al., 2025; Ritter et al., 2025).

Despite widespread use of CRHR1 inhibitors and GR modulators, several issues have arisen regarding their use. The use of CRHR1 inhibitors or GR agonists is usually single-targeted, thus may lack the integrated HPA-axis-balancing concept. This implication is supported by the fact that cortisol secretion is highly dependent on the complex interactions among HPA-axis components (Göver & Slezak, 2024; Herman et al., 2020). These may contribute to mixed results in clinical trials when compared with the seemingly successful results in preclinical studies, mostly in experimental setting (Kwako et al., 2015). *Centella asiatica* is emerging as a promising option to address this problem, with the plant already known for its calming effects and cognitive-supporting properties (Sun et al., 2020).

C. asiatica contains various major bioactive constituents such as madecassoside, asiaticoside, madecassic acid, asiatic acid, and other compounds that may enhance neuronal functions. Given the various compounds contained in *C. asiatica*, the plant extract may modulate multiple proteins, in this context HPA-axis (Bandopadhyay et al., 2023). Although *C. asiatica* has been associated with stress-relieving and neuroprotective effects, the direct, target-specific interactions of its phytoconstituents with HPA-axis receptors, such as CRHR1 and GR, remain poorly characterized. To address this gap, this study investigates the phytoconstituents of *C. asiatica*, particularly asiatic acid and madecassic acid, as candidate multi-target antistress modulators using in silico molecular docking against CRHR1 and GR, with the aim of characterizing CRHR1-antagonistic binding profiles and GR-selective modulatory interactions that may collectively restore balanced HPA-axis regulation.

METHODS

Ligand Collection and Preparation

In this study, the screening and collection of data on bioactive compounds in *C. asiatica* were conducted based on evidence from various literature sources (Khotimah et al., 2015). The bioactive constituents of *C. asiatica* with reported antistress potential were systematically gathered from the literature and verified using the PubChem database. The collected information includes compound names, PubChem Compound Identification (CID) numbers, molecular formulas, canonical SMILES, and chemical structures obtained from the PubChem platform (<https://pubchem.ncbi.nlm.nih.gov/>). These data were used as a reference for subsequent in silico analyses to evaluate structural characteristics and to support the drug design process for the selected compounds.

ADME and Toxicity

ADMET analysis represents a crucial stage in drug discovery, encompassing the evaluation of Absorption, Distribution, Metabolism, Excretion, and Toxicity to determine the interaction of compounds with biological systems. This assessment provides insight into how compounds are absorbed, distributed across tissues, metabolized, eliminated from the body, and their potential toxicological effects. In this study, ADME profiling was performed using

the SwissADME web server (<http://www.swissadme.ch/>). Bioactive compounds derived from *C. asiatica* were screened to assess their potential as antistress drug candidates based on absorption characteristics, solubility, pharmacokinetic behavior, and physicochemical properties. The suitability of each compound as a drug candidate was evaluated according to Lipinski's rule of five, which considers molecular weight, lipophilicity (LogP), hydrogen bond donors and acceptors, and molar refractivity. Canonical SMILES structures of asiatic acid and madecassic acid were obtained from the PubChem database and subsequently submitted to the SwissADME platform to predict the ADMET profiles of each compound.

Biological Activity Analysis and Cytotoxic Prediction

The bioactive compounds of *C. asiatica* were evaluated using the PASS (Prediction of Activity Spectra for Substances) server to predict their potential biological activities, particularly those related to antistress mechanisms, and the CLC-Pred (Cell Line Cytotoxicity Predictor) tool to estimate cytotoxic effects. Canonical SMILES of the selected compounds were obtained from the PubChem database (Table 1) and subsequently submitted to the PASS online platform (<https://www.way2drug.com/PassOnline/>). The predicted biological activities and cytotoxic profiles were expressed as Pa (probability of activity) and Pi (probability of inactivity) values. In this study, threshold criteria of $Pa > 0.7$ were applied for PASS predictions, while $Pa > 0.3$ was used to determine significant outcomes in CLC-Pred analysis. Higher Pa values indicate a greater likelihood of biological activity.

Protein Interaction and Network Analysis of Protein Targets

Network analysis of stress-related signaling pathways was performed by examining protein-protein interactions involving corticotropin-releasing hormone receptor 1 (CRHR1) and the glucocorticoid receptor (GR) using the STRING database (<https://string-db.org/>). This analysis was conducted to identify key molecular pathways associated with stress regulation and hypothalamic-pituitary-adrenal (HPA) axis modulation. Visualization of the protein interaction networks enabled the identification of interacting proteins and signaling pathways linked to CRHR1 and GR, providing insights into the molecular mechanisms underlying stress responses and potential targets influenced by *C. asiatica* bioactive compounds.

Molecular Docking

The preparation of ligands and target receptors constituted the initial step of the molecular docking procedure. The PyRx platform was used to generate ligand conformations with minimum binding energy. Protein structures of corticotropin-releasing hormone receptor 1 (CRHR1) and the glucocorticoid receptor (GR) were prepared using BIOVIA Discovery Studio Visualizer 2019 by removing water molecules and co-crystallized ligands, while ligand structures were generated and optimized through the Open Babel module integrated in PyRx. Molecular docking was performed by defining the grid box dimensions and center coordinates based on the active binding sites of CRHR1 and GR. Binding affinity values between asiatic acid or madecassic acid and the target receptors were calculated using the AutoDock Vina engine via the Vina Wizard in PyRx. Post-docking analysis and visualization of ligand-receptor interactions were subsequently conducted using BIOVA.

RESULTS AND DISCUSSION

ADME and Toxicity of Selected Compounds

The molecular characterization of selected compounds derived from *C. asiatica* (Table 1) indicates that asiatic acid and madecassic acid are pentacyclic triterpenoids exhibiting highly analogous molecular structures, as evidenced by their molecular formulas ($C_{30}H_{48}O_5$ and

C₃₀H₄₈O₆, respectively) and canonical SMILES representations. These compounds differ solely by the presence of an additional hydroxyl group in madecassic acid, which may increase polarity and hydrogen-bonding potential. This structural similarity correlates with their comparable physicochemical properties, both of which display relatively high molecular weights (~500 Da), approaching the upper limit specified by Lipinski's rule of five, and elevated lipophilicity, particularly in asiatic acid (LogP 5.9), which marginally exceeds the recommended threshold, whereas madecassic acid (LogP 5.3) remains closer to acceptable parameters (Kralj et al., 2023; Murthy et al., 2021). Nonetheless, both molecules possess acceptable counts of hydrogen bond donors and acceptors, along with molar refractivity values indicative of favorable polarizability for ligand–receptor interactions.

Regarding ADME-related characteristics (Table 3), the topological polar surface area (TPSA) values for asiatic acid (74.60 Å²) and madecassic acid (94.83 Å²) are below the 140 Å² threshold, implying satisfactory intestinal absorption (Odhiambo et al., 2025). Furthermore, the low number of rotatable bonds (one for each compound) suggests limited molecular flexibility, which may favor binding specificity but reduce conformational adaptability (Wekesa et al., 2023). The increased hydrogen bonding capacity observed in madecassic acid could facilitate stronger interactions with polar residues. However, this may also impact membrane permeability relative to asiatic acid.

Table 1. Molecular Profile Of Selected Compounds From *Centella Asiatica*

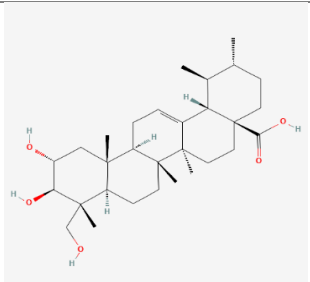
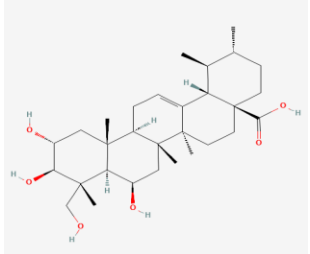
No	Compound	Pubchem CID	Molecular formula	Canonical Smiles	Chemical structure
1	Asiatic acid	119034	C ₃₀ H ₄₈ O ₅	CC1(C)CCC2(C)C1CCC1(C)C2 (CC(C1)O)CCC2(C)C1 (CC(CC2O)C(=O)O)C	
2	Madecassic acid	73412	C ₃₀ H ₄₈ O ₆	CC1(C)CCC2(C)C1CCC1 (C)C2(CC(C1)O)CCC2(C) C1(CC(CC2O)C(=O)O)C(O)C	

Table 2. Physicochemical Properties and Lipinski's Rule Parameters Of Selected Compounds

Ligand	Molecular mass (Dalton)	H-bond donors	H-bond acceptors	LogP	Molar refractivity
Asiatic acid	488.70	2	4	5.9*	132.61*
Madecassic acid	504.70	3	5	5.3*	134.02*

* indicates that the results meet the criteria selectivity index

Table 3. ADME Properties and Oral Bioavailability Parameters Of Selected Compounds

Ligand	LogP	TPSA (Å ²)	Total H bond	Rotatable bond
Asiatic acid	5.9*	74.60*	6*	1*
Madecassic acid	5.3*	94.83*	8*	1*

*indicates that the result meets the selectivity index criteria

Biological Activity and Cytotoxicity Prediction of Selected Compounds

The PASS prediction results indicate that both asiatic acid and madecassic acid exhibit high probabilities of biological activity ($P_a > 0.7$) across several pharmacological effects. Both compounds show strong potential as wound-healing agents and hepatoprotectants, with notable anti-inflammatory activity. Additionally, the predicted stimulation of transcription factors, including NF- κ B, suggests possible involvement in immune and inflammatory regulation (Hernayanti et al., 2021). The compounds also demonstrate potential as apoptosis agonists and antiulcerative agents, indicating a broad therapeutic profile. These predicted activities, particularly anti-inflammatory effects, NF- κ B modulation, and nitric oxide-related mechanisms, are closely associated with stress-related biological pathways, suggesting that both compounds may contribute to potential antistress effects (Jiang et al., 2025).

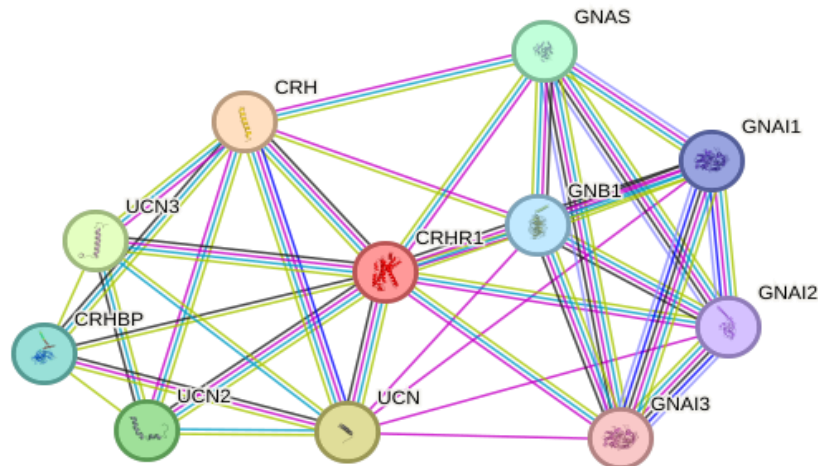
Table 4. Predicted Biological Activities Of Asiatic Acid and Madecassic Acid Based On PASS Analysis

Asiatic acid	Medecassic acid		pa
0,972	0,965	Wound healing agent	0,10
0,97	0,982	Hepatoprotectant	0,20
0,918	0,906	Antiinflammatory	0,30
0,896	0,914	Transcription factor stimulant	0,40
0,896	0,914	Transcription factor NF kappa B stimulant	0,50
0,89	0,83	Apoptosis agonist	0,60
0,848	0,887	Antiulcerative	0,70
0,848	0,859	Hepatic disorders treatment	0,80
0,846	0,812	Nitric oxide antagonist	0,90

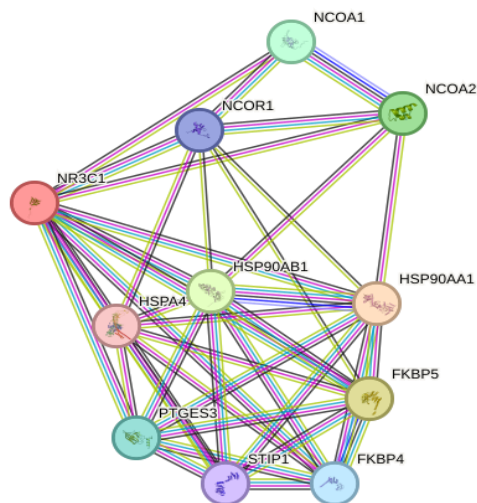
Protein-Protein Interaction and Network Analysis of Protein Target

The protein-protein interaction (PPI) network of CRHR1 demonstrates strong associations with key regulators of the stress response, notably corticotropin-releasing hormone (CRH), urocortins (UCN, UCN2, and UCN3), and CRH-binding protein (CRHBP), highlighting its central role in the modulation of the hypothalamic-pituitary-adrenal (HPA) axis. The proteins involved are essential in the initiation and regulation of stress signaling pathways within the central nervous system. Moreover, the interaction between CRHR1 and G-protein subunits (GNAS, GNAI1, GNAI2, GNAI3, and GNB1) indicates its involvement in intracellular signaling pathways that mediate neuronal responses to stress-related stimuli (Kageyama et al., 2021). The PPI network of CRHR1 indicates the role of CRHR1 as a critical upstream regulator in the neuroendocrine stress response pathway.

The PPI network of GR shows its interaction with several proteins involved in glucocorticoid signaling and stress adaptation. Notably, FKBP5 and FKBP4 are key regulators of GR sensitivity and have been widely linked to stress-related disorders, including anxiety and depression (Mahon et al., 2013). The presence of heat shock proteins such as HSP90AA1 and HSPA4 suggests their role in stabilizing and activating the receptor, which is essential for proper glucocorticoid signaling. Additionally, nuclear receptor co-regulators (NCOA1, NCOA2, and NCOR1) indicate GR's involvement in the transcriptional regulation of stress-responsive genes. Overall, this network highlights GR's role as a downstream effector in the HPA axis, mediating cellular and physiological responses to stress.



Picture 1. Interaction Network Of CRHR1 with Other Proteins In The STRING Database

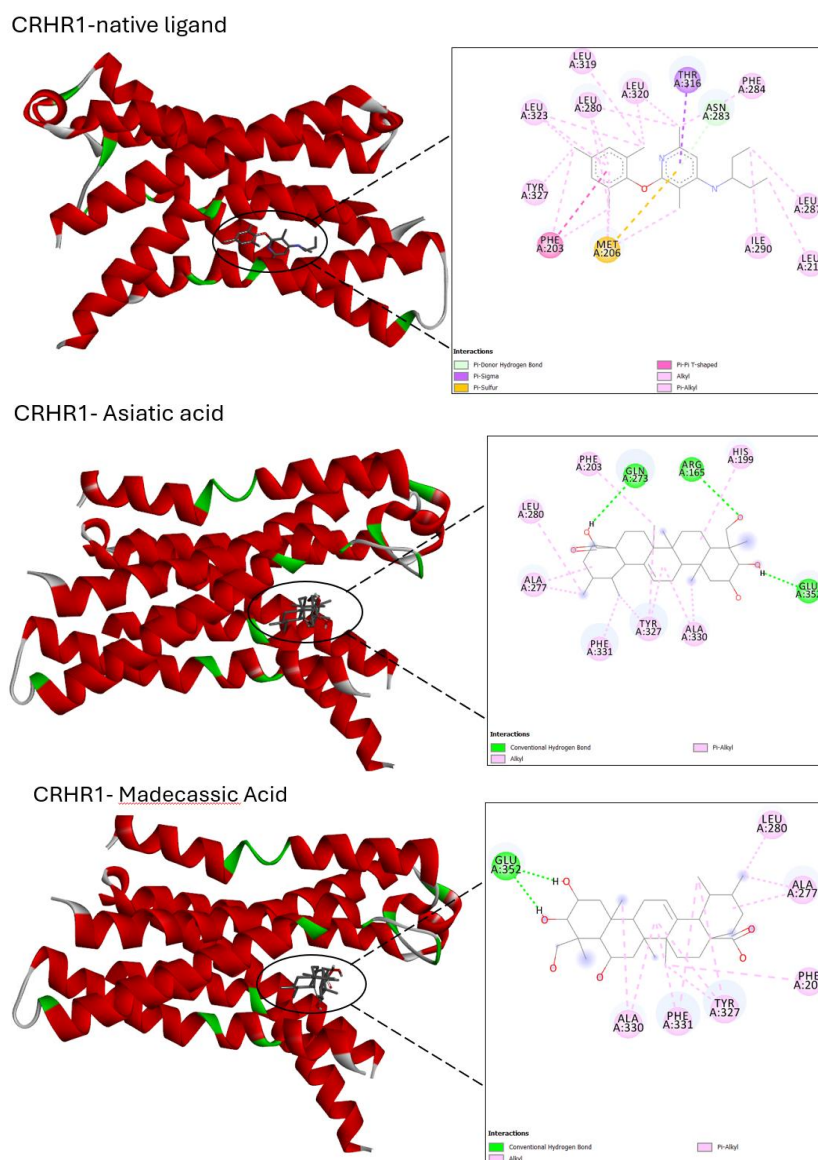


Picture 2. Interaction Network Of NR3C1 (GR) With Other Proteins In The STRING Database

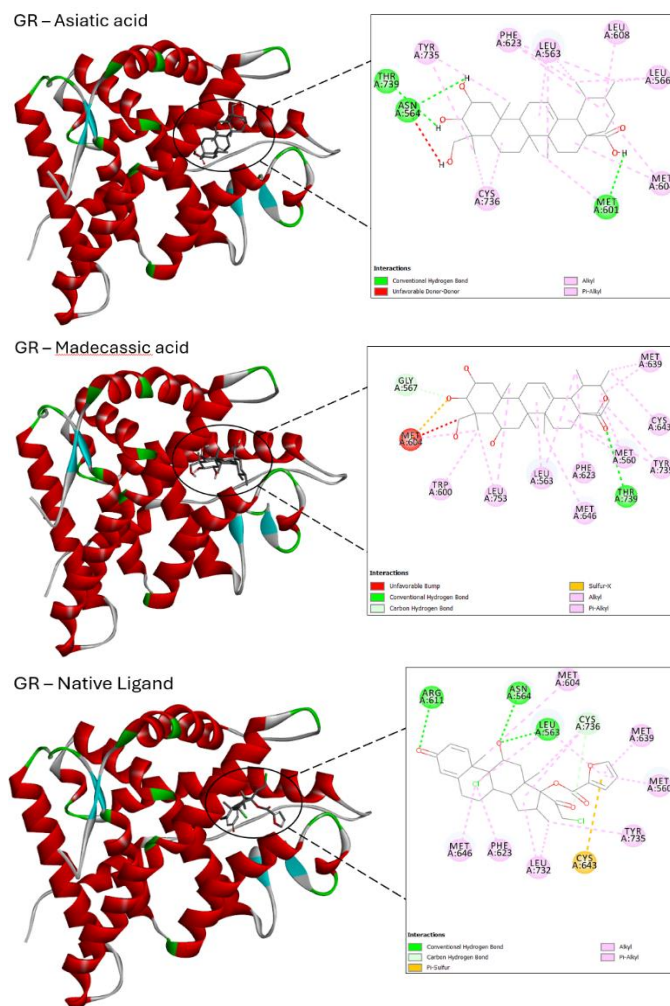
Molecular Docking of Potential Compounds with Protein

Molecular docking analysis of CRHR1 showed that the native ligand binds deeply within the transmembrane binding pocket with a binding affinity of -8.9 kcal/mol, forming key interactions with residues such as Asn283, Thr316, and multiple hydrophobic residues including Phe203, Leu280, and Leu323. This interaction pattern represents the optimal binding mode within the receptor. In comparison, both asiatic acid and madecassic acid exhibited moderate binding affinities of -7.7 kcal/mol and were also able to occupy the transmembrane region, although with slightly shifted binding orientations relative to the native ligand. Asiatic acid demonstrated a more extensive interaction network, forming hydrogen bonds with residues such as Arg165, Gln273, and Glu352, along with hydrophobic interactions involving Phe203, Tyr327, and Leu280, suggesting a relatively stable binding conformation. In contrast, madecassic acid formed fewer hydrogen bonds, primarily interacting with Glu352, and displayed a less extensive interaction profile. Notably, both compounds did not interact with Asn283, indicating a difference in binding mode compared to the native ligand. These findings suggest that while both asiatic acid and madecassic acid are capable of binding within the CRHR1 pocket, asiatic acid exhibits a more favorable interaction pattern, whereas madecassic acid shows a comparatively weaker and less optimal binding conformation.

Molecular docking analysis of the Glucocorticoid receptor demonstrated that the native ligand binds strongly within the ligand-binding domain, exhibiting a binding affinity of -12.9 kcal/mol and forming key interactions with important residues such as Asn564 and Arg611, along with extensive hydrophobic contacts involving Leu563, Met560, Met604, and Phe623. This interaction pattern represents the optimal binding mode within the receptor. In comparison, asiatic acid and madecassic acid showed significantly lower binding affinities of -4.5 kcal/mol and -2.2 kcal/mol, respectively. Asiatic acid was able to maintain interactions with key residues, including hydrogen bonds with Asn564 and Thr739, as well as hydrophobic interactions with Leu563, Phe623, and Tyr735, suggesting a moderate binding capability within the receptor. However, an unfavorable donor-donor interaction was observed, indicating minor instability in the binding conformation. In contrast, madecassic acid exhibited a weaker interaction profile, with limited hydrogen bonding primarily involving Thr739 and the presence of an unfavorable steric interaction with Met604, suggesting a less optimal binding conformation. Overall, although both compounds were able to occupy the ligand-binding domain, their interaction profiles were less favorable compared to the native ligand, with asiatic acid demonstrating a relatively better binding pattern than madecassic acid, indicating its potential to interact with the receptor in a weaker or modulatory manner.



Picture 3. Molecular Docking Results Of CRHR1



Picture 4. Molecular Docking Results Of GR

CONCLUSION

This study indicates that asiatic acid and madecassic acid from *Centella asiatica* have potential as multi-target antistress agents through their interactions with CRHR1 and the glucocorticoid receptor (GR). Both compounds exhibited moderate binding to CRHR1, with asiatic acid showing a more favorable interaction profile, while their binding to GR was weaker, suggesting a possible modulatory rather than strong agonistic effect. Overall, asiatic acid demonstrated better interaction characteristics than madecassic acid, indicating its higher potential to contribute to the regulation of the HPA axis, although further experimental validation is required.

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