OPEN ACCESS

Research Article

Computational Identification of GPCR-Targeting Bioactive Compounds from *Ageratum conyzoides* for Anti-Aging

William Yustian,[1] Marvel R. Suwitono✉,[1] Titin Sulastri, [2]

[1] Affiliation 1; Department of Pharmacy, Universitas Advent Indonesia, Bandung 40559, Indonesia
[2] Affiliation 2; Department of Biology, Universitas Advent Indonesia, Bandung 40559, Indonesia

Abstract

Aging is a complex biological process impacted by genetic, environmental, and lifestyle variables. Natural bioactive chemicals are becoming recognized as possible anti-aging agents, and *Ageratum conyzoides* is a medicinal herb rich in flavonoids, terpenoids, and sterols. In this study, bioactive compounds from *Ageratum conyzoides* that target GPCRs associated with aging mechanisms were investigated using computational methods. Using molecular docking, forty phytochemicals were evaluated against the beta-2 adrenergic, angiotensin AT1 receptor, and adenosine A2A receptors. Strong binding affinities were shown by several substances, including quercetin-3,7-diglucoside (-10.0 kcal/mol), friedelin (-9.0 kcal/mol), and stigmasterol (-10.3 kcal/mol). These results imply that prospective ligands for GPCR regulation in aging-related pathways are present in Ageratum conyzoides. To verify biological significance, additional validation using molecular dynamics and experimental testing is needed.

Keywords

Computational; Virtual Screening; Docking; Ageratum conyzoides; GPCRs; Anti-Aging.

Introduction

Recent advances demonstrate how the incorporation of novel approaches and cutting-edge technologies is increasingly influencing modern medicinal chemistry. The field now accepts computational tools, receptor-based drug design, and natural product exploration as complementary pathways rather than depending exclusively on conventional experimental methods. This change offers new prospects for the discovery of therapeutic agents with anti-aging potential and reflects a larger trend toward efficiency and precision in identifying bioactive compounds [1].

Virtual Screening has developed into an important method, a crucial approach in modern drug discovery, offering a cost-effective and time-efficient substitute to standard high-throughput techniques. By computationally filtering vast molecular libraries, VS enables researchers to identify compounds with the greatest likelihood of interacting with therapeutic targets, while simultaneously eliminating those with unfavorable properties. This strategy not only expedites the initial phases of drug development but also lessens dependence on extensive laboratory facilities, establishing it as a valuable instrument for driving pharmaceutical progress [2].

Scientists highlight that human aging is not an easy process but rather an intricate combination of genetic tendencies, environmental factors, and lifestyle selected, all of which steadily reduce physical abilities, weaken the body, and make the body more prone to sickness. A few biological factors play a key role in this decline, cell that cease dividing but cause inflammation, the destructive imbalance between free radicals and the antioxidant defenses in the body's, the gradual shortening of telomeres that reduces cell regeneration, and decreased ability of mitochondria to make energy. Notably, these processes are not independent, their connections linked to hormone activity, genetic makeup, and immune system activity are seen as vital for explaining how aging develops [3].

Oxidative stress is generally accepted as a central factor in aging. It occurs when production of reactive oxygen and nitrogen molecules exceeds the body's antioxidant defenses, causing gradual damage to proteins, lipids, and other cellular components [4], this imbalance accelerates the aging of cells and contributes to the age-related conditions, such as cardiovascular disease conditions, neurodegenerative disease, and tumors. Other studies emphasize that aging is defined by reduction in internal antioxidant defences and mitochondrial function, which increases risk to oxidative injury. Collectively, these insights show that aging is not only a timeline-based process but a biological progression influenced by oxida-

* Corresponding Author:
Marvel Reuben Suwitono, Department of Pharmacy. Universitas Advent Indonesia, Bandung 40559, Indonesia, rsuwitono@unai.edu
Tel.: +62818420735

tive stress. The gathering of oxidative injury weakens tissue strength, immune defences, and metabolic stability, eventually lowering resilience and making older adults raising vulnerability to chronic illness [5].

Antioxidants have an important role in neutralizing free radicals and sustaining redox equilibrium, thereby decreasing cell injury that speeds up functional decline. Several studies, such as Qarani et al. [6] and Meulmeester et al. [7] emphasize that increased antioxidant function is typically related to stronger resistance to degenerative changes and might contribute to delaying aging. Even though clinical trials have not regularly proven clear benefits, the wider finding indicate that antioxidants play role in lowering oxidative stress and may prove to be useful elements in anti-aging efforts.

Ageratum conyzoides L commonly known as Billy goat weed (Babadotan in Indonesia) is a tropical seasonal plant from the Asteraceae family. It is broadly spread throughout Asia, Africa, and the Americas, recognized by its straight scented stem, opposite oval saw-toothed leaves, and bunches of tiny blooms varying from white to purple [8].

The ethanol extract of *Ageratum conyzoides* has been found to contain several bioactive compounds such as flavonoids, alkaloids, tannins, and polyphenols, which jointly contribute to its antioxidant and anti-aging effects. Even though its capacity to counteract free radicals and block enzymes such as elastase and collagenase was less effective than pure quercetin, the extract still showed clear protective effects in protecting against skin aging, pointing to its potential as a natural source of anti-aging agents [9], [10].

G protein-coupled receptors (GPCRs) represent the biggest receptor family in the human genome, with around 800 unique members. In terms of structure, they are defined by seven transmembrane helices, allowing them to act as flexible molecular sensor [11]. These receptors can detect a wide variety of stimuli such as photons, lipids, amines, and complex proteins and transform them into intracellular signals that regulate almost all physiological processes. After being activated, these receptors connect to transducer proteins such as heterotrimeric G-proteins, GPCR kinases, and β-arrestins, thus starting multiple intracellular signaling pathways [12]. Due to their broad distribution and crucial functions, GPCRs have become major targets in current pharmacology, with therapeutic uses ranging from cardiovascular disease treatment to approaches targeting aging and cellular decline [13].

Adenosine A2A receptors (A2AARs) act as important regulators in skin fibroblast function, especially in directing extracellular matrix restructuring. When activated, A2AARs boost cyclic AMP signaling, which in turn triggers downstream kinases like ERK1/2 and p38 MAPK. These signaling pathways specifically boost the formation of collagen type I and type III, with a stronger tendency toward type III deposition. This selective regulation plays a vital role in tissue repair and scar development, where temporary collagen type III is eventually remodeled into mature collagen type I. In the context of aging, reduced fibroblast performance and decreased collagen formation cause dermal deterioration and wrinkle development. By opposing these pathways, A2AARs activation brings back structural integrity, flexibility, and skin moisture. Moreover, the receptor's contribution to connective tissue growth factor expression and (Wingless Protein) Wnt/β-catenin signal transduction highlights its complex role in sustaining skin homeostasis. Taken together, these mechanisms show why topical adenosine treatments directed at A2AARs can noticeably reduce wrinkles and enhance skin tone in people of middle age [14], [15].

Another receptor in the GPCR family is Angiotensin AT1. Recent research has shown that inhibition of the angiotensin type I (AT1) receptor not only exerts activity in blood pressure control and organ protection, but also has the potential to slow the aging process. Deletion of the angiotensin AT1 receptor in mice can extend lifespan by up to 28% compared to the control group. This response is linked to enhanced cardiovascular integrity, lower generation of reactive oxygen species (ROS), diminished mitochondrial degradation, and elevated expression of pro-survival genes such as Nampt and Sirtuin-3. In contrast, exposure to angiotensin II in vitro results in reduced levels of both proteins. These findings indicate that activation of the angiotensin AT1 receptor contributes to aging through increased oxidative stress and damage to mitochondria, while AT1 blockers have the potential to extend lifespan through cellular protective mechanisms [16], [17], [18].

The β2-adrenergic receptor (β2AR) serves an essential function in regulating physiological activities across multiple organs, which experience alterations during the aging process. Decreased responsiveness to catecholamines, including β2AR-mediated vascular relaxation, has been observed in elderly individuals. This results in decreased vasodilation and increased vasoconstriction, contributing to increased total peripheral resistance. Since β2AR is present on both vascular smooth muscle cells (VSMCs) and endothelial cells (ECs), the reduction in receptor activity has a direct effect on vascular responsiveness, which progressively diminishes with aging. Furthermore, decreased β2AR activity and the associated cAMP production are common factors underlying various aging-related conditions, such as hypertension, atherosclerosis, vascular insufficiency, and orthostatic hypotension. cAMP itself is known to inhibit VSMC proliferation, so βAR-mediated cAMP reduction may accelerate the occurrence of restenosis and atherosclerosis in the elderly. These observations indicate that β2AR is involved not only in the biological mechanisms of aging but also car-

ries significant clinical relevance for the selection and optimization of pharmacological treatments, particularly regarding the administration of βAR agonists and antagonists in older individuals [19], [20], [21].

This study aims to investigate the anti-aging potential of bioactive compounds derived from *Ageratum conyzoides* through virtual screening approaches. By using these computational strategies, the research attempts to pinpoint to promising molecular candidates that can interact with GPCR receptors, thus providing therapeutic opportunities for reducing age-related changes.

Materials and methods

System Configuration

The software for performing Molecular Docking used in this study is PyRx 0.8 from The Scripps Research Institute which is directly integrated with AutoDock Vina as a docking simulation tool [22] and Open Babel as a tool for preparing ligands imported from the SDF (Structure Data File) ligand file format, minimizing the energy of chemical compounds, and converting the format to pdbqt.

This docking process predicts the binding affinity of the active compounds contained in *Ageratum conyzoides* with the target protein and is visualized using BIOVIA Discovery Studio 2020 Visualizer software to see the interaction between ligands and receptors structurally [23]. All research processes were carried out using a personal computer (PC) with an Intel Core i3-10105F Processor (10th Gen – Comet Lake), NVIDIA GeForce RTX 2060 6GB GDDR6 Graphics Card, and 16GB RAM.

Ligand Preparation

The ligand profiles of *Ageratum conyzoides* were retrieved from the Dr. Duke's Phytochemical and Ethnobotanical Databases maintained by the U.S. Department of Agriculture (https://phytochem.nal.usda.gov/) [24]. From the 84 bioactive constituents identified in *Ageratum conyzoides*, a total of 40 compounds were selected as listed in Table S1. The selection was based on the exclusion of non-relevant molecules such as fats, duplicate or structurally similar compounds, and those lacking complete structural information. These chosen molecules were subsequently retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in both 3D Conformer and SDF file formats [25].

Table 1. Classification of Bioactive Compounds

Category	Compound Name	Chemical Formula	CID	Benefits
Flavonoids	Kaempferol	$C_{15}H_{10}O_6$	5280863	Neutralize free radicals, reduce inflammation, and protect neurons
	Kaempferol-3,7-diglucoside	$C_{27}H_{30}O_{16}$	6325460	
	Quercetin	$C_{15}H_{10}O_7$	5280343	
	Quercetin-3,7-diglucoside	$C_{27}H_{30}O_{17}$	10121947	
Phenols	Eugenol	$C_{10}H_{12}O_2$	3314	Natural antioxidants
Terpenes	Bornyl Acetate	$C_{12}H_{20}O_2$	93009	Anti-inflammatory often linked to stress reduction and immune balance.
	Humulene	$C_{15}H_{24}$	5281520	
	Alpha-Cubebene	$C_{15}H_{24}$	442359	
	Alpha-Humulene	$C_{15}H_{24}$	24798693	
	Alpha-Muurolene	$C_{15}H_{24}$	12306047	
	Alpha-Pinene	$C_{10}H_{16}$	6654	
	Beta-Cubebene	$C_{15}H_{24}$	93081	
	Beta-Elemene	$C_{15}H_{24}$	6918391	
	Beta-Farnesene	$C_{15}H_{24}$	5281517	
	Beta-Myrcene	$C_{10}H_{16}$	31253	
	Beta-Pinene	$C_{10}H_{16}$	14896	
	Camphene	$C_{10}H_{16}$	6616	
	Terpinolene	$C_{10}H_{16}$	11463	
	Beta-Cadinene	$C_{15}H_{24}$	10657	
	Beta-Selinene	$C_{15}H_{24}$	442393	
	Germacrene-D	$C_{15}H_{24}$	5317570	
	Cadinol	$C_{15}H_{26}O$	6428423	
	Gamma-Cadinene	$C_{15}H_{24}$	6432404	
Heteroorganics	Demethoxyencecalin	$C_{13}H_{14}O_2$	177040	

	Caryophyllene-Oxide	$C_{15}H_{24}O$	1742210	Modulate enzymes, act as antioxidants and support vascular function
	Coumarin	$C_9H_6O_2$	323	
Olefin	Alpha-Bergamotene	$C_{15}H_{24}$	86608	Provide antioxidant and anti-inflammatory effects
	Alpha-Terpinene	$C_{10}H_{16}$	7462	
	Limonene	$C_{10}H_{16}$	22311	
Terpenoids	2-Carene	$C_{10}H_{16}$	79044	Immune defense, promote tissue repair, and act as antioxidants
	Borneol	$C_{10}H_{18}O$	64685	
	Friedelin	$C_{30}H_{50}O$	91472	
Alcohols	Farnesol	$C_{15}H_{26}O$	445070	Stabilize membranes, fight microbes, and provide calming effects, supporting resilience against age-related stress.
	Linalool	$C_{10}H_{18}O$	6549	
	Beta-Sitosterol	$C_{29}H_{50}O$	222284	
	Stigmasterol	$C_{29}H_{48}O$	5280794	
Lipid	Linoleic Acid	$C_{18}H_{32}O_2$	5280450	Essential for energy, reduce inflammation, and maintain skin integrity vital in anti-aging
	Oleic Acid	$C_{18}H_{34}O_2$	445639	
	Palmitic Acid	$C_{16}H_{32}O_2$	985	
Propene Phenyl	Ethyl-Eugenol	$C_{12}H_{16}O_2$	6429690	Retains antioxidant

The active compounds are categorized into 9 groups and accompanied by their respective CIDs, as shown in Table 1. These categories include Flavonoids, Phenols, Terpenes, Heteroorganics, Olefins, Terpenoids, Alcohols, Lipids, and Phenyl Propenes.

Each ligand structure's energy was minimized using Open Babel, integrated directly with PyRx 0.8, with Universal Force Field (UFF) parameters [26]. This computational approach estimates the energy of a molecular system by accounting for multiple types of interactions, including bond stretching, angle deformation, torsional (dihedral) rotations, as well as non-covalent forces such as Van der Waals and electrostatic interactions. The algorithm optimization process was set using Conjunct Gradients, with a Total Step count of 1,000 [27]. This step is crucial to ensure that the ligand is in a stable conformation before docking. The energy minimization process contributes to the accuracy of subsequent docking simulation results.

Receptors Preparation

The receptors used in this study were three receptors from the GPCR family that have anti-aging activity. Each receptor was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/). Unnecessary molecules or compounds are removed from each receptor using BIOVIA Discovery Studio 2020 Visualizer.

Ligands and receptors were loaded into PyRx to perform molecular docking simulations using the AutoDock Vina algorithm. To enhance both computational efficiency and docking accuracy, the grid box parameters (including size and dimensions) and the exhaustiveness value (set to 8) were carefully adjusted. The grid box defines the three-dimensional region surrounding the target protein where ligand docking is permitted and calculations are executed. Each GPCR protein utilized in the study was assigned a distinct grid box configuration, as summarized in Table 2.

Table 2. Grid Box Parameters

Receptors Name	PDB ID	Center			Dimensions (Angstrom)		
		X	Y	Z	X	Y	Z
Adenosine A2A	5G53	-28.41	1.94	16.53	138.98	124.28	78.37
β2-Adrenergic	6KR8	-4.36	2.26	-2.91	54.22	49.65	75.53
Angiotensin AT1	7F6G	124.40	104.83	152.08	91.38	88.80	115.34

Results

Docking Analysis

Table 3 shows the docking results of 10 active compounds from *Ageratum conyzoides* that exhibit the moderated binding affinity values (-7.0 kcal/mol and above) with GPCR receptors. A binding affinity of -7.0 kcal/mol or more negative is thought to be in the level of strong

precision [28].The complete docking output files are provided in Supplementary S1. Binding energy measured in kcal/mol, show how thermodynamically favorable a molecular interaction is. In molecular docking such as computational experiments using PyRx - Virtual Screening Tool, binding affinity is commonly applied to describe these interactions.

Table 3. Top 10 Docking Result (Binding Affinity kcal/mol) of *Ageratum conyzoides* bioactive with GPCRs Target

Rank	Adenosine A2A (5G53)	β2-Adrenergic Reseptor (6KR8)	Angiotensin AT1 (7F6G)
1	Stigmasterol (-10.3)	Friedelin (-9.0)	Quercetin-3,7-diglucoside (-10.0)
2	Quercetin (-9.2)	Stigmasterol (-8.6)	Kaempferol-3,7-diglucoside (-9.7)
3	Friedelin (-8.8)	Quercetin (-8.2)	Stigmasterol (-9.5)
4	Quercetin-3,7-diglucoside (-8.6)	Kaempferol (-8.1)	Quercetin (-9.2)
5	Gamma-cadinene (-8.5)	Beta-sitosterol (-8.1)	Kaempferol (-9.0)
6	Kaempferol-3,7-diglucoside (-8.4)	Cadinol (-8.0)	Beta-sitosterol (-8.7)
7	Beta-sitosterol (-7.7)	Quercetin-3,7-diglucoside (-7.8)	Friedelin (-8.0)
8	Beta-cadinene (-7.6)	Demethoxyencecalin (-7.6)	Germacrene-D (-7.8)
9	Limonene (-7.0)	Beta-cadinene (-7.5)	Beta-cubebene (-7.7)
10	Germacrene-D (-7.0)	Beta-farnesene (-7.4)	Alpha-cubebene (-7.7)

Binding affinity is crucial in determining which bioactive compound binds effectively to GPCR target proteins. It shows the strength of the interaction (bond) between the ligand and protein. The docking result indicated varying of binding affinities among three receptor, Adenosine A2A (5G53), β2-adrenergic Receptor (6KR8), and Angiotensin AT1 receptor (7F6G) which may suggest their potential involvement in aging-related signalling pathways [29].

From the evaluated compounds, stigmasterol demonstrated the strongest binding affinity for Adenosine A2A receptor (-10.3 kcal/mol), friedelin showed binding strength with β2AR (-9.0 kcal/mol), and quercetin-3,7-diglucoside showed binding strength with Angiotensin AT1 (-10.0 kcal/mol). These strong binding interactions indicate that each compound may deliver selective influence based on the receptor target. Interestingly, stigmasterol and quercetin showed consistent presence in all three receptors with relatively high binding scores, highlighting their potential as multi-target ligands with the ability to modulate various GPCR-linked pathways [30].

GPCRs play a central role in modulating inflammatory activity, oxidative stress, vascular tone, and cell equilibrium all of which are related to aging mechanisms [31], [32]. The potential of these bioactive compounds to bind with such receptors indicates a promising path make for regulating age-related dysfunctions [32], [33], [34], [35]. As an illustration, flavonoids such as quercetin and kaempferol-3,7-diglucoside are noted for their antioxidant and anti-inflammatory agents [36], [37], which could support their receptor-binding mechanisms and improve their pharmacological relevance in anti-aging approaches.

The differences in binding affinities also indicate the unique molecular characteristics of each compound, determined by factors such as polarity, molecular dimensions, and the potential to create hydrogen bonding or hydrophobic effects. These structural properties are essential for defining how well a compound can bind to receptor sites and induce downstream biological processes [30], [38].

Taken Together, the result presents a molecular justification for recognizing *Ageratum conyzoides* may as a source of phytochemicals with anti-aging potential [9], [39]. Importantly, additional validation through molecular dynamics simulations, in vitro experiments, and pharmacokinetic studies is necessary to establish the biological function, safety, and therapeutic potential of these candidates [40], [41], [42].

Analysis Ligand-Protein Interactions

The ligand-protein interaction analysis was executed to explain how bioactive compounds from *Ageratum conyzoides* bind to GPCRs that play crucial roles in aging-related pathways [9], [39], [43]. Docking studies showed that certain phytochemicals like stigmasterol, quercetin, friedelin, and kaempferol-3,7-diglucoside showed strong binding affinities with Adenosine A2A, β2-Adrenergic, and Angiotensin AT1 receptors [38], [44], [45]. These molecular interactions are defined not just by binding energy values but also by the properties of the noncovalent bonds engaged such as hydrogen bonding, hydrophobic interactions, van der waals interactions, and π–π stacking [46], [47].

In the case of the Adenosine A2A receptor, stigmasterol and quercetin showed interactions with essential residues such as MET270, TYR271, and LEU267, which are necessary for supporting the ligand-receptor complex [48], [49]. Regarding the β2AR, friedelin and stigmasterol created hydrophobic interactions with residues such as LEU155, VAL317, and TRP158, stabilizing the binding by means of alkyl and π-alkyl interactions [44], [50]. For the Angiotensin AT1 receptor, quercetin-3,7-diglucoside and kaempferol-3,7-diglucoside created classical hydrogen bonds interacting with residues such as SER147, THR102, and ARG150, thus improving the stability of the complex and pointing the potential biological value [51], [52], [53].

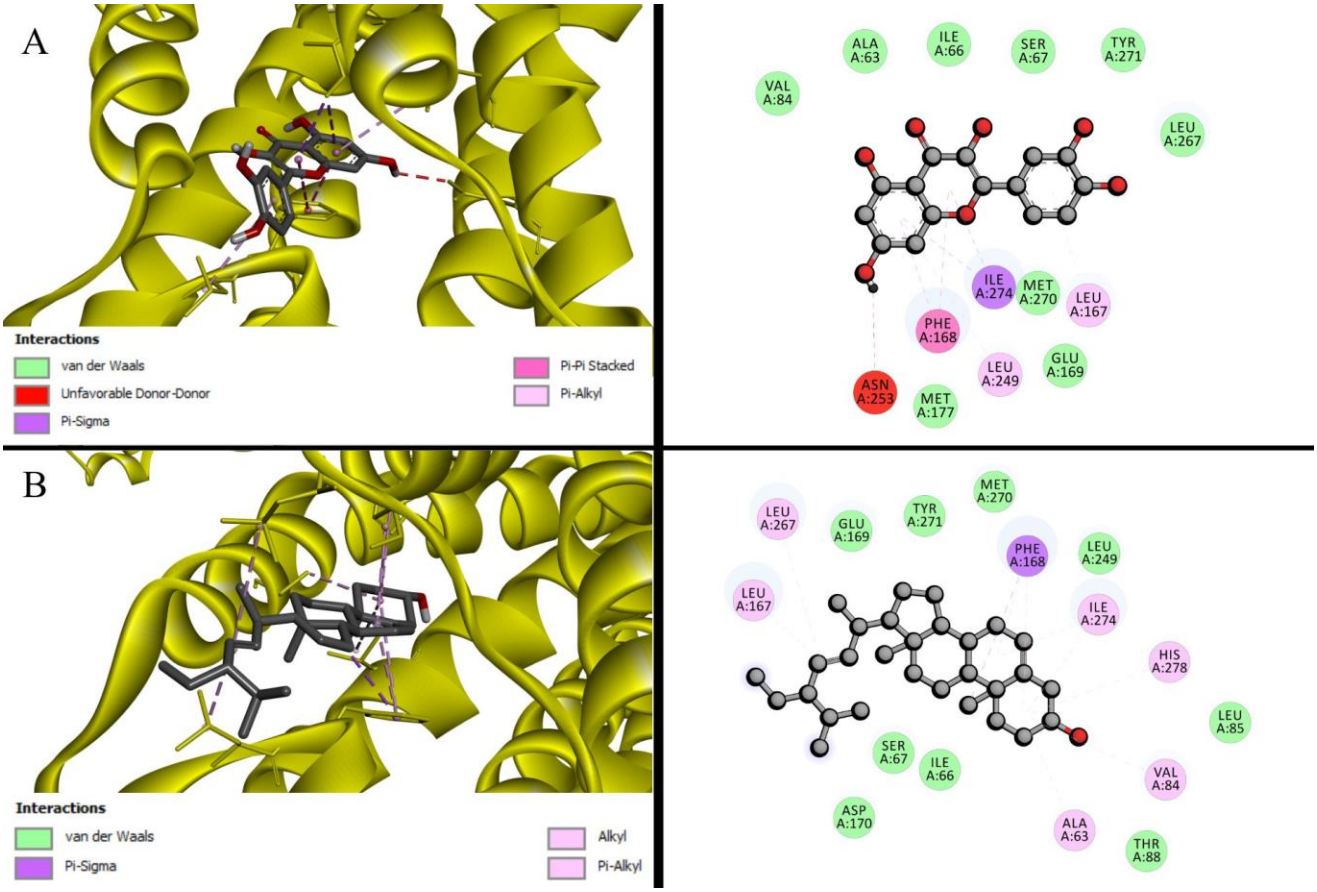

Figure 1. Adenosine A2A (5G53) Interactions. (A) Quercetin, (B) Stigmasterol.

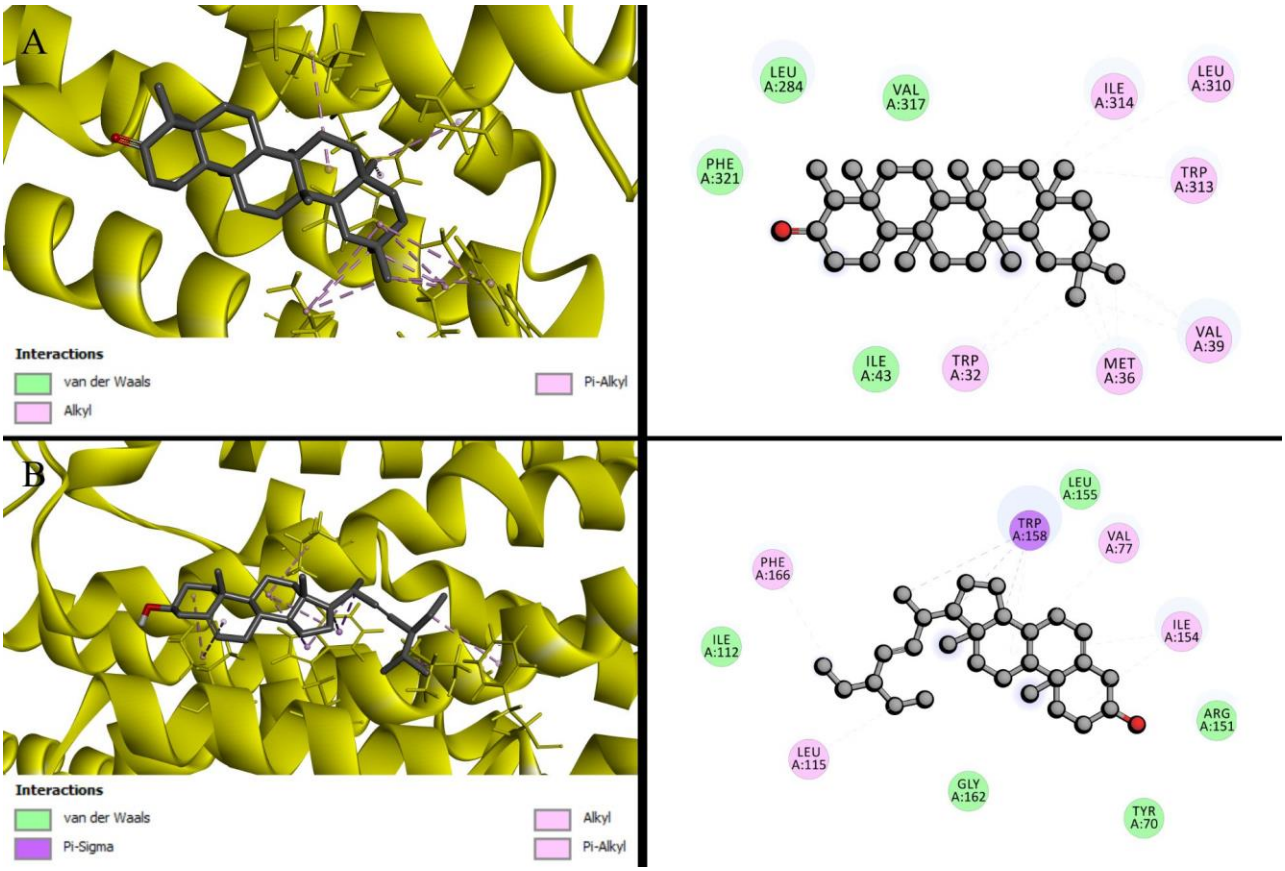

Figure 2. β2-Adrenergic Receptor (6KR8) Interactions. (A) Friedelin, (B) Stigmasterol.

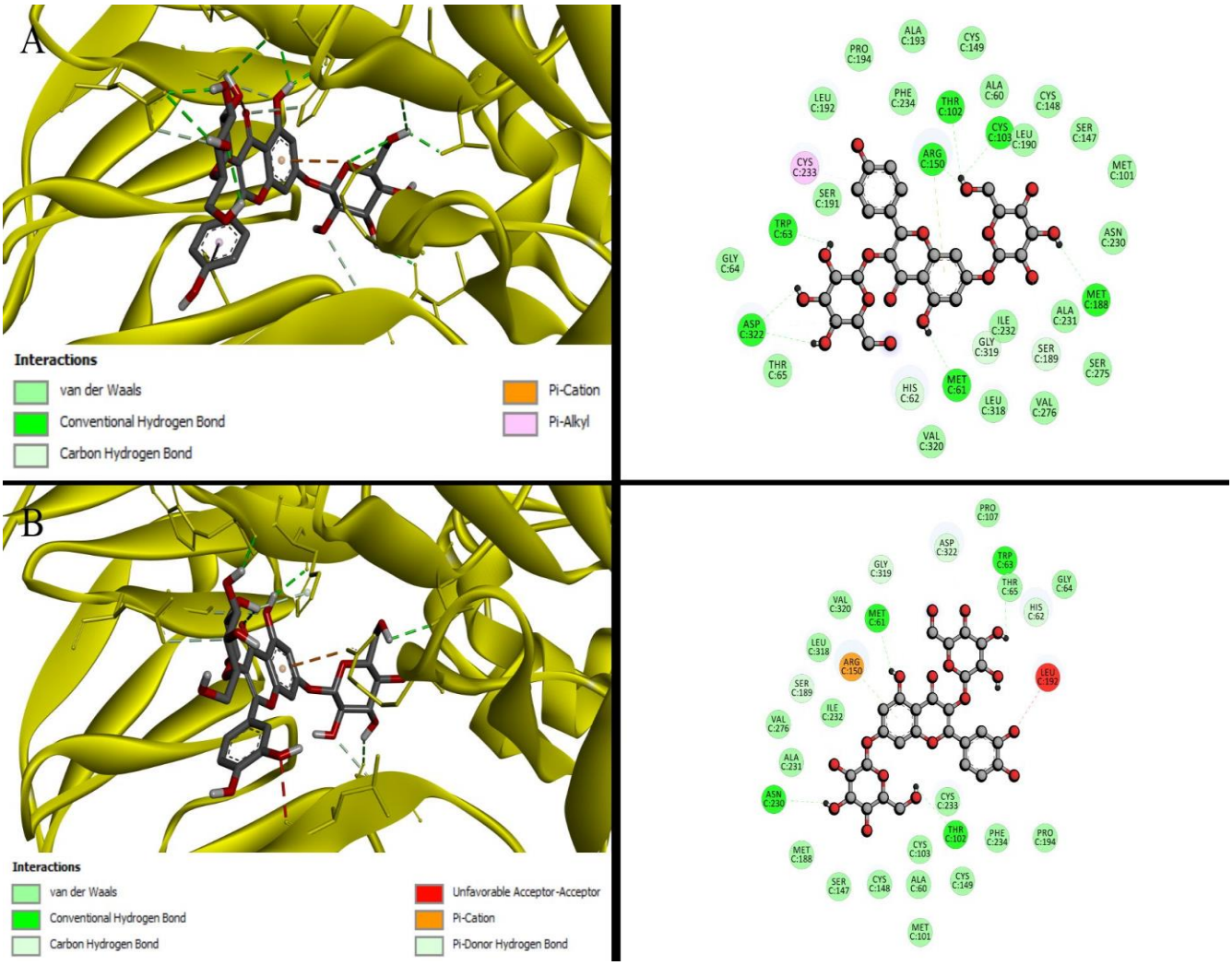

Figure 3. Angiotensin AT1 – 7F6G Interactions. (A) Kaempferol-3,7-diglucoside, (B) Quercetin-3,7-diglucoside.

The interaction profiles presented in Figures 1–3 provide preliminary molecular perspectives suggesting that these phytochemicals may potentially exert anti-aging effects through GPCR-mediated pathways, although further validation is required.

Stigmasterol and quercetin showed significant hydrophobic and π–π bonding with residues such as TYR271 and PHE168 [48], [49], which are positioned within the transmembrane binding pocket. These interactions are may important because A2A receptor activation is connected to collagen formation and dermal fibroblast modulation [54], [55]. By stabilizing these residues, stigmasterol may improve receptor signaling, potentially recovering extracellular matrix integrity, and counteracting age-related skin degeneration. The hydrogen bond of quercetin with GLU169 additionally points to potential to regulate receptor responses through oxidative stress responses pathways, matching its established antioxidant activity [56].

Friedelin and stigmasterol engaged in significant hydrophobic interactions with residues such as LEU155, VAL317, and TRP158. These residues may crucial for β2AR activity in vascular relaxation and cAMP pathway regulation [57]. The preservation of hydrophobic pockets by sterols may reduce the age-relate reduction in β2AR responsiveness, thus strengthening vascular tone and decreasing peripheral resistance in older adults. Furthermore, π-alkyl bonding with aromatic residues such as PHE166 and TRP313 imply that these ligands may stabilize receptor structures essential for efficient catecholamine binding, emphasizing their potential in cardiovascular aging therapies [58].

Quercetin-3,7-diglucoside and kaempferol-3,7-diglucoside engaged in multiple hydrogen bonds with amino acid residues such as SER147, THR102, and ARG150, coupled with π-cation bonding with aromatic residues. These

interactions may highly significant as AT1 receptor activation is closely linked to oxidative stress and mitochondrial dysfunctions [59]. Here, establishing stabilizing hydrogen bonds, flavonoids glycosides may behave as competitive inhibitors, reducing angiotensin II signaling and consequently decreasing ROS production. This observation matches with experimental findings that AT1 blockade enhances lifespan in animal models, indicating that these natural ligands may emulate pharmacological AT1 antagonists [60], [61], [62].

In summary, the ligand-protein interaction analysis emphasizes distinct yet synergistic mechanisms by which *Ageratum conyzoides* phytochemical may control GPCR responses. Sterols like stigmasterol and friedelin mainly stabilize hydrophobic pockets, maintaining receptor configurations connected to cutaneous and vascular regulation. On the other hand, flavonoids such as quercetin and kaempferol derivatives take part in hydrogen bonding and π-cation interactions, conferring antioxidant and anti-inflammatory properties while reducing pro-aging receptor signalling.

This combined mode of action, hydrophobic domain stabilization by sterols and hydrogen-bond regulation by flavonoids indicates a cooperative potential for multi-target anti-aging therapies [63], [64]. The findings yield a molecular explanation for identifying *Ageratum conyzoides* as potential source of GPCR-targeting compounds for anti-aging agents. Upcoming research should include a molecular dynamics simulations and analyses to confirm binding stability, together with in vitro and in vivo studies to validate biological activity and pharmacokinetic applicability.

Discussion

This project was conceived as an experimental investigation concentrating on the virtual screening and visualization of ligand–receptor interactions. We recognize that the docking approach was not confirmed using redocking or RMSD analysis, which constitutes a limitation of the present study, especially for GPCR targets displaying many conformational states. The absence of such validation means that the docking scores given here should be regarded cautiously, since they provide only preliminary signals of putative binding interactions rather than clear evidence of biological control or potential anti-aging implications. In addition, ADMET prediction was not performed in this investigation, which further constitutes a limitation in terms of biological relevance and translational applicability. Accordingly, the data offered in this publication are aimed to serve as a starting point for hypothesis formulation, and future research will add docking validation, ADMET prediction, and experimental assays to strengthen the reliability and biological importance of the results.

Conclusion

This study emphasizes the anti-aging potential of bioactive compounds from *Ageratum conyzoides* using in silico docking approaches (computational) with key GPCR targets such as Adenosine A2A, β2-Adrenergic, and Angiotensin AT1 receptors. Stigmasterol, friedelin, and quercetin-3,7-diglucoside identified as the most active ligands showing predicted binding affinities, may suggest potential involvement in pathways and associated with collagen production, vascular relaxation, and oxidative stress regulation. The sustained effects of flavonoids across various receptors highlights their possible role as multi-target agents in managing the complex nature of aging. These findings provide molecular data supporting *Ageratum conyzoides* as a potential natural source for GPCR-targeting compounds and emphasize the relevance of virtual screening as a low-cost strategy in early drug discovery. However, because the result is restricted to computational predictions, additional validation using molecular dynamics, in vitro and in vivo experiments and pharmacokinetic analyses is necessary to confirm biological efficacy, safety, and therapeutic practically. In summary, this research contributes to the expanding research on natural agents in anti-aging pharmacology and provides novel viewpoints for advancing multi-target strategies.

List of abbreviations

AMP Adenosine Monophosphate
ADMET Absorption, Distribution, Metabolism, Excretion, and Toxicity.
AT1 Angiotensin Receptor 1
β2AR Beta-Adrenergic Receptor 2
ARG Arginine
cAMP Cyclic Adenosine Monophosphate
CIDs Compound Identifiers
DNA Deoxyribonucleic Acid
ECs Endothelial Cells
ERK Extracellular Signal-Regulated Kinase
GLU Glutamate
GPCRs G Protein-Coupled Receptors
LEU Leucine
MAPK Mitogen-Activated Protein Kinase
MET Methionine
PDB Protein Data Bank
PHE Phenylalanine
ROS Reactive Oxygen Species
SDF Structure Data File
SER Serine
THR Threonine
TRP Tryptophan
TYR Tyrosine
VAL Valine
VS Virtual Screening

VSMCs Vascular Smooth Muscle Cells

Author Contributions

W.Y.: Conceptualization, methodology, software, investigation, data curation, formal analysis, visualization, writing – original draft. M.R.S., T.S.: Supervision, critical review, and manuscript refinement. All authors have read and agreed to the published version of the manuscript.

ORCID:

Marvel Reuben Suwitono: 0000-0002-0466-4774
Titin Sulastri: 0000-0002-7471-8563
William Yustian: 0009-0008-7617-3449

Availability of Data and Materials

The datasets underpinning the findings of this research can be accessed upon reasonable request to the corresponding author.

Consent for Publication

No publication consent was necessary since the manuscript contains no individual-related data, figures, or multimedia materials.

Supplementary Materials

The following supporting information can be downloaded in this article. Figure S1: Grid Box Parameters (Vina Search Space). Table S1: Binding Affinity Scores (40 Ligand). Supplementary S1: Ligand & Receptor File (Raws and docking result).

Consent for Publication

No publication consent was necessary since the manuscript contains no individual-related data, figures, or multimedia materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Funding

No external funding was received for this research.

Acknowledgments

Declared none.

AI Declaration

The authors acknowledge the use of Microsoft Copilot (GPT-5) as a supportive tool for language refinement and editorial assistance. The scientific content, interpretation, and conclusions remain solely the responsibility of the authors.

References

[1] M. R. Suwitono and T. Sulastri, *Revolusi Kimia Medisinal: Strategi Cerdas dan Teknologi Terkini*. Jakarta: Bukuloka Literasi Bangsa, 2025.

[2] T. Oliveira, M. Silva, E. Maia, A. Silva, and A. Taranto, "Virtual Screening Algorithms in Drug Discovery: A Review Focused on Machine and Deep Learning Methods," *DDC*, vol. 2, no. 2, pp. 311–334, May 2023, doi: 10.3390/ddc2020017. [CrossRef]

[3] M. R. Suwitono, I. A. I. Wahyuniari, H. Luo, M. C. Suwitono, and T. Sulastri, "Identification of Sirtuin-Activating Bioactive from Taraxacum Officinale Through Virtual Discoveries for Anti-Aging and Stress Resistance Applications," *J. Fac. Pharm. Ankara*, vol. 49, no. 3, pp. 790–808, 2025, doi: https://doi.org/10.33483/jfpau.1588979. [CrossRef]

[4] I. Liguori *et al.*, "Oxidative stress, aging, and diseases," *CIA*, vol. Volume 13, pp. 757–772, Apr. 2018, doi: 10.2147/CIA.S158513. [CrossRef] [PubMed]

[5] R. Militello, S. Luti, T. Gamberi, A. Pellegrino, A. Modesti, and P. A. Modesti, "Physical Activity and Oxidative Stress in Aging," *Antioxidants*, vol. 13, no. 5, p. 557, May 2024, doi: 10.3390/antiox13050557. [CrossRef] [PubMed]

[6] W. Qarani *et al.*, "Antioxidant and antiaging activity of Cinnamomum burmannii and Michelia champaca extract and combinations," *Narra J*, vol. 3, no. 2, p. e111, Aug. 2023, doi: 10.52225/narra.v3i2.111. [CrossRef] [PubMed]

[7] F. L. Meulmeester, J. Luo, L. G. Martens, K. Mills, D. Van Heemst, and R. Noordam, "Antioxidant Supplementation in Oxidative Stress-Related Diseases: What Have We Learned from Studies on Alpha-Tocopherol?," *Antioxidants*, vol. 11, no. 12, p. 2322, Nov. 2022, doi: 10.3390/antiox11122322. [CrossRef] [PubMed]

[8] R. Chahal *et al.*, "*Ageratum conyzoides* L. and Its Secondary Metabolites in the Management of Different Fungal Pathogens," *Molecules*, vol. 26, no. 10, p. 2933, May 2021, doi: 10.3390/molecules26102933. [CrossRef] [PubMed]

[9] A. B. Sutjiatmo *et al.*, "Antioxidant and Antiaging Assays of *Ageratum conyzoides* (L.) Ethanolic Extract," *psr*, vol. 7, no. 3, pp. 145–152, Dec. 2020, doi:

10.7454/psr.v7i3.1061. [CrossRef]
[10] A. T. Adeoye, S. A. Koleosho, O. J. Okuneye, E. O. Ogbaji, and O. C. Nwufoh, "Phytochemical, Proximate and In-Vitro Antioxidant Analyses of *Ageratum conyzoides* Leaf," *ojas*, vol. 4, no. 2, pp. 17–28, Dec. 2023, doi: 10.52417/ojas.v4i2.517. [CrossRef]
[11] C. S. Odoemelam *et al.*, "G-Protein coupled receptors: structure and function in drug discovery," *RSC Adv.*, vol. 10, no. 60, pp. 36337–36348, 2020, doi: 10.1039/D0RA08003A. [CrossRef]
[12] D. Hilger, M. Masureel, and B. K. Kobilka, "Structure and dynamics of GPCR signaling complexes," *Nat Struct Mol Biol*, vol. 25, no. 1, pp. 4–12, Jan. 2018, doi: 10.1038/s41594-017-0011-7. [CrossRef] [PubMed]
[13] A. Grogan, E. Y. Lucero, H. Jiang, and H. A. Rockman, "Pathophysiology and pharmacology of G protein-coupled receptors in the heart," *Cardiovascular Research*, vol. 119, no. 5, pp. 1117–1129, May 2023, doi: 10.1093/cvr/cvac171. [CrossRef] [PubMed]
[14] G. Marucci, M. Buccioni, V. Varlaro, R. Volpini, and F. Amenta, "The possible role of the nucleoside adenosine in countering skin aging: A review," *BioFactors*, vol. 48, no. 5, pp. 1027–1035, Sep. 2022, doi: 10.1002/biof.1881. [CrossRef] [PubMed]
[15] G. Shaikh, J. Zhang, M. Perez-Aso, A. Mediero, and B. Cronstein, "Adenosine A_{2A} receptor promotes collagen type III synthesis via β-catenin activation in human dermal fibroblasts," *British J Pharmacology*, vol. 173, no. 23, pp. 3279–3291, Dec. 2017, doi: 10.1111/bph.13615. [CrossRef] [PubMed]
[16] J. K. Ho, "Memory is preserved in older adults taking AT1 receptor blockers," *Alz Res Therapy*, vol. 9, no. 1, p. 33, Dec. 2017, doi: 10.1186/s13195-017-0255-9. [CrossRef] [PubMed]
[17] J. Tchekalarova, P. Ivanova, and D. Krushovlieva, "Age-Related Effects of AT1 Receptor Antagonist Losartan on Cognitive Decline in Spontaneously Hypertensive Rats," *IJMS*, vol. 25, no. 13, p. 7340, Jul. 2024, doi: 10.3390/ijms25137340. [CrossRef] [PubMed]
[18] A. Ramalingam, S. B. Budin, N. Mohd. Fauzi, R. H. Ritchie, and S. Zainalabidin, "Angiotensin II Type I Receptor Antagonism Attenuates Nicotine-Induced Cardiac Remodeling, Dysfunction, and Aggravation of Myocardial Ischemia-Reperfusion Injury in Rats," *Front. Pharmacol.*, vol. 10, p. 1493, Dec. 2019, doi: 10.3389/fphar.2019.01493. [CrossRef] [PubMed]
[19] N. Akhiyat *et al.*, "Endothelial dysfunction as a feature of vascular aging," *European Journal of Preventive Cardiology*, 2025, doi: https://doi.org/10.1093/eurjpc/zwaf544. [CrossRef] [PubMed]
[20] W. Parichatikanond, R. Duangrat, H. Kurose, and S. Mangmool, "Regulation of β-Adrenergic Receptors in the Heart: A Review on Emerging Therapeutic Strategies for Heart Failure," *Cells*, vol. 13, no. 20, p. 1674, Oct. 2024, doi: 10.3390/cells13201674. [CrossRef] [PubMed]
[21] C. D. Lucia, A. Eguchi, and W. J. Koch, "New Insights in Cardiac β-Adrenergic Signaling During Heart Failure and Aging," *Front. Pharmacol.*, vol. 9, p. 904, Aug. 2018, doi: 10.3389/fphar.2018.00904. [CrossRef] [PubMed]
[22] O. Trott and A. J. Olson, "AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *J Comput Chem*, vol. 31, no. 2, pp. 455–461, Jan. 2010, doi: 10.1002/jcc.21334. [CrossRef] [PubMed]
[23] BIOVIA, Dassault Systèmes, *Discovery Studio Visualizer*. (2020). San Diego.
[24] J. A. Duke, "Dr. Duke's Phytochemical and Ethnobotanical Databases. Ag Data Commons."
[25] S. Kim *et al.*, "PubChem 2025 update," *Nucleic Acids Research*, vol. 53, no. D1, pp. D1516–D1525, Jan. 2025, doi: 10.1093/nar/gkae1059. [CrossRef] [PubMed]
[26] A. K. Rappe, C. J. Casewit, K. S. Colwell, W. A. Goddard, and W. M. Skiff, "UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations," *J. Am. Chem. Soc.*, vol. 114, no. 25, pp. 10024–10035, Dec. 1992, doi: 10.1021/ja00051a040. [CrossRef]
[27] M. R. Hestenes and E. Stiefel, "Methods of conjugate gradients for solving linear systems," vol. 49, no. 6, 1952, doi: https://doi.org/10.6028/jres.049.044. [CrossRef]
[28] Y. Khalak *et al.*, "Alchemical absolute protein–ligand binding free energies for drug design," *Chem. Sci.*, vol. 12, no. 41, pp. 13958–13971, 2021, doi: 10.1039/D1SC03472C. [CrossRef]
[29] J. Kim and C. Choi, "Orphan GPCRs in Neurodegenerative Disorders: Integrating Structural Biology and Drug Discovery Approaches," *CIMB*, vol. 46, no. 10, pp. 11646–11664, Oct. 2024, doi: 10.3390/cimb46100691. [CrossRef] [PubMed]
[30] S. Hashem, A. Dougha, and P. Tufféry, "Ligand-Induced Biased Activation of GPCRs: Recent Advances and New Directions from In Silico Approaches," *Molecules*, vol. 30, no. 5, p. 1047, Feb. 2025, doi: 10.3390/molecules30051047. [CrossRef] [PubMed]
[31] Y. Li, B. Li, W.-D. Chen, and Y.-D. Wang, "Role of G-protein coupled receptors in cardiovascular diseases," *Front. Cardiovasc. Med.*, vol. 10, Jun. 2023, doi: 10.3389/fcvm.2023.1130312. [CrossRef] [PubMed]
[32] J. Ya and U. Bayraktutan, "Vascular Ageing: Mechanisms, Risk Factors, and Treatment Strategies," *IJMS*, vol. 24, no. 14, Jul. 2023, doi: 10.3390/ijms241411538. [CrossRef] [PubMed]

[33] D. Öz-Arslan, M. Yavuz, and B. Kan, "Exploring orphan GPCRs in neurodegenerative diseases," *Front. Pharmacol.*, vol. 15, Jun. 2024, doi: 10.3389/fphar.2024.1394516. [CrossRef] [PubMed]

[34] S. Maudsley *et al.*, "Intersection of the Orphan G Protein-Coupled Receptor, GPR19, with the Aging Process," *IJMS*, vol. 23, no. 21, Nov. 2022, doi: 10.3390/ijms232113598. [CrossRef] [PubMed]

[35] L. M. Luttrell, S. Maudsley, and L. M. Bohn, "Fulfilling the Promise of 'Biased' G Protein–Coupled Receptor Agonism," *Molecular Pharmacology*, vol. 88, no. 3, pp. 579–588, Sep. 2015, doi: 10.1124/mol.115.099630. [CrossRef] [PubMed]

[36] H. Speisky, M. F. Arias-Santé, and J. Fuentes, "Oxidation of Quercetin and Kaempferol Markedly Amplifies Their Antioxidant, Cytoprotective, and Anti-Inflammatory Properties," *Antioxidants*, vol. 12, no. 1, Jan. 2023, doi: 10.3390/antiox12010155. [CrossRef] [PubMed]

[37] B. Salehi *et al.*, "Therapeutic Potential of Quercetin: New Insights and Perspectives for Human Health," *ACS Omega*, vol. 5, no. 20, pp. 11849–11872, May 2020, doi: 10.1021/acsomega.0c01818. [CrossRef] [PubMed]

[38] V. Katritch, V. Cherezov, and R. C. Stevens, "Structure-Function of the G Protein–Coupled Receptor Superfamily," *Annu. Rev. Pharmacol. Toxicol.*, vol. 53, no. 1, pp. 531–556, Jan. 2013, doi: 10.1146/annurev-pharmtox-032112-135923. [CrossRef] [PubMed]

[39] F. R. Patel and N. R. Modi, "Antioxidant activity and FTIR analysis of *Ageratum conyzoides* L," *International Journal of Botany Studies*, vol. 6, no. 5, pp. 1152–1161, 2021.

[40] S. Mohanasundaram *et al.*, "Molecular Docking, Dynamics Simulations, ADMET, and DFT Calculations: Combined In Silico Approach to Screen Natural Inhibitors of 3CL and PL Proteases of SARS-CoV-2," *Cellular Microbiology*, vol. 2024, pp. 1–19, Jan. 2024, doi: 10.1155/2024/6647757. [CrossRef]

[41] O. M. H. Salo-Ahen *et al.*, "Molecular Dynamics Simulations in Drug Discovery and Pharmaceutical Development," *Processes*, vol. 9, no. 1, Dec. 2020, doi: 10.3390/pr9010071. [CrossRef]

[42] S. Kumar, B. H. Kumar, R. Nayak, S. Pandey, N. Kumar, and K. S. R. Pai, "Computational screening and molecular dynamics of natural compounds targeting the SH2 domain of STAT3: a multitarget approach using network pharmacology," *Mol Divers*, vol. 29, no. 6, pp. 5607–5633, Dec. 2025, doi: 10.1007/s11030-024-11075-5. [CrossRef]

[43] U. S. Khandekar, "Investigation of Bioactive Chemical Constituents of *Ageratum conyzoides* Leaves Extract," *International Journal of Advance Research and Innovative Ideas in Education*, vol. 3, no. 3, pp. 2395–4396, 2017.

[44] S. Jafari and J. Bojarska, "Molecular Docking Study of Natural Compounds Targeting the β2-Adrenergic Receptor (β2-AR)," in *ECB 2025*, MDPI, Jul. 2025, p. 3. doi: 10.3390/msf2025034003. [CrossRef]

[45] A. P. Nugraha *et al.*, "Stigmasterol, Quercetin, and Anthocyanin in Eichhornia crassipes as Host Modulation Therapy Candidate: A Bioinformatic Approach," *Journal of International Dental and Medical Research*, vol. 16, no. 3, pp. 1067–1075, 2023.

[46] R. Ferreira De Freitas and M. Schapira, "A systematic analysis of atomic protein–ligand interactions in the PDB," *Med. Chem. Commun.*, vol. 8, no. 10, pp. 1970–1981, 2017, doi: 10.1039/C7MD00381A. [CrossRef] [PubMed]

[47] L. Barrientos, S. Miranda-Rojas, and F. Mendizabal, "Noncovalent interactions in inorganic supramolecular chemistry based in heavy metals. Quantum chemistry point of view," *Int J of Quantum Chemistry*, vol. 119, no. 2, Jan. 2019, doi: 10.1002/qua.25675. [CrossRef]

[48] B. Carpenter and G. Lebon, "Human Adenosine A2A Receptor: Molecular Mechanism of Ligand Binding and Activation," *Front. Pharmacol.*, vol. 8, p. 898, Dec. 2017, doi: 10.3389/fphar.2017.00898. [CrossRef] [PubMed]

[49] A. W. Pradana, S. R. D. Purnaningtyas, D. R. Andaririt, and L. A. M. Putri, "The Potential of Quercetin as Parkinson's Disease Therapy Assessed From Binding Affinity To Adenosine A2a Receptor Using In-Silico," *Strada Journal of Pharmacy*, vol. 7, no. 1, pp. 1–7, 2025.

[50] M. A. Islam *et al.*, "Screening of β1- and β2-Adrenergic Receptor Modulators through Advanced Pharmacoinformatics and Machine Learning Approaches," *IJMS*, vol. 22, no. 20, p. 11191, Oct. 2021, doi: 10.3390/ijms222011191. [CrossRef] [PubMed]

[51] J. A. Canuto *et al.*, "Protective Effect of Quercetin on Renal Tubular Cells and the Involvement with the Renin-Angiotensin-Aldosterone Axis," *Braz. arch. biol. technol.*, vol. 64, p. e21210202, 2021, doi: 10.1590/1678-4324-2021210202. [CrossRef]

[52] R. Jan, M. Khan, S. Asaf, Lubna, S. Asif, and K.-M. Kim, "Bioactivity and Therapeutic Potential of Kaempferol and Quercetin: New Insights for Plant and Human Health," *Plants*, vol. 11, no. 19, p. 2623, Oct. 2022, doi: 10.3390/plants11192623. [CrossRef] [PubMed]

[53] P. M. K. Sundaramoorthy and K. K. Packiam, "Extraction, Isolation and Identification of Kaempferol 3,7 – Diglucoside in the Leaf Extracts of Evolvulus alsinoides (Linn.) and its Inhibition Potency against α-Amylase, α-Glucosidase, Acetylcholinesterase and Amyloid Aggregation," *Pharmacogn. Mag.*, vol. 16, no. 69, pp. 227–234, 2020, doi: 10.4103/pm.pm_583_19. [CrossRef]

[54] X. Zhang *et al.*, "Inhibition of adenosine/A2A receptor signaling suppresses dermal fibrosis by enhancing fatty acid oxidation," *Cell Commun Signal*, vol. 23, no. 1, p. 206, Apr. 2025, doi: 10.1186/s12964-025-02210-2. [CrossRef] [PubMed]
[55] E. S. Chan *et al.*, "Adenosine A2A receptors promote collagen production by a Fli1- and CTGF-mediated mechanism," *Arthritis Res Ther*, vol. 15, no. 3, p. R58, May 2013, doi: 10.1186/ar4229. [CrossRef] [PubMed]
[56] İ. Gulcin, "Antioxidants: a comprehensive review," *Arch Toxicol*, vol. 99, no. 5, pp. 1893–1997, May 2025, doi: 10.1007/s00204-025-03997-2. [CrossRef] [PubMed]
[57] C. Boularan and C. Gales, "Cardiac cAMP: production, hydrolysis, modulation and detection," *Front. Pharmacol.*, vol. 6, Oct. 2015, doi: 10.3389/fphar.2015.00203. [CrossRef] [PubMed]
[58] M. Abdellatif, S. T. Schmid, A. Fuerlinger, and G. Kroemer, "Anti-ageing interventions for the treatment of cardiovascular disease," *Cardiovascular Research*, vol. 121, no. 10, pp. 1524–1536, Aug. 2025, doi: 10.1093/cvr/cvae177. [CrossRef] [PubMed]
[59] X. C. Li, C.-H. Wang, A. P. O. Leite, and J. L. Zhuo, "Intratubular, Intracellular, and Mitochondrial Angiotensin II/AT1 (AT1a) Receptor/NHE3 Signaling Plays a Critical Role in Angiotensin II-Induced Hypertension and Kidney Injury," *Front. Physiol.*, vol. 12, p. 702797, Aug. 2021, doi: 10.3389/fphys.2021.702797. [CrossRef] [PubMed]
[60] W. Yi *et al.*, "Role of angiotensin II in aging," *Front. Aging Neurosci.*, vol. 14, p. 1002138, Dec. 2022, doi: 10.3389/fnagi.2022.1002138. [CrossRef] [PubMed]
[61] R. Timaru-Kast *et al.*, "Angiotensin II Receptor 1 Blockage Limits Brain Damage and Improves Functional Outcome After Brain Injury in Aged Animals Despite Age-Dependent Reduction in AT1 Expression," *Front. Aging Neurosci.*, vol. 11, p. 63, Apr. 2019, doi: 10.3389/fnagi.2019.00063. [CrossRef] [PubMed]
[62] F. P. Chatzipieris, E. Petsas, G. Lambrinidis, J. M. Matsoukas, and T. Mavromoustakos, "Structural and Computational Insights into the Angiotensin II Type 1 Receptor: Advances in Antagonist Design and Implications for Hypertension Therapy (2020–2024)," *Biomolecules*, vol. 16, no. 1, p. 20, Dec. 2025, doi: 10.3390/biom16010020.
[63] W. Li, L. Zhang, X. Xie, X. Shi, and Q. Ma, "Advancements in research on the anti-aging effects and mechanisms of flavonoids in natural products," *Front. Med.*, vol. 12, p. 1637992, Aug. 2025, doi: 10.3389/fmed.2025.1637992. [CrossRef] [PubMed]
[64] B. Păcularu-Burada, A.-I. Cîrîc, and M. Begea, "Anti-Aging Effects of Flavonoids from Plant Extracts," *Foods*, vol. 13, no. 15, p. 2441, Aug. 2024, doi: 10.3390/foods13152441. [CrossRef] [PubMed]