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Research

Molecular Docking Study of Bioactive Compounds from *Averrhoa bilimbi* L. Targeting the Serotonin Transporter (SERT) as Potential Therapeutic Candidates for Phobic Disorders

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Abstract:

Introduction — Social anxiety disorder and phobic conditions are associated with dysregulation of serotonergic neurotransmission, in which serotonin transporter (SERT) plays a key role in serotonin reuptake at the presynaptic membrane. SERT is a molecular target for selective serotonin reuptake inhibitors (SSRIs), making it a target for the identification of inhibitors from natural products using computational approaches.

Methods — Twenty-two compounds retrieved from the KNApSAcK database were screened using PASS Online to predict biological activity. Drug-likeness was evaluated based on Lipinski's Rule of Five. Molecular docking was performed against SERT to determine binding affinity, RMSD, and ligand–receptor interactions, using paroxetine as a reference ligand. ADMET properties were assessed to evaluate pharmacokinetic and toxicity profiles, including absorption, distribution, metabolism, excretion, and toxicity parameters.

Results — PASS screening identified 15 compounds with higher probability of activity than inactivity, with tetradecylamine showing the highest Pa value (0.939). Molecular docking revealed that anapheline exhibited the strongest binding affinity (-7.1 kcal/mol) among the tested compounds, although paroxetine demonstrated superior binding stability (-8.7 kcal/mol). Key interacting residues included TYR95, ILE172, PHE335, VAL501, ASP98, and GLU494. ADMET analysis indicated that most compounds possessed high intestinal absorption (85.76–94.93%), favorable blood–brain barrier permeability, and low predicted toxicity.

Conclusion — Bioactive compounds from *A. bilimbi* L. demonstrated potential inhibitory activity against SERT based on integrated in silico analysis. Among the tested compounds, anapheline showed the most favorable combination of binding affinity and pharmacokinetic properties, suggesting its potential as a lead compound for further development as a SERT inhibitor for phobia-related disorders.

Keywords: *Averrhoa bilimbi* L.; Serotonin Transporter (SERT); Molecular Docking; ADMET; Phobia

1. Introduction

Social Anxiety Disorder (SAD) is an anxiety disorder characterized by an intense and persistent fear of social situations in which individuals may be scrutinized or negatively evaluated by others. Individuals with SAD commonly experience excessive anxiety, fear of negative evaluation, and avoidance of social interactions, which can substantially impair academic, social, and occupational functioning [1]. SAD is one of the most prevalent mental health disorders across different age groups. Globally, its prevalence has been reported to be approximately 4.7% among children, 8.3% among adolescents, and up to 17% among young adults [2]. These findings highlight the substantial public health burden of SAD and emphasize the need for the development of more effective therapeutic strategies.

The pathogenesis of SAD involves a complex interplay of genetic, environmental, and neurobiological factors. Among the neurotransmitter systems implicated in this disorder, the serotonergic system plays a central role in regulating emotion, anxiety, and stress responses. Dysregulation of serotonergic neurotransmission has been recognized as one of the major mechanisms underlying the development of social anxiety symptoms. Within this system, the serotonin transporter (SERT), encoded by the SLC6A4 gene, mediates the reuptake of serotonin from the synaptic cleft into presynaptic neurons, thereby maintaining serotonin homeostasis. Alterations in the expression or function of SERT have been associated with several anxiety disorders, including SAD, making this transporter an important pharmacological target for the development of novel therapeutic agents [3].

Selective serotonin reuptake inhibitors (SSRIs), particularly paroxetine, are considered first-line pharmacological therapy for SAD. Paroxetine exerts its therapeutic effect by inhibiting SERT activity, thereby increasing serotonin availability within the synaptic cleft. A meta-analysis of multiple clinical trials demonstrated that paroxetine effectively reduces the severity of social anxiety symptoms and improves the quality of life of individuals with SAD [4]. Nevertheless, the long-term use of synthetic drugs remains limited by interindividual variability in treatment response and the potential for adverse effects, highlighting the need to identify alternative therapeutic candidates.

Recent advances in computational approaches have promoted the application of molecular docking as an essential strategy in modern drug discovery. This method enables rapid and cost-effective prediction of molecular interactions between candidate compounds and target proteins prior to experimental validation. Several studies have employed molecular docking to investigate the inhibitory potential of natural compounds against SERT, demonstrating that a number of bioactive molecules exhibit promising binding affinities toward this therapeutic target [5].

Averrhoa bilimbi L. (bilimbi) is one of the medicinal plants with considerable potential as a source of bioactive compounds. Traditionally, this plant has been widely used in herbal medicine and is known to contain diverse secondary metabolites with various biological activities [6]. Recent phytochemical investigations have identified several bioactive constituents in *A. bilimbi*, including flavonoids such as myricetin and quercetin, as well as the phytosterol β-sitosterol [7]. Furthermore, numerous studies have reported its antioxidant, antidiabetic, antibacterial, and anti-inflammatory properties. However, the potential of *A. bilimbi* bioactive compounds to target neurobiological mechanisms associated with anxiety disorders remains largely unexplored.

Based on the aforementioned evidence, studies investigating the interaction of *A. bilimbi* bioactive compounds with the serotonin transporter (SERT) as a therapeutic target for SAD are still limited. Therefore, this study aimed to evaluate the interaction of bioactive compounds from *A. bilimbi* with SERT (PDB ID: 5I6X) using a molecular docking approach, with paroxetine employed as the reference ligand. The findings of this study are expected to provide preliminary evidence regarding the potential of *A. bilimbi* bioactive compounds as candidate therapeutic agents for SAD and to serve as a scientific basis for future in silico and experimental investigations.

2. Method

2.1 Data Collection

This in silico study was conducted to evaluate the potential of bioactive compounds from *Averrhoa bilimbi* L. as candidate therapeutic agents for Social Anxiety Disorder (SAD) through their interactions with the serotonin transporter (SERT). The three-dimensional structure of the target protein was retrieved from the Protein Data Bank (PDB) under the accession code 5I6X. Bioactive compounds of *A. bilimbi* were obtained from the KNApSAcK database, which identified a total of 22 secondary metabolites reported in this plant.

All retrieved compounds were subsequently screened using PASS Online (Way2Drug) based on the predicted biological activity for Phobic Disorders Treatment. The screening process was performed by comparing the Probability of Activity (Pa) and Probability of Inactivity (Pi) values, where compounds with Pa values greater than their corresponding Pi values were selected for further analysis. Based on this criterion, 15 compounds were identified as potential candidates and used as test ligands in subsequent analyses. The three-dimensional structures of the selected compounds, together with the reference ligand paroxetine, were downloaded from the PubChem database in Structure Data File (SDF) format.

2.2 Model Design

The study workflow consisted of protein and ligand preparation, docking protocol validation, molecular docking simulation, protein–ligand interaction analysis, and the prediction of pharmacokinetic and toxicity properties.

Protein preparation was performed using Discovery Studio Visualizer (DSV) by removing water molecules and other non-essential components that were not involved in protein–ligand interactions. Subsequently, the selected ligands and the reference ligand were prepared prior to the docking simulation. The docking protocol was validated by redocking the native ligand (8PR) into the binding site of the target protein to assess the reliability of the docking procedure. The validity of the docking protocol was evaluated based on the Root Mean Square Deviation (RMSD) value, where an RMSD of less than 2 Å was considered indicative of acceptable docking reproducibility.

Molecular docking simulations were performed using PyRx integrated with the AutoDock Vina docking engine. The fifteen selected compounds and the reference ligand, paroxetine, were docked against the SERT protein (PDB ID: 5I6X) to determine their binding affinities. Ligand–protein interactions were evaluated based on the calculated binding affinity values, where lower binding energies indicate stronger and more stable interactions between the ligand and the target protein. Protein–ligand interaction analysis was subsequently conducted using DSV to identify the amino acid residues involved in complex formation. The analyzed interactions included hydrogen bonds, hydrophobic interactions, π-alkyl interactions, π–π interactions, and other non-covalent interactions that contribute to the stability of the protein–ligand complex.

Compounds exhibiting the most favorable binding affinities were further subjected to *in silico* pharmacokinetic and toxicity evaluations. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles were predicted using ADMETlab 2.0 and pkCSM. In addition, toxicity assessments were performed using ProTox and Toxtree to predict toxicity endpoints, toxicity classes, and potential toxicological hazards based on the structural characteristics of each compound.

2.3 AI Disclosure

ChatGPT (OpenAI) was used in a limited capacity to assist with language editing and manuscript formatting. Artificial intelligence was not used for study design, data collection, molecular docking

analysis, ADMET prediction, data interpretation, or the development of the study conclusions. All scientific content, analyses, and interpretations were critically reviewed and verified by the authors, who take full responsibility for the accuracy, integrity, and originality of the manuscript.

2.4 Ethical Clearance Statement

This study was conducted entirely using publicly available databases and in silico computational approaches. No human participants, patient data, or experimental animals were involved in this study. Therefore, ethical approval (ethical clearance) and informed consent were not required.

3. Analysis

3.1 Prediction of Biological Activity

To identify bioactive compounds with the highest potential for phobic disorders treatment, all secondary metabolites retrieved from *Averrhoa bilimbi* were initially screened using the PASS Online platform. The prediction was based on the Probability of Activity (Pa) and Probability of Inactivity (Pi), where compounds with Pa values exceeding their corresponding Pi values were considered more likely to exhibit the predicted biological activity. The prediction results for all selected compounds are summarized in Table 1.

A total of 22 compounds were retrieved from the KNApSAcK database. Based on PASS Online screening, 15 compounds exhibited higher Probability of Activity (Pa) values than their corresponding Probability of Inactivity (Pi) values and were subsequently selected for further analysis.

Table 1. PASS Online Prediction of Probability of Activity (Pa) and Probability of Inactivity (Pi) of Bioactive Compounds from *Averrhoa bilimbi* for Phobic Disorders Treatment

No.	Compound	Formula	Pa Value	Pi Value
1.	Anapheline	$C_{13}H_{24}N_2O$	0.689	0.081
2.	Elaeokanine C	$C_{12}H_{21}NO_2$	0.347	0.278
3.	Pentadecanal	$C_{15}H_{30}O$	0.860	0.014
4.	Palmitic Acid Amide	$C_{16}H_{33}NO$	0.899	0.005
5.	14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	0.892	0.007
6.	2-Ethyldodecanoic acid	$C_{14}H_{28}O_2$	0.916	0.004
7.	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	0.874	0.011
8.	7-Hexadecen-1-ol	$C_{16}H_{32}O$	0.891	0.007
9.	Diglycidyl resorcinol ether	$C_{12}H_{14}O_4$	0.722	0.066
10.	4-Hydroxy-8-sphingenine	$C_{18}H_{37}NO_3$	0.696	0.078
11.	Ethyl 3-(N-butylacetamido) propionate	$C_{11}H_{21}NO_3$	0.755	0.052
12.	Enigmol	$C_{18}H_{39}NO_2$	0.774	0.044
13.	Linoleamide	$C_{18}H_{33}NO$	0.833	0.022
14.	Tetradecylamine	$C_{14}H_{31}N$	0.939	0.003
15.	Xestoaminol C	$C_{14}H_{31}NO$	0.825	0.025

3.2 Drug-Likeness Evaluation

Following biological activity screening, the selected compounds were evaluated for their drug-likeness properties according to Lipinski's Rule of Five. This assessment was performed to determine whether the physicochemical characteristics of the compounds were consistent with those generally associated with orally active drug candidates. The evaluated parameters included molecular weight, lipophilicity (Log P), hydrogen bond donors, hydrogen bond acceptors, and molar refractivity. The results of the drug-likeness evaluation are presented in Table 2.

Table 2. Drug-Likeness Evaluation of Bioactive Compounds from *Averrhoa bilimbi*

No.	Compound	Mass (g/mol)	Log P	HBD	HBA	MR
1.	Anapheline	224.34	2.78	2	3	74.01
2.	Elaeokanine C	211.30	2.19	1	3	63.74
3.	Pentadecanal	226.40	3.84	0	1	74.42
4.	Palmitic Acid Amide	255.44	3.87	1	1	81.93
5.	14-Methyl-8-hexadecen-1-ol	254.45	4.48	1	1	84.52
6.	2-Ethyldodecanoic acid	228.37	3.35	1	2	71.18
7.	2-Hydroxyhexadecanoic acid	272.42	3.09	2	3	81.96
8.	7-Hexadecen-1-ol	240.42	4.32	1	1	79.71
9.	Diglycidyl resorcinol ether	222.24	2.63	0	4	56.60
10.	4-Hydroxy-8-sphingenine	315.49	3.79	4	4	94.36
11.	Ethyl 3-(N-butylacetamido) propionate	215.29	2.81	0	3	59.37
12.	Enigmol	301.51	4.18	3	3	93.67
13.	Linoleamide	279.46	5.29	1	1	90.60
14.	Tetradecylamine	213.40	3.89	1	1	72.12
15.	Xestoaminol C	229.40	3.61	2	2	73.28

The drug-likeness assessment demonstrated that the majority of the selected compounds complied with Lipinski's Rule of Five. A single violation was observed for linoleamide, which exhibited a Log P value of 5.29, exceeding the recommended threshold.

3.3 Molecular Docking

To investigate the inhibitory potential of the selected bioactive compounds toward the serotonin transporter (SERT), molecular docking simulations were subsequently performed using the validated docking protocol. The docking analysis evaluated binding affinity, docking reproducibility (RMSD), interacting amino acid residues, and the types of non-covalent interactions contributing to ligand binding. The molecular docking results for the native ligand, paroxetine, and all tested compounds are presented in Table 3.

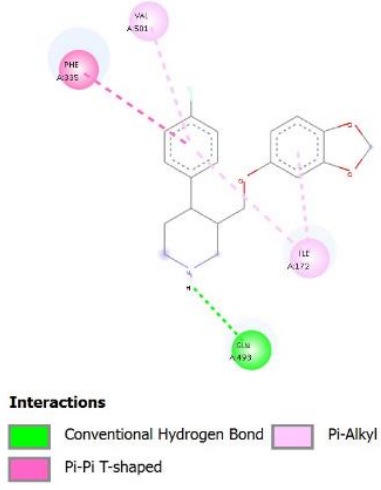
Table 3. Interaction characteristics of the native ligand, paroxetine, and bioactive compounds of *Averrhoa bilimbi* L. against the serotonin transporter (SERT) based on molecular docking analysis.

No.	Compound	Binding Affinity (Kcal/mol)	RMSD	Amino Acid Residues	Bonds
1.	8PR	-8.5	0	GLU493	Hydrogen Bond
				PHE335	Pi-Pi t-Shaped
				VAL501; ILE172	Pi-Alkyl
2.	Anapheline	-7.1	0	SER438; TYR95	Carbon Hydrogen Bond
				ILE172; ALA173; VAL501	Alkyl
				TYR176; PHE341	Pi-Alkyl
3.	Elaeokanine C	-6.9	0	TYR95	Hydrogen Bond
				GLY338; SER438	Carbon Hydrogen Bond
				ILE172; VAL501	Alkyl

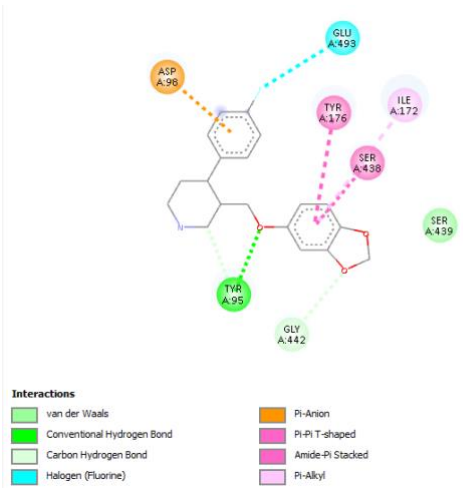
				PHE341; PHE335	Pi-Alkyl
4.	Pentadecanal	-5.4	0	LEU557; PRO499; PRO561; PRO560	Alkyl
				TYR579; TYR495; PHE556	Pi-Alkyl
5.	Palmitic acid amide	-5.9	0	PHE335; ASP98	Hydrogen Bond
				ILE172; VAL501	Alkyl
				TYR176; TYR95; PHE341	Pi-Alkyl
6.	14-Methyl-8-hexadecen-1-ol	-6.5	0	PHE586	Pi- Sigma
				PRO499	Alkyl
				TYR495; TYR579; PHE556	Pi-Alkyl
7.	2-Ethyldodecanoic acid	-5.7	0	ASP98; TYR95	Hydrogen Bond
				TYR176	Pi-Sigma
				ILE172	Alkyl
				PHE341; PHE335	Pi-Alkyl
8.	2-Hydroxyhexadecanoic acid	-5.9	0	TYR495	Hydrogen Bond
				PRO560; PRO499; LEU557; ALA500	Alkyl
				PHE586; TYR579	Pi-Alkyl
9.	7-Hexadecen-1-ol	-6.0	0	ASP98; TYR95	Hydrogen Bond
				TYR176	Pi-Sigma
				ILE172	Alkyl
				PHE341; PHE335	Pi-Alkyl
10.	Diglycidyl resorcinol ether	-6.1	0	TYR495	Hydrogen Bond
				PRO560; PRO499; LEU557; ALA500	Alkyl
				PHE586; TYR579	Pi-Alkyl
11.	4-Hydroxy-8-sphingenine	-5.7	0	THR497; ARG104	Hydrogen Bond
				GLN332; ASP328	Carbon Hydrogen Bond
				PHE335	Pi-Pi Stacked
12.	Ethyl 3-(N-butylacetamido) propionate	-5.2	0	PHE556; GLU494; TYR495	Hydrogen Bond
				TYR579	Pi-Donor Hydrogen Bond
				PRO561; ALA331	Alkyl
				PHE336	Pi-Alkyl
13.	Enigmol	-6.7	0	TYR95	Hydrogen Bond

				ILE172; VAL501	Alkyl
				PHE335	Pi-Alkyl
14.	Linoleamide	-6.3	0	PHE556; GLU494	Hydrogen Bond
				PRO560	Carbon Hydrogen Bond
				ALA500; PRO499	Alkyl
				PHE586; TYR579; TYR495	Pi-Alkyl
15.	Tetradecylamine	-5.3	0	SER438	Hydrogen Bond
				VAL501; ILE172	Alkyl
				TYR176; TYR95; PHE341; PHE335	Pi-Alkyl
16.	Xestoaminol C	-5.9	0	TYR495; GLU494	Hydrogen Bond
				PHE335	Pi-Sigma
				PRO561	Alkyl
				PHE556	Pi-Alkyl
17.	Paroxetine	-8.7	0	TYR95	Hydrogen Bond
				GLY442	Carbon Hydrogen Bond
				GLU493	Halogen (Fluorin)
				ASP98	Pi-Anion
				TYR176	Pi-Pi T-Shaped
				SER438	Amide-Pi Stacked
				ILE172	Pi-Alkyl

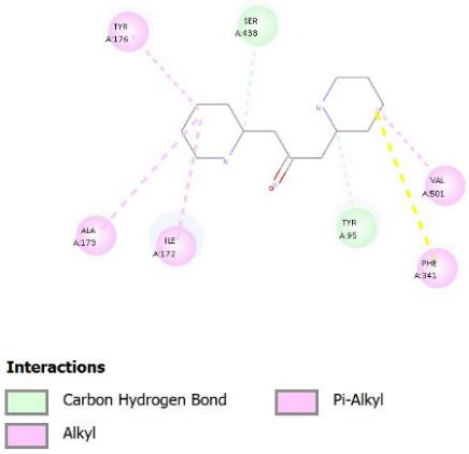
Representative docking poses were subsequently visualized to compare the binding orientations and interaction patterns of the native ligand, the reference ligand (paroxetine), and the best-performing bioactive compound. These visualizations facilitate structural interpretation of the ligand–protein interactions and provide additional evidence supporting the docking results. The interaction profiles are illustrated in Fig. 1.



(a)



(b)



(c)

Fig. 1. Visualization of (a) the redocking result between SERT (PDB ID: 5I6X) and the native ligand (8PR), (b) the molecular docking result between SERT (PDB ID: 5I6X) and paroxetine (positive control), and (c) the molecular docking result between SERT (PDB ID: 5I6X) and anapheline.

The interaction visualization revealed that the native ligand (8PR) interacted with GLU493, PHE335, VAL501, and ILE172 within the SERT binding pocket. Paroxetine, used as the positive control, formed interactions with TYR95, ASP98, ILE172, TYR176, SER438, GLY442, and GLU493. Meanwhile, anapheline interacted with TYR95, SER438, ILE172, ALA173, VAL501, TYR176, and PHE341. Several amino acid residues, including TYR95, ILE172, SER438, and TYR176, were shared between the anapheline–SERT and paroxetine–SERT complexes, whereas ILE172 and VAL501 were also observed in the native ligand (8PR)–SERT complex.

3.4 ADMET Analysis

To complement the molecular docking analysis, the pharmacokinetic behavior and toxicity profiles of the selected bioactive compounds were predicted using in silico ADMET analysis. Parameters related to absorption, distribution, and toxicity were evaluated to assess the suitability of the compounds as potential drug candidates and to identify any potential safety concerns. The predicted ADMET properties are summarized in Table 4.

Table 4. In Silico ADMET Prediction of Bioactive Compounds from *Averrhoa bilimbi*

Compound	Absorption		Distribution			Toxicity		
	$CaCO_2$	HIA	PPB	BBB	Pgp Inhibition	Kroes TTC	Carcinogen	Mutagen
Anapheline	1.336	93.738	8.2	0.91	-	safe	-	-
Elaeokanine C	1.472	85.76	39.7	0.84	-	safe	-	-
Pentadecanal	1.479	92.676	99.3	0.99	-	Low risk	-	+
Palmitic Acid Amide	1.525	90.399	97.1	0.99	-	safe	-	-
14-Methyl-8-hexadecen-1-ol	1.484	90.923	97.7	0.93	-	safe	-	-
2-Ethyldodecanoic acid	1.563	94.023	97.1	0.94	-	safe	-	-
2-Hydroxyhexadecanoic acid	1.511	90.023	98.3	0.63	-	safe	-	-

7-Hexadecen-1-ol	1.471	90.31	98.3	0.91	-	safe	-	-
Diglycidyl resorcinol ether	1.25	92.38	91.6	0.84	-	safe	-	+
4-Hydroxy-8-sphingenine	0.467	94.746	97.9	0.54	-	safe	-	-
Ethyl 3-(N-butylacetamido) propionate	1.399	94.929	52.1	0.90	-	safe	-	-
Enigmol	0.838	90.891	99.4	0.57	-	safe	-	-
Linoleamide	1.54	90.724	97.9	0.99	-	safe	-	-
Tetradecylamine	1.325	89.411	92.0	0.97	-	safe	-	-
Xestoaminol C	1.499	90.372	99.4	0.74	-	safe	-	-

The ADMET prediction results for the 15 selected compounds showed $CaCO_2$ permeability values ranging from 0.467 to 1.563 and Human Intestinal Absorption (HIA) values ranging from 85.76% to 94.93%. Distribution parameters indicated plasma protein binding (PPB) values of 8.2–99.4% and blood–brain barrier (BBB) permeability values ranging from 0.54 to 0.99. All compounds were predicted to be non-inhibitors of P-glycoprotein (P-gp). Based on the toxicity prediction, most compounds were classified as safe, whereas pentadecanal was predicted to present a low toxicological risk and diglycidyl resorcinol ether was predicted to be positive for mutagenicity.

4. Discussion

The selection of *Averrhoa bilimbi* L. as the source of bioactive compounds in this study was based on its diverse secondary metabolite profile. Setyawan et al. (2021) reported that various parts of the plant, particularly the fruits and leaves, contain phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, and triterpenoids. These phytochemicals have been associated with a wide range of biological activities, including antioxidant, antibacterial, antiviral, and cholesterol-lowering effects [6]. The diversity of these bioactive constituents suggests that *A. bilimbi* represents a promising natural source for the discovery and development of novel therapeutic agents.

A total of 22 compounds were retrieved from the KNApSAcK database. The structural diversity of these compounds may contribute to differences in their biological activities and binding characteristics toward specific protein targets. Therefore, an initial screening step was performed to identify compounds with the highest potential biological activity prior to molecular docking analysis [8]. As presented in Table 1, PASS Online screening identified 15 of the 22 compounds with Probability of Activity (Pa) values exceeding their corresponding Probability of Inactivity (Pi) values. Among these compounds, tetradecylamine exhibited the highest Pa value (0.939).

In the PASS Online prediction system, the Pa value represents the probability that a compound exhibits a particular biological activity, whereas the Pi value indicates the probability that the compound is inactive for the predicted activity [9]. In general, compounds with Pa values greater than 0.7 are considered to have a high likelihood of exhibiting the predicted biological activity and often share structural features with previously reported active compounds. Compounds with Pa values between 0.5 and 0.7 are still regarded as promising candidates for further investigation, whereas compounds with Pa values below 0.5 are considered less likely to exhibit the predicted activity [10].

However, a high Pa value alone does not necessarily indicate that a compound is suitable for drug development. The predicted biological activity should be supported by favorable physicochemical properties to ensure adequate absorption, distribution, and target accessibility in vivo. Therefore, the 15 compounds that passed the PASS Online screening were subsequently subjected to drug-likeness evaluation to assess their compliance with the general characteristics of orally active drug candidates

prior to molecular docking analysis. The drug-likeness evaluation was performed according to Lipinski's Rule of Five [8], which includes a molecular weight (MW) ≤500 g/mol, hydrogen bond acceptors (HBA) ≤10, hydrogen bond donors (HBD) ≤5, and a Log P value ≤5. These criteria are widely used as an initial indicator of the oral bioavailability of drug candidates because they are closely associated with intestinal absorption and membrane permeability [10].

As presented in Table 2, the majority of the evaluated compounds complied with all Lipinski criteria. The molecular weights ranged from 211.30 to 315.49 g/mol**,** well below the recommended upper limit of 500 g/mol. Relatively small molecular size has been associated with improved passive diffusion across biological membranes, thereby favoring oral bioavailability. Furthermore, the HBA and HBD values ranged from 1 to 4 and 0 to 4**,** respectively, indicating an appropriate hydrogen-bonding capacity. Maintaining a balanced number of hydrogen bond donors and acceptors is considered important for achieving an optimal balance between aqueous solubility and membrane permeability, both of which influence oral absorption.

The Log P values ranged from 2.19 to 5.29, indicating that most compounds possessed lipophilicity within the recommended range for oral drug candidates. A single Lipinski violation was observed for linoleamide, which exhibited a Log P value of 5.29, slightly exceeding the recommended threshold. Elevated Log P values generally reflect increased lipophilicity, which may enhance membrane permeability but can also reduce aqueous solubility [11]. Nevertheless, a single violation of Lipinski's Rule of Five does not necessarily preclude a compound from further drug development, particularly when the remaining physicochemical properties remain within the acceptable range and are supported by favorable pharmacokinetic characteristics [12].

Overall, the drug-likeness assessment demonstrated that the selected compounds possess physicochemical properties consistent with orally active drug candidates. Accordingly, all compounds that satisfied the selection criteria were further evaluated using molecular docking to investigate their binding affinity and interaction profiles with the serotonin transporter (SERT), the therapeutic target associated with SAD.

The SERT was selected as the target protein because it plays a critical role in serotonergic neurotransmission by mediating the reuptake of serotonin from the synaptic cleft into presynaptic neurons [13]. Dysregulation of the serotonergic system has been implicated in the pathophysiology of several anxiety disorders, including SAD. Clinically, SERT serves as the primary pharmacological target of Selective Serotonin Reuptake Inhibitors (SSRIs), such as paroxetine, which increase serotonin availability in the synaptic cleft by inhibiting serotonin reuptake [14]. Therefore, evaluating the interactions of bioactive compounds from *Averrhoa bilimbi* with SERT is essential for identifying potential inhibitors that may modulate transporter activity and serve as prospective therapeutic candidates for SAD.

Molecular docking analysis was subsequently performed to evaluate the binding affinity and interaction profiles of *Averrhoa bilimbi* bioactive compounds against the serotonin transporter (SERT). The docking parameters included binding affinity, root mean square deviation (RMSD), and interactions with amino acid residues within the protein binding pocket. Binding affinity was used to estimate the stability of the ligand–protein complex, where lower binding energy indicates stronger binding affinity and a more stable complex formation [15]. Paroxetine was employed as the positive control to compare the binding performance of the tested compounds toward the target protein.

The docking protocol was validated by redocking the native ligand (8PR), which yielded an RMSD value of 0 Å, indicating excellent agreement between the predicted docking pose and the crystallographic ligand conformation. RMSD is commonly used to assess the ability of a docking protocol to reproduce the experimentally observed ligand orientation within the protein binding site [16]. In general, an

RMSD value of ≤2 Å is considered acceptable for docking validation, indicating that the protocol is capable of reliably reproducing ligand binding poses. Therefore, the RMSD value obtained in this study confirms the validity of the docking protocol for predicting ligand–protein interactions. The docking results demonstrated that all tested compounds exhibited binding affinity values ranging from –5.2 to –7.1 kcal/mol. Among them, anapheline (–7.1 kcal/mol), elaeokanine C (–6.9 kcal/mol), and enigmol (–6.7 kcal/mol) displayed the strongest binding affinities. Nevertheless, these values remained higher than that of paroxetine (–8.7 kcal/mol), indicating that the paroxetine–SERT complex possesses greater binding stability. More negative binding affinity values indicate a more favorable and energetically stable ligand–receptor interaction [17]. These findings suggest that anapheline, elaeokanine C, and enigmol exhibit relatively favorable binding affinities toward SERT, although their affinities remain lower than that of paroxetine.

Analysis of amino acid interactions revealed that several residues involved in the binding of the tested compounds, including TYR95, ILE172, SER438, PHE335, and VAL501, were also observed in the paroxetine–SERT complex. The presence of these shared residues suggests that the bioactive compounds of *A. bilimbi* may interact within the same or adjacent binding region as the reference ligand. Anapheline formed carbon hydrogen bond interactions with TYR95 and SER438, alkyl interactions with ILE172, ALA173, and VAL501, and π-alkyl interactions with TYR176 and PHE341. These interactions are predicted to contribute to the stabilization of the ligand–protein complex through hydrophobic and aromatic contacts within the SERT binding pocket.

Interaction visualization further demonstrated that anapheline shared several binding residues with both the native ligand (8PR) and paroxetine. Residues ILE172 and VAL501, which were involved in the binding of the native ligand, were also identified in the anapheline–SERT complex. Likewise, TYR95, ILE172, TYR176, and SER438 were common to both the anapheline–SERT and paroxetine–SERT complexes. The presence of these shared residues suggests that anapheline recognizes a structurally relevant binding region within SERT. This observation is consistent with its relatively favorable binding affinity and supports its potential as a candidate SERT inhibitor.

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis was subsequently performed to evaluate the pharmacokinetic properties and preliminary safety profiles of the bioactive compounds following molecular docking. Such evaluation is an important consideration during the early stages of drug discovery, as pharmacokinetic behavior and toxicity largely determine the success of drug development [18]. Regarding absorption, all compounds exhibited positive $CaCO_2$ permeability values ranging from 0.467 to 1.563 and Human Intestinal Absorption (HIA) values between 85.76% and 94.93%. $CaCO_2$ permeability is widely used to predict intestinal membrane permeability, whereas high HIA values indicate favorable oral absorption [19]. These findings suggest that the evaluated compounds possess the potential for adequate oral bioavailability.

For the distribution profile, plasma protein binding (PPB) values ranged from 8.2% to 99.4%, indicating substantial variation in plasma protein binding capacity among the evaluated compounds. Such variation may influence the distribution and free circulating fraction of each compound. In addition, the predicted blood–brain barrier (BBB) permeability values ranged from 0.54 to 0.99, suggesting that most compounds are capable of penetrating the central nervous system. This characteristic is particularly relevant because SERT is predominantly expressed in the central nervous system, making BBB permeability an important consideration for compounds targeting this transporter [20]. Furthermore, all compounds were predicted to be non-inhibitors of P-glycoprotein (P-gp), suggesting a lower likelihood of P-gp-mediated efflux that could otherwise reduce drug accumulation at the target site. Regarding toxicity, most compounds were classified as safe according to the Kroes Threshold of Toxicological Concern (TTC). However, pentadecanal was predicted to present a low toxicological risk, whereas diglycidyl resorcinol ether was predicted to exhibit mutagenic potential, warranting further toxicological evaluation [18].

Overall, the ADMET analysis demonstrated that most bioactive compounds of *A. bilimbi* possess favorable pharmacokinetic characteristics, including high intestinal absorption, adequate BBB permeability, and acceptable preliminary safety profiles. When considered together with the molecular docking results, anapheline emerged as the most promising candidate due to its relatively strong binding affinity toward SERT and favorable predicted ADMET properties. Nevertheless, further experimental validation is required to confirm its pharmacological activity and safety.

Conclusion

Based on the integrated results of drug-likeness screening, molecular docking, and ADMET analysis, the bioactive compounds of *Averrhoa bilimbi* L. demonstrated potential as serotonin transporter (SERT) inhibitors. Although tetradecylamine exhibited the highest predicted biological activity in PASS screening (Pa = 0.939), anapheline showed the strongest binding affinity toward SERT (–7.1 kcal/mol), followed by elaeokanine C and enigmol. ADMET prediction further indicated that these compounds possess favorable pharmacokinetic characteristics, including high intestinal absorption, predicted blood–brain barrier permeability, and acceptable preliminary safety profiles. Among the evaluated compounds, anapheline emerged as the most promising candidate based on its favorable binding affinity and pharmacokinetic properties. Nevertheless, further in vitro and in vivo studies are required to validate its biological activity and therapeutic potential.

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