

Dual Inhibition of SERT and MAO-A by a Novel Phytoconstituent from *Premna esculenta*: A Convergence of Behavioral, Computational, and ADMET Studies

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Abstract

Depression is a common psychiatric problem that coexists with several neurodegenerative diseases, including Alzheimer's disease (AD), which was the most significant pathophysiological mechanism, including imbalance of neurotransmitters and oxidative stress. They are commonly linked to negative sides like adverse effects and resistance to treatment, but the current antidepressants, like SERT and MAO-A, are good. This research paper examined the antidepressant property of ethanolic *Premna esculenta* (EEPE), a medicinal plant, through a multidimensional study involving phytochemical, behavioral studies with established murine models, specifically the Forced Swim and Tail Suspension Tests, where the extracts substantially reduced immobility in a dose-dependent manner under varied conditions, and in silico studies. Also, an acute toxicity test was conducted, and it revealed the safety of using different doses. In terms of docking, GC-MS analysis recognized 1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline as a lead compound, which had strong dual binding potential with serotonin transporter and monoamine oxidase A docks. Additional insights on ADMET and ProToxII showed a favourable blood-brain barrier permeability and lower toxicity than the conventional drugs. Remarkably, anxiolytic and neuroprotective properties of relevance to Alzheimer's disease were also proposed by the PASS prediction regarding THPAC. Lastly, density functional theory (DFT) calculations confirmed this observation with a low value of HOMO-LUMO gap and molecular softness, leading features that are indicative of a high degree of biological interaction. Altogether, the findings allow one to suppose that EEPE can become a multifunctional and safe potential agent in treating depression and even neurodegeneration, due to the presence of THPAC.

Introduction

Neurodegenerative diseases produce profound deficits in emotional, cognitive, and motor functioning. Patients become increasingly impaired and dependent on caregivers for assistance as these diseases progress. For several patients, close loved ones play a primary caregiving role, which is well developed, including up to a fourfold increase in rates of depression. This depression is one of the most common psychiatric disorders, and its prevalence has been intensifying steadily in both the young and the senior citizen populations across the world (Lépine and Briley 2011; Sørensen et al. 2022). More than 300 million people are affected universally, making it a major contributor to disability and reduced quality of life (Fonseca et al. 2023). Clinically, depression is noticeable by persistent low mood, loss of interest in day-to-day activities, cognitive decline, and several physical symptoms, including variations in appetite, fatigue, and sleep disturbances (Devita et al. 2022). The disorder arises from a complex interaction of genetic, environmental, and neurobiological factors. Within the serotonergic system, two molecular targets, the monoamine oxidase A (MAO-A) and serotonin transporter (SERT), are involved in pathology and treatment response (Naoi et al. 2018). Studies using positron emission tomography have shown minimum effective doses, which helps explain both the delayed onset and the therapeutic ceiling often seen in patients (Giraud et al. 2020).

The burden of depression is often enlarged during social and environmental crises. During the COVID-19 pandemic, for instance, around 53.2 million new cases of major depressive disorder were reported in 2020 (Zhang et al. 2025). Even one year after recovery, individuals who had experienced COVID-19 continued to be at greater risk of depression and cognitive impairment, including brain fog (Hampshire et al. 2021). Such findings highlight the urgent need for treatments that are effective, safe, and widely accessible.

serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs). Although SSRIs are considered first-line therapy due to their safety, tolerability, and wide availability, they are not without limitations. Several patients experienced delayed therapeutic effects, partial symptom relief, or complete resistance, specifically in strategies that combine modulation of SERT with inhibition of MAO-A. The molecular dock study of these two proteins is an opportunity to investigate such dual mechanisms and could have a new therapeutic avenue (Hampshire et al. 2021). Nonetheless, systematic studies have not been carried out on its possible impact on depression, thus posing a gap in the existing research.

Premna esculenta, a small shrub from the Lamiaceae family, is traditionally utilized by tribal communities in Bangladesh and India for various ailments, including stress-related conditions, gout, jaundice, and infections. Its leaves are applied to treat arthritis and infections, and the plant is recognized for its analgesic, anti-inflammatory, and antioxidant properties (Yousuf et al.

2025). Despite its extensive traditional use, scientific evaluation of its antidepressant potential remains limited, making it a compelling subject for further research (Yousuf et al. 2025).

To address this gap, the present study aims to determine the antidepressant properties of EEPE through animal behavior assessments of the Forced Swimming Test and Tail Immersion Test. The safety of the extract was ensured through the performance of an acute toxicity study. In parallel, molecular docking analysis was conducted to explore bioactive compounds in the extract that have possible interaction with monoamine oxidase A (MAO-A) and SERT depression-targeting proteins, besides conducting in vivo assessments. ADMET (absorption, distribution, metabolism, excretion, and toxicity) predictions analyzed the pharmacokinetic characteristics along with safety aspects of the phytocompounds and DFT calculations to assess compound stability. To our knowledge, this is the first comprehensive evaluation of EEPE in the context of depression, integrating in vivo and in silico approaches to provide mechanistic insight into its therapeutic potential.

Materials and methods

Plant Collection and Extraction

Fresh leaves of *Premna esculenta* Roxb. were collected from the Chittagong Hill Tracts, Bangladesh, and authenticated by the Department of Botany, University of Chittagong. After thorough washing, the leaves were shade-dried at room temperature for ten days, followed by coarse grinding. A total of 500 grams of powdered material was extracted using 95% ethanol in a Soxhlet apparatus for 72 hours. The collected extract was filtered, evaporated under reduced pressure using a rotary evaporator, and the concentrated residue was preserved at 4°C for further use.

Phytochemical Screening

Preliminary study of qualitative phytochemical screening was carried out to identify the major bioactive constituents (Ouandaogo et al. 2023). The tests included Dragendorff's and Mayer's reagents for alkaloids, ferric chloride and lead acetate for phenolics and tannins, the frothing test for saponins, Shinoda and alkaline reagent tests for flavonoids, and Liebermann-Burchard test for steroids and triterpenoids. These analyses helped to infer the therapeutic potential of the plant based on its chemical profile.

Experimental Animals and Ethical Approval

Adult Swiss albino mice of either sex (weighing 20–25 g) were obtained from the animal house of the Department of Pharmacy, University of Chittagong. The animals were housed under standard laboratory conditions (12-hour light/dark cycle, $22 \pm 2^\circ\text{C}$ temperature, $55 \pm 5\%$ humidity) with ad libitum access to food and water. All animal protocols were reviewed and approved by the Committee of Clinical Pharmacy & Pharmacology, Department of Pharmacy, Dhaka International University (Ref: CPP/DIU/EC/006).

In Vivo Antidepressant Activity

The antidepressant activity of the ethanolic extract was evaluated using two behavioral models: The Forced Swim Test (FST) and Tail Suspension Test (TST) (Castagné et al. 2010). Mice were divided into five groups ($n = 5$): vehicle control (saline), standard drug-treated (diazepam mg/kg), and extract-treated groups receiving 50, 100, and 200 mg/kg orally. After seven days of treatment, FST was performed by placing each mouse in a glass cylinder filled with water ($25 \pm 1^\circ\text{C}$) and recording the immobility time during the final 4 minutes of a 6-minute session. TST was conducted by suspending the mice from a horizontal bar and observing immobility for 6 minutes. A significant reduction in immobility time compared to the control group was considered indicative of antidepressant-like activity.

Acute Toxicity Study

To evaluate the safety of the ethanolic extract, an acute oral toxicity test was performed following OECD Guideline 423. Female mice were fasted overnight and administered a single oral dose of 300 mg/kg, 2000 mg/kg, and 3000mg/kg of extract. This method has been done with some modifications (Bruce 1985; Mahmud et al. 2025). Observations for behavioral changes, signs of toxicity, and mortality were made for the first four hours and then daily for 2 days. No mortality or significant behavioral abnormalities were observed, indicating a high safety margin.

Compound Identification through GC-MS

In accordance with the sample preparation section, dried leaves of *Premna esculenta* were ground into a powder and extracted using methanol. Before analysis, a 0.22 μm syringe filter was used to filter the concentrated extract. The Clarus® 690 gas chromatograph (PerkinElmer, CA, USA) used for the GC-MS analysis was outfitted with a Clarus® SQ8 C mass spectrometer (PerkinElmer, CA, USA) and an Elite-35 GC column (30 m length, 0.25 mm diameter, 0.25 μm film thickness). At 70 eV, the mass spectrometer used the electron ionization (EI) mode. The column's oven temperature was first set at 60°C for 0 minutes, then increased to 240°C at a rate of 4°C per minute, and it remained there for 15 minutes (Gomathi et al. 2015). By comparing the compounds' mass spectra with those of the NIST library, they were identified.

Ligand Preparation

Based on GC-MS profiling, compounds 42 and 4 were selected due to their CNS-relevant two standards. Their chemical structures were downloaded from PubChem <https://pubchem.ncbi.nlm.nih.gov/>. The 3D structures were exported as an SDF format. These structures were further converted to PDBQT format using AutoDock Tools. For validation and comparison, standard inhibitors were used against each protein: Clorgyline (4380) for MAO-A, and Paroxetine (3386) for SERT. These standards were also subjected to the same ligand preparation pipeline.

Protein Preparation

Four target proteins associated with depression and neurological modulation were selected: MAO-A (PDB ID: 2Z5X) and Serotonin Transporter (PDB ID: 5I6X). Each structure was retrieved from the RCSB Protein Data Bank <https://www.rcsb.org/>. Water molecules and heteroatoms were removed using Discovery Studio Visualizer (Visualizer 2005). Polar hydrogens were added, and Kollman charges were assigned to the proteins.

Molecular Docking Studies

The three-dimensional crystal structures of SERT (PDB ID: 5I6X) and MAO-A (PDB ID: 2Z5X) were retrieved in PDB format from the Protein Data Bank (Berman et al. 2000). Those models were then subjected to energy minimization through the steepest descent and conjugate gradient to remove bad contact of protein atoms. Computation was carried out in vacuo with the GROMOS 96 parameters set, with execution using the Swiss-PDB Viewer. For docking analysis, AutodockVina was employed, and AutoDock Tools (ADT) of the MGL software package was used to convert PDB into a pdbqt format to input protein and ligands. Then, AutoDockVina was implemented through the shell script provided by the AutoDockVina developers. The binding affinity of the ligand was observed in kcal/mole as a unit for a negative score (Trott and Olson 2010). Through literature studies, PyMol, Discovery Studio, Uniprot, and CATSp for getting active sites, binding sites, and gatekeeper residues.

ADME and Toxicity Predictions

The drug-likeness, pharmacokinetics, and toxicity of ligands 42 and 4 were assessed using SwissADME <http://www.swissadme.ch/>, pkCSM <https://biosig.lab.uq.edu.au/pkcsml/>, Protox-II, and PASS prediction web servers. Parameters such as molecular weight, LogP, hydrogen bond donors/acceptors, gastrointestinal absorption, blood-brain barrier (BBB) permeability, cytochrome P450 enzyme inhibition, and oral bioavailability were evaluated. Toxicity assessments included hepatotoxicity, skin sensitization, and AMES mutagenicity tests. Compounds adhering to Lipinski's rule of five and demonstrating favorable BBB penetration were considered optimal for CNS activity.

DFT Analysis

For the analysis, density functional theory (DFT) analysis, this study focused on the hardness and softness of all compounds, which were determined from the energies (ϵ) of frontier HOMOs and LUMOs. Considering the Parr and Pearson interpretation (Parr 1989) of DFT and Koopmans' theorem (Pearson 1986), hardness (η) and softness (S) of the drugs were calculated according to the following equation.

$$\eta = (\text{HOMO} - \text{LUMO})/2$$

$$S = 1/\eta$$

Results and Discussion

Phytochemical Screening Test

The findings of phytochemical screening of the extracts showed that the leaf extracts of *Premna esculenta* included combined reducing sugar, tannins, flavonoids, saponins, gums, alkaloids, proteins, terpenoids, and acidic compounds (Table 1).

In this investigation, a computational study was undertaken to evaluate the antidepressant characteristics of the phytoconstituents of *Premna esculenta*, and the results are presented both graphically and numerically. Numerous depression treatments exist in the market, yet their success rates are about 50–60%, leaving a significant number of patients insensitive to initial medication (Nestler et al. 2002; Kiss 2008). Additionally, most depression drugs cause intolerable side effects such as arrhythmias, sexual dysfunction, insomnia, and hypotension (Demyttenaere 1997; Guadarrama-Cruz et al. 2008). Initially, the overall evaluation began with phytochemical studies, where the EEPE confirmed the presence of tannins, reducing sugars, flavonoids, saponins, gums, protein, alkaloids, and terpenoids, all of which are well-documented for their neuropharmacological effects. Particularly, flavonoids and alkaloids have been broadly reported to exert antidepressant and anxiolytic characteristics through modulation of monoaminergic systems, often via inhibition of monoamine oxidase (MAO) or serotonin transporters (SERT), resulting in preeminent synaptic levels of serotonin, norepinephrine, and dopamine (Carradori et al. 2016; Wang et al. 2019a; Yan et al. 2022). Saponins and tannins may further contribute to mood stabilizing effects through mitigating oxidative stress and neuroinflammation, although it has less direct links to neurotransmitter regulation, two key methods implicated in depressive disorder (Ikram et al. 2021; Sehati et al. 2021; Díaz et al. 2022; Zhao et al. 2024).

Table 1
Result of the chemical group test of *P. esculenta*

Phytochemical Group	Result
Reducing sugar	-
Combined reducing sugar	+
Tannins	+
Flavonoids	+
Saponin	+
Gums	+
Steroids	-
Alkaloids	+
Glycoside	-
Proteins	+
Terpenoids	+
Acidic compounds	-

+ indicates = Presence and - indicates = Absence

GC-MS analysis

The following chemicals were found in the sample by GC-MS analysis. In Fig. 3, the unique GC-MS chromatogram is shown. Table 1 lists the retention time (RT), molecular weight, and peak area (%) of the bioactive compounds detected in the sample. Of particular interest within this chemical profile is the nitrogen-containing alkaloid and aromatic amide class, exemplified by the identified phytoligand 1,2,3,4-tetrahydro-3-(phenylacetamido)quinoline (THPAC). Structurally, THPAC possesses features such as an aromatic moiety capable of π - π stacking and an amide group, which enables hydrogen bonding, that are ideally suited for interactions with monoaminergic target sites (Zhang et al. 2012; Gospodinova and Tomšík 2015; Gao et al. 2020). The attributes provide a credible explanation for both the antidepressant-like effects detected in the in vivo models and the robust target binding suggested by the computational analysis.

Time (Min)

Figure 1

GC-MS chromatogram of *Premna esculenta*

Table 2
Phytochemical composition of *Premna esculenta*

Serial No.	Retention time (RT)	Name of the compounds	Molecular weight	% Area
1	3.01	2,2'-bioxirane	86	0.05
2	8.07	Sydnone, 3,3'-tetramethylenedi-	226	0.30
3	12.87	Methyl 8-methyl-nonanoate	186	2.53
4	14.89	3-chloropropionic acid, benzyl ester	198	1.04
5	17.75	Methyl stearate	298	1.68
6	22.34	2,4-dihydroxybenzoic acid	154	1.13
7	26.39	Suberohydroxamic acid	204	0.74
8	28.97	4-(4-chloro-2-methylphenoxy) butyric acid, octadecyl ester	480	0.89
9	29.89	1,3-dioxolane, 2-heptyl	172	0.48
10	30.42	Cyclohexane, 1-methyl-4-(1-methylethenyl)-	138	0.29
11	31.52	Heptacosanoic acid, 25-methyl-, methyl ester	438	1.84
12	33.07	1,3-dioxolane, 2-heptyl	172	0.31
13	33.48	1,3-dioxolane, 2-pentadecyl	284	0.29
14	33.97	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270	17.92
15	35.45	1,3-dioxolan-4-one, 2-(1,1-dimethylethyl)-5-(1-methylethyl)-	186	0.79
16	35.91	Docosanoic acid	340	19.93
17	38.38	Neophytadiene	278	8.89
18	38.53	Methyl 2-o-methyl-. Beta. -d-xylopyranoside	178	8.53
19	39.12	9-azabicyclo [6.1.0] non-4-en-9-amine, (1. Alpha.,4z,8. Alpha.)	138	1.01
20	39.71	Cyclohexanecarboxylic acid, 1-cyclohpentylethyl ester	224	1.03
21	39.94	Methoxyflurane	164	0.81
22	40.44	Cyclopentanecarboxylic acid, 2-ethylcyclohexyl ester	224	1.44
23	41.09	3-hexene, 1-(1-ethoxyethoxy)	172	0.32
24	41.32	2-octen-4-one	126	0.67
25	42.54	Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl-	252	0.74
26	43.42	Butyl 14-methylhexadecanoate	326	8.83
27	45.40	Cyclohexanamine, n-cyclohexyl-n-nitroso	210	0.99
28	46.24	1,2,3,4-tetrahydro-3-(phenylacetamido)quinoline	266	2.56
29	47.74	Dichloroacetic acid, 2,2-dimethylpropyl ester	198	1.01
30	48.63	1-hexanol, 3,5,5-trimethyl-	144	0.78
31	50.81	2-n-propylthiolane, s,s-dioxide	162	0.29
32	58.08	Cyclobutanone, 2-(1,1-dimethylethyl)	126	0.65

Antidepressant activity

Forced swimming test.

The ethanolic extract of *Premna esculenta* (EEPE) exhibited a dose-dependent decrease in immobility period (6 min) in mice after being administered the extracts during the test of FST, as represented in Table 3 and Fig. 2, compared with the mice in the control group, which received a saline solution. 200 mg/kg body weight of EEPE extract showed a very significant reduction in the period of immobility (33.4 ± 2.61) when it was administered to the mice in the EEPE 100 and 50 mg/kg groups, though all these groups showed noteworthy effects compared to CNS depressant drugs (176.2 ± 3.11).

Table 3
Effect of *Premna esculenta* extract and diazepam on the forced swimming test.

Treatment	Dose mg/kg	Immobility Time (sec) $\pm$ SD
Control	0.1 mL/mouse	63.6 ± 1.82
Diazepam	1	176.2 ± 3.11 ***
EEPS	50	50.4 ± 0.89 ***
EEPS	100	42.2 ± 1.64 ***
EEPS	200	33.4 ± 2.61 ***
Values are presented as mean $\pm$ SD (n = 5). EEPE = Ethanolic extract of <i>Premna esculenta</i> ***p < 0.001, vs. control, SD = Standard Deviation		

Tail-suspension test

The ethanolic extract of *Premna esculenta* (EEPE) demonstrated a dose-dependent decrease of the immobility period (5 min) in mice after being administered the extracts during the test of TST, as represented in Table 4 and Fig. 3, compared with the mice in the control group, which received a saline solution. 200 mg/kg body weight of EEPE extract indicated a very significant reduction in the period of immobility (48.2 ± 2.17) when it was administered to the mice in the EEPE 100 and 50 mg/kg groups, though all these groups exhibited noteworthy effects compared to CNS depressant drugs (165.07 ± 1.95).

Table 4
Effects of *Premna esculenta* extract and diazepam on the tail suspension test.

Treatment	Dose mg/kg	Immobility Time (sec) $\pm$ SD
Control	0.1 mL/mouse	87.4 ± 1.52
Diazepam	1	180.6 ± 1.95 ***
EEPE	50	72.4 ± 0.55 *
EEPE	100	61.8 ± 1.30 **
EEPE	200	48.2 ± 2.17 ***
Values are presented as mean $\pm$ SD (n = 5). EELS = Ethanolic extract of <i>Premna esculenta</i> ***p < 0.001, vs. control, SD = Standard Deviation		

Figure 3. Effects of the ethanolic leaves extract administration on the immobility time in the Tail suspension test.

The forced swimming test (FST) and tail suspension test (TST) are widely recognized methods for identifying antidepressant activity. For this study, the behavior assessment characteristic of immobility is considered analogous to human depression. In mice, the capability to decrease immobility periods is close to the drug's antidepressant potential (Wesołowska et al. 2007; Rosas-Sánchez et al. 2022). In behaviour studies, the ethanolic extract of *Premna esculenta* (EEPE) significantly and dose-dependently reduced the time of immobility in both methods of FST and TST. At the highest tested dose of 200 mg/kg, the immobility time was reduced to 33.4 ± 2.61 seconds in the FST, and TST showed 48.2 ± 2.17 seconds, suggesting an extensive increase in active behaviours when compared to control animals. These consequences are considered highly predictive of antidepressant activity, specifically for the agents that increase monoaminergic neurotransmission by blocking SERT, the

norepinephrine transporter (NET), or inhibiting MAO-A (Haenisch and Bönisch 2011; Wang et al. 2019b; Martín-Hernández et al. 2021). Given the phytochemical constituents of EEPE and the molecular docking data for THPAC, it is reasonable to conclude that the antidepressant-like effects may result from the simultaneous inhibition of serotonin reuptake and monoamine degradation. This dual effects mechanism is significant because most existing antidepressants concentrate on a single primary target. SSRIs like paroxetine act primarily at SERT, and MAOIs like clorgyline, MAO-A, where THPAC shows potential for high-affinity binding to both (Pacher and Kecskemeti 2004; Sharma et al. 2023).

Evaluation of Acute Toxicity Test

The acute toxicity evaluation exposed no significant alterations in mean body weight between the experimental groups and the control on both the first and second days of observation. Extract-I (300 mg/kg) demonstrated a slight decrease in mean weight on the first day, with no potential toxicity at this dosage. Similarly, Extract-II (2000 mg/kg) induced no significant reduction in mean weight, indicating no toxicity compared to the control. Conversely, Extract-III (5000 mg/kg) showed a minimal increase in mean weight on the first day, hinting that there is no toxicity effect. These results highlight a dose-dependent toxicity pattern, with all extracts exhibiting no significant toxicity (Tables 4 and 5 and Fig. 3), and no behavioral change was found. Further investigation into the underlying mechanisms driving these effects is crucial for understanding the safety profile of these extracts.

Table 4
Effects of ethanolic extract of *Premna esculanta* leaves on the body weight of mice.

Group	1st day Mean Wt ± SEM	2nd day Mean Wt ± SEM
Conrol	26.6 ± 0.97	26.3 ± 0.75
Extract-I (300 mg/kg)	18.2 ± 0.68	18.5 ± 0.71
Extract-II (2000mg/kg)	15.8 ± 0.50	15.6 ± 0.86
Extract-III (5000mg/kg)	22.0 ± 1.41	22.9 ± 0.66

Table 5
General appearance and behavioural observations of the acute toxicity study for control and treated groups

Observation	Group-I (control)	Group-II (300 mg/kg)	Group-III (2000mg/kg)	Group-IV (5000mg/kg)
Food intake	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Moderate
Drowsiness	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal

In silico studies

Molecular Docking Analysis

The current study aimed to identify the most promising phytoligand with antidepressant potential through molecular docking and pharmacokinetic evaluation against two major targets implicated in depression: the serotonin transporter (SERT) and monoamine oxidase A (MAO-A). Paroxetine and Clorgyline served as standard reference drugs for SERT and MAO-A, respectively. Among the tested ligands, 1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline (THPAC) demonstrated the highest binding affinity toward both SERT and MAO-A, with a docking score of -9.3 kcal/mol for each, thereby establishing itself as a dual inhibitor in

Tables 6 and 7. For SERT, Paroxetine showed a slightly better docking score of -10.8 kcal/mol, while Clorgyline exhibited a weaker affinity of -7.5 kcal/mol for MAO-A. The binding energy of 1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline to MAO-A was more favorable than the standard, indicating potential for enhanced inhibitory efficacy (Owoloye et al. 2022). We have taken the cut-off value for the SERT top 8, as the standard (paroxetine) showed the best binding affinity. On the other hand, for the MAO-A, we selected the docking score of phytocompound before the standard docking score, as we saw that the standard (clorgyline) demonstrated a comparatively low score.

In terms of docking, which provided further insight into the molecular basis of this activity. THPAC bound strongly with the protein of SET (−9.3 kcal/mol) and MAO-A (−9.3 kcal/mol), values almost similar to the standard of paroxetine for SERT (−10.8 kcal/mol) and higher than the affinity for clorgyline for MAO (−7.5 kcal/mol). At the SERT site, THPAC showed in carbon-hydrogen bonding with ASP98 and SER438, π–π T-shaped interactions with TYR176 and PHE341, and π–alkyl contacts with ILE172, ALA169, ALA173, and VAL501. The phenylacetamido group likely acts as an aromatic pharmacophore, enabling hydrophobic and stacking interactions, whereas the tetrahydroquinoline framework occupies hydrophobic pockets, reinforcing binding stability. In MAO-A, THPAC formed π–sulfur bonds with CYS406, π–π T-shaped stacking with PHE352, TRP397, and TYR407, as well as amide-π stacking with GLY66 residues essential for orienting ligands within the flavin adenine dinucleotide (FAD) containing active site (Son et al. 2008; Hakeem et al. 2025). This outline of interactions advocates the classification of THPAC as a potential dual inhibitor, which is capable of reducing serotonin clearance both by transport blockade and by enzymatic degradation.

Table 6
Paroxetine and Pytoligand docking score with SERT

Ligand	Binding Affinity (kcal/mol)
Paroxetine	-10.8
1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	-9.3
Cyclohexanecarboxylic acid, 1-cyclohpentylethyl ester	-7.7
Cyclopentanecarboxylic acid, 2-ethylcyclohexyl ester	-7.6
N-Nitrosodicyclohexylamine	-7.3
Tetradecanoic acid, 10,13-dimethyl-, methyl ester	-7.1
Sydnone, 3,3'-tetramethylenedi-	-7
Neophytadiene	-6.9

Table 7
Clorgyline and Pytoligand docking score with MAO-A

Ligand	Binding Affinity (kcal/mol)
1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	-9.3
Cyclohexanecarboxylic acid, 1-cyclohpentylethyl ester	-8.3
Neophytadiene	-8
1-(1,2-Dimethylpropyl)-1-methyl-2-nonylcyclopropane	-7.9
Sydnone, 3,3'-tetramethylenedi-	-7.7
N-Nitrosodicyclohexylamine	-7.6
Clorgyline	-7.5

ADME & Toxicity Analysis

The assessment of pharmacokinetic and toxicity of standard drugs and all screened phytoligands, as summarized in Tables 8 and 9, shows that most compounds follow Lipinski's Rule of Five, except Neophytadiene, Tetradecanoic acid, and 1-(1,2-Dimethylpropyl)-1-methyl-2-nonylcyclopropane, which each exhibited a single violation due to high LogP values, indicating elevated lipophilicity and potential bioavailability constraints. Among all the identified candidates, THPAC emerged as the most promising phytocompound, with an optimal molecular weight (266.34 g/mol) compared to paroxetine (329.37 g/mol) and clorgyline (272.17 g/mol), and well-balanced hydrophobicity (LogP 2.47). A favorable topological polar surface area (TPA 41.13 Å²), all of these results indicate that it has efficient membrane permeability and good oral absorption.

The compound demonstrated high intestinal absorption (95.80%), and strong Caco-2 cell permeability (1.352), values close to Paroxetine (1.755) and only slightly lower than Clorgyline (1.352), while upholding moderate water solubility (LogS – 3.648), superior to highly lipophilic ligands such as Neophytadiene, which has the value of LogS – 8.559. In terms of distribution insights, it demonstrated a BBB permeability of 0.246, which is slightly higher than Paroxetine (0.223) but well below Clorgyline (0.690), suggesting that it has adequate central nervous system penetration, potentially with fewer CNS-related side effects. Its CNS permeability index (-1.589) was also closely similar to Paroxetine (-1.443). Metabolism forecasts indicated that neither these phytocompounds nor the reference drugs are likely to be CYP450 substrates, reducing the threat of rapid clearance and drug-drug interactions. For excretion, the total clearance rate of THPAC (0.271 mL/min/kg) was notably lower than both standards of Paroxetine (0.829 mL/min/kg) and Clorgyline (0.821 mL/min/kg), indicating a potentially prolonged systemic half-life and more sustained plasma concentration levels. Furthermore, toxicity assessment predicted no hepatotoxicity or hERG channel inhibition for the phytoligands and the standards, and the acute toxicity value (LD₅₀ 2.298 mol/kg) also fell within the non-toxic range. Collectively, THPAC showed a balanced ADME and toxicity profile compared to standards.

Although Cyclohexanecarboxylic acid, 1-cyclopentylethyl ester exhibited acceptable pharmacokinetic properties, its affinity to bind receptors (–7.7 kcal/mol with SERT and –8.3 kcal/mol with MAO-A) was lower as compared to that of 1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline (–9.3 kcal/mol with both targets), which showed higher interaction with the receptors. Additionally, the chemical and metabolic instability of the ester functional group causes it to be chemically and metabolically unstable as esters are highly hydrolyzed by esterases, thereby resulting in poor stability, short half life and unpredictable pharmacokinetics. It also has a low solubility (LogS – 4.539) that further restricts dissolution and oral absorption. Conversely, 1,2,3,4-tetrahydro-3-(phenylacetamido)quinoline had a balanced pharmacokinetic profile, good solubility and high intestinal absorption (95.79%), and high target binding, which indicated a high level of efficacy and stability. Thus, it was chosen as the most promising one, and the ester form was avoided because of low stability, low solubility, and low binding affinity.

The pharmacokinetics predictions also enhance the THPAC profile. The compound fulfilled all the requirements of the Lipinski Rule of Five, with the following properties: a molecular weight of 266.34 g/mol, a balanced LogP of 2.47, and a topological polar surface area (TPSA) of 41.13 Å²; parameters associated with good oral bioavailability and membrane permeability (Martin 2005; Ma et al. 2021; Caminero Gomes Soares et al. 2023). Its estimated intestinal absorption was high (95.8%), and Caco-2 permeability (1.352) was comparable to paroxetine (1.755) and clorgyline (1.352). It indicated a moderate permeability rate to the blood-brain barrier (BBB) (0.246), which is greater than that of paroxetine (0.223) or clorgyline, therefore possibly giving adequate access to the CNS without excessively high central adverse effects (Gupta et al. 2019; Sánchez-Dengra et al. 2023). The predictions of clearance (0.271 mL/min/kg) were significantly lower than those of paroxetine (0.829) and clorgyline (0.821), and hence their tendency to have a longer systemic residence (Smith et al. 2018). Predictions of toxicity were especially favourable: THPAC was non-hepatotoxic, non-carcinogenic, and non-immunotoxic as compared with paroxetine, predicted to be hepatotoxic and immunotoxic, and clorgyline predicted to be carcinogenic and immunotoxic. The absence of the hERG channel prediction among all compounds is a positive indication with regard to cardiovascular safety (Kalyaanamoorthy and Barakat 2018; Creanza et al. 2021).

Table 8
Physicochemical and Absorption Properties of Standard and Phytoligands

Molecule Name	MW (g/mol)	LogP	HBD	HBA	MR	TPSA (Å ²)	Lipinski	Solubility	Caco2 (P)	Int. Abs (%)
Paroxetine	329.37	3.5	1	5	92.1	39.72	Yes; 0 violation	-4.082	1.755	96.879
Clorgyline	272.17	3.71	0	2	72.9	12.47	Yes; 0 violation	-3.411	1.602	91.972
1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	266.34	2.47	2	1	83.4	41.13	Yes; 0 violation	-3.648	1.352	95.795
Cyclohexanecarboxylic acid, 1-cyclopentylethyl ester	224.34	3.64	0	2	66.5	26.3	Yes; 0 violation	-4.539	1.537	98.134
Cyclopentanecarboxylic acid, 2-ethylcyclohexyl ester	224.34	3.52	0	2	66.5	26.3	Yes; 0 violation	-4.058	1.5	98.134
N-Nitrosodicyclohexylamine	210.32	3.12	0	2	63.2	32.67	Yes; 0 violation	-3.845	1.177	96.745
Tetradecanoic acid, 10,13-dimethyl-, methyl ester	270.45	5.4	0	2	85.1	26.3	Yes; 1 violation	-6.691	1.631	92.96
Sydone, 3,3'-tetramethylenedi-	228.21	-2.7	2	4	53.3	99.76	Yes; 0 violation	-2.402	-0.214	62.283
Neophytadiene	278.52	7.07	0	0	97.3	0	Yes; 1 violation	-8.559	1.425	92.85
1-(1,2-Dimethylpropyl)-1-methyl-2-nonylcyclopropane	252.48	6.48	0	0	86.3	0	Yes; 1 violation	-8.121	1.405	94.586

MW = Molecular Weight, HBD = H-Bond Donors, HBA = H-Bond Acceptors, MR = Molar Refractivity, TPSA = Topological Polar Surface Area, P = Permeability, Int. Abs = Intestinal Absorption

Table 9
Distribution, Metabolism, Excretion & Toxicity of Standard and Phytoligands

Molecule Name	BBB (P)	CNC (P)	Metabolism	Clearance	Rat Toxicity	Hepatotoxicity	hERG Inhib.
Paroxetine	0.223	-1.443	No	0.829	2.486	No	No
Clorgyline	0.69	-1.829	No	0.821	2.805	No	No
1,2,3,4-Tetrahydro-3-(phenylacetamido)quinoline	0.246	-1.589	No	0.271	2.298	No	No
Cyclohexanecarboxylic acid, 1-cyclopentylethyl ester	0.334	-1.202	No	1.04	1.975	No	No
Cyclopentanecarboxylic acid, 2-ethylcyclohexyl ester	0.318	-1.896	No	1.072	2.187	No	No
N-Nitrosodicyclohexylamine	0.317	-3.021	No	1.365	2.753	No	No
Tetradecanoic acid, 10,13-dimethyl-, methyl ester	0.732	-1.562	No	1.632	1.609	No	No
Sydnone, 3,3'-tetramethylenedi-	-0.897	-3.61	No	0.725	2.151	No	No
Neophytadiene	0.983	-1.299	No	1.764	1.473	No	No
1-(1,2-Dimethylpropyl)-1-methyl-2-nonylcyclopropane	0.947	-1.366	No	1.386	1.566	No	No

BBB (P) = Blood–Brain Barrier Permeability, CNC (P) = Central Nervous System Permeability, Clearance = Total Clearance, hERG Inhib. = hERG Channel Inhibition, Hepatotox = Hepatotoxicity

Post-docking analysis

Detailed analysis of the binding interactions revealed that THPAC engaged with several critical amino acid residues at the SERT binding site. It formed carbon-hydrogen bonds with ASP98 and SER438, and hydrophobic π - π T-shaped interactions with TYR176 and PHE341 (Table 10, Fig. 4, and Fig. 5). Additional π -alkyl interactions with ILE172, ALA169, ALA173, and VAL501 further stabilized the binding. These interactions are crucial as they overlap with key residues involved in serotonin reuptake inhibition (Ferreira et al. 2018). In contrast, Paroxetine formed strong conventional hydrogen bonds with TYR95 and ALA96, as well as substantial amide- π stacking interactions with SER438 and SER439, which explain its higher affinity (Coleman et al. 2020).

In the case of MAO-A, THPAC quinoline demonstrated significant binding interactions, including π -sulfur bonding with CYS406, π - π T-shaped stacking with PHE352, TRP397, and TYR407, and amide- π stacking with GLY66. These residues are integral to the MAO-A active site, and their engagement is indicative of potent inhibition. Clorgyline, although effective, displayed comparatively fewer and less diverse interactions, primarily hydrophobic π -alkyl contacts with TYR407 and PHE352 (Negi et al. 2025).

Table 10 Binding interactions of compounds with SERT and MAO-A, including standard references.

Ligand / Standard	Name	Distance (Å)	Category	Type / Types
THPAC (SERT)	SER438	3.425	Hydrogen Bond	Carbon-Hydrogen Bond
	ASP98	3.458	Hydrogen Bond	Carbon-Hydrogen Bond
	ILE172	3.384	Hydrophobic	Pi-Sigma
	TYR176	5.625	Hydrophobic	Pi-Pi T-shaped
	PHE341	5.016	Hydrophobic	Pi-Pi T-shaped
	SER439:N	5.117	Hydrophobic	Amide-Pi Stacked
	SER438:C,O	5.117	Hydrophobic	Amide-Pi Stacked
	ILE172	5.209	Hydrophobic	Pi-Alkyl
	VAL501	4.961	Hydrophobic	Pi-Alkyl
	ALA169	5.241	Hydrophobic	Pi-Alkyl
	ALA173	5.225	Hydrophobic	Pi-Alkyl
Paroxetine (SERT)	TYR95	1.890	Hydrogen Bond	Conventional Hydrogen Bond
	ALA96	2.714	Hydrogen Bond	Conventional Hydrogen Bond
	SER336	3.489	Hydrogen Bond	Carbon-Hydrogen Bond
	ALA169	3.492	Hydrogen Bond	Carbon-Hydrogen Bond
	ILE172	3.663	Hydrophobic	Pi-Sigma
	ILE172	3.819	Hydrophobic	Pi-Sigma
	TYR176	4.788	Hydrophobic	Pi-Pi T-shaped
	PHE341	5.102	Hydrophobic	Pi-Pi T-shaped
	SER438:C,O	4.298	Hydrophobic	Amide-Pi Stacked
	SER439	4.298	Hydrophobic	Amide-Pi Stacked
	ALA169	4.572	Hydrophobic	Pi-Alkyl
	ALA173	4.482	Hydrophobic	Pi-Alkyl
	VAL501	5.350	Hydrophobic	Pi-Alkyl
THPAC (MAO-A)	CYS406	4.793	Other	Pi-Sulfur
	PHE352	5.117	Hydrophobic	Pi-Pi T-shaped
	TRP397	5.765	Hydrophobic	Pi-Pi T-shaped
	TYR407	4.728	Hydrophobic	Pi-Pi T-shaped
	GLY66:C,O	3.658	Hydrophobic	Amide-Pi Stacked
	GLY66:N	3.658	Hydrophobic	Amide-Pi Stacked
	ALA68	4.991	Hydrophobic	Alkyl
	TYR407	4.890	Hydrophobic	Pi-Alkyl
	VAL303	5.302	Hydrophobic	Pi-Alkyl
	LYS305	5.109	Hydrophobic	Pi-Alkyl
	ILE335	5.296	Hydrophobic	Pi-Alkyl

Ligand / Standard	Name	Distance (Å)	Category	Type / Types
Clorgyline (MAO-A)	GLY443:O	3.419	Hydrogen Bond	Carbon-Hydrogen Bond
	GLY443:O	3.459	Hydrogen Bond	Carbon-Hydrogen Bond
	GLY443	3.227	Halogen	Halogen (Cl, Br, I)
	TYR407	3.927	Hydrophobic	Pi-Pi Stacked
	ARG51	4.764	Hydrophobic	Alkyl
	CYS406	4.586	Hydrophobic	Alkyl
	ALA448	3.977	Hydrophobic	Alkyl
	ILE23	4.196	Hydrophobic	Alkyl
	MET445	5.165	Hydrophobic	Alkyl
	TYR69	4.967	Hydrophobic	Pi-Alkyl
	PHE352	4.910	Hydrophobic	Pi-Alkyl
	TYR407	5.368	Hydrophobic	Pi-Alkyl
	TYR407	5.100	Hydrophobic	Pi-Alkyl

Deep Toxicity Analysis and Pass Prediction

Toxicological profiling using the ProTox-II platform indicated that THPAC possessed a favorable safety profile compared to the reference drugs. It was predicted as non-hepatotoxic and non-carcinogenic, and exhibited no immunotoxic potential (Probabilities: 0.76, 0.71, and 0.99, respectively). In contrast, paroxetine was predicted to be hepatotoxic (0.69), immunotoxic (0.96), and mildly neurotoxic (0.87). Clorgyline showed carcinogenic potential (0.54) and moderate immunotoxicity (0.80). All three compounds were predicted to be non-mutagenic and non-cytotoxic; however, the phytoligand THPAC consistently demonstrated a lower risk across multiple endpoints. These findings suggest that, despite its modest predicted antidepressant activity, THPAC offers a promising alternative due to its anxiolytic action and superior toxicological safety (Tables 11 and 12 and Fig. 6).

PASS prediction analysis revealed distinct differences in the predicted pharmacological activities of the standard drugs and the phytoligand (THPAC) derived from *Premna esculenta* Table 12. Paroxetine exhibited the highest probability for neurotransmitter uptake inhibition (Pa = 0.775) and antidepressant activity (Pa = 0.667), indicating its strong potential in classical antidepressant pathways. Clorgyline, in contrast, showed moderate probability for neurotransmitter inhibition (Pa = 0.516) and exhibited the highest likelihood for Alzheimer's disease-related activity (Pa = 0.723). Interestingly, THPAC displayed the most promising anxiolytic potential (Pa = 0.618), surpassing both paroxetine and clorgyline, and also showed moderate Alzheimer-related activity (Pa = 0.366). However, its predicted antidepressant activity was comparatively lower (Pa = 0.200), suggesting it may exert its effects primarily through anxiety-relieving and neuroprotective mechanisms rather than classic serotonergic pathways.

The concerns of the structure-activity relationship (SAR) highlight the activity of the functional groups of the THPAC. This compound is a derivative of 1,2,3,4-tetrahydroquinoline, which has lots of pharmacological activities such as anticancer, antidepressant, anti-inflammatory, and antimicrobial activities (Bobowski et al. 1980; Ivanov et al. 2011; Marella et al. 2013; Meléndez et al. 2024). The phenylacetamido substituent adds aromatic 2-p stacking possibilities and hydrogen bond donor/acceptor groups, increasing the binding to both SERT and MAO-A (Wheeler 2011; Zhuang et al. 2019; Ortore et al. 2020; Suryawanshi et al. 2022). The tetrahydroquinoline central motif enhances lipophilicity and leads to a scaffold that associates in hydrophobic areas existing in each target. The fluorophenyl ether and a piperidine ring of Paroxetine contribute to its SERT affinity, and by inserting a propargylamine group, the combination of clorgyline irreversibly binds to the MAO-A FAD cofactor (Owens et al. 2001; Finberg and Rabey 2016). The hybrid pharmacophore structure of THPAC combines structures observed in each of the classes of drugs, and this is the reason why it has dual-target activity.

PASS prediction analysis demonstrated that the estimated THPAC (Pa = 0.200) antidepressant probability was lower (Pa = 0.667) than the paroxetine case. Nonetheless, the anxiolytic activity outcome (Pa = 0.618) was better than both standards. It brings into question a wider possibility of psychotropic effect profile potentially including secondary effects at GABAergic or glutamatergic transmission in addition to monoaminergic pathways (Micó and Prieto 2012; Kaygisiz et al. 2015). The relatively high possibility of Alzheimer-related effects (Pa = 0.366) in the compound also corresponds with the earlier findings of the ability of alkaloid and flavonoid derivatives to mediate neuroprotective effects with the help of antioxidant, anti-inflammatory, and anti-amyloid properties (Ayaz et al. 2019; Calderaro et al. 2022; Utpal et al. 2025). This multi-target potential may have value in patients with mood disorders compounded by cognitive worsening. These results showed there is a possibility of becoming an alternative drug for Alzheimer's disease, as the THPAC natural compound has good activity against MAO-A and SERT, which are related to the generation of Alzheimer's disease (Tian et al. 2023).

Table 11
Pro-Tox II Toxicological parameters of the identified phytoconstituents and ligands

Classification	Target	Paroxetine		Clorgyline		THPAQ	
		Pre	Pro	Pre	Pro	Pre	Pro
Organ toxicity	Hepatotoxicity	A	0.69	I	0.83	I	0.76
	Neurotoxicity	A	0.87	A	0.78	A	0.77
Toxicity end points	Carcinogenicity	I	0.62	A	0.54	I	0.71
	Immunotoxicity	A	0.96	A	0.8	I	0.99
	Mutagenicity	I	0.97	I	0.65	I	0.55
	Cytotoxicity	I	0.93	I	0.76	I	0.64
Tox21-Nuclear receptor signalling pathways	AhR	I	0.97	I	0.92	I	0.52
	AR	I	0.99	I	0.99	I	0.91
	AR-LBD	I	0.99	I	0.99	I	0.96
	Aromatase	A	1	I	0.92	I	0.86
	ER	A	0.99	A	0.89	I	0.84
	ER-LBD	A	1	I	0.98	I	0.98
	PPAR-Gamma	I	0.99	I	0.99	I	0.96
Tox21-Stress response pathways	nrf2/ARE	I	0.88	I	0.97	I	0.95
	HSE	I	0.88	I	0.97	I	0.95
	MMP	I	0.7	I	0.81	I	0.83
	p53	I	0.96	I	0.96	I	0.87
	ATAD5	I	0.99	I	0.98	I	0.92

A: Active; **I:** Inactive; **Pre:** Predicted class; **Pro:** Probability; **AhR:** Aryl hydrocarbon receptor; **AR:** Androgen receptor; **AR-LBD:** Androgen receptor–ligand binding domain; **ER:** Estrogen receptor; **ER-LBD:** Estrogen receptor–ligand binding domain; **PPAR-γ:** Peroxisome proliferator-activated receptor gamma; **nrf2/ARE:** Nuclear factor erythroid 2–related factor 2/Antioxidant response element; **HSE:** Heat shock element; **MMP:** Mitochondrial membrane potential; **p53:** Tumor protein p53; **ATAD5:** ATPase family AAA domain-containing protein 5

Table 12
PASS prediction of standard drug and selected bioactive compounds
of *Premna esculenta*

Molecule	Characteristic	Pa	Pi
Paroxetine	Neurotransmitter uptake inhibitor	0.775	0.005
	Antidepressant	0.667	0.009
	Anxiolytic	0.388	0.043
	Alzheimer's disease	0,217	0.005
Clorgyline	Neurotransmitter uptake inhibitor	0.516	0.055
	Antidepressant	0.171	0.132
	Anxiolytic	0.294	0.112
	Alzheimer's disease	0.723	0.003
THPAC	Neurotransmitter uptake inhibitor	0.263	0.263
	Antidepressant	0.2	0.108
	Anxiolytic	0.618	0.063
	Alzheimer's disease	0.366	0.036
Pa: Probability "Active"; Pi: Probability "Inactive"			

Density Functional Theory insights

The frontier molecular orbital energies and related quantum chemical descriptors of Paroxetine, Clorgyline, and the phytocompound THPAC were computed to evaluate their electronic behavior and reactivity (Table 13 and Fig. 7). Paroxetine exhibited a HOMO energy of -0.29178 Hartree and a LUMO energy of -0.03553 Hartree, resulting in an energy gap (ΔE) of 0.25625 Hartree. Clorgyline showed a HOMO of -0.23227 Hartree and a LUMO of -0.03563 Hartree, corresponding to a slightly smaller gap of 0.19664 Hartree. Remarkably, THPAC demonstrated the highest HOMO (-0.19575 Hartree) and the least negative LUMO (-0.00780 Hartree), yielding the lowest energy gap of 0.18795 Hartree among the three compounds.

A lower HOMO–LUMO gap generally indicates higher chemical reactivity and better potential for electron transfer, which may enhance the interaction between a ligand and its biological target. The softness (S) and hardness (η) values further support this trend. THPAC exhibited the lowest hardness (0.09398 Hartree) and the highest softness (10.6411 Hartree $^{-1}$), indicating greater molecular polarizability and reactivity compared to Clorgyline ($\eta = 0.09832$, $S = 10.1709$) and Paroxetine ($\eta = 0.12813$, $S = 7.8049$). These values suggest that THPAC has a more favorable electronic profile for bioactivity.

Collectively, these findings reveal that THPAC surpasses both standard compounds in terms of chemical softness and frontier orbital behavior, highlighting its potential as a promising ligand. Its superior electronic characteristics suggest a greater ability to participate in binding interactions, warranting further *in silico* and experimental validation.

Such results were advocated by electronic structure simulations based on density functional theory (DFT). The HOMO-LUMO gap was the least in the case of THPAC (0.18795 Hartree) compared to the rest of the compounds, which implied more chemical activity and potential to transfer electrons upon binding to the target (De Lile et al. 2020; Sim et al. 2022). There is high softness (10.6411 Hartree $^{-1}$) and low hardness (0.09398 Hartree) signatures, which show a high polarizability of molecules, which may have benefited its versatility to various conformations of the binding sites. Its structural pharmacophore enhances the likelihood of effective binding to transporter and enzyme targets, together with these features.

Table 13
Energy of HOMOs, LUMO, gap, hardness, and softness (all units are in Hartree) of Standard and Phytoligand

Name	ϵ HOMO	ϵ LUMO	Gap	η (Hardness)	S (Softness)
Paroxetine	-0.29178	-0.03553	0.25625	0.12813	7.8049
Clorgyline	-0.23227	-0.03563	0.19664	0.09832	10.1709
THPAC	-0.19575	-0.00780	0.18795	0.09398	10.6411

Altogether, the convergence of in vivo, docking, ADMET, and quantum chemical data supports the potential of THPAC as a dual-action antidepressant candidate. By simultaneously inhibiting serotonin reuptake and MAO-A activity, it could achieve faster onset and broader efficacy compared to single-target SSRIs or MAOIs, while maintaining a superior predicted safety profile. The phenylacetamido and tetrahydroquinoline groups appear central to this pharmacological versatility and could provide a foundation for further medicinal chemistry optimisation.

However, the findings are not without caveats. The relatively modest PASS predicted the probability of antidepressants, which indicates that further behavioral studies, particularly in learned helplessness models or chronic stress, are needed to validate the efficacy profile of extracts. In addition, there is the possibility of irreversible inhibition of MAO-A, as has been seen with some compounds of the quinoline, which needs to be investigated in enzyme kinetics studies in order to eliminate safety issues. The anxiolytic and neuroprotective properties of the compound, as predicted, are also open to further study in terms of polypharmacology through receptor profiling and validation using disease models. Future investigation into its actions on neurotrophic signalling pathways (including brain-derived neurotrophic factor (BDNF) expression) as well as its metabolic stability in human liver microsomes will be necessary to drive clinical translation.

Overall, THPAC provides a unique blend of powerful dual-target occupancy and a favourable pharmacokinetic and toxicological characterization of the compound. This hybridisation, in terms of structure and mechanism, places it as a prospective scaffold in the formulation of next-generation antidepressants. Its individual activity profile protects full preclinical investigation, and its contribution can increase the range of depression and comorbid neuropsychiatric diseases of treatment.

Conclusion

The current study reveals that the ethanolic extract of *Premna esculenta*, particularly the alkaloid-amide phytoligand 1,2,3,4-tetrahydro-3-(phenylacetamido)quinoline (THPAC), has potential dual-action antidepressant properties. By integrating in vivo, in silico, ADMET, and DFT studies, it was demonstrated that THPAC displays a high affinity with SERT and the MAO-A FAD-bound catalytic center, providing synergistically effective boosting of monoamine levels. Its desirable pharmacokinetics, envisaged safety, and the combination of pharmacophores of SERT and MAOIs in a functional group design justify its presence as a new lead in the neuropsychiatric drug development arena. In addition to the effect of reducing depression, anxiolytic and neuroprotective properties have been predicted, which indicates the possible usefulness in mood and/or cognitive comorbidities. The results are indicative of a good reason to progress THPAC to fully preclinical experiments to come up with a next-generation antidepressant with greater efficacy and tolerability.

Declarations

CRedit authorship contribution statement

Md Foyzur Rahman, Md Shawon Miah, As-Sazzad Mahmud: Writing-original draft & editing, Software, Validation, Methodology, Formal analysis, Resources, Lab work, Investigation, Data curation, Visualization, and editing. They contributed equally. **Md. Mostaque Hasnat:** Writing-original draft & editing, Data curation, Visualization. **Al Mamun Shohag, Hafijul Yousuf:** Writing-editing, Resources, Formal analysis, Resources under the supervision of As-Sazzad Mahmud. **As-Sazzad Mahmud:** Supervised the whole project, Methodology, review & editing, Validation, Evaluation, Data curation, and finalization. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be available after providing the request.

Declaration of competing interests

The authors state that they have no known competing financial benefits or personal relationships that could have appeared to impact the work described in this paper.

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Figures

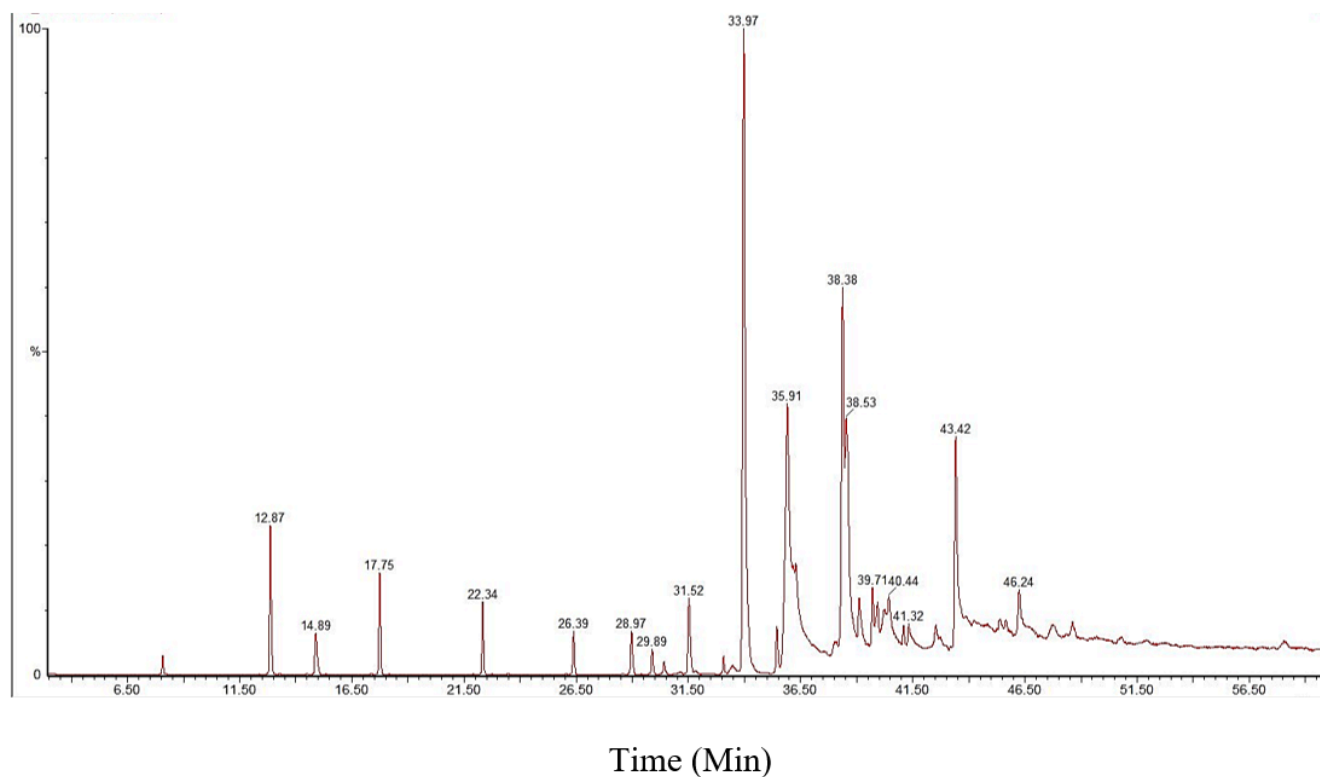


Figure 1

GC-MS chromatogram of *Premna esculenta*

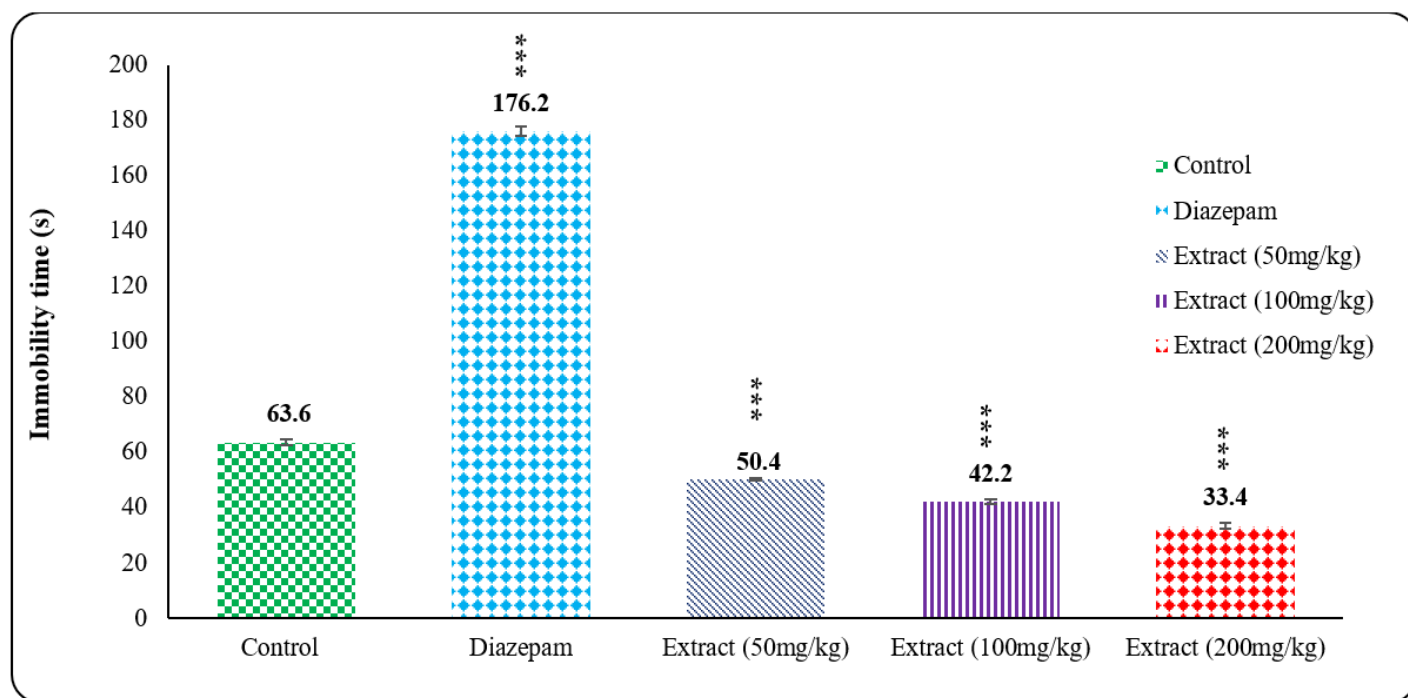


Figure 2

Effects of extract administration on the immobility time in the Forced Swim Test.

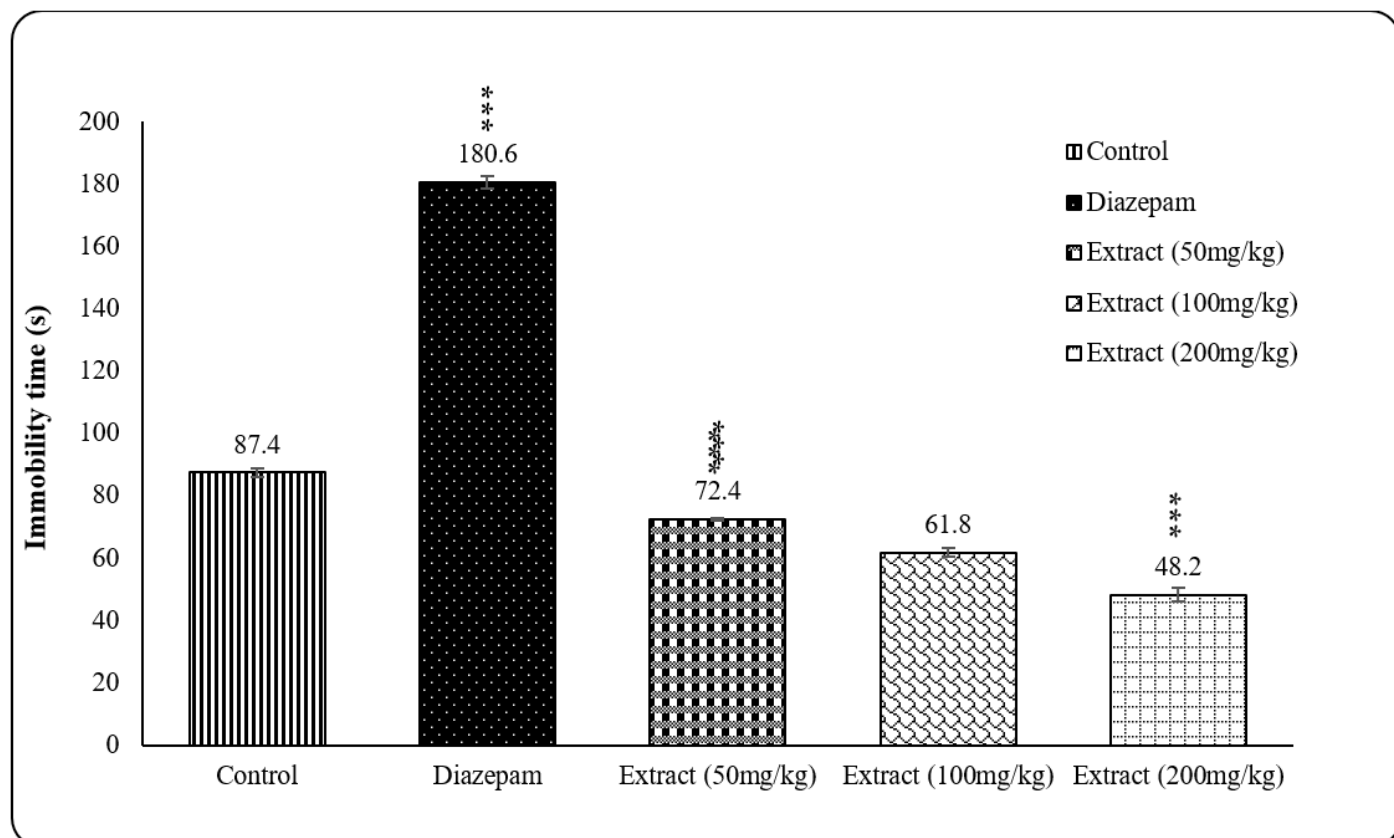


Figure 3

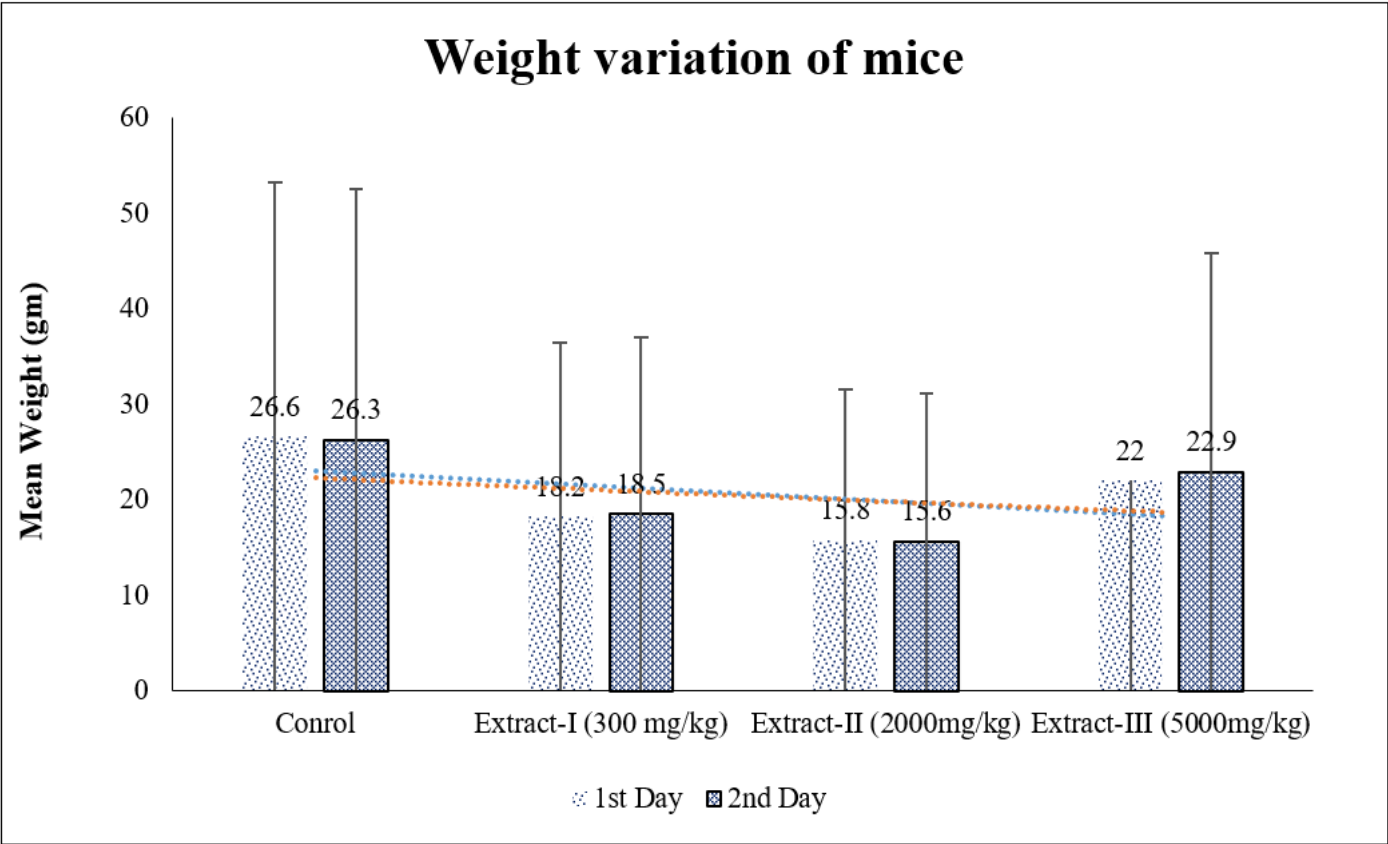


Figure 4

Fig. 3. Effects of ethanolic extract of *Premna esculanta* leaves on the body weight of mice

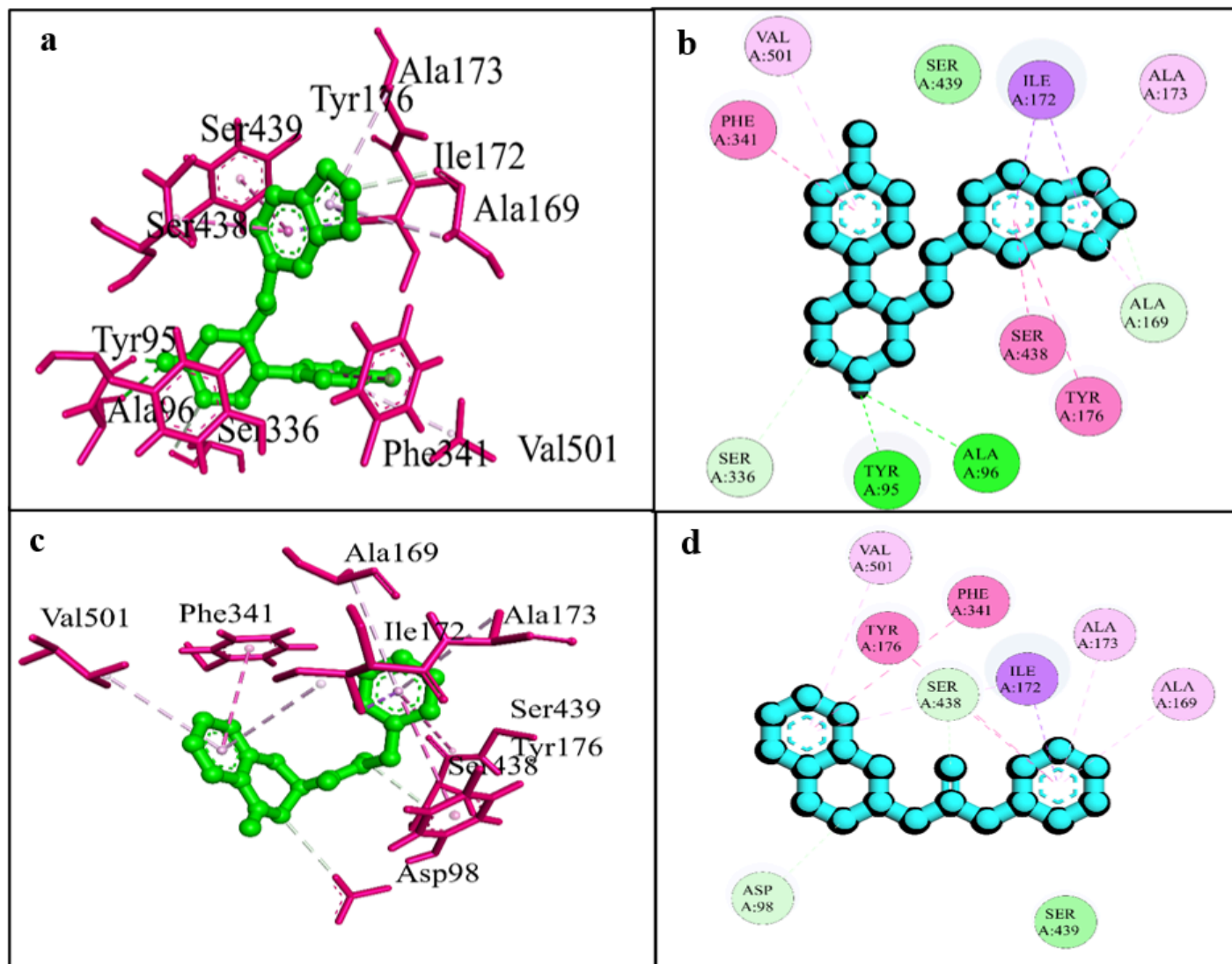


Figure 5

Fig 4. Three-dimensional (3D) docking poses (left) and two-dimensional (2D) interaction diagram (right) for paroxetine with SERT (**a** and **b**) and THPAC with SERT (**c** and **d**)

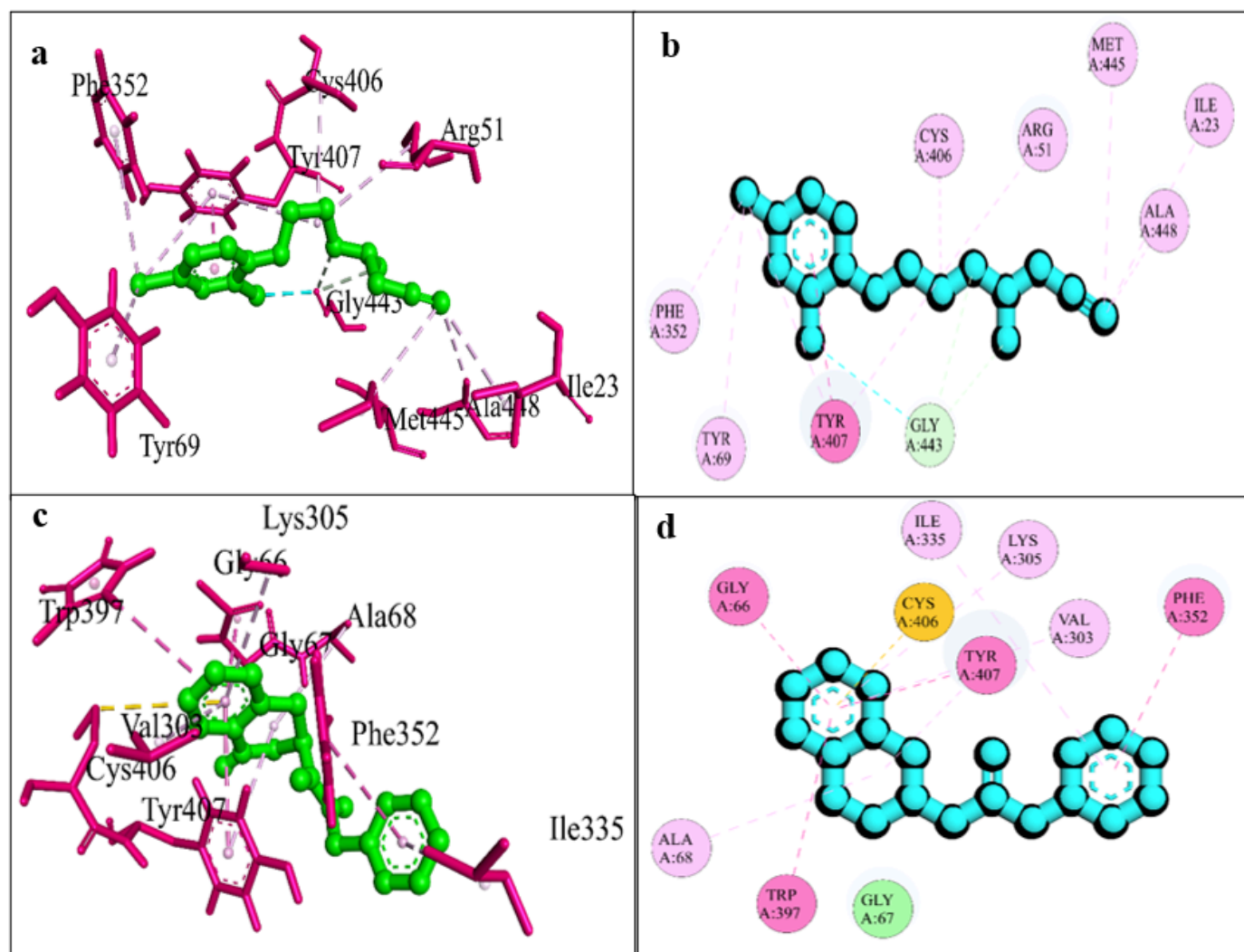


Figure 6

Fig 5. Three-dimensional (3D) docking poses (left) and two-dimensional (2D) interaction diagram (right) for paroxetine with MAO-A (**a** and **b**) and THPAC with MAO-A (**c** and **d**)

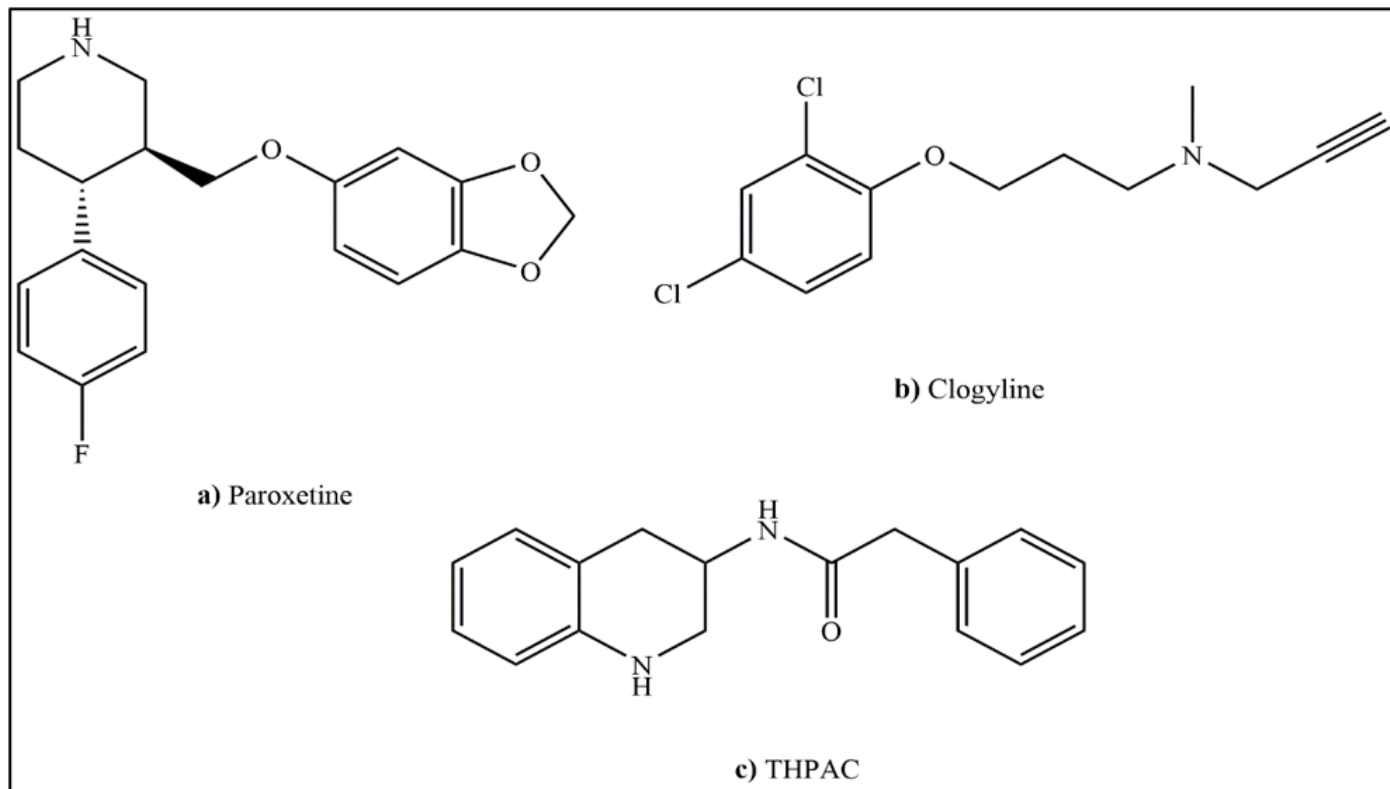


Figure 7

Fig. 6. Molecular structure of standards (a, b) and phytoligand (c).

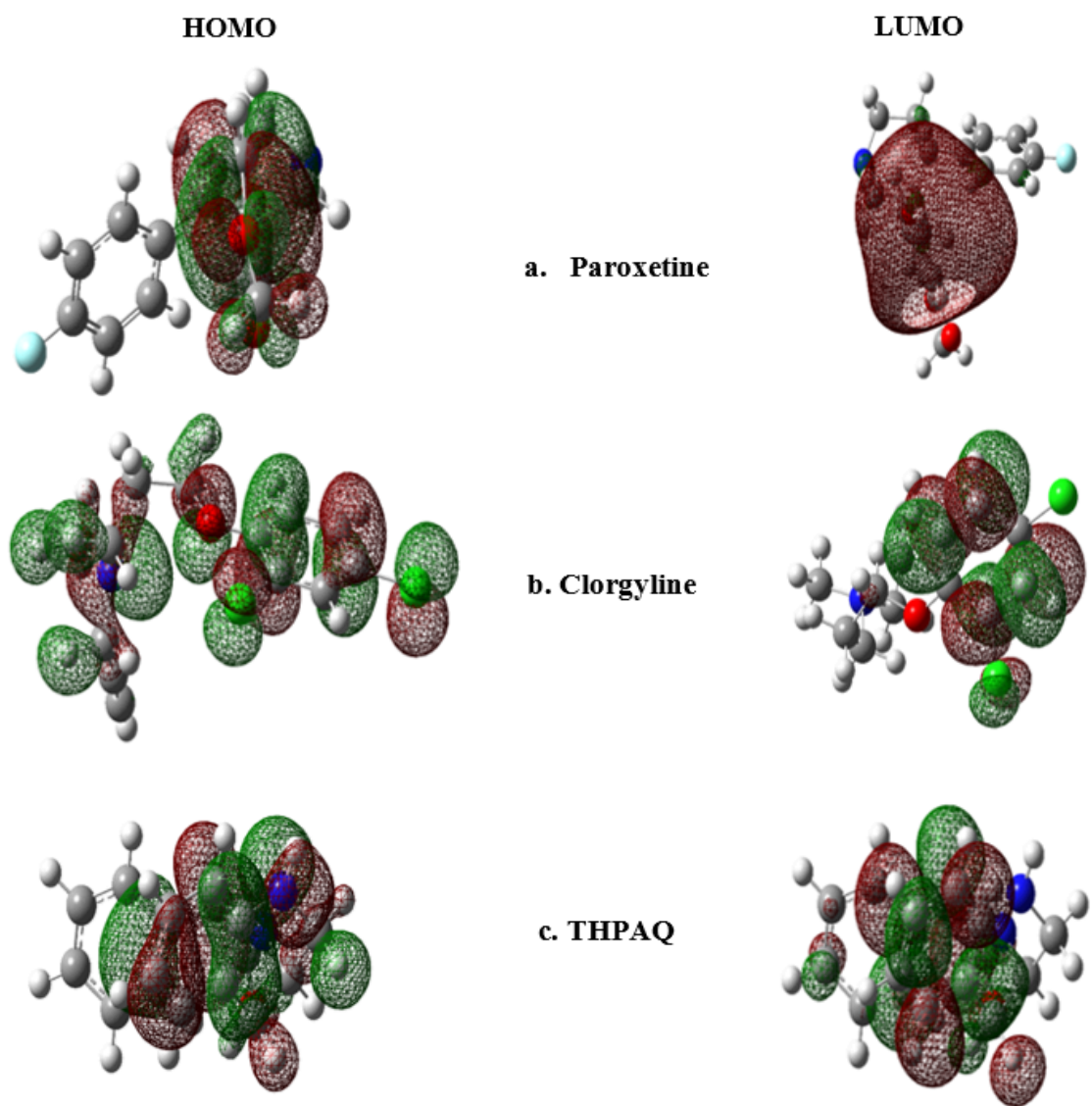


Figure 8

Fig 7. Molecular orbital distribution plots of HOMO and LUMO of Standard (**a** and **b**) and Phytoligand (**c**)

Supplementary Files

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