

Preliminary Investigation of the Neurobehavioral Effects of *Cinnamomum tamala* Leaf Extract Through Behavioral Models and Molecular Docking

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Abstract

Cinnamomum tamala is a traditionally used medicinal plant for many medical purposes; however, its neuropharmacological potential has not been properly studied. Using *in vivo* behavioral models and *in silico* molecular docking this study investigated the potential antidepressant and anxiolytic effects of the ethanolic leaf extract of *C. tamala*. Preliminary phytochemical screening identified the presence of alkaloids, flavonoids, phenols, tannins, saponins and glycosides. Behavioral evaluations were conducted using the tail suspension test (TST), forced swim test (FST) and open field test (OFT). While extract-treated groups showed trends toward decreased immobility in the TST and FST and no statistically significant differences were found when compared to the control group ($p > 0.05$). The extract also did not produce significant anxiolytic-like effects in the OFT. In contrast, favorable binding affinities and stable interactions of specific phytoconstituents (e. g. β -Caryophyllene, coumarin and α -humulene) with key amino acid residues were shown by molecular docking analysis against the human serotonin transporter (PDB ID: 5I6X) which is comparable to the reference drug, fluoxetine. Overall, even though *C. tamala* extract did not exhibit any notable behavioral effects in the current experimental setup but the *in-silico* findings suggest potential serotonergic involvement warranting further investigation with optimized experimental designs.

Key words: Ethanolic extract, phytochemical screening, antidepressant, anxiolytic, FST, TST, OFT, hSERT, 5I6X, Cinnamomum tamala, molecular docking.

Introduction

Depression and anxiety are some of the most common mental diseases globally today, affecting the quality of life and causing a substantial public health burden. Although current antidepressants such as selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants are found to be beneficial but they are found to be frequently linked with side effects and limited efficacy in most occasions (Cipriani *et al.*, 2018). This has led the researchers to focus into natural items as alternative or supplementary therapy.

Plants are historically been recognized as a rich source of bioactive chemicals having neuroprotective

and mood-regulating abilities (Dandapat *et al.*, 2014; Panche *et al.*, 2016). Such a way, *Cinnamomum tamala* is widely been used in traditional medicine due to its versatile therapeutic properties which include anti-inflammatory, antioxidant and digestive effects (Chaudhary *et al.*, 2022). The phytoconstituents such as flavonoids, alkaloids and phenols are found to have effect in the central nervous system function in preliminary research. Nevertheless, thorough assessments are required for its neurobehavioral effects. To meet this gap, the current study assessed the antidepressant and anxiolytic characteristics of *C. tamala* ethanolic leaf extract by using *in vivo* behavioral models such as the

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Forced Swim Test (FST), Tail Suspension Test (TST) and Open Field Test (OFT) etc. Key phytochemicals were evaluated through in silico molecular docking against the receptor 5I6X to achieve mechanistic insights. This integrated method aims to evaluate both the behavioral and molecular evidence of *C. tamala's* neuroactive potential (Xiao *et al.*, 2022).

Materials and Methods

Plant materials and preparation of extracts:

Fresh *C. tamala* leaves were collected from Tangail, Bangladesh in September 2025 and identified by the experts of the Bangladesh National Herbarium, where

a voucher specimen (DACB 135508) has been deposited. After shade drying for 8-10 days the leaves were grinded into a fine powder to improve extraction. 200 g powdered leaves were soaked by 800 mL ethanol (1:4) at RT for 5 days by the cold extraction procedure. The mixture was then filtered using a vacuum filter and condensed with a rotary evaporator (RE-200, Thomas Scientific) (Figure 1), yielding crude extract weighing 4.52 g. In order to detect several bioactive compounds having medicinal effects in the extract, a follow-up phytochemical screening was conducted.

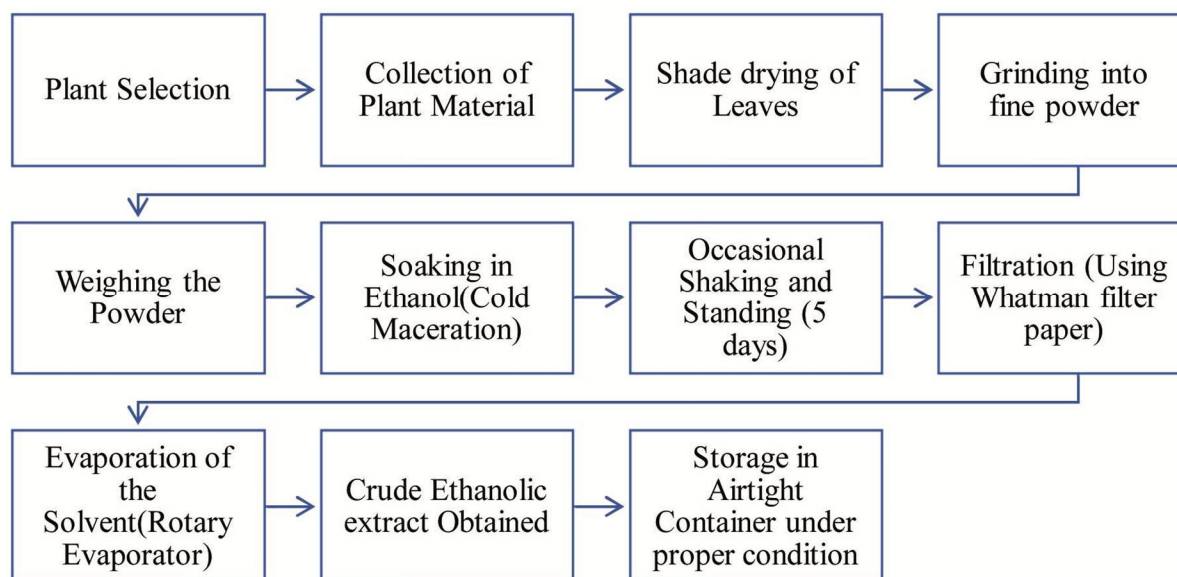


Figure 1. Schematic diagram of extraction.

Animals: The tests were conducted utilizing Swiss-albino mice, weighing 20-25 g and were kept in standard conditions having a 12-hour light/dark cycle and ensured unlimited access to food and water. The ethical authorization was issued by Ethical Review Committee, Faculty of Life and Earth Sciences, Jagannath University, Dhaka, Bangladesh (Approval Number: JnU/ERC/2025/38, Dated:

24.12.2025). With the 3Rs and humane treatment norms in mind and acclimatized the mice for 3 days at a temperature of 22 ± 2 °C and RH of 50-60 %. They were observed every day to check any signs of illness or stress that are shown in Table 1.

Drugs: Fluoxetine (Prodep®) was purchased from a local pharmacy in Dhaka, Bangladesh as the reference standard for antidepressant activity.

Table 1. Different groups of animal model for control, standard and test sample administration.

Test sample	Group	No. of mice	Purpose	Dose (ml/ kg and mg/Kg)	Route of administration
Normal saline	I	5	CG	10	Oral
Fluoxetine	II		SG	10	
EE (<i>C. tamala</i>) 200 mg/kg	III		TS	200	
EE (<i>C. tamala</i>) 400 mg/kg	IV		TS	400	

EE: Ethanolic extract, CG: Control group, SG: Standard group, and TS: Test sample.

Neuropharmacological activity evaluation

Antidepressant activity

Forced Swim Test: A clear cylindrical water tank (height: 25 cm; diameter: 10 cm) filled with water at 25 ± 1 °C to a depth of 15 cm was utilized (Aslam, 2016a; Can *et al.*, 2012) and Mice were placed in the water filled container after being habituated to

laboratory conditions. The test was conducted for six minutes of which two minutes were for acclimatization and four minutes were for behavioral recording (Kara *et al.*, 2018; Yankelevitch-Yahav *et al.*, 2015). The reduction in immobility time in treated groups established antidepressant efficacies are presented in Figure 2.

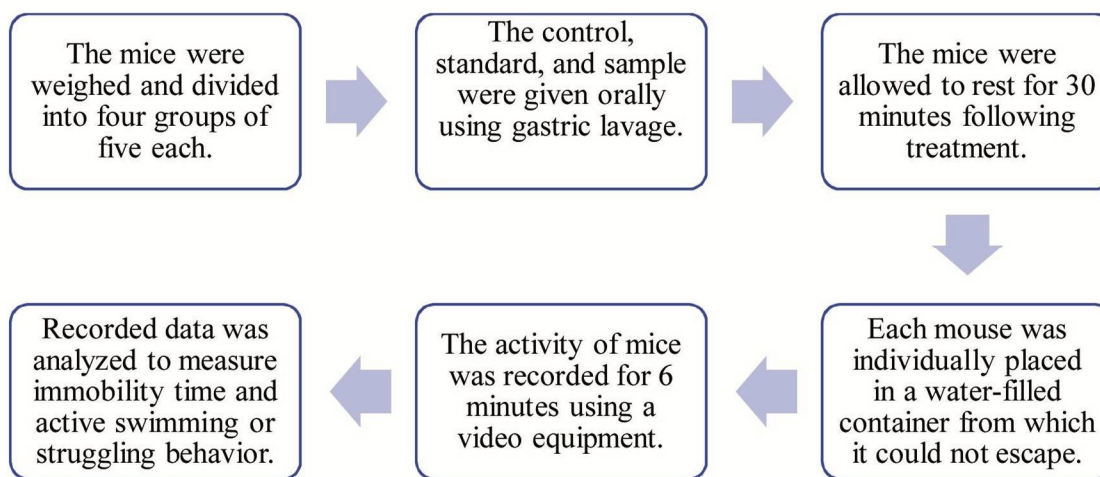


Figure 2. Schematic diagram of forced swimming test procedures for CNS activity evaluation.

Tail suspension test: Mice were individually strung by the tail with adhesive tape about 1.0 cm from the tip and placed about 50 cm above the floor to avoid contact with any surface. Each mouse was observed for six minutes and immobility time, defined as the lack of limb and body movements other than those required for balance was measured (Aslam, 2016b; Steru *et al.*, 1985). Reduced

immobility time in treated groups suggested potential antidepressant effects (Figure 3).

Antianxiety test

Open field test: The open field test is conducted to evaluate the neurobiological effects of anxiolytic medications by observing mouse locomotor activity in an open arena (Kraeuter *et al.*, 2018). The mice were placed and freed to explore the equipment in a

peripheral zone. The apparatus consisted of a square arena (40 x 40 cm for mice) with 30 cm high walls and the floor was divided into equal squares to generate core and periphery (Seibenhener & Wooten, 2015). The number of movements, mouse raising

frequency and grooming time were observed. Increased central zone activity and exploration in the treated groups indicates the anxiolytic action (Rani *et al.*, 2024). To prevent carry-over effects, behavioral tests are conducted in a certain order (Figure 4).

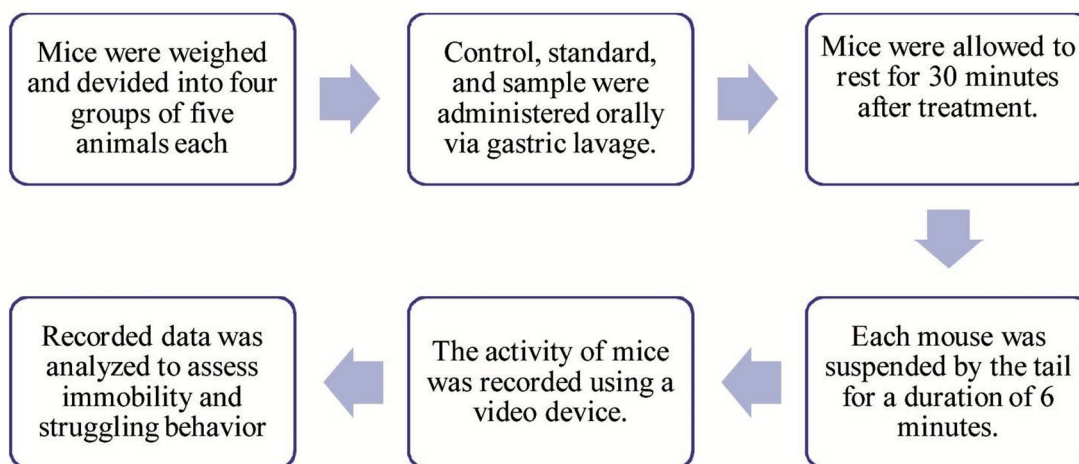


Figure 3. Schematic diagram of tail suspension test procedures for CNS activity evaluation.

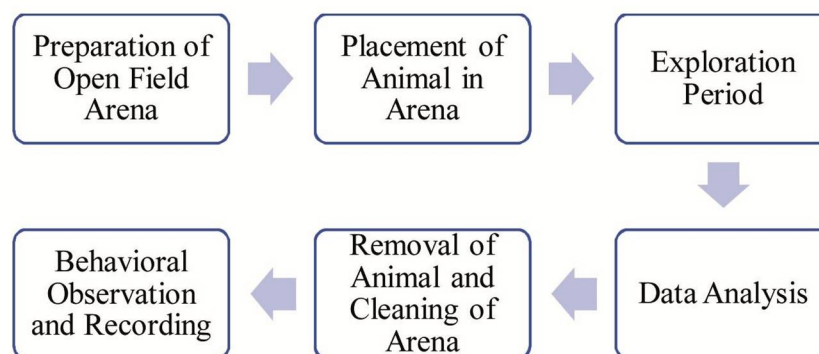


Figure 4. Schematic diagram of open field test procedures for CNS evaluation.

Molecular docking

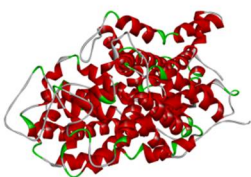
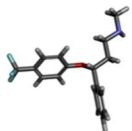
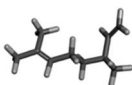
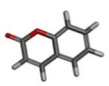
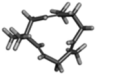
Major phytochemicals found in prior GC-MS studies of *C. tamala* leaves were chosen as ligands for molecular docking based on preliminary laboratory phytochemical screening (Kumar *et al.*, 2012; Sharma & Dang, 2021; Tandukar *et al.*, 2022). The human serotonin transporter (hSERT) is a significant therapeutic target for antidepressant medicines especially selective serotonin reuptake inhibitors such as fluoxetine which exert their action by blocking serotonin reuptake. The crystal structure

of hSERT (PDB ID: 5I6X), resolved in association with an antidepressant inhibitor exhibits the physiologically relevant binding site of SSRIs and provides insight into inhibitor binding at the central pocket. Although fluoxetine is not co-crystallized in this structure, it shares the same mechanism and binding pocket making 5I6X suitable for docking standard and plant-derived compounds. The 3D structure of the antidepressant receptor 5I6X was collected from the Protein Data Bank (PDB) and optimized for docking by eliminating water

molecules and adding hydrogens (Coleman et al., 2016; Nagarajan *et al.*, 2023). Ligand structures were retrieved in SDF format from the PubChem Compound Database (<https://pubchem.ncbi.nlm.nih.gov/>) before docking and converted into PDB format using Discovery Studio. They were then subjected to energy-minimization and were protonated correctly. Molecular docking was performed by using Autodock Vina to predict the maximum binding affinity between the ligand and protein using

Autodock Vina and BIOVIA Discovery Studio software was used to discover the kind of amino acids residues interaction with the ligand. Fluoxetine was used as a reference standard to evaluate the relative binding capability of the chosen phytochemicals (Wang *et al.*, 2022). Key interactions were conducted to obtain mechanistic insights of the potential antidepressant functions of *C. tamala* components (Table 2).

Table 2. Structures of the target receptor (5I6X) and selected ligands used for molecular docking.

Receptor/ligand	Structure
5X6I	
Fluoxetine	
Linolol	
Coumarin	
α -Humelene	

Result and Discussion

Preliminary phytochemical screening: Preliminary phytochemical analysis of the ethanolic leaf extract of *C. tamala* indicated the presence of phenols, flavonoids, steroids, terpenoids, tannins, proteins, glycosides and carbohydrates but alkaloids and saponins were not detected. The high levels of phenolic and flavonoid chemicals suggest that they have substantial antioxidant and neuroprotective

properties (Calderaro *et al.*, 2022; Vaishnav & Shahi, 2025). Terpenoids and steroids are also known for their CNS-modulating properties that may contribute to the observed neuropharmacological activity (Calderaro *et al.*, 2022). These findings back up the traditional use of *C. tamala* in neurological and therapeutic applications (Panche *et al.*, 2016; Vaishnav & Shahi, 2025). These results are summarized in table 3.

Table 3. Preliminary phytochemical screening result.

Phytochemicals	Standard test name	Result
Phenol	Ferric chloride test	+++
Flavonoid	Alkaline reagent test	+++
Alkaloid	Mayer's, wagner's test	--
Saponin	Foam test	-
Steroid	Liebermann-burchard test	++
Terpenoid	Salkowski interface test	+++
Tannin	Ferric chloride test	+++
Protein	Biuret test	++
Glycoside	Keller-killiani test	+
Carbohydrate	Molisch's, benedict's test	+++

Signs (+) and (-) indicate the presence and absence of phytochemical classes.

+++ = strongly present, ++ = moderately present, + = weakly present (trace), - / -- = absent

Neuropharmacological activity evaluation

Effect of the *C. tamala* extract on forced swim test: During the Forced Swim Test (FST), the immobility time of rats was measured to evaluate the possible antidepressant activities of the ethanolic extract of *C. tamala* leaf. The followings were the immobility lengths- Control: 157.6 seconds; fluoxetine (10 mg/kg): 184.6 seconds; EE 200 mg/kg: 158 seconds and EE 400 mg/kg: 138.4 seconds. Compared to the control group, the 400 mg/kg extract group showed a slight reduction in immobility while the 200 mg/kg did not show any virtual change. Fluoxetine showed an increased immobility time which was unexpected. This could be due to the FST's sensitivity to experimental conditions and the known variability of SSRI responses in acute behavioral models (Petit-Demouliere et al., 2005). One-way ANOVA statistical analysis revealed no statistically significant differences between the treatment groups and the control group ($p > 0.05$). These results show that under the current experimental conditions *C. tamala* extract did not exhibit a statistically significant antidepressant-like effect in the FST (Table 4 and Figure 5).

Table 4. Result of ethanolic extract of *C. tamala* in forced swim test.

Group	Immobility time (Seconds) (Mean \pm SD)
Control	157.6 \pm 48.75
Standard	184.6 \pm 16.21
EE (200 mg/kg)	158 \pm 20.84
EE (400 mg/kg)	138.4 \pm 43.47

Effect of the *C. tamala* extract on tail suspension test: The tail suspension test (TST) was conducted to evaluate the antidepressant effect of the ethanolic leaf extract of *C. tamala*. The immobility times were: Control: 118.6 s, Fluoxetine (10 mg/kg): 113 s, EE 200 mg/kg: 55.8 s and EE 400 mg/kg: 92.6 s. When compared to the control both extract-treated groups displayed decreased immobility with the 200 mg/kg group showing the greatest reduction. Nevertheless, these decreases were not statistically significant ($p > 0.05$) according to one-way ANOVA. Additionally, fluoxetine did not significantly reduce immobility time which may be due to the TST's sensitivity to experimental conditions and the known variability of SSRI responses (Cryan et al., 2005). Although significant effects were not observed the trend toward decreased immobility suggests that *C. tamala* extract may have potential antidepressant-like activity (Table 5, Figure 5)

Table 5. Result of ethanolic extract of *C. tamala* in tail suspension test.

Group	Immobility time (Seconds, Mean±SD)
Control	118.6 ± 75.54
Standard	113 ± 30.55
EE (200 mg/kg)	55.8 ± 46.28
EE (400 mg/kg)	92.6 ± 56.24

Effect of the Cinnamomum tamala extract on open field test: According to the open field test ($p > 0.05$, one-way ANOVA), the doses of ethanolic extract of *Cinnamomum tamala* of 200 and 400 mg/kg did not significantly change exploratory or anxiety related behaviors in mice. Vertical

exploratory activity remained steady as the number of raising responses (Control: 43.2; EE 200: 41.4; EE 400: 43.2) was comparable to the vehicle-treated controls. Similarly, there was no significant difference between the groups time spent in the center (Control: 18.2 s; EE 200: 13.2 s; EE 400: 18.0 s) and admissions into the central zone (Control: 11.2; EE 200: 8.2; EE 400: 10.0). The reference drug fluoxetine (10 mg/kg) showed only a small increase in time spent in the center (21.0s). Moreover, this finding demonstrates that *C. tamala* extract has no observable anxiolytic-like effects in the open field test under the current experimental conditions (Table 6 and Figure 6).

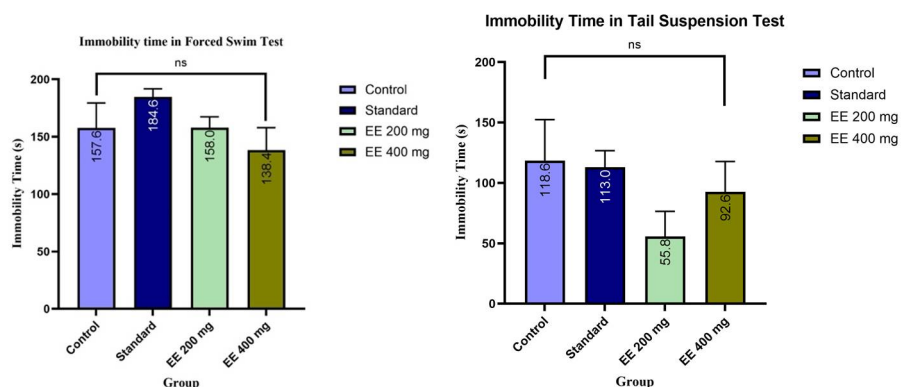
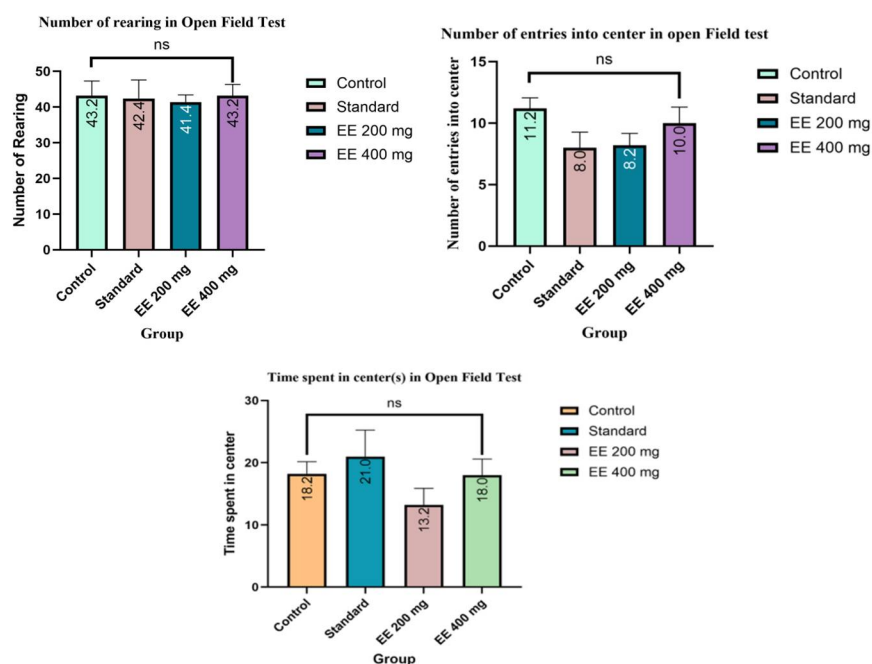
**Figure 5. Neuropharmacological activity by FST and TST examination.****Figure 6. Neuropharmacological activity evaluation by open field test.**

Table 6. Result of ethanolic extract of *C. tamala* in open field test.

Group	Number of rearing (Mean \pm SD)	Number of entries into center (Mean \pm SD)	Time spent in center (Mean \pm SD)
Control	43.2 \pm 9.20	11.2 \pm 1.92	18.2 \pm 4.38
Standard	42.4 \pm 11.63	8 \pm 2.82	21 \pm 9.51
EE (200 mg/kg)	41.4 \pm 4.50	8.2 \pm 2.16	13.2 \pm 6.01
EE (400 mg/kg)	43.2 \pm 7.04	10 \pm 2.91	18 \pm 5.83

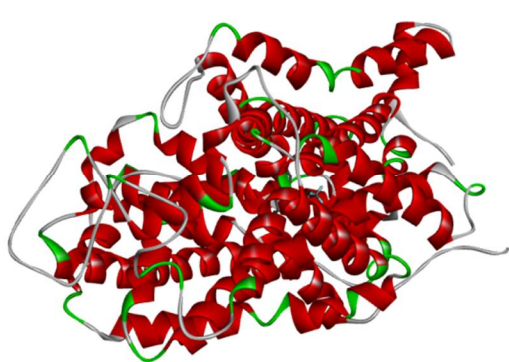
Molecular docking evaluation

The standard SSRI fluoxetine demonstrated the strongest binding affinity (-9.2 kcal/mol) and the greatest number of favorable interactions (10) confirming the docking reliability. Among the phytochemicals from *C. tamala* Eugenol formed many interactions (8) but had weaker binding (-6.0 kcal/mol) suggesting that binding strength is not solely determined by interaction number. Despite having fewer contacts, α -Humulene (-8.4 kcal/mol) and β -Caryophyllene (-8.6 kcal/mol) showed relatively strong binding highlighting the significance

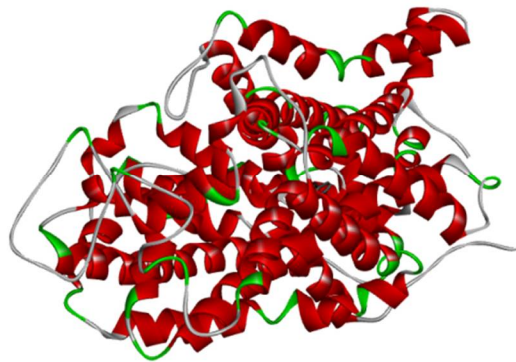
of hydrophobic stabilization and interaction quality. Docking studies suggested stable binding of the phytoconstituents within the hSERT active site through hydrophobic and π - π interactions with key amino acid residues showing a binding profile similar to fluoxetine. These results imply that *C. tamala* compounds may partially mimic the binding of fluoxetine supporting their possible modulatory role on hSERT and warranting further investigation. The relative binding potential of major phytoconstituents of *C. tamala* leaves against 5I6X is summarized in table 7 and figures 7-9.

Table 7. Relative binding potential of major phytoconstituents from *C. tamala* leaves against 5I6X.

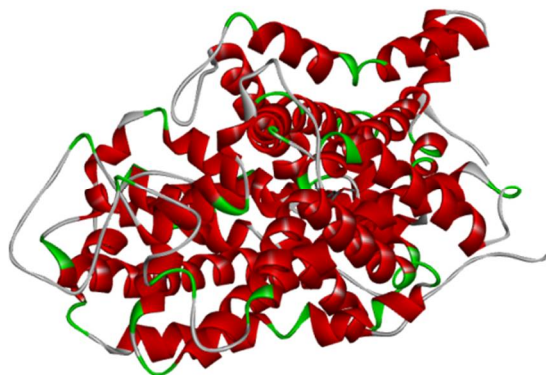
Ligands	No. of total favorable non-bonds	Hydrogen bonds		Binding site residue	Binding Affinity (Kcal/mole)
		Strong H-bond D(H)...A (max distance)	Weak H-bond D(H)...A (max distance)		
Fluoxetine	10	3.4	3.8	ASP98, ALA96, SER336, ALA173, SER439, ALA169, TYR176, SER438, ILE172, VAL501.	-9.2
Linalool	6	3.4	3.8	ILE172, VAL501, TYR95, PHE334, PHE335, PHE341.	-6
Coumarin	6	3.4	3.8	ASN177, ALA173, SER439, TYR176, SER438, ILE172.	-7.1
α -Humulene	6	3.4	3.8	ILE172, VAL501, TYR95, PHE334, PHE335, PHE341.	-8.4
Eugenol	8	3.4	3.8	ALA169, TYR176, SER438, SER439, ILE172, ALA173, LEU443, PHE341.	-6.4
β -Caryophyllene	6	3.4	3.8	ILE172, VAL501, TYR95, PHE334, PHE335, PHE341.	-8.6



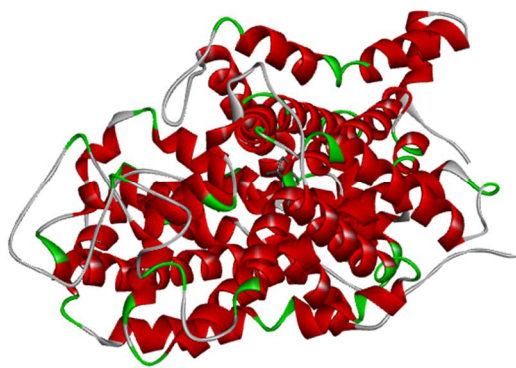
3D structure of Fluoxetine-hSERT Complex



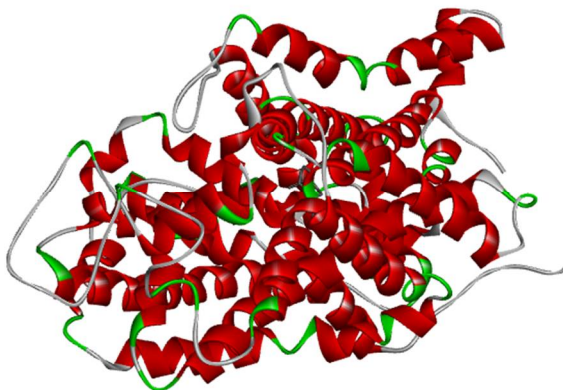
3D structure of Coumarin-hSERT complex



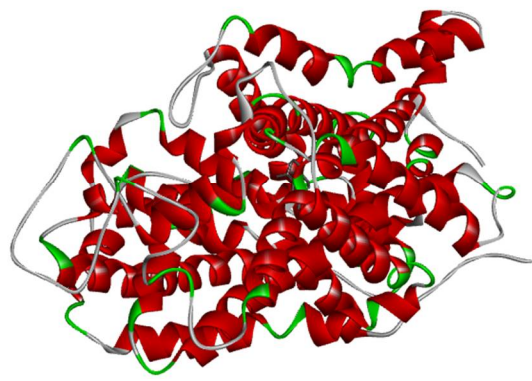
3D structure of Eugenol-hSERT Complex



3D structure of Linalool-hSERT Complex

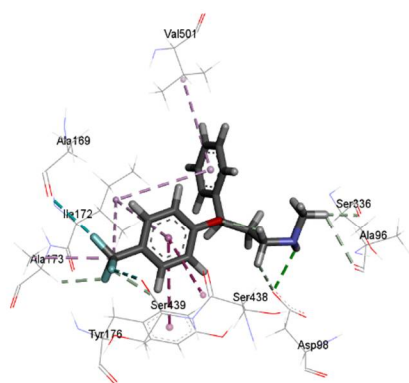


3D structure of β -Carophyllene -hSERT Complex

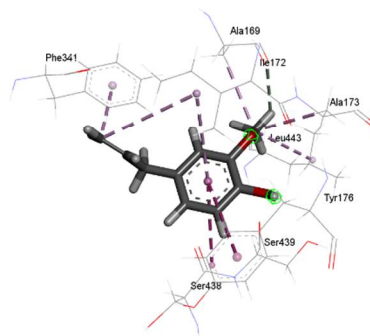


3D structure of α -Humelene-hSERT Complex

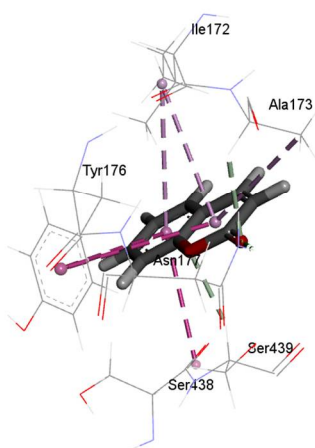
Figure 7. 3D structure of Receptor-Ligand complex.



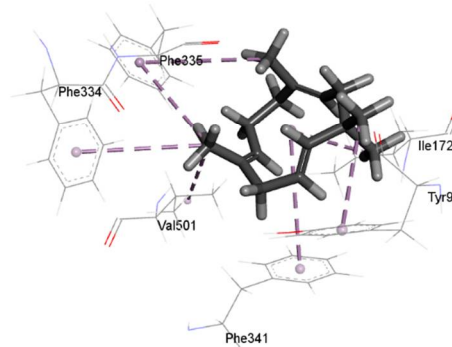
3D interaction profile of Fluoxetine-hSERT



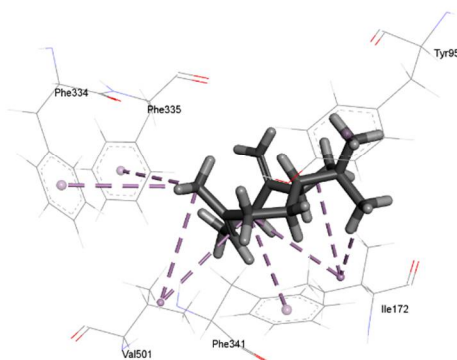
3D interaction profile of Eugenol-hSERT



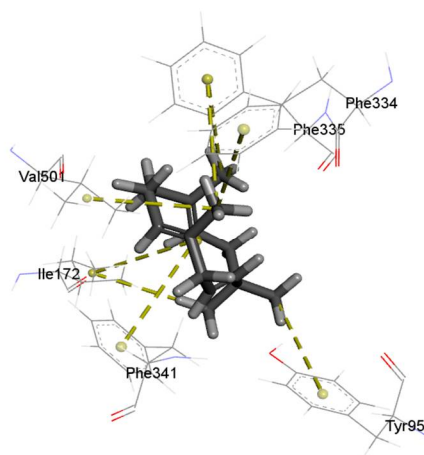
3D interaction profile of Coumarin-hSERT



3D interaction profile of Linalool-hSERT

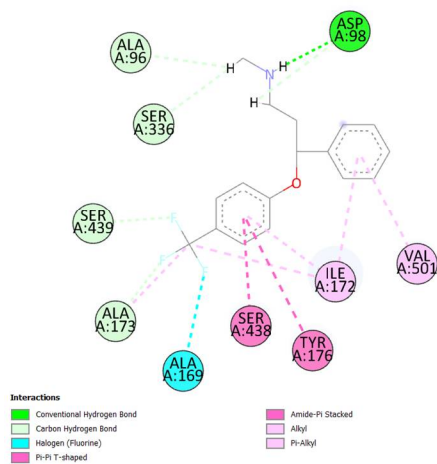


3D interaction profile of β -Caryophyllene-hSERT

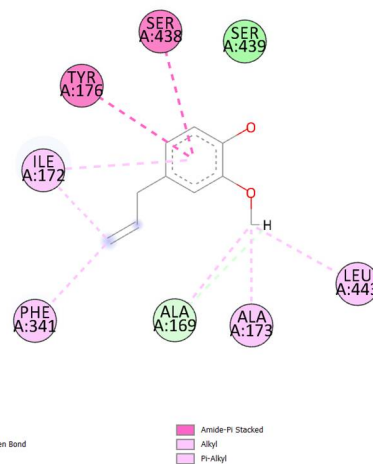


3D interaction profile of α -Humulene-hSERT

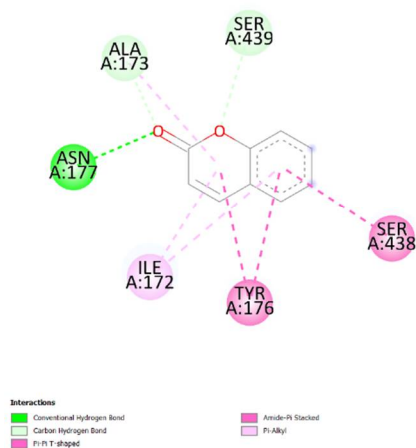
Figure 8. 3D interaction profile of ligand and protein.



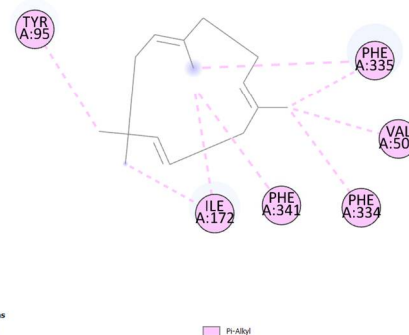
2D interaction profile of Fluoxetine-hSERT



2D interaction profile of Eugenol-hSERT



2D interaction profile of Coumarin-hSERT



2D interaction profile of Linalool-hSERT

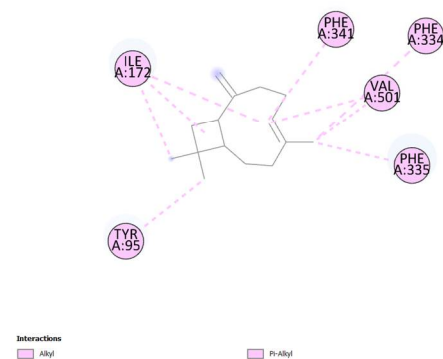
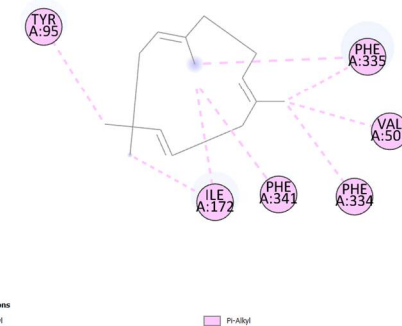
2D interaction profile of β -Caryophyllene-hSERT2D interaction profile of α -Humulene-hSERT

Figure 9. 2D interaction profile of ligand and protein.

Conclusion

Docking studies of bioactive components of *C. tamala* against the antidepressant target receptor 5I6X revealed that some compounds notably linolol, Coumarin and α -Humelene exhibited lower to moderate binding affinities compared to the standard drug Fluoxetine which exhibited the strongest receptor interactions. *In vivo*, the ethanolic leaf extract showed a decent and non-significant trend toward anxiolytic and antidepressant-like effects in the Open Field, Forced Swim and in the Tail Suspension tests with a dose-dependent reduction in immobility in the TST that suggests potential mood-enhancing activity. Collectively, these results indicate that though *C. tamala* contains phytochemicals that are capable of interacting with antidepressant targets but their behavioral efficacy *in vivo* is still insignificant. Additional research with optimized dosages, expanded groups and more behavioral models is necessary to elucidate its neurobehavioral capabilities.

Authors contribution

Bilkis performed the entire research work under the supervision of M.M. Hussain. Bilkis has written the draft of the work and M.M. Hussain corrected the manuscript. After that the both authors revised this manuscript to publish in this journal.

Conflict of interest

The authors declared that no conflict of interest in this research work.

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