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Research Article

Phytochemistry, Neuropharmacological Insight, and Computer-Aided Docking Studies of a of *Litsea glutinosa*

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Abstract

Introduction: Phytochemicals are related to the synthesis of crucial drugs for treatments. The current study aimed to evaluate phytochemistry, biopotential activities of methanolic extract of *Litsea glutinosa*, and *in-silico* molecular docking studies.

Method: The phytochemical analysis was estimated by total flavonoid and total phenolic content, and GC-MS test. Multiple biological activities were assessed such as antioxidant activity by DPPH and superoxide scavenging test, anxiolytic activity by plus maze and hole-board test, and antidepressant activity by and forced swimming and tail suspension test. *In-silico* binding interaction of phytoconstituents with their targets was virtually screened using PyRx. Drug-likeness properties were assessed by SwissADME server.

Results: Using GC and MS, eight phytochemical compounds were identified. The extract contained significant total flavonoid and phenolic content (41.996 ± 1.754 mg of QE/g, 13.264 ± 2.451 mg of GAE/g, respectively) that confirms its anti-oxidant activity (DPPH scavenging 59.12 ± 1.78% and superoxide scavenging activity 55.62 ± 1.21%). In the anxiolytic test, with the increasing dose of the plant extract, time spent in open arms, number of head dipping, and latency of first head dipping were increased whereas in antidepressant tests, with the increasing dose of the plant extract, immobile time decreased compared to the control. The computational studies confirmed several bioactive lead compounds showed strong binding abilities with the receptors of anxiolytic and antidepressant activities and revealed the Drug-likeness properties of *Litsea glutinosa.*

Conclusion: Our results yield that *L. glutinosa* extract has antioxidant activity and better neuropharmacological activity including anxiolytic and antidepressant activity.

Keywords: *Litsea glutinosa; Photochemistry; Antioxidant; GC-MS; Anxiolytic; Antidepressant; Neuropharmacological Activity; Molecular Docking*

Introduction

Plants are considered the main sources of biologically active constituents that are used for disease purposes [1]. Researchers search for novel drugs or chemical components with improved therapeutic activity. This is because of cost-effectiveness with a high safety profile and used for drug screening and discovery in lower animals to higher animals [2]. Different types of medicinal plants contain tannins,

steroids, flavonoids, phenolic compounds, saponins, volatile oils, alkaloids etc. that has potential pharmacological actions and applied for the treatment of different diseases states [3].

Herbal-based medicines have been used to treat and cure a wide range of illnesses [4]. Additionally, Indian traditional medicine has a wide range of prescriptions for treating conditions like ulcers, leprosy, scabies, diarrhea, inflammation, skin infections, wounds, and snake bites.

A traditional medicinal plant is an essential plant in the health sector. These medicinal plants are used for various pharmacological actions with a few side effects. Numerous phytochemicals with a wide range of biological activities are used in traditional medicine to treat a variety of human diseases. These activities include antibacterial, antidiabetic, antifungal, antifertility, antitumor, anti-inflammatory, and cardiovascular effects, as well as central nervous system depressant, cytotoxicity, and diuretic effects [5]. In the era of new technology, we can identify new drug molecules from traditional medicinal plants to minimize the cost of medicine and to get effective cures. *L. glutinosa* is a moderately sized tree. The bark of this plant is thin, grey or pale brown [6]. This plant's leaves are 7-15 × 3-7 cm, the flower is umbels numerous, fruit is 6 mm in diameter, globose, and purplish black. *Laur.* family is distributed throughout tropical and subtropical regions, principally Southeast Asia and tropical America, particularly in Brazil [7]. In Bangladesh, *L. glutinosa* leaves are traditionally used in diarrhea and dysentery, excessive semen flow for young boys, and also applied poultices for wounds and bruises. In another study, it is used for insomnia and neurosis [8]. The oil of this plant is used for rheumatism [9]. It has antimicrobial and hepatoprotective properties [8]. The previous findings give a clue that may be the leaf extract of *L. glutinosa* could contain variety of bioactive compounds for recovery of the specific diseases. The current project was designed to evaluate antidepressant, anxiolytic, and antioxidant activities of methanolic extract of *L. glutinosa* in different *in vivo*, *in vitro* model, and computer-aided model for characterizing bioactive lead compounds.

Materials and Methods

Collection of plant materials

Leaves of *L. glutinosa* were collected from Bogura district of Bangladesh in August 2021. After collection the plant it was identified by the Bangladesh Nation Herbarium, Mirpur-1, Dhaka-1216. One voucher specimen was deposited in Bangladesh National Herbarium (BNH). The voucher specimen (Accession number: DACB-65511).

Drying and grinding

The collected plant parts (leaves) were separated from undesirable materials. They were dried in open air under shade for two weeks. The shade-dried plant's part is ground into coarse powder [10]. The powder was stored in an air-tight container and kept in cool, dark and dry place until analysis commenced.

Preparation of plant extract

Extracts were prepared in methanol at room temperature by simple extraction method [11]. About 800 gm of powder of leaf were placed in a neat glass container and immersed in 2400 ml (2.4L) of 99% methanol. After securing the vessel, the contents were maintained for 15 days with sporadic shaking and mixing. The complete mixture was then subjected to coarse filtration on a piece of clean Whatman® filter paper. The obtained filtrate was evaporated under Rotary evaporator machine under pressure for 30 minutes [12].

Experimental animals

Approximately 6 - 7 weeks aged Swiss-albino mice of both male and female sexes (Around 80 mice), an average weight of 25 - 35g [13] taken from central animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. They were utilized for screening anxiolytic and antidepressant activity. The guidelines governing the use of laboratory animals were followed during the research project.

GC-MS

GC/MS analysis was carried out using Perkin Elmer auto system XL with turbo mass system equipped with PE 5 MS 30m X 250-micron silica capillary. Overall runtime was 32 minutes. They have compared with their standards (more than 4000 patterns) and identified chemical compounds.

Determination of total flavonoid content

The plant extract's total flavonoid content was determined by the well-known aluminum chloride colorimetric method [14]. Maximum absorbance is 420 nm.

Determination of total phenolic content

The total phenolic content test was done using materials like Folin-Ciocalteu reagent (10-fold diluted), Gallic acid, Na_2CO_3 solution (7.5%). The TPC was evaluated using Folin-Ciocalteu reagent [15]. After incubating the solution for 20 minutes at 25°C, the absorbance at 765 nm was measured in triplicate. In the TPC calculation of milligrams of Gallic Acid Equivalents (GAE) per gram, gallic acid served as the benchmark [16].

Determination of total antioxidant activity

DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extract. DPPH (1, 1-diphenyl-2-picrylhydrazyl), Methanol, Ascorbic Acid. The scavenging activity of *L. glutinosa* was evaluated using the standard protocol [17]. Of each dilution, 0.1 ml was added to 3 ml of 0.004% DPPH Solution (4 mg DPPH in 100 ml of 95% methanol), followed by incubation for 30 minutes (25°C). Three samples were made for each concentration. The absorbance was detected at 517 nm by an ultra-violate (UV spectrophotometer).

Determination of superoxide scavenging activity

Superoxide radical scavenging activity of the extract was determined by the alkaline DMSO [18]. Alkaline DMSO (Dimethyl sulfoxide), NaOH, Nitro-blue tetrazolium (NBT). The absorbance was measured at 560 nm.

Acute oral toxicity test

Twin 80 (1%) was used for the test [19]. The mice were kept overnight fasted for the study [20]. The crude extract was weighted and dissolved in required amount of Tween 80 (1%) to prepare 4000 mg/kg and 2000 mg/kg and it was orally administered. Individual animal was kept in close observation during at first 30 minutes after dosing, periodically first 24 hr special attention for the first 4 hr. The effective therapeutic dose was taken as one-tenth of median lethal dose [21] which is 400 mg/kg and doses used for *in vivo* were 400 mg/kg, 200 mg/kg, and 100 mg/kg.

Evaluation of neuropharmacological activity

Anxiolytic effect

Elevated plus maze (EPM) and Hole Board test (HBT) were carried out to assess the neuropharmacological activity of the methanolic leaves extract of *L. glutinosa* [22].

Elevated plus maze method

The test animals in the EPM were split up into five groups, each with five mice: control, standard, and three test samples. While the test groups received extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg body weight of mice, the control groups received just vehicle (1% tween-80 water). Diazepam (1 mg/kg) was used as a standard drug. At 60 minutes after treatment, the mice were placed individually in the central square section of the EPM. The observation was recorded for 5 minutes [23].

Hole board method

Hole board test is a generally used method for screening the potential anxiolytic character of drugs [24]. The test subjects for the Hole Board method were split up into five groups, each with five mice: control, standard, and three test samples. While the test groups received extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg body weight of mice, the control groups were given just vehicle (1% tween-80 water). The device is made up of a grid-patterned enclosed area with sixteen holes. Each hole is numbered so that the number of times a mouse explores a specific hole may be counted [25]. The number of head dipping by the animal was counted for 5 minutes. Diazepam (1 mg/kg) was used as a standard drug.

Antidepressant activity

Forced swim method

The forced swim test is a rodent behavioral test used for the evaluation of antidepressant [26] drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states [27]. The animals used in this experiment were split up into five groups: three test samples, standard, control, and one batch of five mice apiece. While the test groups received extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg body weight of mice, the control groups received just vehicle (1% tween-80 water). Mice are placed in an inescapable transparent tank that is filled with water and their escape related mobility behavior is measured. Fluoxetine (10 mg/kg) was as a standard drug.

Tail suspension method

The tail-suspension test is a mouse behavioral test useful in the screening of potential antidepressant drugs [26] and assessing other manipulations that are expected to affect depression related behaviors. Tape is used to suspend mice by their tails so they are unable to escape or cling to surrounding objects. Duration of the test was typically six minutes [28]. The test involved the division of the animals into five groups, each with five mice: control, standard, and three test samples. While the test groups received extract doses of 400 mg/kg, 200 mg/kg, and 100 mg/kg body weight of mice, the control groups were given just vehicle (1% tween-80 water). The standard medication used was fluoxetine (10 mg/kg).

In silico studies

Protein preparation and virtual screening

The protein targets were scrutinized and downloaded from the RCSB PDB (https://www.rcsb.org/) website. The 3D structures of the available phytochemicals were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database and saved as SDF format [29]. The downloaded protein structures were prepared by cleaning all the heteroatoms. Even, some proteins were found to be incomplete and missing a few residues, so their full structures were predicted using homology modeling through SWISS-MODEL (https://swissmodel.expasy.org/). The virtual screening was performed using PyRx (version 0.8). The protein structure was loaded into the PyRx application and converted into macromolecules. The ligands were loaded in SDF format into Open Babel at the control panel, optimized using the UFF force field and steepest descent algorithm, and converted into PDBQT format. Finally, the grid was positioned to cover the whole structure and virtual screening was performed. The results were documented in a CSV file. For visualization and interaction inspection purposes, BIOVIA 2021 discovery studio was used [30].

Drug-likeness and toxicity evaluation

"Drug-likeness" is a parameter that describes how a compound is prone to be a drug substance, and toxicity criteria are important for assessing a drug's pharmacological potential. The Available phytochemical structures were saved as Mol files (*.mol) and were ready for uploading as a list of SMILES into the SwissADME (http://www.swissadme.ch/) server, and one by one in the admetSAR (http://lmmd.ecust.edu.cn/admetsar2) server. The drug-likeness results were saved in CSV files. For toxicity studies, each result was copied into the clipboard of the local computer and pasted into a spreadsheet for later analysis.

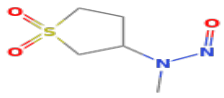
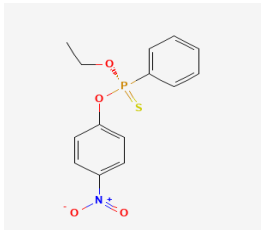
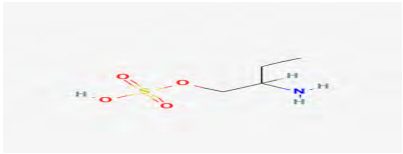
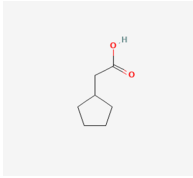
Statistical analysis

Statistical comparisons were performed using GraphPad prism 9. All the data are expressed as mean ± SEM (Standard Error of Mean). The differences between multiple group means were evaluated by one-way ANOVA with Tukey's post hoc tests for pair-wise comparisons. All comparisons were between a specific dose and the control unless otherwise indicated. $p < 0.05$ were considered statistically significant.

Results

Phytochemical screening

Gas chromatography and Mass spectroscopy (GC-MS) were used to identify the phytochemical compounds and 8 compounds were identified from the methanolic extract of *L. glutinosa* (Table 1 and figure 1).

SN	Phytoconstituents	Chemical Structure	RT (min)	m/z	Peak Area (%)
1	Z,Z-3,13-Octadecedien-1-ol		25.665	207	9
2	3-Thiophenamine, tetra-hydro-N-methyl-N-nitroso-,1, 1-d		26.615	50	384
3	Pentaborane		27.990	50	427
4	2-Aminobutylhydrogensul-fate		-	50	-
5	Cyclopentaneacetic acid		-	50	-
6	Methyl 9-tetradecenoate		-	207	-

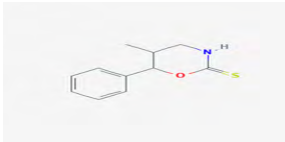
7	1-Amininonadecane, N-trifluoroacetyl-		207	-	-
8	5-Methyl-6-phenyltetra-hydro-1,3-oxazine-2-thione		207	-	-

Table 1: *The obtained phytochemical compounds by GC-MS.*

**RT: Retention Time.*

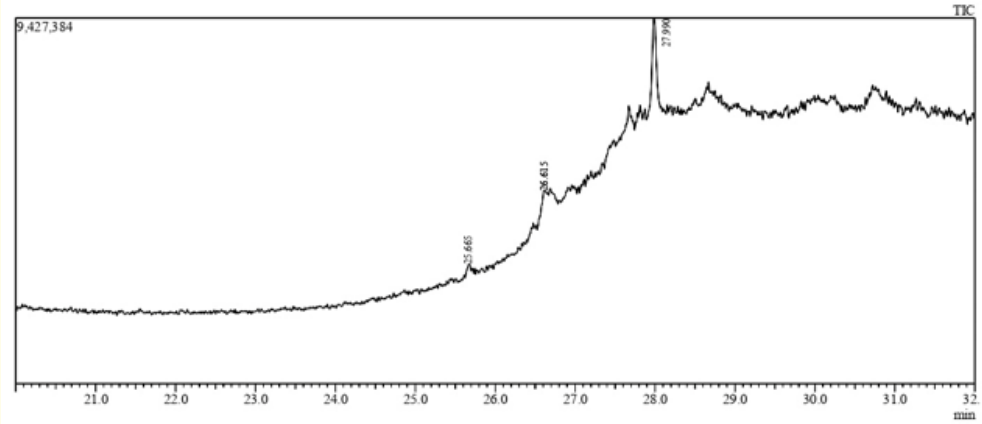


Figure 1: *GC-MS analysis graph of methanolic extract of L. glutinosa.*

Determination of total phenolic and flavonoid contents

The observed total phenolic contents (TPC) and total flavonoid contents (TFC) in the methanolic extract of *L. glutinosa* are below.

Plant of extract	TPC	TFC
L. glutinosa	13.264 ± 2.451 mg of GAE/gm	41.996 ± 1.754 mg of QE/gm

Table 2: *The values of TPC and TFC.*

Determination of free radical scavenging and superoxide scavenging activity

Methanolic extract of *L. glutinosa* exhibited a moderate free radical scavenging activity in the DPPH assay. The antioxidant DPPH scavenging activity of *L. glutinosa* was significantly lower than that of ascorbic acid (Table 3). Among all six concentrations of *L. glutinosa* extract, 500 µg/mL showed the highest scavenging activity while this concentration of ascorbic acid exhibited 94.3 ± 2.3% (Table 3) scavenging activity. As well as, for the superoxide scavenging activity the alcoholic extract showed dose dependent scavenging activity where standard ascorbic acid scavenging activity is 81.2 ± 2.5%.

L. glutinosa (µg/ml)	DPPH Scavenging (%)	Superoxide Scavenging (%)
25	42.73 ± 0.81*	27.67 ± 0.92*
50	45.81 ± 0.55*	35.34 ± 0.33*
100	47.55 ± 0.75**	51.84 ± 0.45**
200	49.21 ± 0.75**	52.20 ± 0.12**
300	53.67 ± 1.2**	55.77 ± 0.49**
400	56.40 ± 1.5**	53.26 ± 0.99**
500	59.12 ± 1.78**	56.62 ± 1.2**
Ascorbic acid (Standard)	94.3 ± 2.3**	81.2 ± 2.5**

Table 3: *The values of DPPH scavenging and superoxide scavenging at different concentration.*

*Data are mean ± standard deviation values (n = 3); *p < 0.005, **p < 0.01.*

Acute toxicity test

The effective therapeutic dose was taken as one-tenth of median lethal dose (LD_{50}>4000 mg/kg), which is 400 mg/kg and doses used for *in vivo* are 400 mg/kg, 200 mg/kg, 100 mg/kg. 4000 mg/kg dose showed severe itching problem and highly sedative activity with no mortality record, whereas 2000 mg/kg dose showed no itching, sedative effect, and mortality record.

Anxiolytic activity test

Determination of anxiolytic activity we have two main tests e.g. EPM and HBT. In the EPM graph (Figure 2a) the methanolic extract at 400 mg/kg (35.07 ± 1.255) dose showed significant increase in the number of entries into the open arms compared to the positive control, while all other doses of the extract did not significantly affect the parameter compared. On the other hand, none of the doses of the extract or diazepam significantly affected the number of closed arm entries. The time the mice spent in the open arms was significantly increased in animals treated with 100 mg/kg (19.02 ± 0.9135) and 200 mg/kg doses of the extract compared to the control group.

Mice treated with 100 and 200 mg/kg (26.87 ± 0.7344) doses significantly increased the percentage of open arm entry compared to the control (15.82 ± 0.7828). Statistical significance was calculated using one-way ANOVA; ***p < 0.001; error bars denote SEM.

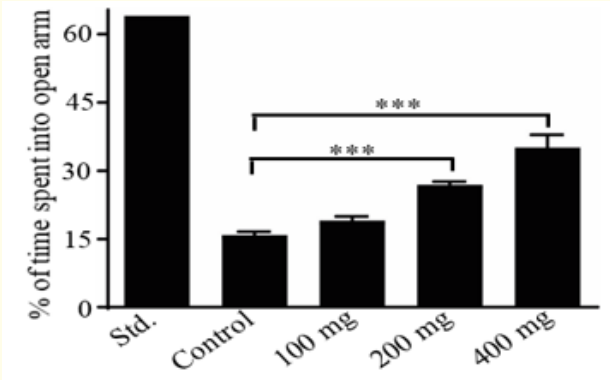


Figure 2a: *Comparison of % of time spent in open arm of different dose with standard and control. ***p < 0.001; **p < 0.01; *p < 0.05.*

On the same way for HBT the amount of head dipping increased with dose and the summarized (Figure 2b). The 400 mg/kg of the extract treatment significantly increased the amount of head dipping (40 ± 1.02) compared to the control (25 ± 3.00) and 100 mg/kg, 200 mg/kg shows (25.60 ± 1.03) and (30 ± 1.81) respectively. In here diazepam was used as standard. Statistical significance was calculated using one-way ANOVA; ***p < 0.001; error bars denote SEM.

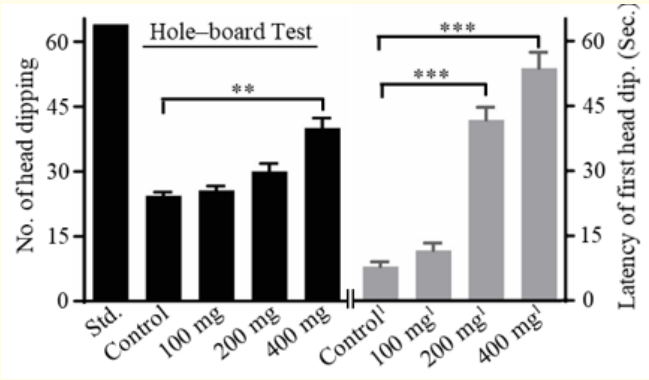


Figure 2b: *Comparison of number of head dipping of different doses with standard and control. ***p < 0.001; **p < 0.01; *p < 0.1.*

Antidepressant activity test

Antidepressant activity study was also done by two main tests such as FST and TST. The antidepressant efficiency of plant extract (400, 200 and 100 mg/kg) was observed by force swimming test was displayed in the figure 3a. Over the observation period during FST test, the depressive behavior, such as time of immobility (sec) was decreased in a significant dose-dependent manner. In comparison with control (116 ± 1.720) the highest dose of extract 400 mg/kg shows (78.60 ± 1.778) which is too much less than 200 mg/kg (90.60 ± 1.03) and 100 mg/kg (104.4 ± 1.364). Finally, increasing the extract dose time of immobilization is decreased and finally, it has defined that the extract has antidepressant activity. Statistical significance was calculated using one-way ANOVA; ***p < 0.001; error bars denote SEM.

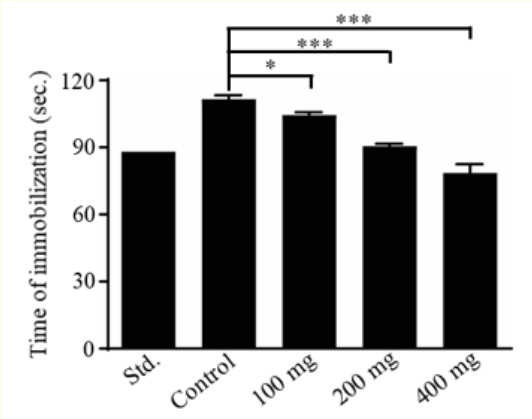


Figure 3a: *Comparison of immobilization (FST) of different doses with standard and control. ***p < 0.001; **p < 0.01; *p < 0.1.*

The outcome of the tail suspension test is shown in figure 3b. In the test, treatment of the extract at 400, 200, and 100 mg/kg doses exhibited antidepressant like action. The most significance decreased immobile time was recorded (55 ± 1.000) for 400 mg/kg, (65 ± 2.5) sec for 200 mg/kg, and (75 ± 1.5) for 100 mg/kg in comparison to control (50.00 ± 1.7503). However, as a standard drug fluoxetine (10 mg/kg) was used. Statistical significance was calculated using one-way ANOVA; ***p < 0.001; error bars denote SEM (Standard Error of Mean).

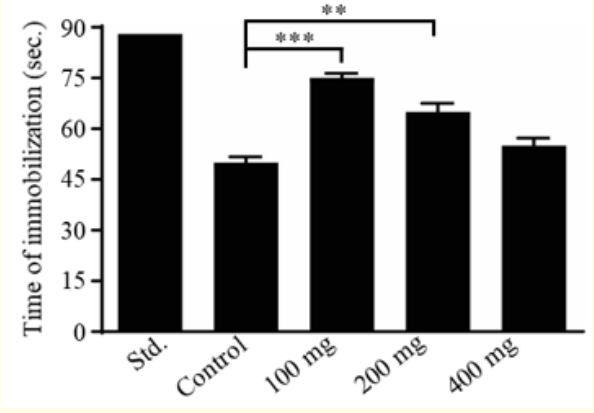


Figure 3b: *Comparison of immobilization (TST) of different doses with standard and control. ***p < 0.001; **p < 0.01; *p < 0.1.*

Virtual screening

The virtual screening of available phytoconstituents was done with the transmembrane receptors. Receptors were searched based on their contribution to anxiolytic and antidepressant activity. These receptors are the serotonin transporter (6BQG), norepinephrine transporter (6M2R), dopamine transporter (4M48), GABA receptor (1KJT), monoamine oxidase A (2Z5X), and monoamine oxidase B (2V5Z) [31-34]. To validate the docking study, reference drug fluoxetine (PubChem CID: 3386) was used. Binding affinity of *L. glutinosa's* phytoconstituents were presented in table 4. A negative value in kcal/mol was used to represent the docking outcomes, with lower scores denoting a more beneficial binding relationship [35]. Additionally, toxicity and drug-likeness analyses were performed on these compounds to determine which one was the most promising drug candidate.

PDB	Serotonin transporter (6BQG)	Norepinephrine transporter (6M2R)	Dopamine transporter (4M48)	GABA receptor (1KJT)	Monoamine oxidase A (2Z5X)	Monoamine oxidase B (2V5Z)
Ligand	**Binding affinity (Kcal/mol)**					
3-thiophenamine	-3.6	-3.5	-3.6	-3.2	-3.5	-3.6
5-Methyl-6- phenyltetrahydro-1,3-oxazine-2- thione	-6.1	-5.7	-5.9	-5.5	-5.4	-5.6
Cyclopentylacetic acid	-5.4	-6	-5.1	-4.4	-5.2	-5.1
Pentaborane	-7.2	-6.4	-7.4	-5.6	-6.7	-6.5
Tetrahydro-N- methyl-N-nitroso-, 1,1-dioxide	-5.2	-5.1	-4.8	-4.4	-5.6	-5.2
Z,Z-3,13-Octadecedien-1-ol	-5.9	-5.1	-4.7	-4.4	-5	-5.4

Table 4: *Binding affinity by phytoconstituents of L. glutinosa.*

Drug-likeness and toxicity

The SwissADME server was used to assess the drug-likeness properties. Lipinski's rule of five is a potential parameter in drug-likeness that states a potential drug candidate must have a molecular weight ≤500 Da, a cLogP value (logarithm of its partition coefficient between n-octanol and water log (coctanol/cwater) ≤4.15, hydrogen bond acceptors ≤10, hydrogen bond donors' ≤5 and rotatable bonds ≤10 [36]. The drug-likeness and toxicity study are shown in table 5, where among the available 3D structure phytochemicals in the PubChem database Z, Z-3, 13-Octadecedien-1-ol has a greater cLogP and a higher number of rotatable bond (nRB). Only three chemicals 3-thiopheamine, 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione, and cyclopentylacetic acid, have passed blood-brain barrier. Toxicity prediction was done by admetSAR.

Compounds	Drug-likeness parameters							Toxicity				
	cLog-P	MW (g/mol)	HBA	HBD	nRB	BBB	BA	Carcinogens	AMES	Acute Oral	Biodegradation	hERG inhibition
3-thiophenamine	1.24	99.15	0	1	0	Yes	0.55	Non-carcinogens	AMES toxic	II	Not ready	Weak inhibitor
5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	2.3	207.29	1	1	1	Yes	0.55	Non-carcinogens	Non-AMES toxic	III	Not ready	Weak inhibitor
Cyclopentyl acetic acid	1.5	128.17	2	1	2	Yes	0.85	Non-carcinogens	Non-AMES toxic	III	Ready	Weak inhibitor
Pentaborane	3.18	323.3	4	0	6	No	0.55	Carcinogens	Non-AMES toxic	I	Not ready	Weak inhibitor
Tetrahydro-N-Methyl-N-Nitroso-, 1,1-Dioxide	0.19	178.21	4	0	2	No	0.55	Non-carcinogens	AMES toxic	III	Not ready	Weak inhibitor
Z,Z-3,13-Octadecedien-1-ol	5.61	266.46	1	1	14	No	0.55	Non-carcinogens	Non-AMES toxic	III	Ready	Weak inhibitor

Table 5: *ADMET profile of the L. glutinosa phytoconstituents.*

Binding interaction analysis of pentaborane and reference drug fluoxetine

Selective serotonin reuptake inhibitors (SSRIs) are mostly prescribed and commonly used as antidepressants. Fluoxetine is one of the potential SSRIs, and was used in this study as control and selected for comparative binding mode analysis [37]. Figure 4 describes the molecular docking study representation of the serotonin transporter with fluoxetine. The binding affinity of serotonin transporter and fluoxetine was calculated to be -8.2 Kcal/mol. Fluoxetine shows strong binding interaction with two conventional hydrogen bonds with the Asp121, Leu196 residues. It also formed a carbon-hydrogen bond with Val195, a pi-anion bond with Asp121, a pi-sigma bond with Phe365, a pi-pi stacked with Phe201, and an alkyl bond with Val122 and Ile118.

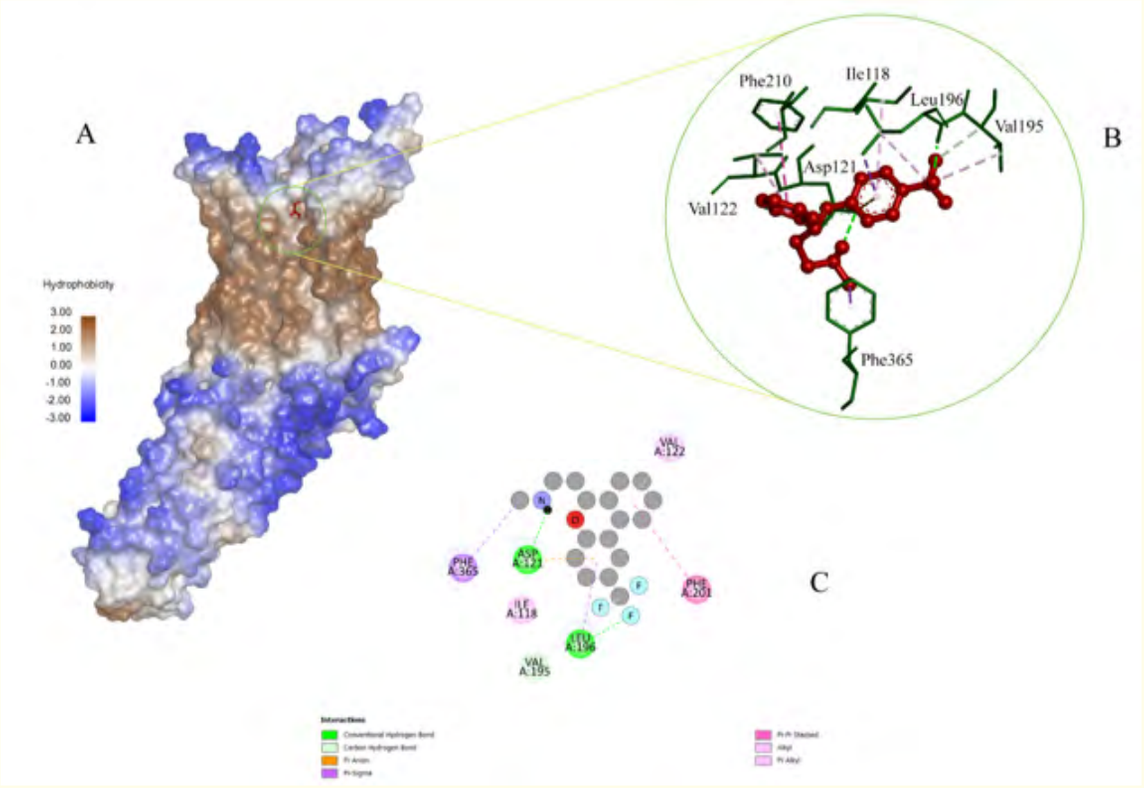


Figure 4: *Visualization of docking analysis of fluoxetine with serotonin transporter (PDB ID: 6BQG). (A) Surface representation. (B) 3D interaction. (C) 2D interaction of binding residues in active site.*

On the other hand, among our investigated *L. glutinosa* phytoconstituents, pentaborane was found to have the highest binding score with monoamine neurotransmitter receptors. In figure 5, pentaborane is shown to have a pi-anion bond with Asp121, a pi-sulfur bond with Phe365, a pi-pi stacked bond with Phe365, and an alkyl bond with Val392, Leu196, and Ile118.

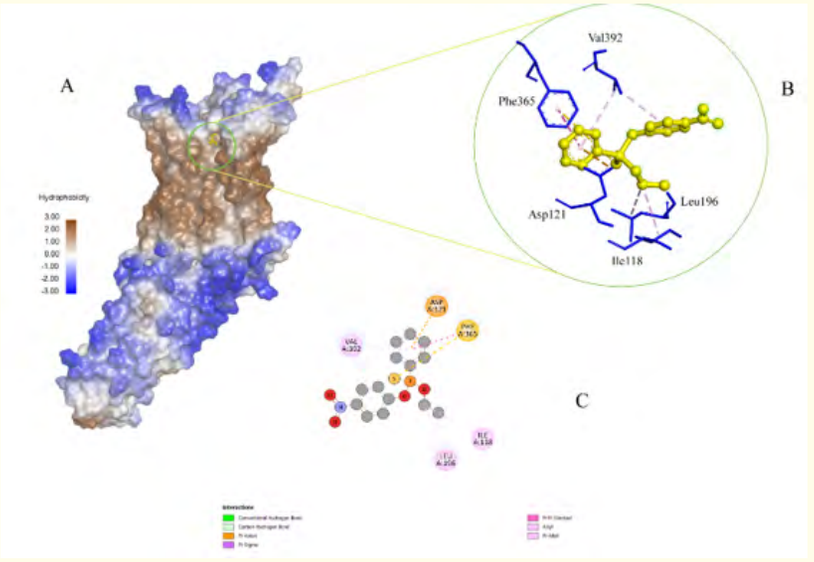


Figure 5: *Visualization of docking analysis of pentaborane with serotonin transporter (PDB ID: 6BQG). (A) Surface representation. (B) 3D interaction (C) 2D interaction of binding residues in the active site.*

Discussion

At the *in-vitro* analysis of the methanolic extract of *Litsea glutinosa* several tests were performed to ensure antioxidant properties of the medicinal plant. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms [38]. Phenolic flavonoids are naturally present in plants. It has positive effects on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities [39,40]. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [41]. Naturally, the electron donation ability of natural products can be measured by 2, 20-diphenyl-1- picrylhydrazyl radical (DPPH) purple-colored solution bleaching [42]. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [43]. Superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [44]. The extract of the plant also presents the superoxide activity. To ensure the safe dose of the extract we have done the acute toxicity test and finally we got safe dose. Then we did EPM, HBT, FST, and TST. EPM and HBT help to determine the anxiety. The EPM has been widely used as a tool in the investigation of the psychological and neurochemical bases of anxiety for screening anxiety-modulating drugs. Hole board test is a generally used method for screening the potential anxiolytic character of drugs. The test is based on the assumption, that head-dipping activity of the animals is inversely proportional to their anxiety state. The forced swim test is a rodent behavioral test used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states [45]. The tail-suspension test is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant compounds.

The highest binding affinity of pentaborane for the serotonin transporter suggests its greater ability to inhibit the reuptake of serotonin by the transporter, leading to increased levels of serotonin in the brain. It is also thought that pentaborane has a role in depleting monoamine neurotransmitters such as serotonin, norepinephrine, and dopamine by regulating the metabolism [46]. The study compared the binding interaction of pentaborane with that of fluoxetine, a well-known SSRI used as a reference drug [47]. The comparative molecular docking interaction study of fluoxetine and pentaborane with serotonin transporter identified Asp121, Leu196, Phe365, and Ile118 residues as common and active site residues of the receptor. However, pi-sulfur interaction was mostly uncommon in pentaborane interaction.

Conclusion

The methanolic extract of *L. glutinosa* contains flavonoid and phenolic compounds having statistically significance anxiolytic, antioxidant, antidepressant, and superoxide scavenging properties. It can further be investigated in the molecular and genomic level.

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All experiments including animal handling and experiments followed by ethical guidelines approved by Research monitoring Committee of the Department of Pharmacy, Manarat International University (Approval No. 2022061392).

Consent for Publication

Not applicable.

Competing Interests

The authors have declared no conflict of interest.

Bibliography

1. Sasidharan S., *et al*. "Extraction, isolation and characterization of bioactive compounds from plants' extracts". *African Journal of Traditional, Complementary and Alternative Medicines* 8.1 (2011): 1-10.

2. Di Lorenzo C., *et al*. "Adverse effects of plant food supplements and botanical preparations: a systematic review with critical evaluation of causality". *British Journal of Clinical Pharmacology* 79.4 (2015): 578-592.

3. Tungmunnithum Duangjai, *et al*. "Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview". *Medicines* 5.3 (2018): 93.

4. Sarkar C., *et al*. "*Heliotropium indicum* L.: From farm to a source of bioactive compounds with therapeutic activity". *Evidence-Based Complementary and Alternative Medicine* (2021): 9965481.

5. Mondal M., *et al*. "Phytochemical profiling and evaluation of bioactivities of methanolic and ethyl acetate extracts of *Marsdenia tenacissima* leaves". *Journal of Herbs, Spices and Medicinal Plants* 26.4 (2020): 405-422.

6. Bhowmick R., *et al*. "*In vivo* analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from *Litsea glutinosa* leaves". *Biological Research* 47.1 (2014): 56.

7. Mohammad N., *et al*. "Genetic diversity and population structure of *Litsea glutinosa* (Lour.) in Central India". *Physiology and Molecular Biology of Plants* 24.4 (2018): 655-663.

8. Jamaddar S., *et al*. "Ethnomedicinal uses, phytochemistry, and therapeutic potentials of *Litsea glutinosa* (Lour.) C. B. Robinson: A literature-based review". *Pharmaceuticals* 16.1 (2022): 3.

9. Agrawal N., *et al*. "Butanolides from methanolic extract of *Litsea glutinosa*". *Chemistry and Biodiversity* 10.3 (2013): 394-400.

10. Mohammed MMD., *et al*. "Comprehensive chemical profiling of *Bassia indica* wight. aerial parts extract using UPLC-ESI-MS/MS, and its antiparasitic activity in *Trichinella spiralis*-infected mice: *in silico* supported *in vivo* study". *BMC Complementary Medicine and Therapies* 23.1 (2023): 161.

11. Erdogrul ÖT. "Antibacterial activities of some plant extracts used in folk medicine". *Pharmaceutical Biology* 40.4 (2002): 269-273.

12. Alabri THA., *et al*. "Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L". *Journal of King Saud University - Science* 26.3 (2014): 237-243.

13. Aziz MAI., *et al*. "Possible neuropharmacological effects of *Adenia trilobata* (Roxb.) in the Swiss albino mice model". *Future Journal of Pharmaceutical Sciences* 6 (2020): 72.

14. Mahboubi A., *et al*. "Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L. var. Pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases". *BMC Complementary and Alternative Medicine* 15 (2015): 366.

15. Ainsworth EA and KM Gillespie. "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent". *Nature Protocols* 2.4 (2007): 875-877.

16. Saeed N., *et al*. "Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L". *BMC Complementary and Alternative Medicine* 12 (2012): 221.

17. Bouslamti M., *et al*. "Total polyphenols content, antioxidant and antimicrobial activities of leaves of *Solanum elaeagnifolium* Cav. from Morocco". *Molecules* 27.13 (2022): 4322.

18. Ganapaty S., *et al*. "Free radical scavenging activity of Gossypin and Nevadensin: An *in-vitro* evaluation". *Indian Journal of Pharmacology* 39 (2007): 281-283.

19. Kalantari H., *et al*. "Evaluation of sub-acute oral toxicity of lithium carbonate microemulsion (nano size) on liver and kidney of mice". *Jundishapur Journal of Natural Pharmaceutical Products* 10.1 (2015): e22312.

20. Fan Y., *et al*. "Acute, subacute oral toxicity and AMES test of py-mulin: an antibacterial drug candidate". *BMC Pharmacology and Toxicology* 23.1 (2022): 2.

21. Finkelstein Y., *et al*. "Colchicine poisoning: the dark side of an ancient drug". *Clinical Toxicology (Philadelphia)* 48.5 (2010): 407-414.

22. Adebesin IF., *et al*. "Evaluation of neuropharmacological effects of aqueous leaf extract of *Albizia glaberrima* (Leguminosae) in mice". *Journal of Ethnopharmacology* 160 (2015): 101-108.

23. Bello-Arroyo E., *et al*. "MouBeAT: A new and open toolbox for guided analysis of behavioral tests in mice". *Frontiers in Behavioral Neuroscience* 12 (2018): 201.

24. Ahmadu U., *et al*. "Studies on some neuropharmacological properties of nevirapine in mice". *IBRO Neuroscience Reports* 12 (2021): 12-19.

25. Brown GR and C. Nemes. "The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia?" *Behavioural Processes* 78.3 (2008): 442-448.

26. Castagné V., *et al*. "Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice". *Current Protocols in Pharmacology* 5 (2010): 8.

27. Yankelevitch-Yahav R., *et al*. "The forced swim test as a model of depressive-like behavior". *Journal of Visualized Experiments* 97 (2015): 52587.

28. Can A., *et al*. "The tail suspension test". *Journal of Visualized Experiments* 59 (2011): e3769.

29. Feldman HJ., *et al*. "A complete small molecule dataset from the protein data bank". *FEBS Letters* 580.6 (2006): 1649-1653.

30. Kemmish H., *et al*. "Fully automated antibody structure prediction using BIOVIA tools: validation study". *PLOS One* 12.5 (2017): e0177923.

31. Xu H., *et al*. "A computational drug-target network for Yuanhu Zhitong prescription". *Evidence-Based Complementary and Alternative Medicine* (2013): 658531.

32. Gibbons AS., *et al*. "Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects". *Journal of Affective Disorders* 116.3 (2009): 184-192.

33. Zhao F., *et al*. "Dopamine receptors: is it possible to become a therapeutic target for depression?" *Frontiers in Pharmacology* 13 (2022): 947785.

34. Jeon WJ., *et al*. "The role of muscarinic receptors in the pathophysiology of mood disorders: a potential novel treatment?" *Current Neuropharmacology* 13.6 (2015): 739-749.

35. Uzzaman M., *et al*. "Physicochemical, spectral, molecular docking and ADMET studies of bisphenol analogues; a computational approach". *Informatics in Medicine Unlocked* 25 (2021): 100706.

36. Lipinski CA., *et al*. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". *Advanced Drug Delivery Reviews* 46.1-3 (2001): 3-26.

37. Mandrioli R., *et al*. "Selective serotonin reuptake inhibitors (SSRIs): Therapeutic drug monitoring and pharmacological interactions". *Current Medicinal Chemistry* 19.12 (2012): 1846-1863.

38. Umamaheswari M and TK Chatterjee. "*In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract". *African Journal of Traditional, Complementary and Alternative Medicines* 5.1 (2007): 61-73.

39. Di Carlo G., *et al*. "Flavonoids: Old and new aspects of a class of natural therapeutic drugs". *Life Sciences* 65.4 (1999): 337-353.

40. Montoro P., *et al*. "Structure-antioxidant activity relationships of flavonoids isolated from different plant species". *Food Chemistry* 92.2 (2005): 349-355.

41. Bravo L. "Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance". *Nutrition Reviews* 56.11 (1998): 317-333.

42. Pereira X., *et al*. "Biological oxidations and antioxidant activity of natural products". Phytochemicals as Nutraceuticals, InTech (2012).

43. Krishnaiah D., *et al*. "A review of the antioxidant potential of medicinal plant species". *Food and Bioproducts Processing* 89.3 (2011): 217-233.

44. Meyer AS and A Isaksen. "Application of enzymes as food antioxidants". *Trends in Food Science and Technology* 6.9 (1995): 300-304.

45. Paloucek FP and JB Leikin. "Poisoning and toxicology handbook". 4th edition, CRC Press (2007).

46. Mandrioli R., *et al*. "Selective serotonin reuptake inhibitors (SSRIs): Therapeutic drug monitoring and pharmacological interactions". *Current Medicinal Chemistry* 19.12 (2012): 1846-1863.

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