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Evaluation of Potential Antidepressant Activity of *Laserpitium latifolium* Plant: *In Silico* Molecular Docking, ADMET, and *In Vivo* Analysis

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Abstract

Background: Globally, depression affects over 300 million people and is a leading cause of disability, yet current synthetic antidepressants often come with significant side effects such as weight gain, sexual dysfunction, and insomnia or suffer from a slow onset of action. This has driven a search for safer, plant-derived alternatives that can modulate the monoaminergic system (serotonin, norepinephrine, and dopamine) or target the neuro-inflammatory pathways. **Aim:** This study aims to investigate the antidepressant potential of an Ethanolic Extract of *Laserpitium latifolium* L (EELL). **Methods:** The plant material was extracted by maceration using ethanol as solvent. The obtained EELL was analysed by Gas Chromatography – Mass Spectrometry (GC-MS) to identify the active phytoconstituents. The selected phytoconstituents were selected as ligands, and human monoamine oxidase A (2Z5Y) was selected as the protein. Docking studies were performed, and finally, the extraction was used to perform *in vivo* antidepressant activity by using the Forced Swim Test (FST) and Tail Suspension method. **Results:** 29 compounds were identified through GC-MS analysis and *in silico* docking studies performed for 8 compounds using Monoamine Oxidase A (MAO-A; PDB ID: 2Z5Y) protein. out of 8 compounds, Blebbistatin exhibited superior binding affinity (-10.2 kcal/mol) compared to the standard MAO-A inhibitor, Imipramine (-8.0 kcal/mol). In the *in vivo* antidepressant study, 24 male Wistar albino rats were randomly divided into four groups: Group I (Normal control), Group II (Standard Imipramine, 10mg/kg), and Groups III and IV (Treatment groups, 200 and 400mg/kg, respectively). No morbidity and mortality were observed up to the dose of 2000 mg/kg. The antidepressant results showed that the extract significantly reduced immobility time in both the Forced Swim Test (FST) and Tail Suspension Test (TST) at doses of 200 and 400 mg/kg. **Conclusion:** The above findings suggest that *L. latifolium* L. exhibits antidepressant properties, supported by both *in-silico* and *in vivo* evidence.

Major Findings: *Laserpitium latifolium* L. (EELL) demonstrated significant antidepressant-like effects in animal models, with the compound Blebbistatin showing higher binding affinity for MAO-A than the standard antidepressant Imipramine. Additionally, the extract exhibited a high safety profile, with no toxicity, morbidity, or mortality in rats at doses up to 2000 mg/kg, and GC-MS analysis identified 29 distinct bioactive compounds.

Keywords: ADMET, Depression, Forced Swim Test, Imipramine, Depression, Molecular Docking, Tail Suspension Method

Abbreviations: MAO: Monoamine oxidase; OECD: The Organisation for Economic Cooperation and Development; FST: Forced Swim Test and TSM: Tail Suspension Test; WHO: World Health Organisation; HPLC: High-Performance Liquid Chromatography; EELL: Ethanolic Extract of *Laserpitium latifolium* L; GC-MS: Gas Chromatography-Mass Spectrometry.

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1. Introduction

Depression is a widespread illness that has a major impact on behaviour, emotion, and physical health. It is one of the main causes of disability and early death in the world[1]. Depression is a widespread disorder affecting a significant portion of the global population. According to the World Health Organisation (WHO), approximately 3.8% of the population experiences depression, with 5% of adults (4% of men and 6% of women) and 5.7% of adults over 60 years old being affected. Globally, around 280 million people suffer from depression[2]. and symptoms include suicide thoughts[3], feelings of powerlessness, decreased physical activity, and bad emotions. The human enzyme MAO catalyses oxidative deamination, which is partly responsible for the condition's correlation with low levels of key neurotransmitters that contain monoamine in the brain and peripheral tissue, including dopamine, serotonin, and norepinephrine[4,5]. This is partly explained by the oxidative deamination process that is facilitated by the human enzyme MAO[6,7].

MAO-A and MAO-B are the two forms of MAO isoenzymes that humans have[8]. While MAO-B is in charge of deaminating phenethylamine and benzylamine, MAO-A primarily deaminates serotonin, norepinephrine, melatonin, and epinephrine. MAO-A and MAO-B catalyse dopamine, tyramine, and tryptamine equally[9]. Current depression therapies include a variety of medications, including tricyclic antidepressants, selective and nonselective monoamine reuptake inhibitors, Monoamine Oxidase Inhibitors (MAOI), and selective serotonin reuptake inhibitors[10]. Nevertheless, these drugs frequently have drawbacks, such as side effects and ineffectiveness[11,12].

Laserpitium latifolium L., a member of the Apiaceae family, is commonly known as broad-leaved laserwort and is native to Europe. It primarily grows in temperate biomes and is widespread across various countries in Europe and the Western part of Asia. It has been traditionally used for various medicinal purposes, including as a diuretic, purgative, and tonic for belly sicknesses, pulmonary tuberculosis, fever, and rheumatism, as well as in gynaecology, coronary heart and liver issues, and externally for toothache and skin conditions[13]. The plant is known to contain biologically active compounds like quercetin and sesquiterpene lactones. Chemical investigations of *L. latifolium* have revealed the presence of daucane derivatives (laserpitin, laserpitinol, isolaserpitine, and deoxodehydrolaserpitine) and essential oils containing monoterpenoids (sabinene, α-pinene, and limonene). Additionally, phenolic compounds isolated from the leaves include quercetin derivatives such as avicularin, isoquercitrin, quercitrin, and rutin, confirmed through paper chromatography and High-Performance Liquid Chromatography (HPLC) analysis[14]. The study aimed to evaluate the antidepressant potential of *L. latifolium* plant extraction by *in-silico* molecular docking, ADMET and *in vivo* analysis.

2. Materials and Methods

2.1 Plant Authentication

Laserpitium latifolium L. was authenticated by Dr K. Madhava Chetty, Assistant Professor, Department of Botany, from Sri Venkateswara University, Tirupati, Andhra Pradesh.

2.2 Preparation of the Extract

The plant material was collected, cleaned with distilled water, and shade-dried. The dried plant was then ground into a coarse powder. One kilogram of the powder was placed in a stoppered container and extracted with 95% ethanol for 7 days, with frequent stirring. The mixture was filtered using a muslin cloth or net, and the filtered solvent was evaporated using a rotary evaporator. The remaining extract was dried in a desiccator to remove any residual ethanol. The resulting ethanolic extract of *L. latifolium* L. (EELL) was stored in a sealed container for further research.

2.3 Initial Identification for Phytochemicals

The preliminary phytochemicals of EELL were identified according to the procedures of Fathima *et al*[15].

2.4 GC-MS Investigation

GCMS analysis was performed for EELL. A JMS 600H JEOL mass spectrometer and an Agilent gas chromatograph (model number 6890 N) were used for the detection. Utilising a fusion capillary SPBI column (30m, 0.32mm, 0.25µ m film thickness) and a temperature program that ranged from 50 to

256°C at a rate of 4°C/min, with a 2-minute hold, the phytoconstituents of the extract were successfully separated. Helium was used as the carrier gas, and the injector was adjusted to 260°C. The flow rate was 1 mL per minute. The ionisation voltage, electron emission, ion source temperature, and analyser temperature of the EI mode JMS 600 H JEOL mass spectrometer were 70Ev, 100Ma, and 250°C, respectively. In split mode, 1 µL of ethanolic extract was manually injected at a sample ratio of 20:1. GC-MS study of phytochemicals in an ethanolic extract of *L. latifolium* L. The method involved computer examination of sample mass spectra utilising the National Institute of Standards and Technology (NIST), peak and retention time comparison, computer matching, and observing the typical fragmentation patterns of mass spectra from a certain class of molecules.

2.5 Evaluation of *In Silico* Docking Studies

2.5.1 Software Tools

UCSF Chimera, Auto Dock Vina, Discovery Studio Visualiser 2020 (BIOVIA), Drug Banking, MGL instruments, Protein Data Bank (PDB), and PubChem were among the technologies used in this study.

2.5.2 Protein Target Selection

Potential treatment targets for melancholy (depression) have been identified by a systematic evaluation of the literature. The PDB, a vast collection of chemical molecules, was used to validate the identified targets.

2.5.3 The Target Protein

Using the Research Collaboratory for Structural Bioinformatics (RCSB) Proteins Data Bank, human monoamine oxidase A (2Z5Y) (resolution: 2.17Å) was acquired. These structures, which have been used in recent research initiatives and are published in several reputable journals, have been accepted as being related to humans. This guarantees that the outcomes can be reproduced using the appropriate tools. An established receptor for sadness and activated anxiety is PDB ID 2Z5Y. Using the appropriate tools guarantees the reproducibility of the results. Recognised receptors for anxiety and depression are PDB ID 2Z5Y. First, the Biochemical Studio Visualizer (BIOVIA) was used to extract protein ligands from complexes. Proteins were optimised and prepared for docking using UCSF Chimaera. Polar hydrogen was added to the necessary H-atoms after water molecules were extracted from the structures. After determining Gasteiger charges, proteins were converted to PDBqt format using AutoDock Vina docking[16].

2.5.4 Sorting Virtually

The binding agents [(2mercaptoethyl) guanidine, (+)-Blebbistatin, caryophyllene, aztreonam, phytol, Neophytadine, ipriflavone, and oleic acid] of *L. latifolium* were reduced and optimised using PyRX-autodock vina software to finish the virtual screening. The binding sites of the receptor's target pockets have been covered by the ligand-centred grid box in the PyRx autogrid program. The grid box permits unrestricted movement of the ligand inside the designated planes (X, Y, and Z). Using the Discovery Studio Visualizer, the coordinate values for x, y, and z were obtained from the corresponding PDB crystal structure (2z5y). As in previous research, the grid scale was 60 Å × 60 Å x 60 Å. The BIOVIA Discovery Studio Visualiser 2020 was used to visualise the docking (ligand-protein interactions).

2.6 Statistical Analysis

The results were presented as Mean ± SEM. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test, utilising GraphPad Prism 5.0. Error bars represent the 95% confidence interval.

2.7 Animals

Both sexes of Wistar albino rats weighing 180–210 grams were used in this investigation. The animals were kept in a 12:12 light/dark cycle at 24±2°C. They have limitless access to food and beverages. Before the examination, the animals were acclimated for seven days. The CMR College of Pharmacy's IAEC approved all animal experiments under Protocol No. CPCSEA/ 1657/ IAEC/ CMRCP/ COL-23/124.

2.8 Assessment of Antidepressant Activity

2.8.1 Grouping of Animals

Wistar male albino rats were used for the study. The animals (n=6) were separated into four groups of six animals each.

2.8.2 Treatment Groups

Group I (Normal Control): Received normal saline (5 ml /kg b. wt, *p.o*) for 14 days

Group II (Standard Imipramine): Received imipramine (10mg/kg b. wt, *p.o*) for 14 days

Group III (EELL 200mg/kg): Received EELL (200mg/kg b. wt, *p.o*) for 14 days

Group IV (EELL 400mg/kg): Received EELL (400mg/kg b. wt, *p.o*) for 14 days

2.8.3 Forced Swim Test (FST)

The forced swim test is a behavioural assessment conducted on rodents to evaluate the effectiveness of antidepressant medications, the efficacy of new compounds, and experimental interventions aimed at inducing or preventing depressive-like behaviours.

In this test, Rats are placed in a transparent tank filled with water from which they cannot escape, and their mobility related to escape is monitored. The procedure is simple to execute with consistent results and requires minimal specialised equipment. All animal groups underwent the forced swim test following the administration of their respective drug solutions. On the initial day, during the training session, rats were individually placed in a vertical Plexiglas cylinder (height: 40cm; diameter: 18cm) filled with fresh water up to 15cm, maintained at 25°C for 15 minutes, during which they were observed for 6 minutes. In this assessment, after a brief period of active swimming, the animals typically exhibit a state of immobility, characterised by floating motionlessly in the water, making only the necessary movements to keep their heads above the surface. This immobility is indicative of a depressive state. Each mouse underwent this procedure 24 hours before and 1 hour after receiving their respective drugs for a duration of 5 minutes during the test session, with the duration of immobility recorded during the last 4 minutes. Actual test recordings were conducted on the 1st, 7th, and 14th days of treatment. After the mobility-immobility times were recorded, each mouse was removed, dried with a cloth, and allowed to dry before being returned to their home cages[17,18].

2.8.4 Tail Suspension Method

The tail-suspension test serves as a behavioural assessment for rats, instrumental in the evaluation of potential antidepressant medications and other interventions anticipated to influence behaviours associated with depression. In this procedure, rats are suspended by their tails using tape, preventing them from escaping or grasping nearby surfaces. The test, which lasts approximately six minutes, measures the resulting escape-oriented behaviours. This method is a significant asset in drug discovery, particularly for high-throughput screening of potential antidepressant agents. All animal groups underwent this test by being suspended from a string attached to a metal stand, with adhesive tape affixed 1cm from the tail's tip, and the string positioned 58cm above the tabletop. The mobility and immobility durations of the rats were recorded during the final 4minutes of a 5min observation period. Rats were deemed immobile when they hung passively and remained completely still. Throughout the experiment, each subject was acoustically and visually isolated from others. Measurements were taken on the 1st, 7th, and 14th days of treatment[19,20].

3. Results and Discussion

3.1 Phytochemical Screening

The phytochemical screening of the EELL was found to be alkaloids, flavonoids, phenolic compounds, steroids, and saponins as chemical constituents.

3.2 Evaluation of GC-MS Compounds

GC-MS analysis was done through aqueous method GC-MS SCAN B.amx and the analysis method *1.pmx the column of Agilent 19091S-433UI and the dimensions of the column through uncalibrated method was 30m x 250µm x 0.25µm and the The column's rate of flow was 1.2ml per minute, and its temperature ranged from -60°C to -325°C(350°) the pressure of the column includes 11.367psi and holdup time of solvent was 1.2376 min and the average velocity was 40.402cm/sec. In this, the highest peak was observed at the time of 18.418(min).

In the process of GC-MS analysis, we have evaluated the Compounds, Retention Time (RT), Peak Area (PPA), Molecular weight, and molecular formula of every individual compound. (2-mercaptoethyl) guanidine having a RT of 3.394(min) the PA was 7.61%. (+)- Blebbistatin having a RT of 3.475(min) the PA was

Table 1. Compounds identified through GC-MS analysis

S. No.	Retention Time (min)	Compound	Molecular Formula	Molecular Weight	Peak Area %
1	3.394	(2-mercaptoethyl) guanidine	$C_3H_9N_3S$	119	7.61
2	3.475	(+)- Blebbistatin	$C_{18}H_{16}N_2O_2$	292	6.71
3	17.084	Caryophyllene	$C_{15}H_{24}$	204	5.33
4	22.747	Aztreonam	$C_{13}H_{17}N_5O_8S_2$	435	1.33
5	29.630	Phytol	$C_{20}H_{40}O$	296	5.39
6	29.630	Neophytadiene	$C_{20}H_{38}$	278	5.39
7	37.000	Ipriflavone	$C_{18}H_{16}O_3$	280	5.26
8	38.481	Oleic acid	$C_{18}H_{34}O_2$	282	8.42
10	3.394	Glycerin	$C_3H_8O_3$	92	7.6
11	3.475	Benzaldehyde	C_7H_6O	106	6.71
12	3.475	Benzeneacetonitrile, α-hydroxy-	C_8H_7NO	133	6.71
13	5.694	Try ala	$C_{12}H_{16}N_2O_4$	252	1.50
14	5.694	Benzoic acid, hydrazide	$C_7H_8N_2O$	136	1.50
15	5.694	Ethanone,2-hydroxy-1-phenyl	$C_8H_8O_2$	136	1.50
16	15.821	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	1.46
17	15.821	Cyclopentane Carboxamide, N-(4-Fluorophenyl)	$C_{12}H_{14}FNO$	207	1.46
18	17.084	Cycloartenol acetate	$C_{32}H_{52}O_2$	468	5.33
19	17.084	Bicyclo (7.2.0) undec-4-ene,4,11,11-trimethyl-8-methylene-	$C_{15}H_{24}$	204	5.33
20	18.421	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	53.07
21	18.421	Myo-inositol,4-c-methyl-	$C_7H_{14}O_6$	194	53.07
22	22.603	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	1.05
23	22.603	Bicyclo(3.1.1) heptane,2,6,6-trimethyl-, (1α,2β,5α)-	$C_{10}H_{18}$	138	1.05
24	22.603	16-Heptadecenal	$C_{17}H_{32}O$	252	1.05
25	22.747	4-Methoxy-6-methyl-6,7-dihydro-4H-furo(3,2-c) pyran	$C_9H_{12}O_3$	208	1.33
26	26.835	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	252	2.86
27	29.630	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.86
28	37.000	Des iso propyldisopyramide	$C_{18}H_{23}N_3O$	297	5.26
29	38.481	2,4-thiazolidinedione,5-((4-(4-pyridinyl)-6-quinolinyl) met	$C_{18}H_{11}N_3O_2S$	333	8.42

6.71%. Caryophyllene, having an RT of 17.084(min), the PA was 5.33%. Aztreonam had an RT of 22.747(min) the PA was 1.33%. Phytol and Neophytadiene have the same RT of 29.630(min) and the same PA was 5.39%. Ipriflavone had an RT of 37.000 (min), and the PA was 5.26%. Oleic acid, having RT of 38.481(min), the PA was 8.42%. Figure 1 shows the compound peaks, and Table 1 shows the compounds identified.

3.3 Evaluation of *In Silico* Studies

3.3.1 Docking Analysis

The ligand-protein complex's binding orientation and structural stability are predicted via molecular docking, calculating Root Mean Square Deviation (RMSD) of every ligand. 2Z5Y R-value observed 0.196. During the docking process, a search algorithm is used to generate several poses, and a scoring function is employed to assess how well certain poses and proteins bond together. Only the static protein-ligand interaction can be predicted by molecular docking. In order to comprehend the manner in which ligands interact with the dynamic protein in a setting akin to physiological settings, Dihydrozeatin (DHZ) underwent 30-NS MD simulation research**.** The creation of a water bridge and an H-bond between residue G110A and the hydroxyl group of DHZ. With residue, stable interactions were

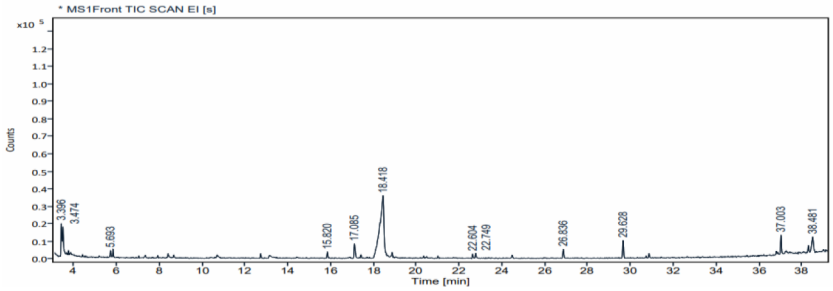


Figure 1. GC-MS analysis of EELL.

Table 2. List of GC-MS compounds interactions and docking score

S. No.	Compound	Docking Score	H -Bonds	Vanderwall Interaction
1	(2mercaptoethyl) guanidine	-8.0	TYR-407, ALA-68	ILE-180, GLN-215, MET-350, TYR-69, LYS-305, GLY-66, GLY-67, MET-445, GLY-443, ARG-51, CYS-406, TYR-444, ASN-181.
2	(+)- Blebbistatin	-10.2	TYR-407, ALA-68	ILE-180, GLN-215, MET-350, TYR-69, LYS-305, GLY-66, GLY-67, MET-445, GLY-443, ARG-51, CYS-406, TYR-444, ASN-181.
3	Caryophyllene	-6.5	TYR-407, ALA-68	ILE-180, GLN-215, MET-350, TYR-69, LYS-305, GLY-66, GLY-67, MET-445, GLY-443, ARG-51, CYS-406, TYR-444, ASN-181.
4	Aztreonam	-5.2	GLN-215, ILE-207, PHF-208	ASN-181, GLY-214, TYR-69, ALA-68, IYR-444, MET-445, GLY-67, GLY-443, PHF-352, MET-350.
5	Phytol	-8.1	GLY-67	ARG-51, CYS-406, VAL-303, GLY-66, MET-350, GLN-215, PHE-208, ILE-180, ALA-68, GLY-443, MET-445.
6	Neophytadiene	-7.7	-	GLY-55, GLY-67, ALA-68, TYR-444, TYR-69, ILE-180, PHE-708, GLY-443, MET-350, GLN-215.
7	Ipriflavone	-9.7	-	GLY-67, PHE-352, TYR-69, ALA-68, GLY-67, GLY-443, TYR-444, ASN-181.
8	Oleic acid	-7.6	TYR-69, ALA-68	GLY-67, MET-445, GLY-443, GLN-74, ILE-180, GLN-215, MET-350, ALA-111, SER-209.
9	Imipramine	-8.9	TYR-407, VAL-65	ILE-180, GLN-215, PHE-352, TYR-69, LYS-305, GLY-66, TRP-397, VAL-303, CYS-406, GLY-67, ALA-68, GLY-443.

seen, mostly hydrophobic and water bridge. In this study, nine compounds were used for docking studies. All compounds were interacted and bound with protein to show the structural activity relationship, the protein that initiates the interaction and binding to Human Mono Amine Oxidase A (PDB ID: 2Z5Y) (resolution:2.17Å). The individual compounds' docking score and interaction with hydrogen bonds and van der Waals interactions are listed below in Table 2

Human Monoamine Oxidase A (2Z5Y) resolution of the protein 2.17Å and binding to every individual component to show their activity. nine components for docking studied for and they indicated the binding interaction between (2-mercaptoethyl) guanidine against HMAO-A the resulted docking score -8.0, (Figure 2) indicates the binding interaction between (+)-blebbistatin against HMAO-A the resulted docking score -10.2, (Figure 3) indicates the binding interaction between caryophyllene against HMAO-A the resulted docking sore -6.5, (Figure 4) indicates the binding interaction between aztreonam against HMAO-A the resulted docking score -5.2,(Figure 5) indicates the binding interaction between phytol against HMAO-A the resulted docking score -8.1, (Figure 6) indicates the binding interaction between neophytadiene against HMAO-A the -7.7, (Figure 7) indicates the binding interaction between ipriflavone against HMAO-A the resulted docking score -9.7, (Figure 8) indicates the binding interaction between oleic acid against HMAO-A the resulted docking score -7.6, (Figure 9) indicates the binding interaction between imipramine (standard drug) resulted the docking score of -8.9 (Figure 10). In this (+)-Blebbistatin has the highest docking score compared to the remaining compounds,

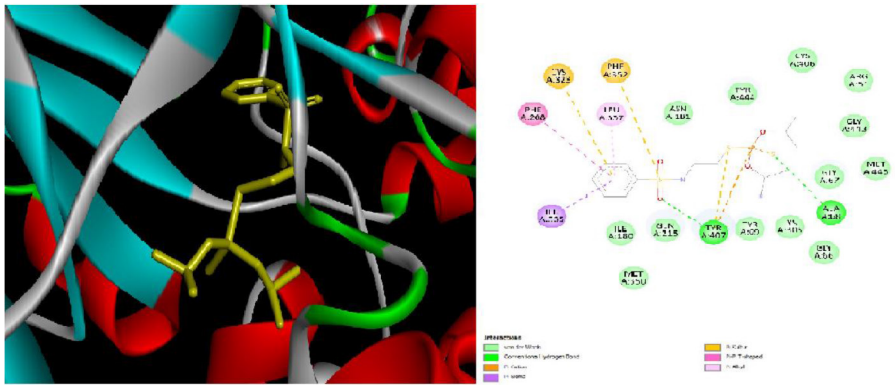


Figure 2. The binding interaction of (2-Mercaptoethyl) Guanidine against MAO A (PDB ID: 2Z5Y).

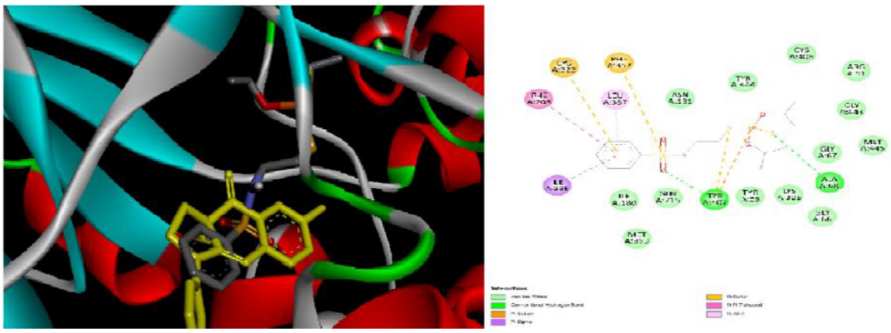


Figure 3. The binding interaction of (+)-Blebbistatin against MAO A (PDB ID: 2Z5Y).

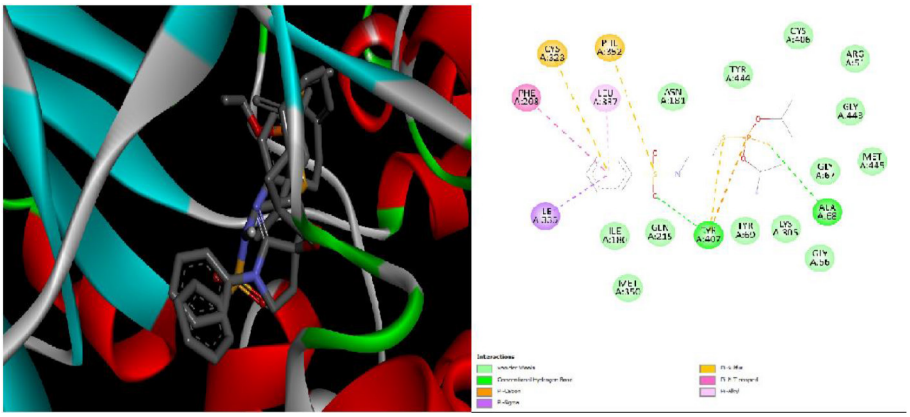


Figure 4. The binding interaction of Caryophyllene against MAO A (PDB ID: 2Z5Y).

and also more Vanderwall interactions occurred. This shows the antidepressant activity in EELL extract through docking studies.

3.4 Blebbistatin ADME/T Analysis by the pkCSM Tool

The structure of Blebbistatin, as shown in Figures 11 and 12, contains a radar plot of Blebbistatin to elucidate the physicochemical characteristics. The Absorption parameters include Caco-2 Permeability, which are the human epithelial colorectal adenocarcinoma cell lines, to study the absorption-related characteristics. The reported value, which is > 0.90, is considered the highest permeability. Intestinal absorption is the primary site of the absorption of a drug molecule into the systemic circulation. Blebbistatin has more than 90% intestinal absorption. Water solubility is reflected by the logS, which is an indication of greater absorption

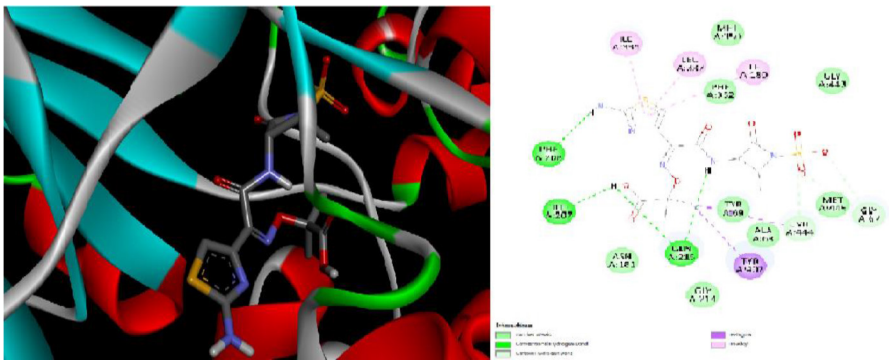


Figure 5. The binding interaction of Aztreonam against MAO A (PDB ID: 2Z5Y).

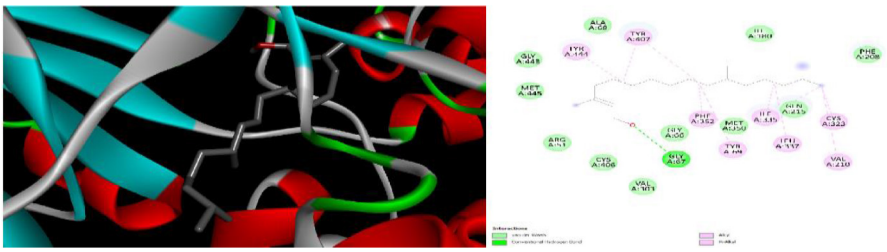


Figure 6. The binding interaction of Phytol against MAO A (PDB ID: 2Z5Y).

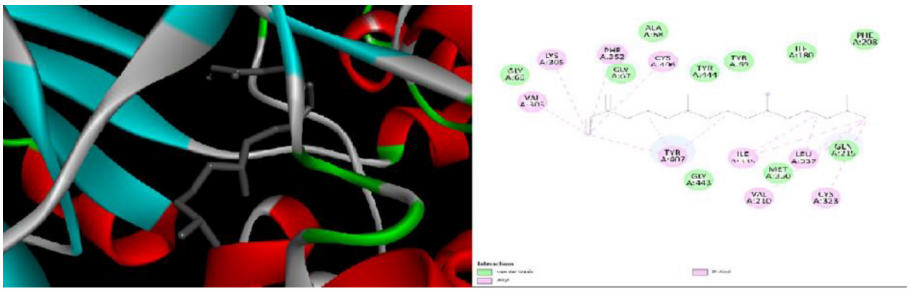


Figure 7. The binding interaction of Neophytadiene against MAO A (PDB ID: 2Z5Y).

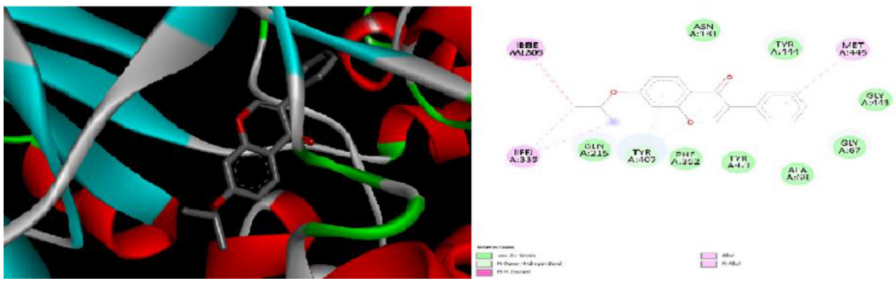


Figure 8. The binding interaction of Ipriflavone against MAO A (PDB ID: 2Z5Y).

when compared with the lipid-soluble drugs. -4.269 log Mol/L indicates less water solubility and increases lipid absorption. Pgp substrate indicates the expulsion of the toxins by binding with the Adenosine Triphosphate Binding (ATP) cassette. Log Kp is the sign of skin permeability, which is > -2.5, indicating less permeation through skin when it is given the transdermal delivery route of administration. Results were shown in Table 3.

Distribution characteristics are termed the Volume of Distribution (VDss), which is > 0.45, and is likely

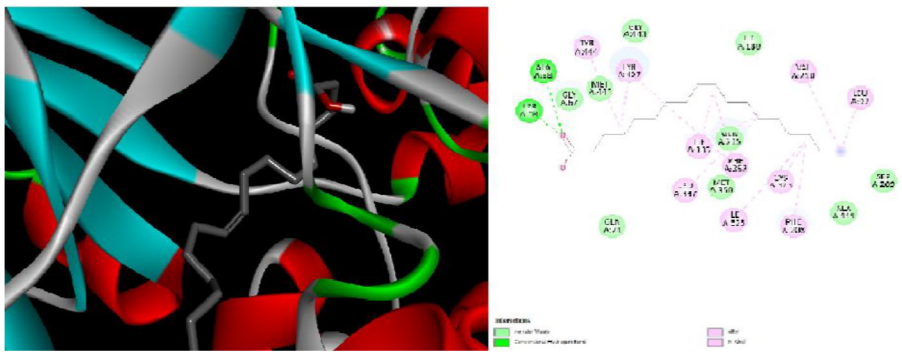


Figure 9. The binding interaction of Oleic acid against MAO A (PDB ID: 2Z5Y).

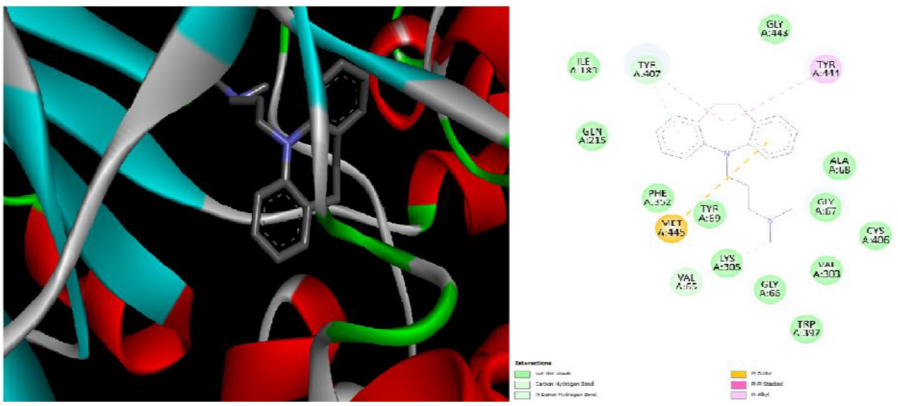


Figure 10. The binding interaction of Imipramine against MAO A (PDB ID: 2Z5Y).

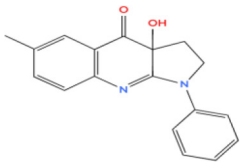


Figure 11. Structure of Blebbistatin.

to be more distributed in tissues rather than plasma levels. The blood-brain barrier is the obstacle system that protects the brain from exogenous particles. A Blood Brain Barrier (BBB) value of less than 0.3 indicates it is less permeable through the BBB. The fraction of unbound concentration of the drug particles is calculated to measure the value of unbound drug particles in the bloodstream. Higher bound concentration leads to less in circulation. logPS is the Central Nervous System (CNS) permeability; it should be >-2 to cross the barrier to increase the brain product surface area[21].

Metabolism is the general process of excreting the xenobiotics through the excretion process. Cytochrome P450 (CYP450) is the major metabolic enzyme that detoxifies drugs. CYP 2D6 and CYP 3A4 are both considered as the isoforms of the CYP 450 in nature. Inhibition of these isoforms may affect the pharmacokinetics of the other drugs. Excretion of the drugs includes the OCT2 (organic cationic transporter 2), which is the main transporter for the uptake of the drugs to facilitate the excretion process. Drug clearance includes the hepatic and renal clearance parameters to measure the dosage concentration within steady state levels. Renal drug clearance is measured in ml/min/kg, and the higher the value, the more likely the drug is to be cleared from the body.

Toxicity parameters are considered to measure the drug's toxic levels to harm the body organs. The Ames test is a measure of mutagenicity using bacteria. Ames toxicity YES indicates there is a chance of mutations in the genes when our body is exposed to that particular drug. Hepatotoxic concern is the measure of the disruption of the liver functions, and skin sensitisation is the damage caused by the drug when

Table 3. Molecule descriptors specifications of Blebbistatin

Name of ADME/T Property	Model Name	Reported Value	Units
Absorption	Water Solubility	-4.269	(log mol/L)
	CaCo2 Permeability	1.314	(log Papp in 10^{-6} cm/s)
	Intestinal Absorption	97.711	(% absorbed)
	Skin Permeability	-3.119	(log Kp)
	p-Glycoprotein Substrate	Yes	
	p-Glycoprotein I Inhibitor	No	
	p-Glycoprotein II Inhibitor	No	
Distribution	VDss	-0.102	(log L/kg)
	Fraction Unbound	0.15	(Fu)
	BBB Permeability	0.167	(log BB)
	CNS Permeability	-1.834	(log PS)
Metabolism	CYP2D6 Substrate	No	
	CYP3A4 Substrate	Yes	
	CYP1A2 Inhibitor	Yes	
	CYP2C19 Inhibitor	Yes	
	CYP2C9 Inhibitor	No	
	CYP2D6 Inhibitor	No	
	CYP3A4 Inhibitor	No	
Excretion	Total Clearance	0.208	(log ml/min/kg)
	Renal OCT2 Substrate	No	
Toxicity	AMES Toxicity	Yes	
	Max. Tolerated Dose	0.814	(log mg/kg/day)
	hERG I Inhibitor	No	
	hERG II Inhibitor	No	
	Hepatotoxicity	Yes	
	Skin Sensitisation	No	
	Oral Rat Acute Toxicity (LD_{50})	2.444	(mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	1.691	(log mg/kg bw/day)

it is given by the topical route of administration[22]. Maximum Recommended Tolerable Dose (MRTD) is the dose of a drug to be taken. If it is less than 0.477, it is considered a low-dosage drug. Inhibition of the hERG I and II enzymes leads to a disturbance in the QT interval of the Electrocardiogram (ECG) of the heart[23].

3.5 Drug Likelihood Properties of Blebbistatin

Drug likeliness is the analysis of the possibilities of the drug being delivered through the oral route of administration. Lipinski's rule of five indicates the drug-like properties by assessing the drug molecular descriptor values, all of which are limited to a factorial of five. Molecular weight of the drug should be < 500 Daltons, partition coefficient <5, Hydrogen bond acceptors < 5, Hydrogen bond donors < 10 to deliver the drug through the oral route. More than 2 violations in the molecular descriptor led to it being impossible to deliver by the oral route. Results are shown in Table 4.

The shaded region of the plot indicates the controlled molecular descriptors, and these values are within the range of prediction values. Lipophilicity <5, molecular weight < 500 daltons, total polar surface area

20 to 130A^0 range, insolubility (LogS) is between -6 to 0 range, instauration should be 0.25 to 1, flexibility > 0, and number of rotatable bonds is > 9. The present radar plot violated the ranges with the instauration parameter, as shown in Figure 12[24].

Table 4. Molecular descriptor reported values by the SWISSADME

S. No.	Molecular Descriptor*	Reported Value
1	Molecular Weight	292.33
2	Partition Coefficient	3.45
3	HBA	3
4	HBD	1

* No violation of Lipinski's rule of five.

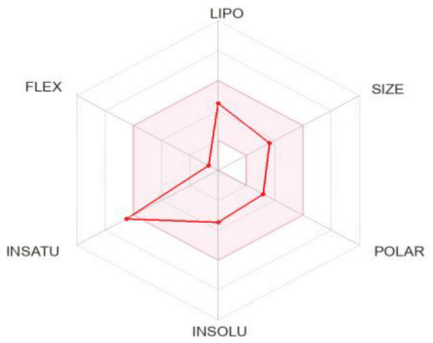


Figure 12. Radar plot of Blebbistatin to elucidate the physicochemical characteristics.

3.6 Evaluation of *In Vivo* Studies

The average effectiveness of the many depression treatments that are now on the market is between 50 and 60 per cent, and many patients do not respond to the initial dose of medicine[25,26]. Furthermore, hypotension, arrhythmias, sleeplessness, and sexual dysfunction are among the unbearable adverse effects of the majority of depressive drugs[27,28]. This emphasises how urgently better, more palatable, and more potent antidepressants are needed. Therefore, the main purpose of the present study was to study the effect of *L. latifolium* against the FST and TST-induced depression. Results were shown in Tables 5 and 6. The acute toxicity of EELL did not show any toxicity up to 2000mg/kg b.wt. Based on the study, selected 1/10th and 1/20th of the tolerable doses for screening of antidepressant activity.

Gamma-Aminobutyric Acid (GABA) serves as the primary inhibitory neurotransmitter in the central nervous system. Various antidepressants, muscle relaxants, and sedative-hypnotic medications exert their effects through GABAergic inhibition in the CNS, resulting in either a reduction in the firing rates of essential neurons in the brain or direct activation of GABA receptors by the extracts. This outcome indicates a significant reduction in the immobility duration in the FST and TST due to EASL. It is plausible that our plant extracts operate through GABAergic and/

Table 5. Effect of EELL immobility time in the forced swim test

Name of the Group	DAY-1	DAY-7	DAY-14
Immobility in Sec			
Normal Control	146.83±3.291	141.0.93±0.93	137.6±2.17
Standard Imipramine (10mg/kg)	111.5±1.19***	108.33±1.33***	105.23±1.38***
EELL (200mg/kg)	135.5±3.41**	131.33±4.4**	128.16±2.6**
EELL (400mg/kg)	128.16±4.76***	122.3±2.41***	118.16±1.94***

where, ***p<0.001 and **p<0.01 in comparison as normal control.

Table 6. Effect of EELL immobility time in the tail suspension test

Name of the Group	DAY-1	DAY-7	DAY-14
Immobility in Sec			
Normal Control	185.66±2.1	189.83±1.13	183.33±1.66
Standard Imipramine (10mg/kg)	101.16±2.94***	89.33±2.55***	70.33±2.58***
EELL (200mg/kg)	138.83±1.74**	128.16±2.60**	95.66±1.13**
EELL (400mg/kg)	121.66±2.98***	108.83±1.24***	86.37±2.74***

Where, ***p<0.001 and **p<0.01 in relation to normal control

or glutamatergic transmission, and alterations in cytokines or steroids cannot be dismissed[29-32].

The chemicals (2-mercaptoethyl)-guanidine, (+)-blebbistatin, caryophyllene, aztreonam, phytol, neophytadiene, ipriflavone, and oleic acid interact with human monoamine oxidase A (PDB ID: 2Z5Y), as illustrated in Figures 1-9. (+)-Blebbistatin is more effective than all other substances, demonstrating antidepressant activity and perhaps producing antidepressant effects through the inhibition of MAO-A, even when the results are compared to the aforementioned criteria. Strong interactions (hydrogen bonds, hydrophobic contacts, and electrostatic interactions) with particular residues in the MAO-A enzyme's binding site cause this inhibition. Blebbistatin functions as an MAO-AI, boosting the availability of neurotransmitters in peripheral tissues, including dopamine, serotonin, and norepinephrine[33-35]. This may lessen the symptoms of sadness and anxiety. Blebbistatin may work better than imipramine, a common MAO-A inhibitor, which has a much lower MAO-A docking score of -10. 2 kcal/mol, (-8. 9 kcal/mol).

The FST and TST are widely used behavioural models for screening potential antidepressant agents, including herbs. These tests measure immobility in rodents, which is interpreted as a state of despair or lowered mood, analogous to human depression. Kumar *et al*., reported antidepressant activity of hydroalcoholic extract of *Sedum lineare* Thumb at a dose of 100, 250 and 500 mg/kg b.wt against FST and TST induced depression. The administered hydroalcoholic extract of *S. lineare* improved mobility and normalised behaviour of the mice in a dose-dependent manner[36].

In our study, EELL demonstrated a reduction in the duration of immobility during the forced swimming and tail suspension tests compared to the control group, exhibiting a dose-dependent antidepressant effect. The behaviour assessed in these tests, referred to as immobility, is thought to represent behavioural despair akin to human depression; thus, any decrease in this measure indicates antidepressant efficacy. A significant correlation exists between the clinical effectiveness of antidepressant medications and their potency in the forced swim test, a relationship not observed in other models. Notably, our findings suggest that a dose of 400 mg/kg showed a greater effectiveness than a 200mg/kg dose in both the forced swim and tail suspension tests. The present study results were correlated to the reports of Kumar *et al.*,[36] and Arraey *et al*[37].

4. Conclusion

Phytochemical analysis of the Ethanolic extract of *L. latifolium* plant revealed the presence of various compounds, including alkaloids, flavonoids, and phenolic compounds. GC-MS analysis identified 29 compounds, with 8 selected for docking studies. In-silico studies using Human Mono Amine Oxidase A (HMAO-A) protein showed that (+)-Blebbistatin had a high docking score and promising activity. *In vivo* studies, FST and TST demonstrated the extract's potential antidepressant effects, comparable to the standard drug imipramine. The study suggests that *L. latifolium* plant extract exhibits promising therapeutic action in treating depression.

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V. V. Rajesham: Conceptualisation, Methodology, Investigation, Data Analysis, Writing – Original Draft Preparation. P. Kishore Kumar: Data Curation, Methodology Support, Literature Review. P. Narendra: Supervision, Project Administration, Resources, Writing – Review, Ashok Morsu: Editing, V. Uma Maheswara Rao: Final Approval of the Manuscript. V. Sai Kiranmai: Performed phyto-constitution tests of the drug sample and other studies. All authors have read and agreed to the published version of the manuscript.