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# *In-Vivo* and *In-silico* Study of *Ludwigia perennis* L. Leaf Extract: A Promising Source of Anti-depressant Agents

**Research Article**

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## Abstract

*Ludwigia perennis* is used in India to treat several ailments in the traditional system of medicine. The chloroform leaf extract of *Ludwigia perennis* was evaluated for depression and anxiety using *in vivo* and *in silico* studies. Wistar albino rats were divided into groups based on parameters like control, standard, 20, and 30 mg/kg b.w. chloroform leaf extract groups for drug administration using gastric intubation. The tail suspension test (TST) and forced swim test (FST) were used to assess the antidepressant activity. Molecular docking against monoamine oxidase A (MAO-A), ADME analysis, toxicity tests, and pass prediction studies were among the *in silico* investigations. A delayed onset of immobility and lowered immobility time were seen at both the treatment doses (FST: 38.49±2.04 and 35.55±2.95 s; TST: 30.23±1.73 and 26.72±2.26 s) and the standard drug fluoxetine (FST: 31.26±1.76 and TST: 25.54±1.08 s), indicative of its antidepressant ability. While 30 identified phytochemicals were docked with monoamine oxidase A proteins, six compounds mainly showed higher binding affinity. It is stated that γ-sitosterol has a binding affinity of -8.5. The binding affinity of five compounds, namely stigmasterol, ergosterol, dibutyl phthalate, campesterol, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione is -8.2, -8.2, -8.0, -7.8, and -7.4 are like this. The results of the molecular docking studies indicate that the six chemicals that were successfully docked have an anti-depressive impact. Apart from docking, pharmacokinetic and PASS tests validated their drug-likeness, predicted safety after consumption, and predicted pharmacological effects. The chloroform leaf extract of *Ludwigia perennis* is a rich source of bioactive compounds with strong antidepressant properties.

**Keywords:** Forced Swim Test, Tail Suspension Test, Docking, ADME, Toxicity, Pass Prediction.

## Introduction

Depression is a prevalent mental illness that poses a global health risk (1). Sadness, boredom, lack of interest, difficulty in concentrating, low self-esteem, fatigue and guilt feelings, irregular sleep and/or eating patterns, and other symptoms are indicative of depressive disorders (2). Although depression is classified as a mental illness, it also has an impact on physical health. It could affect the health of the heart, kidneys, brain system, and immune system, among other systems. Anxiety disorders affect 38 million Indians, or 3.5% of the population, and depression affects 56 million, or 4.5% of the population, according to recent World Health Organization research (3).

Severe cardiovascular disease is more likely to occur in people with depression. On the other hand, depression may develop as a result of physical restrictions and disabilities that occur in chronic diseases. It can also be regarded as a risk factor for chronic diseases (4). Pharmacological treatment for depression includes the use of monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, norepinephrine and dopamine reuptake inhibitors, and serotonin antagonists. Tricyclic antidepressants are also included in this class of drugs (5), (6). While some patients gain more from the currently available treatments, others gain little or nothing at all (7). Additionally, these drugs may have a number of negative side effects, including postural hypotension, anxiety, impotence, weight gain, seizures, drowsiness, and cardiac dysrhythmias (8).

Indigenous cultures use a wide variety of medicinal plants to cure a wide range of illnesses. Through oral communication, this knowledge has been passed down from generation to generation and has ingrained itself into the cultures of our nations. Many plants, including *Melissa officinalis* L. (Lamiaceae), *Humulus lupulus* L. (Cannabaceae), *Crocus sativus* L. (Iridaceae), and *Hypericum perforatum* L. (Hypericaceae), have shown pharmacological promise for use as an antidepressant in humans (9,10,11,12,13,14). *Ludwigia perennis* is a plant belonging to the wetland category. It is a plant belonging to the Onagraceae family. Plants belonging to this family also have antidepressant activity (15).

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*Ludwigia perennis* grows in India, Tropical Africa, Madagascar, Southeast Asia, Malaysia, Tropical Australia, and New Caledonia. It is used for anti-oxidant, anti-diabetic, anti-nutrient, anti-cancer, anti-inflammatory, and anti-pyretic activities (16). Anti-depressant screening models include the tail suspension test, forced swim test, chronic unpredictable stress test, sucrose preference test, monoamine oxidase inhibition assay, learned helplessness test, open field test, hole board test, etc.; phytochemical compounds demonstrating anti-depressant activity include flavonoids, steroids, saponins, sugars, lectins, alkaloids, etc. (17). The phytochemicals alkaloids, flavonoids, tannins, steroids, terpenes, carbohydrates, saponins, phenols, reducing sugars, and cardiac glycosides are contained in the plant *Ludwigia perennis* (18). There is no much information on this plant's antidepressant properties at the moment. Therefore, the main objective of this study was to determine the antidepressant activity of the chloroform leaf extract of the *Ludwigia perennis* plant.

## Materials and Methods

### Plant materials

*Ludwigia perennis* was collected from a marshy area close to the village of Nandipulam in the Thrissur district of Kerala, India, and was identified at the Durva Herbal Centre in Pammal, Chennai (Voucher specimen number SK 3564).

### Extract Preparation

After cutting off sections of *Ludwigia perennis* leaves, they were thoroughly cleaned under running water to remove any leftover dirt. The components were air-dried in the shade after being cleaned with distilled water. All moisture was removed from the dry leaf fragments before they were ground into a coarse powder in a home grinder. The powder was extracted using chloroform utilizing Soxhlet apparatus (19).

### Antidepressant activity

### Animals

For the investigation, Wistar albino rats weighing between 180 and 200 grams of either sex were employed. The animals were acquired from the Sri Lakshmi Narayana Institute of Medical Sciences animal house in Osudu, Pondicherry. The animals were housed in polypropylene cages with bedding made of rice husk and randomly assigned to treatment groups. The animals were housed in cages with six animals per group under conventional environmental conditions that included a 12-hour light/dark cycle, a temperature of 27 ± 2ºC, and a relative humidity of 30–70%.All of the animals were given commercial pelleted rat chaw (M/s. Hindustan Lever Ltd., Mumbai) and had unrestricted access to water. The Institutional Animal Ethics Committee (938/Po/Pe/S/06/CPCSEA) evaluated the study's use of animals and experimental methodologies and found that everything followed the institution's ethical principles (Proposal Number: SLIMS/06/IAEC/ 2022-23).

### Groupings and Dosage Schedule

Four groups of six rats each were randomly selected from the study's sample of rats. Group I, acting as the vehicle control, was given an oral dose of 10 ml per kg of normal saline. Groups II were served as reference controls, administered with 20 mg/kg of fluoxetine as an antidepressant drug. Groups III and IV were served as test controls, administered with 20 and 30 mg/kg of chloroform leaf extract of *Ludwigia perennis*, respectively. All test drugs were administered only once per day via gastric intubation for 7 consecutive days. The animals were subjected to forced swim and tail suspension tests 1 hour after the last drug treatment.

### Forced Swimming Test

The forced swimming test was based on this method (20). The rats were forced to swim individually in a glass cylinder (20 cm × 14 cm) containing fresh water up to a height of 10 cm at 25 ± 1°C. The rats were individually forced to swim for a period of 6 minutes, and the total duration of immobility was recorded during the last 4 minutes. Rats were considered immobile when they floated in the water without struggling and made only those movements necessary to keep their heads above the water.

### Tail Suspension Test

The tail suspension test was conducted (21). The rats were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during the last 4 minutes of the 6-minute period. An animal was considered immobile when it did not show any movement of the body, hung passively, or was completely motionless.

### Statistical Analysis

The data were presented as Mean ± S.E.M., and the one-way ANOVA was used to analyze the results before Dunnett's "t" test was used statistically. When $P<0.05$ was reached, differences between groups were deemed statistically significant.

### GCMS analysis

Triple-axis detector model 7890 A GC for the apparatus Column: 5MS Database 30 meters long, 0.250 mm wide, and 0.25 µm thick. Using a split ratio of 5:1, 2 L of the material was injected for examination. 99.9995 percent helium made up the carrier gas, which flowed at a rate of 1 mL/min. An ionization energy of 70 eV was used while working in the electron impact (EI) mode. Temperature control was kept constant at 280 °C for the injector.

### Softwares Used

The RCSB Protein Data Bank and PubChem provided the structures of the phytochemicals discovered during the GC-MS investigation. Using Swiss ADME, Protox 2, and PASS, the obtained

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structures' physical, chemical, biological, and pharmacological properties were examined.

**Molecular Docking Studies**

**Protein structure**

Following an extensive search of the literature and database analysis, since monoamine oxidase A (MAO-A) controls the levels of the three primary monoamines in the brain—serotonin, norepinephrine, and dopamine—it makes sense to look into this enzyme in relation to depression (22).The structure details were downloaded from RCSB's Protein Data Bank (PDB) (https://www.rcsb.org). Docking investigations were carried out using the crystal structures of the monoamine oxidase A protein 2z5y (23). The protein synthesis process made use of Biovia Studio Visualizer. Following a hierarchical examination of the protein structure, ligands and water molecules were eliminated. The raw protein crystal structure was the aim of the docking investigations.

**Ligand structure**

The NIST (Mass Spectrometry Database), IMPPAT (Indian Medicinal Plants and Phytochemistry Database), and ChEMBL were consulted for ligand structures that were not found in PubChem (24),(25), (26). To decrease the energy of the ligand, the Open Babel tool's conjugate gradients method and force field were utilized (27).

**Docking**

Molecular docking is an efficient computational technique that is commonly used to forecast the affinities and binding modalities of molecular recognition events *in silico* (28). Molecular docking was utilized to ascertain the most likely mode of action of a few selected ligand molecules for the monoamine oxidase-A enzyme. PyRx software, which makes use of enhanced Autodock Vina capabilities, was utilized for flexible docking. Upon identifying the protein's active site, the PyRx program was used to generate a second receptor grid (29). The target protein atoms' distances from the ligand and the grid center are listed in Table 2. The target protein in the docking studies was 2z5y. The docked conformations were obtained in PDB format for further investigation. BIOVIA Discovery Studio was used to meticulously visualize and compare the docking positions of ligands with target proteins (30). The docking energy of the receptor and the ligand, sometimes referred to as the dock score interaction, is minimized when a molecule attaches to a protein (31).

***In-Silico* ADME Study**

Lipinski's rule of five was followed in evaluating the best docked compounds utilizing Swiss ADME for their ADME properties (32),(33). A compound is considered to possess drug-like properties if it satisfies Lipinski's criteria. The first five requirements are molar refractivity 40 and less than 130, lipophilicity 5, number of hydrogen bond donors 5, number of hydrogen bond acceptors 10, and MW 500. Those who fit the Lipinski criteria become excellent drug candidates (34).

***In-Silico* Toxicity Prediction Study**

ProTox-II was used to forecast the compounds' LD50 and level of toxicity (35).

***In-Silico* PASS Prediction Study**

An online software called Prediction of Activity Spectra for Substances (PASS) is used to evaluate the probable bioactivities of compounds that have the strongest supporting data. PASS estimates a molecule's potential bioactivities up to 3750 times using a chemical structural analysis (36). With Pa and Pi values ranging from 0.000 to 1.000, the researchers referred to the results as "probable activity" (Pa) and "probable inactivity" (Pi). To determine a substance's bioactivity, the Pa > Pi and Pa > 0.700 criteria were employed (37).

## Results and Discussion

**Antidepressant activity**

As shown in Table 1 and Figure 1a,1b shows the effects of the chloroform leaf extract of *Ludwigia perennis* on the duration of immobility in the forced swim test and tail suspension test for antidepressant activity. In the forced swim test, the immobility time in the control group was 125.35±8.77 sec, but the reference control fluoxetine (20 mg/kg) and chloroform leaf extract (20 mg/kg) both significantly (P<0.001) reduced immobility. The times were 31.26±1.76 and 38.49±2.04 seconds, respectively. Chloroform leaf extract (30 mg/kg) also significantly (P<0.001) decreased the immobility time to 35.55±2.95, indicating that chloroform leaf extract has fluxoteine-like activity. In the tail suspension test, the immobility time in the control group was 106.22±5.35 seconds, whereas the reference control fluoxetine (20 mg/kg) and chloroform leaf extract (20 mg/kg) significantly (P<0.001) reduced the immobility time to 25.54±1.08 and 30.23±1.73 seconds, respectively. The immobility time of chloroform leaf extract (30 mg/kg) was also significantly (P<0.001) reduced to 26.72±2.26. All the above facts indicate that the chloroform leaf extract has similar anti-depression activity to the control drug, fluoxetine

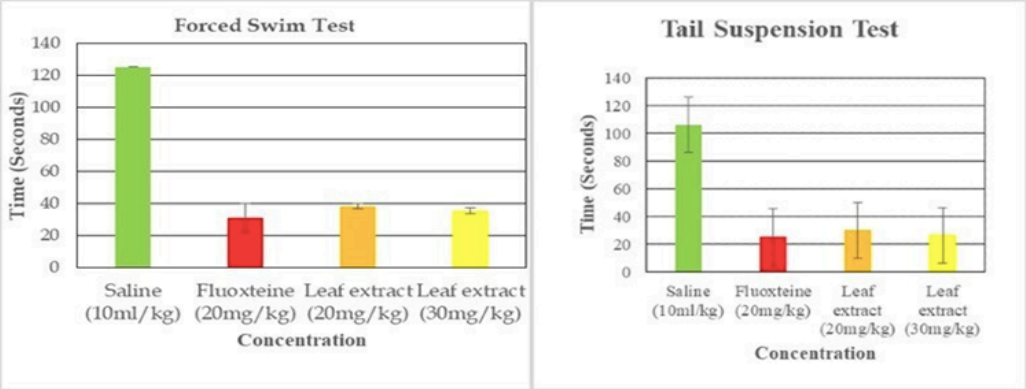
**Table 1: Effect of chloroform leaf extract of *Ludwigia perennis* on immobility time in forced swim test and tail suspension test.**

| Groups | Drug Treatment | Forced Swim Test Immobility Time (Secs) | Tail Suspension Test Immobility Time (Secs) |
|---|---|---|---|
| I | Vehicle Control Saline (10ml/kg) | 125.35 ±8.77 | 106.22 ±5.35 |
| II | Reference Control Fluoxetine (20mg/kg) | 31.26 ±1.76*** | 25.54 ±1.08*** |
| III | Leaf extract (20mg/kg) | 38.49 ±2.04*** | 30.23 ±1.73*** |
| IV | Leaf extract (30mg/kg) | 35.55 ±2.95*** | 26.72 ±2.26*** |

Values are in mean±SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001 Vs Vehicle Control.

**Figure 1a and 1b: Effect of isolated Chloroform leaf extract on immobility time in forced swim test and tail suspension test**

**Figure 1a** **Figure 1b**




## GCMS analysis

The GC-MS findings showed that *Ludwigia perennis* chloroform leaf extract included thirty bioactive components. The substances were identified using the chemical formula, retention time (RT), and peak area. Table 2 and Figure 2 display the active principle along with its MW, percentage peak area, RT, and chemical formula.

**Table 2: GC-MS profile of chloroform leaf extract of *Ludwigia perennis***

| S. No. | RT | Compound Name | Molecular formula | Molecular weight | Peak Area % |
|---|---|---|---|---|---|
| 1 | 25.012 | Phenol, 2,4-bis(1,1-dimethylethyl)- | $C_{14}H_{22}O$ | 206 | 1.874% |
| 2 | 26.601 | 1-Hexadecene | $C_{16}H_{32}$ | 224 | 0.787% |
| 3 | 30.636 | 1-Nonadecene | $C_{19}H_{38}$ | 266 | 1.012% |
| 4 | 31.349 | 1-Hexadecanol, 3,7,11,15-tetramethyl | $C_{20}H_{42}O$ | 298 | 0.578% |
| 5 | 31.494 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 23.175% |
| 6 | 31.596 | 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- | $C_{20}H_{40}$ | 280 | 1.626% |
| 7 | 31.970 | Phthalic acid, isobutyl octadecyl ester | $C_{30}H_{50}O_4$ | 474 | 7.729% |
| 8 | 31.970 | 1,4-Eicosadiene | $C_{20}H_{38}$ | 278 | 6.734% |
| 9 | 32.420 | 1-Hexadecanol | $C_{16}H_{34}O$ | 242 | 1.673% |
| 10 | 32.879 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | $C_{17}H_{24}O_3$ | 276 | 2.497% |
| 11 | 33.218 | Pentadecanoic acid, 14-methyl-, methyl ester | $C_{17}H_{34}O_2$ | 270 | 0.647% |
| 12 | 33.813 | Dibutyl phthalate | $C_{16}H_{22}O_4$ | 278 | 2.945% |
| 13 | 34.144 | Phthalic acid, butyl 2-pentyl ester | $C_{17}H_{24}O_4$ | 292 | 0.647% |
| 14 | 34.535 | 1-Nonadecene | $C_{19}H_{38}$ | 266 | 0.709% |
| 15 | 34.807 | 2,5-di-tert-Butyl-1,4-benzoquinone | $C_{14}H_{20}O_2$ | 220 | 0.616% |
| 16 | 35.708 | Hexanoic acid, 2-ethyl-, anhydride | $C_{16}H_{30}O_3$ | 270 | 13.498% |
| 17 | 36.268 | 17-Pentatriacontene | $C_{35}H_{70}$ | 490 | 0.568% |
| 18 | 36.362 | 9,12-Octadecadienoic acid, methyl ester, (E,E)- | $C_{19}H_{34}O_2$ | 294 | 0.673% |
| 19 | 36.472 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- | $C_{21}H_{36}O_4$ | 352 | 0.820% |
| 20 | 36.693 | Phytol | $C_{20}HZ_{40}O$ | 296 | 8.893% |
| 21 | 38.188 | 1-Hexacosanol | $C_{26}H_{54}O$ | 382 | 0.593% |
| 22 | 40.796 | 8-Ethoxy-4,5-dihydro-1-[(4-isopropylphenyl)imino]-4,4-dimethyl-1H-[1,2]dithiolo[3,4-c]quinoline | $C_{23}H_{26}N_2OS_2$ | 410 | 2.418% |
| 23 | 43.710 | 1,2-Benzenedicarboxylic acid, diisooctyl ester | $C_{24}H_{38}O_4$ | 390 | 2.456% |
| 24 | 47.864 | Squalene | $C_{30}H_{50}$ | 410 | 2.190% |
| 25 | 48.501 | γ-Sitosterol | $C_{29}H_{50}O$ | 414 | 1.279% |
| 26 | 50.005 | Trilinolein | $C_{57}H_{98}O_6$ | 878 | 0.779% |
| 27 | 53.429 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 2.514% |
| 28 | 55.043 | Ergosterol | $C_{28}H_{44}O$ | 396 | 5.025% |
| 29 | 55.689 | Campesterol | $C_{28}H_{48}O$ | 400 | 2.298% |
| 30 | 56.394 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 2.747% |

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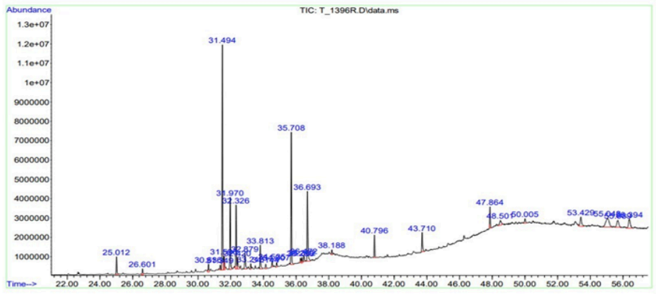
**Figure 2: Chromatogram (GC-MS) of chloroform leaf extract of *Ludwigia perennis* L.**




## Molecular Docking

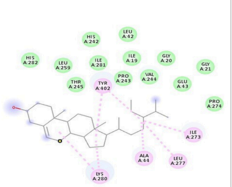
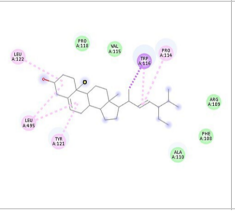
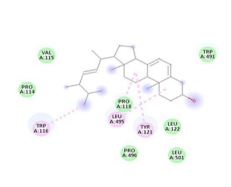
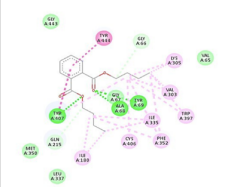
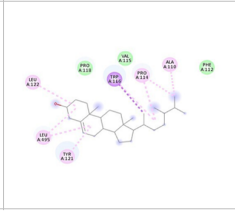
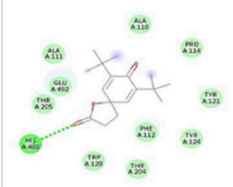
The protein's docking potential was examined using PyRx and Auto Dock Vina, a virtual screening tool. Out of the 30 phytochemical compounds initially screened, only six ligands—γ-Sitosterol, Stigmasterol, Ergosterol, Dibutyl phthalate, Campesterol, and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione—were selected for molecular docking studies with the target protein Monoamine Oxidase A (PDB ID: 2z5y). These compounds were chosen based on their superior docking scores and predicted binding affinities, indicating their potential as effective inhibitors in comparison to the others evaluated. Grid center and dimensions between ligand and target protein atoms is shown in Table 3.The binding energy (in kcal/mol) ascertained by molecular docking is shown in Table 4. The protein-ligand interaction molecule demonstrates that, after inhibitory ligand docking, the active sites of the docked ligands are located in the proper protein active sites (Figure 3-8). The protein that causes depression and the kind of interaction that takes place between a protein and a ligand are the main subjects of these studies on protein-ligand interactions, which are included in Table 4. Although the residues that stabilize proteins and those that cause major conformational changes in proteins are not fully understood, this information contributes to the unraveling of this puzzle (38). Table 4 presents the docked complexes binding affinities, interacting residues, and interaction types.

**Table 3: Grid center and dimensions between ligand and target protein atoms**

| Protein Structure | Grid center | | | Dimensions (Angstrom) | | |
|---|---|---|---|---|---|---|
| | X | Y | Z | X | Y | Z |
| 2z5y | -32.71 | -23.57 | -10.45 | 117.93 | 83.42 | 84.69 |

**Table 4: Protein-ligand Docked complex and their binding affinity generated through molecular docking method, Interacting residues and interaction type**

| Protein-Ligand Complex Name | Binding affinity (kal/mol) | Interacting residues | Interaction type |
|---|---|---|---|
| 2z5y_ γ-Sitosterol | -8.5 | ILE A:19, GLY A:20, GLY A:21, LEU A:42, GLU A: 43, ALA A:44, HIS A:242, PRO A:243, VAL A:244, THR A:245, LEU A:259, ILE A:273, PRO A:274, LEU A:277, LYS A:280, ILE A:281, HIS A:282, TYR A:402 | Van der Waals, Alkyl, Pi-Alkyl |
| 2z5y_ Stigmasterol | -8.2 | PHE A:108, ARG A:109, ALA A:110, PRO A:114, VAL A:115, TRP A:116, PRO A: 118, TYR A:121, LEU A:122, LEU A:495 | Van der Waals, Pi-Sigma, Alkyl, Pi-Alkyl |
| 2z5y_ Ergosterol | -8.2 | PRO A:114, VAL A:115, TRP A:116, PRO A:118, TYR A:121, LEU A:122, TRP A:491, LEU A:495, PRO A:496, LEU A:501 | Van der Waals, Alkyl, Pi-Alkyl |
| 2z5y_ Dibutyl phthalate | -8.0 | VAL A:65, GLY A:66, GLY A:67, ALA A:68, TYR A:69, ILE A:180, GLN A:215, VAL A:303, LYS A:305, ILE A:335, LEU A:337, MET A:350, PHE A:352, TRP A:397, CYS A:406, TYR A:407, GLY A:443, TYR A:444 | Van der Waals, Conventional Hydrogen Bond, Carbon-Hydrogen Bond, Pi-Pi stacked, Alkyl, Pi-Alkyl |
| 2z5y_ Campesterol | -7.8 | ALA A:110, PHE A:112, PRO A:114, VAL A:115, TRP A:116, PRO A:118, TYR A: 121, LEU A:122, LEU A: 495 | Van der Waals, Alkyl, Pi-Sigma, Pi-Alkyl |
| 2z5y_7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | -7.4 | ALA A:110, ALA A:111,PHE A:112, PRO A:114,TYR A:121,TYR A:124, TRP A:128, THR A:204, THR A:205, HIS A: 488, GLU A:492 | Van der Waals, Conventional Hydrogen Bond |

| Figure 3: 2z5y_ γ-Sitosterol | Figure 4: 2z5y_ Stigmasterol | Figure 5: 2z5y_ Ergosterol | Figure 6: 2z5y_ Dibutyl phthalate | Figure 7: 2z5y_ Campesterol | Figure 8: 2z5y_7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

**ADME analysis**

Using the web application SwissADME, the ADME characteristics of the best-docked compounds were examined in order to learn more about their pharmacokinetics, drug-likeness, and physiochemical characteristics (Table 5). According to Lipinski's rule of five, all the compounds except dibutyl phthalate, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione violated the rules of lipophilicity, and compounds γ-Sitosterol and stigmasterol violated the rule of molecular refractivity. Conversely, Lipinski's requirements were met by dibutyl phthalate, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, and these substances are thought to have the best possible drug-like character. Compounds γ-Sitosterol and stigmasterol break multiple rules. The remaining compounds do not violate more than one rule.

Drugs that break two or more of these rules are regarded as non-orally accessible drugs. But in this study, all the compounds except γ-Sitosterol and stigmasterol reported 0 or 1 violations that suggested these are bioavailable or orally available drugs. Apart from the two docked compounds mentioned above, the remaining four docked compounds have orally active drug-like properties according to the Lipinski rule. Compounds with lower molecular weight, hydrogen bond capacity, and lipophilicity are said to have higher permeability, better absorption, and higher bioavailability (39), (40). This analysis does not, however, determine if a chemical has a specific pharmacological action.

**Table 5: ADME property prediction for the best docked compounds**

| Compound | Molecular Weight[1] | HB Acceptor[2] | HB Donor[3] | Lipophilicity[4] | Molar Refractivity[5] | Rule of Five[6] |
|---|---|---|---|---|---|---|
| γ-Sitosterol | 414 | 1 | 1 | 7.19 | 133.23 | 1 |
| Stigmasterol | 412 | 1 | 1 | 6.97 | 132.75 | 1 |
| Ergosterol | 396 | 1 | 1 | 6.49 | 127.47 | 1 |
| Dibutyl phthalate | 278 | 4 | 0 | 3.69 | 77.84 | 0 |
| Campesterol | 400 | 1 | 1 | 6.90 | 128.42 | 1 |
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 276 | 3 | 0 | 3.40 | 79.66 | 0 |

[1]Molecular weight (acceptable range: <500). [2]HB, Hydrogen bond acceptor (acceptable range: ≤10). [3]HB, Hydrogen bond donor (acceptable range: ≤5). [4]Lipophilicity (expressed as Log Po/w, acceptable range: <5). [5]Molar refractivity should be between 40 and 130. [6]Rule of five: Number of violations of Lipinski's rule of five; recommended range: 0–4.

**Toxicity prediction**

One crucial element in the drug discovery process is the assessment of toxicity. Table 6 displays the findings of Protox-II's analysis of the ligands' hazardous characteristics. The server has classified the results into six classes using Protox-II, a free in silico toxicity predictor that estimates the lethal dose 50 (LD50) value in mg/kg body weight. The level of a substance at which 50% or half of the test population will perish is known as the lethal dosage (LD50) (41). Protox-II indicates that compounds in Classes 5 and 6 are generally safe for consumption and non-toxic; in particular, Class 6 is designated as the safest non-toxic class of compounds for oral consumption due to its compounds LD50 value above 5000 mg/kg. Dibutyl phthalate is regarded as harmless and is classified as class 5 of the Protox classification with an LD50 value of 3474 mg/kg. Since the ligands cleared every toxicity filter, they may be employed as possible antidepressants.

**Table 6: Toxicity prediction of best-docked compounds by ProTox-II**

| Compound | Predicted LD50, mg/kg[a] | Predicted Toxicity Class[a] | Predicted Toxicity |
|---|---|---|---|
| γ-Sitosterol | 890 | 4 | Immunotoxicity |
| Stigmasterol | 890 | 4 | Immunotoxicity |
| Ergosterol | 10 | 2 | Immunotoxicity |
| Dibutyl phthalate | 3474 | 5 | Carcinogenicity |
| Campesterol | 890 | 4 | Immunotoxicity |
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8- | 900 | 4 | Inactive |

[a]ProTox (http://tox.charite.de/protox_II) Class 1: deadly if consumed (LD50 ≤ 5); Class 2: deadly if consumed (5 < LD50 ≤ 50); Class 3: lethal if consumed (50 < LD50 ≤ 300); Class 4: harmful if consumed (300 < LD50 ≤ 2000); Class 5: maybe harmful if consumed (2000 < LD50 ≤ 5000); Class 6: non-lethal (LD50 > 5000)

**Biological Activity Prediction**

An online technique that uses structural features to predict bioactivity Prediction of Activity Spectra for Substances was used to evaluate the likely biological activity (PASS) of the best-docked compounds. Table 7 shows Five biological activities were assessed for each compound using the parameters Pa > Pi and Pa > 7. The results revealed several notable activities with Pa > 0.9, suggesting a larger potential for the species.

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**Table 7: Prediction of biological activity of best-docked compounds**

| Compound | Pa a | Pi b | Biological Activity |
|---|---|---|---|
| γ-Sitosterol | 965 | 1 | DELTA14-sterol reductase inhibitor |
| | 960 | 2 | Antihypercholesterolemic |
| | 959 | 2 | Prostaglandin-E2 9-reductase inhibitor |
| | 957 | 1 | Cholesterol antagonist |
| | 952 | 2 | Alkenylglycerophosphocholine hydrolase inhibitor |
| Stigmasterol | 970 | 2 | Antihypercholesterolemic |
| | 965 | 1 | Cholesterol antagonist |
| | 933 | 1 | Oxidoreductase inhibitor |
| | 915 | 5 | Testosterone 17beta-dehydrogenase (NADP+) inhibitor |
| | 913 | 4 | Prostaglandin-E2 9-reductase inhibitor |
| Ergosterol | 951 | 1 | Cholesterol antagonist |
| | 926 | 3 | Antihypercholesterolemic |
| | 901 | 6 | Testosterone 17beta-dehydrogenase (NADP+) inhibitor |
| | 896 | 4 | Caspase 3 stimulant |
| | 889 | 5 | Prostaglandin-E2 9-reductase inhibitor |
| Dibutyl phthalate | 939 | 3 | Sugar-phosphatase inhibitor |
| | 929 | 4 | Alkenylglycerophosphocholine hydrolase inhibitor |
| | 912 | 3 | Pullulanase inhibitor |
| | 908 | 3 | Eye irritation, inactive |
| | 906 | 5 | Phobic disorders treatment |
| Campesterol | 973 | 0 | DELTA14-sterol reductase inhibitor |
| | 962 | 2 | Prostaglandin-E2 9-reductase inhibitor |
| | 962 | 2 | Antihypercholesterolemic |
| | 955 | 1 | Cholesterol antagonist |
| | 955 | 2 | Alkenylglycerophosphocholine hydrolase inhibitor |
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 883 | 8 | Ubiquinol-cytochrome-c reductase inhibitor |
| | 813 | 30 | Aspulvinone dimethylallyltransferase inhibitor |
| | 793 | 27 | Testosterone 17beta-dehydrogenase (NADP+) inhibitor |
| | 743 | 38 | Mucomembranous protector |
| | 705 | 9 | CYP2B5 substrate |

[a]Pa = Possibility of activity; b Pi = Possibility of inactivity

## Conclusion

The results of this investigation indicate that *Ludwigia perennis* leaf extract in chloroform has considerable potential for use in natural medicine. This study discovered that the extract has a high concentration of bioactive phytochemicals with antidepressant properties. Six compounds of particular interest that have been identified in this experiment are γ-Sitosterol, Stigmasterol, Ergosterol, Dibutylphthalate, Campesterol, and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, which may have the potential to serve as anti-depressive drugs. Nevertheless, more thorough research is required to evaluate these substances' effectiveness as antidepressants. In conclusion, the chloroform leaf extract of *Ludwigia perennis* has shown significant promise as a natural remedy for depression studies. The extract's bioactive phytochemicals exhibit antidepressant qualities that may be used to develop therapeutic interventions. In order to create safe and efficient therapy alternatives for patients, further research is required to fully comprehend the therapeutic potential of these molecules.

### Conflict of Interest

The authors declared no conflict of interest in the manuscript.

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