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In Silico anticancer analysis of compounds from *Ludwigia perennis*

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Abstract

Cancer related deaths has been on an increase world-wide and necessitates urgent action to investigate anticancer treatments. Natural substances are likely to be a viable source. In this study, 18 phytochemicals were identified using GC-MS analysis after a phytochemical examination of a chloroform root extract of *Ludwigia perennis*. Protein Data Bank accession number 4GIZ was used to attach these phytochemicals onto the E6 protein. While 18 identified phytochemicals were docked with cervical cancer proteins, six compounds mainly showed higher binding affinity. The binding affinity of 1,1'-(1,2-cyclobutanediyl)bis-trans benzene is reported to be -8.2. The other five molecules have binding affinities of -6.5 and are phytol, geranyl isovlerate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, phthalic acid, and butyl tetradecyl ester. The six compounds that were successfully docked show anticancer effect, according to the molecular docking research findings. Their drug-likeness, anticipated safety after ingestion, and anticipated pharmacological effects were all validated by pharmacokinetic and PASS investigations in addition to docking.

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Introduction

The most prevalent cancer among women in low-income nations is cervical cancer (Yang *et al.,* 2004). When cervical cancer reaches a deadly stage, the majority of current therapies are no longer effective (Stuver and Adami, 2002). The human papillomavirus (HPV) is one of the major risk factors for the development of cervical cancer (Okunde, 2020). More than 200 different forms of HPV have been found over time. There are two varieties of HPV: low-risk, which is less likely to cause cancer, and high-risk, which can cause cancer (Park and Chang, 2019; Kumar *et al.,* 2015). High-risk HPV-18 and -16 are the only viruses that cause genital warts; they are also the source of anogenital cancer, accounting for 15.7 percent and 62.6 percent of cervical malignancies, respectively (Merkhover and Maslow, 2015; Kumar *et al.,* 2015). Consequently, the primary targets for the development of anticancer medications are HPV 16 and 18.

The double-stranded virus HPV invades the epithelium (Bharatha, 2015). The HPV genome consists of three main parts. Initially, the long control region (LCR), which manages DNA replication, regulates the transcription of viral genes. Non-structural proteins that are essential for both oncogenesis (E5, E6, E7) and viral replication (E1, E2, E4) are encoded by the early region (E). It is believed that the structural proteins (L1 and L2) required for viral replication, dissemination, and propagation are encoded in the late portions of viruses (Sanjose *et al.,* 2018; Pinidis *et al.,* 2016; Fernandes and Fernandes, 2012). HPV-16 and -18 both express the oncoprotein E6, which has been linked to carcinogenesis, immortality, and malignant transformation (Nabati *et al.,* 2020). p53, a critical tumour suppressor and regulator of the cell cycle checkpoint (Kharisma *et al.,* 2020), is the direct target of this oncoprotein. Through the ubiquitination route, E6 forms a heterotrimeric complex with p53 and E6-associated protein (E6AP), which results in p53's destruction (Messa *et al.,* 2018). When p53 levels are low, the body cannot repair DNA damage, which leads to cancer (Nabati *et al.,* 2020). Although HPV has been known to cause cervical cancer for more than three decades, there is still a need to identify effective treatments to combat HPV infection (Marimuthu *et al.,* 2017).

Existing HPV infections cannot be cured at this time. Since HPV is a virus, it cannot be treated with antibiotics intended for bacterial illnesses. There are currently no antiviral medicines approved for use in treating HPV. In recent years, the discovery of phytochemicals and their application to the prevention and treatment of cancer have become more promising (Proboningrat *et al.,* 2021).When it comes to healing the human body, medicinal plants are still a powerful resource. The bioactive compounds that may enhance health and be used as medicines are abundant in medicinal and aromatic plants (Vaou *et al.,* 2021; Msseddi *et al.,* 2020; Jamal *et al.,* 2018; Al-Haidari *et al.,* 2016; Aminzare *et al.,* 2016). Traditional medicine is now again receiving attention, and the demand for medications derived from plant sources is rising. Compared to synthetic drugs, plant-based products are frequently thought to be less hazardous and are less likely to cause negative effects (Poongothai and Annapoorani, 2019; Singh *et al.,* 2019). Because of their pharmacological and biological activity, they are frequently exploited in the development of new drugs (Vishit and Chaturvedi, 2012).

Ludwigia perennis, a wet-land weed, is a plant of great medicinal value. Very little has been discovered about this plant's medicinal properties. This study will analyse a chloroform extract of *Ludwigia perennis* roots using gas chromatography-mass spectrometry (GC-MS), in silico docking studies, absorption, distribution, metabolism, excretion, and toxicity (ADME/T), and substance-activity prediction spectra (PASS). The purpose of this research is to examine the possible anticancer inhibition by analysing the interaction of discovered phytochemicals from *Ludwigia perennis* with target proteins such E6 cervical cancer protein.

Material 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).

Extraction and isolation

Pieces of *Ludwigia perennis* roots were cut off and rinsed under running water to get rid of any remaining dirt. After being rinsed in distilled water, the parts were air-dried in the shade. Dry root pieces were crushed into a coarse powder in a household grinder after all moisture had been removed. Soxhlet apparatuses were used to extract the powder using choloroform, as is customary procedure (Syamkumar *et al.,* 2023). The phytochemical compunds in the extract were then determined by GC-MS analysis.

GC-MS analysis

A Model 7890 A GC with a triple axis detector and a DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Metre Thick column were used to examine the root extract. To inject 2 L of the sample for analysis, a split ratio of 5:1 was used. "The carrier gas flowed at a rate of 1 mL/min and was 99.9995 % helium gas. The investigation was conducted in the electron impact (EI) mode, with an ionisation energy of 70 eV. The injector's temperature was maintained at 280˚C (constant). After comparing the observed spectrum configurations with those of the existing mass spectral database, the chemicals were identified (NIST-08 SPECTRAL DATA).

Software used

After the GC-MS analysis, the structures of the phytochemicals that were found were obtained from the RCSB Protein Data Bank and PubChem. The retrieved structures were analyzed for their physical, chemical, biochemical and pharmacological parameters using Swiss ADME, Protox 2, and PASS.

Molecular docking studies

Protein structure

After a thorough literature search and database analysis, it was shown that the HPV protein E6 was crucial to the development of cervical cancer. Protein Data Bank (PDB) of the RCSB (https://www.rcsb.org) Crystal structures of 4GIZ, an E6 protein, were gathered (Mohan *et al.,* 2023) in order to conduct docking studies. Biovia Studio Visualizer was utilised in the protein synthesis. The protein structure was analysed hierarchically, and ligands and water molecules were then removed. For docking studies, the target was determined to be the raw protein crystal structure (Tyagi *et al.,* 2020).

Ligand structure

For docking investigations, the crystal structures of 4GIZ, an E6 protein, were gathered (Yim and Park, 2005). To create the proteins, we utilised Biovia Studio Visualizer. After doing a hierarchical analysis of the protein structure, ligands and water molecules were removed. The objective for docking studies was the unprocessed protein crystal structure (Tyagi *et al.,* 2020). For those compounds where the structure is not available in PubChem, references were taken from NIST (Mass Spectrometry Database), IMPPAT (Indian Medicinal Plants and Phytochemistry Database), and ChEMBL(Vivek-Ananth *et al.,* 2023; Papadatos and Overington, 2014; Linstrom and Mallard, 2001). The force field and conjugate gradients algorithm of Open Babel tool were employed to minimize the ligand energy (Rappe *et al.,* 1992).

Molecular docking

For the purpose of forecasting the affinities and binding modalities of molecular recognition events in silico, molecular docking is a widely used and efficient computational technique (Hasanuddin *et al.*, 2022). Using flexible docking with PyRx software, which leverages improved Autodock Vina capabilities, molecular docking was used to determine the most likely mechanism of action of a few chosen ligand molecules for the cervical cancer protein. After the protein's active site was determined, a second receptor grid was created using the PyRx application (Opo *et al.,* 2021).

Table 2 lists the grid centre and the distances between the target protein atoms and the ligand. Docking experiments were performed using 4GIZ as the target protein. For additional study, we retrieved the docked conformations in PDB format. The docking positions of ligands with target proteins were rigorously visualised and contrasted using BIOVIA Discovery Studio (Pawar and Rohane, 2021). When a molecule binds to a protein, the docking energy of the receptor and the ligand, also known as the dock score interaction, is minimised (Abdulfatai *et al.*, 2018).

In Silico ADME study

The finest docked compounds were assessed using Swiss ADME (Daina *et al.,* 2017; Lipinski *et al.,* 2001) for their ADME attributes in compliance with molar refractivity 40 and less than 130, lipophilicity 5, number of hydrogen bond donors 5, number of hydrogen bond acceptors 10, and MW 500 are the first five conditions. Excellent medication candidates are those that meet the Lipinski criteria (Lipinski, 2004).

In Silico toxicity prediction study

The toxicity and LD50 of the compounds were predicted using ProTox-II (Banerjee *et al.,* 2018).

In Silico PASS prediction study

Prediction of Activity Spectra for Substances (PASS) is an online web programme used to assess the potential bioactivities of compounds with the most supporting evidence. PASS uses a chemical structural analysis to estimate up to 3750 possible bioactivities of a molecule (Lagunin *et al.,* 2000). The findings were referred to by the researchers as "probable activity" (Pa) and "probable inactivity" (Pi), with Pa and Pi values ranging from 0.000 to 1.000. The Pa > Pi and Pa > 0.700 criteria were used to establish a substance's bioactivity (Goel *et al.,* 2011).

Results and discussion

GCMS analysis

The chloroform root extract of *Ludwigia perennis* included eighteen bioactive components, according to the GC-MS data.

The chemical formula, retention time (RT), and peak area were used to confirm the compounds' identification. The active principle is shown in Table 1 and Fig. 1, together with their MW, peak area in percentage, RT, and molecular formula.

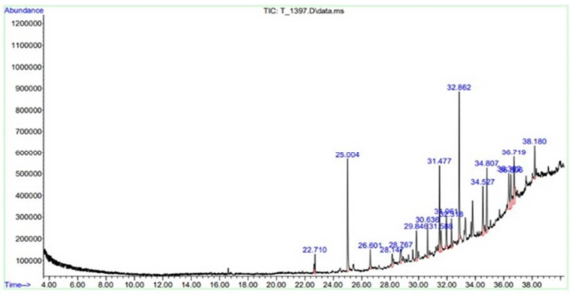

Fig. 1. Chromatogram (GC-MS) of chloroform root extract of *Ludwigia perennis*

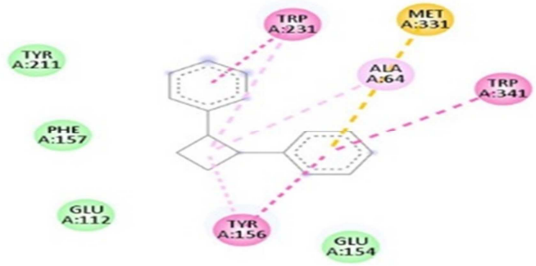

Fig. 2. 4giz_Benzene,1,1'-(1,2-cyclobutanediyl)bis-, trans

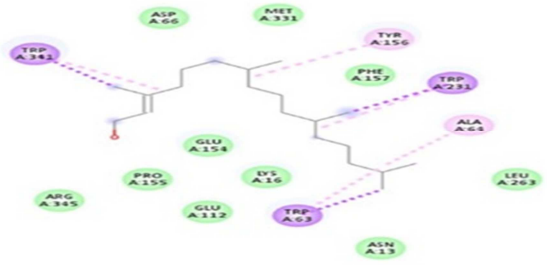

Fig. 3. 4giz_Phytol

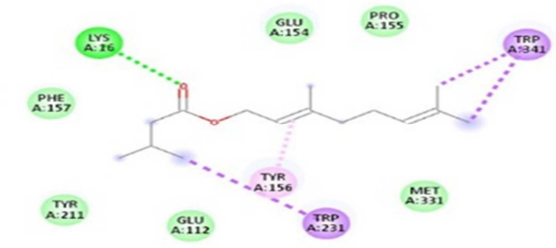

Fig. 4. 4giz_Geranyl isovlerate

Table 1. GC-MS profile of chloroform root extract of *Ludwigia perennis*

SL	RT	Compound name	Molecular formulae	Molecular weight	Peak area %
1	22.710	Tripentyl orthoformate	$C_{16}H_{34}O_3$	274	1.643%
2	25.004	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	12.244%
3	26.601	Acetic acid n-octadecyl ester	$C_{20}H_{40}O_2$	312	2.438%
4	28.147	Phorbol	$C_{20}H_{28}O_6$	364	2.093%
5	28.767	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	2.731%
6	29.846	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans	$C_{16}H_{16}$	208	4.174%
7	30.636	Behenic alcohol	$C_{22}H_{46}O$	326	3.705%
8	31.477	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	8.590%
9	31.588	1-Dodecanol, 3,7,11-trimethyl	$C_{15}H_{32}O$	228	3.679%
10	31.961	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	418	3.704%
11	32.318	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.897%
12	32.862	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	15.454%
13	34.527	1-Docosene	$C_{22}H_{44}$	308	6.157%
14	34.807	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	220	8.000%
15	36.362	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	5.768%
16	36.506	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	5.881%
17	36.719	Phytol	$C_{20}H_{40}O$	296	6.829%
18	38.180	17-Pentatriacontene	$C_{35}H_{70}$	490	4.012%

Table 2. 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
4giz_Benzene,1,1'-(1,2-cyclobutanediyl)bis-, trans	-8.2	ALA A:64, GLU A:112, GLU A:154, TYR A: 156, PHE A:157, TYR A:211, TRP A:231, MET A:331, TRPA:341	Van der Waals, Pi-Pi T-shaped, Pi-Sulfur, Alkyl, Pi-Pi Stacked, Pi-Alkyl
4giz_Phytol	-6.5	ASN A:13, LYS A:16, TRP A:63, ALA A:64, ASP A:66, GLU A:112, GLU A:154, PRO A:155, TYR A:156, PHE A:157, TRP A:231, LEU A:263, MET A:331, TRP A:341, ARG A: 345	Van der Waals, Pi-Sigma, Alkyl, Pi-Alkyl
4giz_Geranyl isovlerate	-6.3	LYS A:16, TYR A:156, TRP A:231, TRP A:341	Pi-Sigma, Pi-Alkyl, Conventional Hydrogen bond
4giz_3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-6.5	LYS A:16, TRP A:63, ALA:64, ASP A:66, GLU A:112, GLU A:154, PRO A:155, TYR A:156, TRP A:231, LEU A:263, MET A:331, TRP A:341	Van der Waals, Alkyl, Pi-Sigma, Pi-Alkyl
4giz_7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-7.2	ASN A:13, ASP A:15, LYS A:16, TRP A:63, ALA A:64, ASP A:66, GLU A:112, TYR A: 156, TRP A:231, LEU A:263, MET A:331	Van der Waals, Pi-Sigma, Conventional Hydrogen Bond, Pi-Alkyl
4giz_ Phthalic acid, butyl tetradecyl ester	-6.3	GLU A:45, TRP A:63, ALA A:64, ASP A:66, ARG A:67, GLU A:112, GLU A:154, PRO A:155, TYR A:156, PHE A:157, TRP A:231, MET A:331, TRP A:341	Van der Waals, Pi-Pi-Stacked, Carbon-Hydrogen bond, Alkyl, Pi-Sigma, Pi-Alkyl

Molecular docking studies

PyRx was used to analyse the protein's docking potential using Auto Dock Vina, a virtual screening technology. Table 2 displays the binding energy (in kcal/mol) determined via molecular docking. The protein-ligand interaction molecule shows that the docked ligands' active sites are situated in the appropriate protein active sites following inhibitory ligand docking (Fig. 2-7). These studies on protein-ligand interactions, which are described in Table 2, focus on the protein that causes cervical cancer and the type of interaction that occurs between a protein and a ligand.

The residues responsible for stabilising proteins and those involved in significant conformational changes to proteins are not well understood, but this knowledge helps to answer this mystery (Beg and Athar, 2020). Table 2 displays the binding affinities, interacting residues, and interaction types of the docked complexes.

ADME analysis

Table 3 shows the best-docked compounds' pharmacokinetics, drug-likeness, and physiochemical characteristics were further characterized using the SwissADME online program. Benzene, trans, 7,9-Di-tert-butyl-1-oxaspiro, 1,1'-(1,2-cyclobutanediyl)bis-, and geranyl isovlerate(4,5)The Lipinski rule of five can be met by these compounds (deca-6,9-diene-2,8-dione). The Lipinski rule of lipohilicity prohibits the following substances: phthalic acid, butyl tetradecyl ester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, and phytol. The substances phytol, phthalic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and butyl tetradecyl ester satisfied Lipinski's requirements. The ideal drug-like properties was estimated and it was found that no compound violated more than one requirement.

Table 3. 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]
Benzene,1,1'-(1,2- cyclobutanediyl)bis-, trans	208	0	0	4.34	68.20	1
Phytol	296	1	1	6.22	98.94	1
Geranyl isovlerate	238	2	0	4.28	74.56	0
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	1	1	6.22	98.94	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
Phthalic acid, butyl tetradecyl ester	418	4	0	6.83	125.91	1

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.

Table 4. Toxicity prediction of best-docked compounds by ProTox-II

Compound	Predicted LD50, mg/kg[a]	Predicted toxicity class[a]	Predicted toxicity
Benzene,1,1'-(1,2- cyclobutanediyl)bis-, trans	6430	6	inactive
Phytol	5000	5	inactive
Geranyl isovalerate	10660	6	inactive
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5000	5	inactive
7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	900	4	inactive
Phthalic acid, butyl tetradecyl ester	1340	4	Carcinogenicity

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)

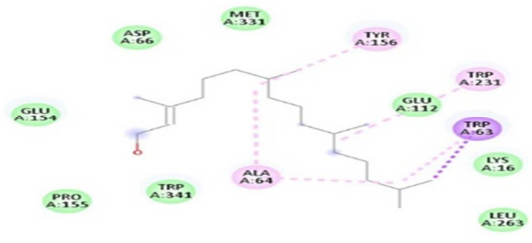

Fig. 5. 4giz_3,7,11,15-Tetramethyl-2-hexadecen-1-ol

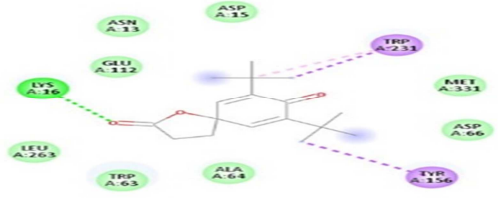

Fig. 6. 4giz_7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione

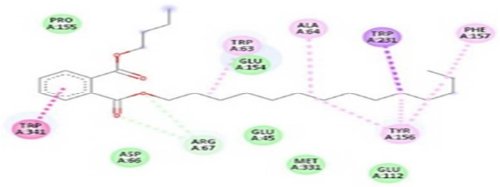

Fig. 7. 4giz_ Phthalic acid, butyl tetradecyl ester

No drug can be regarded as an oral drug if it breaks two or more of the Lipinski Rules. All the docked compounds in this study did not violate Lipinski's rules more than once. Therefore, these compounds can be administered orally. Therefore, all docked compounds have drug-like properties. It has been noted that substances with reduced hydrogen bonding capacity, molecular weight, and lipophilicity have higher permeability, better absorption, and higher bioavailability (Duffy *et al.,* 2015; Daina *et al.,* 2014).

Toxicity prediction

In ProTox-II, compound phthalic acid, butyl tetradecyl ester, is predicted to have carcinogenicity. Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans-, and geranyl isovlerate are the only substances in this inquiry that were expected to have minimal toxicity; the other chemicals are assumed to be non-toxic. The LD50 value and anticipated toxicity class for several substances are provided in Table 4.

Table 5. Prediction of biological activity of best-docked compounds

Compound	Pa a	Pi b	Biological activity
Benzene,1,1'-(1,2-cyclobutanediyl)bis-, trans	0,943	0,003	Phobic disorders treatment
	0,919	0,004	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
	0,903	0,003	Nicotinic alpha6beta3beta4alpha5 receptor antagonist
	0,910	0,010	CYP2C12 substrate
	0,897	0,003	Nicotinic alpha2beta2 receptor antagonist
Phytol	0,911	0,002	Prenyl-diphosphatase inhibitor
	0,907	0,001	Retinol dehydrogenase inhibitor
	0,905	0,005	Ubiquinol-cytochrome-c reductase Inhibitor
	0,893	0,007	Phobic disorders treatment
	0,885	0,002	Undecaprenyl-phosphate mannosyltransferase inhibitor
Geranyl isovalerate	0,960	0,003	Mucomembranous protector
	0,947	0,003	Lipid metabolism regulator
	0,935	0,001	Prenyl-diphosphatase inhibitor
	0,913	0,002	All-trans-retinyl-palmitate hydrolase inhibitor
	0,910	0,001	Undecaprenyl-phosphate mannosyltransferase inhibitor
3,7,11,15-Tetramethyl -2-hexadecen-1-ol	0,911	0,002	Prenyl-diphosphatase inhibitor
	0,907	0,001	Retinol dehydrogenase inhibitor
	0,905	0,005	Ubiquinol-cytochrome-c reductase Inhibitor
	0,893	0,007	Phobic disorders treatment
	0,885	0,002	Undecaprenyl-phosphate mannosyltransferase inhibitor
7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	0,883	0,008	Ubiquinol-cytochrome-c reductase inhibitor
	0,813	0,030	Aspulvinone dimethylallyltransferase Inhibitor
	0,793	0,027	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
	0,743	0,038	Mucomembranous protector
	0,705	0,009	CYP2B5 substrate
Phthalic acid, butyl tetradecyl ester	0,945	0,002	Sugar-phosphatase inhibitor
	0,935	0,003	Alkenylglycerophosphocholine inhibitor
	0,919	0,003	Pullulanase inhibitor
	0,914	0,004	Phobic disorders treatment
	0,911	0,002	Gluconate 5-dehydrogenase inhibitor

Biological activity prediction

An online method for predicting bioactivity based on structural factors Utilizing Prediction of Activity Spectra for Substances, Table 5 shows the best-docked compounds were assessed for their likely biological activity (PASS).Pa > Pi and Pa > 7 were used to evaluate five biological activities for each chemical. Indicating larger potential for the species, the results showed a number of noteworthy activities with Pa > 0.9.

Conclusion

In conclusion, GC-MS analysis of a root extract from the plant *Ludwigia perennis* revealed that it contains phytoconstituents with a high binding affinity for the E6 protein, a protein implicated in cervical cancer through docking studies. Research into ADME and toxicity verified the druglike properties". The PASS analysis for phytoconstituents showed several significant roles for the investigated phytochemicals. Docking studies suggest that 1,1'-(1,2-cyclobutanediyl)bis-, trans benzene might be an effective natural medication for treating the E6 protein. Even though the bioactivity that has been found is promising, further research is needed to identify the factors that are causing it, as well as to define its toxicity profile and long-term safety**.**

Recommendation(s)

Based on the results, the compounds from chloroform root extract were recommended for treating cervical cancer and could possibly develop new drug formulations for treating cervical cancer from the *Ludwigia perennis* plant.

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