Article

In Silico Investigation of *Pajanelia longifolia* (Willd.) K. Schum Bark Extract against NSCLC Targets: Potential Involvement in Apoptotic Pathways

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Abstract: Nature provides innumerable answers to human problems, but our knowledge is restricted. The use of medicinal plants to treat health problems dates back to ancient times., It has evolved into contemporary techniques that combine traditional knowledge with modern medicine. Cancer, the biggest cause of mortality worldwide, remains difficult to treat properly. This study focusses on non-small cell lung cancer (NSCLC)., The most common type of lung cancer, accounting for 85–90% of occurrences and associated with factors such as smoking and pollution. *Pajanelia longifolia*, an Indian traditional medicinal herb, has therapeutic potential and has historically been used to cure a variety of diseases. This study examines the phytochemical elements of *P. longifolia* bark using metabolite profiling. It evaluates its anti-NSCLC activity using computational methods. The key compounds were identified using liquid chromatography-mass spectrometry (LC-MS), and molecular docking was performed against protein B-Raf and EGFR, both linked to cancer proliferation. The findings emphasise the potential of *P. longifolia* as a source of bioactive chemicals for cancer therapy. They highlight the need for additional investigation into its medicinal potential, particularly in combination with proven medicines such as irinotecan.

Keywords: *Pajanelia longifolia*; antioxidant; anticancer; irinotecan; metabolites profiling

1. Introduction

There is a belief that nature contains the solution to every problem, it is we, the living creatures, who need to discover them. Our knowledge about nature is very limited, we have managed to utilise the blessings of nature to meet our needs from daily essentials to life-saving drugs. In ancient times, when modern medical sciences were unavailable, people treated various ailments using the medicinal plants. Today, by combining traditional knowledges of medicinal plants with modern medical science, numerous life-saving drugs are curing millions lives.

Currently, cancer is the disease that worries the world. This deadly disease is the second most notable cause of mortality after cardiovascular disease [1] Cancer is characterized by uncontrolled mitosis and cell proliferation [2]. Lung cancer, colorectal cancer, prostate cancer, and stomach cancer are the leading types of cancer in male. In contrast, breast cancer, colorectal cancer, lung cancer, and cervical cancer are predominant in females [1,3]. The proper therapy for cancer is unavailable. The existing therapies include chemotherapy and radiation therapy. However, these therapies have unwanted side effects and do not promise an optimistic prognosis. Thus, it is essential to develop alternative treatment strategies against cancer. In this work, we focus on the non-small cell lung cancer (NSCLC), one of the most predominantly diagnosed cancer types. NSCLC is the most common form of lung cancer, accounting for 85–90% of cases, and is strongly associated with smoking, exposure to certain industrial substances, family history, and high air pollution. NSCLC includes adenocarcinomas (LUADs), large cell cancers, and squamous cell cancers (LUSCs), which show a reduced sensitivity to radiation and chemotherapy

[4]. The NSCLC results in severe morbidity and mortality each year, with millions of new cases and deaths worldwide. Globally, lung cancer is among most common cancers, with NSCLC making up about 85% of cases [5]. More than 2.2 million new cases of lung cancer are diagnosed annually, and the disease causes over 1.8 million deaths world-wide [5].

Pajanelia longifolia is an important medicinal plant traditionally used to cure the various complexities [6]. This plant belongs to the family Bignoniaceae and varies from small to medium evergreen type. This plant is commonly distributed in the Eastern Bengal and Western Ghats in India and other tropical countries, such as Myanmmar, Burma and Bangladesh. This plant is so alued that it is recorded in Charaka Samhita (1000 BCE) for treating diseases such as urinary disorders, arthritis, stomach disorder etc. [7,8]. In local folklore practitioners also used this plant for treatment. In Karnataka, this plant is used for obesity, in Tripura and Cachar district of Assam, India. This plant is used for liver disease, such as jaundice, stomach ulcer, etc. In southern Assam, the leaves of *P. longifolia* are used on the skin for treating the infections [6,7,9]. Different studies have revealed the presence of phenolics and flavonoid compounds in the plant. It is also mentioned that, the bark of this plant is noted for its hepatoprotective and antimicrobial activity [8,10,11]. However, a comprehensive phytochemical screening has not yet been conducted. In this study, we have carried out the metabolites profiling of the bark extract and predicted its anti-NSCLC activity through computational methods.

2. Materials and Method

2.1. Preparation of Plant Extracts

The bark of the plant was collected from Silchar, Southern Assam, in the Cachar district, Northeast India during the month of June and July. It was then washed thoroughly with water and then air-dried in shade. Once moisture-free, it was crushed into a fine powder using an electric grinder for extract preparation. A powdered of the bark sample (150 g) was used for the extraction by following the maceration process [12], using the increasing solvents polarity as petroleum ether, ethyl acetate, acetone and methanol. The filtrate was first dried using a rotary evaporator under the reduced pressure and then with a lyophilizer. The extracts were stored at 4 °C for further experiments. Consequently, the four extracts were named as petroleum ether extract (PL-PE), ethyl acetate extract (PL-EA), acetone extract (PL-AC) and methanolic extract (PL-ME).

2.2. Quantitative Phytochemical Screening

2.2.1. Total Phenolic Content (TPC) Estimation

The total phenolic content of the various extracts of the plant was determined using a modified version of the method originally described in the literature [13]. In brief, the sample was prepared at a concentration of 1 mg/mL in methanol. From this stock solution, 0.5 mL of the sample was taken, and 0.1 mL of Folin–Ciocalteu reagent along with 2.4 mL of distilled water was added. The mixture was thoroughly mixed and allowed to stand for 3 min. Subsequently, 2 mL of a 2% Na_2CO_3 solution was added, and the mixture was kept in complete darkness for 60 min. The absorbance was then measured at 750 nm, and the results were expressed as Gallic acid equivalents (GAE/mg) of the plant extract.

2.2.2. Total Flavonoid Content (TFC) Estimation

To quantify the total flavonoids present in the extracts, a slightly modified standard protocol was implemented [14]. An equal volume of plant extracts (1mg/mL) and $AlCl_3$ (2%) was mixed properly and incubated at dark for a period of 15 min. After the incubation period, the absorbance was recorded at 415 nm and the results were expressed as quercetin equivalents (QE/mg) of the plant extract.

2.3. In Vitro Antioxidant Assay

Determination of DPPH Free-Radical Scavenging Activity

Antioxidant properties of all the four extracts of the plant sample was determined using DPPH free radicals scavenging activity following the protocol described in the literature [15]. Briefly, 80 µg/mL DPPH solution is prepared in methanol and kept it in dark. Then six serial dilutions of each extract were carried out from stock solution of 1 mg/mL. An equal volume of each sample solution and the stock DPPH solution was mixed and kept it in dark for 30 min. The absorbance was taken at 517 nm after the incubation period. The DPPH solution in

methanol was used as a control and 95% methanol was used as a blank. The results were compared with standard ascorbic acid. The percent inhibition of the free radicals was calculated using the following formula:

$$\% \text{ inhibition} = [(Ac - As)/Ac] \times 100$$

where 'Ac' is the absorbance of control and 'As' is the absorbance of the sample. The IC_{50} value, which is the concentration of the test material that reduces 50% of the free-radical concentration, was calculated through sigmoidal dose-response curve.

2.4. Metabolites Profiling

A detailed metabolite profiling was performed for the plant extract to identify the active compounds. Liquid Chromatography-Mass Spectrometry (LC-MS) were employed for this analysis at the Sophisticated Analytical Instrument Facility (SAIF) at IIT Bombay. LC-MS analysis was performed using a Varian Inc. 410 Prostar Binary LC system, equipped with 500 MS IT PDA Detectors. The separation was achieved using an RRHT C18 column (2.1 mm × 100 mm, 1.8 µm). The mobile phase consisted of two solvent systems: Solvent A (water with 0.1% formic acid) and Solvent B (acetonitrile with 10% water and 0.1% formic acid). The injection volume was set at 5 µL, with a flow rate of 0.300 mL/min, and the column temperature was maintained at 40 °C.

2.5. In Silico Analysis

2.5.1. Target

Malignant cells are characterized by their rapid proliferation, driven by uncontrolled cell proliferation. In this study, the focus was placed on two specific targets, B-Raf (PDB id: 4R5Y) and EGFR (PDB id: 6LUB) responsible for cell proliferation identified through comprehensive literature research. These proteins, when mutated, play a critical role in cancer development. The 3D structures of these mutated proteins from the Protein Data Bank (www.rcsb.org/pdb; accessed on 30 August 2024) and utilized as drug targets.

2.5.2. Ligand

The identified phytocompounds from the plant extract, as determined through LCMS analysis, were used as ligands for this study. The SMILES format of these compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/; accessed on 23 July 2024) and generated using ACD/ChemSketch (version 2021.1.2, Advanced Chemistry Development, Inc., Toronto, ON, Canada) whichever compounds are not available in PubChem database. Since molecular docking requires the compounds in mol format, the conversion from SMILES to mol was carried out using Open Babel software v 3.1.1.

2.5.3. Molecular Docking

Molecular docking is a computational method used to estimate how well a ligand can bind to the active site of a target protein. This technique not only predicts the binding efficiency but also provides insights into the binding conformation and orientation. In this analysis, Molegro Virtual Docker (MVD) version 6.0 was used for performing the docking simulations. The receptor proteins were prepared by removing any bound inhibitors, cofactors, and water molecules before loading them into the software. The protonation states of amino acids were adjusted using the built-in protein preparation tool. MVD's cavity detection feature facilitated the identification of the active sites of the receptors, which were designated as the docking sites. After conducting energy minimization and optimizing hydrogen bonds, the software generated key metrics, such as the MolDock score, hydrogen bond score, and the geometry of ligand binding at the active site.

2.5.4. Prediction of ADME Profile and Drug-Likeness

ADMET analysis was performed using the SwissADME server (https://www.swissadme.ch/) provided by the Swiss Institute of Bioinformatics. The compounds were input in SMILES format, and the server's algorithm generated data on physicochemical properties, lipophilicity, water solubility, pharmacokinetics, medicinal chemistry, and drug-likeness characteristics.

3. Result

3.1. Crude Yield of Plant Extracts

A powdered of the bark sample (150 g) was extracted and upon drying, the yield of crude extracts obtained from their respective solvents is presented in Table 1 below.

Table 1. Crude yield of the extracts per 100 g powdered sample.

Crude Extract Yield per 100 g Powdered Sample			
PL-PE	PL-EA	PL-AC	PL-ME
1.5 g	1.77 g	2.35 g	19.4 g

3.2. Total Phenol and Flavonoid Content

The results indicate that the acetonic extract of *P. longifolia* contains the highest levels of total phenolic content (TPC) and total flavonoid content (TFC) compared to other extracts. The acetonic extract has a TPC of 109 GAE/mg and a TFC of 135 quercetin/mg (Figure 1). The TPC was calculated using the gallic acid standard curve equation and the TFC was determined using the quercetin standard curve equations. The equations were as follows:

$$y = 0.0045x + 0.4498,\ R^2 = 0.9867$$

$$y = 0.0002x + 0.117;\ R^2 = 0.9999$$

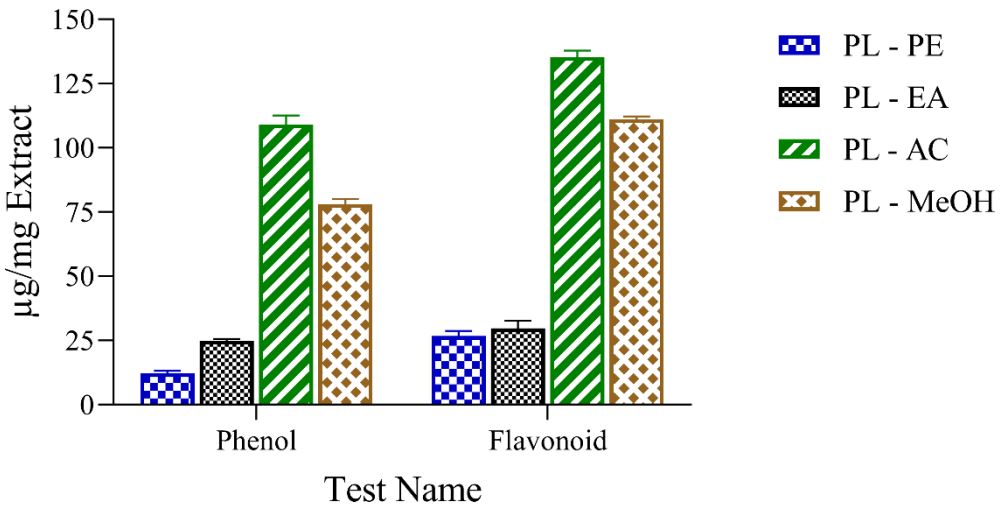

Figure 1. Comparative TPC and TFC in the different extracts of *Pajanelia longifolia*.

3.3. Antioxidant Activity

The results of the DPPH radical scavenging activity for *P. longifolia* and the standard ascorbic acid are presented in the Figure 2. The percentage inhibitory activity of free radicals, particularly the ability to inhibit by 50%, is widely used as a parameter to measure antioxidant activity. In this study, both the plant extract and standard significantly scavenged the DPPH radical with increasing concentrations. The Figure 2 showed the dose response curve of DPPH radical scavenging activity IC_{50} (µg/mL) of the acetone extract (10.54 ± 0.01 µg/mL) was found to be the lowest, while the IC_{50} for the methanolic extract (13.85 ± 0.01 µg/mL) of *P. longifolia*is ranked the second lowest among all extracts analysed. Both the acetone and methanolic extracts demonstrated better DPPH radical scavenging activity compared to the standard ascorbic acid (12.50 ± 0.01 µg/mL).

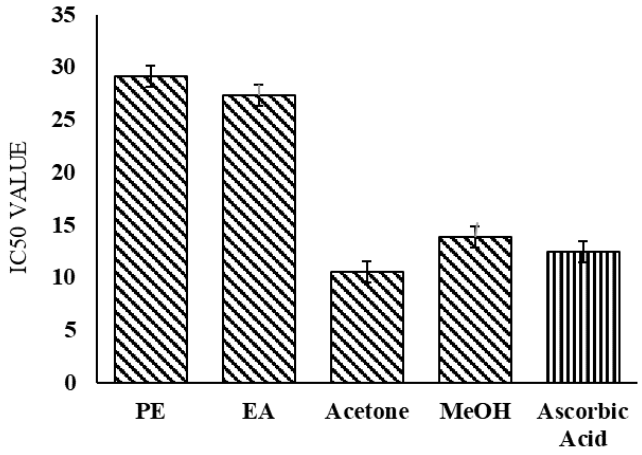

Figure 2. Graph showing the IC_{50} concentration in µg/mL of inhibiting DPPH free radicals by the different extract of P. longifolia and standard ascorbic acid.

3.4. Metabolites Profiling

Based on the results from the TPC, TFC, and DPPH free radical scavenging assays, the acetonic extract of *P. longifolia* (PL-AC) was selected for further phytochemical analysis using LC-MS techniques. These metabolomic analyses provide insights into the phytochemicals present in the plant, facilitating their identification. The compounds detected in the LC-MS chromatograms (Figure 3) of PL-AC are listed in the Table 2.

Table 2. (**A**) Compounds detected from PL-AC extract of bark extract of *P. longifolia* through LC-MS analysis (‘+’ ve ESI). (**B**) Compounds detected from PL-AC extract of bark extract of *P. longifolia* through LC-MS analysis (‘−’ ve ESI).

(A)				
Name	**Score**	**Mass**	***m/z***	**RT**
D-Lombricine	74.41	270.07	293.06	1.14
Myoinositol 1-phosphate	76.04	260.03	261.04	1.28
5-Hydroxy-3,3′,7,8-tetramethoxy-4′,5′-methylenedioxyflavone	79.52	402.09	425.08	1.99
Tiracizine	69.27	367.19	390.17	3.30
Ricinine	95.08	164.06	165.07	3.41
1,5-Dibutyl methyl hydroxycitrate	88.84	334.16	357.15	3.63
3,4,5-Trimethoxycinnamic acid	85.94	238.08	239.09	3.68
2,4,6-Trihydroxytoluene	87.03	140.05	141.05	4.02
Sulprostone	89.66	465.18	466.19	4.34
(R)-Cryptone	86.17	138.10	139.11	4.40
Funtumine	80.53	317.27	340.26	4.68
Pivmecillinam	40.91	439.21	462.20	4.73
Methylergonovine	63.92	339.19	362.18	4.97
Puromycin	48.68	471.23	494.22	5.02
Alfuzosin	81.49	389.20	390.21	5.03
Istamycin C1	85.37	431.27	432.28	5.26
Netilmicin	87.91	475.30	476.31	5.61
3-Oxo-12,18-ursadien-28-oic acid	45.04	452.33	475.32	6.42
Vernodalin	92.63	360.12	361.13	6.87
16,17-Dihydro-16a,17-dihydroxygibberellin A4 17-glucoside	96.95	528.22	551.21	7.08
Cubebin	85.51	356.13	357.13	7.13
Methyl trans-*p*-methoxycinnamate	83.95	192.08	193.09	8.69
N_1,N_5,N_{10}-Tricoumaroyl spermidine	59.11	583.26	584.27	9.38
Dihydrodeoxystreptomycin	92.07	567.29	568.30	10.18
Manumycin A	55.04	550.26	573.25	10.26
Neuraminic acid	55.79	267.09	290.08	10.50
N-(1-Deoxy-1-fructosyl)serine	56.61	267.09	290.08	10.79
Protorifamycin I	59.33	639.31	640.32	10.88
Cortolone	97.94	366.24	367.25	11.02

Eugenol	94.65	164.08	165.09	11.25
Glycine, *N*-[(3a,5b,7a)-3-hydroxy-24-oxo-7-(sulfooxy)cholan-24-yl]-	67.6	529.28	552.27	11.39
1-(b-*D*-Ribofuranosyl)-1,4-dihydronicotinamide	78.82	256.10	279.09	11.47
Sulfadimidine	73.4	278.09	279.09	11.74
7-Hydroxyflavanone beta-*D*-glucopyranoside	97.58	402.13	403.14	11.83
Prunetin	84.97	284.07	285.08	12.14
5,6,7,8,3′,4′,5′-Heptamethoxyflavone	92.22	432.14	433.15	12.29
(9*Z*,11*E*,13*E*,15*Z*)-4-Oxo-9,11,13,15-octadecatetraenoic acid	81.72	290.19	291.19	12.33
Gingerenone C	96.48	326.15	327.16	13.06
Mitoxantrone	74.04	444.20	445.21	13.38
Butyl 2-aminobenzoate	98.49	193.11	194.12	13.41
Kanamycin	81.02	484.24	507.23	15.31
Cycloate	85.5	215.13	238.12	15.35
Medroxyprogesterone	75.06	344.24	345.25	16.51
Gingerglycolipid C	96.35	680.40	703.39	16.80
Irinotecan	86.84	586.28	609.27	19.98
Oxidized dinoflagellate luciferin	90.41	602.28	625.26	20.26
Pheophorbide a	96.4	592.27	593.27	21.01

(B)

Name	**Score**	**Mass**	***m/z***	**RT**
Vanillylmandelic acid	84.16	198.05	197.05	3.07
Diethyl L-malate	93.61	190.09	235.08	3.11
MeIQ	94.25	212.11	257.10	3.66
8-*D*-Olivosyl-landomycin	59.95	468.14	467.14	4.06
Aspirin	90.11	180.04	179.04	4.36
Esculetin	81.46	178.03	177.02	4.60
Byakangelicin	63.36	334.11	379.11	4.65
Inumakilactone A glycoside	85.22	526.17	525.16	4.88
4′,7-Di-*O*-methylcatechin	81.35	318.11	363.11	5.45
Phloroacetophenone 6′-[xylosyl-(1->6)-glucoside]	83.74	490.17	489.16	5.53
Silandrin	53.14	466.13	525.14	5.54
Isoacteoside	81.27	624.21	623.20	6.51
Lindleyin	73.51	478.15	523.15	6.51
(2*S*,2′*R*,3*S*,3′*R*,4*S*)-3,4′,5,7-Tetrahydroxyflavan(2->7,4->8)-3,3′,5,5′,7-pentahydroxyflavan	62.79	560.13	559.13	6.51
Glaucolide A	76.01	464.17	509.17	6.81
Guibourtinidol-(4alpha->6)-catechin	62.09	546.15	545.15	6.82
Aloesin	79.52	394.13	393.12	6.96
Artonol B	61.55	420.12	479.14	6.98
Ethofumesate	91.67	286.09	345.10	7.04
Methyl 3,4-dihydroxy-5-prenylbenzoate 3-glucoside	78.96	398.16	443.16	7.04
Aromadendrin 4′-methyl ether 7-rhamnoside	72.89	448.14	507.15	7.28
(2S,2′‘S,3S,3′‘R,4S)-3,4′,5,7-Tetrahydroxyflavan(2->7,4->8)-3,4′,5,7-tetrahydroxyflavan	63.4	544.14	543.13	7.29
Salfredin B11	91.17	232.08	231.07	7.36
Mahuannin D	63.55	528.14	573.14	7.67
2-(3,4-Dihydroxyphenylethyl)-6-*epi*-elenaiate	74.57	378.13	377.13	7.75
Vernolide	68.25	362.14	361.13	8.07
Morusignin B	75.36	328.10	327.09	8.96
Galactopinitol A	66.45	356.13	401.13	9.30
Elephantin	77.4	374.14	373.13	10.74
9*Z*-Octadecenedioic acid	86.8	312.23	311.22	16.01

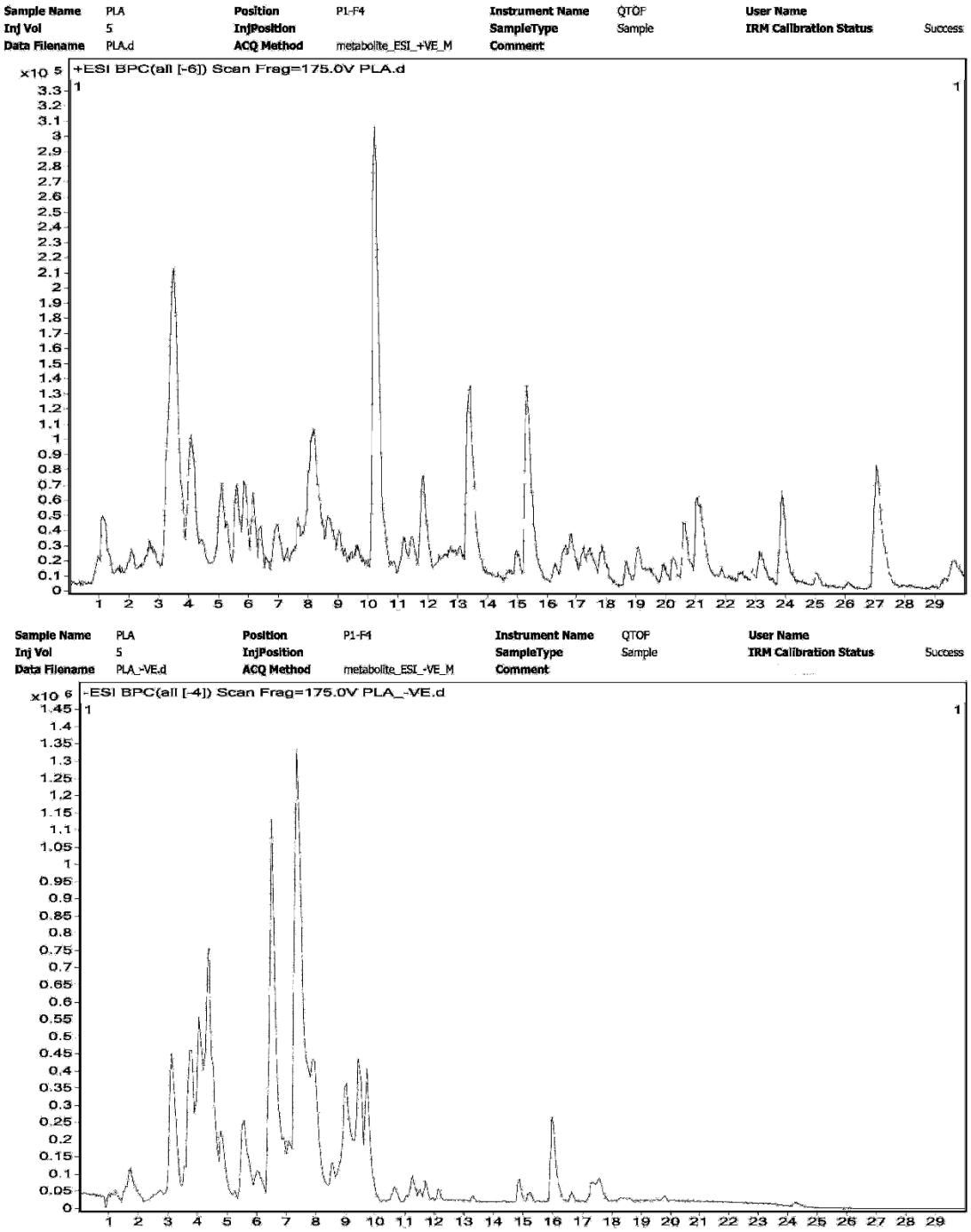

Figure 3. LCMS chromatogram of PL-AC.

3.5. Docking Scores and Inhibition of Receptors

Among the two selected targets, several identified compounds demonstrated superior binding efficacy compared to the respective positive controls. In the case of the EGFR triple mutant protein, Pheophorbide A exhibited the strongest binding, with a MolDock score of −182.13, surpassing the positive control gefitinib, which had a score of −118.65. In addition to Pheophorbide A, 18 other phytocompounds from this plant also showed stronger binding than the positive control (Table 3). Similarly, the docking results for the B-Raf V600E mutant protein revealed that the compound Manumycin A had the highest binding affinity, followed by four additional compounds, which outperformed the positive control Dabrafenib, with a MolDock score of −193.48. Both proteins are implicated in cancer cell proliferation, and binding to these targets could potentially reduce cancer cell growth and the formation of malignant tumours.

Table 3. Provides a comparison of docking scores of ligands against the targets, alongside the positive control, i.e., market-approved drugs for these targets.

Compound Name	EGFR		BRAF	
	Moldock Score	H-Bond Score	Moldock Score	H-Bond Score
Gefitinib (Positive Control of EGFR)	−118.651	−7.31214	-	-
Dabrafenib (Positive control of BRAF)	-	-	−154.12	0
Pheophorbide A	−192.13 *	−8.84	−186.93	4.19
Manumycin A	−171.62	−9.09	−193.48 *	−5.90
Irinotecan	−141.10	−4.06	−157.87	−1.91
Sulprostone	−139.38	−9.39	−155.77	−4.19
Isoacteoside	−137.83	−4.73	−159.60	−20.26
Lindleyin	−146.16	−15.70		
Elephantin	−138.44	−5.28		
Cubebin	−137.38	−3.54		
(9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic_acid	−135.28	−7.90		
Puromycin	−133.78	−5.17		
Dihydrodeoxystreptomycin	−131.71	−13.26		
Glaucolide_A	−127.96	−6.26		
Vernodalin	−126.43	−5.99		
Mahuannin_D	−125.87	−10.89		
Glycine,N-[(3a,5b,7a)-3-hydroxy-24-oxo-7-(sulfooxy)cholan-24-yl]-	−123.63	−9.58		
8-D-Olivosyl-landomycin	−121.38	−9.31		
Protorifamycin_I	−120.47	−3.32		
Methyl_3,4-dihydroxy-5-prenylbenzoate_3-glucoside	−119.94	−9.77		
Manumycin_A	−171.62	−9.09		

* Highest binding affinity.

After reviewing the data, it was observed that the five compounds, namely Manumycin A, Pheophorbide A, Isoacteoside, Irinotecan and Sulprostone (Figure 4), demonstrated the potential to inhibit the selected target proteins., This suggests their use as possible anti-NSCLC drugs. The docking poses and 2D interactions of these five compounds, along with the positive controls, are displayed in Figure 5.

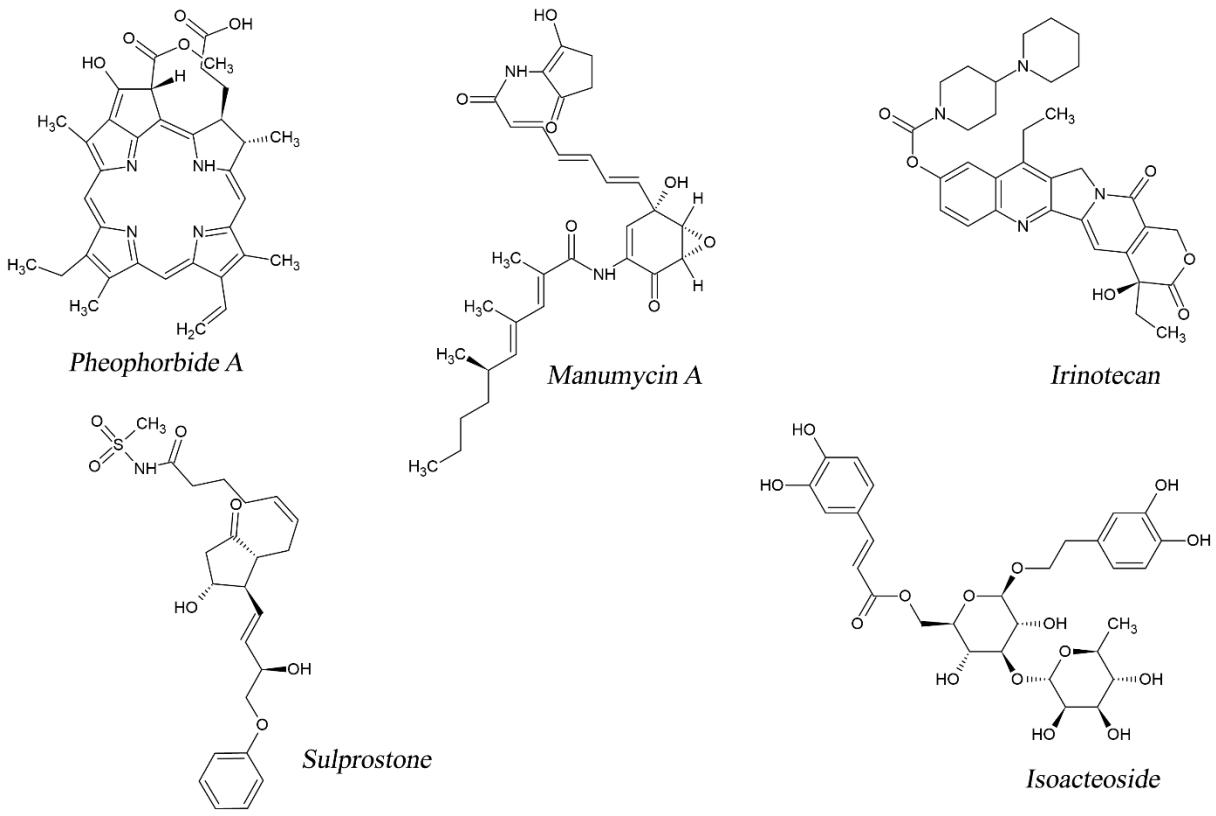

Figure 4. Chemical structure of best five compounds.

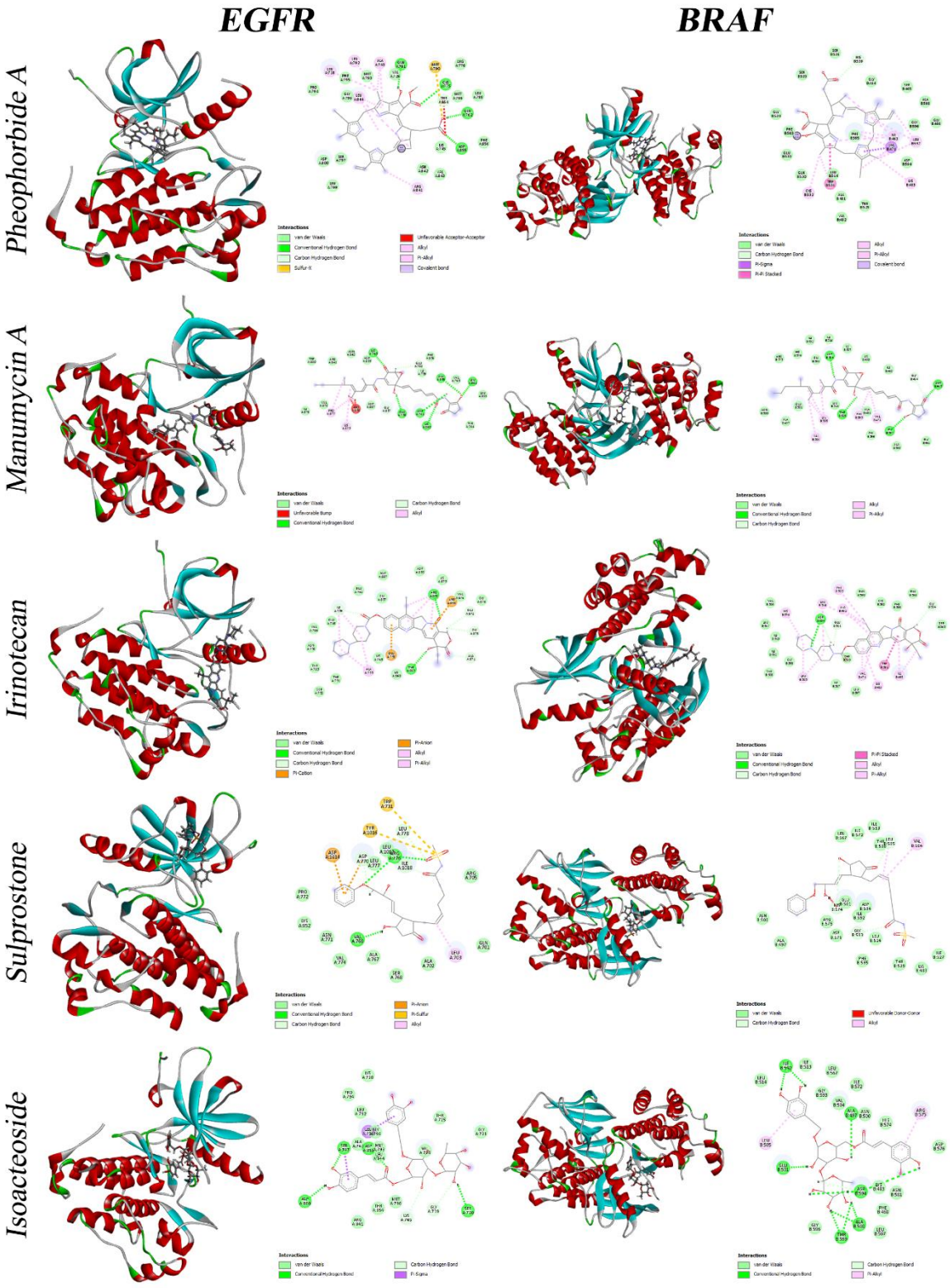

Figure 5. Docking pose of best five compounds with EGFR and BRAF protein.

3.6. ADMET Profile Analysis

Among the compounds evaluated for potential drug-likeness, irinotecan emerged as the most promising candidate based on an analysis of its physicochemical and pharmacokinetic properties. This assessment was conducted using widely accepted criteria, including Lipinski's Rule of Five, solubility, gastrointestinal (GI) absorption, bioavailability score, and safety alerts. Irinotecan displayed a moderate bioavailability score of 0.55, suggesting reasonable potential for oral bioavailability, and its GI absorption was classified as high, a desirable trait for orally administered drugs. Additionally, it demonstrated acceptable drug-like characteristics with only one

Lipinski violation, and it was free from PAINS and Brenk alerts, which indicates a lower likelihood of promiscuous binding or toxicity issues. This is particularly advantageous, as compounds with fewer alerts are less likely to cause off-target effects or adverse reactions. In contrast, other compounds such as Pheophorbide A, Manumycin A, Sulprostone, and Isoacteoside exhibited limitations, including low GI absorption, multiple rule violations, or safety alerts. Notably, Isoacteoside had a very low bioavailability score (0.17) and multiple Lipinski violations, making it an unsuitable candidate. Although irinotecan is a P-glycoprotein (Pgp) substrate, which may limit its bioavailability in certain tissues due to potential drug efflux, its overall profile, high GI absorption, moderate bioavailability score, and minimal rule violations—positions it as the most favorable compound for further investigation as a potential therapeutic agent. The pharmacokinetics and drug-likeness scores for all the compounds are detailed in Table 4 and boiled egg illustration at Figure 6

Table 4. Calculated pharmacokinetics and drug-likeness parameters of the ligands.

Molecule	Pheophorbide A	Manumycin A	Irinotecan	Sulprostone	Isoacteoside
Molecular Weight	592.68	550.64	586.68	465.56	624.59
H-bondacceptors	8	7	8	7	15
H-bond donors	3	4	1	3	9
ESOL Class	Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Soluble
Ali Class	Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Moderately soluble
GI absorption	Low	Low	High	Low	Low
BBB permeant	No	No	No	No	No
Pgp substrate	Yes	Yes	Yes	Yes	Yes
Lipinskiviolations	1	1	1	0	3
Ghoseviolations	3	3	3	0	4
Veberviolations	0	0	0	1	2
Egan violations	1	1	0	1	1
Muegge violations	0	0	0	0	4
Bioavailability Score	0.56	0.56	0.55	0.55	0.17
PAINS alerts	0	0	0	0	1
Brenk alerts	0	0	0	1	2

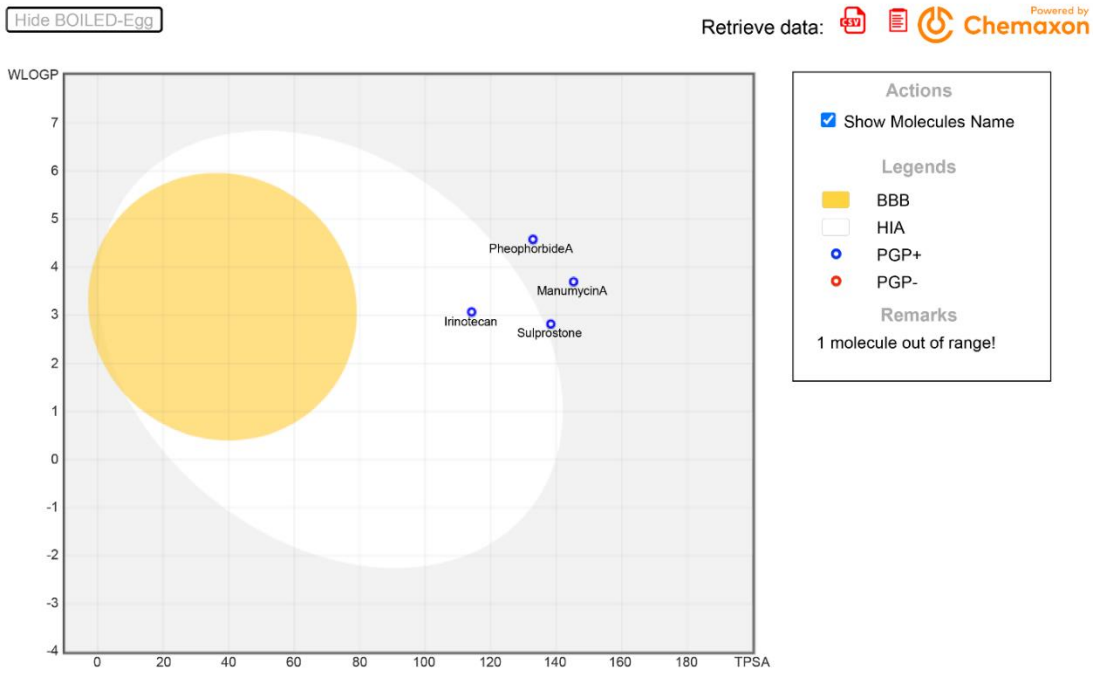

Figure 6. The BOILED-EGG MODEL is used to study gastrointestinal absorption and brain penetration. Molecules in the yolk of boiled eggs are considered capable of passing through the blood-brain barrier (BBB). Molecules in the white of a boiled egg are assumed o be passively absorbed through the gastrointestinal tract. P-glycoproteins are believed to actively remove the blue-dotted molecules from the Central nervous system (CNS).

4. Discussion

The use of natural compounds in cancer research has gained significant interest as researchers seek alternative therapies that present fewer side effects than conventional approaches. This study focusses on the possible

anticancer effects of *P. longifolia*, an ethnomedicinal plant traditionally been used to treat numerous diseases [6]. Notably, the acetone extract (PL-AC) of *P. longifolia* showed significant antioxidant activity, displaying a DPPH radical scavenging potential of 10.54 μg/mL, lower than the standard ascorbic acid (IC_{50} = 12.50 μg/mL). This data implies that *P. longifolia* contains active compounds with high free radical scavenging capacities, which gives the clue for the selection of potential extracts [15]. Further phytochemical screening using LC-MS identified various bioactive components in the PL-AC extract, including flavonoids and phenolics, which are known to have antioxidant and anticancer activities [10]. Flavonoids have been linked to the modulation of signalling pathways in cancer cells, causing apoptosis, and the inhibition tumour development [16]. The high concentration of these phytochemicals is consistent with previous studies highlighting the anticancer properties of *P. longifolia* and supports its traditional medicinal use for treating liver and skin diseases [7].

The anticancer activity of the isolated compounds was also verified by in silico molecular docking studies focusing on B-Raf and EGFR proteins as two main oncogenes associated with NSCLC. In many cancers, B-Raf and EGFR are often mutated, resulting in an overproduction of cells [4]. The favourable scores obtained in our study with MolDock indicate that compounds from *P. longifolia* may effectively interact with the active sites of these proteins, inhibiting their activity. This inhibition could be vital in controlling the growth and proliferation of NSCLC cells that are usually less responsive to conventional therapies [5].

After analysing the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles of selected five drugs, irinotecan was found as the most viable option for further development. This evaluation used known criteria such as Lipinski's Rule of Five, solubility, gastrointestinal (GI) absorption, bioavailability score, and safety alerts. Notably, irinotecan, a camptothecin analogue, is a strong topoisomerase I inhibitor used to treat metastatic colorectal cancer [17]. It has a moderate bioavailability score of 0.55, indicating a fair potential for oral administration, as well as high GI absorption, an important characteristic for medications intended for oral usage [4]. Furthermore, it followed Lipinski's guidelines with only one violation and was free of both PAINS and Brenk alerts, indicating a lower risk of harmful effects and promiscuous binding [18,19]. In contrast, other tested compounds, including Pheophorbide A, Manumycin A, Sulprostone, and Isoacteoside, have severe limitations such as limited GI absorption, multiple rule violations, and safety alerts. Isoacteoside was particularly problematic, with a very low bioavailability score of 0.17 and three Lipinski violations, making it an unsuitable candidate. Despite beinga P-glycoprotein (Pgp) substrate, which may reduce its bioavailability due to drug efflux mechanisms, irinotecan remains the best option due to its favorable pharmacokinetic profile. The comprehensive pharmacokinetic and drug-likeness data for all substances, highlighting their relative strengths and shortcomings, are presented in Table 4.

The research findings contribute to the growing body of research supporting the therapeutic benefit of plant-based drugs in oncology. The high incidence and mortality rates associated with lung cancer, particularly NSCLC, highlight the need for innovative therapeutic modalities that are both effective and safe. This study serves as a foundation for future research using plant-derived therapeutics on NSCLC, combining ancient knowledge with modern scientific methodologies to improve patient outcomes through reduced toxicity from treatments such as chemotherapy or radiation [20]. Further in vitro and in vivo studies are necessary to validate the findings and to investigate the pharmacokinetics and bioavailability compounds derived from *P. longifolia.*

5. Conclusions

The research findings indicate that *P. longifolia* possesses significant anticancer capabilities, particularly against non-small cell lung cancer (NSCLC). Through extensive phytochemical investigation and metabolite profiling, a diverse array of active compounds in the bark extracts has been identified, contributing to their medicinal potential. The high antioxidant activity of the extracts suggests a mechanism by which these substances protect against oxidative stress, a condition frequently linked to cancer development. Moreover, the results highlight the importance of irinotecan, a well-known chemotherapeutic drug, implying that its efficacy may be amplified by the synergistic effects of bioactive components obtained from *P. longifolia*. This study emphasises the potential of traditional medicinal plants as a source of new therapeutic molecules. Further research is necessary to explore the unique mechanisms of action and clinical applications of these compounds, as this could lead to the development of more effective and targeted cancer treatments. This research findings contribute to the expanding body of evidence supporting the use of natural ingredients in modern oncology, opening up the way for future study and drug develop.

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