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Computational Analysis of Bioactive Phytocompounds from Methanolic Extract of *Pajanelia longifolia* (Willd.) K. Schum against EGFR and TGF-β Cancer Targets

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Abstract: *Pajanelia longifolia* (Willd.) K. Schum., a medicinal plant traditionally used in India, exhibits significant therapeutic potential and has long been employed in the treatment of various ailments. In this study, a target-based in silico strategy was applied to explore the interaction of metabolites from the bark of *P. longifolia* with two key proteins, EGFR and TGFβRI, identified through network pharmacology. These proteins are crucial regulators in the development and progression of various cancers. Alongside computational analysis, phytochemical screening, antioxidant activity, and metabolite profiling were performed on *P. longifolia* bark extract with different types of solvents. The GC-MS analysis was conducted on the methanolic extract of the plant and GC-MS-identified metabolites, along with compounds previously documented in literature, were subjected to molecular docking analysis against the selected target proteins. Several metabolites demonstrated prominent MolDock scores than the standard reference inhibitors against targets. These phytocompounds, such as Lindleyin, Pheophorbide a, irinotecan, silandrin and rescinnamine emerging as the most promising docking results with respect to their positive control against targets. Rescinnamine appears to be the most suitable and potentially bioactive compound from the methanolic extract of this plant. Pharmacokinetic and physicochemical evaluation further indicated that these bioactive molecules possess favorable drug-like properties, suggesting their potential as leads for novel therapeutic agents against cancer. The findings emphasize the importance of *P. longifolia* as a valuable source of anticancer metabolites. Future work should include molecular dynamics simulations to confirm binding stability, followed by in vitro and in vivo validation to assess biological efficacy and safety. These steps may ultimately support the development of plant-derived therapeutic agents for the management of various cancers.

Keywords: *Pajanelia longifolia* (Willd.) K. Schum; methanolic extract; in silico; molecular docking; bioactive compound; rescinnamine.

1. Introduction

Historically, traditional medicine has long been used to treat several diseases. The compounds isolated and sourced from natural sources remain a key source of new drugs, new bioactive entities and new pharmacological leads [1]. Medicinal plants have been used as pharmaceutical intermediates, food supplements, nutraceuticals, and chemical templates for the creation of synthetic drugs [2]. Plant secondary metabolites have demonstrated significant potential in the treatment of various cancers. The global percentage of various types of cancer occurrence is shown in Section 3.2 [3–9]. Cancer cells are especially susceptible to oxidative stress, which plays a crucial role in promoting cancer cell growth. Plant secondary metabolites can protect the liver by exerting anti-inflammatory and antioxidant actions that influence pathways like EGFR, TGF-β, NF-κB/TLR4, PI3K/AKT, and Nrf2/HO-1. Additionally, certain Phenols, polyphenols and flavonoids can demonstrate anti-cancer effects against several cancers by limiting tumor growth, metastasis, and EMT via MAPK, PI3K-Akt and Wnt pathways inhibition [10]. In addition, phenolics target key molecular pathways implicated in cancer progression, such as inhibiting the NF-κB pathway, which regulates inflammation and tumor development, and modulating the PI3K/Akt/mTOR and MAPK signaling pathways, both of which are important for cancer cell survival and proliferation [11,12]

Pajanelia longifolia (Willd.) K. Schum is one of such medicinal plants that carries the potential to affect the specific targets that cause serious diseases. *P. longifolia* has been historically utilized to address various health issues [13]. This plant is mentioned in the Charaka Samhita, dating back to 1000 BCE, because of its high value and for its use in treating urinary issues, arthritis, and gastrointestinal disorders [14]. In Karnataka, this plant has importance among folklore practitioners for reducing excess body fat [15]. The effects of bark decoction of this plant in liver ulcer and jaundice were acclaimed by the tribal communities of Cachar district, Assam, India. The bark of the plant has subsequent potential to have hepatoprotective properties [16,17]. In Southern Assam, *P. longifolia* leaf crude extract is applied to the skin for treating infections [14]. The antibacterial activity of the leaf extract was shown to be due to the presence of the polar components [18]. Further hepatoprotective activity has also been reported for this plant's stem bark [19]. The main components of today's early pharmaceutical research include the target and lead discovery; in silico approaches are now an integral part of bioinformatics, which has established itself as an important tool for target discovery, facilitating the selection of the most prominent targets for any disease under study [20]. In silico methods, such as virtual screening (VS), can be used to discover novel hit compounds once the targets are validated [21]. When the hit compounds are identified, *ADME* analysis (absorption, distribution, metabolism, excretion) should be considered, as unfavorable pharmacokinetics of drugs often become the reason for failure in clinical trials [22]. Computational screening and approaches to discover new drugs have maximized the potential outcome in recent years. The advancement of technology and the power of computational approaches have reduced the time and resources required in drug discovery processes [23]. Network pharmacology underlines a synergistic approach, combining systematic medicine and information science developed by Andrew L. Hopkins, and paving the way for deciphering multiple targets with computational modeling [24]. Worldwide, hepatic diseases pose a severe threat, accounting for morbidity and mortality, with nearly 1.3 million deaths occurring annually from acute and chronic viral hepatitis [25,26].

The transforming growth factor-β (TGF-β) superfamily consists of structurally related proteins, including TGF-β isoforms, activins/inhibins, and bone morphogenetic proteins (BMPs). These molecules play critical roles in regulating diverse cellular processes such as proliferation, apoptosis, differentiation, epithelial–mesenchymal transition (EMT), and cell migration. The first characterized member, TGF-β, has been extensively associated with the pathogenesis of various human diseases, including vascular disorders, autoimmune conditions, and cancer development [27–29]. Transforming growth factor-beta (TGF-β) suppresses tumours in the early stages of cancer by causing cell cycle arrest and encouraging apoptosis. TGF-β, on the other hand, changes to a pro-tumorigenic function in later stages, increasing the invasiveness and propensity for metastasis of tumour cells. Furthermore, TGF-β signalling modulates a variety of cellular functions by interacting either antagonistically or synergistically with various cellular pathways [30,31]. Transforming growth factor-β (TGF-β) plays a context-dependent dual role in colorectal cancer. While it supports intestinal homeostasis by maintaining immune tolerance and epithelial integrity, its dysregulation in advanced tumors drives chronic inflammation, epithelial transformation, and tumor-stroma interactions that facilitate cancer progression, a phenomenon termed the TGF-β paradox [32]. A multipurpose cytokine, transforming growth factor-β (TGF-β) controls a variety of biological functions, especially in the pulmonary system. The development of lung cancer has been closely linked to TGF-β signalling dysregulation. Notably, TGF-β is essential for promoting the epithelial–mesenchymal transition (EMT), which in turn promotes tumour invasiveness and the spread of metastases. As a result, a TGF-β-induced EMT signature is becoming more widely acknowledged as a possible predictive biomarker in lung cancer, and research conducted in animal models has shown that blocking TGF-β-mediated EMT can successfully prevent metastasis [33]. The

onset of hepatocellular carcinoma (HCC) begins with liver damage characterized by inflammation that causes death of liver cells and their subsequent regeneration. This ongoing liver condition progressively develops through stages of fibrosis and cirrhosis before culminating in hepatocellular carcinoma [34,35]. Networking multiple proteins is responsible for regulating HCC. The two most prominent targets, EGFR (Epidermal Growth Factor Receptor), and TGFβRI (Transforming Growth Factor Beta Receptor 1), play significant roles in promoting the disease. EGFR is a transmembrane receptor protein that plays an integral role in tumor development, promoting many cellular mechanisms such as cellular specialization and multiplication, increased cell invasion and an inflammatory environment [36]. The epidermal growth factor receptor, or EGFR, is essential for preserving tissue homeostasis and epithelial growth. The importance of EGFR in both normal physiology and illness is highlighted by the fact that it plays a major role in carcinogenesis in malignancies, including lung, breast, and glioblastoma and is increasingly connected to treatment resistance through amplification and secondary mutations [37]. EGFR, the prototypical member of the receptor tyrosine kinase family, plays a critical role in tumorigenesis. Brain tumors often exhibit EGFR overexpression with large deletions, while lung cancers predominantly show kinase-domain point mutations or insertions, making EGFR and its heterodimeric partner HER2/ERBB2 key therapeutic targets [38]. Despite the clinical benefits of EGFR tyrosine kinase inhibitors (EGFR-TKIs), patients with advanced non-small cell lung cancer (NSCLC) harboring EGFR-activating mutations often face poor prognosis. This is primarily attributed to the emergence of intrinsic or acquired resistance mechanisms [39,40]. It modulates epithelial cell growth and viability, thus contributing to organ formation and tissue repair under normal circumstances [41]. The oncogene EGFR, when mutated, participates in the development of liver tumorigenesis [37]. The downstream pathways, like Raf-ERK and PI3K-mTOR signaling pathways, get upregulated by the activation of the EGFR signaling pathway [42]. However, the TGF-β signaling pathway involves two serine/threonine kinase receptors-the TGF-β type I receptor (TGFβRI) and TGF-β type II receptor (TGFβRII), which are ubiquitously expressed across all cell types [43]. Progressive stages of HCC and adverse outcomes are linked with high TGF-β levels in patients having HCC [44]. Ras/Raf/MEK/ERK and PI3K/ AKT/mTOR cellular signaling cascades promote HCC development with elevated TGF-β levels [45]. Thus, by regulating either EGFR or TGF-β, cancers could be controlled. The current study was undertaken to investigate the phytoconstituents of methanolic extract from *P. longifolia* and their drug-like effects against cancer targets using in silico approaches.

2. Methodology

2.1. Collection of Plant Sample

The bark of *P. longifolia* was collected from the campus of Assam University, Silchar (Latitude 24.686748°, Longitude 92.751486°), Cachar district of Southern Assam, Northeast India and submitted to Assam University Silchar Central Herbarium (AUSCH) for identification with Voucher number *M. Das 1* and Accession number *8645*.

2.2. Preparation of Plant Extract

Collected bark was washed thoroughly with water and dried at ambient temperature. It was then ground to fine powder using a mechanical grinder. A powdered bark sample (100 g) was used for the extraction using methanol (ME) [46]. The powdered sample was first soaked in methanol (ME) for 3 days and filtered. The extract was prepared after the filtrate's solvent was dried and lyophilized to remove any water content from the sample. The extract was then kept at ambient temperature for subsequent investigations.

2.3. Phytochemical Analysis

2.3.1. Quantitative Phytochemical Screening

For quantitative analysis, PL-ME was prepared at a concentration of 1 mg/mL in methanol as stock solutions for the following experiments in a set of triplicates.

Total Phenolic Content

The total phenolic content was assessed using a modified protocol of the originally reported procedure [47]. In brief, the extract was prepared at a concentration of 1 mg/mL in methanol. Gallic acid served as a standard; a 100 μL aliquot of each sample was combined with 500 μL of 10% Folin Ciocalteu solution and 400 μL of 7.5% sodium carbonate. Following incubation for 60 min in dark conditions, optical density (OD) was measured at 750 nm. The data were calculated as gallic acid equivalents (GAE/mg) of each plant extract.

Total Flavonoid Content

For the quantification of total flavonoids present in the extract, a slightly modified standard protocol from the method described in the literature [48]. Quercetin was used as a standard; 100 µL of plant extracts was mixed with 5% 30 µL $NaNO_3$, 30 µL 10% $AlCl_3$, 200 µL 1 mM NaOH and 640 µL distilled water and incubated in the dark for 30 min. Once the incubation period was over, the absorbance was checked at 415 nm and the data were quantified as quercetin equivalents (QE/mg) of the plant extract.

Determination of DPPH Free-Radical Scavenging Activity

Antioxidant (free-radical scavenging) properties of the plant sample was assessed by the DPPH free-radical scavenging assay, following the protocol mentioned in the literature [49]. Initially, five different concentrations were prepared from the stock solutions of each solvent, 300 µL of each of the diluted plant extract dissolved in 300 µL DPPH (4 mg in 50 mL methanol solvent) solution and then 1 mL volume was made using methanol. The mixture was left in a dark place for incubation for 15–30 min. The absorbance of the resulting mixture was measured at 517 nm. Ascorbic acid was used as a standard and the same procedure was followed for it. The percent inhibition was calculated using the following formula:

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

where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample. A concentration-response curve was used to figure out the IC_{50} value for each extract.

2.4. Metabolite Profiling

A thorough metabolite profiling of PL-ME extracts was performed using gas chromatography-mass spectrometry (GC-MS) to elucidate and identify bioactive compounds.

2.5. In Silico Analysis

2.5.1. Network Pharmacology of Target Identification

Target Prediction and PPI (Protein–Protein Interaction) Network Construction

Target identification for the phytochemicals was studied using the Swiss target prediction web-based tool (https://www.swisstargetprediction.ch/predict.php, accessed on 20 May 2025) using the SMILES of the compounds with "Homo sapiens" as the species model. The probability of the query compound having a protein as a target was considered >0.7% in the database which implied higher prediction reliabilty [50]. Protein-protein interaction (PPI) was analyzed using STRING v.12 (https://string-db.org/) using "Homo sapiens" as the species and a confidence score > 0.9. The output TSV file from the STRING software was built using evidence from many sources and loaded into Cytoscape v3.8.3 for further analysis [51]. The network was created and visualized by Cytoscape v3.8.3, a tool often used in network pharmacology investigations. The blueprint of the signaling pathway was observed using the KEGG pathway database (https://www.genome.jp/kegg/pathway.html, accessed on 17 May 2025), where the signaling pathways have been studied for the prominent targets, which led us to find out about the genes responsible for the regulation of cancer [52].

Clustering and Gene Network Analysis

The Molecular Complex Detection (MCODE) tool in Cytoscape v3.8.2 was used to identify highly interacting areas (clusters) in the huge gene network. The clusters are ranked using the MCODE score, which considers the number and distance between edges [51,53]. The whole network was chosen for cluster analysis with MCODE, with the following default parameters: degree cutoff at 2, node score cutoff at 0.2, k-score at 2, and maximum depth at 100.

2.5.2. Preparation of Targets

Analyzing the signaling pathways, two prominent targets, EGFR (PDB ID: 4LQM), and TGFβRI (PDB ID:5E8U), were determined to be responsible for the upregulation of various cancers were selected. The PDB ID and the 3D structures of the selected targets were taken from the RCSB Protein Data Bank (www.rcsb.org/pdb; accessed on June 2025) database and utilized as drug targets, which were again verified from the respective literature [54,55].

2.5.3. Preparation of Ligands

The phytoconstituents of *P. longifolia* revealed by GC-MS analysis, along with those previously documented from different solvent-based extracts of this plant, were considered collectively as ligands for this study. The smiles codes of these compounds were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/; accessed on May 2025) and 3D conformers of the compounds were downloaded in SDF format. Minimization of the 3D conformers was done using the Chimera software (version 1.19) and was converted into SDF format using Discovery Studio Software (2025 Client). The energy minimization step is important before molecular docking to facilitate accurate binding interactions of ligands to the target protein.

2.5.4. Molecular Docking

The Molegro Virtual Docker (MVD) version 6.0 software was used for the docking of ligands in the active sites of the target protein [56]. Molecular docking is a computer-based approach that helps determine how well the ligand fits into the active site of the protein, predicting both the strength of binding and the way the ligand is positioned. 3D protein structure of the targets was loaded in the MVD 6.0 interface and co-factors, water molecule, and attached molecules were removed from the structure. 3D SDF files of all the ligands, along with the considered positive control, were loaded. On the determination of active sites of each protein target, the dimension grid for EGFR (x: −56.08, y: −3.82, z: −22.47) and TGFβRI (x: 14.71, y: 3.07, z: 10.43) was set to 0.3. The software optimizes the necessary H-bond required for docking and the default run for the process was set to be 10 for each compound. Post-docking analysis requires the analysis of the binding score (MolDock score) of ligands with the target against the positive control considered for the study.

2.5.5. Prediction of ADME Profile and Drug-Likeness

SwissADME web server (https://www.swissadme.ch/) was used for ADME (absorption, distribution, metabolism, excretion) analysis [57]. Using the compound's canonical smiles, the server generated data including physicochemical properties, lipophilicity, solubility, pharmacokinetics, medicinal chemistry, and drug-likeness. *ADME* analysis provides us with the traits for the compounds and identifies whether the compound is feasible as a drug, and provides its drug likeness properties.

3. Results

3.1. Phytochemical Analysis of P. longifolia Extracts

Methanolic extract of *P. longifolia* was found to contain phenolic and flavonoid contents. TPC of PL-ME extracts was found to be 363.7 ± 5 mg GAE/g, which was calculated using the regression equation y = 0.0007x + 0.1141, R2 = 0.971 obtained from the gallic acid standard curve equation. The TFC of PL-ME was measured to be 300.12 ± 5 mg QE/g weight of the bark of *P. longifolia* plant, calculated using the regression equation obtained from the quercetin standard curve, y = 0.0008x + 0.2089, R2 = 0.9932. The presence of phenolic and flavonoid content signifies the antioxidant capacity of this plant. We then measured the antioxidant activity of the extract using the DPPH assay. Impressively, we found that the methanolic extract showed the scavenging potential with a minimal IC_{50} value of (348.1 ± 0.01 µg/mL) in comparison with the standard ascorbic acid with an IC_{50} value of (714.5 ± 0.01 µg/mL). The results of preliminary phytochemical analysis of the extract have been provided in Supplementary Table S1.

3.2. Metabolite Profiling

Based on the studies and obtained results from phytochemical screening, the methanolic extract of *P. longifolia* (PL-ME) was further analyzed using GC-MS techniques. The compounds detected from the methanolic extracts in the GC-MS analyses are listed in the supplementary file with the chromatogram (Supplementary Table S2, Figure 1). The compounds previously reported from this plant of the acetone and methanolic extracts were also selected along with the PL-ME extract for the In-silico study (Lists are provided in Supplementary Table S3).

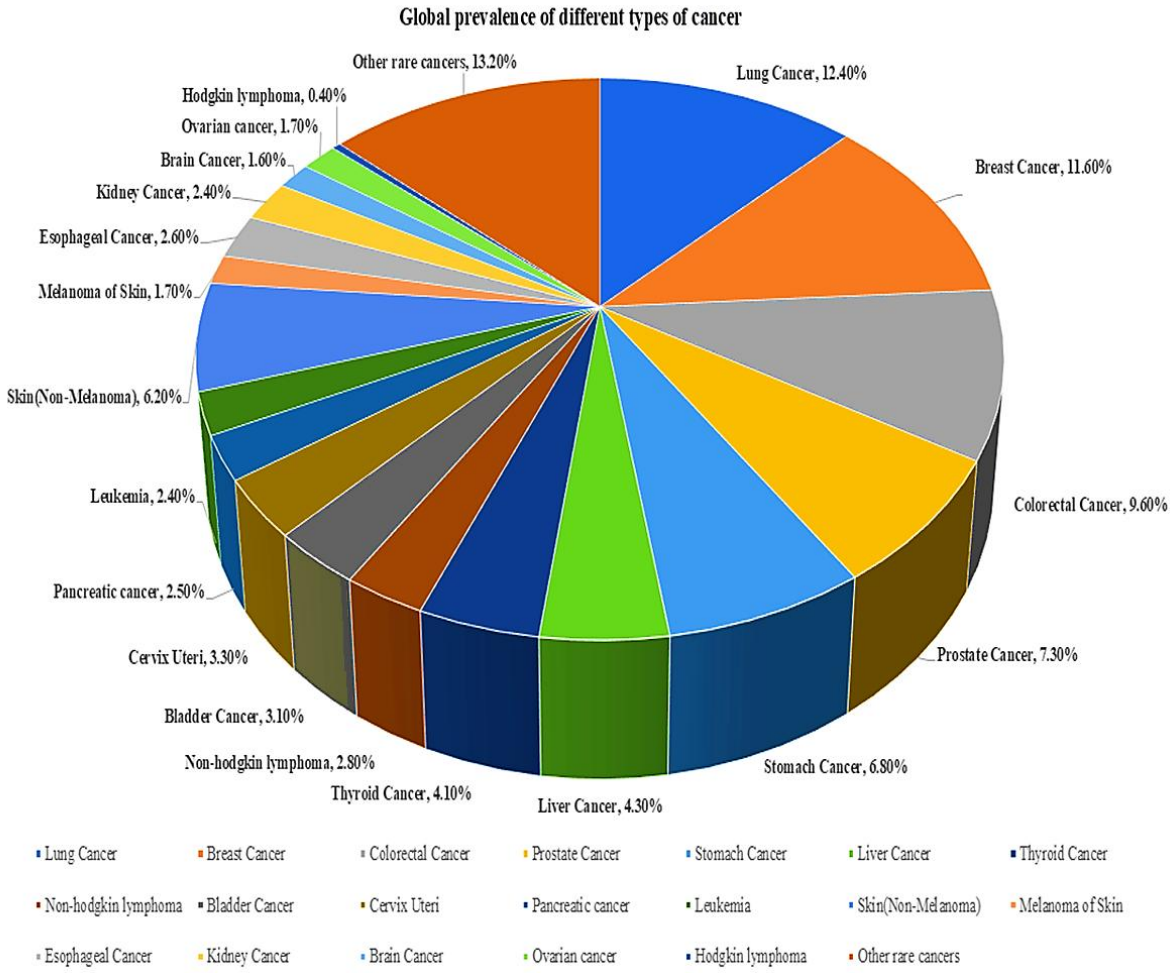

Figure 1. Percentage of different types of cancer around the globe.

3.3. In Silico Investigation

3.3.1. Networking and Target Identification

All selected compounds were uploaded to the SWISS Target Prediction web tool using SMILES in succession to identify the relevant targets. After removing the duplicates, a total of 308 targets were identified and PPI network analysis was conducted using STRING (The list of 308 genes and PPI network is provided in the Supplementary Table S4 and Figure 2, respectively). Using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database and literature reports 24 prominent genes were analyzed. The clustering of these 24 targets was made using MCODE of Cytoscape. The PPI network and gene cluster have been provided in Figure 2. From these 24 targets, EGFR and TGF-β are the two most prominent proteins responsible for the development and progression of various cancers and have been considered as study targets in the present study for analyzing the bioactivity of these compounds. Table 1 represents the identified common 24 targets, and Figure 2 presents the networking of 24 genes with clustering of the key targets of major cancers.

The two most prominent targets selected for this study were EGFR (PDB ID: 4LQM) and TGFβR1 (PDB ID: 5E8U), which were found to be responsible for cell proliferation and selected based on the reported literature and KEGG pathway analysis of different cancers. An illustration of the signaling pathways of EGFR & TGFβR1 in cancer is shown in Figure 3.

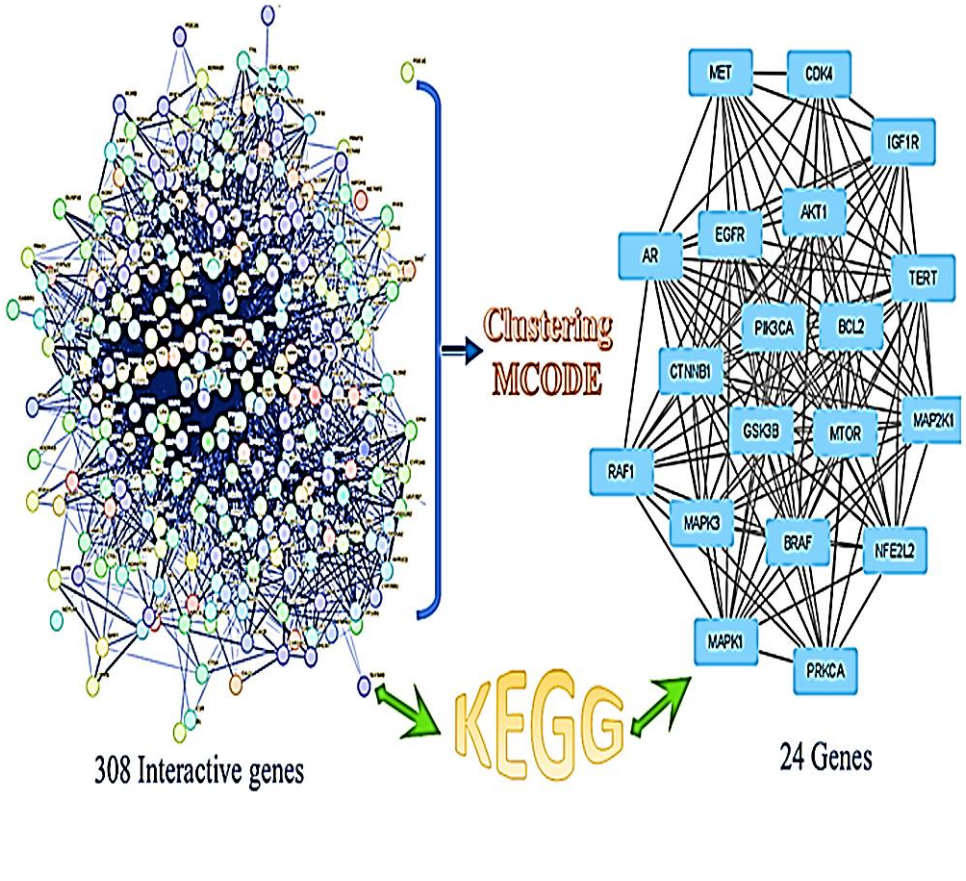

Figure 2. PPI Network analysis and showing prominent genes using different associations of cancers.

Table 1. List of target genes identified from the PPI network.

Sl. No.	Identified Targets
1	PIK3CB
2	PIK3CA
3	NFE2L2
4	EGFR
5	MAPK1
6	CDK4
7	GSK3B
8	MAP2K1
9	AR
10	PRKCA
11	CTNNB1
12	AKT1
13	BRAF
14	MET
15	RAF1
16	BCL2
17	HMOX1
18	TERT
19	MAPK3
20	IGF1R
21	MTOR
22	PIK3CD
23	PRKCG
24	TGFβR1

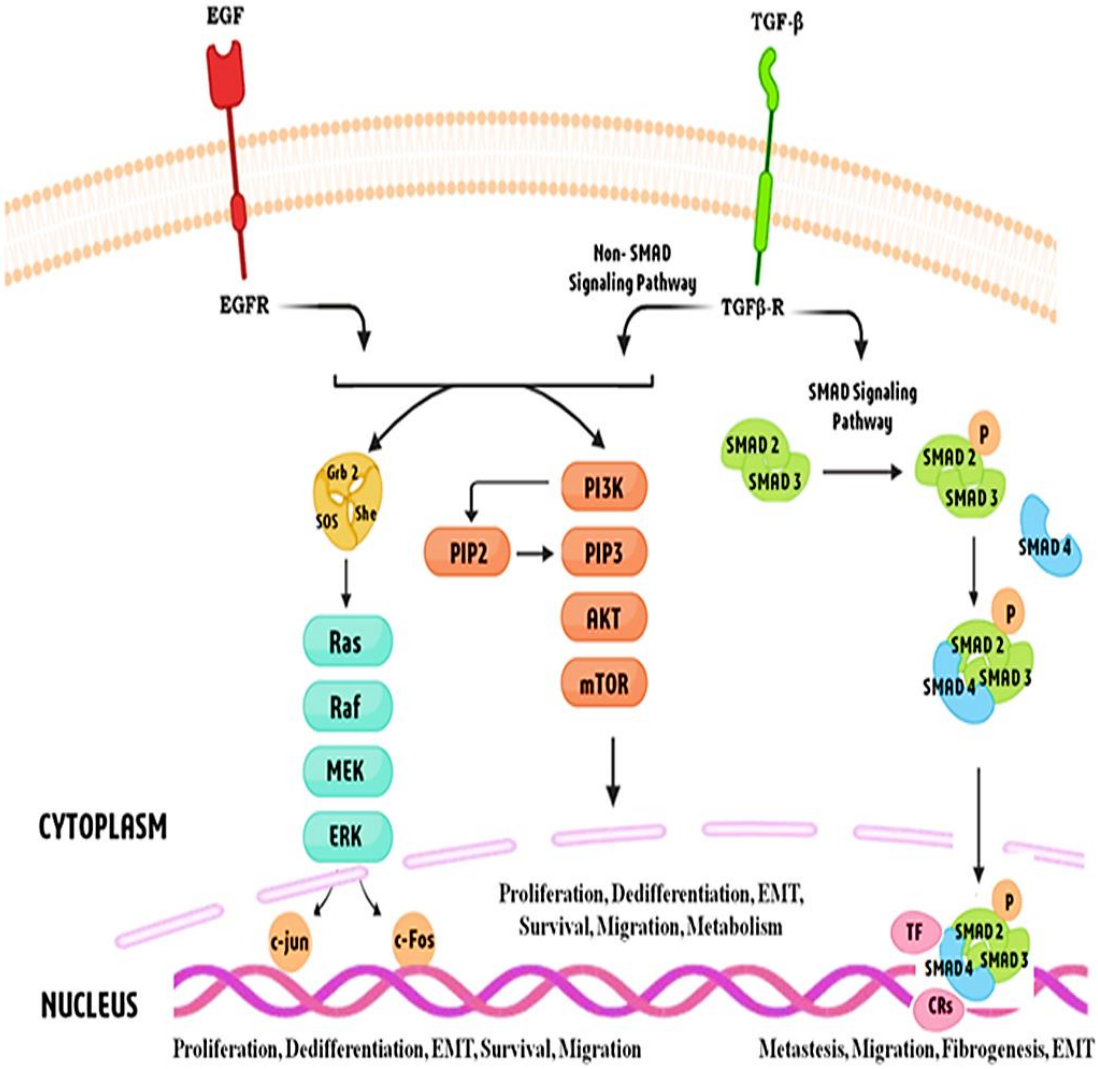

Figure 3. Illustration of the signaling pathways of the selected targets EGFR and TGFβRI, Ras/Raf/MEK/ERK and the PI3K/AKT/mTOR cascades, mainly involved in transmission of signals to the cell nucleus, which ultimately influence the transcription of specific genes.

3.3.2. Docking Score and Inhibition of Receptors

Among the two chosen targets, multiple compounds identified displayed superior binding strength relative to the positive controls. MolDock of the best docked compounds surpassed the considered positive control, which is listed in Table 2A,B. In the case of TGFβRI mutant protein, LY-2109761 was used as a positive control, whose MolDock score was −163.67 and the prominent compounds with a MolDock Score surpassing the positive control are listed in Table 2B. Both targets are involved in cancer proliferation and thus binding with these targets may significantly reduce the occurrence of the diseases. Docking pose of the most prominent compounds rescinnamine, irinotecan & silandrin for the targets EGFR and the prominent compound rescinnamine, Lindleyin & Pheophorbide for the target TGFβRI, based on *ADME* analysis, along with their interactions, are shown in Figures 4 and 5. Structures of these compounds were drawn using ChemSketch (Figure 6). From the result we found that rescinnamine has the strongest binding affinity and appears to be the best in both targets, having a more negative mol. dock score compared with the respective positive control. Bonded residues of the most prominent compounds, rescinnamine, with both the selected targets, the amino acids and their interactions with the respective compounds are presented in Table 3A,B.

Table 2. (A): Comparative account of docking scores of the Top 3 ligands against the target EGFR, along with the positive control. (B): Comparative account of docking scores of the Top 3 ligands against the target TGF-βR1, along with the positive control

(A)				
Target	**Positive Control**			
EGFR	Name	MolDock Score	Rerank score	Hbond
	Gefitinib (Positive Control EGFR)	−121.915	−100.622	−4.687
	Ligand			
	Rescinnamine	−149.315	−125.011	−1.57
	Irinotecan	−139.31	−107.008	−5.69
	Silandrin	−135.844	−112.535	−3.68
(B)				
Target	**Positive Control**			
TGF−βR1	Name	MolDock Score	Rerank score	Hbond
	LY−2109761 (Positive Control TGF−βR1)	−163.67	−109.904	−1.57813
	Ligand			
	Rescinnamine	−170.489	−126.032	−4.56111
	Lindleyin	−169.529	−142.884	−13.9486
	Pheophorbide	−179.976	−131.159	−4.96138

Table 3. (A): Bonded residue of the best docked compounds with target EGFR. (B): Bonded residue of the best docked compounds with target TGFβ R1.

(A)			
	Bonded Residues with Target EGFR		
Compound Name	**Carbon H-Bonds**	**Conventional H-Bonds**	**Van der Waals Interactions**
Rescinnamine	Ile 759	Glu 758	Asp 761
	Phe 723	Lys 745	Lys 757
	Arg 841		Glu 762
			Ala 755
			Leu 747
			Cys 797
			Asn 842
			Thr 854
			Lys 857
			Arg 836
			Ala 859
			Lys 860
(B)			
	Bonded Residues with Target TGFβ R1		
Compound Name	**Carbon H- Bonds**	**Conventional H-Bonds**	**Van der Waals Interactions**
Rescinnamine	Asp 290	Lys 232	Leu 352
	Ile 211	His 283	Val 231
	Glu 284		Ser 200
	Tyr 282		Ala 230
	Asp 333		Ser 287
			Gly 286
			Arg 294
			Asp 281
			Leu 340
			Gly 212
			Lys 337
			Asn 330
			Lys 335
			Thr 375
			Lys 213
			Gly214
			Val 219
			Thr 249

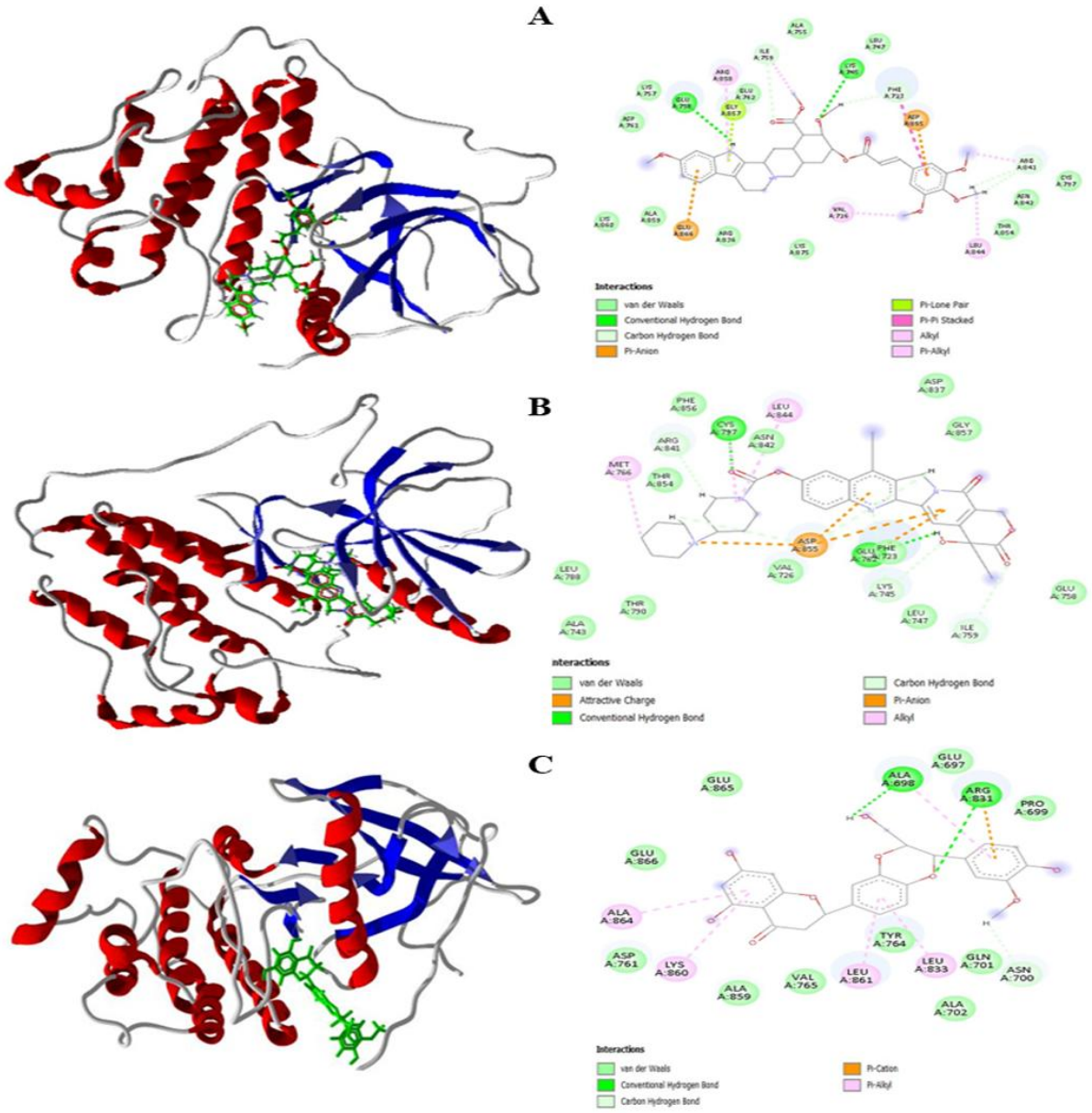

Figure 4. Docking Pose of compound along with 2D Interaction with target EGFR (**A**) rescinnamine, (**B**) irinotecan, (**C**) silandrin. The respective colored lines, as indicated, represent H-bonds and other interactions with the compounds.

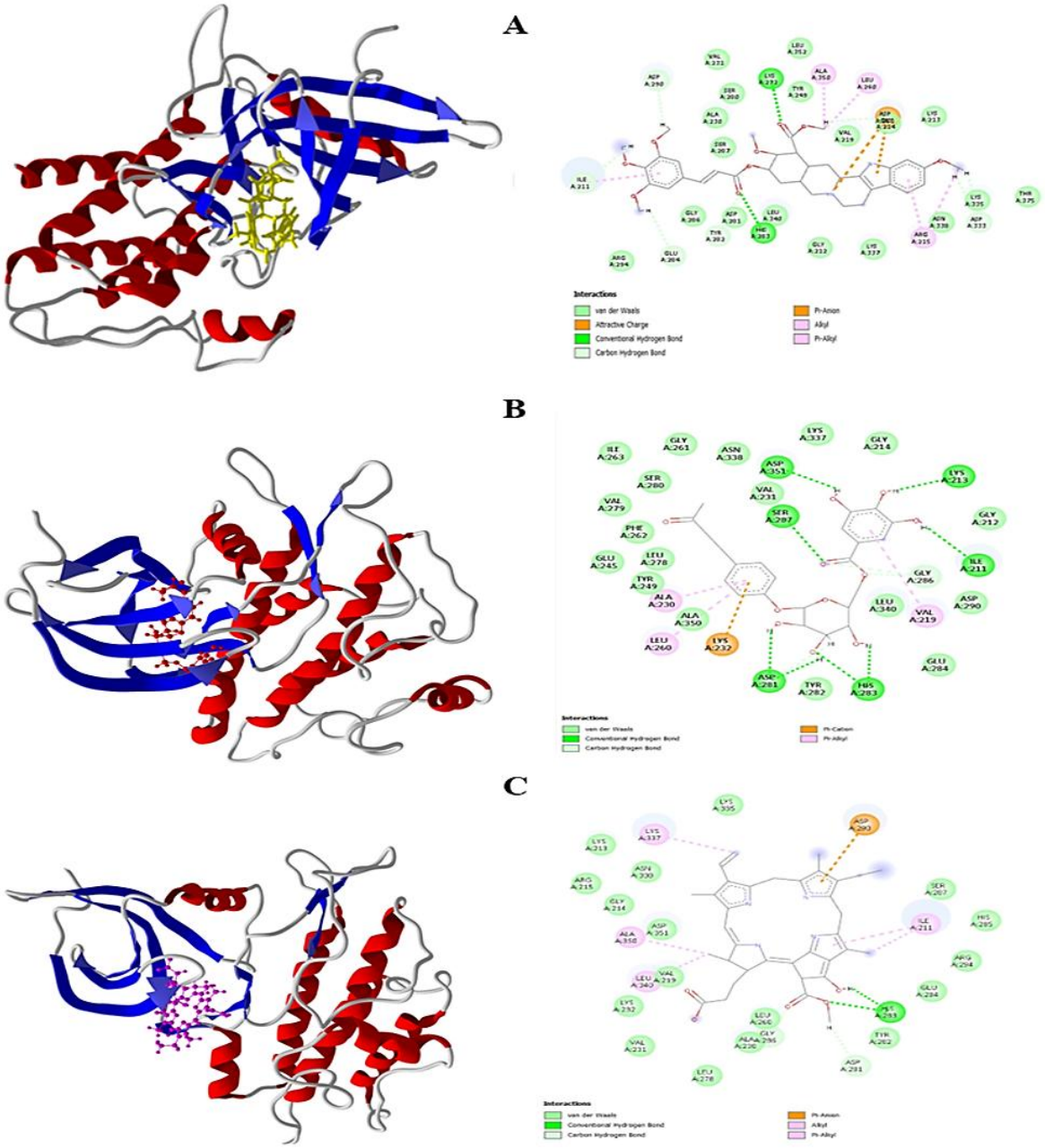

Figure 5. Docking Pose of compound along with 2D Interaction with target TGF-βR1 (**A**) rescinnamine. (**B**) Lindleyin, (**C**) Pheophorbide. The respective colored lines, as indicated, represent H-bonds and other interactions with the compound.

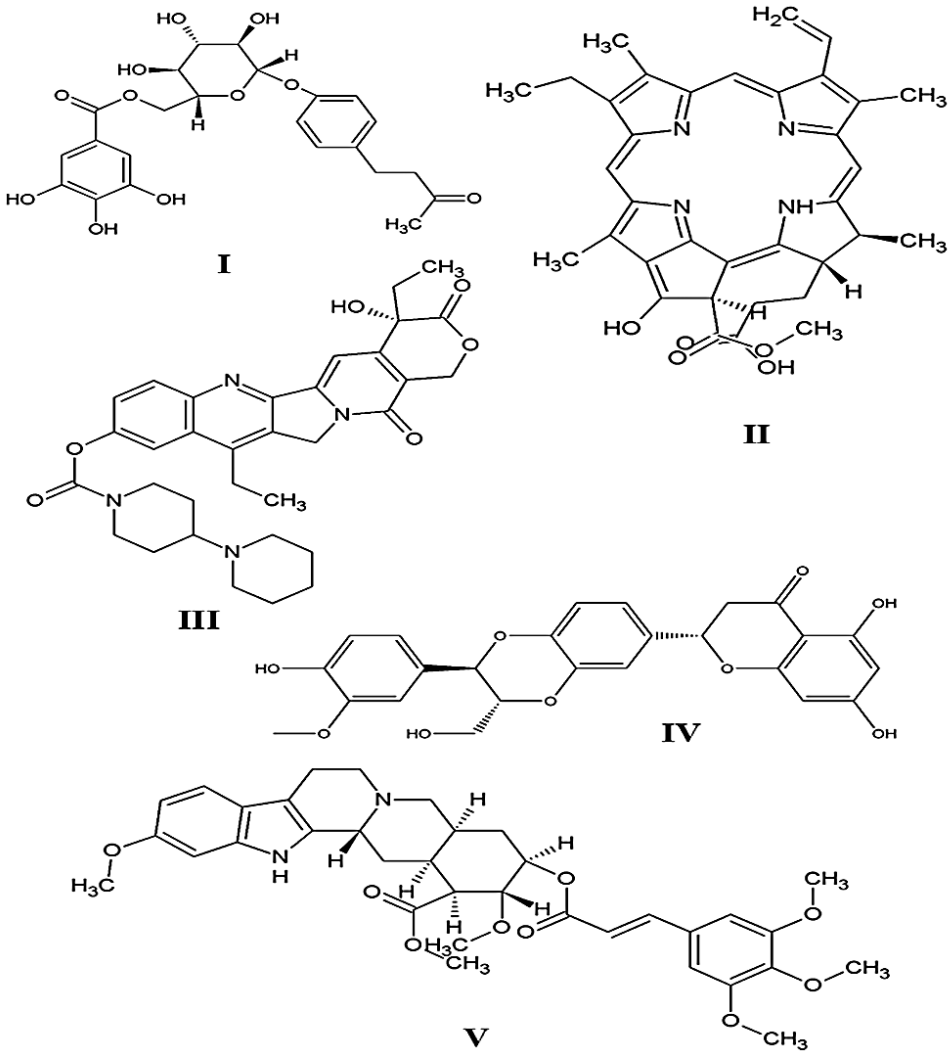

Figure 6. Chemical structure of the most prominent compounds, where (**I**) Lindleyin, (**II**) Pheophorbide a, (**III**) irinotecan, (**IV**) silandrin. (**V**) rescinnamine.

3.3.3. ADME Profile Analysis

Based on their physicochemical and pharmacokinetic activities, the evaluated compounds exhibit potential drug-like properties. The analysis was conducted through the widely accepted criteria, including Lipinski's Rule of Five, solubility, gastrointestinal (GI) absorption, bioavailability score, and safety alerts. All the compounds evaluated showed a significant bioavailability score, suggesting significant potential for oral bioavailability, and each GI absorption was identified, which is a preferable trait for oral drugs. Among the several compounds Lindleyin, Pheophorbide a, irinotecan, silandrin and rescinnamine showed high gastrointestinal (GI) absorption, indicating a desirable trait for oral drugs as shown. However, other prominent compounds exhibited significant results in all aspects with minimal Lipinski violations. The detailed pharmacokinetics and drug-likeness scores of the evaluated compounds for both targets, EGFR and TGFβR1 were listed in Table 4. Thus, these evaluated compounds profile with a significant bioavailability score and minimal rule violations marks as favorable compounds for further investigation as potent therapeutic agents.

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

Molecule	Molecular Weight	H-Bond Acceptor	H-Bond Donor	Solubility	GI Absorption	BBB Permeant	Pgp Substrate	Lipinski Violation
Irinotecan	586.68	8	1	Moderately soluble	High	No	Yes	1
Rescinnamine	634.72	10	1	Poorly soluble	High	No	Yes	2
Silandrin	466.44	9	4	Moderately soluble	High	No	No	0
Lindleyin	478.45	11	6	Soluble	Low	No	Yes	2
Pheophorbide a	592.68	8	3	Moderately soluble	Low	No	Yes	1

4. Discussion

The present study highlights about the therapeutic potential of *P. longifolia* through molecular docking and ADME profiling of compounds against EGFR and TGFβRI, two key targets that influence various signaling pathways in cancerous cells. The docked compounds, which outperformed the reference ligands, with Pheophorbide a, Irinotecan, Rescinnamine, Silandrin, and Lindleyin emerged as the most promising candidates due to favorable docking scores and high gastrointestinal absorption. Irinotecan (CPT-11) has been developed as a semi-synthetic derivative of plant alkaloid camptothecin with improved water-solubility and reduced toxicity [58]. It acts as DNA Topoisomerase I inhibitor and has been clinically approved as prodrug for treating colorectal, ovarian, and lung cancers [59]. Also, Irinotecan has been identified in the acetone extract of *P. longifolia* using liquid chromatography-mass spectrometric technique [56]. In a reported study, Pheophorbide a, which is a photosensitizer phytochemical, has shown anticancer activity along with the ability to overcome MDR (multidrug resistance) in conjunction to chemotherapy drugs, regardless of PTD (photodynamic therapy) [60]. Rescinnamine is a pharmacologically active alkaloid isolated from *Rauwolfia serpentina* and it has been reported to exhibit antipsychotic, anticancer and antihypertensive activities [61,62]. Silandrin and its diastereomers have been isolated and structurally elucidated from the fruits of white- flowered variant *Silybum marianum* L. [63] while Lindleyin has been isolated from *Rhei rhoma* as a major phytoestrogen component, contributing to the estrogenic property through estrogen receptor (Erα & Erβ) [64]. As it can be observed plant plant-derived compounds have essential pharmacologically active properties with low systemic toxicity and the ability to modulate target-based signaling networks, tumor microenvironment or drug efflux pumps [65,66]. Phytochemicals offer an alternative approach by overcoming resistance to conventional drugs and could complement existing approaches for treating cancer. In the present study, the presence of high phenolic, flavonoid content and maximum free radical scavenging activity with the lowest IC50 has been observed in the methanolic extract of *P. longifolia*. Interestingly, the acetone extract of this plant also exhibited a potent ability to neutralize free radicals in comparison to the standard reference taken, suggesting its potential antioxidant ability. Similar antioxidant efficiency with a minimum IC_{50} value of different extracts of *P. longifolia* has been observed in reported studies [14,15,67]. EGFR and TGF-β proteins have been selected through networking and clustering of genes that play a pivotal role in invasion and progression of cancer, as shown in Figure 2. Table 1 represents the list of common 24 genes identified from phytochemicals-based predicted targets and cancer-promoting signaling pathways. A similar study for the identification of targets from the phytochemicals for non-small cell lung cancer has been reported [56]. EGFR signalling activates Raf/MEK/ERK and PI3K/AKT/mTOR pathways, influencing the [68]. In this study, molecular docking revealed 3 compounds with better binding to EGFR than Gefitinib shown in Table 3A. Docking pose of these compounds with EGFR and 2D interaction of these compounds with the target are provided in Figure 4. A study reported about molecular docking analysis and molecular dynamics simulation (MDS) on *Dalbergia sissoo* plant parts revealed bioactive metabolites, namely Prunetin, Prunetin 4′-O-Galactoside and Tectorigenin, with better binding affinity to EGFR protein as anticancer agents [69]. Again, another study revealed Berberine, Emodin and Coptisine with promising binding affinity to EGFR kinase domain with binding energies ranging from −1.96 kcal/mol to −7.11 kcal/mol than FDA-approved reference drug Erlotinib with −8.5 kcal binding energy, suggesting these phytochemicals as potent alternatives as anticancer inhibitors to Erlotinib [70]. Molecular docking with TGFβR1 revealed three compounds viz., Pheophorbide a, Lindleyin & Rescinnamine, with a higher docking score than LY-2109761 in this study and their 2D interactions have been shown in Figure 5. A similar report with screening of SB202190, silymarin, SB203580 & cryptotanshinone showed good binding affinities to TGFβR1 using MD, DFT (density functional therapy) & MD simulation and they were recommended as TGFβR1 inhibitors for targeted drug development against prostate cancer [71]. However, a notable study has reported that the influence of EGFR expression on TGF-β regulation has been seen in many types of carcinomas. EGFR downregulation triggers TGF-β, which promotes more pro-migratory and invasive properties in cancerous cells. The balance of EGF/TGF-β synergistic effect is essential to regulate cellular homeostasis and their positive correlation could be observed in aberrant migration and local spread of cancerous cells. Canonical Smad3 and ERK/Sp1 signaling of TGF-β induced EGFR upregulation, promoted the aggressiveness and migration in breast cancer cells [72]. In a reported study, TGF-β has been observed to upregulate the expression of RHOC/CDC42, promoting actomyosin contractibility in EGFR-silenced cells [73]. Of note, the report highlighted the tumor-suppressing function of EGFR counterbalancing the actions of TGF-β-induced epithelial-amoeboid alterations in HCC [73]. Another study investigated the potential of diabetes and the prescribed drug Metformin, predicted to inhibit TGFβR1 kinase protein of cancer cells [74]. Using pharmacophore mapping, MD, ADMET and MDS, three new lead compounds viz., CID 72473, 10316977 and 45140078 revealed metformin–like properties with efficient binding energies with TGFβR1 and has been predicted to act as anti-cancer agents [74]. In this study, ADME analysis indicated good GI

absorption, no BBB interference, with limited violations reinforcing pharmacological relevance of the five compounds. Rescinnamine exhibited consistent activity against both targets, suggesting its value as a potent lead molecule. Nevertheless, the identification of Irinotecan and Rescinnamine as P-gp substrates indicates possible efflux-mediated reduction in intracellular concentration, which could compromise therapeutic efficacy and warrants further optimization. Network pharmacology, MD & ADME analysis thus revealed five compounds viz., Pheophorbide a, Irinotecan, Rescinnamine, Lindleyin and Silandrin identified from different extracts of *P. longifolia* with good GI absorption, pharmacokinetic properties and solubility, predicting them as lead compounds for anticancer activity, and as EGFR and TGF-β inhibitors. Collectively, this work provides a mechanistic insight that bridges traditional claims with computational evidence, while emphasizing the need for in vitro and in vivo validation to confirm the translational potential of these compounds as anticancer agents. The pharmacokinetic analysis suggested optimal gastrointestinal absorption and acceptable solubility for the studied compounds, alongside minimal BBB penetration and limited violations, thereby reinforcing their therapeutic relevance. Among them, rescinnamine showed reliable dual-target activity, underscoring its suitability as a lead structure. On the other hand, the designation of irinotecan and rescinnamine as substrates of P-glycoprotein may pose challenges related to efflux and reduced intracellular concentration, indicating the need for chemical or formulation-based improvements. Through the integration of ADME screening, molecular dynamics, and network pharmacology approaches, five bioactive were recognized from *P. longifolia* extracts with strong pharmacokinetic promise and predicted anticancer potential via EGFR and TGF-β inhibition. Where Rescinnamine appears to be the most suitable and potentially bioactive compound from the methanolic extract of this plant. Pharmacokinetic and physicochemical evaluation further indicated that these bioactive molecules possess favorable drug-like properties, suggesting their potential as leads for novel therapeutic agents against cancer. Altogether, present work bridges ethnomedicinal relevance with computational validation, while emphasizing the need for in vitro and in vivo validation to confirm the translational potential of these compounds for HCC therapy.

5. Conclusions

The present study highlights the therapeutic promise of *P. longifolia* (Willd.) K. Schum, where five key phytocompounds such as Lindleyin, Pheophorbide a, irinotecan, silandrin and rescinnamine demonstrated remarkable interactions with critical targets. These phytocompounds, such as Lindleyin, Pheophorbide a, irinotecan, silandrin and rescinnamine emerging as the most promising docking results with respect to their positive control against targets. In-silico docking revealed their strong binding affinities toward EGFR and TGFβRI, two major regulators implicated in cancer progression. The favorable MolDock scores, along with supportive physicochemical and pharmacokinetic profiles, further reinforce their potential as effective modulators of these targets. Rescinnamine appears to be the most suitable and potentially bioactive compound from the methanolic extract of this plant. Pharmacokinetic and physicochemical evaluation further indicated that these bioactive molecules possess favorable drug-like properties, suggesting their potential as leads for novel therapeutic agents against cancer. The findings emphasize the importance of *P. longifolia* as a valuable source of anticancer metabolites. These findings provide initial evidence that *P. longifolia* contains bioactive molecules capable of influencing pathways relevant to cancer. Continued investigation and validation of these compounds could lead to the identification of new lead molecules for drug development. Overall, the study emphasizes *P. longifolia* as a promising natural source of phytochemicals for future cancer therapies.

Supplementary Material

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/2510231041562797/NPA-25090022-SI-for-online.pdf. Table S1. Quantitative analysis of methanolic extract of *P. longifolia* Bark (PL-ME). Table S2. Compounds detected from the PL-ME extract of bark of *P. longifolia* through GC-MS analysis. Table S3. (A): List of GC-MS reported methanolic extract compounds of *P. longifolia*. (B): List of LC-MS reported methanolic extract compounds of *P. longifolia*. List of LC-MS reported acetone extract compounds of *P. longifolia.* Table S4. List of 308 target genes identified from metabolites of *P. longifolia* using the STRING database. Figure S1. GC-MS chromatogram of PL-ME. Figure S2. PPI Network image of 308 common targets.

Author Contributions

P.N. & B.N.D.: Data curation, data analysis, writing- original draft & preparation. T.A., D.N., M.D. & L.N.: Writing—reviewing and editing, S.K. & A.D.T.: Writing—original draft, methodology, software, Formal Analysis, Writing—reviewing and editing, Conceptualization, visualization, methodology, investigation, supervision,

software, writing—reviewing and final editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data that support the findings of this study are available in the supplementary material of this article

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

Abbreviation

ADME	Absorption, Distribution, Metabolism, Excretion
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
GC-MS	Gas Chromatography-Mass Spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
PL-ME	*Pajanelia longofolia* Methanol
MVD	Molegro Virtual Docker
MD	Molecular Docking
MDS	Molecular Dynamics Simulation
NAFLD	Non-alcoholic Fatty Liver Disease
TBW	Total Body Weight
TGFβ	Transforming Growth Factor Beta
TGFβRI	Transforming Growth Factor Beta Receptor 1
VS	Virtual Screening

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