Journal of Young Pharmacists, 2026; 18(2):457-466.
https://www.jyoungpharm.org

Original Article

In silico Evaluation of *Pergularia daemia* Steroidal Lactone Phytochemicals as Potential Multi-Target Modulators of PIK3CA and STAT3 Signaling Pathways in Pancreatic Cancer

Santhiya Gopalsamy*, Muthukumaran Mylasalam, Pughazendhi Ravi, Gowrishankar Jayabalan

School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal, Puducherry, INDIA.

ABSTRACT

Background: Pancreatic cancer remains a lethal disease, largely because of its aggressive biology and scant therapeutic options. Central to its progression are dysregulated oncogenic pathways-most notably PI3K/AKT/mTOR and STAT3, which drive tumor growth, survival, and resistance to therapy. Natural products, with their ability to target multiple nodes in these networks, offer a promising source of novel anticancer agents. **Objectives:** Here we investigated a set of steroidal lactone phytochemicals isolated from *Pergularia daemia* for their potential interactions with key pancreatic cancer associated targets. **Materials and Methods:** Five compounds-Calotropin, Calactin, Calotropagenin, Coroglaucinin, and Corotoxigenin-were screened using an integrative network-pharmacology pipeline. SwissTargetPrediction identified 45 putative targets, and pathway enrichment (KEGG and GO) highlighted a strong signal for the pancreatic cancer pathway (9 genes, fold enrichment ≈ 150, -log10 FDR ≈ 12). The PI3K/AKT/mTOR axis emerged as the most enriched network. Drug-likeness and pharmacokinetic predictions (Swiss ADME) confirmed favorable ADME profiles, with high gastrointestinal absorption and low predicted toxicity. Molecular docking against six pancreatic-cancer-associated proteins (PIK3CA, STAT3, AKT1, EGFR, mTOR, BRAF) using SwissDock revealed favorable predicted binding affinities (-4.84 to -8.87 kcal mol^{-1}). **Results:** Calotropagenin and Coroglaucinin showed the strongest interactions with PIK3CA (-8.87 and -8.81 kcal mol^{-1}, respectively), while Calactin and Calotropin showed favorable docking interactions with STAT3 (-7.65 and -7.44 kcal mol^{-1}). **Conclusion:** Collectively, these findings suggest that steroidal lactones from *Pergularia daemia* may exhibit potential multi-target interactions with pancreatic cancer-associated proteins particularly within the PI3K/AKT/mTOR and STAT3 signaling pathways. However, these findings are based on computational predictions and require further experimental validation.

Keywords: *In silico* study, Molecular docking, Network pharmacology, Pancreatic cancer, *Pergularia daemia*, Phytochemicals, PIK3CA, STAT3.

Correspondence:
Dr. Santhiya Gopalsamy
Assistant Professor, School of Pharmacy, SBV Karaikal Campus, Sri Balaji Vidyapeeth (Deemed to be University), Karaikal-609609, Puducherry, INDIA.
Email: santhiyagopalsamy31@gmail.com
ORCID: 0009-0008-9370-917X

Received: 24-12-2025;
Revised: 07-01-2026;
Accepted: 16-04-2026.

INTRODUCTION

Pancreatic cancer is also among the deadliest cancers in the world with delayed diagnosis, advanced progression, and ineffectiveness of therapy (Bray *et al.*, 2018). Although the surgical and chemotherapeutic approaches have improved, the five-year survival rate has been appallingly low, and new therapeutic approaches are highly needed (Siegel *et al.*, 2020). The involvement of oncogenic signalling cascades including PI3K/AKT/mTOR and STAT3 through their dysregulation has been strongly implicated in the growth, metastasis, angiogenesis, and resistance to anti-apoptotic in pancreatic tumour (Fresno *et al.*, 2004; Yu *et al.*, 2014).

PIK3CA, which is the gene encoding one of the catalytic subunits of phosphoinositide 3-kinase, is central to cellular survival and proliferation. STAT3, at the same time, is a transcription factor, which facilitates tumour progression and evasion of immunity (Yu *et al.*, 2014). Simultaneously targeting these pathways is thus considered as an encouraging approach to improve the treatment outcome in pancreatic cancer. PIK3CA alternations have been reported in a subset of pancreatic cancer cases although the reported frequency varies across studies (Mehra *et al.*, 2021; Mortazavi *et al.*, 2022; Hind *et al.*, 2024). Similarly, the constitutive activation of STAT3 through cytokine signalling especially IL-6, also plays a role in tumour progression, immune evasion and therapeutic resistance (Hamel *et al.*, 2024).

Natural products have a long history of involvement in the discovery of oncology drugs due to their structural diversity

DOI: 10.5530/jyp.20260040

and multi-target action. *Pergularia daemia* is a medicinal herb rich in steroidal lactones and cardiac glycoside-like compounds which have been reported to display a range of pharmacological activity which includes anticancer potential. Major cardenolides that include calotropin, calactin, calotropagenin, corotoxigenin and coroglaucinin are found in the plant and have been shown to display cytotoxic activity in different types of cancer cell lines (Pergolizzi *et al.*, 2024). However, the molecular pathways that mediate their action against pancreatic cancer-related targets have not been properly investigated.

Network pharmacology, which is a new field of research incorporating systems biology, polypharmacology and computational methods, offers a broad platform to examine the sophisticated interactions between natural compounds and the biology system (Fakih *et al.*, 2025). This method allows the co-identification of several targets and pathways which is especially relevant to natural products, which often present polypharmacological effects (Gopinath *et al.*, 2025). Molecular docking, a computational technology projecting the favoured position of a ligand on binding a goal protein, provides information of the atomic-mechanism of drug-target interactions.

As such, the current study is based on a combined network pharmacology and molecular docking approach used to determine the binding affinity and activity of selected *Pergularia daemia* phytochemicals on major pancreatic cancer-related molecular targets. The overall aim of this study was to computationally evaluate the potential interactions of selected *Pergularia daemia* phytochemicals with key pancreatic cancer associated proteins particularly within the PI3K/AKT/mTOR and STAT3 signaling.

Although network pharmacology and molecular docking approaches are increasingly used in phytochemical research only limited studies have explored steroidal lactones from *Pergularia daemia* against important pancreatic cancer associated targets such as PIK3CA and STAT3. In particular the possible involvement of these phytochemicals in the modulation of PI3K/AKT/mTOR and STAT3 signaling pathways remains insufficiently investigated. Therefore, the present study was designed to integrate network pharmacology, molecular docking, and ADMET prediction to evaluate the potential interactions of selected *Pergularia daemia* phytochemicals with major pancreatic cancer-related proteins.

MATERIALS AND METHODS

Phytochemical selection

The current study has chosen five steroidal lactone phytochemicals of *Pergularia daemia* based on the reported relevance in anticancer in relation to their structures and structural diversity. These compounds were selected in order to analyse them: Calotropin, Calactin, Calotropagenin, Coroglaucinin and Corotoxigenin (Chen *et al.*, 2021).

All ligands (two-dimensional and three-dimensional structures) were obtained and converted to SDF format, after which the PubChem database was searched to retrieve all of them in PDB format by using Open Babel (Campos *et al.*, 2025).

Network Pharmacology Analysis

An analysis of network pharmacology was done to determine the possible proteins targets of the phytochemicals that were chosen. The Swiss Target Prediction web server (Daina *et al.*, 2017) was utilized to forecast on the most likely macromolecular targets of each compound. Swiss Target Prediction uses a mixture of 2D and 3D similarity measures to predict protein associations. The targets were further analyzed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) to enrich KEGG pathways, Gene Ontology Biological Processes (GO-BP), Gene Ontology Cellular Components (GO-CC), and Gene Ontology Molecular Functions (GO-MF) as predicted targets were passed through the database (Grosdidier *et al.*, 2011).

KEGG and GO enrichment analyses were performed using DAVID Bioinformatics Resources (version 6.8) with Homo sapiens selected as the background organism. Pathways and GO terms with $p<0.05$ and Benjamini -Hochberg adjusted False Discovery Rate (FDR)<0.05 were considered statistically significant.

The interaction between proteins was plotted using the STRING database to create protein-protein interaction networks among the proteins of interest identified. Protein-protein interaction analysis was performed using STRING database with a minimum required interaction score of 0.4 (medium confidence). To describe the interactions between the phytochemicals and their predicted targets, the compound-target network was created with the help of Cytoscape. These networks were interrogated to determine core targets and important signalling pathways that are involved in pancreatic cancer.

Target Protein Selection

Upon the network pharmacology analysis and a thorough literature review, six proteins that are related to pancreatic cancer were chosen to be included into the molecular docking studies:

- Phosphatidylinositol-4, 5-bisphosphate 3 -kinase catalytic subunit alpha (PIK3CA): a key regulator of the PI3K / AKT / mTOR pathway, and commonly mutated in pancreatic neoplasm.
- Signal Transducer and Activator of Transcription 3 (STAT3): It is a transcription factor that is constitutively activated in pancreatic cancer, which helps in tumour progression and immune evasion.
- An example of downstream signalling effectors of PI3K is the AKT serine/threonine kinase 1 (AKT1): a crucial protein in cell survival and growth.

- Epidermal Growth Factor Receptor (EGFR): This is a receptor tyrosine kinase that is also overexpressed in pancreatic carcinoma and is linked to a poor prognosis.
- Mammalian Target of Rapamycin, (mTOR): a serine/threonine kinase that regulates cellular growth and proliferation in pancreatic cancer conditions.
- B-Raf proto-oncogene serine/threonine kinase (BRAF): is a common pancreatic tumour mutated kinase that activates the MAPK signalling pathway constitutively.

Protein Data Bank (PDB) was used to obtain the three-dimensional crystallised structures of these target proteins with the following accession codes: PIK3CA (7R9V), STAT3 (6NJS), AKT1 (7APJ), EGFR (8PO3), mTOR (4DRH), and BRAF (3OGT). Before docking, any extraneous water molecules, heteroatoms, and co-crystallised ligands were removed and the proteins refined using standard structural optimization procedures.

ADMET Prediction and Drug-Likeness

Swiss ADME was used to determine drug-like behaviour and pharmacokinetic properties of the chosen phytochemicals. The parameters analysed were molecular weight, number of hydrogen bond acceptors and donors, lipophilicity (LogP) and oral bioavailability. Corroboration was done by target prediction with Swiss Target Prediction to confirm that it is relevant to oncogenic pathways. The ProTox-II was used to project the toxicity profile and this offers a detailed information about various endpoints, including cytotoxicity, genotoxicity, and organ toxicity (Huang *et al.*, 2009).

Molecular Docking Studies

The Swiss Dock web server was used to perform the molecular docking of the phytochemicals with respect to the interaction profiles and binding affinities of the phytochemicals with the selected targets. Swiss Dock is a computer application using the EA Dock DSS engine which uses Lamarckian genetic algorithm to search the ligand conformational space and predict binding poses at the active sites on the target proteins. The positioning of the ligands was done through blind docking in order to have no bias. The results of docking were scored in regards to the estimated binding free energy (ΔG, kcal/mol), and the most favourable binding poses were chosen to be further studied (Peng *et al.*, 2019).

Due to the exploratory computational nature of the present study extensive docking protocol validation including redocking of co-crystallized ligands and RMSD calculations was not performed. However, docking interpretations were based on experimentally resolved protein structures and reported active site regions available in the literature.

Interaction Analysis and Visualization

The interaction between the protein and its ligand (hydrogen bonds, hydrophobic contacts, and electrostatic interactions) was viewed and examined in Discovery Studio Visualizer. Patterns of interaction had been studied in order to gain clarity about the binding stability and the molecular process of inhibition. To determine the residual amino acids that play important roles in the interactions and to understand the structural basis of their binding affinity, the binding poses of the compounds were analysed (Hind *et al.*, 2024).

RESULTS

Network Pharmacology Analysis

The analysis of network pharmacology recognised 45 possible protein targets of the five chosen phytochemicals of *Pergularia daemia*. In the compound-target network, it was found that all the five compounds have numerous targets associated with them, which reinforced the observation of a potential multi-target interaction profile. The central nodes found on the network were AKT1, STAT3, MTOR, ESR[1], EGFR, PIK3CA, MDM2 and PPARG, BRAF (Hamel *et al.*, 2024; Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025; Gopinath *et al.*, 2025; Chen *et al.*, 2021; Campos *et al.*, 2025; Daina *et al.*, 2017).

Pathway Enrichment Analysis

KEGG Pathway Enrichment

Analysis of the predicted targets using the KEGG pathway enrichment analysis showed that the predicates were greatly enriched by various cancer-related pathways. Figure 1 demonstrates the most enriched pathways.

The pathway of pancreatic cancer had been enriched significantly with nine genes and a fold-enrichment of about 150 (-log10 FDR=12) confirming the interest of the study in the area of pancreatic cancer. The other pathways that were enriched significantly included glioma, EGFR tyrosine kinase inhibitor resistance, acute myeloid leukaemia, endocrine resistance, prostate cancer, non-small cell lung cancer, melanoma, ErbB signalling pathway and colorectal cancer. These observations indicate that the phytochemicals can have widespread anticancer effects in other instances than pancreatic cancer (Daina *et al.*, 2017).

GO Biological Process Enrichment

GO biological process enrichment analysis showed that the predicted targets were highly enriched with cancer progression processes. Figure 2 shows the most enriched biological processes.

Figure 2 Bar plot of the 15 enriched GO biological processes. The x-axis indicates the fold enrichment, the y-axis indicates the biological process terms, the circle size indicates the number of genes involved and the colour indicates -log10

FDR values. The prominent enriched processes are regulation of miRNA transcription, miRNA transcription, regulation of miRNA metabolic process, response to peptide hormone and cellular response to hormone stimulus, response to hormone, response to organic cyclic compound, positive regulation of cell differentiation, growth, and negative regulation of apoptotic process (Peng *et al.*, 2009). The processes have great relevance to cancer biology especially on hormone response, cell proliferation and apoptosis control (Fresno *et al.*, 2004; Yu *et al.*, 2014).

GO Cellular Component Enrichment

The enrichment analysis of GO cellular components showed that the predicted targets are strongly enriched by certain cellular structures. Figure 3 describes the most enriched cellular components.

Figure 3 Bar plot depicting the 15 enriched GO cellular components. The x -axis indicates the fold enrichment, the y -axis is the list of cellular component terms, the size of the circles indicates the number of affected genes, and the colour -log1 - FDR. The main enriched elements are phosphatidylinositol 3 -kinase complex class III -B, Torc1 complex, multivescular body internal vesicle and phosphatidylinositol 3 -kinase complex class III-A. Other levels of enrichment included phosphatidylinositol 3-kinase complex class I, Torc2 complex (Peng *et al.*, 2019), Tor complex, lamellipodium, transcription regulator complex, and cell leading edge. The hyperenrichment of PI3K and mTOR complexes is a strong indication of the role of PI3K/AKT/mTOR signalling axis in the action of these phytochemicals (Fresno *et al.*, 2004; Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025).

GO Molecular Function Enrichment

GO molecular function enrichment analysis showed that the predicted targets were significantly enriched with certain molecular functions Figure 4 is a representation of the most enriched molecular functions.

Figure 4 Bar plot that represents the 15 most enriched GO molecular functions. The x-axis is fold enrichment, the y-axis is molecular function terms, the size of circles is the number of genes involved and the colour is the -log10 FDR. Important enriched functions involve nitric-oxide synthase regulator activity, oestrogen response element binding, transcription coactivator binding and protein serine kinase activity. Other enhanced functions included nuclear receptor activity, ligand-activated transcription factor activity, and general transcription initiation factor binding, AtPase binding, transcription coregulator binding, protein serine/threonine kinase activity (Peng *et al.*, 2019), and protein serine/threonine kinase activity. These activities are of great relevance to cancer biology, especially in the context of the signalling of kinase activity, transcription, and nuclear receptor signalling (Fresno *et al.*, 2004; Yu *et al.*, 2014; Daina *et al.*, 2017).

Protein-Protein Interaction Network

The Protein-Protein Interaction (PPI) network built with the help of the STRING database showed that the identified potential target proteins have a high connectivity Figure 5. The targets core and noncore consisted of AKT1, STAT3, MTOR, EGFR, PIK3CA, MDM2, BRAF, and other targets including KDR, PPARG, ESR[1], CHEK1, PTGS2, GLI1, AR, CTRC, RET, and NTRK1. The multi-target inhibitory phenotype of the phytochemicals is supported by the high degree of interaction and indicates that they might have a simultaneous influence on several interlocking

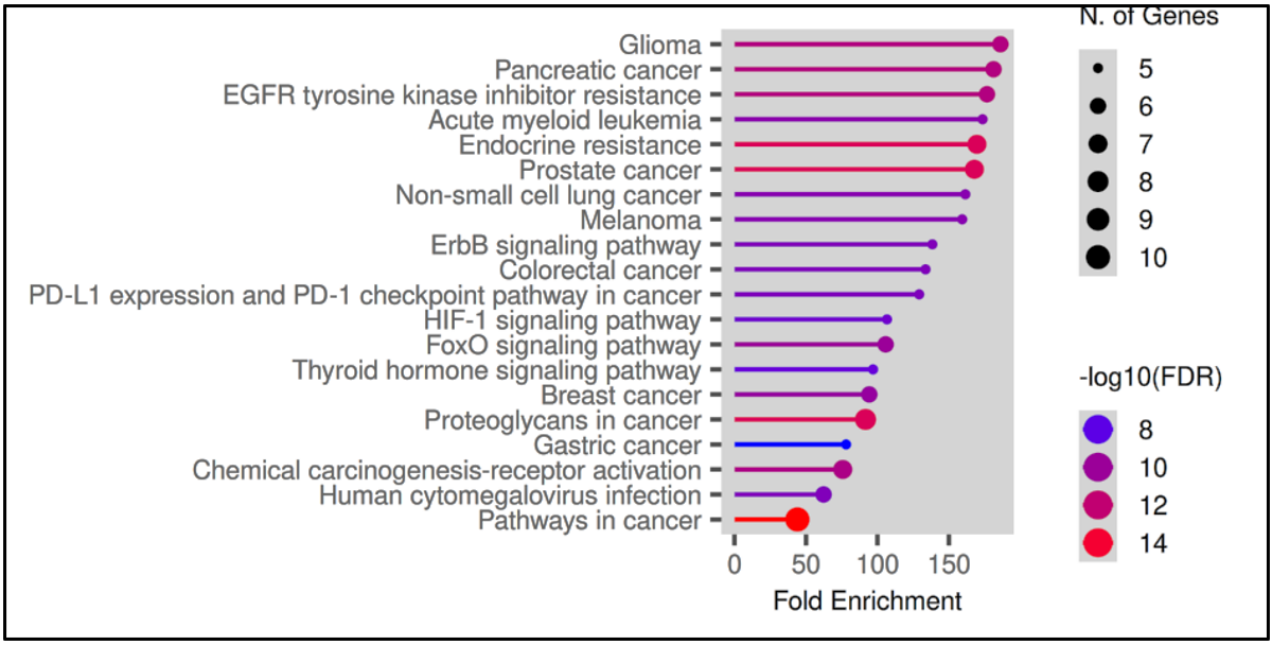

Figure 1: KEGG pathway enrichment analysis of predicted target genes.

signalling pathways (Hamel *et al.*, 2024; Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025; Gopinath *et al.*, 2025; Chen *et al.*, 2021; Campos *et al.*, 2025; Daina *et al.*, 2017).

Figure 5 STRING PPI network showing interactions between predicted target proteins. Nodes represent proteins, and edges represent Protein-Protein Interactions. The network shows strong connectivity between core targets involved in pancreatic cancer signaling pathways.

Drug-Likeness and ADMET Analysis

Swiss ADME analysis revealed that the chosen phytochemicals present in the plant matter of the selected plant species, that is, *Pergularia daemia* showed good physicochemical characteristics in line with bioactive natural products. The five compounds all met Lipinski's Rule of Five, which means that they have favourable oral bioavailability. The MWs were between 388.50 $gmol^{-1}$ (Corotoxigenin) and 532.62 $gmol^{-1}$ (Calotropin, Calactin, and Coroglaucinin). The logP was between 2.18 and 3.14 indicating moderate lipophilicity. The gastrointestinal absorption of all the compounds was high, and this made them effective orally. All the compounds were not expected to cross the blood-brain barrier, and this is positive to attacking peripheral tumours. The predictability of toxicity with ProTox-II indicated that all the compounds belong to Class 2 toxicity, which is acceptable with anticancer lead compounds (Table 1) (Mehra *et al.*, 2021).

Molecular Docking

The docking experiment indicated moderate to favorable predicted binding affinities of the target phytochemicals with several pancreatic-cancer-linked molecular targets. The binding free energies calculated had a range between -4.839 to -8.872 mol^{-1} which had increasingly negative values as the affinity increased (Table 2).

PIK3CA

Calotropagenin and Coroglaucinin showed the greatest binding affinities (-8.872 -8.808 mol^{-1} respectively), which is indicative of potential interaction with the PI3K signaling pathway inhibition (Figure 6). Calotropin was also a very good binder (-8.132 0 mcal). Such observations are of interest especially since PIK3CA is one of the most commonly mutated oncogenes in pancreatic cancer and that its silencing has been reported to check tumour growth

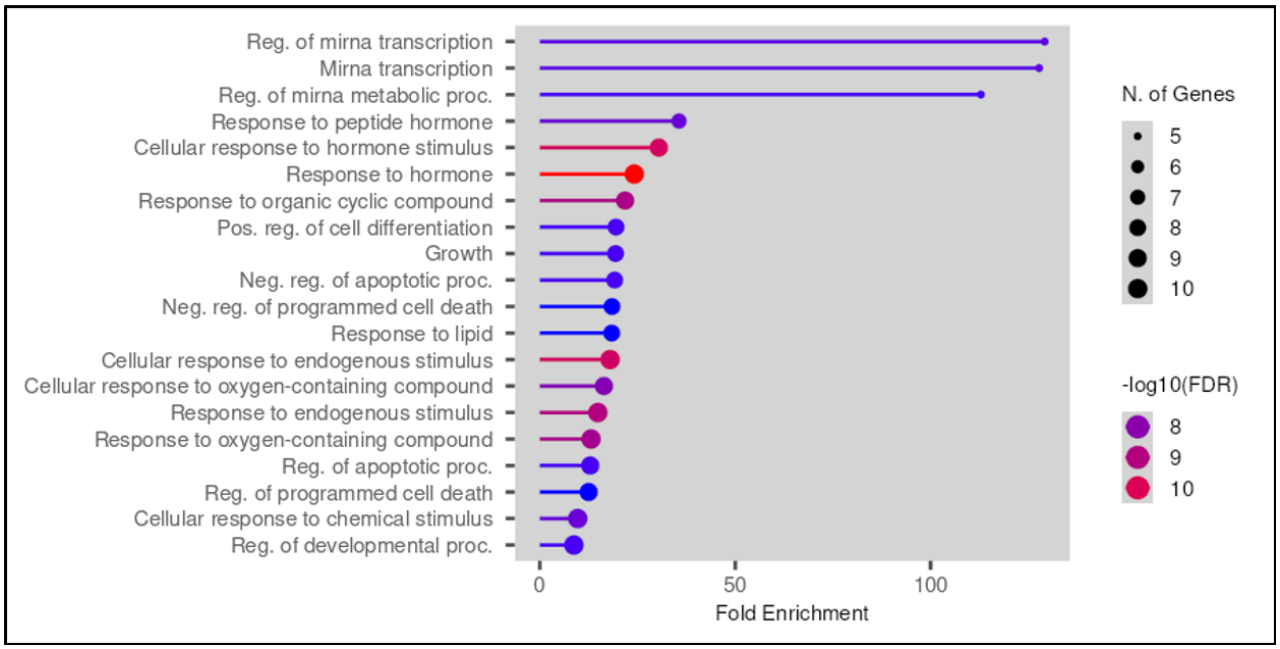

Figure 2: Gene Ontology biological process enrichment of predicted targets related to cancer progression and apoptosis.

Table 1: ADME and Toxicity profile of *Pergularia daemia* phytochemicals.

Phytochemical	Molecular Weight (g/mol)	H-bond Donors	H-bond Acceptor	LogP	Lipinski's Rule of 5	GI Absorption	BBB Permeation	Toxicity class	Drug-likeness
Calotropin	532.62	3	9	3.14	Pass	High	No	Class 2	0.47
Calotropagenin	404.50	3	6	2.18	Pass	High	No	Class 2	0.88
Coroglaucigenin	390.51	3	5	2.77	Pass	High	No	Class 2	1.06
Calactin	532.62	3	9	2.94	Pass	High	No	Class 2	0.47
Corotoxigenin	388.50	2	5	2.59	Pass	High	No	Class 2	0.29

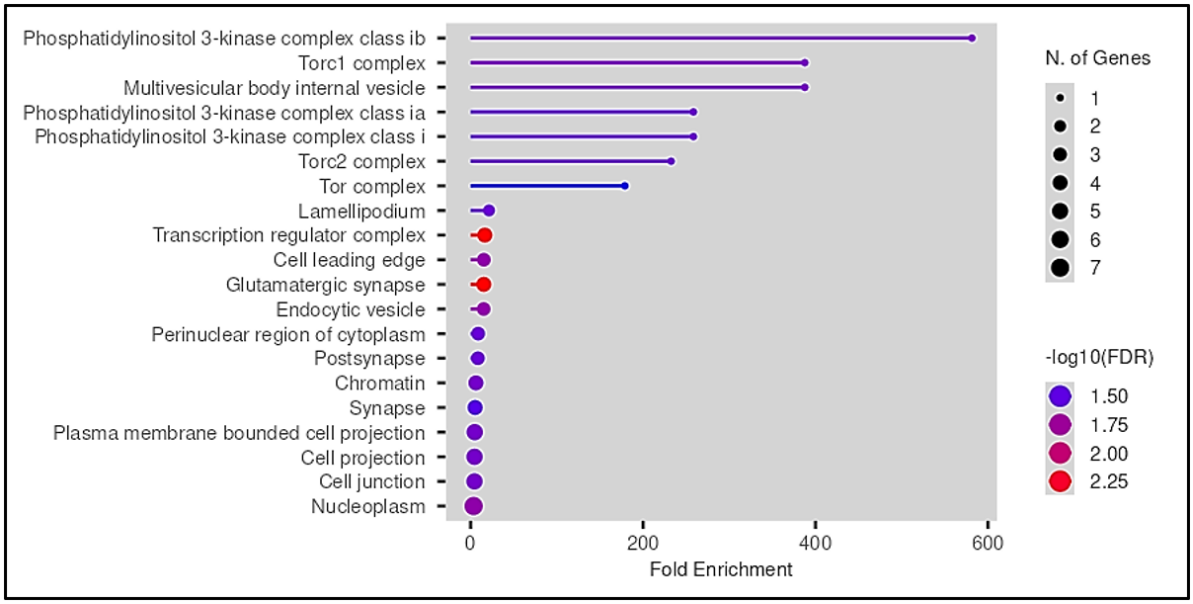

Figure 3: GO cellular component enrichment analysis.

and enhance the effectiveness of traditional chemotherapeutic agents (Fresno *et al.*, 2004; Hamel *et al.*, 2024).

STAT3

Calactin and Calotropin exhibited positive binding energies (-7.652 kcal/mol^{-1} and -7.440 kcal/mol^{-1}, respectively) and stable interaction networks, suggesting that the compounds have potential modulation of STAT3 associated signaling pathways (Figure 7). There was also good binding of calotropagenin (-7.446 kcal mol^{-1}). STAT3 is a therapeutic target that has been demonstrated to be a valid one, its inhibition decreases the growth of tumours and enhances the effect of chemotherapy, and maintained its constitutive activity in pancreatic cancer through cytokine signalling (Yu *et al.*, 2014; Gopinath *et al.*, 2025; Chen *et al.*, 2021).

AKT1, EGFR, mTOR, and BRAF

The phytochemicals exhibited moderate to high binding affinities all, which favour a potential multi-target interaction profile. Corotoxigenin and Coroglaucinin showed the highest affinity with EGFR and mTOR respectively (7.214 -7.244 -7) (Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025; Daina *et al.*, 2017). The docking scores, on the whole, have always revealed positive interactions between the phytochemicals and oncogenic proteins involved in the progression of pancreatic cancer.

The conformations produced by Swiss Dock under docked structures were ranked by the binding free energy values and the conformation with the lowest binding energy was considered in the further interaction analysis (Mortazavi *et al.*, 2022).

Protein-Ligand Interaction Analysis

The analysis of the interaction showed that the compounds were bound by three or more hydrogen bonds and three or more hydrophobic interactions with the main amino-acid residues in the active or regulatory regions of the target proteins, which contribute to the binding stability and may disrupt downstream oncogenic signalling pathways.

Calotropin

The ligand formed stable hydrogen bond interactions with essential residues in active sites of PIK3CA and STAT3 such as Lys802, Asp933 and Arg214. Moreover, hydrophobic interactions were also noted with Phe934, Ile848 and Val851.

Calactin

The compound was found to have high affinity towards STAT3 through hydrogen bonding of the compound with Arg609, Ser611 and Thr620. It also π-stacked with Phe613 and was structurally hydrophobically bound with Leu614 and Val637 (Hind *et al.*, 2024).

Calotropagenin

The phytochemical was most bound to PIK3CA and it was able to form numerous hydrogen bonds with Lys802, Asp933 and Glu849. It also exhibited favorable hydrophobic interactions between itself and Phe934, Ile848 and Val851.

Corotoxigenin

The compound was showed favorable predicted interactions with EGFR with hydrogen bonds to Thr790, Met793, and Asp855 and hydrophobic contacts were formed with Leu792, Phe856, and Leu858.

Coroglaucinin

It showed favorable predicted interactions with PIK3CA and mTOR; hydrogen bond was made with Lys802, Asp933 and Glu849 PIK3CA and with Asp2195, Ser2196, and Tyr2225 mTOR. It was also found that significant hydrophobic interactions existed involving Phe934, Ile848 and Val851 of PIK3CA, and Leu2185, Leu2187 and Ile2354 of mTOR.

DISCUSSION

Intricate molecular aberrations involving many signalling cascades characterise pancreatic adenocarcinoma (Fresno *et al.*, 2004; Yu *et al.*, 2014). Inhibition of individual protein often results in therapeutic resistance and thus multi-target inhibition is a more beneficial approach (Hamel *et al.*, 2024; Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025). In this paper, the steroidal lactones of *Pergularia daemia* have been shown to have strong and consistent affinities to key oncogenic proteins including PIK3CA and STAT3, which has been supported by network pharmacology and molecular docking studies (Grosdidier *et al.*, 2011; Mortazavi *et al.*, 2022). Inevitably, differences in the docking scores as reported in different studies are expected, due to differences in docking algorithms, grid parameters, and scoring functions (Mortazavi *et al.*, 2022).

Network Pharmacology Findings

The study of network pharmacology has revealed 45 potential protein targets of the five phytochemicals with pronounced enrichment of the pancreatic cancer pathway (9 genes, fold enrichment ij150, -log10(FDR)=12 (Peng *et al.*, 2019). This fact supports the focus on pancreatic cancer and suggests that these compounds can have extensive antineoplastic effects. PI3K/AKT/mTOR were also prominently represented in the KEGG and GO enrichment analysis, and a large number of PI3K and mTOR complexes were involved. This has been especially noteworthy considering that PI3K/AKT/mTOR signalling cascade is one of the most commonly dysregulated pathways in pancreatic cancer, with PIK3CA mutations found in about 15-20% cases (Fresno *et al.*, 2004; Hamel *et al.*, 2024).

The interaction between the five phytochemicals and a multiple number of proteins was demonstrated by the compound-target network, which supports a multi-target interaction paradigm (Grosdidier *et al.*, 2011). The major identified targets included AKT1, STAT3, MTOR, ESR[1], EGFR, PIK3CA, MDM2, PPARG, and BRAF. Protein-protein interaction network showed a strong connection between these entities suggesting that the phytochemicals might simultaneously regulate an ensemble of interconnected signalling pathways (Huang *et al.*, 2009). This multi-targeting approach gives an edge in the pancreatic cancer disease, which is typified by the coordination of multiple signalling pathways and resistance to monotherapies (Pergolizzi *et al.*, 2024; Fakih *et al.*, 2025).

Molecular Docking Findings

The molecular docking analysis mapped moderate to favorable predicted binding affinities of the phytochemicals to various cancer-related targets of the pancreas. The binding affinities were between -4.839 to -8.872 kcal/mol and the more negative the interaction strength. Calotropagenin and Coroglaucinin demonstrated the largest binding affinities in PIK3CA (-8.872 kcal/mol and -8.808 kcal/mol, respectively), which is of particular importance considering that PIK3CA is one of the most commonly mutated oncogenes in pancreatic cancer (Fresno *et al.*, 2004; Hamel *et al.*, 2024).

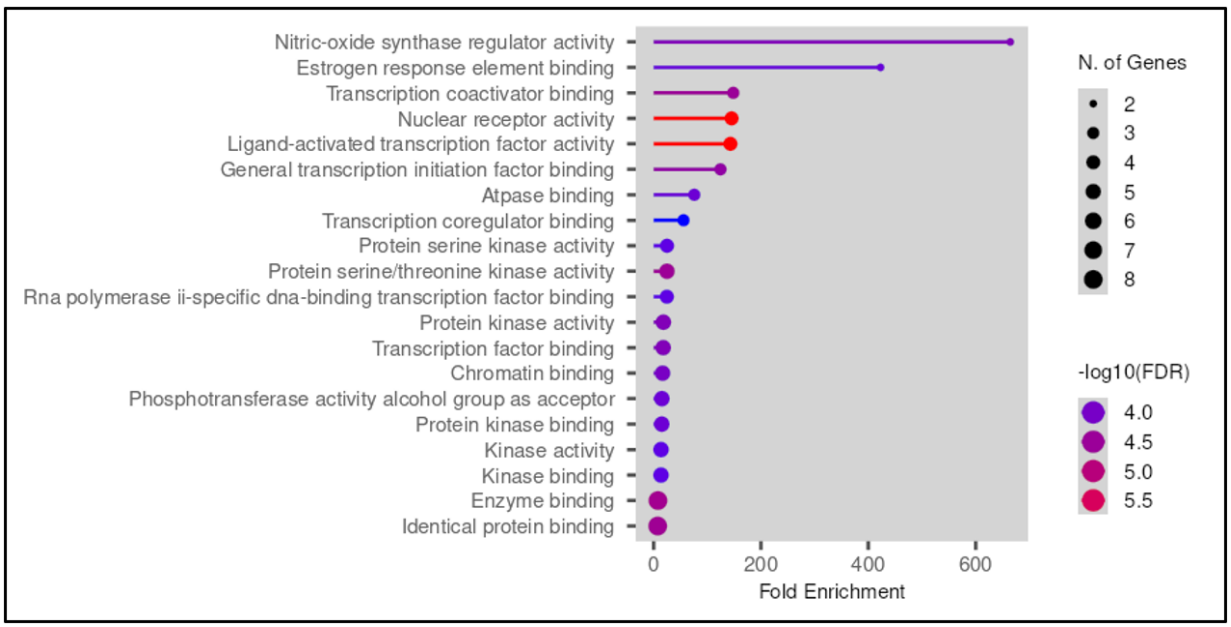

Figure 4: GO molecular function enrichment analysis.

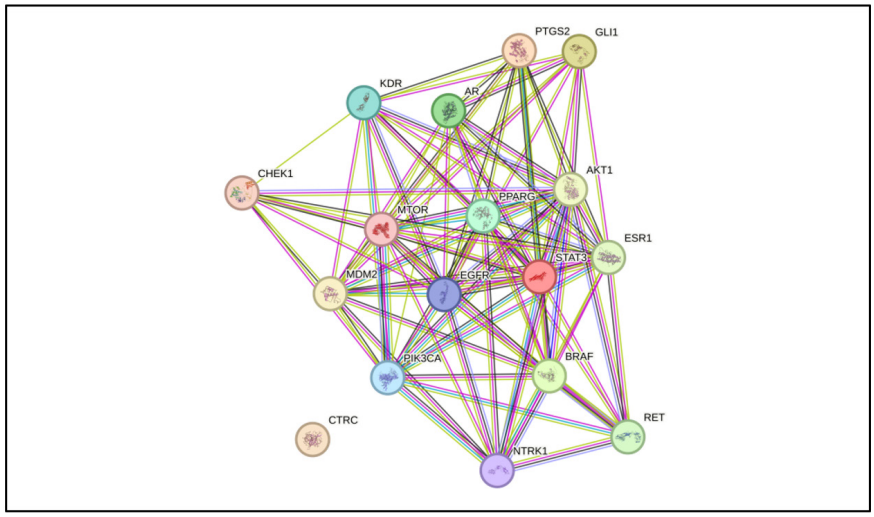

Figure 5: Protein-protein interaction network.

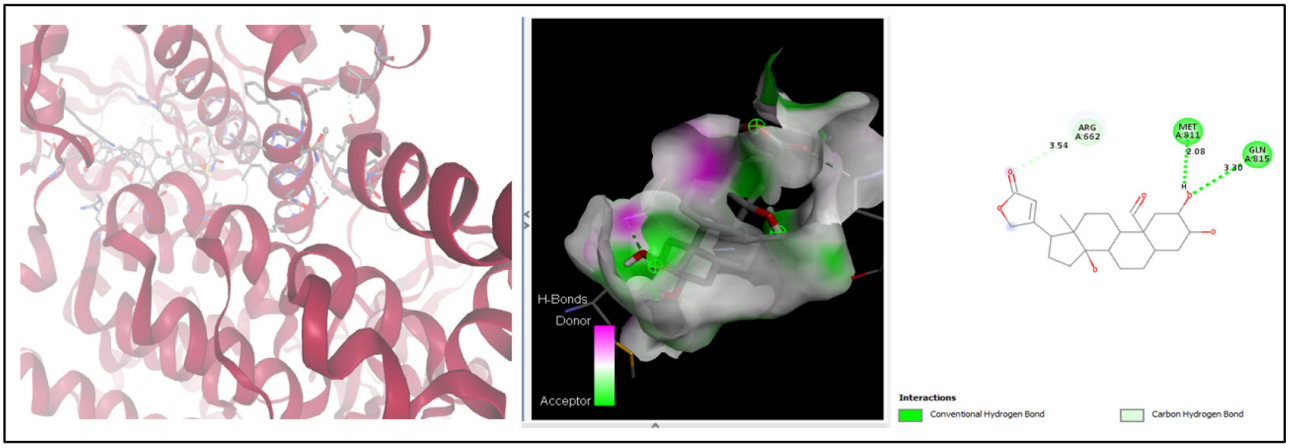

Figure 6: Molecular docking interaction analysis of Calotropagenin with PIK3CA (PDB ID: 7R9V).

Table 2: **Binding affinity (Kcal/mol) of *Pergularia daemia* phytochemicals against pancreatic cancer targets.**

Phytochemical	PIK3CA (7R9V)	STAT3 (6NJS)	AKT1 (7APJ)	EGFR (8PO3)	mTOR (4DRH)	BRAF (3OGT)
Calotropin	-8.132	-7.440	-7.032	-6.032	-4.869	-6.685
Calactin	-7.947	-7.652	-4.839	-6.101	-4.947	-6.498
Calotropagenin	-8.892	-7.446	-6.307	-7.146	-5.145	-5.425
Corotoxigenin	-7.517	-7.055	-6.183	-7.214	-4.949	-5.443
Coroglaucinin	-8.808	-6.940	-6.129	-6.879	-7.244	-5.384

Calactin and Calotropin were shown to have good binding energy to STAT3 (-7.652 kcal/mol and -7.440 kcal/mol, respectively). Pancreatic cancer IL-6 signalling constitutively activates STAT3 which has demonstrated preclinical promise as an inhibitor. These compounds are showed favorable predicted interactions with STAT3 indicating their potential as STAT3 inhibitors.

The ability of these compounds to act as potential multi-target modulator is a unique strength, and pancreatic cancer has a number of signalling pathways. Concomitant blocking of the PI3K/AKT/mTOR and the STAT3 signalling pathways could overcome therapeutic resistance and increase anticancer effects. These compounds have good ADME/Tox properties such as large gastrointestinal absorption and toxicity acceptability, which further justify their suitability as lead molecules in drug development.

The predicted docking scored observed in the present study were within ranges commonly reported for several references inhibitors of PI3K and STAT3 in previous computational studies. However, direct comparisons with established inhibitors should be interpreted cautiously as docking scores may vary depending on docking algorithms, scoring functions, and protein preparation methods.

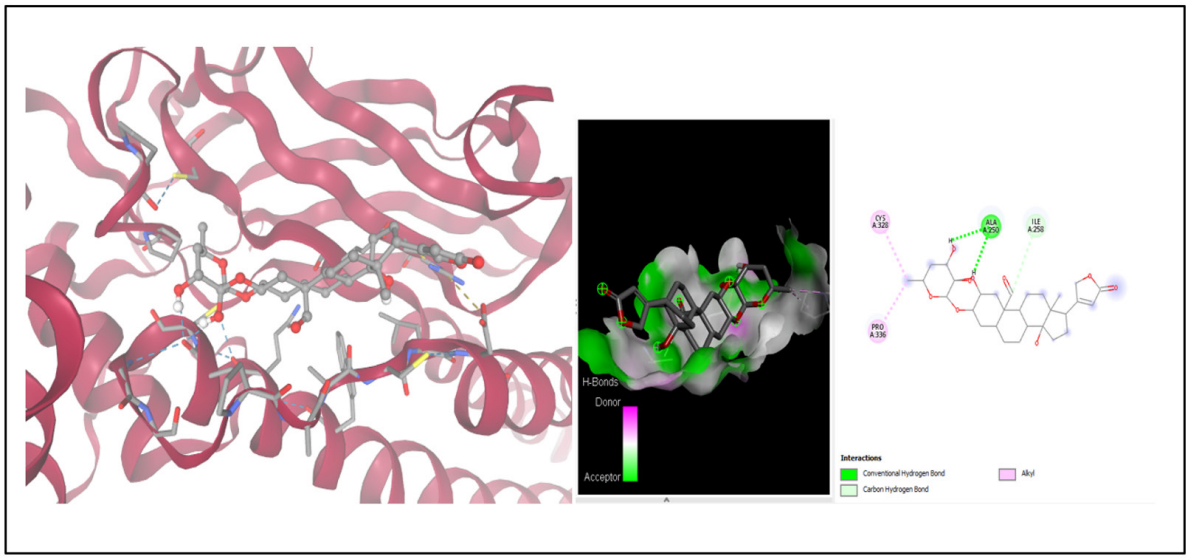

Figure 7: The analysis of the molecular interactions between Calactin and STAT3 (PDB ID: 6NJS) visualised in the Discovery Studio Visualizer and showing the important hydrogen-bonding and hydrophobic interactions.

Biological Significance

The strong binding of Calotropagenin and Coroglaucinin to PIK3CA suggests that the two may potentially modulate PI3K/AKT signalling, which plays a role in the survival and proliferation of tumour cells. PIK3CA is mutated in about 15-20% of pancreatic cancer cases and PI3K inhibitors have shown promise in preclinical (Hamel *et al.*, 2024; Pergolizzi *et al.*, 2024). Calotropagenin (-8.872 kcal/mol) and Coroglaucinin (-8.808 kcal/mol) demonst rated favorable predicted interactions with PIK3CA in the present computational analysis suggesting their possible relevance as exploratory lead molecules for future investigation.

In the same manner, the effective interaction of Calactin and Calotropin with STAT3 enhances their future application in the prevention of transcriptional regulation of tumour growth, inflammatory and immune evasion related genes (Yu *et al.*, 2014; Gopinath *et al.*, 2025). STAT3 is constitutively phosphorylated in pancreatic cancer through IL 6 signalling, and potential lead molecules for future STAT3 targeted studies have been shown to be effective in preclinical research. Calactin (-7.652 kcal/mol) and Calotropin (-7.440 kcal/mol) also demonstrated favorable predicted interactions with STAT3 suggesting their relevance as potential lead molecules for future STAT3 targeted investigations.

Potential multi-target interaction profile of such compounds can be defined as a strategic benefit in consideration of the fact that there are multiple signalling pathways that maintain pancreatic cancer. PI3K/AKT/mTOR and STAT3 cascade inhibition can be used simultaneously to relieve therapy resistance and enhance anticancer efficacy. Their promising prospects as lead molecules in the development of drugs are supported by their favourable ADME/Tox profiles, which are high gastrointestinal absorption and predicted toxicity profile (Mehra *et al.*, 2021).

Comparison With The Existing Therapies

Existing pancreatic cancer therapies including gemcitabine, nab-paclitaxel, FOLFIRINOX, and targeted therapies remain associated with therapeutic resistance and limited long-term efficacy. In the present study, the investigated phytochemicals demonstrated computationally predicted interactions with multiple pancreatic cancer associated targets suggesting their possible relevance as exploratory lead compounds for future investigation. However direct therapeutic comparisons with established anticancer agents cannot be made without comprehensive experimental and clinical validation.

LIMITATIONS

The current study is limited to computational analyses. Docking scores alone cannot confirm biological inhibition or therapeutic efficacy. Anticancer efficacy and safety require experimental validation through cell-based assays, enzyme inhibition studies, and *in vivo* models. In addition, molecular dynamics simulations and binding free binding free energy calculations such as MM-PBSA/MM-GBSA were not performed in the present study. Therefore, the long-term stability of the predicted protein-ligand complexes requires further investigation.

CONCLUSION

The present *in silico* study suggests that steroidal lactone phytochemicals from *Pergularia daemia* may exhibit potential interactions with multiple pancreatic cancer associated proteins in computational analyses. Analysis of network pharmacology revealed 45 potential protein targets, the pancreatic cancer pathway was significantly enriched (9 genes, fold enrichment ≈150, -log10 FDR=12). Both KEGG and GO enrichment analyses showed the PI3K/AKT/mTOR signaling axis as a prominent one. Molecular docking showed good binding affinities of

-4.839 to -8.872 kcal/mol; Calactin and Calotropin were found to have good interactions with STAT3 (-7.440 and -7.652 kcal/mol, respectively); Calotropagenin and Coroglaucinin showed good interactions with PIK3CA (-8.872 and -8.808 kcal/mol, respectively). All tested compounds had good ADME/Tox profiles, good gastrointestinal absorption and predicted toxicity profiles. These results indicate that phytochemicals in *Pergularia daemia* may serve as potential lead molecules for future experimental investigation in pancreatic cancer research, which can work by combining to block the PI3K/AKT/mTOR and STAT3 pathways. More *in vitro and in vivo* studies are justified to confirm these observations and to undertake the therapeutic uses of these compounds in clinical practice.

ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity; **AKT1:** AKT Serine/Threonine Kinase 1; **AR:** Androgen Receptor; **ATPase:** Adenosine Triphosphatase; **BRAF:** B-Raf Proto-Oncogene, Serine/Threonine Kinase; **BBB:** Blood–Brain Barrier; **CHEK1:** Checkpoint Kinase 1; **DAVID:** Database for Annotation, Visualization and Integrated Discovery; **ΔG:** Binding Free Energy; **EA Dock DSS:** EADock Dihedral Space Sampling; **EGFR:** Epidermal Growth Factor Receptor; **ESR1:** Estrogen Receptor 1; **FDR:** False Discovery Rate; **GI:** Gastrointestinal; **GLI1:** GLI Family Zinc Finger 1; **GO:** Gene Ontology; **GO-BP:** Gene Ontology–Biological Process; **GO-CC:** Gene Ontology–Cellular Component; **GO-MF:** Gene Ontology–Molecular Function; **H-bond:** Hydrogen Bond; **IL-6:** Interleukin-6; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **KDR:** Kinase Insert Domain Receptor.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUTURE DIRECTIONS

Future research work efforts should be focused on the following:

1. Experimental validation: Perform cell-based tests, enzyme inhibition tests, and *in vivo* models that ensure anticancer efficacy and safety.
2. Structural optimization: The medicinal chemistry strategies include application to improve selectivity, decrease toxicity and preserve potent anticancer activity.
3. Mechanistic studies: Conduct an in-depth study concerning the molecular mechanistic action of PI3K/AKT/mTOR and STAT3 inhibition by the latter phytochemicals.
4. Combination studies: Study synergistic actions upon combination with already established pancreatic cancer therapeutics.
5. Clinical translation: Start mass preclinical tests and clinical trials to determine the therapeutic effectiveness on patients with pancreatic cancer.

REFERENCES

BIOVIA. Dassault systèmes [Discovery Studio visualizer]. Dassault Systèmes [Software].

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394–424. https://doi.org/10.3322/caac.21492

Campos, A. D. et al. (2025). A STAT3/integrin axis accelerates pancreatic cancer progression. Cell Reports, 54(1), Article 114001. https://doi.org/10.1016/j.celrep.2025.114001

Chen, H. et al. (2021). Targeting STAT3 by a small molecule suppresses pancreatic cancer progression. Oncogene, 40(1), 169–180. https://doi.org/10.1038/s41388-020-01547-1

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7, Article 42717. https://doi.org/10.1038/srep42717

Fakih, T. M., Syabihah, H., Gusti, T. A. S., Oktafira, T., Sri Mulyani, N. O., Wisnuwardhani, H. A., & Muchtaridi, M. (2026). Network pharmacology and molecular simulation-based analysis of Citrus aurantium metabolites targeting breast cancer pathways. Intelligent Pharmacy, 4(1), 67–87. https://doi.org/10.1016/j.ipha.2025.11.001

Fresno Vara, J. A., Casado, E., de Castro, J., Cejas, P., Belda-Iniesta, C., & González-Barón, M. (2004). PI3K/Akt signalling pathway and cancer. Cancer Treatment Reviews, 30(2), 193–204. https://doi.org/10.1016/j.ctrv.2003.07.007

Gopinath, R., Ashok, C., Rajasekaran, N. K., Sekar, M., Pavadai, P., Shing, W. L., Subramaniyan, V., & Jeyabalan, S. (2025). Computational discovery of the neuroprotective potential of karanjin against Parkinson's disease. ChemistrySelect, 10(38). https://doi.org/10.1002/slct.202503043

Grosdidier, A., Zoete, V., & Michielin, O. (2011). SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Research, 39(Web Server issue)(Web Server Issue), W270–W277. https://doi.org/10.1093/nar/gkr366

Hamel, Z. et al. (2024). Role of STAT3 in pancreatic cancer. Frontiers in Oncology, 14, Article 1332456. https://doi.org/10.3389/fonc.2024.1332456

Hind, C. et al. (2024). Inhibition of the PI3K/AKT/mTOR pathway in pancreatic cancer. World Journal of Gastroenterology, 30(15), 2001–2015. https://doi.org/10.3748/wjg.v30.i15.2001

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using David bioinformatics resources. Nature Protocols, 4(1), 44–57. https://doi.org/10.1038/nprot.2008.211

Liu, X., Riquelme, M. A., Tian, Y., Zhao, D., Acosta, F. M., Gu, S., & Jiang, J. X. (2021). ATP inhibits breast cancer migration and bone metastasis through down-regulation of CXCR4 and purinergic receptor P2Y11. Cancers, 13(17), Article 4293. https://doi.org/10.3390/cancers13174293

Mortazavi, M. et al. (2022). Prospects of targeting PI3K/AKT/mTOR pathway in pancreatic cancer. Critical Reviews in Oncology/Hematology, 170, Article 103589. https://doi.org/10.1016/j.critrevonc.2021.103589

Peng, Q., Shen, Y., Lin, K., Zou, L., Shen, Y., & Zhu, Y. (2019). Identification of microRNA-92a and related biomarkers in colorectal cancer. Journal of Cancer, 10(13), 2946–2954. https://doi.org/10.7150/jca.30273

Pergolizzi, R. G., & Brower, S. T. (2024). Molecular targets for the diagnosis and treatment of pancreatic cancer. International Journal of Molecular Sciences, 25(19), Article 10843. https://doi.org/10.3390/ijms251910843

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. CA: A Cancer Journal for Clinicians, 70(1), 7–30. https://doi.org/10.3322/caac.21590

STRING database. String: Functional Protein Association Networks [Internet]. https://string-db.org/

SwissTargetPrediction: Target prediction of bioactive small molecules [Internet]. SwissTargetPrediction. http://www.swisstargetprediction.ch/

Yu, H., Lee, H., Herrmann, A., Buettner, R., & Jove, R. (2014). Revisiting STAT3 signalling in cancer: New and unexpected biological functions. Nature Reviews. Cancer, 14(11), 736–746. https://doi.org/10.1038/nrc3818

Cite this article: Santhiya G, Muthukumaran M, Pughazendhi R, Gowrishankar J. *In silico* Evaluation of *Pergularia daemia* Steroidal Lactone Phytochemicals as Potential Multi-Target Modulators of PIK3CA and STAT3 Signaling Pathways in Pancreatic Cancer. J Young Pharm. 2026;18(2):457-66.