JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/41950
RESEARCH ARTICLE

Exploring the Anticancer Potential of *Saussurea costus* Phytoconstituents Against Prostate Cancer Using Virtual Screening and Molecular Docking

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Abstract

Background: Cancer remains a leading cause of death globally, necessitating the development of novel and targeted therapies. Natural products derived from traditional medicinal plants, such as *Saussurea costus*, offer promising anticancer potential due to their bioactive compounds. Virtual screening provides an efficient in silico approach to identify potential drug candidates by targeting specific pathways and molecular mechanisms associated with cancer. **Objectives:** This study aimed to identify bioactive compounds in *S. costus* with potential anticancer properties and to elucidate their mechanisms of action using virtual screening, molecular docking, and pathway enrichment analyses. **Methods:** Compounds from the IMPPAT database were screened for drug-likeness and pharmacokinetics using SwissADME and PASS webserver tools. Toxicity predictions were conducted using ProTox-II. Differentially Expressed Genes (DEGs) associated with prostate cancer were identified through GEO2R analysis of the GSE47108 dataset. Overlapping genes between DEGs and compound targets were subjected to Protein-Protein Interaction (PPI) network analysis using STRING and Cytoscape to identify hub genes. Molecular docking of selected compounds with hub genes was performed using AutoDock Vina, and pathway analysis was conducted using ShinyGO and KEGG databases. **Results:** Three compounds—stigmasterol, isoboldine, and beta-sitosterol—were identified with favorable ADME and toxicity profiles. Hub genes such as SRC, FGFR1, and HSP90AA1 were strongly associated with prostate cancer pathways. Docking studies revealed significant binding affinities, with stigmasterol showing the strongest interaction with HSP90AA1. Pathway enrichment highlighted the involvement of PI3K/AKT and cancer-related pathways. **Conclusion:** *Saussurea costus* exhibits multi-targeted anticancer potential, particularly against prostate cancer. These findings warrant further *in vitro* and *in vivo* studies to validate the therapeutic efficacy of its bioactive compounds.

Keywords: Anticancer Activity, Bioactive Compounds, Prostate Cancer, *Saussurea costus*, Virtual Screening

1. Introduction

Cancer is a complex and uncontrolled growth and spread of abnormal cells. It is the one of the main causes of death throughout the world, though there are advancements in cancer treatment, the development of more effective and targeted therapies is still an urgent need. Traditional medicine has provided a valuable source of potential anticancer agents, and natural products derived from medicinal plants have shown promising therapeutic effects[1]. *Saussurea costus*, commonly known as *costus*, belongs to the *Asteraceae* family. It is widely distributed in the Himalayan region and has been used for centuries in traditional medicine systems such as *Ayurveda* and *Unani* for the treatment of various ailments, including cancer. Several studies have reported the presence of bioactive compounds in *S. costus* with potential anticancer properties. These compounds include sesquiterpene lactones, flavonoids, phenolic acids, and alkaloids, which have demonstrated

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Article Received on: 06.03.2024 Revised on: 07.10.2024 Accepted on: 25.10.2024

diverse biological activities, including antioxidant, anti-inflammatory, and cytotoxic effects[2].

Virtual screening, also known as *in silico* screening, is a computational approach that plays a crucial role in drug discovery and development. It involves the use of computer algorithms and molecular modelling techniques to screen large chemical libraries and identify potential drug candidates. Virtual screening methods can significantly reduce the time and cost associated with traditional experimental screening approaches. They enable the identification of lead compounds that can be further optimized and developed into effective drugs. The identification of targeted pathways is essential for understanding the mechanism of action of potential anticancer agents. By elucidating the specific pathways or molecular targets affected by a compound, researchers can gain insights into the underlying biological processes involved in cancer development and progression. This knowledge can guide the design of more effective therapeutic strategies[3].

In this study, we propose to utilize virtual screening techniques to identify the targeted pathways of *S. costus* against cancer. The primary objective is to identify potential bioactive compounds from *S. costus* that exhibit anticancer activity through the modulation of specific molecular targets or pathways. By employing a combination of molecular docking, we aim to prioritize the most promising candidates for further experimental validation. The results of this study will help to gain a deeper understanding of their mode of action and explore their potential synergistic effects with existing anticancer drugs. Furthermore, the findings may provide insights into the broader pharmacological properties of *S. costus*, paving the way for its wider utilization in traditional and modern medicine.

2. Methods

2.1 *In Silico* Screening

Structure-based virtual screening was used to screen the potential compounds acquired from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database. Based on the selection criteria such as, compound should accept Lipinski's 5 rules and should be the most promising drug likeness score, molecular weight, and bio availability. Further, selected molecules are screened using PASS webserver.

2.2 Prediction of Activity Spectra for Substances (PASS) Evaluation

The biological characteristics of chemical compounds can be evaluated using PASS analysis, which investigates chemical-biological interactions. The PASS web server employs an internal algorithm and generates two key descriptors: "Probability of being active (Pa)" and "Probability of being inactive (Pi)". A higher Pa value suggests a stronger likelihood that the compound will exhibit the specified biological activity[4].

2.3 Pharmacokinetic Prediction

The solubility of the selected small compounds in the gut, as well as their ability to pass the blood-brain barrier, were determined using Swiss ADME[5].

2.4 Toxicity Risk Prediction

To discover probable toxic effects of a selected compounds, toxicity prediction was performed using the Pro Tox II server, which are used to assess a chemical's toxicity categorization[6].

2.5 Identification of Target Genes

Identification of targets of compounds selected against prostate cancer was done using Swiss target prediction[7].

2.6 Identification of Differentially Expressed Genes (DEGs)

Gene Expression Omnibus (GEO) was used to identify microarray data for prostate cancer, by applying the filter "expression arrays" to shortlist gene dataset. GSE47108 dataset was shortlisted and GEO2R was used to calculate the P value <0.05 and Log Fc values. Venn diagram was constructed for the target genes of selected compounds of *S. costus* and genes from dataset. The overlapped genes were chosen for further investigation[8,9].

2.7 Identification of Hub Genes

We generated a Protein-Protein Interaction (PPI) network by utilizing the Search Tool for the Retrieval of Interacting Genes or Proteins (STRING) database (version 11.0), for intersecting genes between the selected compounds and Differentially Expressed Genes (DEGs). Subsequently, we computed the Maximal

Clique Centrality (MCC) algorithm in Cytoscape to identify the top 10 hub gens[10].

2.8 Gene Enrichment and Pathway Analysis

Hub genes were analysed for enrichment using GO, with a significance threshold of FDR < 0.05. Pathway analysis was performed using Shiny GO, with homo sapiens as the selected species and a significance threshold of p < 0.05 for all pathway and GO enrichment analyses[11].

2.9 *In Silico* Molecular Docking Studies

Molecular docking was performed to determine the binding affinity of selected plant-derived compounds with their top 10 hub genes and a standard drug. The virtual screening software PyRx-v0.8, incorporating the AutoDock-Vina interface, was employed for this purpose. Blind docking was conducted to predict the binding affinity, and Discovery Studio 2020 (BIOVIA) was used to visualise the best protein-ligand complexes in 2D diagrams[12].

3. Results

3.1 Virtual Screening Data Retrieval

Based on drug likeness and bioavailability 3 compounds were selected and further screened down using PASS and ADME.

3.2 PASS Evaluation

Through this activity of selected compounds were predicted and standard drug Copanlisib was used as reference to validate the predicted results. Table 1 shows the activities of compounds with higher Pa.

3.3 ADME Prediction

Good ADME properties are as critical as therapeutic properties we have screened selected molecule for pharmacokinetic prediction using Swiss ADME Tables 2 and 3.

3.4 Target Genes of Compounds Identified from *S. costus*

The structures of compounds and standard drugs are obtained from PubChem. Subsequently, targets genes of compounds are obtained using Swiss target prediction and saved for subsequent use.

Table 1. PASS evaluation of selected compounds

Isoboldine		
Pa	**Pi**	**Activity**
0,171	0,037	Anti-cancer (gastric cancer)
0,235	0,112	Anticancer (small cell lung cancer)
0,115	0,012	Breast cancer-resistant protein inhibitor
0,182	0,106	Anticancer (breast cancer)
0,223	0,214	Cancer associated disorders treatment
0,146	0,123	Anticancer (renal cancer)
0,119	0,093	Anticancer (uterine cancer)
0,197	0,165	Anticancer (bone cancer)
0,220	0,058	Anticancer (non-small cell lung cancer)
Stigmasterol		
0,178	0,062	Anticancer (renal cancer)
0,443	0,016	Prostate cancer treatment
0,406	0,015	Anticancer (pancreatic cancer)
0,282	0,026	Anticancer (liver cancer)
0,247	0,036	Anticancer (bone cancer)
0,261	0,067	Anticancer (breast cancer)
0,208	0,026	Anticancer (thyroid cancer)
0,178	0,062	Anticancer (renal cancer)
Beta Sitosterol		
0,831	0,003	Chemopreventive
0,806	0,005	Caspase 3 stimulant
0,558	0,031	Apoptosis agonist
0,411	0,100	Anticancer
0,337	0,031	Chemoprotective
0,301	0,041	Prostate cancer treatment
0,319	0,046	Anticancer (pancreatic cancer)
0,273	0,014	Anticancer (bone cancer)
0,209	0,025	Anticancer (thyroid cancer)
0,173	0,080	Anticancer (endocrine cancer)
0,162	0,091	Anticancer (lung cancer)
0,161	0,090	Anticancer (renal cancer)
0,153	0,134	Anticancer (breast cancer)
Copanlisib		
0,442	0,089	Anticancer
0,352	0,057	Anticancer (solid tumors)
0,226	0,031	Anticancer (ovarian cancer)
0,178	0,092	Anticancer (glioblastoma multiforme)
0,207	0,131	Anticancer (bone cancer)
0,149	0,090	Anticancer (colorectal cancer)
0,144	0,103	Anticancer (glioma)

Table 2. ADME prediction

Molecule	Canonical SMILES	Formula	MW	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	PAINS #alerts
Copanlisib	COc1c(OCCCN2CCOCC2)ccc2c1nc(=NC(=O)c1cnc(nc1)N)n1c2NCC1	C23H28N8O4	480.52	Low	No	Yes	No	No	Yes	No	Yes	-9	1	2	1	1	0	0.55	0
Isoboldine	COc1cc2c(cc1O)CC1c3c2c(O)c(OC)cc3CCN1C	C19H21NO4	327.37	High	Yes	Yes	Yes	No	No	Yes	Yes	-6.71	0	0	0	0	0	0.55	0
Beta- Sitosterol	CCC(C(C)C)CCC(C1CCC2C1(C)CCC1C2CC=C2C1(C)CCC(C2) O) C	C29H50O	414.71	Low	No	No	No	No	No	No	No	-2.2	1	3	0	1	2	0.55	0
Stigmasterol	CCC(C(C)C)C=CC(C1CCC2C1(C)CCC1C2CC=C2C1(C)CCC(C2) O) C	C29H48O	412.69	Low	No	No	No	No	Yes	No	No	-2.74	1	3	0	1	2	0.55	0

Table 3. Toxicity prediction

Name of the Compound	Class of Toxicity	Predicted LD50 (mg/kg)	Known Toxicity
Isoboldine	Class 4	450 mg/kg	Immunotoxicity
Beta sitosterol	Class 4	890 mg/kg	Immunotoxicity
Stigmasterol	Class 4	890mg/kg	Immunotoxicity
Copanlisib	Class 4	1500mg/kg	Immunotoxicity

Table 4. Top 10 hub genes

Gene name	MCC	Degree	Betweenness
SRC	124838456	73	4355.12445
FGFR1	124274364	66	3292.1731
MET	121238616	41	393.23934
MAPK3	119667451	50	1884.3078
HSP90AA1, EL52	112044187	34	464.45539
PTPN11	107138686	64	3396.51289
PIK3CA	101799470	53	2230.69267
ESR1	101353453	56	3086.71886
EGFR	62880780	26	522.98766
MTOR	60089064	25	141.73632

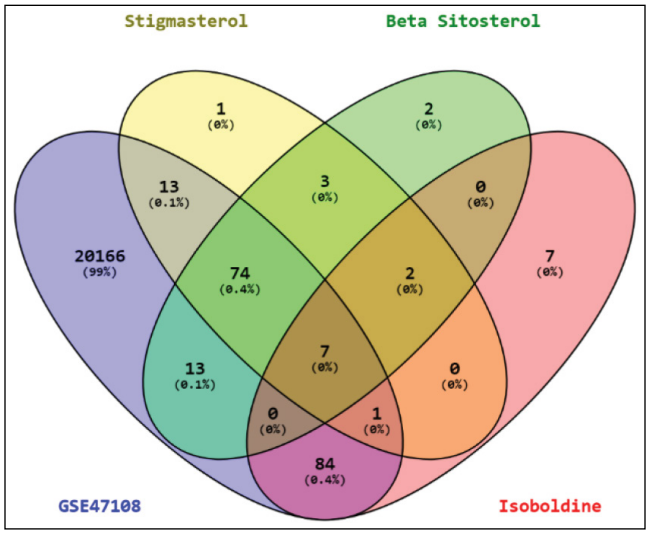


Figure 1. Venn diagram- intersection of genes between selected compounds and gene dataset.

3.5 DEG's of Prostate Cancer

The GSE47108 dataset were filtered using criteria; P-values of 0.05 and log Fold Change (FC) values of -1 and >1. A Venn diagram was drawn to visualize the overlap between the target genes of the selected compounds and the DEGs from the dataset. Figure 1

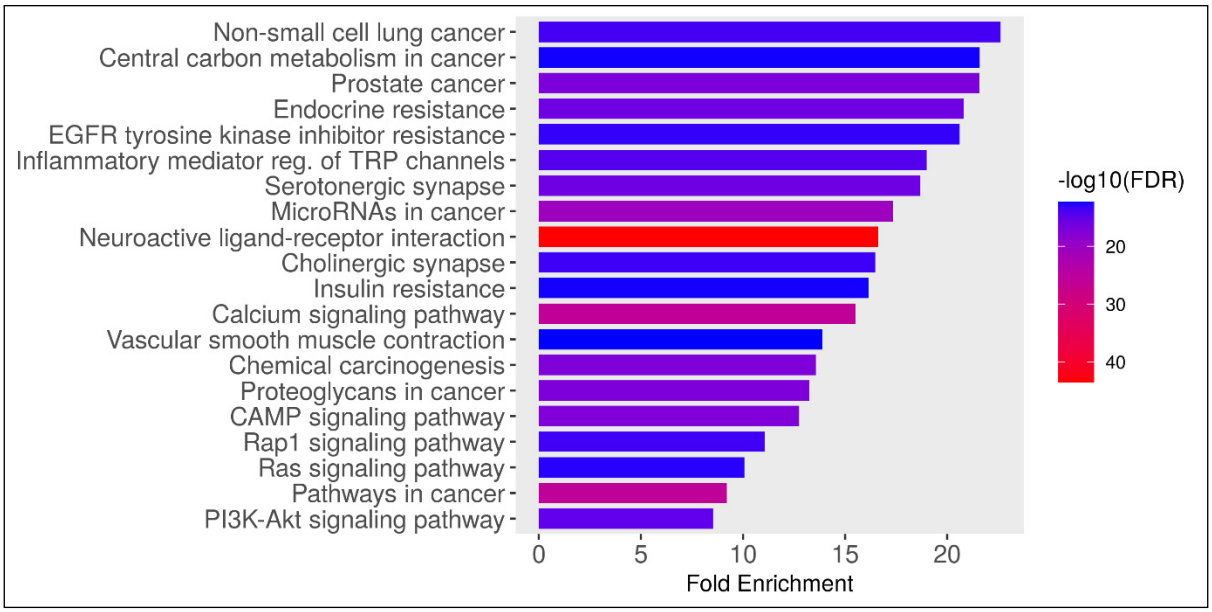


Figure 2. KEGG pathway analysis.

shows that 110 genes were identified as overlapping genes.

Venn diagram illustrating the intersection of genes between the selected bioactive compounds from *S. costus* and the gene dataset obtained from differential gene expression analysis. Overlapping genes represent potential targets shared between the bioactive compounds and the gene dataset, providing insights into the molecular mechanisms underlying the anticancer activity of *S. costus*.

3.6 Protein-Protein Interaction (PPI) and Hub Genes

PPI was constructed in STRING and network was transferred to cytoscape to identify hub genes. Top 10 hub genes are arranged in descending order based on MCC values Table 4.

3.7 Pathway Analysis

GO ontology was utilized to analyze the overlapping genes. GO terms encompass biological processes, molecular functions, and cellular components. Among the top biological processes identified were positive regulation of protein kinase B signaling, epidermal growth factor receptor signaling pathway, trans membrane receptor protein tyrosine kinase signaling pathway, peptidyl-tyrosine phosphorylation, and peptidyl-tyrosine autophosphorylation. As for cellular components, membrane, macromolecular complex, plasma membrane, dendritic growth cone, and perinuclear region of cytoplasm were prominent. The key molecular function processes included protein binding, ATP binding, protein serine or threonine or tyrosine kinase activity, identical protein binding, and protein kinase activity. Furthermore, KEGG analysis, where the P-value was less than 0.05 and the top 20 pathways were retained, revealed that these overlapping genes were primarily associated with cancer-related pathways, as depicted in Figure 2. Examples include prostate cancer and various pathways implicated in cancer.

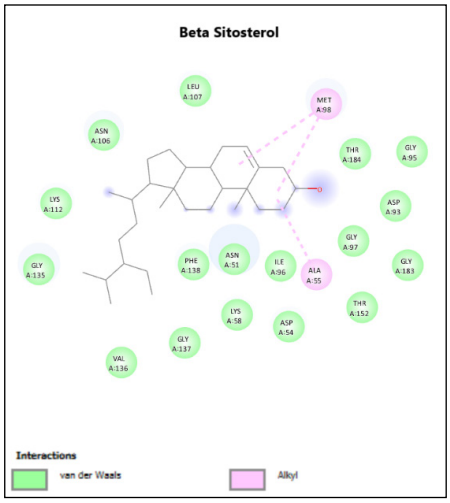
The Figure 2, highlighting pathways significantly associated with the overlapping genes between the selected bioactive compounds from *S. costus* and the gene dataset. The analysis reveals key pathways implicated in cancer, including prostate cancer and various other cancer-related pathways, shedding light on potential molecular mechanisms underlying the anticancer effects of *S. costus* compounds.

3.8 Molecular Docking

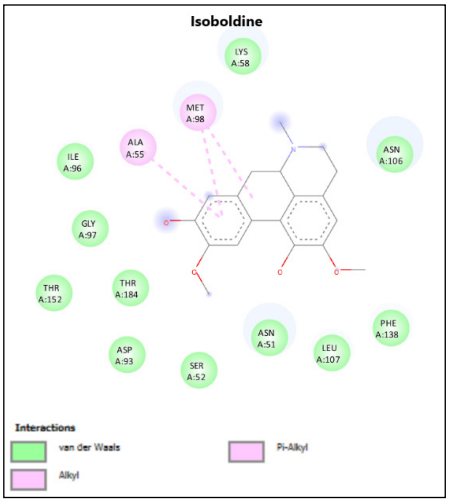
Based on the Hub genes identified KEGG pathway is identified and we selected prostate cancer signaling pathway and different pathways in cancer. Selected phytochemical compounds and standard drug was docked against 10 target proteins and binding affinity is

Table 5. Binding affinity values

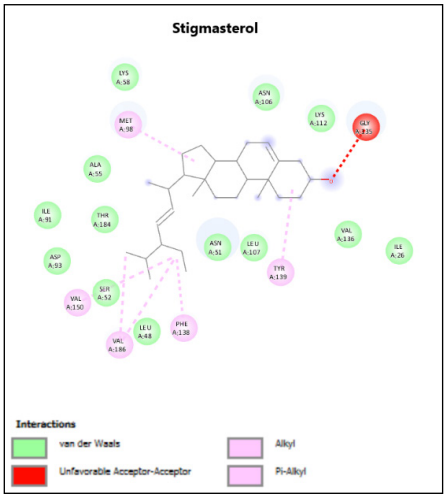
Gene Name	Binding Affinity Values			
	Beta Sitosterol	Stigmasterol	Isoboldine	Copanlisib
PIK3CA	-3	-1.5	-5.1	-5.5
MET	-6	-6.2	-5.3	-6.1
MTOR	-6.8	-7.1	-5.7	-6.7
PTPN11	-6.7	-6.7	-6.3	-7
ESR1	-6.1	-6.6	-7.1	-7.3
FGFR1	-8.1	-8.9	-7.3	-7.8
EGFR	-7.2	-7.7	-7.4	-7.9
MAPK3	-6.8	-7.6	-6.8	-8.2
HSP90AA1, EL52	-7.3	-8.7	-7.5	-8.5
SRC	-7	-7.3	-6.9	-9



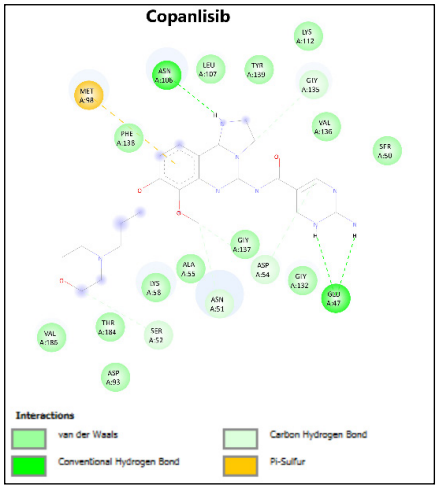
A. Beta sitosterol



B. Isoboldine



C. Stigmasterol



D. Copanlisib

Figure 3. Molecular docking of selected compounds with HSP90AA1.

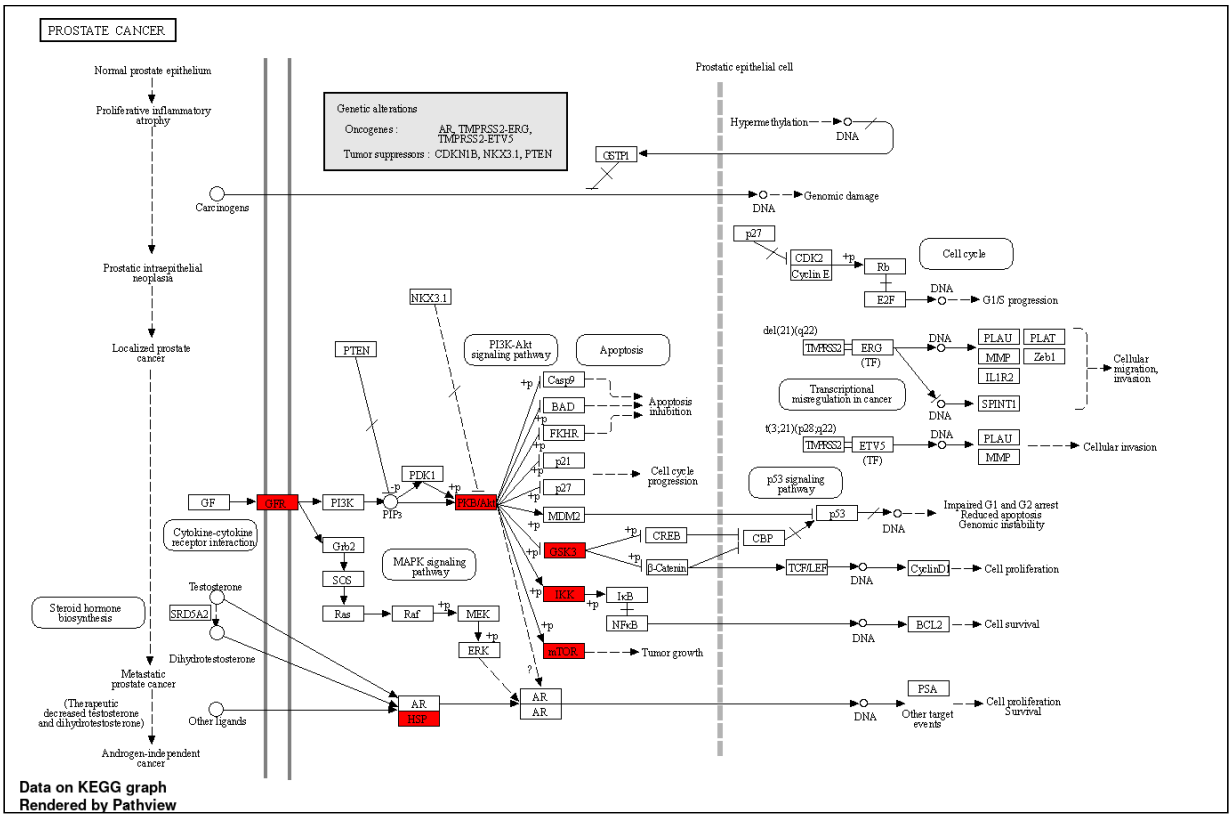


Figure 4. Pathways in cancer.

noted Table 5. The most stable conformation is selected compounds with HSP90AA1, the one with the lowest binding energy as showed in Figures 3A to 3D.

The Figure 3 depicts the molecular docking results showing the interaction between selected bioactive compounds from *S. costus* and the target protein HSP90AA1. The docking simulations reveal the binding affinity and pose of each compound within the active site of HSP90AA1. The results highlight the potential interactions and binding modes of the compounds with the target protein, providing insights into their therapeutic efficacy against cancer, particularly prostate cancer.

4. Discussion

Bioactive compounds have potential impact in human life and has history of proactive activity against many chronic diseases. *S. costus* have different bioactive out of which we selected 3 compounds stigmasterol, isoboldine, and beta sitosterol, based on their ADME and toxicity properties. We have used different online servers and virtual screening tools to identify potential activity of selected compounds. This approach not only saves time and resources but also increases the chances of identifying lead compounds with desired pharmacological properties[13].

We identified top 10 hub genes SRC, FGFR1, MET, MAPK3, HSP90AA1, PTPN11, PIK3CA, ESR1, EGFR, MTOR, each gene is acting in prostate cancer and in different pathways in cancer. The results of our virtual screening analysis selected bioactive compounds from *S. costus* that showed strong binding affinity towards molecular targets associated with cancer and when compared with standard drug copanlisib, selected compounds have shown promising results for ideal drug molecule. HSP90AA1 got lowest binding affinity value for stigmasterolis -8.7 less than standard drug copanlisib-8.5 while, isoboldine and beta sitosterol are having -7.5 and -7.3 which are good scores for drug molecule. The identification of these compounds suggests that *S. costus* may exert its anticancer effects through multiple pathways, highlighting its potential as a source of multi-targeted therapeutics[14].

Pathway enrichment analysis indicated that 15 pathways are closely associated with the 10 hub genes. Majorly, pathways are focused on different types of cancers. In this study we tried to focus on prostate cancer and common pathway for various cancer. Genes acting in prostate cancer pathway HSP, PI3K/AKT signaling pathway are importance and induction of these pathways may reduce chances of prone to cancer. In Figure 4, the identification of specific pathways can guide further research on the potential synergistic effects of *S. costus* compounds with existing anticancer drugs, leading to the development of combination therapies with enhanced efficacy[15].

The pathways highlighted are closely associated with the genes overlapping between the selected bioactive compounds from *S. costus* and the gene dataset. The analysis indicates the involvement of these pathways in various aspects of cancer development and progression, including prostate cancer.

5. Conclusion

The identification of targeted pathways affected by *S. costus* compounds is crucial for understanding the underlying molecular mechanisms involved in cancer development and progression. This knowledge can guide the design of more effective therapeutic strategies and facilitate the development of optimized treatment regimens. However, it is important to note that virtual screening is a computational prediction method, and the results obtained should be further validated through experimental studies. *In vitro* and *in vivo* experiments are necessary to confirm the bioactivity and efficacy of the identified compounds. These experiments should include cell viability assays, apoptosis induction studies, cell cycle arrest analysis, and evaluation of tumour growth inhibition using relevant cancer cell lines and animal models.

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