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Research Article

San Huang Decoction as an effective treatment for oral squamous cell carcinoma based on network pharmacology

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Abstract: Oral squamous cell carcinoma (OSCC) represents a major global health concern due to its aggressive nature, late-stage diagnosis, and resistance to conventional treatments. San Huang Decoction (SHD) is a traditional Chinese medicine formulation consisting of Astragalus membranaceus (Huangqi), Rheum officinale (Dahuang), and Curcuma longa. However, the specific pharmacological mechanisms underlying SHD's effects on OSCC remain inadequately explored. This study aims to delineate the therapeutic potential of SHD in the treatment of OSCC. We identified 11 active compounds within SanHuang decoction, including key components such as calycosin, formononetin, curcumol, and kaempferol. By integrating these compounds with gene expression data from the GSE74530 and GSE23558 datasets, we identified 257 differentially expressed genes (DEGs) that overlap with SHD targets and OSCC-specific mutated genes. Pathway enrichment analysis revealed that these DEGs are involved in critical biological processes and pathways such as "Platinum drug resistance," "EGFR tyrosine kinase inhibitor resistance," and "PI3K-AKT signaling pathway." Furthermore, protein-protein interaction (PPI) network analysis identified five key hub genes, including AKT1, MMP9, EGFR, STAT1, and ERBB2, which play pivotal roles in cancer progression, metastasis, and resistant to therapy. Our findings highlight the value of integrating TCM's holistic principles with the development of novel strategies to enhance immune responses in OSCC patients.

Keywords: Isan Huang Decoction (SHD); Oral Squamous Cell Carcinoma (OSCC); Network Pharmacology; Molecular docking; Chinese medicine

1. Introduction

Oral squamous cell carcinoma (OSCC) represents a major global health concern, accounting for a significant percentage of head and neck cancers. The World Health Organization estimates around 377,713 new cases of oral cavity cancers annually, with OSCC comprising the majority of these diagnoses(1). Known for its aggressive behavior, high recurrence rates, and multifaceted etiologies, OSCC significantly impacts public health(2). OSCC is linked to environmental and lifestyle factors, including tobacco and alcohol use, alongside genetic predispositions(3). The disease is often accompanied by comorbidities such as anxiety, depression, and nutritional deficiencies, which further complicate treatment and reduce patient quality of life(4). Standard treatments—surgery, radiation, and chemotherapy—primarily address localized disease but frequently cause significant side effects and exhibit limited efficacy in advanced stages(5). Notably, resistance to conventional therapies is observed in approximately 30% of patients, underscoring the urgent need for innovative strategies(6). Among chemotherapeutics, 5-fluorouracil(5-FU) is widely employed due to its efficacy in inhibiting DNA synthesis and inducing apoptosis in cancer cells(7). Despite its benefits, 5-FU is associated with resistance mechanisms and adverse effects, necessitating the exploration of complementary therapies(8).

Sanhuang Decoction, a well-known TCM formulation, comprises Astragalus membranaceus (Huangqi), Rheum officinale (Dahuang), and Curcuma longa (Jianghuang), and is recognized for its potential anticancer properties(9). The decoction is believed to exert synergistic effects by modulating immune function, reducing inflammation, and improving blood circulation(10). Preliminary studies has demonstrated the ability of SHD to inhibit triple-negative breast cancer (TNBC) by suppressing the phosphorylation of the JAK2-STAT3 signaling pathway, which may be associated with the modulation of lipid metabolism. This effect highlights the potential of SHD as a therapeutic agent in cancer treatment(11). Furthermore, individual herbal components have demonstrated anti-inflammatory, antioxidant, and immune-enhancing properties that collectively contribute to its anticancer potential(12).

Despite its promising attributes, the pharmacological mechanisms of Sanhuang Decoction in OSCC remain

underexplored. Tools like molecular docking offer powerful methodologies to study interactions between 5-FU and active herbal compounds, elucidating binding affinities and modes of action(13). Additionally, network pharmacology provides a comprehensive framework for understanding the complex multi-component and multi-target mechanisms characteristic of TCM formulations. By integrating systems biology and pharmacology, network pharmacology highlights how individual compounds within Sanhuang Decoction act synergistically to influence cancer-related pathways(14). Through the identification of active compounds, the construction of disease-related interaction networks, and molecular docking studies, we aim to delineate the mechanisms underlying Sanhuang Decoction's effects on OSCC. By bridging traditional herbal knowledge with modern scientific approaches, these findings hold promise for improving therapeutic outcomes in OSCC management.

2. Methods

2.1. Identification of Active Ingredients and Targets for OSCCs: To investigate the active ingredients of Sanhuang Decoction and their therapeutic targets for oral squamous cell carcinoma(OSCC), a systematic methodology was employed. Initially, a comprehensive literature review was conducted utilizing databases such as PubMed, Web of Science, and the China National Knowledge Infrastructure(CNKI). The search utilized keywords including "Sanhuang Decoction," "active ingredients," and "oral squamous cell carcinoma" to compile a list of chemical constituents recognized for their pharmacological effects. Following this, the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) was utilized to identify potential targets associated with these active ingredients. The identified target names were subsequently converted to gene symbols using the UniProt database for further analysis. To ensure the selection of viable therapeutic candidates, Lipinski's rule of five was applied to filter the active compounds based on drug-likeness criteria. Ultimately, 11 active compounds were selected, comprising 10 from Huangqi and one from Dahuang, as illustrated in (Figure 1).

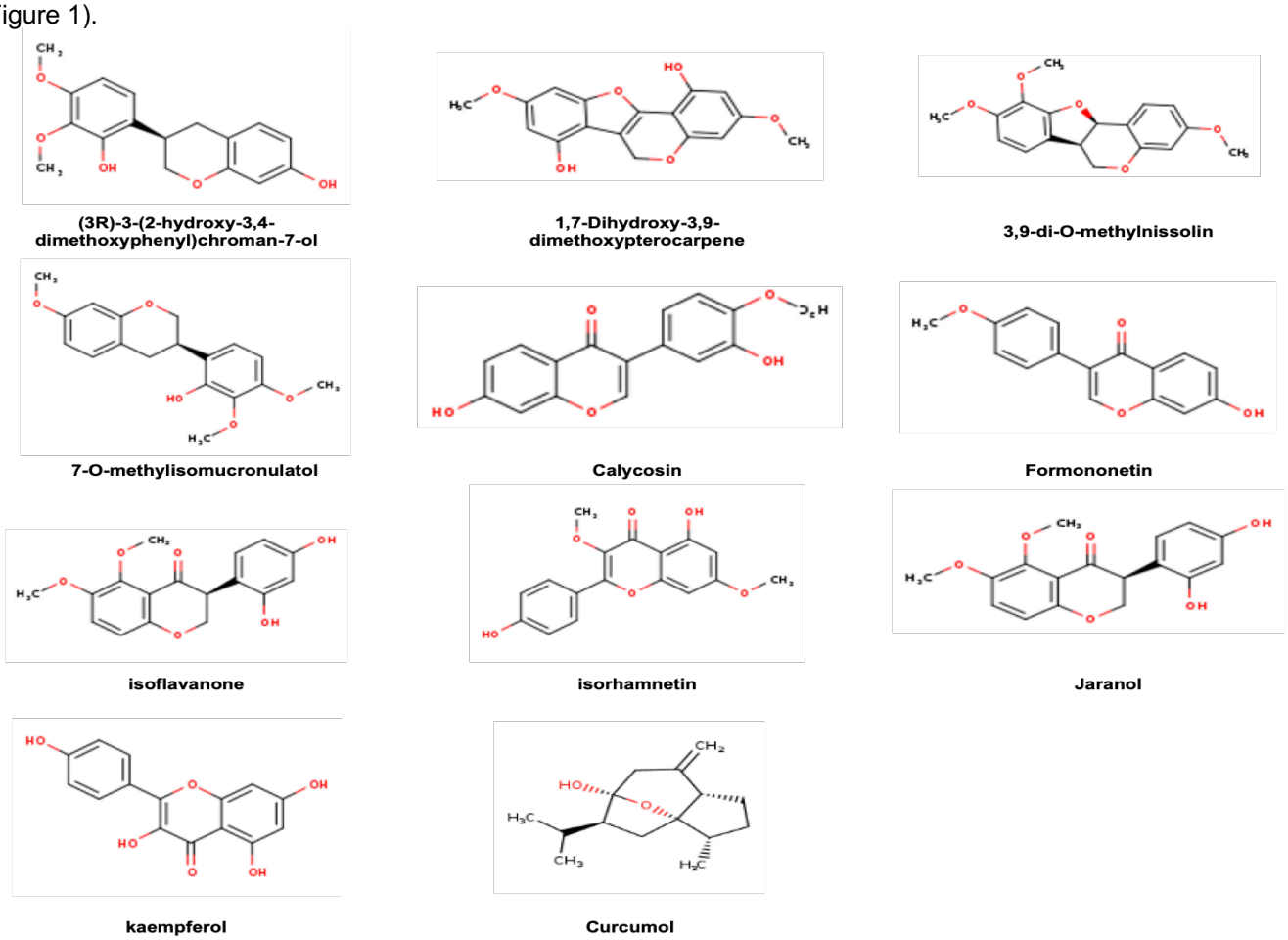

Figure 1. Structures of active compounds in Sanhuang Decoction (SHD).

2.2. Identification of Differentially Expressed Genes: To identify differentially expressed genes (DEGs) in OSCC, we employed the GEO2R tool, a user-friendly web application for analyzing gene expression data from the Gene Expression Omnibus (GEO)(15). We analyzed two datasets: GSE74530, which consists of gene expression profiles from oral squamous cell carcinoma (OSCC) tissues obtained using the Affymetrix Human Genome U133 Plus 2.0 Array, and GSE23558, which compares OSCC patients to healthy controls using Agilent microarrays. Both datasets were accessed

through the GEO database. Subsequently, the Fragments per Kilobase Million (FPKM) data were normalized to transcripts per kilobase million (TPM) values. For our analysis, we set a significance threshold of adjusted p-value < 0.05 and log2 fold change > 1 to robustly identify significant gene alterations relevant to OSCC pathology (GSE74530: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74530; GSE23558: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23558).

2.3. Identification of Common Genes Across Gene Expression Datasets and OSCC Mutated Genes: To identify common genes among the gene expression datasets GSE74530, GSE23558, the targets of SHD active compounds, and mutated genes in oral squamous cell carcinoma (OSCC), we utilized Venny 2.0 (https://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html), an interactive tool designed for visualizing relationships among multiple gene sets. This tool allows users to input lists of gene identifiers, which it processes to generate Venn diagrams that effectively display overlaps and unique elements among the datasets. The mutated genes specific to OSCC were sourced from cBioPortal, facilitating a comprehensive comparison. By employing Venny, we were able to visualize and analyze the common targets across these datasets, thus enhancing our understanding of the molecular underpinnings of OSCC mentioned in (Table 1).

2.4. Pathway Enrichment Analysis: To perform gene enrichment analysis, we utilized the ShinyGO (http://bioinformatics.sdstate.edu/go/). We input a list of common genes into the tool, selecting appropriate organism settings for analysis. The analysis included functional enrichment for Gene Ontology (GO) terms, KEGG pathways, and Reactome pathways. We set significance thresholds at a p-value of <0.05 and employed Benjamini-Hochberg correction for multiple testing. The results were visualized through charts and dot plots, highlighting enriched biological functions and pathways associated with our gene list(16).

2.5. PPI network and hub gene identification: To identify hub genes, we utilized Cytoscape in conjunction with the STRING database and the CytoHubba plugin(17). Initially, we constructed a protein-protein interaction (PPI) network by retrieving interaction data for our common gene targets list from the STRING database, setting a minimum interaction score of 0.4 to ensure reliable connections. The resulting network was imported into Cytoscape for visualization and further analysis. We employed three algorithms available in CytoHubba: EPC (Edge Percolated Component), Degree, and Bottleneck, to evaluate the importance of each node based on their connectivity and centrality measures. The top-ranked genes from these analyses were considered hub genes, representing key regulatory nodes within the network.

2.6. External validation UALCAN: To analyze the expression of hub genes, we utilized the UALCAN tool (https://ualcan.path.uab.edu/), a comprehensive online resource for cancer data analysis. Initially, we accessed the UALCAN platform and selected our cancer type of interest from the left panel. We then input the official symbols of the identified hub genes into the search area and selected the relevant TCGA dataset for analysis. UALCAN provided us with detailed expression profiles of these hub genes across various cancer subtypes and normal tissues, allowing us to visualize differences in expression levels through box plots and heat maps. Additionally, we evaluated survival data associated with these genes to assess their prognostic significance in cancer patients(18).

2.7. Molecular Docking: To perform molecular docking we used CB-Dock2.0 (https://cadd.labshare.cn/cb-dock2/index.php) and Discovery Studio 3.5 (https://discover.3ds.com/discovery-studio-visualizer-download). for analyzing the interactions between ligands and key proteins associated with oral squamous cell carcinoma (OSCC). The initial step involved preparing the protein structures of the hub genes: AKT1 (PDB ID: 6HHG), EGFR (PDB ID: 1WT5), MMP9 (PDB ID: 1L6J), ERBB2 (PDB ID: 2A91), and STAT1 (PDB ID: 8D3F) and ensuring it was free of water molecules and heteroatoms. Using CB-Dock2, we uploaded the protein structure along with the ligand files in MOL2 or SDF format. The tool automatically detected binding cavities and performed blind docking, integrating both structure-based and template-based docking methods to enhance accuracy in binding pose prediction. Subsequently, we utilized Discovery Studio for further analysis and visualization of the docking results, allowing us to assess binding affinities and interactions between the ligand and protein(19).

3. Results

3.1. Identification of Active Ingredients and Targets for OSCCs: The investigation into the active components of Sanhuang Decoction revealed a total of 150 components from Astragalus membranaceus (Huangqi), 460 from Rheum officinale (Dahuang), and 200 from Curcuma longa (Jianghuang). From this extensive analysis, we focused on selecting 11 key active compounds based on their compliance with Lipinski's rule of five, which assesses drug-likeness and potential bioavailability. The selected compounds include:(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol,1,7-dihydroxy-3,9 dimethoxypterocarpene, 3,9-di-O-methylnissolin, 7-O-methylisomucronulatol, calycosin, formononetin, isoflavanone, isorhamnetin, Jaranol, kaempferol, and Curcumol. These compounds were chosen for their promising pharmacological profiles and potential therapeutic effects against oral squamous cell carcinoma (OSCC), as indicated

by their favorable properties in the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) its compound target network was made as in (Figure 2).

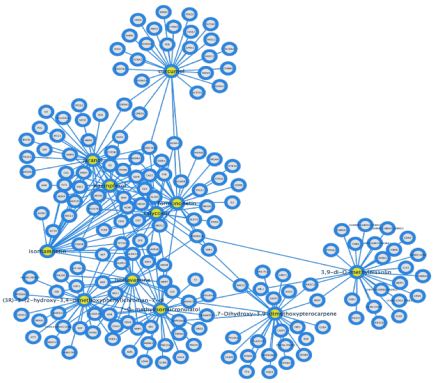

Figure 2. **Compound-Target Network of Selected TCM Compounds.** Yellow nodes represent Traditional Chinese Medicine (TCM) compounds, and blue nodes represent target proteins. The connections indicate interactions between these compounds and their corresponding protein targets, showcasing the potential multi-target effects of key compounds like isorhamnetin, kaempferol, and isoflavanone.

3.2. Pharmacological Insights into Active Compounds from Astragalus membranaceus and Rheum officinale: We identified a total of 11 active compounds from the herbal components of Sanhuang Decoction, specifically from Astragalus membranaceus (Huangqi) and Rheum officinale (Dahuang). These compounds include (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol, 1,7-dihydroxy-3,9-dimethoxypterocarpene, 3,9-di-O-methylnissolin, 7-O-methylisomucronulatol, calycosin, formononetin, isoflavanone, isorhamnetin, Jaranol, kaempferol, and Curcumol. Each compound exhibits unique molecular characteristics that suggest potential therapeutic benefits. For instance, (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol has a molecular weight of 302.32 g/mol and a logP of 2.57, indicating moderate lipophilicity which may enhance its bioavailability (score: 0.55). Similarly, 1,7-dihydroxy-3,9-dimethoxypterocarpene and 3,9-di-O-methylnissolin possess favourable properties with bioavailability scores of 0.55 and 0.48 respectively. Notably, compounds like isorhamnetin and kaempferol have lower logP values (1.65 and 1.58) but high TPSA values (120.36 Å² and 111.13 Å²), suggesting they may interact effectively with biological targets while maintaining solubility. The presence of multiple hydrogen bond donors and acceptors across these compounds indicates potential for strong molecular interactions within biological systems. Curcumol from Dahuang stands out with a molecular weight of 236.35 g/mol and a logP of 2.90, highlighting its potential as an effective therapeutic agent due to its favorable absorption characteristics (Table 1). Collectively, these findings emphasize the pharmacological potential of the selected compounds in Sanhuang Decoction for treating conditions such as oral squamous cell carcinoma (OSCC), warranting further investigation into their mechanisms of action and clinical efficacy in cancer therapy.

Table 1. The ADME details of the *active compounds in SHD* used.

S.No	Name	Source	Molecular Weight (g/mol)	LogP	TPSA (Å²)	Bioavailability Score	H-bond Donors	H-bond Acceptors	OB	DL
1	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	Huangqi	302.32 g/mol	2,57	68.15 Å²	0,55	2	5	67,67	0,26
2	1,7-Dihydroxy-3,9-dimethoxypterocarpene	Huangqi	314.29 g/mol	2.55	81.29 Å²	0,55	2	6	39,05	0,48
3	3,9-di-O-methylnissolin	Huangqi	314.33 g/mol	2,84	46.15 Å²	0,55	0	5	53,74	0,48
4	7-O-methylisomucronulatol	Huangqi	316.35 g/mol	2,98	57.15 Å²	0,55	1	5	74,69	0,3

5	calycosin	Huangqi	284.26 g/mol	2,3	79.90 Å²	0,55	2	5	47,75	0,24
6	formononetin	Huangqi	268.26 g/mol	2,66	59.67 Å²	0,55	1	4	69,67	0,21
7	isoflavanone	Huangqi	316.31 g/mol	2,01	85.22 Å²	0,55	2	6	109,99	0,3
8	isorhamnetin	Huangqi	316.26 g/mol	1,65	120.36 Å²	0,55	4	7	49,6	0,31
9	Jaranol	Huangqi	314.29 g/mol	2,36	89.13 Å²	0,55	2	6	50,83	0,29
10	kaempferol	Huangqi	286.24 g/mol	1,58	111.13 Å²	0,55	4	6	41,88	0,24
11	Curcumol	Jianghuang	236.35 g/mol	2,9	29.46 Å²	0,55	1	2		

3.3. Analysis of Common Genes Across OSCC Gene Expression Datasets: The analysis of differentially expressed genes (DEGs) in oral squamous cell carcinoma (OSCC) utilized two datasets, GSE74530 and GSE23558, to identify significant gene alterations associated with the disease. GSE74530 included samples from six OSCC patients and six normal adjacent tissues, revealing 885 upregulated and 315 downregulated genes based on a significance threshold of $P < 0.05$ and $|\log2 \text{ fold change}| \geq 1.0$ (Figure 3A). In contrast, GSE23558, which comprised 27 OSCC patients and five healthy controls, yielded a much larger set of DEGs, with 2805 upregulated and 3863 downregulated genes (Figure 3B). The integration of these datasets using Venny 2.0 facilitated the identification of common genes across the datasets, targets of SHD active compounds, and mutated genes in OSCC (Figure 3C). A total of 257 overlapping genes were found. Notably, three genes were shared among GSE74530, SHD compound targets, and OSCC genes, while 21 genes were common to the compound targets and OSCC genes alone. Additionally, five genes overlapped between GSE74530 and OSCC genes, and ten were shared between GSE23558 and OSCC genes. The datasets also exhibited extensive overlap, with 154 common genes identified between GSE23558 and GSE74530. Among the significant overlapping genes were EPHB2, CDK4, IL18, BCL2, SLC2A1, PIK3CD, MMP7, MMP1, PLAUR, and MTOR (Table 2).

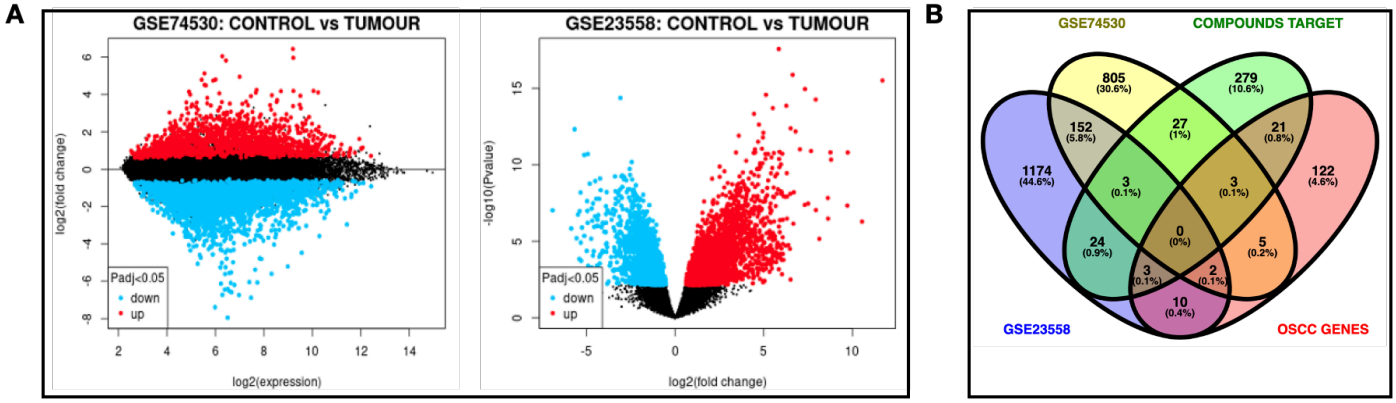

Figure 3. Differential Gene Expression and Overlap in OSCC samples. (A) The figure shows volcano plots for two datasets (GSE74530 and GSE23558), comparing gene expression in tumor versus control samples. Upregulated genes are indicated in red, downregulated genes in blue, and non-significant genes in black. (B) The Venn diagram below displays the overlap of differentially expressed genes between the two datasets, their intersection with compound targets, and OSCC genes, highlighting shared and unique genes across the categories.

Table 2. This table highlights genes identified as common across datasets and categories analyzed via Venn diagrams: GSE23558 and GSE74530: Gene expression datasets. COMPOUNDS TARGET: Genes targeted by therapeutic agents and OSCC GENES: Genes linked to oral squamous cell carcinoma. Each row shows genes shared among two or three datasets, representing significant overlaps relevant to gene function, therapy, or disease pathways.

3 common elements in "GSE74530", "COMPOUNDS TARGET" and "OSCC GENES":	21 common elements in "COMPOUNDS TARGET" and "OSCC GENES":	5 common elements in "GSE74530" and "OSCC GENES":	10 common elements in "GSE23558" and "OSCC GENES":	2 common elements in "GSE23558", "GSE74530" and "OSCC GENES":	3 common elements in "GSE23558", "COMPOUNDS TARGET" and "OSCC GENES":	3 common elements in "GSE23558", "GSE74530" and "COMPOUNDS TARGET":	154 common elements in "GSE23558" and "GSE74530":	27 common elements in "GSE23558" and "COMPOUNDS TARGET":	30 common elements in "GSE74530" and "COMPOUNDS TARGET":
EPHB2	CDK4	IL18	BCL2	SLC2A1	PIK3CD	SLC6A4	SLITRK5	MME	HPGD
MMP7	MMP1	FILIP1L	ARHGAP24	PLAUR	MMP9	PTK2	CRYM	CD38	AR
MMP2	MTOR	RECK	SOX2		TERT	NEK2	CYSLTR1	BCHE	PTK6
	PIK3CB	RASGRP3	HPSE				LOX	PRKCB	AKR1A1
	PIK3CG	CCL2	KLF4				ZNF254	PDE2A	LRRK2
	PIK3CA		ZNF582				SLC25A23	VCAM1	ACVR1
	CASP3		XRCC3				CD24	LNPEP	VCP
	EGFR		PSMD9				GRHL1	MAPT	CTSS
	AKT1		VEGFA				FAM189A2	SPHK1	INSR
	MAPK1		FAT1				YOD1	PDE7A	MET
	CA9						CIART	ADAMTS4	IDO1
	ABCB1						ABHD5	CDC25B	APP
	PARP1						PKIB	HDAC9	SLC1A3
	CYP1A1						FOXE1	HSD17B1	PDE10A
	KDM5B						ARHGAP20	SLC22A12	EPHB2
	TLR9						NFIX	CDC25A	ADAM10
	ERBB2						TNFRSF11A	CDK6	KDR
	IL2						MPP7	HCRTR1	CA2
	TNF						CDH11	PIK3CD	BMP1
	PTGS2						RHOJ	PRKCA	CSNK1D

	MDM2						CNFN	AURKB	PDGFRB

3.4. Visualization and Interpretation of Hub Gene Connectivity in the PPI Network: The Protein-Protein Interaction (PPI) network analysis, conducted using the STRING database, provided significant insights into the interactions among proteins associated with oral squamous cell carcinoma (OSCC). The constructed network comprised 151 nodes and 551 edges, indicating a dense interaction pattern with an average node degree of 7.3, suggesting that each protein interacts with approximately 7.3 other proteins. This high interconnectivity reflects a robust and complex network, as evidenced by an average local clustering coefficient of 0.494, which indicates a moderate level of clustering where proteins tend to form tightly interconnected groups.

Statistical analysis revealed that the expected number of edges for a random network of this size was only 258, while the actual network exhibited 551 edges, demonstrating a significantly higher degree of connectivity than would be expected by chance. The PPI enrichment p-value was calculated to be less than 1.0e-16, confirming that the observed interactions are statistically significant and unlikely to have occurred randomly (Figure 4). These findings suggest that the proteins within this network are functionally associated and likely play coordinated roles in OSCC-related biological processes, including tumor progression, signaling pathways, and therapeutic resistance. The substantial number of interactions observed indicates the biological complexity inherent in OSCC and highlights the cooperative nature of these proteins in disease mechanisms. Consequently, these proteins may represent potential targets for therapeutic interventions aimed at improving patient outcomes in OSCC.

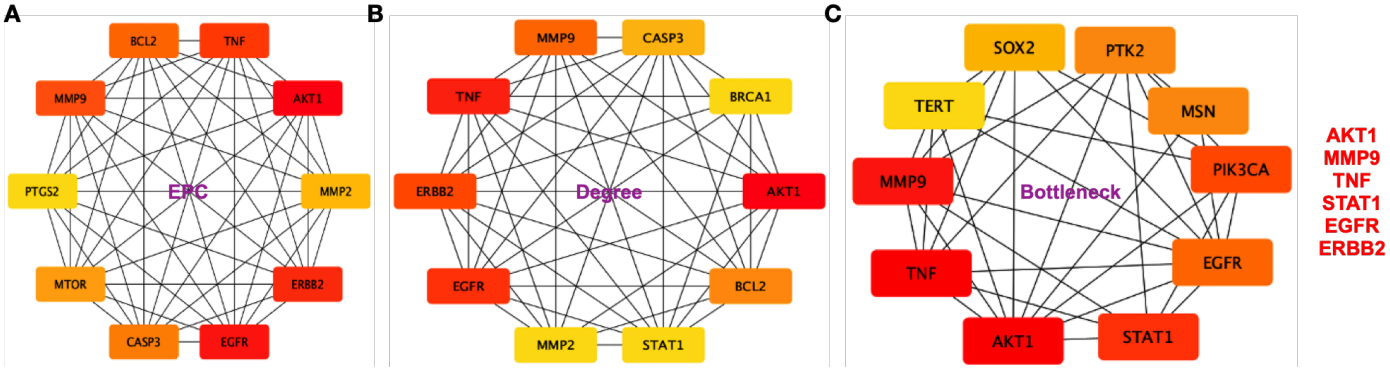

Figure 4. Network Analysis of Key Genes in OSCC. This figure illustrates the identification of hub genes from gene interaction networks generated using Cytoscape. (A) EPC Network (Left): Displays the Enhanced PageRank Centrality (EPC) of genes, highlighting hub genes (e.g., AKT1, MMP9) by color gradients representing their centrality in the network. (B) Degree Network (Middle): Illustrates gene connectivity, pinpointing AKT1 as a prominent hub, indicating its significant role in the network's structure. (C) Bottleneck Network (Right): Identifies bottleneck genes crucial for maintaining network integrity, with highlighted genes such as AKT1 and MMP9 suggesting potential therapeutic targets.

To identify key regulatory nodes within this network, we employed three algorithms from the CytoHubba plugin: EPC (Edge Percolated Component), Degree, and Bottleneck (Figure 3). These algorithms evaluate the importance of each gene based on its connectivity and centrality within the network. The results from this analysis pinpointed several hub genes, including AKT1, MMP9, EGFR, STAT1, and ERBB2, as the most influential nodes in the network. These hub genes are highly interconnected and play central roles in various cellular processes such as cell signaling, inflammation, and immune response, making them particularly valuable for further investigation. Their identification as hub genes suggests that they could be key targets for therapeutic strategies aimed at modulating these biological pathways.

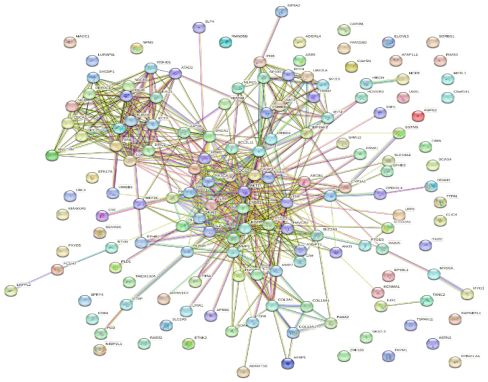

Figure 5. Protein-Protein Interaction (PPI) Network. This Figure displays a protein-protein interaction (PPI) network generated using STRING. Nodes represent proteins, with node size indicating interaction strength; edges represent established interactions.

3.5. GO gene enrichment analysis and KEGG pathway annotation: The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed significant biological insights related to oral squamous cell carcinoma (OSCC). In the GO analysis, the term "Response to oxygen-containing compound" was associated with 39 observed genes out of a background of 1547, yielding a strength of 0.52 and a false discovery rate (FDR) of 4.87E-07, with notable proteins including MAPK1, MMP2, IL2, and EGFR. The "Cellular response to organonitrogen compound" term showed 23 observed genes with an FDR of 1.22E-06, highlighting proteins such as MAPK1, MDM2, and PIK3CA. Additionally, "Intracellular signal transduction," with 36 observed genes and an FDR of 5.71E-06, included key proteins like MAPK1 and EGFR. Other significant terms included "Cellular response to endogenous stimulus," "Response to organic substance," and "Regulation of programmed cell death," each demonstrating substantial gene involvement and low FDRs. In the KEGG pathway analysis, the "Platinum drug resistance" pathway was identified with 9 observed genes and an FDR of 2.25E-06, featuring proteins such as MAPK1, MDM2, and PIK3CA. The "C-type lectin receptor signaling pathway" also emerged as significant, with 10 observed genes and an FDR of 2.25E-06, including MAPK1 and STAT1. Furthermore, the "AGE-RAGE signaling pathway in diabetic complications" showed similar significance with 10 observed genes and an FDR of 2.25E-06. Notably, the "EGFR tyrosine kinase inhibitor resistance" pathway included 8 observed genes with an FDR of 1.02E-05, reinforcing the role of EGFR in therapeutic resistance mechanisms. Overall, these analyses underscore the complex regulatory networks involved in OSCC development and highlight potential therapeutic targets that could be explored for intervention strategies aimed at improving patient outcomes. (Figure 5).

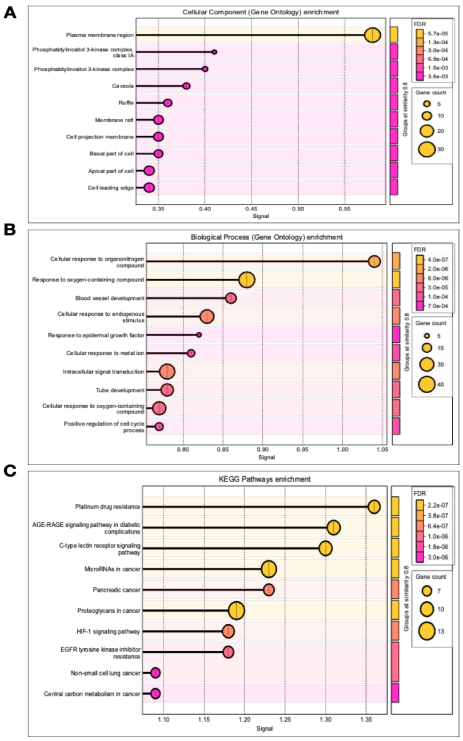

Figure 6. **Functional evaluation.** (A) and (B) belong to Gene Ontology terms enrichement analysis and (C) presents KEGG Pathway Enrichment Analysis. This Figure presents the results of gene enrichment analysis, highlighting significant Cellular Component (A), Biological Process (B), and KEGG Pathway enrichments (C). The x-axis represents signal values, while the size of the circles indicates the number of genes associated with each category. The false discovery rate (FDR) is also indicated, demonstrating the statistical significance of each enrichment.

3.6. External validation UALCAN: The mRNA expression and survival analyses for AKT1, MMP9, EGFR, STAT1, and ERBB2 were performed using the UALCAN platform. This analysis revealed that elevated expression levels of these genes are significantly associated with various clinical outcomes in patients with oral squamous cell carcinoma (OSCC) (Figure 7). For AKT1, increased mRNA expression was correlated with poorer overall survival rates, suggesting its role in promoting tumor growth and progression. MMP9 also exhibited similar trends, with high expression levels linked to worse survival outcomes, indicating its involvement in extracellular matrix remodeling and metastasis. EGFR showed a strong association between high expression and reduced survival rates, highlighting its critical role in signaling pathways that drive OSCC progression. STAT1's expression was similarly linked to unfavorable survival outcomes, suggesting its potential involvement in immune evasion mechanisms within the tumor microenvironment. Finally, ERBB2 was found to have elevated expression levels associated with poor prognosis, reinforcing its significance as a therapeutic target in OSCC (Figure 8).

3.7. Molecular modelling: Docking of the active components Molecular docking studies were conducted to evaluate the binding affinities of various compounds to key proteins associated with oral squamous cell carcinoma (OSCC), specifically AKT1, EGFR, MMP9, STAT1, and ERBB2 (Table 3). The results indicated that the compound (3R)-3-(2-hydroxy-3,4-

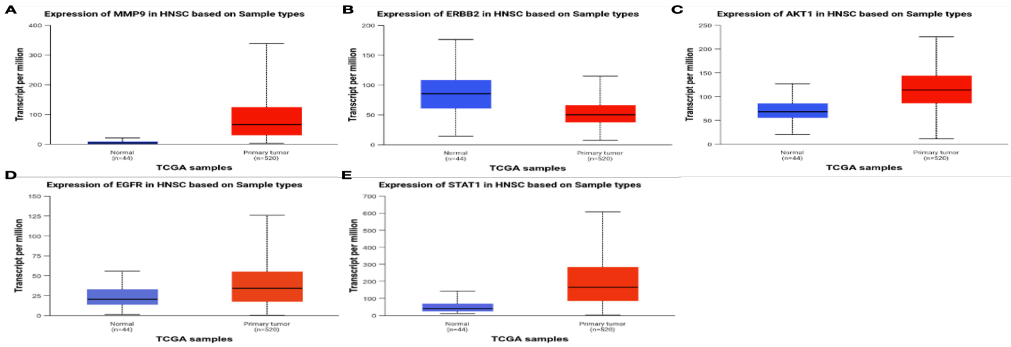

Figure 7. Expression of Key Genes in Tumor vs. Normal Samples. Boxplots showing the expression levels of (A) MMP9, (B) AKT1, (C) ERBB2, (D) IGF1R, and (E) STAT1 in head and neck squamous cell carcinoma (HNSCC)) samples from TCGA. Blue boxes represent normal samples, while red boxes indicate primary tumor sample.

dimethoxyphenyl)chroman-7-ol exhibited the strongest binding affinity across multiple proteins, with values of -9.2 kcal/mol for AKT1, -8.5 kcal/mol for EGFR, -9.7 kcal/mol for MMP9, -6.7 kcal/mol for STAT1, and -8.2 kcal/mol for ERBB2. In comparison, the standard chemotherapeutic agent 5-fluorouracil (5FU) displayed lower binding affinities of -5.2 kcal/mol for AKT1 and -5.7 kcal/mol for EGFR, indicating that the tested compounds may offer superior binding potential to these targets than 5FU.Among the other compounds tested, Calycosin and formononetin also showed promising affinities, particularly with MMP9 (-10.1 kcal/mol and -9.9 kcal/mol respectively), suggesting their potential as effective inhibitors in OSCC treatment strategies (Figure 9). Overall, the docking results highlight the potential of these natural compounds to interact more favorably with critical proteins involved in OSCC compared to 5FU, which may lead to novel therapeutic avenues for enhancing treatment efficacy in this malignancy.

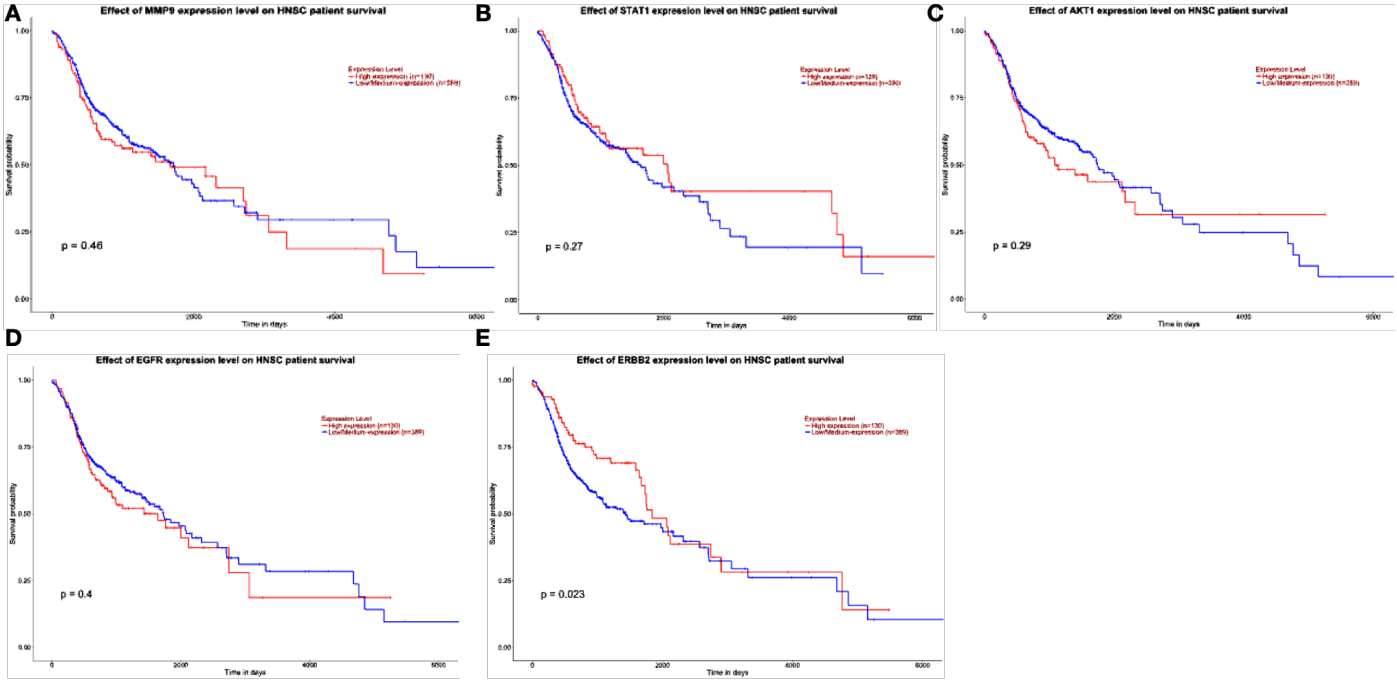

Figure 8. Kaplan-Meier Survival Curves for Selected Protein Expression Levels in HNSC Patients. Each plot shows the effect of different protein expression levels on BRCA patient survival. The red and blue lines represent high and low expression groups, respectively. The proteins analyzed are (A) ERBB2, (B) EGFR, (C) AKT1, (D) STAT1, and (E) MMP9. p-values are indicated for each plot, showing the statistical significance of survival differences between expression groups. Notably, ERBB2 shows a significant effect on survival with a p-value of 0.023.

Table 3. It compares the docking scores of active compounds from Sanhuang Decoction with key hub genes (AKT1, EGFR, MMP9, STAT1, and ERBB2) against the chemotherapeutic agent 5-fluorouracil (5-FU).

Protein name	PDB ID	Compound	Binding affinity (kcal/mol)		
AKT1	6HHG	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	-9.2	5FU	-5.2
		1,7-Dihydroxy-3,9-dimethoxypterocarpene	-9.1		
		3,9-di-O-methylnissolin	-8.8		
		7-O-methylisomucronulatol	-8.5		
		Calycosin	-6.7		
		formononetin	-6.8		
		isoflavanone	-8.9		
		isorhamnetin	-7.5		
		Jaranol	-6.7		
		kaempferol	-7.7		
		curcumol	-7.7		

EGFR	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	-8.5	5FU	-5.7
	1,7-Dihydroxy-3,9-dimethoxypterocarpene	-9		
	3,9-di-O-methylnissolin	-7.2		
	7-O-methylisomucronulatol	-6.5		
	Calycosin	-8.1		
	formononetin	-7.8		
	isoflavanone	-7.7		
	isorhamnetin	-7.6		
	Jaranol	-8.7		
	kaempferol	-9.2		
	curcumol	-6.7		
MMP9	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	-9.7	5FU	-6.1
	1,7-Dihydroxy-3,9-dimethoxypterocarpene	-8.4		
	3,9-di-O-methylnissolin	-9		
	7-O-methylisomucronulatol	-6.8		
	Calycosin	-10.1		
	formononetin	-9.9		
	isoflavanone	-10.3		
	isorhamnetin	-8.8		
	Jaranol	-8		
	kaempferol	-9.2		
	curcumol	-7		
STAT1	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	-6.7	5FU	-4.6
	1,7-Dihydroxy-3,9-dimethoxypterocarpene	-7.1		
	3,9-di-O-methylnissolin	-6.9		
	7-O-methylisomucronulatol	-6.9		
	Calycosin	-6.9		
	formononetin	-7.1		
	isoflavanone	-7.1		
	isorhamnetin	-7.4		
	Jaranol	-6.6		
	kaempferol	-7.1		
	curcumol	-5.9		

ERBB2	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	-8.2	5FU	-5.2
	1,7-Dihydroxy-3,9-dimethoxypterocarpene	-8.3		
	3,9-di-O-methylnissolin	-8		
	7-O-methylisomucronulatol	-7.7		
	Calycosin	-7.8		
	formononetin	-7.8		
	isoflavanone	-7.9		
	isorhamnetin	-9.2		
	Jaranol	-7.7		
	kaempferol	-8.6		
	curcumol	-7.4		

4. Dicussion: The aggressive nature of oral squamous cell carcinoma (OSCC), its late-stage identification, and its resistance to standard treatments make it a significant health concern. Even though 5-fluorouracil (5-FU) is a commonly used chemotherapeutic drug, tumor resistance, serious side effects, and patient response variability are the reasons for its limited effectiveness(20,21,22,23,24). In an effort to overcome these constraints, recent studies have looked at the processes behind 5-FU resistance. Changes in the enzymes that are in charge of 5-FU metabolism can lead to resistance. For example, resistant cancer cells have been shown to have lower activity of orotate phosphoribosyltransferase (OPRT) and thymidine phosphorylase (TP), which results in a diminished conversion of 5-FU into its active metabolites(25,26,27.28). Resistance is linked to elevated levels of TS, an enzyme that catalyzes the transformation of deoxyuridine monophosphate into thymidine monophosphate. Intrinsic resistance in cancers can result from TS gene amplification and polymorphisms(29,30,31,32,33). In order to withstand the DNA damage caused by 5-FU, cancer cells may improve their capacity for DNA repair. This process has been associated with checkpoint kinase 1 (Chk1) activation, indicating that blocking Chk1 may make resistant cells more sensitive to 5-FU(34,35,36,37). Through the promotion of a more aggressive cancer phenotype, the EMT process can potentially lead to chemoresistance. Chemotherapy frequently results in increased survival and proliferation capacity for cells undergoing EMT(38,39,40,41,42,43). 5-FU resistance has been linked to the presence of cancer stem cells in tumors. It has been demonstrated that elements such phosphorylated c-Fos enhance resistance by promoting stemness traits(44, 45). In individuals with colorectal cancer, resistance to 5-FU and a poor prognosis have been linked to increased expression of extracellular matrix protein 1 (ECM1). Compared to patients who responded well to treatment, resistant individuals had higher ECM1 levels(46, 47). Resistance may result from mutations that impair the mismatch repair (MMR) system's ability to identify and fix DNA damage brought on by 5-FU. For instance, the misincorporated nucleotides from 5-FU are not recognized in individuals with MLH1 gene loss(48,49,50,51). Cellular stress responses like autophagy and oxidative stress control can also act as mediators of resistance. For example, resistant cells can lessen the harmful effects of 5-FU by upregulating antioxidant enzymes(52,53). Changes that prevent apoptosis, such as elevated levels of anti-apoptotic proteins or decreased expression of pro-apoptotic proteins, are seen in many resistant cancer cells. Through a variety of mechanisms, such as hypoxia-induced alterations that modify drug delivery and boost survival pathways,

the tumor microenvironment can have a substantial impact on drug resistance(54,55). Traditional Chinese Medicine (TCM) combines herbal remedies, acupuncture, and other therapies to treat various health conditions. It emphasizes holistic treatment based on the balance of Yin and Yang and the flow of Qi. Network pharmacology is a modern approach that integrates systems biology and pharmacology, aiming to understand how drugs interact within biological networks rather than focusing on single-target interactions. In TCM, network pharmacology helps identify the molecular mechanisms of herbal formulas, uncovering complex interactions between active compounds and biological targets, which provides a more comprehensive understanding of TCM's therapeutic effects and potential for personalized medicine(56,57,58). A traditional Chinese herbal recipe called SanHuang decoction has drawn interest recently due to its potential as a treatment for a number of illnesses. Curcuma longa, Rheum officinale, and Astragalus membranaceus make up the majority of this decoction, which is known for its detoxifying and heat-clearing qualities. Its efficacy against medication resistance in the treatment of cancer has been demonstrated by recent studies. The ingredients of San Huang Decoction (SHD), a traditional Chinese medicine formulation, include Curcuma longa (Jianghuang), Rheum officinale (Dahuang), and Astragalus membranaceus (Huangqi). Its possible therapeutic benefits for a range of malignancies have been investigated(59,60,61). Here are some conclusions from current research on its application to various cancer kinds. By altering the tumor microenvironment and enhancing patient quality of life during endocrine therapy, SHD has demonstrated potential in the treatment of breast cancer. According to a clinical investigation, patients who received SHD in addition to normal treatment had improved results in terms of inflammatory variables and chronic stress(62). Targeting Aurora Kinase A and downregulating the ERK signaling pathway may help the decoction's anti-cancer effects by preventing tumor angiogenesis(63). According to network pharmacology research, 39 of SHD's active components interact with 185 putative targets linked to breast cancer, pointing to a complex mode of action(64).

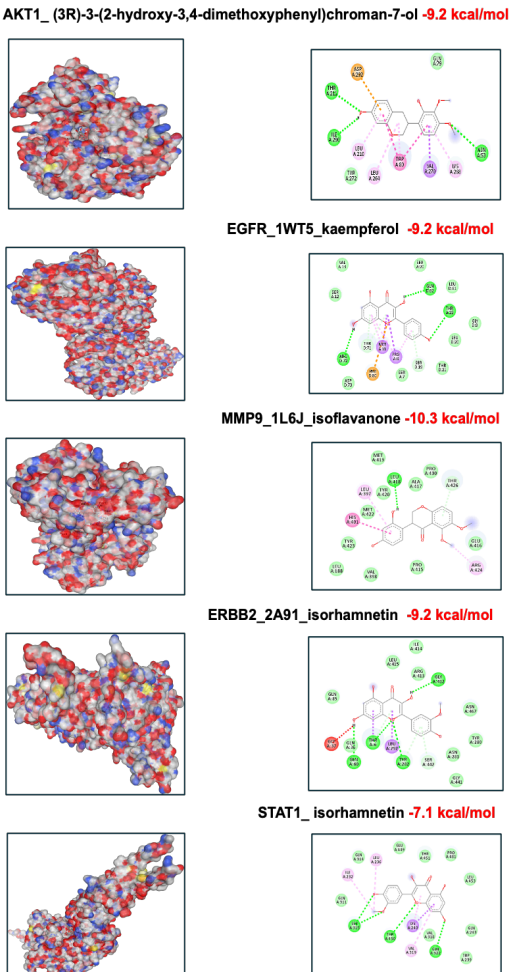

Figure 9. Protein-Ligand Interaction Analysis. Each panel displays a protein-ligand interaction, showing the 3D binding conformation (left) and the corresponding 2D interaction map (right). The binding affinities are labeled in red for each interaction. The proteins and their respective ligands are: AKT1 bound to (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol with a binding affinity of -9.2 kcal/mol, EGFR (1WT5) bound to kaempferol with a binding affinity of -9.2 kcal/mol, MMP9 (1L6J) bound to isoflavanone with a binding affinity of -10.3 kcal/mol, ERBB2 (2A91) bound to isorhamnetin with a binding affinity of -9.2 kcal/mol and STAT1 bound to isorhamnetin with a binding affinity of -7.1 kcal/mol. The 2D diagrams illustrate key molecular interactions such as hydrogen bonds, hydrophobic contacts, and the amino acid residues involved in ligand stabilization. Lower (more negative) binding energies indicate stronger interactions.

The precise pharmacological processes by which SHD affects OSCC are still not well understood, despite these encouraging characteristics. Strong procedures for examining interactions between 5-FU and active herbal substances are offered by molecular docking techniques, which also help to clarify binding affinities and mechanisms of action. Additionally, network pharmacology provides a thorough framework for comprehending the intricate multi-target and multi-component mechanisms found in formulations used in traditional Chinese medicine. In this study, we demonstrated how individual substances within SHD function in concert to impact cancer-related pathways by combining systems biology with pharmacology, specifically network pharmacology. In order to comprehend the therapeutic potential of Sanhuang Decoction (SHD) in the treatment of oral squamous cell carcinoma (OSCC), this study provides an integrative approach. By combining the principles of Traditional Chinese Medicine (TCM) with contemporary network pharmacology, gene expression analysis, and molecular docking, we have shed light on the multi-target mechanisms underlying the anticancer effects of SHD.

Eleven active chemicals were found in SHD by our analysis, including important constituents like kaempferol, curcumol, formononetin, and calycosin. These substances showed great promise in modifying pathways linked to OSCC and were chosen based on their drug-likeness characteristics. These chemicals were combined with gene expression data from the GSE74530 and GSE23558 datasets, and we found 257 DEGs that coincide with mutant genes specific to OSCC and SHD targets. According to pathway enrichment analysis, these DEGs are implicated in important biological pathways and processes, including "PI3K-AKT signaling pathway," "EGFR tyrosine kinase inhibitor resistance," and

"Platinum drug resistance." These results imply that SHD can be useful in overcoming typical chemoresistance mechanisms seen in patients with OSCC. Additionally, five important hub genes—AKT1, MMP9, EGFR, STAT1, and ERBB2—that are crucial for cancer progression, metastasis, and treatment resistance were found by our protein-protein interaction (PPI) network analysis. Strong binding affinities between the active components of SHD and these hub proteins were confirmed by molecular docking experiments, pointing to a possible mechanism through which SHD has anticancer effects. Our results demonstrate the possibility of SHD as an adjunctive treatment approach for patients with OSCC, especially those who show resistance to traditional treatments like 5-FU. Future clinical research aiming at confirming the effectiveness and safety of SHD in human populations will be based on the identification of important pathways and hub genes. Furthermore, to fully comprehend the therapeutic potential of SanHuang decoction's active ingredients and maximize their clinical uses, further research into their pharmacokinetics and bioavailability is essential. Clarifying how these substances are absorbed, transported, metabolized, and eliminated in the body is crucial for choosing the right dosage plans and optimizing effectiveness while reducing adverse effects, therefore such research should try to accomplish just that. It is also crucial to comprehend the synergistic effects of combining SanHuang decoction with currently available chemotherapeutic agents. According to early research, the decoction may increase the cytotoxic effects of traditional therapies, possibly enabling lower dosages and fewer side effects. Furthermore, investigating the immunomodulatory effects of SanHuang decoction can provide further information about its potential therapeutic advantages. Combining immunotherapy with TCM's holistic principles may offer new ways to boost immune responses in OSCC patients, as the immune system's function in cancer treatment is becoming more widely acknowledged. The complex biological networks impacted by SanHuang decoction may be revealed by combining systems biology with conventional herbal therapy using cutting-edge techniques like single-cell sequencing and proteomics. The discovery of novel therapeutic targets and combination tactics that make use of both conventional and traditional methods may result from this information. In the end, it is impossible to overestimate the promise of SanHuang decoction as a comprehensive therapy approach for OSCC. We may gain a more thorough grasp of its workings and uses by combining conventional wisdom with state-of-the-art scientific study. In addition to respecting the tenets of Traditional Chinese Medicine, this integrative approach is in line with contemporary therapeutic paradigms that strive to improve patient outcomes when treating oral squamous cell carcinoma, especially when traditional therapies have failed. As the area develops, more research and cooperation between conventional and contemporary medical procedures could result in discoveries that greatly enhance the quality of life for people with OSCC and other difficult cancers.

5. Conclusions: Oral squamous cell carcinoma (OSCC) poses significant challenges in treatment due to its aggressive nature and resistance to conventional therapies. The exploration of Sanhuang Decoction as a complementary therapeutic strategy offers promising insights into overcoming these challenges, particularly through its multi-target mechanisms and potential to enhance the efficacy of existing treatments like 5-fluorouracil.

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