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RESEARCH ARTICLE

Investigating the therapeutic potential of *Tabernaemontana alternifolia* bark for managing lung squamous cell carcinoma

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Abstract

The high global incidence and mortality rates of Lung Squamous Cell Carcinoma (LUSC) are extremely concerning. Current therapeutic strategies face significant challenges, including drug toxicity and the growing resistance to Food and Drug Administration approved medications, underscoring the urgent need for novel treatment options. Notably, natural alkaloids extracted from the plant *Tabernaemontana alternifolia* have exhibited promising anticancer effects across various cancer types. In this work, we focused on the molecular targets of LUSC for phytochemicals from *T. alternifolia* bark (TAB) and evaluated their potential as a therapeutic line for its treatment. A network pharmacology analysis was conducted to identify the molecular targets and pathways relevant to LUSC therapy. Additionally, validation of these findings through docking studies have been done. Results revealed that in-silico docking tests using AutoDock Vina, the plant's compounds 9-methoxycamptothecin, camptothecin and heyneanine demonstrated the ability to inhibit LUSC cell proliferation and induce apoptosis. These compounds suppressed genes involved in crucial cellular processes such as proliferation, angiogenesis, DNA repair and cell cycle control, which contribute to cancer management. Additionally, they also have the potential to inhibit the expression of key oncogenic factors, including MET, KDR and MMPs. This work provides significant insights into the molecular mechanisms underlying the anticancer effects of these compounds in LUSC. It suggests that they could be a promising novel therapeutic approach for combating LUSC soon. However, the safety and efficacy of TAB phytochemicals for LUSC treatment must be thoroughly validated through adequate preclinical and clinical trials.

Keywords: alkaloids; cancer; pharmacology; phytochemistry; *Tabernaemontana alternifolia*

Introduction

Lung cancer happens to be among the most prevalent forms of cancer which is frequently fatal worldwide. It is estimated that around 2 million lung cancer cases are diagnosed annually, with approximately 1.8 million resulting in death worldwide (1). The second most common histological subtype of lung cancer, LUSC, is known to be a major cause of morbidity and mortality worldwide (2). Despite significant advancements in the detection and treatment of LUSC, its prognosis remains poor, with a high rate of recurrence and metastasis and a 5-year survival rate of less than 20 % among all reported cases (3). Radiation therapy, chemotherapy and surgical resection are the recommended management lines for lung LUSC. However, the effectiveness of these therapies is limited and they frequently come with serious adverse effects. This emphasises the need for innovative and potent treatment approaches to increase lung cancer patients' chances of survival and quality of life.

Since ancient times, traditional medicine has utilized natural substances derived from plants to treat a variety of illnesses, including cancer. Many plants have been exploited as sources for novel medicinal compounds and have been reported to possess anti-cancer effects. The plant species *Tabernaemontana alternifolia*, commonly known as tree jasmine, is widely distributed across Asia and Africa. Native to the Western Ghats of India and locally referred to as 'nagkuda,' it has been used in traditional medicine to treat various conditions, including cancer, inflammation and fever (4). Recent investigations have demonstrated the potential anti-cancer effects of *T. alternifolia* bark (TAB) extracts against various cancer types, including lung cancer (4-6). These studies have shown the ability of the extracts to inhibit cell growth and induce apoptosis in a range of cancer cell lines. Bark was chosen as the target plant part because of its generally high concentration of secondary metabolites, which act as natural defence mechanisms and frequently have strong biological anti-cancer properties. These metabolites include tannins, flavonoids and phenolic compounds. Compared to other plant parts like leaves or roots, bark can be harvested with little effect on plant survival and maintains a more consistent phytochemical composition throughout the seasons, which further benefits standardization and sustainability. While TAB may have potential targets in LUSC, the underlying mechanisms of action remain unclear.

Network pharmacology is an emerging field that explores the complex interactions between medications and their targets within biological systems, integrating principles from network biology, systems biology and pharmacology (7). Lately, network pharmacology has emerged as a powerful tool for identifying new therapeutic targets and understanding the mechanisms that regulate the actions of natural compounds. It allows for the exploration of the molecular processes underlying the therapeutic effects of natural products, enabling the discovery of potential therapeutic targets and drug development pathways. Network pharmacology has gained popularity in cancer research as a valuable approach for uncovering new therapeutic targets and developing novel anti-cancer medications.

This study utilized network pharmacology to investigate the anti-cancer potential of TAB against lung squamous cell carcinoma (LUSC). We aimed to: (1) construct a comprehensive network linking TAB phytochemicals with LUSC-associated targets and genes, (2) identify key therapeutic targets and molecular pathways through which TAB exerts its anti-cancer effects and (3) elucidate the underlying mechanisms of TAB's therapeutic action in LUSC. The findings will provide mechanistic insights into TAB's anti-cancer properties and support the development of evidence-based therapeutic approaches for LUSC treatment. This research contributes to the scientific validation of traditional medicine while exploring TAB as a potential natural therapeutic alternative for lung cancer management.

Materials and Methods

Dataset collection and Pre-processing

This study utilized a wide range of in-silico tools and publicly available databases (Table 1) to collect, screen and prepare bioinformatics and molecular data for further analysis.

Bioactive identification

The Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database was used to obtain information about the phytochemicals present in TAB (8, 9). A manually curated database, IMPPAT, was created by digitizing data from over 100 books on traditional Indian medicine, 7000+ published research articles and other available sources. To present, IMPPAT is the biggest online resource on the phytochemicals of Indian medicinal plants. The IMPPAT IDs corresponding to these phytochemicals were recorded for further investigation. All the phytochemicals were checked for ADME characteristics.

Potential gene target prediction

The PUBCHEM database was used to obtain the unique identifying code (Canonical SMILES) of the phytochemicals (10). Then, Swiss Target Prediction, a web tool, was used to predict the specific proteins or molecules that these phytochemicals might interact with in the body (11).

Identification of differentially expressed genes (DEGs) of squamous cell lung cancer (LUSC)

A list of DEGs was obtained for LUSC from the GEPIA2 web tool (12).

Identification of intersection gene targets

The Venny 2.1 web tool was utilized to identify shared genes between the gene targets linked to LUSC and the putative gene targets of the bioactive phytochemicals in TAB https://bioinfogp.cnb.csic.es/tools/venny/index.html. Possible anti-LUSC gene targets were the genes that coincided in both groups.

Protein-protein interaction analysis

The STRING database was accessed for putative anti-LUSC gene targets to do an investigation of protein-protein interactions (13). The STRING PPI analysis results were then imported into Cytoscape 3.9.1 software in the tab-separated values (tsv) file format to investigate possible anti-LUSC core targets (14). Only targets with a moderate confidence score > 0.4 and specific to the Homo sapiens species were included in the analysis.

Prime targets identification using MCODE

The key components of the protein-protein interaction network for potential anti-LUSC primary targets were identified using the Cytoscape MCODE plug-in. The MCODE analysis was conducted with the following parameters: a maximum depth of 100, a degree cutoff of 2, a node score cutoff of 0.2 and a k-core of 2, which allowed for the identification of clusters across the entire network.

Core targets identification using CYTOHUBBA

CYTOHUBBA is used in Cytoscape version 3.9.1 to filter the top 10 targets (15). By utilizing the four strategies of Degree, Maximal Clique Centrality (MCC), Maximum Neighbourhood Component (MNC) and Closeness to find the intersection of the goals obtained. Thus, the primary targets were identified.

Building a network between prospective (prime and core) targets and bioactive targets

Cytoscape software was used to build the network between TAB active phytochemicals and the LUSC-related (prime and core) targets (16).

Table 1. Links of databases used in the study

Databases	Links
IMPPAT	https://cb.imsc.res.in/imppat/
PUBCHEM	https://pubchem.ncbi.nlm.nih.gov/
UNIPROT	https://www.uniprot.org/
SWISS TARGET PREDICTION	http://www.swisstargetprediction.ch/
GEPIA2	http://gepia2.cancer-pku.cn/#index
VENNY 2.1	https://bioinfogp.cnb.csic.es/tools/venny/
STRING DB	https://string-db.org/
FUNRICH	http://www.funrich.org/
CYTOSCAPE	https://cytoscape.org/
AUTO DOCK VINA	https://vina.scripps.edu/
RCSB PDB	https://www.rcsb.org/
CYTOSCAPE [MCODE PLUG-IN]	https://apps.cytoscape.org/apps/mcode
CYTOSCAPE [CYTOHUBBA PLUG-IN]	https://apps.cytoscape.org/apps/CYTOHUBBA
MGL TOOLS	https://ccsb.scripps.edu/mgltools/
BIOVIA DISCOVERY STUDIO	https://discover.3ds.com/discovery-studio-visualizer-download
DAVID	DAVID Functional Annotation Bioinformatics Microarray Analysis (ncifcrf.gov)
OPEN BABEL GUI	https://openbabel.org/docs/current/GUI/GUI.html

GO and KEGG enrichment analysis

Further analysis was done on prospective core targets for anti-LUSC utilising GO functional and KEGG pathway enrichment approaches, while keeping the "Homo sapiens" species threshold. GO keywords fall into three categories: molecular function (MF), biological process (BP) and cellular component (CC). The top 30 KEGG pathways and the top 10 GO analysis data (BP, CC and MF) were shown as an enrichment dot bubble when the data was uploaded to a bioinformatics platform http://www.bioinformatics.com.cn/. The conventional hypergeometric test was employed to ascertain statistical significance. We used the adjusted $p< 0.05$ as the significant level in our research after utilizing the Benjamini-Hochberg approach to control the false discovery rate (FDR) for multiple hypothesis testing.

Molecular docking

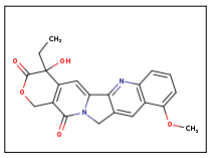
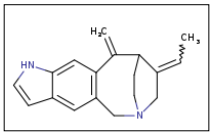
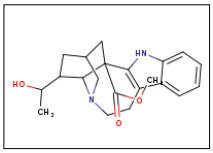
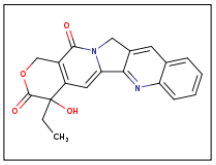
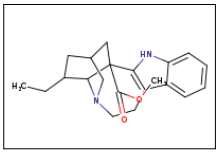
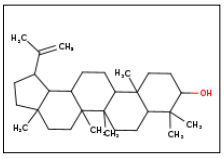
The current study links the active phytochemicals in TAB with potential anti-LUSC core targets. The top ten probable anti-LUSC core targets' binding affinities with the top three active phytochemicals from TAB were evaluated (MMP9, KDR, MMP2, JAK2, NOS3, MET, KIT, PDGFRB, FGFR1, PARP1). All the primary targets' crystal structures (PDB IDs: 6ESM, 3WZE, 7XGJ, 8BAK, 1M9J, 2WD1, 4U0I, 3MJG, 4ZSA, 7KK2), respectively, were obtained from the RCSB PDB in PDB format (17). Three of the active phytochemicals in TAB's active phytochemicals (9-Methoxycamptothecin, Heyneanine and Camptothecin) were sourced from NCBI PubChem (10). OPEN BABLE GUI was then used to convert their three-dimensional (3D) PDBQT file-format structures (18). Following the submission of all primary targets' PDB-formatted crystal structures to BIOVIA Discovery Studio Visualizer, 2023. The polar hydrogens were introduced after the heteroatoms, water and other ligands were eliminated. The resultant proteins were then loaded into AutoDock tools, stored in PDBQT format and given the Kollman partial charges (19). After being loaded into AutoDock Vina 1.2.0 (20) and tested for torsion, the formatted 3D structures of the active phytochemicals from TAB were saved in PDBQT format. Proteins and phytochemicals that have been uploaded are chosen as ligands and macromolecules, respectively and then stored in the PDBQT format. Subsequently, a grid box was generated for each protein using AutoDock Vina 1.2.0 to perform blind docking. Molecular docking scripts were then executed via the command prompt, with the resulting data presented as binding affinities. The docked complexes were visualized in both 2D and 3D using the BIOVIA Discovery Studio Visualizer.

Results

Screening of *T. alternifolia's* active phytochemicals

According to the IMPPAT database, the *T. alternifolia* plant contains 38 phytochemicals. Out of which 6 of the active phytochemicals were found in the plant's bark as shown in Table 2.

Table 2. List of phytochemicals found in TAB

S. No.	Name of phytochemical	PubChem ID	IMPPAT ID	Structure
1.	9-methoxycamptothecin	123617	IMPHY012331	
2.	Pericalline	6436240	IMPHY006461	
3.	Heyneanine	15559731	IMPHY001591	
4.	Camptothecin	24360	IMPHY002933	
5.	Coronaridine	73489	IMPHY007011	
6.	Lupeol	259846	IMPHY012473	

Potential gene targets of active phytochemicals of *T. alternifolia*

The Swiss Target Prediction online database was used to identify 401 possible genes target with a probability '< 0'. These genes target was chosen particularly for the 6 active phytochemicals.

LUSC-related gene target

Using the Gepia2 web tool, 5960 gene targets related to LUSC were identified. These targets were determined using the ANOVA differential method to distinguish between over-expressed and under-expressed genes on chromosomes, with a q-value cutoff of 0.01 and a log2FC cutoff of 1.

Intersection gene targets analysis

Fig. 1 illustrates that VENNY 2.1 identified 88 common gene targets shared between the 5960 gene targets associated with LUSC and the 401 putative gene targets of the active phytochemicals in TAB. These 88 gene targets were considered as candidates for further analysis.

PPI network analysis

Using STRING, a protein-protein interaction (PPI) network was analyzed, revealing 88 nodes and 219 edges, as shown in Fig. 2A. The network's average local clustering coefficient is 0.511, with an average node degree of 4.98. The average PPI enrichment p-value is < 1.0e-16 and there are 91 predicted edges. But according to Cytoscape analysis, the PPI network comprises 78 nodes, shown in Fig. 2, because there were 10 non-interacting nodes present in the PPI network with 219 edges and a characteristic path length of 2.473 between every pair of nodes. The following statistics describe the network: density, diameter, average number of neighbours, clustering coefficient and network radius, which are as follows: 0.036, 6, 5.615, 0.224 and 1 respectively. As illustrated in Fig. 3, 29 nodes from the network were selected as the primary anti-LUSC targets based on the degree centrality (DC) criterion, with a threshold of DC ≥ average value (5.6). These 29 nodes, representing the key anti-LUSC gene targets, are listed in Table 2 and ranked by DC in a bar graph.

Cluster network analysis

The network of 78 potential anti-LUSC targets was analyzed using the MCODE plugin on Cytoscape to identify clusters, revealing two cluster networks within the PPI network of prime anti-LUSC targets, as shown in Fig. 4.

Fig. 4A illustrates the first cluster network, which consists of 9 nodes and 21 edges, with a score of 5.250. This cluster includes several genes, such as FGFR1, PDGFRB, MET and KDR, which have multiple gene targets and a degree centrality (DC) value of ≥ 4.667. Fig. 4B depicts the second cluster network, containing 14 nodes and 22 edges, with a score of 3.385. In this cluster, genes like MMP9, JAK2 and MMP2 show high interconnectedness within the network, with a DC value of ≥ 3.143.

Core targets screening

Core targets were identified using the "CYTOHUBBA" plugin in Cytoscape 3.9.1, as shown in Fig. 5. Four distinct methods-Degree, MNC, MCC and Closeness were employed to filter and select the top 10 core targets in each category.

Network of TAB active phytochemicals and anti-LUSC targets

Fig. 6 shown the relationship between the active phytochemicals in TAB and 78 potential anti-LUSC targets, represented by a network consisting of 84 nodes and 114 edges. This network has a diameter and radius of 1, a density of 0.010, a typical path length of 1.000 and an average of 2.714 neighbours per node. The clustering coefficient is 0.000, indicating minimal clustering within the network. The edges depict interactions between the potential anti-LUSC targets and the active phytochemicals, with nodes turning more purple as their degree, or number of connections, increases.

In Fig. 7, a hub network was constructed using Cytoscape to connect 29 potential anti-LUSC primary targets with the active phytochemicals in TAB. This final network comprises 29 nodes and 38 edges, with a radius and diameter of 1 and a density of 0.032. The network reveals that the 29 putative anti-LUSC prime targets interact with all six of TAB's active phytochemicals to varying degrees. The bar graph represents the top phytochemicals that interact with potential anti-LUSC core targets in the hub network. These 6 phytochemicals, namely camptothecin, 9-methoxycamptothecin, heynianine, coronaridine, lupeol and pericalline, are the active compounds found in TAB. They have been ranked based on their edge count, indicating their importance in targeting anti-LUSC properties.

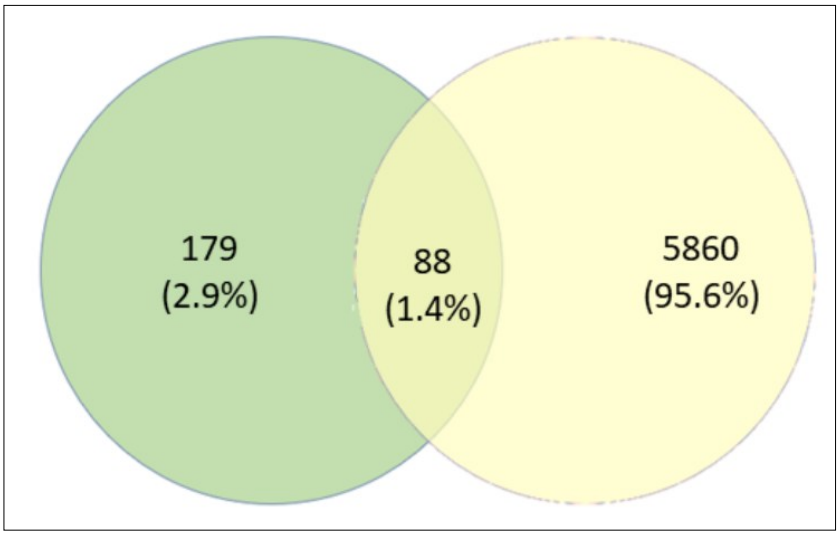


Fig. 1. Venn diagram illustrating shared gene targets.

The overlap between LUSC-related gene targets and predicted gene targets of active phytochemicals from TAB is shown. The intersecting region represents potential anti-LUSC gene targets common to both datasets.

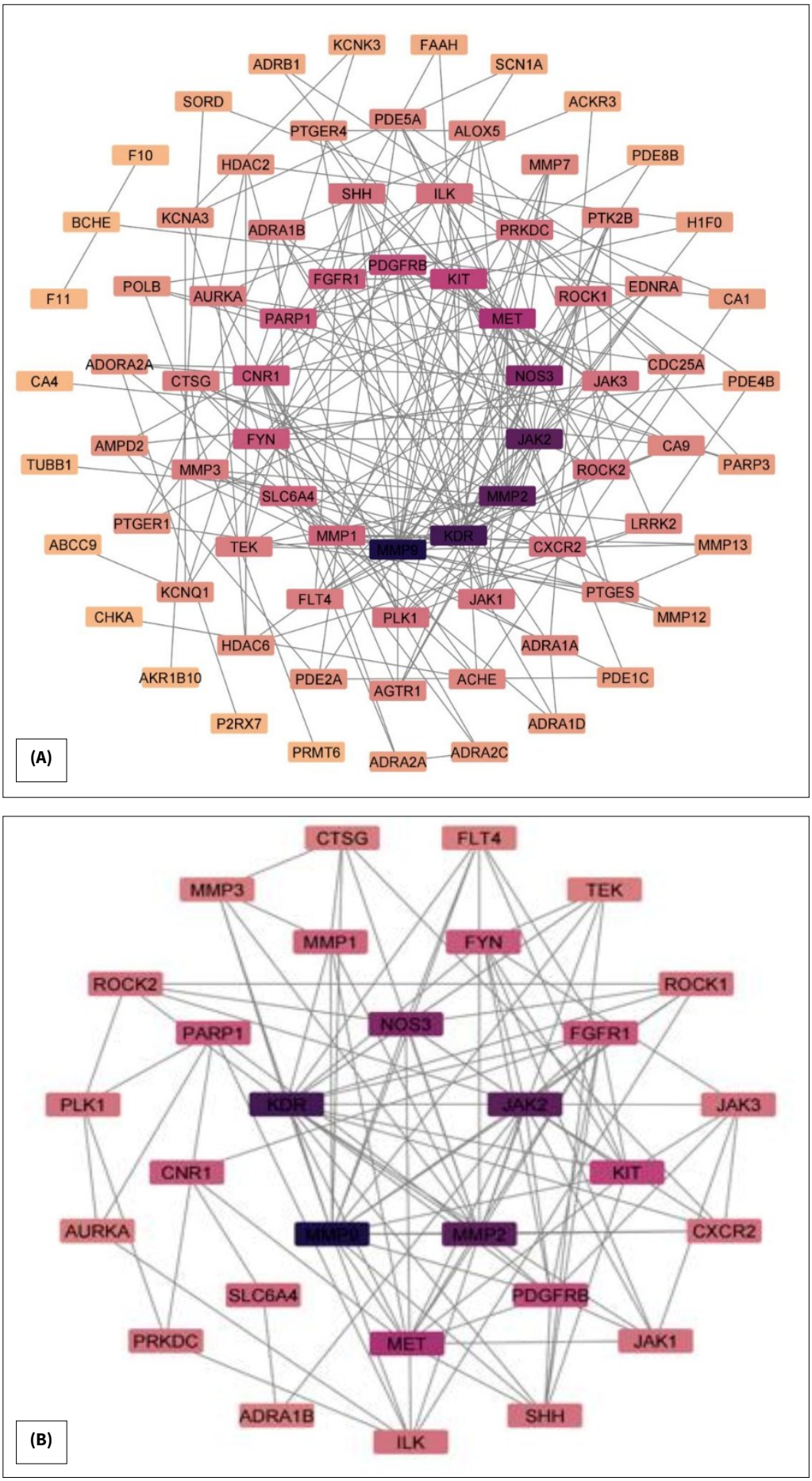


Fig. 2. Protein-protein interaction (PPI) networks derived from STRING.

(A) Interaction network of 78 predicted anti-LUSC prime targets.

(B) Network of 29 core targets filtered from the prime target set.

Node colour intensity represents degree value, transitioning from orange (low) to purple (high).

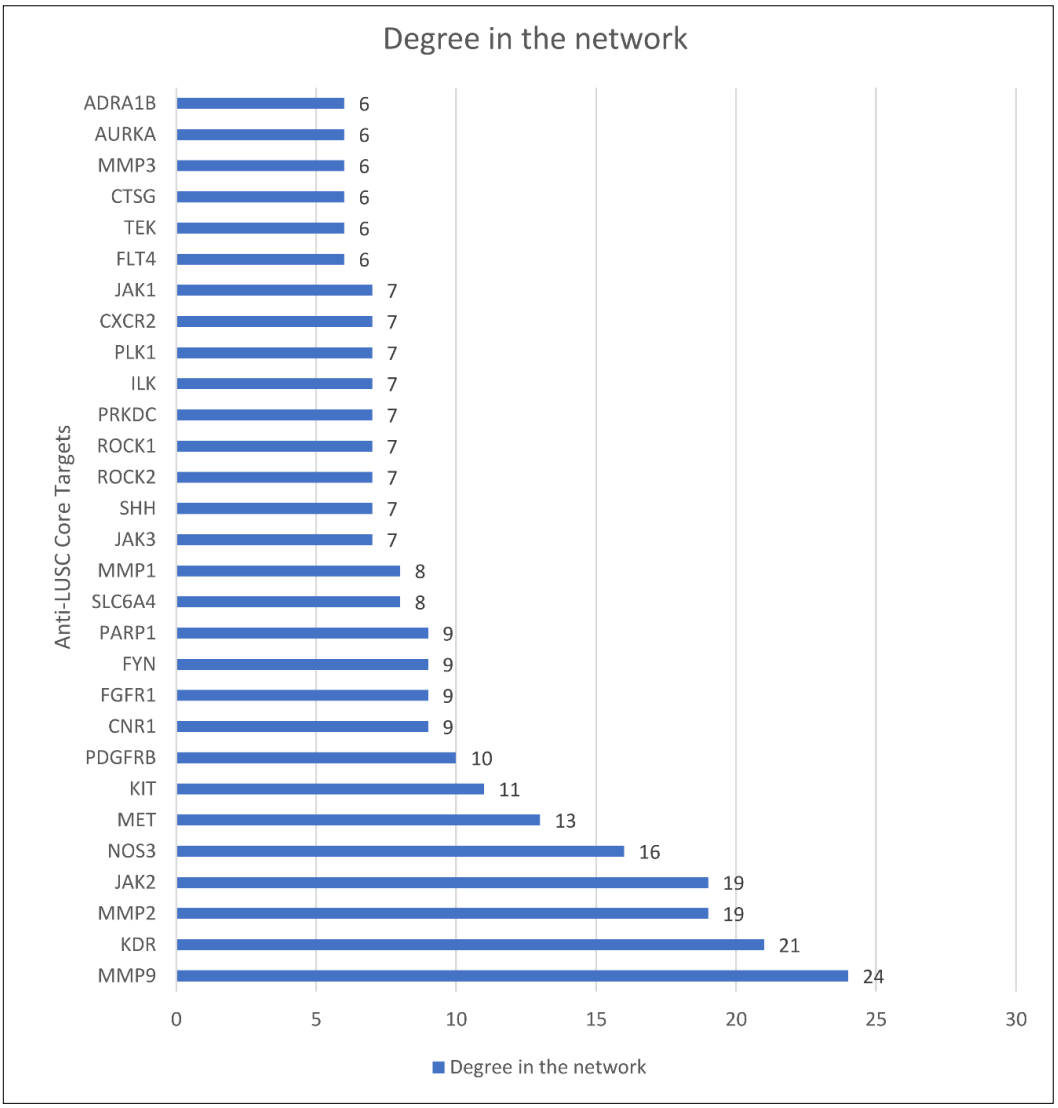


Fig. 3. Degree distribution of 29 anti-LUSC core targets.

Bar graph displaying the degree values (Y-axis) of each core target (X-axis) from the PPI network, representing their centrality within the interaction network.

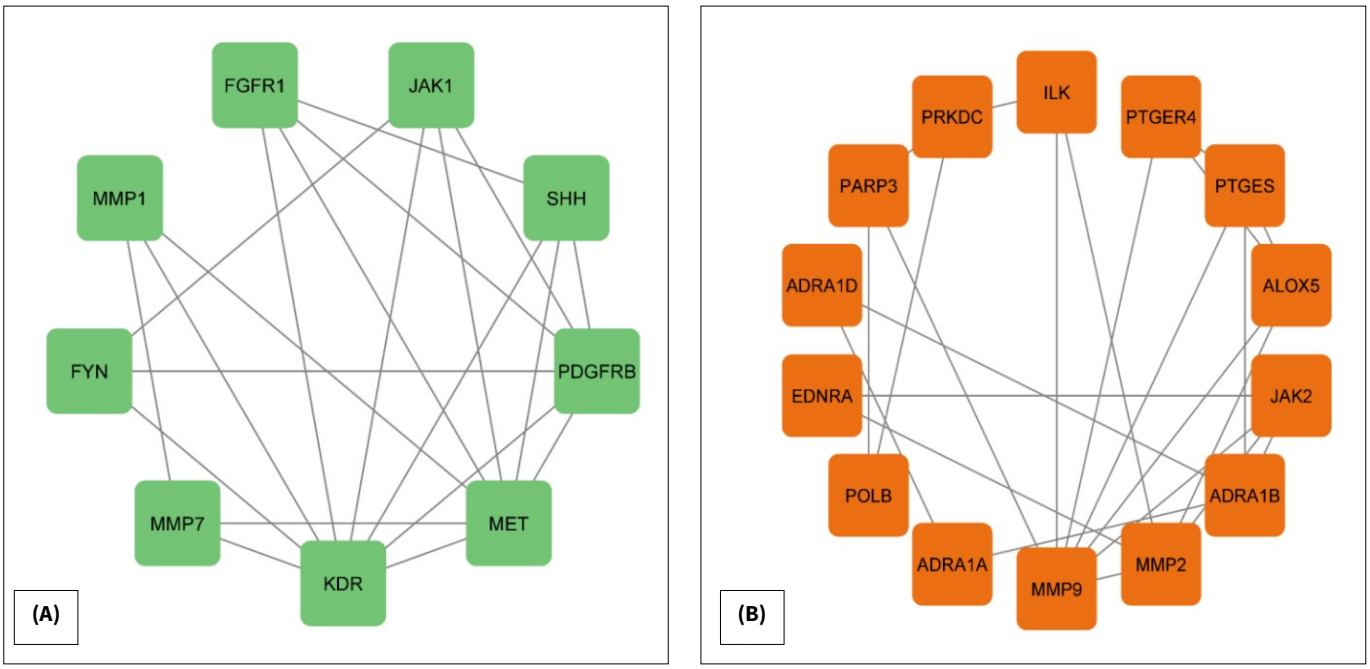


Fig. 4. MCODE clustering analysis of PPI network.

(A) Cluster 1 and (B) Cluster 2 visualized using the MCODE plug-in in Cytoscape. Clusters represent densely connected sub-networks among the 78 anti-LUSC targets, indicating possible functional modules.

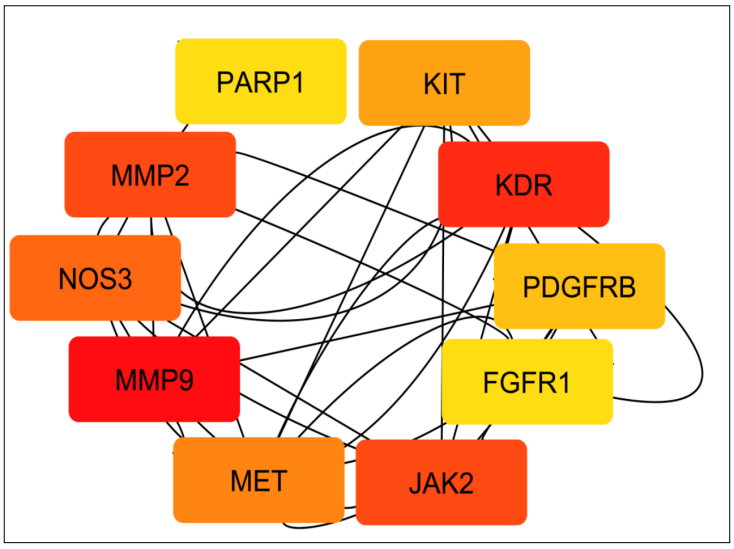


Fig. 5. Hub gene identification using CytoHubba.

Top-ranked hub genes from the PPI network of anti-LUSC targets were identified using four CytoHubba algorithms in Cytoscape 3.9.1.

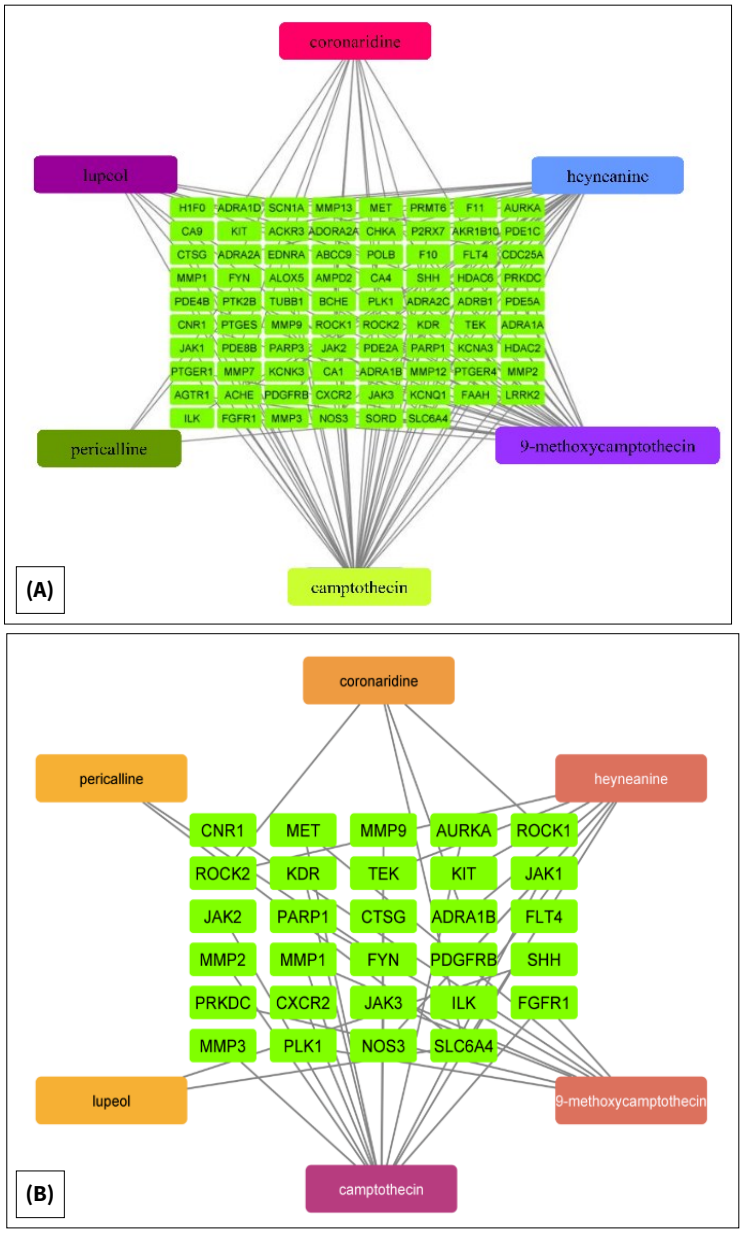


Fig. 6. Interaction networks between phytochemicals and anti-LUSC targets.

(A) Network of active phytochemicals from TAB linked to 78 primary anti-LUSC targets.

(B) Network of active phytochemicals interacting with 29 core anti-LUSC targets.

Node color gradient from orange to blue represents increasing degree centrality.

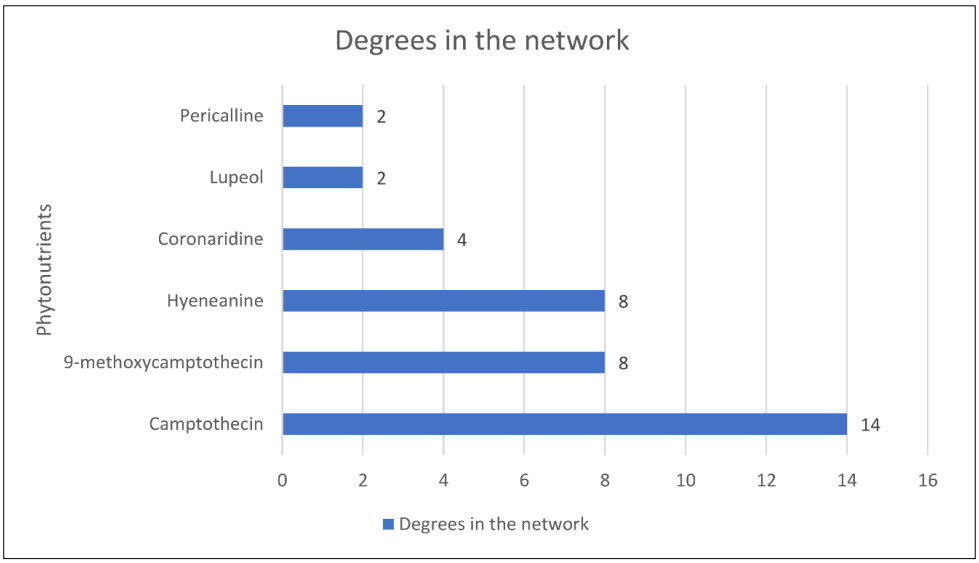


Fig. 7. Top six active phytochemicals of TAB based on network centrality.

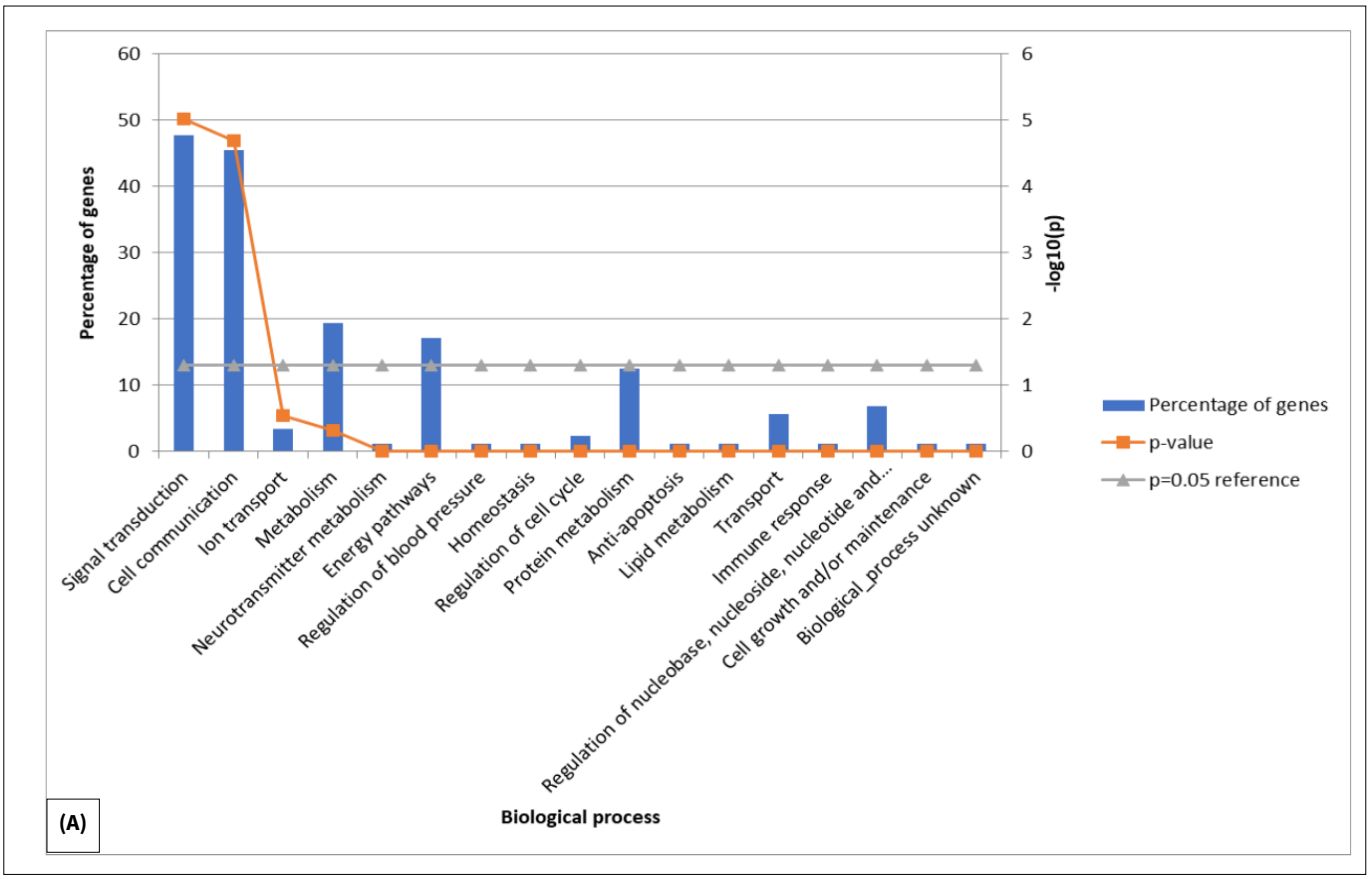
Phytochemicals are ranked by degree value from the compound target interaction network. These represent the most interconnected bioactive components against LUSC.

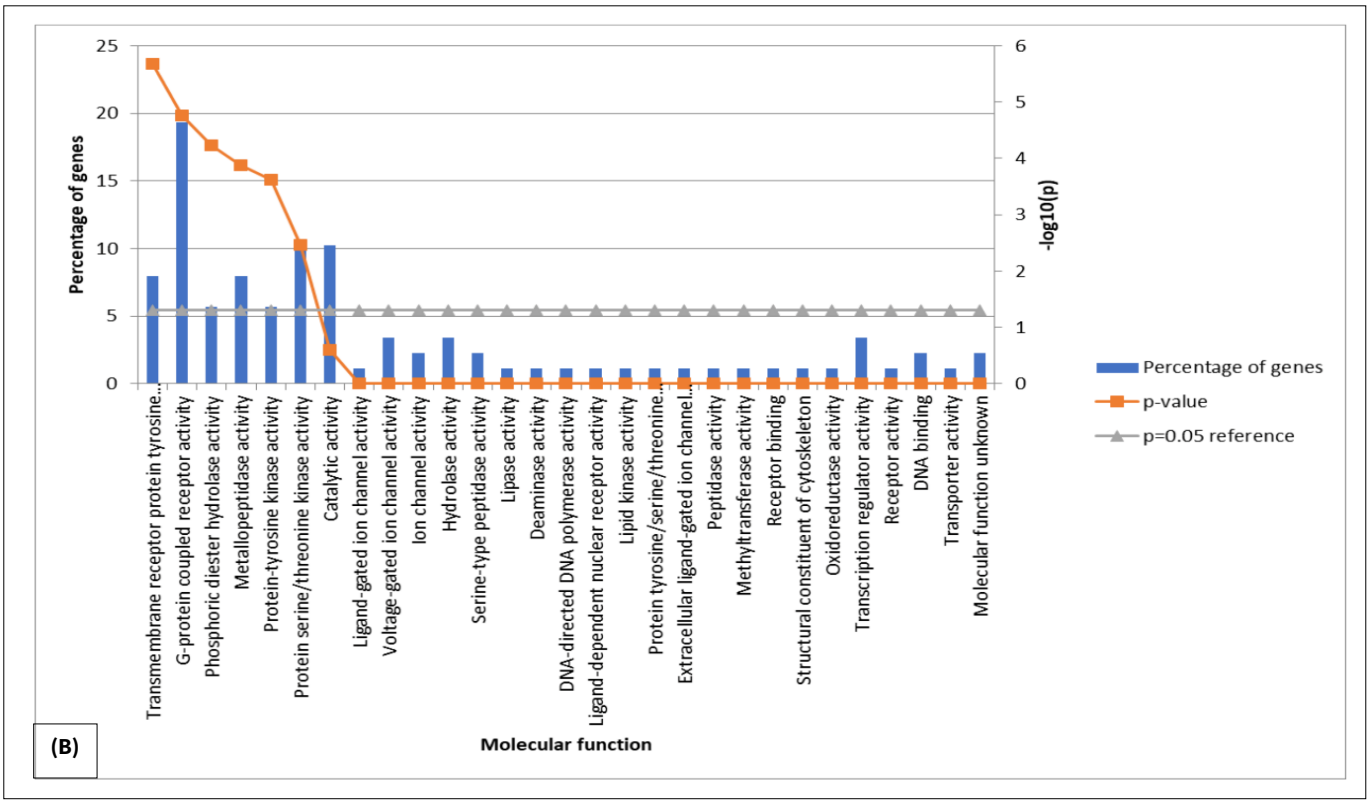
GO enrichment analysis

We conducted a GO enrichment analysis of the 78 potential anti-LUSC targets using Funrich software, identifying the top 10 enrichment terms for Biological Process (BP), Molecular Function (MF) and Cellular Component (CC), as displayed in Fig. 8. The analysis revealed that the gene targets related to BP were involved in key processes such as signal transduction, metabolism and transport. Gene targets in CC were primarily found in locations such as Cytoplasm, plasma membrane, nucleus and exosomes. Moreover, the enriched MF ontology was largely comprised of terms such as G-protein coupled receptor activity, Protein serine / threonine kinase activity, Catalytic activity and Transcription regulator activity.

KEGG analysis

A KEGG pathway analysis was conducted to explore how TAB may contribute to alleviating LUSC. We uploaded 78 potential anti -LUSC targets to the Shiny Go platform (21) and identified the top 30 KEGG pathways most relevant to the study, with a significance level of $p < 0.05$ (Fig. 9). According to the results, TAB can significantly target calcium signalling pathways and CGMP-PKG signalling pathways whereas few genes are involved in nitrogen metabolism, base excision repair etc. which are related to cancer progression. These pathways may play crucial roles in the molecular mechanisms through which TAB appears to be effective in treating LUSC.





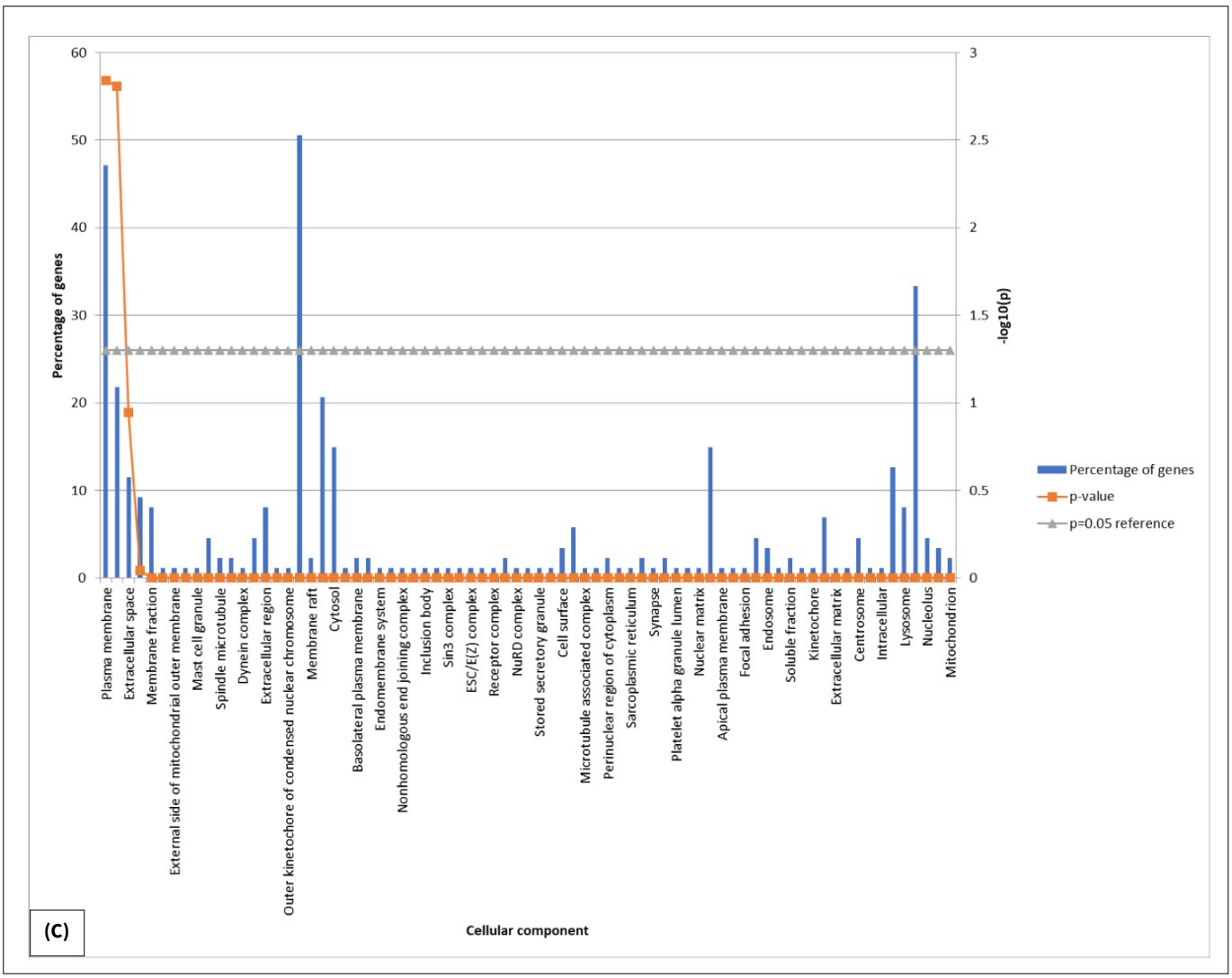


Fig. 8. Gene Ontology (GO) enrichment analysis of 78 anti-LUSC targets.

(A) Biological processes (BP), (B) molecular functions (MF) and (C) cellular components (CC) are shown. The X-axis represents GO terms and the Y-axis indicates the number of associated genes.

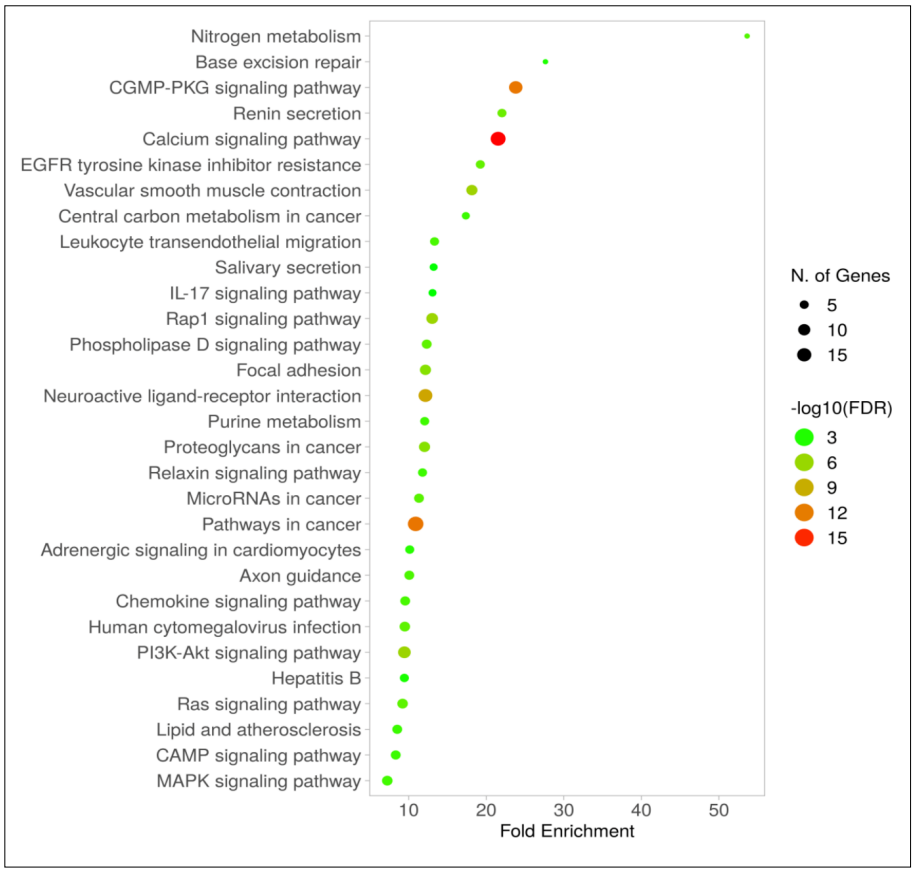


Fig. 9. Top 30 KEGG pathways enriched in anti-LUSC targets.

Bubble plot showing the most significantly enriched pathways. The Y-axis lists pathway names and the X-axis denotes gene counts. Bubble size indicates the number of genes per pathway, while colour intensity reflects adjusted p-values.

Identification of core pathways

The top 24 KEGG pathways and the top 12 LUSC core targets were combined to create a network (Fig. 10). This network was used to investigate the core pathways involved in the anti-LUSC effects of the major active phytochemicals in TAB. The top 24 KEGG pathways and the top 12 LUSC core targets were integrated to create a network (Fig. 10), which was then used to investigate the core pathways underlying the anti-LUSC actions of the major active phytochemicals in TAB. The network has 64 edges and 34 nodes, according to the findings. Furthermore, it was found that the network's density, radius and diameter were 0.057, 1 and 1, respectively. The network analysis reveals that each of the 24 pathways interacts with the 12 anti-LUSC targets in a variety of ways, with an interesting finding that the pathways related to cancer showed the greatest degree of interaction with the targets.

Molecular docking investigation

The top ten anti-LUSC core targets were molecularly docked with the top three active phytochemicals from TAB, including MMP9, KDR, MMP2, JAK2, NOS3, MET, KIT, PDGFRB, FGFR1, PARP1. As shown Fig. 11, the outcomes of the molecular docking study in terms of binding affinity (kcal/mol) scores are displayed as a heatmap. Additionally, Fig. 12 displays the docked complexes in both 2D and 3D formats. The higher binding capacity of the phytochemicals and targets is shown by the decreased binding affinity. In the present study, docking scores ≤ -9.0 kcal/mol are considered strong and -6.4 to -8.9 kcal/mol as moderate binding affinities. The results showed that all three major active phytochemicals in TAB had exhibited moderate to exceptional binding affinity scores with all anti-LUSC core targets, ranging from -6.4 to -11.3 kcal/mol. Heyneanine demonstrated binding affinity scores with NOS3, PARP1, MMP9 and JAK2 (-11.3, -9.3, -7.9 and -7.8 kcal/mol, respectively). Camptothecin, on the other hand, showed superior binding affinity scores (-9.5, -8.8 and -8.5 kcal/mol, respectively) for JAK2, NOS3 and MMP9. MET and FGFR1 showed the same binding affinities with camptothecin, i.e., -7.8 kcal/mol. Despite having lower binding affinity scores than heyneanine and camptothecin, 9-methoxycamptothecin has shown high binding affinity against MMP9, KDR, NOS3 and KIT (<-7.6 kcal/mol). Additionally, 9-methoxycamptothecin demonstrated moderate binding affinity scores against MMP2, JAK2, MET, PDGFRB and PARP1 of <-7.0 kcal/mol. Additionally, the binding affinity scores for camptothecin and 9-methoxycamptothecin against MMP2 (-7.6) and PARP1 (-7.3) were similar. The results of the molecular docking study, therefore, supported the idea that TAB may reduce LUSC by modifying the activities or expressions of these targets.

Discussion

This research work aimed to explore the molecular targets, active phytochemicals and molecular processes involved in the use of TAB for treating LUSC. Six phytochemicals of this plant exhibited promising anti-cancer potential against LUSC as shown in Table 1. The network results showed that several anti-LUSC core targets and several important active phytochemicals in TAB have a synergistic impact on mitigating LUSC pathogenesis.

Numerous genes, including MMP9, KDR, MMP2, JAK2, NOS3, MET, KIT, PDGFRB, FGFR1, PARP1, etc., are shown to be involved in both the pathogenicity of LUSC and the anti-LUSC actions of the main active phytochemicals found in TAB, according to the PPI network analysis (Fig. 2B, 3 & 13). Table 3 presents a summary of key phytochemicals from TAB, key targets

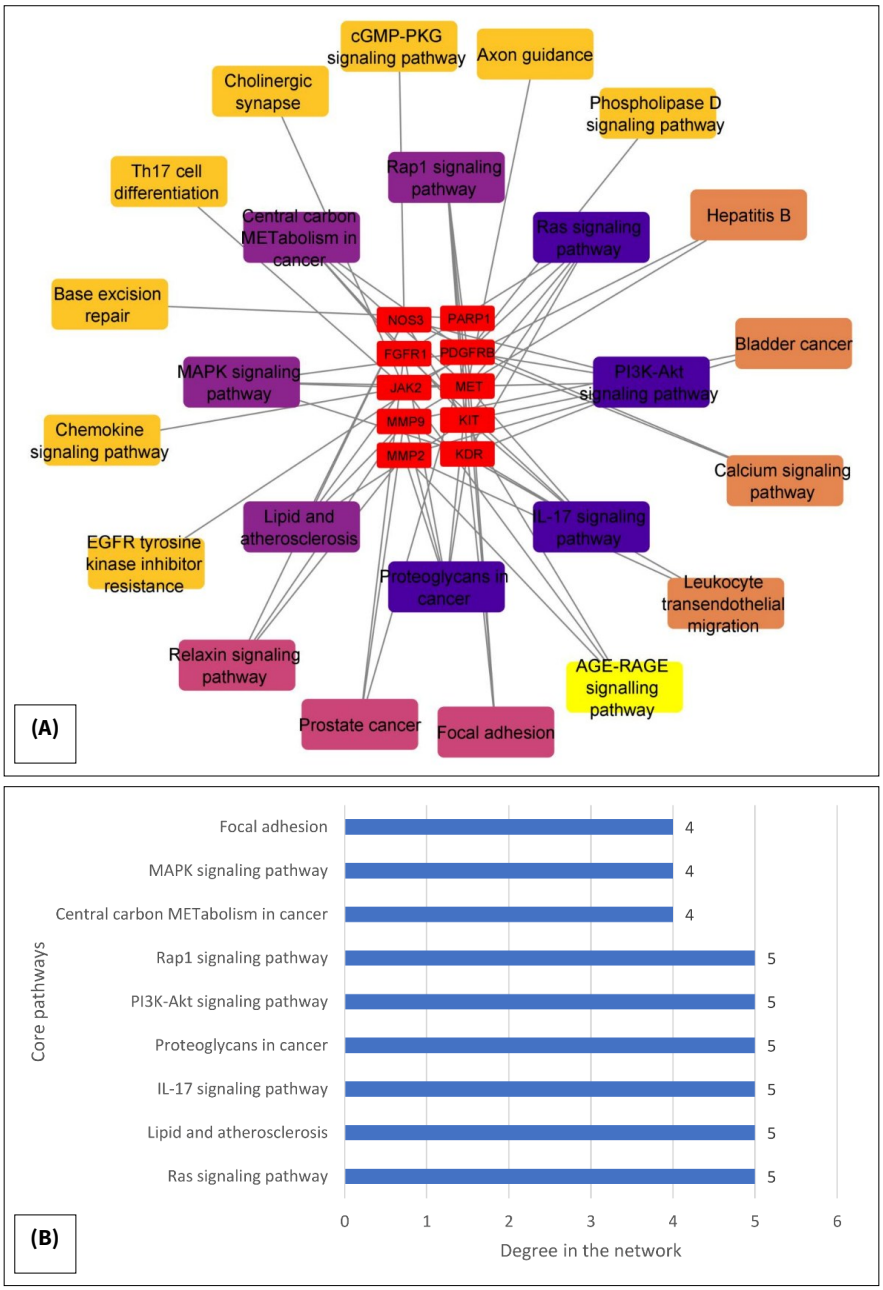


Fig. 10. Pathway-target interaction network.

(A) Network of KEGG pathways and their connected anti-LUSC targets.

(B) Top 9 pathways ranked by degree value.

Node color shifts from orange to blue as pathway connectivity increases.

	9-methxycamptothecin	Heyneanine	Camptothecin
MMP9	-8.7	-7.9	-8.5
KDR	-7.9	-6.4	-7.2
MMP2	-7.6	-7.7	-7.6
JAK2	-7.1	-7.8	-9.5
NOS3	-7.8	-11.3	-8.8
MET	-7.6	-6.5	-7.8
KIT	-7.7	-7.4	-7.3
PDGFRB	-7.1	-6.6	-6.7
FGFR1	-6.9	-6.6	-7.8
PARP1	-7.3	-9.3	-7.3

Fig. 11. Binding affinity heatmap of active phytochemicals against anti-LUSC core targets.

Binding energies (kcal/mol) between three lead phytochemicals (9-methoxycamptothecin, heyneanine and camptothecin) and the top 10 core targets are visualized. Yellow indicates weaker binding; green indicates stronger binding affinities.

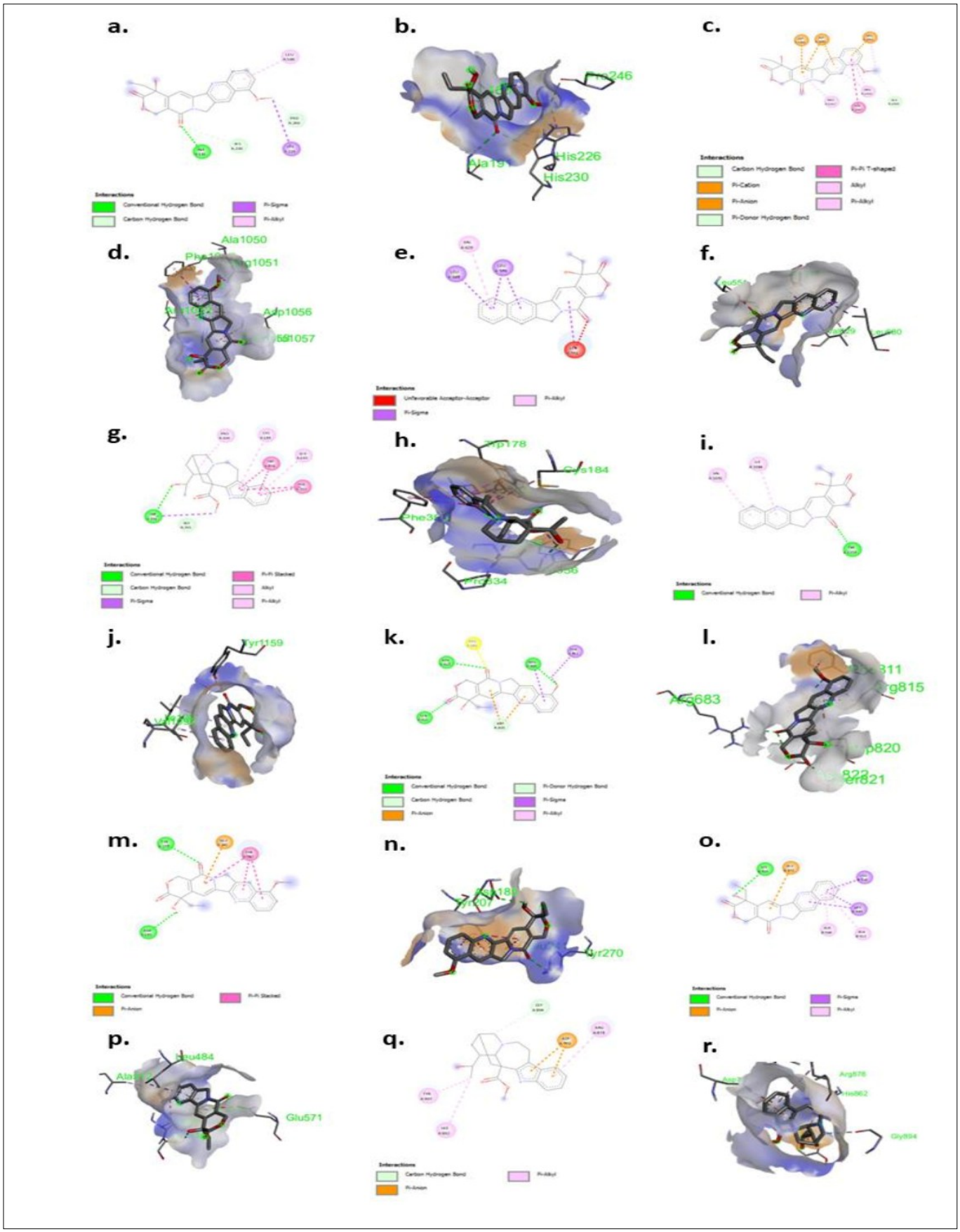


Fig. 12. Molecular docking visualization of phytochemicals with anti-LUSC targets. 2D and 3D interactions are shown for the following:

(a, b) MMP9-9-methoxycamptothecin,

(c, d) KDR-9-methoxycamptothecin,

(e, f) JAK2-camptothecin,

(g, h) NOS3-heyneanine,

(i, j) MET-camptothecin,

(k, l) KIT-9-methoxycamptothecin,

(m, n) PDGFRB-9-methoxycamptothecin,

(o, p) FGFR1-camptothecin,

(q, r) PARP1–heyneanine.

Docking scores, hydrogen bond interactions and key residues are depicted

and associated pathways of LUSC elucidated from the present study. Extracellular matrix proteins, which are essential for tissue repair and remodelling, are broken down by an enzyme called MMP9 & MMP2 (22), also referred to as matrix metalloproteinase-9 & matrix metalloproteinase-2 respectively. They have been discovered to be over-expressed in tumour tissues when LUSC is present as opposed to healthy lung tissues (23). According to studies, MMP9 & MMP2 may be involved in the angiogenesis and development of the tumour as well as the invasion and metastasis of LUSC cells (24). The receptor protein dysregulation has been associated with the onset and development of numerous cancer types, including LUSC. These proteins involve FGFR1, MET (hepatocyte growth factor receptor), KIT, PDGFRB & KDR (VEGF2). However, the precise function of KIT in LUSC is still unclear and more investigation is required to ascertain if KIT could be a suitable therapeutic target for this malignancy. Many signalling pathways, such as the MAPK/ERK and PI3K/Akt pathways, are important for the survival, growth and migration of cancer cells, can be activated by these receptor proteins. They have been found to be over-expressed in LUSC and may hasten the disease's development by promoting angiogenesis, invasion, migration and proliferation. Specifically, KDR, MET and PDGFRB are linked to poor prognosis of LUSC. Several downstream signalling pathways, including the JAK/STAT system, which is essential for controlling cell proliferation, differentiation and survival, are thought to be activated by JAK2. Most of the LUSC tumours contain JAK2 mutations and overexpression, which are linked to a poor prognosis (25). JAK2 has been demonstrated to have a role in boosting angiogenesis, which is necessary for tumour development and metastasis (26), in addition to its direct effects on cancer cells. The NOS3 enzyme, sometimes referred to as endothelial nitric oxide synthase, is essential for producing nitric oxide (NO) in the endothelial cells that line blood arteries. The control of blood pressure, angiogenesis and immunological response are just a few examples of the physiological processes in which nitric oxide plays a key role as a strong vasodilator and signalling molecule (27). The function of NOS_3 in LUSC is not entirely known. According to certain research, NOS3 may limit tumour growth in LUSC by preventing cell proliferation and encouraging apoptosis (28). However, additional research has revealed that NOS_3 may accelerate the growth and progression of LUSC by boosting angiogenesis and tumour cell invasion (29). Poly (ADP-ribose) polymerase 1, or PARP1, is an enzyme that contributes to genomic stability and DNA repair. Studies have revealed that through encouraging tumour cell survival and proliferation, PARP1 may contribute to the onset and progression of LUSC (30). This may happen because PARP1-mediated signalling pathways such the PI3K/AKT pathway, which can encourage cell growth and survival, are activated. According to certain theories, PARP1 has a role in LUSC cells reaction to DNA damage. When DNA damage occurs, PARP1 may be turned on to speed up DNA repair. In contrast, PARP1 activation in LUSC cells may encourage DNA damage and genomic instability (31), which may eventually encourage the cancer.

According to GO enrichment analysis, the main active phytochemicals in TAB that have anti-LUSC activities may be

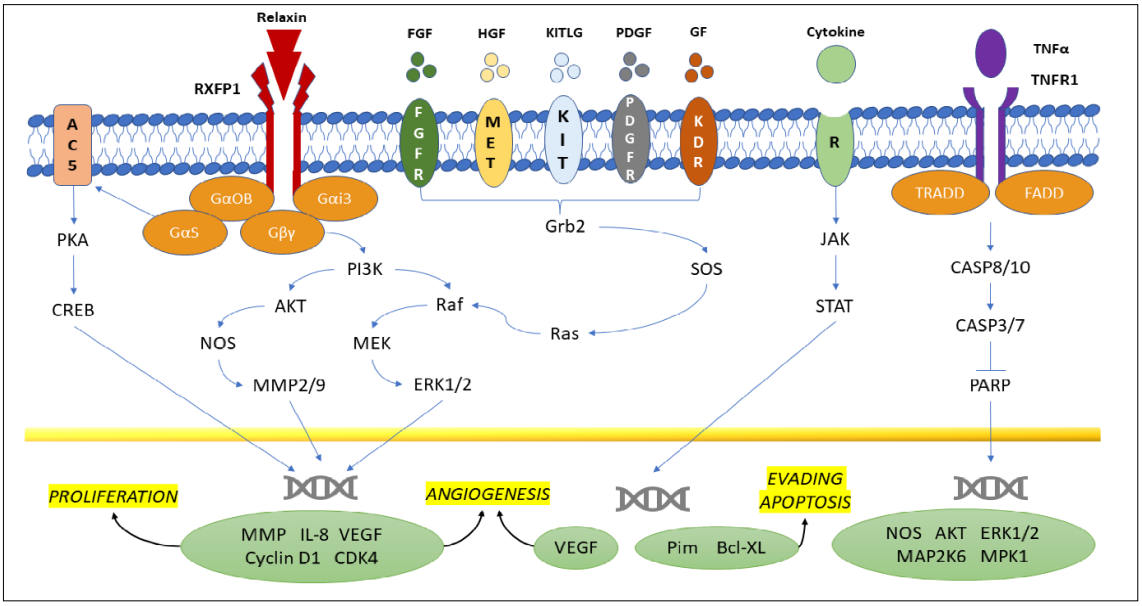


Fig. 13. Proposed molecular mechanism of anti-LUSC activity.

Schematic representation showing how the top 10 core targets, modulated by TAB phytochemicals, are involved in major biological pathways related to lung squamous cell carcinoma suppression.

Table 3. Summary table of phytochemicals from TAB, key targets and associated pathways of LUSC elucidated from the present study

Phytochemical	Key Targets (Docking Score >-8 kcal/mol)	Associated Pathways
Heyneanine	NOS3, PARP1	Relaxin, PI3K-Akt, cGMP-PKG, Calcium, AGE-RAGE signaling pathway and Base excision repair
Camptothecin	JAK2, NOS3, MMP9	Chemokine, MAPK, Relaxin, PI3K-Akt, cGMP-PKG, Calcium, AGE-RAGE, IL-17 signaling pathway, Cholinergic synapse, Leukocyte trans endothelial migration and proteoglycans-related pathway
9-Methoxycamptothecin	MMP9	Relaxin, IL-17 signaling pathway, trans endothelial migration and proteoglycans-related pathway

linked to a few gene targets that are connected to a variety of BP, including cell communication, signal transduction, metabolism, transport and so forth, as shown in Fig. 8. Receptors are important for tumor cells, as reports have shown. As a result, genetic material mutations cause the cancer microenvironment to change, which promotes unchecked development and metastasis (32). G-protein coupled receptors (GPCRs) comprise most of the overexpressed receptors in lung cancer. According to recent clinical reports and emerging investigative evidence, these receptors may be involved in the initiation, growth and metastasis of cancer. Malignant cancer cells have been shown to be able to hijack the regular physiological processes of cell receptors. As a result, they can proliferate on their own, increasing their supply of oxygen and nutrients, evading immune recognition, invading nearby tissues and spreading to distant organs (33, 34). These GPCRs are also essential for angiogenesis, which occurs both during the development of cancer and the spread of cancerous cells to other organs. The main active phytochemicals in TAB may have anti-LUSC actions due to a few different gene targets, most of which are located in the cytoplasm, plasma membrane, nucleus, exosomes, etc. These many gene targets carry out a variety of tasks, including G-protein coupled receptor activity, protein serine/threonine kinase activity, catalytic activity, transcription regulator activity, etc., according to an enriched MF ontology study.

The key active phytochemicals in the bark of TAB may have anti-LUSC effects through the IL-17 signalling pathway, PI3K-Akt signalling pathway, lipid & atherosclerosis, proteoglycans in cancer, central carbon metabolism in cancer, MAPK signalling pathway, Rap1 signalling pathway and other pathways, according to the KEGG pathway enrichment analysis. Furthermore, as demonstrated in Fig. 10B, the network analysis of the top 24 KEGG pathways anti-LUSC core targets revealed the twelve core pathways ranked by DC >= average value of (3.765). The key routes among them include the PI3K-Akt signalling system, the Rap1 signalling pathway, the proteoglycans in cancer, the lipid & atherosclerosis and the Ras signalling pathway. One of the signalling channels that is most often disrupted in human cancer is the PI3K pathway (35), which can be triggered by a variety of growth factors or ligands particular to distinct RTKs, including as the insulin and insulin-like growth factor 1 (IGF-1) receptor, FGF and members of the EGFR family (36). The PI3K pathway is altered often in LUSC; changes were detected in 68 % of LUSC samples (37). Molecular abnormalities that activate the PI3K signalling pathway are crucial in both boosting the growth of tumours and their resistance to anticancer treatments (38, 39). Mutational damage in tumour cells can arise from several sources, leading to aberrant signalling through RAS pathways. Activating point mutations in RAS, most often in KRAS, NRAS and HRAS, are present in around 20 % of human tumours (40).

TAB's potential and present limitations in the context of LUSC are highlighted by a brief comparison with well-known plant-derived therapies like curcumin and berberine. Numerous studies have demonstrated that curcumin, a polyphenolic compound derived from *Curcuma longa*, modulates important cancer-related pathways such as EGFR, NF-κB, JAK/STAT and PI3K/AKT (41). Although its low bioavailability is still a problem, it causes apoptosis, suppresses tumour growth and metastasis and acts as a chemosensitizer in non-small cell lung cancer (NSCLC) models. With encouraging preclinical results but little clinical validation, berberine, an isoquinoline alkaloid present in *Berberis* species, also demonstrates anti-cancer activity by targeting the EGFR, STAT3 and MAPK pathways, encouraging apoptosis and inhibiting the epithelial-mesenchymal transition (EMT) (42). TAB, on the other hand, contains bioactive substances like heyneanine, camptothecin and 9-methoxycamptothecin, which have been shown in the present study to interact with important LUSC-related pathways like Rap1, MAPK and PI3K-AKT. Although it exhibits multi-target potential, particularly in modifying immune and signalling pathways linked to cancer, its therapeutic effects are presently only supported by computational data. Although network pharmacology and molecular docking studies offer insightful information about the possible modes of action of TAB phytochemicals against LUSC, these hypotheses need to be verified empirically. Our in silico approach yielded binding affinities and molecular interactions that are not conclusive evidence of therapeutic efficacy, but rather a basis for generating hypotheses. Consequently, even though it appears to be a promising candidate for additional research, thorough preclinical and clinical studies will be necessary to develop it into a workable anti-LUSC treatment.

Conclusion

Using a network pharmacology analysis, this study investigated the therapeutic potential of TAB for the treatment of LUSC. The findings were indeed encouraging as these provided valuable insights into the molecular targets, pathways and mechanisms involved in the anti-LUSC effects of the phytochemicals present in TAB. The in-silico docking studies revealed that key active phytochemicals, such as 9-methoxycamptothecin, camptothecin and heyneanine, exhibited significant anti-LUSC properties by inhibiting cell growth and inducing apoptosis. It was discovered that these phytochemicals suppressed the expression of significant carcinogenic factors such MMPs, KDR and MET while simultaneously favourably modulating genes linked to angiogenesis, proliferation, DNA repair and cell cycle regulation. The molecular mechanisms through which TAB phytochemicals exert their anticancer effects in LUSC were elucidated, shedding light on potential therapeutic approaches. Furthermore, the GO enrichment analysis revealed the involvement of multiple gene targets associated with various biological processes, while the KEGG pathway analysis highlighted pathways such as Ras signalling, PI3K-Akt signaling, IL-17 signalling, proteoglycans in cancer, etc. These pathways are known to play pivotal roles in cancer development, progression and response to therapy. This in silico study is a first but crucial step in examining TAB phytochemicals' potential as a treatment for LUSC. The lack of experimental validation, however, limits the findings. Future research should focus on the following areas: (1) pharmacokinetic studies to determine the best dosage schedules and bioavailability; (2) *in vivo* studies in suitable animal models to evaluate therapeutic efficacy and safety profiles; (3) molecular validation studies to validate the predicted protein-ligand interactions and pathway modulations found through our computational analyses; and (4) *in vitro* studies using LUSC cell lines to validate the anti-cancer effects of identified phytochemicals.

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Authors' contributions

AS carried out conceptualization, data curation, formal analysis, investigation, methodology, validation, writing original draft. BS carried out conceptualization, data curation, formal analysis, investigation, methodology, validation, writing - original draft. VK provided resources, writing review and editing. AA carried out conceptualization, project administration, validation, supervision, writing review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflicts of interest to declare.

Ethical issues: None

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