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Discovering the Anti-cancer Effects of *Ligusticum Chuanxiong* through Network-based Pharmacology Analysis and Molecular Docking: An Inquiry into Natural Products

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In some cases of head and neck cancers (HNC), surgical interventions may result in the loss of organs and/or changes to their functions, thereby significantly affecting the patient's quality of life. As a result, the surgical treatment of HNC patients is often limited to specific cases, and alternative treatment modalities, such as chemotherapy, are considered. However, serious adverse effects caused by chemotherapy, such as severe nausea and vomiting, necessitate the need for the development of adjunctive methods to minimize patient suffering. Chuanxiong, *Ligusticum chuanxiong* (*L. chuanxiong*), is a natural herb used in Eastern medicine to treat cerebrovascular disorders and headaches. This study aimed to predict the effect and potential of *L. chuanxiong* as an auxiliary anticancer drug through network-based pharmacology and molecular docking analysis. The study results showed that 40 out of 41 genes of *L. chuanxiong* shared common targets of HNC and their proteins could be used to target HNC cells to prevent cancer progression. The results of the functional enrichment analysis confirmed that *L. chuanxiong* is associated with the neuroactive-ligand metabolism and neurotransmitter pathways, indicating its potential medicinal value as an adjuvant in HNC treatment. Lastly, our findings demonstrated that the active ingredient of *L. chuanxiong*, (Z)-Ligustilide, has the ATP binding site of heat shock protein 90, a protein known to promote the activation of cancer cells. These results suggest that *L. chuanxiong* is a promising candidate for developing auxiliary anticancer drugs, and further research could potentially lead to the discovery of newer and safer anti-cancer agents.

Key words : Heat shock protein 90, *Ligusticum chuanxiong*, (Z)-Ligustilide, molecular docking, network pharmacology

Introduction

Head and neck cancer (HNC) is the sixth most frequent cancer in the world, according to Globocan 2020, an online database of the International Agency for Research on Cancer (IARC) that provides global cancer statistics [9]. The human papillomavirus (HPV), immunodeficiency, and exposure to carcinogens, such as those involved in heavy smoking and alcohol consumption are the well-known factors that raise the risk of HNC [29]. Among the cases of HNC, 90% are head and neck squamous cell carcinomas (HNSCC), which has the eighth highest mortality rate among other types of cancer.

HNC located in the oral cavity or oropharynx is visually observable with the naked eye. Further, examination of the pharynx and larynx through nasofibroscopy allows real-time observation and ease in performing biopsies. Despite this, HNC is usually diagnosed only in loco-regionally advanced stages compared to other solid tumors, and more than 50% of the patients die within five years of being diagnosed, making it one of the leading causes of death due to cancer worldwide [15].

Modern conventional treatment options for HNC include surgical intervention, radiotherapy, and chemotherapy. However, in some cases of HNC, the use of surgical interventions may be limited in order to preserve the patient's quality of

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life. Therefore, radiotherapy and chemotherapy are often the preferred alternative treatment modalities. According to the National Comprehensive Cancer Network (NCCN), combining cisplatin and radiation therapy is highly effective and recommended for the treatment of HNC patients. NCCN also suggests the co-administration of cetuximab, an effective radiation sensitizer with radiotherapy, and the weekly administration of carboplatin, an alkylating agent similar to cisplatin [4]. However, the treatments mentioned above induce severe side effects: patients commonly experience dry mouth, anxiety, vomiting, and chronic fatigue. Besides, unlike other cancer patients, HNC patients may also experience difficulty breathing, eating, and speaking, depending on the anatomical location of the primary cancer [6, 11, 16]. The poor clinical outcomes and the high toxicity of the chemotherapeutic agents necessitate the need to develop less toxic therapeutic agents for HNC patients. This comprehensive study explores the medicinal effects and biological mechanisms of the natural product Chuanxiong, *Ligusticum chuanxiong* (*L. chuanxiong*), as a safe and efficacious alternative to currently available anticancer therapies.

For thousands of years, *L. chuanxiong* has been utilized to manage head and neck pain in traditional medicine. It is also the most frequently mentioned herb in the traditional Korean medical book, Donguibogam, for treating diseases associated with the cranial region [7, 26]. In Eastern medicine, this herb is commonly prescribed for treating virus-related diseases and various inflammatory reactions in the head [34]. Recent studies have reported that *L. chuanxiong* exerts its anticancer effects by regulating the oxidative stress pathways and inducing apoptosis in cancer cells [14]. However, its efficacy and safety as an anticancer agent is unclear, and further research is required.

This study predicted and validated the anticancer effects of (Z)-Ligustilide, an active ingredient of *L. chuanxiong* through systematic pharmacological analyses and molecular docking. The findings of this study suggest that this ingredient could contribute to modern anticancer therapeutics activity through the development of an agent which could function alone or synergistically with other chemotherapeutic agents through an alternate mechanism of action offering safety and efficacy for the treatment of HNC.

Materials and Methods

Screening active compounds and fishing the target genes of *L. chuanxiong*

To identify the major bioactive compounds in *L. chuanxiong* we used the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/tcmsp.php) [22]. TCMSP provides the pharmacokinetic properties of each component of the herbal medicines along with information on the correlation between compounds, targets, and diseases. In this study, molecular weight (MW), oral bioavailability (OB), and drug-likeness (DL) values were referenced to identify the active compounds of *L. chuanxiong* [25, 36]. Compounds that satisfied the conditions of 180≤ MW ≤500, OB ≥50%, and DL ≥0.07 were considered the active compounds of *L. chuanxiong*. The predicted target proteins of these selected active compounds were also obtained from the TCMSP database, and the corresponding genes translating the target proteins were selected from the UniProt Knowledgebase (https://www.uniprot.org/) [32]. The compound-target network was constructed using Cytoscape 3.9.1. (https://cytoscape.org/).

Common target genes of HNC and *L. chuanxiong*

The target genes for the treatment of HNC were selected using the DisGeNET (https://www.disgenet.org/) [20, 21], GeneCards database (https://www.genecards.org/) [23, 28] and Comparative Toxicogenomics Database (CTD, https://ctdbase.org/) [5]. A search was conducted on each database using keywords such as 'Head and neck cancer', 'Head and neck neoplasms' and 'Head and neck carcinoma'. Using the FunRich 3.1.4 software tool (http://www.funrich.org) [10, 18, 19], a Venn diagram was drawn to identify genes that were duplicated twice or more among all the genes found in these three databases. From this selection, the genes that intersected with the genes encoding the targets of active compounds of *L. chuanxiong* were chosen as the common target genes.

Protein-protein interaction network construction

To investigate the Protein-Protein Interaction (PPI) between the Common target Genes of *L. chuanxiong* and HNC (CGLHs), we utilized the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/). We set the species as *Homo sapiens* and by inputting CGLHs into the database under these criteria, we sought to investigate their PPIs.

Functional enrichment analysis

The functional analysis of the intersecting genes between HNC and *L. chuanxiong* was conducted using Gene ontology (GO) and the Kyoto encyclopedia of genes and genomes

(KEGG) pathways. We utilized the database for annotation, visualization, and integrated discovery (DAVID) bioinformatics resources for mapping and visualized the GO analysis using the Science and research online plot (SRplot, https://www.bioinformatics.com.cn/en). The genes were analyzed at the biological process (BP), cellular component (CC), and molecular function (MF) levels to verify their functions.

Molecular docking

Docking analysis of molecular-level interactions between ligands and proteins was performed using AutoDock Vina (https://github.com/ccsb-scripps/AutoDock-Vina) [8, 31]. The protein data bank (PDB) structures of (Z)-Ligustilide (Pubchem CID 5319022) and adenosine triphosphate (ATP, Pubchem CID 5957) were downloaded from the Pubchem compound database (https://pubchem.ncbi.nlm.nih.gov). The PDB ID of human heat shock protein 90 (HSP90), 1YES (resolution 2.20 Å) [27], was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank website (https://www.rcsb.org/). Converting the PDB files to Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) files and creating grid boxes for docking was done using the AutoDockTool-1.5.7. The visualization of the results of the protein-ligand binding was performed using the Biovia Discovery Studio Visualizer v17.2, 2021 (BIOVIA, Dssault Systèmes, Waltham, USA).

Results

The active ingredients of *L. chuanxiong*

A total of 22 active compounds of *L. chuanxiong* were identified by setting the criteria of 180≤ MW ≤500, OB ≥ 50%, and DL ≥0.07 in the TCMSP database (Table 1).

Identification of disease-drug common target genes

A total of 41 target proteins of the active compounds of *L. chuanxiong* were identified and converted into gene IDs using the UniProt database (Table 2). The network of the active compounds and target genes are visualized in Fig. 1. The average number of neighbors within this network was 6.476 for each compound. Three compounds had the highest degree score and are assumed to play a central role: Methyl 2-pentanoylbenzoate (22 targets), 4-Hydroxy-3-butylphthalide (21 targets), and (Z)-Ligustilide (18 targets). The centrality rating of targets, by degree score, are ranked as follows: Gamma-aminobutyric acid receptor subunit alpha-1 (*GABRA1*), Prostaglandin G/H synthase 2 (*PTGS2*), Muscarinic acetylcholine receptor M1, 2, 3 (*CHRM1, CHRM2, CHRM3*).

Table 1. Active compounds of *L. chuanxiong* with 180≤ MW ≤500, OB ≥50%, and DL ≥0.07 in the TCMSP database

Mol ID	Molecule Name	MW	OB (%)	DL
MOL001390	49070_FLUKA	222.41	85.51	0.12
MOL000196	L-Bornyl acetate	196.32	65.52	0.08
MOL000208	()-Aromadendrene	204.39	55.74	0.1
MOL002096	(+)-ALPHA-FUNEBRENE	204.39	52.87	0.1
MOL002098	3-Butylidene-7-hydroxyphthalide	204.24	62.68	0.08
MOL002099	Senkyunolide-K	208.28	61.75	0.08
MOL002122	(Z)-Ligustilide	188.24	53.72	0.07
MOL002127	Cnidilide	194.3	77.55	0.07
MOL002136	Neocnidilide	194.3	83.83	0.07
MOL002140	Perlolyrine	264.3	65.95	0.27
MOL002144	Senkyunolide-D	222.26	79.13	0.1
MOL002150	1-Acetyl-beta-carboline	210.25	67.12	0.13
MOL002152	Sinapic acid	224.23	64.15	0.08
MOL002153	1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, (1aR-(1aalpha,4aalpha,7beta,7abeta,7balpha))-	220.39	82.33	0.12
MOL002165	Methyl 2-pentanoylbenzoate	220.29	69.28	0.07
MOL002178	4,7-Dihydroxy-3-butylphthalide	222.26	106.09	0.1
MOL002181	4-Hydroxy-3-butylphthalide	206.26	70.31	0.08
MOL002186	Aromadendrene oxide 2	220.39	65.1	0.14
MOL002190	Cedrene	204.39	51.14	0.11
MOL002191	Carotol	222.41	149.03	0.09
MOL002201	cis-Ligustilide	190.26	51.3	0.07
MOL002207	1(3H)-Isobenzofuranone, 3-butyl-3a,4,5,6-tetrahydro-, cis-(-)-	194.3	65.03	0.07

Table 2. 41 Targets of *Ligusticum chuanxiong*

Target protein name	Gene name	UniProt ID
Alcohol dehydrogenase 1C	*ADH1C*	P00326
Alpha-1A adrenergic receptor	*ADRA1A*	P35348
Alpha-1B adrenergic receptor	*ADRA1B*	P35368
Alpha-2A adrenergic receptor	*ADRA2A*	P08913
Alpha-2B adrenergic receptor	*ADRA2B*	P18089
Alpha-2C adrenergic receptor	*ADRA2C*	P18825
Beta-1 adrenergic receptor	*ADRB1*	P08588
Beta-2 adrenergic receptor	*ADRB2*	P07550
Choline O-acetyltransferase	*CHAT*	P28329
Muscarinic acetylcholine receptor M1	*CHRM1*	P11229
Muscarinic acetylcholine receptor M2	*CHRM2*	P08172
Muscarinic acetylcholine receptor M3	*CHRM3*	P20309
Neuronal acetylcholine receptor subunit alpha-2	*CHRNA2*	Q15822
Sodium-dependent dopamine transporter	*DAT1*	Q6LC27
Dopamine D1 receptor	*DRD1*	P21728
D(2) dopamine receptor	*DRD2*	P14416
Estrogen receptor	*ESR1*	P03372
Gamma-aminobutyric acid receptor subunit alpha-1	*GABRA1*	P14867
Gamma-aminobutyric-acid receptor alpha-2 subunit	*GABRA2*	P47869
Gamma-aminobutyric-acid receptor alpha-3 subunit	*GABRA3*	P34903
Gamma-aminobutyric-acid receptor subunit alpha-4	*GABRA4*	P48169
Gamma-aminobutyric-acid receptor alpha-5 subunit	*GABRA5*	P31644
Gamma-aminobutyric-acid receptor subunit alpha-6	*GABRA6*	Q16445
Glutamate receptor 2	*GRIA2*	P42262
Heat shock protein HSP 90	*HSP90AB1*	P08238
Leukotriene A-4 hydrolase	*LTA4H*	P09960
Lysozyme	*LYZL1*	H0YDZ2
Amine oxidase [flavin-containing] A	*MAOA*	P21397
Amine oxidase [flavin-containing] B	*MAOB*	P27338
Nuclear receptor coactivator 2	*NCOA2*	Q15596
Nitric oxide synthase, inducible	*NOS2*	P35228
Nitric-oxide synthase, endothelial	*NOS3*	P29474
cAMP-dependent protein kinase inhibitor alpha	*PKIA*	P61925
Prostaglandin G/H synthase 1	*PTGS1*	P23219
Prostaglandin G/H synthase 2	*PTGS2*	P35354
Retinoic acid receptor RXR-alpha	*RXRA*	P19793
Sodium channel protein type 5 subunit alpha	*SCN5A*	Q14524
Sodium-dependent noradrenaline transporter	*SLC6A2*	P23975
Sodium-dependent dopamine transporter	*SLC6A3*	Q01959
Sodium-dependent serotonin transporter	*SLC6A4*	P31645
DNA topoisomerase II	*TOP2A*	P11388

To find the CGLHs, we searched the DisGeNET, Gene Cards, and CTD databases to collect genes associated with HNC. We obtained 786 genes from DisGeNET, 1,418 genes from GeneCards, and 35,682 genes from CTD. From these, we selected genes that overlapped across multiple databases and were also related to *L. chuanxiong* (Fig. 2). Out of the 41 *L. chuanxiong*-related genes, 40 genes (except for Dopamine transporter1 [*DAT1*]) overlapped with the target genes of HNC.

PPI network construction

We confirmed that there were 40 CGLHs and put these 40 targets into the STRING database to proceed with the PPI network analysis. In the PPI network, 40 nodes and 166 edges were observed. The thickness of the network edges represents the confidence score from data sources. The active interaction source categories settings for the confidence score mainly focused on the experimental/biochemical data, association in curated databases, and textmining evidence through mention-

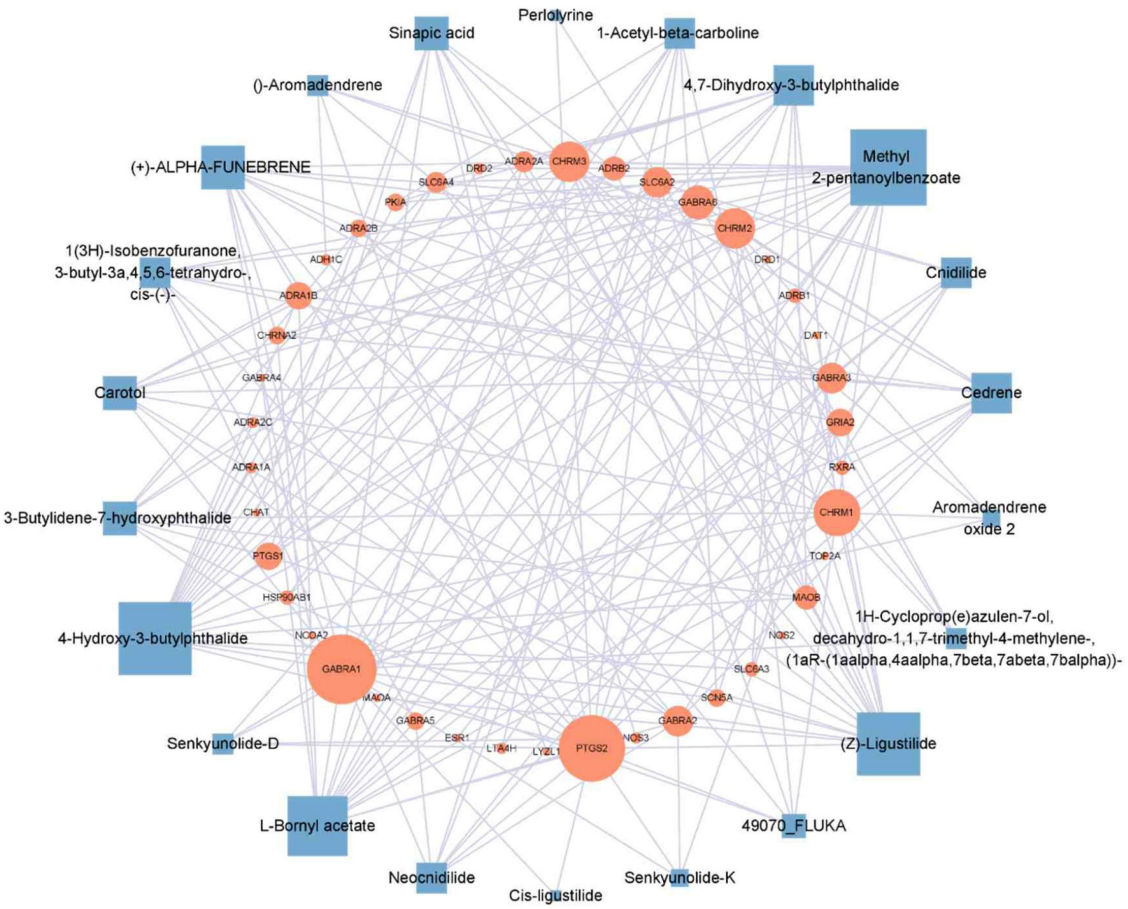


Fig. 1. Network of active compounds of *L. chuanxiong* and their targets. The network shows the multiple targeting characteristics of *L. chuanxiong*. Blue squares represent active compounds, and red circles represent the target genes. The size of the shape of nodes indicates the degree score.

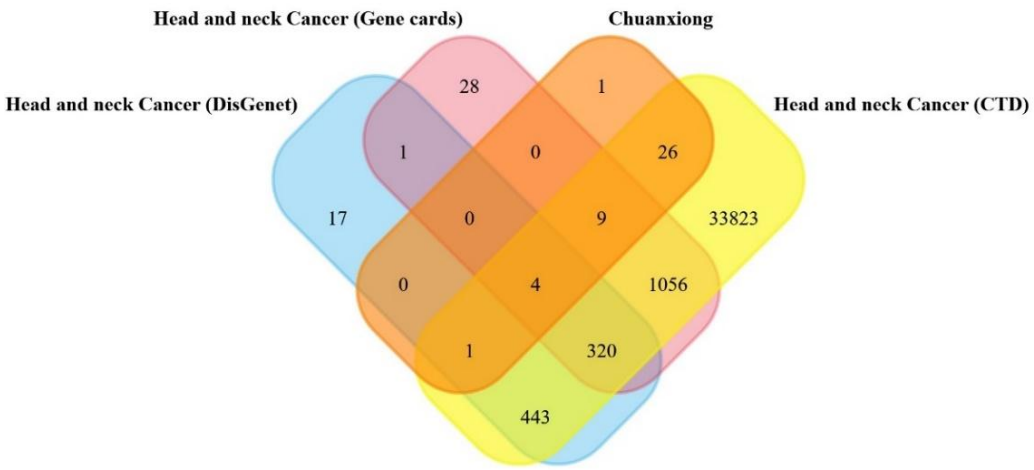
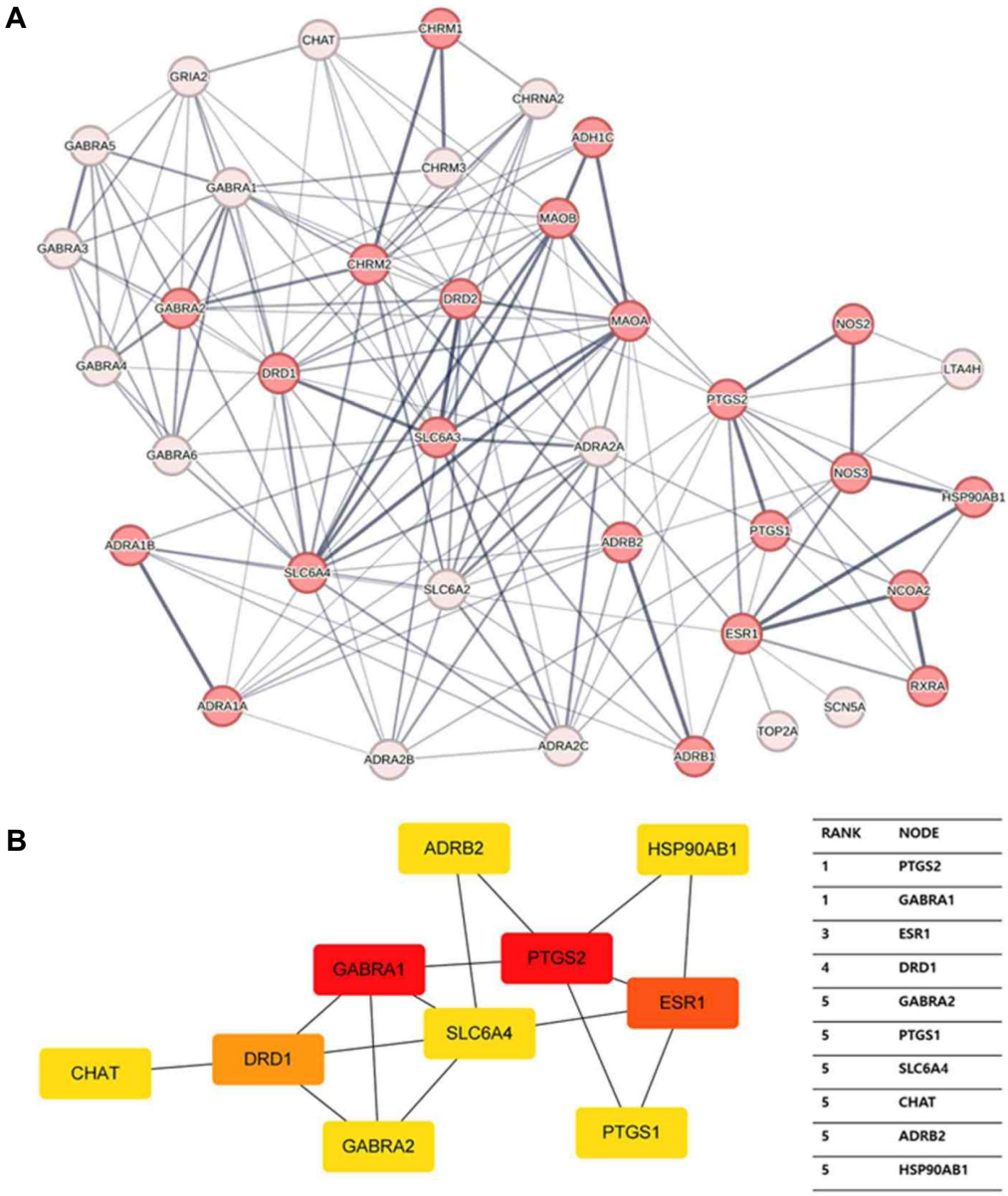


Fig. 2. Identification of the common target genes of *L. chuanxiong* and head and neck cancer. Venn Intersection Analysis by FunRich3.14 showed there were 40 target genes common to *L. chuanxiong* and head and neck cancer.

ing in relevant publications (Fig. 3A). As a result of calculating the importance of genes through the bottleNeck of cytohubba plugin in cytoscpe, *PTGS2, GABRA1*, Estrogen receptor (*ESR1*), Dopamine D1 receptor (*DRD1*), *GABRA2, PTGS1*, Sodium-dependent serotonin transporter (*SLC6A4*), Choline O- Acetyltransferase (*CHAT*), Beta-1 adrenergic re-



RANK	NODE
1	PTGS2
1	GABRA1
3	ESR1
4	DRD1
5	GABRA2
5	PTGS1
5	SLC6A4
5	CHAT
5	ADRB2
5	HSP90AB1

Fig. 3. Protein-protein interaction network construction for common genes between *L. chuanxiong* and head and neck cancer. (A) There were 40 nodes and 166 edges found. The PPI enrichment p-value was less than 1.0e-16. The average node degree was 8.9. The proteins with notably high concentration scores are highlighted in red. (B) Key genes generated from cytoHubba plugin in Cytoscape ranked by BottleNeck.

ceptor (*ADRB2*), and *HSP90AB1* were in the top 10. Intermediation represents the number of the shortest path passing through a node, and nodes with such high mediation nodes are called BottleNecks and are one of the conditions for deriving a hub gene along with the degree (Fig. 3B) [37].

Enrichment analysis for HNC - *L. chuanxiong* common targets

Using the 40 CGLHs, GO and KEGG pathway analyses were conducted. According to the GO analysis, 1,409 results were obtained in the perspective of BP, 142 results in the perspective of CC, and 200 results in the perspective of MF. The most observed terms in all three perspectives were dopamine, gamma-aminobutyric acid (GABA) and synaptic transmission. This indicated that the active compounds in *L. chuanxiong* were associated with the treatment of diseases associated with the head and neck region (Fig. 4A).

In the KEGG pathway analysis, pathways related to neuroactive ligand-receptor interaction were found to be significant. This indicates that the CGLHs are closely related to the sig-

nalling pathway of diseases associated with the human brain (Fig. 4B).

Molecular docking analysis for (Z)-Ligustilide and HSP 90

To validate the molecular binding between one of the active compounds of *L. chuanxiong*, (Z)-Ligustilide and the molecular chaperone HSP90, we employed the PDB code 1YES as the receptor. 1YES is HSP90 N-terminal domain and reported as human HSP90 geldanamycin binding domain (HSP90-GBD), open conformation (residues 9-232). Geldanamycin mimics ATP structure binding in the HSP90 N-terminal nucleotide-binding pocket, and the pocket is made up by the residues such as Leucine (Leu)48, Asparagine (Asn)51, Aspartic acid (Asp)54, Alanine (Ala)55, Lysine (Lys)58, Isoleucine (Ile)91, Asp93, Ile96, Glycine (Gly)97, Methionine (Met)98, Asn106, Leu107, Lys112, Gly135, Phenylalanine (Phe)138, Valine (Val)150, Threonine (Thr)184, and Val186 [27]. The human HSP90 N-terminal ATP-binding sites are reported in the crystal structure of HSP90 N-terminal-ATP complex (PDB code 3T0Z), and the interacting residues are Asn51, Asp54, Asp93, Glu47, Gly135, Gly137, Gly97, Leu48, Met98, Phe138, Ser52, Thr184, and Val136 [13]. Molecular docking results showed a calculated ligand-receptor binding affinity of -6.1 kcal/mol, with the formation of conventional hydrogen bonds, hydrophobic bonds and van der Waals contacts between (Z)-Ligustilide and amino acid residues of 1YES (Fig. 5A). (Z)-Ligustilide interacted with the following amino acid residues: Asp93, Ala55, Asn51, Met98, Gly97, Ile96, Phe138, Leu107, Thr184, Ser52, Asn106, Val150, and Val186. O2 of (Z)-Ligustilide made a conventional hydrogen bond to Gly97. (Z)-Ligustilide made hydrophobic bindings to Asn51, Ala55, Met98, and Val 186, and made van der Waals contacts with Phe138, ASP93, Ser52, Thr184, Ile96, Asn106, Leu107, and Val150 (Fig. 5B). To further compare the (Z)-Ligustilide binding sites and ATP binding sites for Hsp90, we performed molecular docking between ATP and 1YES (Fig. 5C), and the interacting modes are visualized in Fig. 5D.

Using the alignments of HSP-GBD sequence, the binding sites of (Z)-Ligustilide and ATP were compared. Thirteen amino acid residues of (Z)-Ligustilide-HSP90 binding site were in the nucleotide-binding pocket of the HSP90 N-terminal domain. Eleven residues were the same as the ATP-binding site of the ATP-1YES binding result. Eight residues were the same as the ATP-binding site of 3T0Z (Fig. 6).

Lastly, the results of the compound-target network analysis showed that (Z)-Ligustilide has 18 targets (Fig. 1). According to our molecular docking results between (Z)-Ligustilide and HSP90, HSP90 should be added as a target of (Z)-Ligustilide.

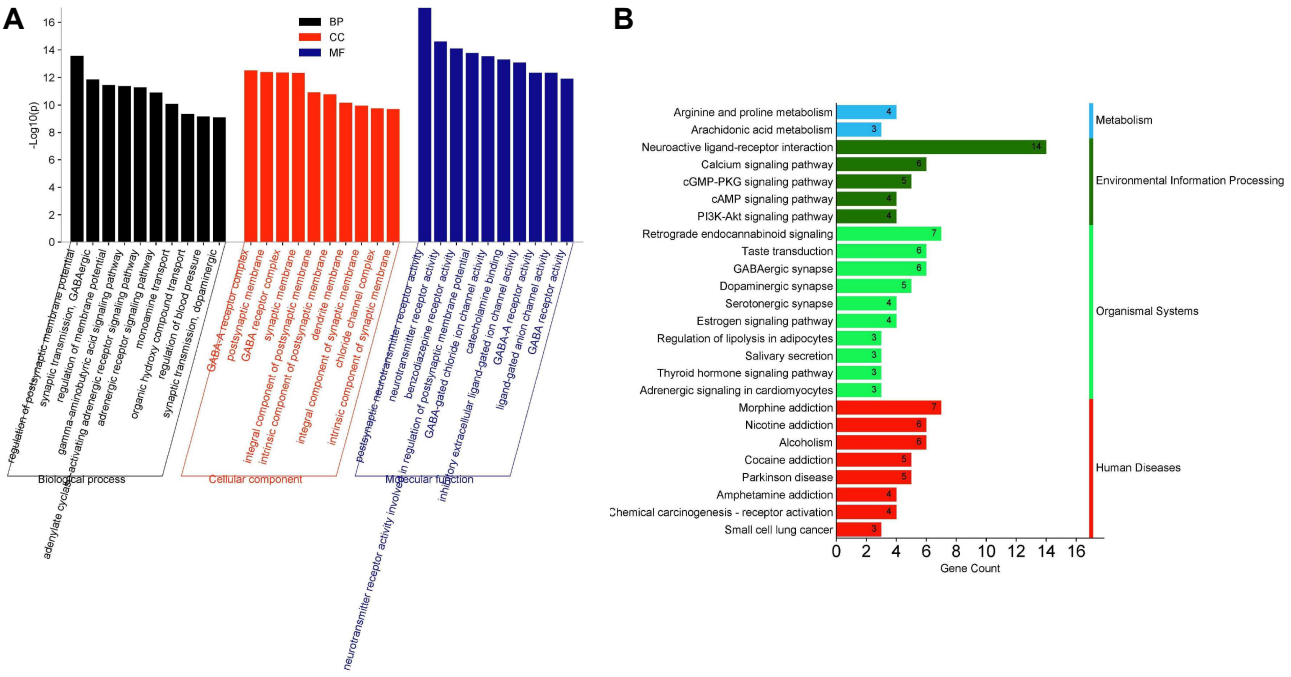


Fig. 4. Enrichment analysis of 40 common targets. (A) Gene Ontology (GO) enrichment analysis at the level of Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). (B) Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment pathways.

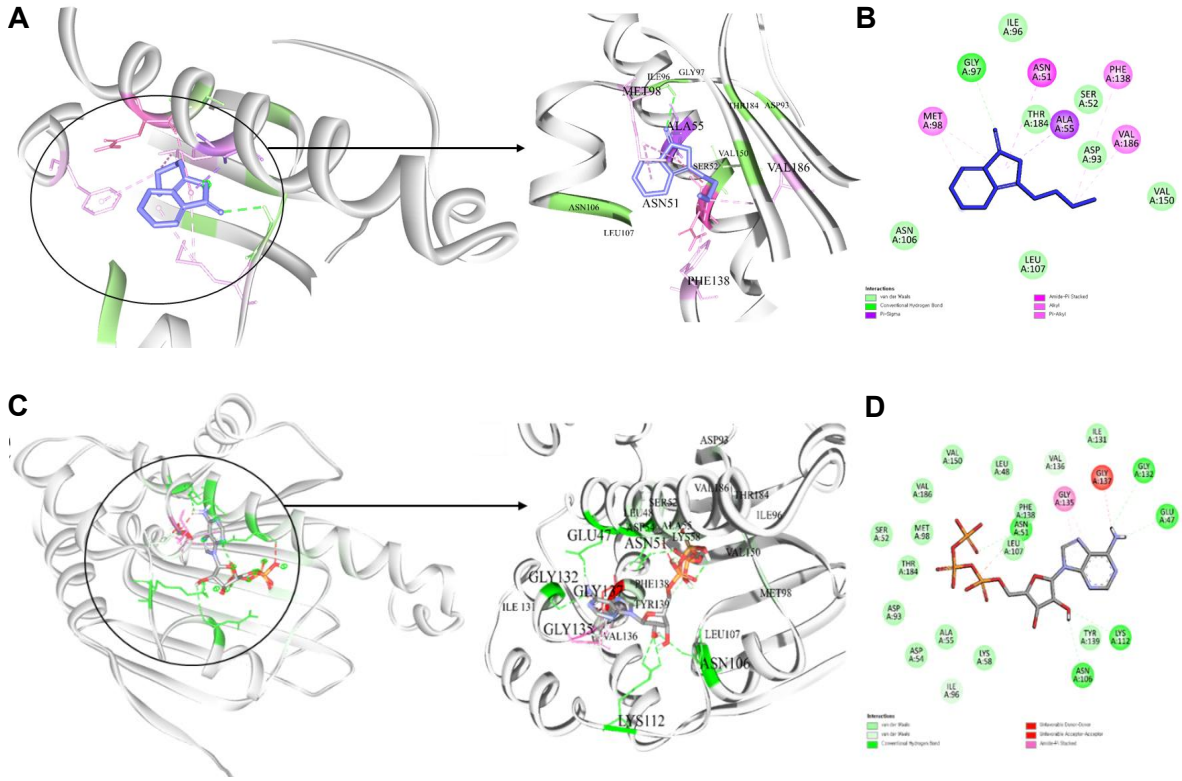


Fig. 5. Molecular docking analysis between HSP90 (PDB code 1YES) and (Z)-Ligustilide and ATP. (A) Molecular docking of (Z)-Ligustilide to 1YES model. (B) Two-dimensional binding diagram of (Z)-Ligustilide and amino acid residues of 1YES. (C) Molecular docking of ATP to 1YES. (D) Two-dimensional visualization of ATP-1YET docking result.

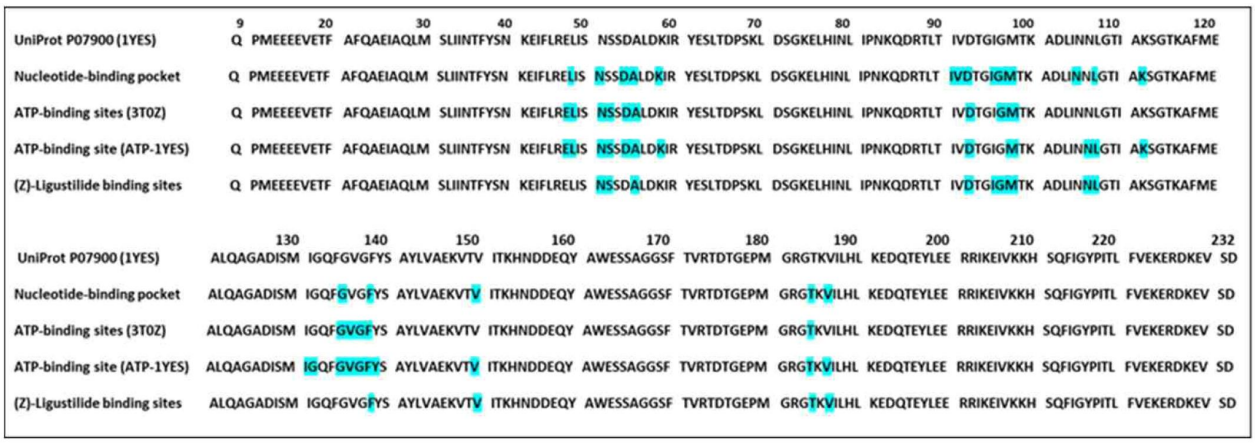


Fig. 6. (Z)-Ligustilide and HSP90 binding site analysis using alignment of HSP90 N-terminal domain sequences (1YES). The amino acid sequences of 1YES were downloaded from UnitProt database. The residues that make up the nucleotide binding pocket in 1YES [27], the ATP-binding sites of 3T0Z [13], the ATP-binding sites of 1YES, and the (Z)-Ligustilide binding sites of 1YES are highlighted (cyan blue).

Discussion

The 22 active compounds of *L. chuanxiong* were identified from the network pharmacology analyses (Table 1). The relevant genes of the compounds were compared with the HNC genes to identify the common genes. It was found that 40 out of the 41 genes relating to *L. chuanxiong* overlapped with the HNC–associated genes (Fig. 2). The key genes generated from PPIs of CGLHs were PTGS2, GABRA1, ESR1, DRD1, GABRA2, PTGS1, SLC6A4, CHAT, ADRB2, and HSP90AB1

(Fig. 3B). We conducted GO and KEGG enrichment analyses on the CGLHs, further exploring the underlying biological mechanisms of *L. chuanxiong*. We approached the studies with three main areas of focus: BP, CC and MF. The GO results showed that the target genes mainly enriched the biological functions that regulate the postsynaptic membrane potential, synaptic transmission GABAnergic, and the regulation of the membrane potential (Fig. 4A). The results support the correlation between *L. chuanxiong* and neurotransmitter transmission – especially the inhibitory signalling pathways. Further research could discover valuable medicinal insights into *L. chuanxiong* to provide a supporting mechanism to reduce the unwanted side effects of modern conventional anti-cancer therapies.

Notably, this study further elucidated the anti-cancer mechanism of *L. chuanxiong* in treating HNC through (Z)-Ligustilide, one of its main active compounds. We focused on its correlation with HSP90 which is an ATP-dependent protein that indirectly affects the processing of cell cycle regulation, proliferation, migration, and apoptosis by folding various regulatory proteins in response to external stress on the cell [30]. Among the isoforms of HSP90, HSP90AB1 was highly expressed in HNSC, regulating HNSC cell proliferation, migration, and glycolysis through the AKT pathway [38]. HSP90 acts as a chaperone, assisting in proper protein folding and maintaining the structure of proteins [24]. It controls the cell cycle and aids in DNA repair in response to damage to the DNA [2]. In addition, HSP90 can also stabilize overexpressed oncogenic proteins that contribute to cancers [17]. In some cases, it also acts as a factor that promotes cancer development. These characteristics have made HSP90 a potential target for cancer therapy [33, 35].

Disrupting the structure of HSP90 complexes, which directly aid in promoting oncoproteins, should facilitate the degradation of target proteins. This is also an important factor that validates the function of anti-cancer drugs [1, 3, 12]. Studies have reported that when Baicalein, an HSP90 ATPase inhibitor, binds to the ATP binding site of HSP90, the HSP90/PTGS2 complex dissociates, leading to the inactivation of HSP90 [39]. Here, when HSP90 activates as it binds with PTGS2, to deactivate HSP90, a drug should work as an ATPase domain inhibitor of HSP90. In this study, (Z)-Ligustilide bound to the HSP90 chaperone, interfering with the function of the signalling molecules. We performed 3D molecular docking using AutoDock Vina, generated a 2D visualization model, and verified the association by examining (Z)-Ligustilide and HSP90 (Fig. 5A, 5B). The ATP binding sites of HSP90 and (Z)-Ligustilide were found to be identical based on sequences comparison with an existing ATP binding model (3T0Z) and molecular docking results of the ATP binding site of 1YES (Fig. 6). This suggests that the underlying anti-cancer mechanisms of *L. chuanxiong* can be further elucidated and future research on this natural product could lead to advancement in anti-cancer therapeutics. However, these results are limited to laboratory-based systematic pharmacology. Further practical validation is needed to precisely analyse the underlying molecular mechanisms for use in nature-based adjuvant therapies or develop a new method in conventional therapeutics with fewer side effects.

We provided insights into the medicinal value of *L. chuanxiong*. Based on system pharmacology, we have suggested that the therapeutic role of the herb in the treatment of cerebrovascular diseases is an important factor that could lead to advancements in the treatment of other diseases including modern anticancer therapeutics by reducing the serious side effects associated with chemotherapeutic agents. We also elucidated the anti-cancer mechanisms of (Z)-Ligustilide, one of the main active compounds of *L. chuanxiong* and we confirmed that (Z)-Ligustilide binds to the ATP binding site of HSP90. Therefore, we suggest that further research may unravel the role of *L. chuanxiong* as a potential agent in adjuvant therapy for the treatment of cancer.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 네트워크 기반 약리학 분석 및 분자 도킹을 통한 천궁의 항암 효과 예측: 천연물에 대한 탐구

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두경부암(HNC)의 경우, 외과적 개입은 환자의 삶의 질에 심각한 영향을 미칠 수 있으며, 화학요법을 병행하게 된다. 그러나 화학요법에는 현저한 부작용이 있으므로 환자의 고통을 최소화하기 위한 보조 방법의 개발이 필요하다. 천궁(*Ligusticum chuanxiong*)은 동양 의학에서 뇌혈관 장애 및 두통에 사용되는 천연 허브이다. 본 연구에서는 네트워크 기반 약리학 및 분자 도킹 분석을 통해 천궁의 근본적인 항암기전을 예측하였다. 본 연구에서 HNC와 관련된 천궁의 공통 유전자를 밝혀내어 신경 활성 리간드의 대사 및 신경 전달 물질 경로와의 연관성을 확인했다. 본 연구는 천궁의 성분 중 하나인 (Z)-ligustilide 가 암세포 활성화에 관련된 heat shock protein 90의 ATP 결합 부위를 공유함을 입증했다. 이 결과는 천궁이 보조 항암제 개발을 위한 유망한 후보임을 시사하며, 향 후 더욱 새롭고 안전한 항암제의 연구개발에 과학적 근거를 제시하는 새로운 발견이다.