Network Pharmacology Approach for Uncovering the Multiple Mechanisms of *Cynomorium songaricum* against New Diseases: A Case Study on Oral Squamous Cell Carcinoma

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Abstract

Cynomorium songaricum is an herbal medicine traditionally used for enhancing kidney function or stimulating immunologic function. However, the new pharmacologic activity of *C. songaricum* and the exploration of its multiple mechanisms is not well understood. Herein, we reported the chemical compositions of *C. songaricum* methanol extract by UHPLC-Q-Exactive and literature collection and then performed molecular docking experiments with targets from the RCSB, STITCH and Uniport databases and literature. We adopted a network pharmacology-based strategy and selected ingredients and targets showing better scores than those of the positive drugs for six diseases (oral squamous cell carcinoma [OSCC], HIV, AD, gastric cancer, melanoma and gastric ulcer). Then, Gene Ontology and pathway analyses on OSCC were conducted. A total of 77 compounds were identified from the methanol extract and papers on *C. songaricum*. Molecular docking indicated that 34 compounds, among which are flavonoids and glycosides, were docked with 18 targets and showed better scores than those of the positive drugs, such as luteolin and ursolic acid together with 3OD4 and 4ACC, which are mainly related to OSCC. The top two biological processes were

phosphorylation and protein phosphorylation, and the representative pathways were the PI3K-Akt, cGMP-PKG and Wnt signalling pathways for OSCC. In conclusion, this research partially explored the potential *C. songaricum* pharmacologic activities, which showed multi-component, multi-target, multi-pathway and multi-disease mechanisms. This work can lay a foundation for further experimental research on the effectivity of *C. songaricum* in OSCC and guidance for the novel study of other herbal medicines.

Keywords: *Cynomorium songaricum*; Molecular docking; Network pharmacology; UHPLC-Q-Exactive; Anti-oral squamous cell carcinoma

1 Introduction

Cynomorium songaricum (Suo Yang) is widely distributed across the northwest provinces of China, Iran and Mongolia. Suo Yang is an edible medicine in Mongolia, a vulnerable plant worldwide and commonly used component in medicine formulas. Similar to Suo Yang Gujing Wan, an herbal formula is used for nourishing the kidney yang with the most important herbal medicine ---Suo Yang[1, 2]. As uncovered by numerous phytochemical studies, chemical ingredients in *C. songaricum* include flavonoids, tannins, organic acids and triterpenoids[3]. Currently, *C. songaricum* is utilised for treating impotence and spermatorrhea, kidney yang deficiency and constipation. Extracts of *C. songaricum* have also been patented in China and prevent dizziness and sonitus, treat female climacteric syndrome and manage cancer in recent years[4-6]. However, the novel pharmacologic activities and multiple mechanisms of *C. songaricum* are unclear.

Molecular docking is a method for understanding the reaction mechanism between proteins or enzymes and ligands with high accuracy, convenience and low cost[7, 8]. Meanwhile, network pharmacology is an emerging approach that captures the mechanism of herbal medicine at the systems level[9, 10]. In this study, we explored the pharmacologic activities of *C. songaricum* by molecular docking, investigated the multiple mechanisms by network pharmacology and conducted Gene Ontology (GO) and pathway analyses. We presented the activity of *C. songaricum* in six diseases, including oral squamous cell carcinoma (OSCC) (a severe health problem ranked as the

sixth most common cancer in the world[11, 12]), Alzheimer's disease (AD; a progressive neurodegenerative disease with high incidence; around 35 million patients were found to suffered from dementia worldwide in 2010[13]), and HIV infection (an emerging public health issue with high morbidity and mortality, according to the analysis of the World Health Organisation). The aim was to develop a novel activity in *C. songaricum* and guide the discovery of new drugs from other herbal medicines[14]. Figure 1 depicts a flowchart for this study.

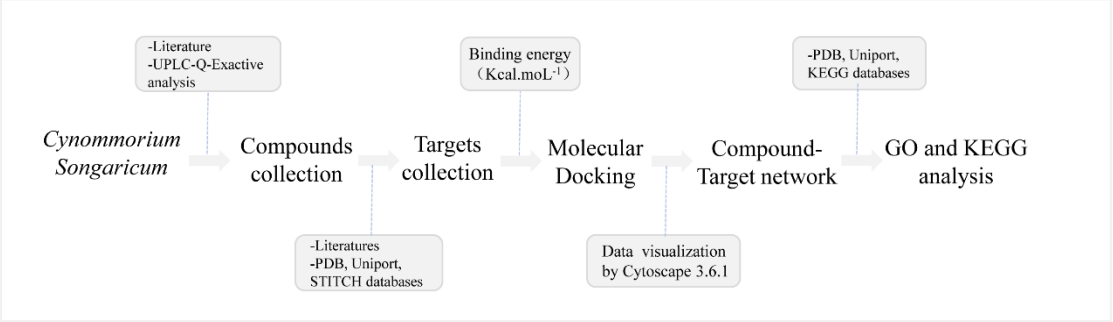

Figure 1 The whole framework of this study

2 Materials and Methods

2.1 Plant material and sample preparation

Samples of *C. songaricum* were collected in Alasan Left Banner, Inner Mongolia in May 2015. The samples were identified by Professor Linfang Huang as the dry fleshy stem of *C. songaricum* with voucher specimens (CMPB06301) deposited in the institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences.

The dried fleshy *C. songaricum* stem samples were crushed in a pulveriser. The powder (1000 mg) that passed through sieve No. 3 was extracted in a conical flask with cover by using 20 mL methanol. The samples were weighed and ultrasonically extracted at a power of 75 W for 35 min. Then, the extract was allowed to stand at room temperature, compensated for the lost weight, mixed well and filtered. The filtrate was collected and further filtered through 0.22 μm micropore membranes before chemical characterisation.

2.2 Chemicals and standard substances

Liquid chromatography (LC)–mass spectrometry (MS)-grade acetonitrile was acquired

from Fisher Scientific (Beijing, China). Deionised water was decontaminated through a Milli-Q system (Millipore, USA). Analytical-grade reagents were used during extraction as received from Beijing Chemical Plant Co. Ltd. (Beijing, China).

Gallic acid, rutin, 3,4-dihydroxybenzoic acid and (−)-catechin and phloridzin were purchased from Chengdu Must Biotechnology Co. Ltd. (Sichuan, China).

2.3 UPLC-Q-Exactive Analysis

2.3.1 LC

UHPLC analysis was executed by an Ultimate 3000 system (Dionex, USA), which was combined with a column compartment with a thermostat, an online vacuum degasser, a quaternary pump and an autosampler. The chromatographic column was ACQUITY UPLC HSS T3, 1.7 μm, 2.1 mm × 100 mm (Waters, USA) with the temperature of 40 °C. The separation conditions consisted of a gradient elution of acetonitrile (phase A) and aqueous formic acid 0.1% (v/v) (mobile phase B) at a 0.3 mL/min flow rate. The gradient was applied as follows: 0–1 min, 0% A; 1–10 min, 0%–100% A; 10–10.1 min, 100%–0% A and 10–10.1 min, 0% A. The injection volume and the injection temperature were 2 μL and 15 °C, respectively.

2.3.2 MS

MS was performed with the Q Exactive Orbitrap mass spectrometer (Thermo Fisher, USA) along with a heated electrospray ionisation source for target compound ionisation under the negative mode. The operating parameters were as follows: assistant gas heater temp, 300 °C; capillary temp, 320 °C; spray voltage, 3700 V; sheath gas pressure, 30 psi; assistant gas pressure, 10 arb; scan mode, full MS (resolution 7 million); and scan range, m/z 100–1500. The data were processed by the Thermo Xcalibur software.

2.4 Data preparation

2.4.1 Chemical compounds in *C. songaricum*

The chemical compositions of the *C. songaricum* extract were characterised by UHPLC-Q-Exactive. On the basis of standard substances, the retention time, molecular ions and major fragments were investigated by MS, online METLIN database and literature; accordingly, 21 chemical compounds were identified to be mainly flavonoids,

organic acids and triterpenoids. The identified components' information is listed in Table 1. The base peak chromatogram of the *C. songaricum* extract is shown in Figure 2.

Table 1 Chemical characterization of *C. songaricum* extract by UPLC-Q-Exactive in negative mode.

Peak	TR(min)	Formula	M/Z calculated	M/Z experimental	MS/MS fragment ions	Tentative identification
1	1.11	$C_4H_4O_4$	115.00259	115.00219	-	Succinic acid
2*	4.09	$C_7H_6O_5$	169.01315	169.01315	125	Gallic acid
3	4.25	$C_{16}H_{12}O_5$	283.0601	283.06827	268, 239, 211	Acacetin
4	4.56	$C_8H_8O_4$	167.03389	167.03394	149, 123	Vanillic acid
5*	4.69	$C_7H_6O_4$	153.01824	153.01816	109	3,4-Dihydroxybenzoic acid
6	4.78	$C_{30}H_{26}O_{12}$	577.13405	577.13495	425, 407, 289	Procyanidin B_3
7	4.92	$C_{45}H_{38}O_{18}$	865.19744	865.19806	577, 425, 407, 289	Procyanidin trimer
8*	5.04	$C_{15}H_{14}O_6$	289.07066	289.07159	245, 203	（-）-Catechin
9*	5.54	$C_{27}H_{30}O_{16}$	609.14501	609.1450111	301, 300, 271	Rutin
10	5.69	$C_{21}H_{20}O_{12}$	463.0871	463.0871024	301, 271	Isoquercetin
11	5.73	$C_{14}H_6O_8$	300.99789	300.99893	284, 245, 229, 201	Ellagic acid
12	5.77	$C_{22}H_{18}O_{10}$	441.08162	441.0827	289, 271, 169, 125	（-）-Epicatechin gallate
13	5.81	$C_9H_8O_3$	163.03897	163.03894	119	2-hydroxycinnamic acid
14	6.09	$C_{21}H_{22}O_{10}$	433.11292	433.11325	271, 151,119	Naringenin 4'-O- glucoside
15*	6.17	$C_{21}H_{24}O_{10}$	435.12857	435.12924	273, 167, 123	Phloridzin
16	7.27	$C_{15}H_{12}O_5$	271.0601	271.0611	253, 227, 151,119, 107	Naringenin
17	11.05	$C_{16}H_{32}O_2$	255.23186	255.23259	-	palmitic acid
18	11.13	$C32H_{50}O_4$	497.36254	497.36331	455, 437	acetyl ursolic acid
19	11.17	$C_{33}H_{50}O_6$	541.35237	541.35345	497, 455, 437	Malonyl ursolic acid hemiester
20	11.43	$C_{18}H_{34}O_2$	281.24751	281.24838	183, 117	Oleic acid
21	11.86	$C_{30}H_{48}O_3$	455.35197	455.35352	-	Ursolic acid

* identified with a standard reference

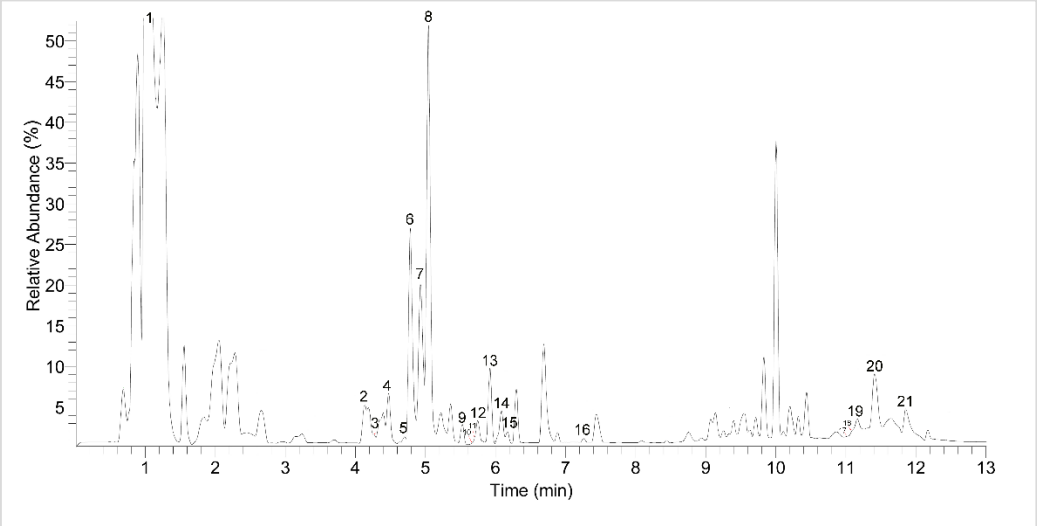

Figure 2 Base peak chromatogram (BPC) of *C. songaricum* extract (negative mode)

2.4.2 Target collection for *C. songaricum*

STITCH (http://stitch.embl.de/), as a public repository, provides information on protein targets for diseases. We searched protein targets by inputting the six diseases into STITCH and literature, obtained the corresponding gene targets by using the Uniport database (http://www.uniport.org/) and retrieved the PDB ID of the protein hypotype and the structure of small molecules by RCSB (http://.rcsb.org/pdbhome/home.do). A total of 43 targets were obtained for the following six diseases: HIV(17), OSCC (12), gastric ulcer(7), AD(3), gastric cancer(2) and melanoma(2). Information on the targets are presented in Table S1.

2.4.3 Molecular docking simulation

To evaluate the binding affinity of compounds in *C. songaricum* to candidate targets, we performed a molecular docking simulation through the software QuickVina 2.0 (www.qvina.org), an open source utility developed by the Alhossary research group. To verify the binding affinity between the targets and the compounds, we calculated a docking score through QuickVina 2.0. The docking scores that exceeded those of the positive drugs (data for every positive drug can be obtained from the corresponding targets in RCSB or literature) indicated a strong binding affinity between candidate targets and the corresponding compounds[15-18].

2.4.4 Network construction

Compound–compound targeting was conducted using the network visualisation

software Cytoscape (http://cytoscape.org/, 3.6.1). The software is well suited for visualising networks of intermolecular interactions. 'Degree' is an important parameter for screening network nodes in network analysis and is defined as the number of edges to a node[19].

2.4.5 GO analysis and pathway analysis

The Uniport ID of targets were inputted into the Uniport database. Then, the GO-BP (GO–biological process), GO-CC (GO–cellular component) and GO-MF (GO–molecular function) were analysed. Pathway analysis was explored by the Uniport database and the KEGG database (http://www.genome.jp/kegg/).

3 Results and Discussions

3.1 Candidate active compounds and targets of the diseases managed with *C. songaricum*

A total of 77 candidate active compounds in *C. songaricum* and 43 targets on six diseases were explored. Among these targets, 34 compounds and 18 candidate targets were screened by Quick Vina 2.0 and provided better scores than those of positive drugs. This result implies that the 34 compounds are in a close relationship with the 18 candidate targets in resisting the six diseases under study. The degree of the network on the compound–target interaction and the scores of the candidate therapeutic active compounds are depicted in Table 2, which also includes 52 nodes (34 active compound nodes and 18 compound target nodes) and 187 edges. Most of the compounds are flavonoids and glycosides. The targets of 3OD4, 4ACC, 4DJU and 3TJP play an important role in resisting the six diseases. Table 3 shows information on the 18 candidate targets related to the six diseases (HIV, gastric cancer, gastric ulcer, AD, melanoma and OSCC). Amongst these diseases, OSCC possessed the 10 most important targets. We also conclude that the common protein targets are the PI3 kinase, MMP-13 and AKT2. Data for positive drugs, such as cyclosporine A, can be obtained from the RCSB database and literature. The results readily uncover that each component is associated with multiple targets and multiple diseases and suggest that the traditional Chinese medicine is equipped with multi-component and multi-target

characteristics.

Table 2 Information of compounds of *C. Songaricum* on six diseases

Compounds	Binding energy(Kcal·moL^{-1})	Diseases	Edge
(-)-Epicatechingallate	-8.2(1M17),-10.2(1HBJ),-8.9(4DJU), -13(1PWM),-8.8(2JDO),-9.2(3OD4),-9(3TJP),-8.7(4ACC)	gastric ulcer, gastric cancer, AD	7
L-epicatechin	-7.3(1M17),-8.1(3OD4)	gastric cancer, OSCC	2
maslinic acid	-9.8(5COK), -8.5(1M17), -9.3(4DJU), -9.3(3G61)	HIV, gastric cancer, OSCC, AD	4
oleuropein	-7.6(1M17),-9.1(2JDO),-7.5(3OD4)	gastric cancer, OSCC	3
polydatin	-7.5(1M17),-8.6(2JDO),-7.2(3OD4)	gastric cancer, OSCC	3
(-)-catechin	-8.1(1M17),-8.5(1R0P),-8.4(2JDO),-9.7(3KRY),-8.3(3OD4),-8.5(3TJP),-8.2(4ACC)	gastric cancer, OSCC, gastric ulcer	7
(-)-lariciresinol	-7.7(1M17),-8.1(1R0P),-8.8(4DJU),-8.2(2JDO),-8(3OD4)	gastric ulcer, gastric cancer, OSCC, AD	5
(+)-catechin	-8(1M17),-8.3(1R0P),-9.6(3KRY),-8.6(3OD4)	gastric ulcer, gastric cancer, OSCC	4
3β-28-dihydroxyoleana-11,13(18)-diene	-10.7(4LL3),-10.9(5COK),-8(1M17),-9.5(4DJU),-9(3G61),-9.6(4ACC)	HIV, gastric cancer, OSCC, AD	6
4-hydroxyphenethhyl-2-acetate	-7.5(1M17),-7.6(3OD4)	gastric cancer, OSCC	2
acacetin	-8.5(1M17),-8.2(1R0P),-8.4(4DJU),-8.4(2JDO),-9.6(3KRY),-9.4(3OD4),-8.4(3TJP),-8.3(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	8
acacetin-7-O-β-D-glucoside	-8.8(1M17),-10.3(1HBJ),-9.2(4DJU),-9.1(2JDO),-9.8(3I16),-9.4(3TJP),-8.6(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	7
acetyl ursolic acid	-9.4(4DJU),-9.1(3G61)	OSCC, AD	2
arachidoside	-8(1M17),-8.5(1R0P),-8.3(3OD4),-8.6(3TJP),-8.2(4ACC)	gastric ulcer, gastric cancer, OSCC	5
betulinic acid	-7.9(1M17),-9(4DJU),-9.3(3G61),-8.5(4ACC)	gastric cancer, OSCC, AD	4
coniferyloside	-7.7(1M17)	gastric cancer	1
daucosterol	-8.9(1M17),-9(4DJU),-9.2(1Y6A),-12.2(1PWM),-8.9(4ACC)	gastric cancer, OSCC, melanoma, AD	5
ellagic acid	-8.9(1M17),-10.4(1HBJ),-8.6(1R0P),-8.4(4DJU),-8.8(2JDO),-7.3(3OD4),-9.2(3TJP),-9.6(3TL5),-9.2(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	9
epiluteoforol	-7.7(3OD4)	OSCC	1
isoconiferyloside	-7.9(1M17),-7.6(3OD4)	gastric cancer, OSCC	2
isolecanol-4-O-β-D-glucoside	-8.5(1M17),-8.5(4DJU),-8.4(2JDO),-7.7(3OD4),-8.7(3TJP)	gastric cancer, OSCC, AD	5
isoquercitrin	-7.9(1M17),-8.3(1R0P),-9(4DJU),-7.6(3OD4),-9.1(3TJP),-8.1(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	6
luteolin	-8.4(1M17),-10.9(1HBJ),-8.6(1R0P),-8.7(2JDO),-9.9(3KRY),-9.6(3OD4),-8.8(3TJP),-8.5(4ACC)	gastric ulcer, gastric cancer, OSCC	8
luteolin -7-O-β-D-glucoside	-9.2(1M17),-10.6(1HBJ),-9.1(1R0P),-9.4(4DJU),-12.2(1PWM),-10.1(2JDO),-7.5(3OD4),-9.6(3TJP),-8.8(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	9
naringenin	-8.5(1M17), -10.4(1HBJ), -8.4(1R0P), -8.5(2JDO),-8.9(3OD4),-8.5(3TJP),-8.4(4ACC),	gastric ulcer, gastric cancer, OSCC	13
naringenin -4'-glucopyranoside	-8.6(1M17),-9.4(4DJU),-9.1(2JDO),-10.6(3KRY),-8.3(3OD4),-9.1(3TJP),-8.4(4ACC)	gastric cancer, OSCC, AD	7
naringin	-8.3(1M17),-9.1(4DJU),-12(1PWM),-9.3(2JDO),-8.8(3OD4),-8.4(3TJP),-8.1(4ACC)	gastric cancer, OSCC, AD	7
nasocitrate	-9(1M17),-9(1R0P),-10.1(4DJU),-12.3(4EY7),-9.4(2JDO),-9.9(2ZJW),-9.6(3KRY),-8.1(3OD4),-8.4(3TJP),-9.1(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	10
rutin	-8.3(1M17),-8.3(1R0P),-10(4DJU),-8.9(2JDO),-10(3I16),-8.5(3OD4),-9.7(3TJP),-9.1(4ACC)	gastric ulcer, gastric cancer, OSCC, AD	8
phlorizin	-8.1(1M17),-8.8(2JDO),-7.8(3OD4)	gastric cancer, OSCC	3
procyanidin B3	-7.6(1M17),-9.5(4DJU),-8.2(2JDO),-8.8(3OD4)	gastric cancer, OSCC, AD	4
quercetin	-8.6(1M17),-10.5(1HBJ),-8.7(1R0P),-8.7(2JDO),-9.9(3KRY),-8.5(3OD4),-8.7(3TJP),-8.3(4ACC)	gastric ulcer, gastric cancer, OSCC	8
ursolic acid	-8.9(1M17),-9.2(4DJU),-12(1PWM),-8.4(2JDO),-9.5(3G61),-8.4(3TJP),-10.2(4ACC)	gastric cancer, OSCC, AD	7

β-sitosterol	-7.3(1M17),-12.3(1PWM),-8.4(2JDO),-8(3OD4),-8.9(4ACC)	gastric cancer, OSCC	5

Table 3 Information of 6 diseases' potential targets of *C. Songaricum*

Numbers	Diseases	PDB ID	Uniport ID	Protein name	Positive Drug	Score of Protein Drug (Kcal.moL^{-1})
1	OSCC	1PWM	P15121	Aldose Reductase	FID	-11.7
2		2JDO	P31751	PKB-BETA (AKT2)	I5S	-8.2
3		2ZJW	P68400	CK2 alpha	REF	-9.7
4		3G61	P21447	P-glycoprotein	Cyclosporine A	-8.9
5		3KRY	P45452	MMP-13	3KR	-9.6
6		3L16	P48736	(Dual) Pan-PI3-Kinase /mTOR	JZX	-9.3
7		3OD4	Q9NWT6	HIF-1 Alpha	8XQ	-7.1
8		3TJP	P48736	PI3K gamma	13K	-8.4
9		3TL5	P48736	PI3 Kinase/mTOR Kinase	980	-9.6
10		4ACC	P49841	GSK3b	7YG	-8.1
11	HIV	4LL3	Q9WFL7	HIV protease	017	-9.7
12		5COK	G0X8E8	HIV-1 protease	52U	-9.6
13	gastric cancer	1M17	P00533	EGFR tyrosine kinase	AQ4	-7.1
14	gastric ulcer	1HBJ	P04058	AchE	FBQ	-10.2
15		1R0P	P08581	HGFR	KSA	-8
16	AD	4DJU	P56817	BACE	0KK	-8.4
17		4EY7	P22303	AchE	E20	-12.3
18	melanoma	1Y6A	P35968	VEGFR 2	AAZ	-9.2

3.2 Compound–target network of *C. songaricum* in diseases

The compound–target network is a bipartite network where the nodes represent the targets and component, whereas the edges (links, connections) are the interactions between components and targets. For improved understanding, this paper established the compound–target network model by Cytoscape 3.6.1; 34 compounds and 18 targets were analysed in Figure 3. In this network, clockwise rotation indicates the strengthening interaction between the component and target. Figure 3 reveals the presence of ten candidate targets related to OSCC; two each to HIV, AD and gastric ulcer; and one to several related to gastric cancer and melanoma. The favourable molecular docking results were obtained between luteolin-7-O-β-D-glucoside and 2JDO, luteolin and 3OD4, rutin and 3TJP and ursolic acid and 4ACC. Figure 4 shows the 3D molecular docking results of four pairs of compounds and targets.

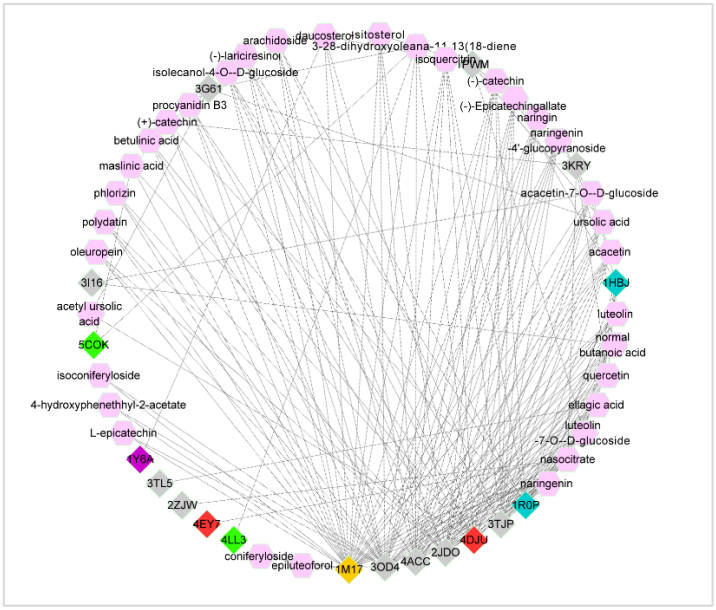

Figure 3 compound-target network of major active components of the *C. Songaricum*

(Pink represent the candidate active ingredients; Gray represent the candidate targets of OSCC; Green represent the candidate targets of HIV; Blue represent the candidate targets of gastric ulcer; Red represent the candidate targets of AD; Purple represents the candidate target of melanoma.)

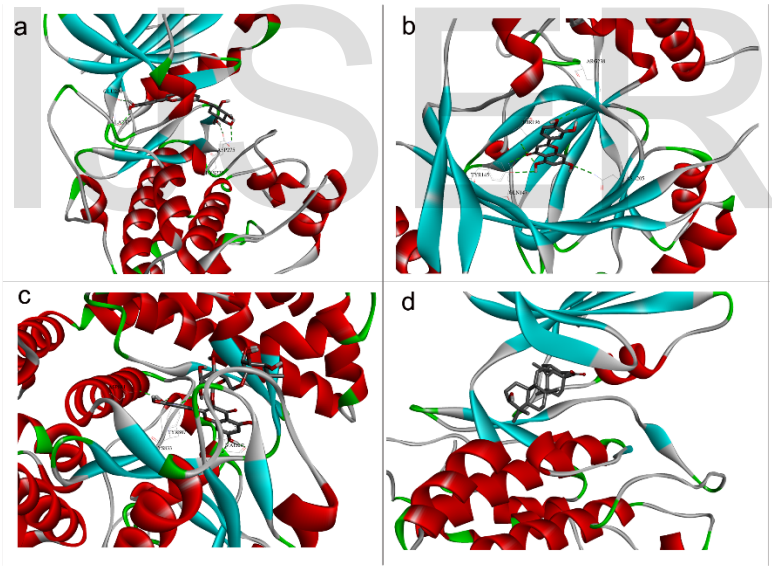

Figure 4 The three-dimensional molecular docking results of between compounds and targets related to OSCC

(luteolin -7-O-β-D-glucoside and 2JDO(3a); luteolin and 3OD4(3b); rutin and 3TJP (3c); ursolic acid and 4ACC(3d).

3.3 GO analysis and pathway analysis of candidate targets

To capture the related biological functions of *C. songaricum*, every target of OSCC was analysed using the Uniport, RCSB and KEGG databases. Given the GO analysis of the candidate targets with good scores on molecular docking, we concluded that the three aspects are molecular function, cellular component and biological process. Firstly, for

the molecular function, protein binding, nucleotide binding and ATP binding were the top three entries with more than 7 degrees in the analysis of molecular function. Secondly, for cellular component, cytosol, cytoplasm and plasma membrane achieved 8, 7 and 7 degrees, respectively, and ranked as the top three entries (Figure 5A). Thirdly, for biological process (Figure 5B), the top two entries were phosphorylation and protein phosphorylation, with 6 degrees each. Most of the other entries, such as adaptive immune response, angiogenesis and carbohydrate metabolic process, scored 3 degrees.

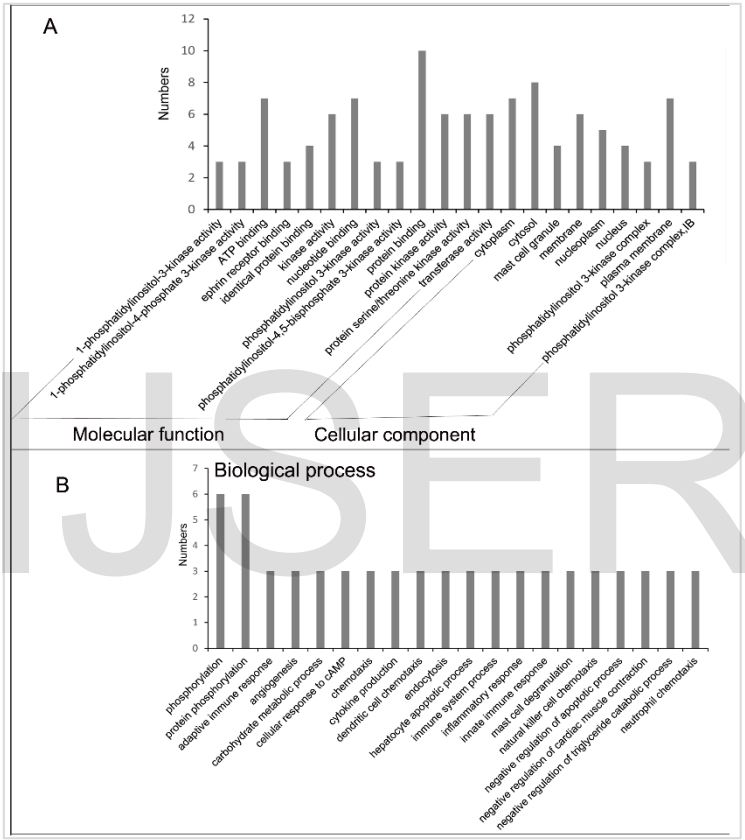

Figure 5 Analysis of gene ontology enrichment of anti-OSCC target of major active ingredients in *C. Songaricum*

(Molecular function and cellular component (A); Biological process (B))

On the basis of the pathway analysis of the related candidate targets on anti-OSCC for *C. songaricum*, nine main targets were involved in the 27 representative pathways (Table 4). The central pathways were the PI3K-Akt, cGMP-PKG, Wnt, NF-kappa B and Jak-STAT signalling pathways.

Table 4 Information of the representative pathways of OSCC on *C.songaricum*

PDB ID	Uniport ID	KEGG pathways
1PWM	P15121	positive regulation of JAK-STAT cascade
2JDO	P31751	Apoptosis
2JDO	P31751	Focal adhesion
2JDO	P31751	FoxO signaling pathway
2JDO	P31751	Glioma
2JDO	P31751	HIF-1 signaling pathway
2JDO	P31751	Jak-STAT signaling pathway
2JDO	P31751	MAPK signaling pathway
2JDO	P31751	Melanoma
2JDO	P31751	Neurotrophin signaling pathway
2JDO	P31751	PI3K-Akt signaling pathway
2JDO	P31751	Ras signaling pathway
2JDO	P31751	TNF signaling pathway
2JDO	P31751	Toll-like receptor signaling pathway
2JDO	P31751	VEGF signaling pathway
2ZJW	P68400	NF-kappa B signaling pathway
2ZJW	P68400	Wnt signaling pathway
3KRY	P45452	extracellular matrix disassembly
3L16	P48736	cGMP-PKG signaling pathway
3OD4	Q9NWT6	negative regulation of Notch signaling pathway
3TJP	P48736	negative regulation of cardiac muscle contraction
3TL5	P48736	T cell activation
4ACC	P49841	Hedgehog signaling pathway
4ACC	P49841	Hippo signaling pathway
4ACC	P49841	mTOR signaling pathway
4ACC	P49841	Pathways in cancer
4ACC	P49841	Prolactin signaling pathway

This paper utilised data mining, molecular docking and network pharmacology methods to explain the characteristics and mechanism of action of *C. songaricum.* A total of 77 ingredients and 43 compound targets were tested by molecular docking. The results showed good interactions between the 34 active ingredients and 18 candidate targets on resisting the six diseases. In particular, luteolin-7-O-β-D-glucoside, luteolin, rutin and ursolic acid were the main ingredients along with 2JDO, 3OD4, 3TJP and 4ACC as the main targets for anti-OSCC.

Molecular docking and network pharmacology analyses unveiled the numerous ingredients in *C. songaricum* that exert pharmacological effects on the six diseases,

especially OSCC. The main activities include regulating a vast number of biological processes, such as phosphorylation and protein phosphorylation. Moreover, the pathway analysis in our study demonstrated that *C. songaricum* simultaneously operates in various signalling pathways, such as the PI3K-Akt, cGMP-PKG and Wnt signalling pathways. This finding implies that *develop a novel activity in C. songaricum* and guide the discovery of new drugs [20, 21].

In summary, the current work is the first to propose and apply molecular docking technology and network pharmacology analyses to illuminate the multiple mechanisms (multi-ingredients multi-target, multi-pathway and multi-disease) of *C. songaricum* against six diseases, especially OSCC. In this research, *C. songaricum* was first uncovered to possess potential pharmacological activity against six diseases, including OSCC, AD and HIV. Despite all the above achievements, further experiments in pharmacological and molecular research are warranted to validate the results. Finally, we hope that our study can provide a reference for anti-OSCC drug discovery and guide the novel research of other herbal medicines.

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Authors' contributions: LFH conceived the ideas; XZ collected and analyzed the data of *C. songaricum* together with XXR and RZ; XZ was a major contributor in writing the manuscript.

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Supplementary Material: Table S1 The 43 PDB ID on six diseases from RCSB

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Supplementary Material

Table S1 The 43 PDB ID on six diseases from RCSB

(PDB ID number: Oral cancer,12; HIV,17; gastric ulcer,7; melanoma,2; gas cancer,2; AD, 3)

Numbers	Diseases	PDB ID
1	oral cancer	3G61
2		3OD4
3		1PWM
4		2JDO
5		2ZJW
6		1TVO
7		4ACC
8		3L16
9		3TL5
10		3KRY
11		3TJP
12		3DBS
13	HIV	1AJV
14		1D4Y
15		1S6Q
16		3KFS
17		1EBZ
18		1D4I
19		1SDT
20		3QO9
21		4KV8
22		2YNH
23		4O4G
24		4IG0
25		1RT2
26		5COK
27		2ZD1
28		4PHV
29		4LL3
30		1HBJ
31	gastric ulcer	3F82
32		1R0P
33		3CP9
34		3BE2
35		1Q84
36		3CE3
37	melanoma	1Y6B
38		1Y6A
39	gastric cancer	1M17
40		1AQ1
41		4DJU

42	AD	4EY7
43		2CKM