ARTICLE
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Predicting bioactive compounds and cancer-related molecular targets of lotus seedpod (*Receptaculum Nelumbinis*) based on network pharmacology and molecular docking

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Competing interests
The authors declare no conflicts of interest.
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Abbreviations
LSP, Lotus seedpod; AKT1, RAC-alpha serine/threonine-protein kinase; ESR1, Estrogen receptor 1; HSP90AA1, Heat shock protein HSP 90-alpha; JUN, Transcription factor AP-1; MAPK1, Mitogen-activated protein kinase 1; MAPK3, Mitogen-activated protein kinase 3; PIK3CA, Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform; PIK3R1, Phosphatidylinositol 3-kinase regulatory subunit alpha; SRC, Proto-oncogene tyrosine-protein kinase Src; STAT3, Signal transducer and activator of transcription 3; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HIF-1, Hypoxia-inducible factor 1; TCMSP, Traditional Chinese Medicine System Pharmacology; SMILES, Simplified Molecular-Input Line-Entry System; GI, Gastrointestinal; DL, Drug-likeness; PPI, Protein-protein interaction; BP, Biological process; CC, Cell composition; MF, Molecular function.
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Abstract
Background: Lotus seedpod (*Receptaculum Nelumbinis*) is the abundant by-products produced during lotus seed processing, and the sources are usually considered to be wastes and are abandoned outdoors or incinerated. This study aims at predicting its bioactive compounds and cancer-related molecular targets against six cancers, including lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer. **Methods:** Network pharmacology and molecular docking methods were performed. **Results:** Network pharmacology results indicated that 14 core compounds (liensinine, tetrandrine, lysicamine, tricin, sanleng acid, cireneol G, ricinoleic acid, linolenic acid, 5,7-dihydroxycoumarin, apigenin, luteolin, morin, quercetin and isorhamnetin) and 10 core targets (AKT1, ESR1, HSP90AA1, JUN, MAPK1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3) were screened for lotus seedpod against the six cancers. Molecular docking analysis suggested that the binding abilities between the core compounds and the core targets were mostly strong. GO analysis revealed that the intersected targets between the bioactive compounds of lotus seedpod and the six cancers were significantly related to biological processes, cell compositions and molecular functions. KEGG analysis showed that PI3K-Akt, TNF, Ras, MAPK, HIF-1 and C-type lectin receptor signaling pathways were notably involved in the anti-cancer activities of lotus seedpod against the six cancers. **Conclusions:** 14 core compounds and 10 core targets were screened for lotus seedpod against lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer. This study supports the application of lotus seedpod in treating cancers, and promotes the recycling and the high-value utilization.

Keywords: Lotus seedpod; Anti-cancer; Bioactive compounds; Molecular targets; Network pharmacology; Molecular docking.

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Background

Cancer is being a serious disease that threatens human life and health. It is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (https://www.who.int/news-room/fact-sheets/detail/cancer). According to the statistical data of China National Cancer Center announced in 2022, lung cancer, gastric cancer, liver cancer and breast cancer are the four most common cancers, with 828,000, 397,000 389,000 and 306,000 new cases in China in 2016. Meanwhile, ovarian cancer and cervical cancer are two prevalent cancers in women [1, 2]. Clinically, treatments of cancers depend on radiotherapy, conventional cytotoxic chemotherapy, hormonal therapy, targeted therapy or immunotherapy with a number of long-term side-effects [3]. Natural products have been increasingly acted as emerging chemo-therapeutic agents, owing to they are readily applicable, inexpensive, accessible and acceptable therapeutic approach with minimum cytotoxicity [4].

Lotus seedpod (*Receptaculum Nelumbinis*), the mature receptacle of lotus house, is the food by-products during lotus seed processing. Lotus seedpod sources are abundant in Asian countries, such as China, India, Korea, Thailand and Japan [5]. However, the sources are usually considered to be wastes and are abandoned outdoors or incinerated, as lack of adequate understanding on theirs nutritional and pharmaceutical values. This will bring environmental pollution and waste of resources. Modern studies have indicated that extracts and bioactive compounds (polyphenols, procyanidins, etc.) from lotus seedpod exerted anti-cancer activities on lung cancer and liver cancer [6–9]. However, whether lotus seedpod has anti-cancer activity on other cancers or not is unclear. With the development of research, more than 94 compounds have been identified in lotus seedpod [10]. From this view, whether there are other anticancer components in lotus seedpod is unknown. Therefore, exploring the anticancer components from lotus seedpod are of significant values for recovery of lotus seedpod sources and are helpful to the treatment of cancers.

Network pharmacology, a promising approach towards drug design, plays important roles in the efficacy, action mechanism, rational design of compatibility, development of new drugs and safety [11]. Molecular docking is a method of designing drugs by simulating the interaction mode between the receptor and the drug, and its application in explaining the relevant action mechanism has become a trend in the research and development of new drugs [12]. The combination of network pharmacology and molecular docking has attracted increasing attention, owing to the complexity of the ingredients and targets of many traditional Chinese medicines [11]. Nowadays, nutritional therapy are being essential to manage progressive cancer, and a healthy diet plays an important role in cancer treatment [13]. Moreover, combination of network pharmacology and molecular docking has been carried out to uncover the active constituents and mechanisms of foods against cancers [14–17]. Similarly, combination of network pharmacology and molecular docking might be a good choice to screen the bioactive compounds and action mechanisms of lotus seedpod against cancers.

In this study, network pharmacology and molecular docking were performed to explore bioactive compounds and underlying mechanisms of lotus seedpod on the treatment of six cancers (lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer) for the first time. The workflow of this research is illustrated in Figure 1. This study may provide theoretical basis for anti-cancer mechanisms of lotus seedpod, and promote the high-value utilization of lotus seedpod sources.

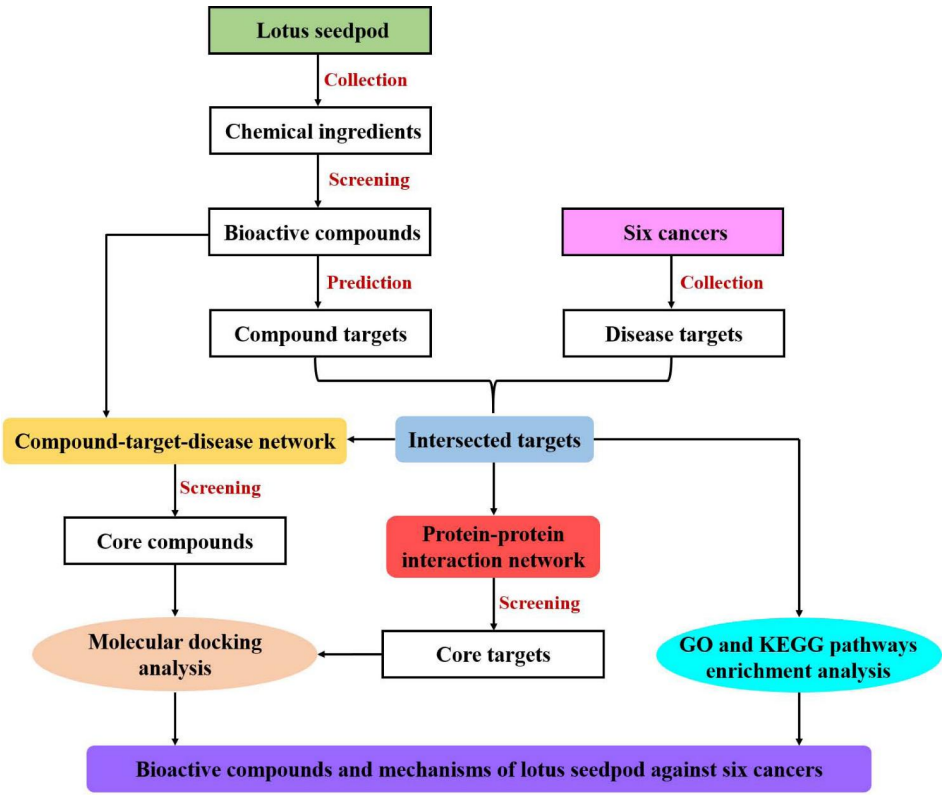

Figure 1 The workflow of this research

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Materials and methods

Acquisition of bioactive compounds from lotus seedpod

Compounds from lotus seedpod were collected from previous literatures [18–24], Traditional Chinese Medicine System Pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php) and HERB database (http://drug.ac.cn/). The information on Canonical Simplified Molecular-Input Line-Entry System (SMILES) and PubChem ID of each ingredient was acquired from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). If this information of compound has not been found in PubChem database, the SMILES of it was gained by drawing the corresponding chemical structure by a StoneMIND Collector software. Then, the SMILES of ingredient was imported into SwissADME tool (http://www.swissadme.ch/) for gastrointestinal (GI) absorption prediction and for drug-likeness (DL) analysis. Those ingredients with "high" GI absorption and two or more models among five DL models (Lipinski, Ghose, Veber, Egan and Muegge) met "Yes" were selected as bioactive compounds [25]. Meanwhile, some ingredients did not satisfy the above criteria but have been demonstrated to possess good anti-cancer activities in previous literatures were also considered to be bioactive compounds [26].

Prediction of targets for bioactive compounds

SMILES of obtained bioactive compounds uploaded into Swiss Target Prediction database (http://www.swisstargetprediction.ch/) to predict the corresponding targets. During this processes, the targets prediction were conducted as the species of "Homo sapiens" and the screening condition of "probability > 0"[27]. All predicted targets were standardized as gene names using Uniprot database (https://www.uniprot.org/).

Collection of targets for cancers

Potential targets for six cancers (lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer) were screened from GeneCards database (https://www.genecards.org/) with relevance score ≥ 10 [28], OMIM database (https://www.omim.org/), DisGeNET database (https://www.disgenet.org/), PharmGKB database (https://www.pharmgkb.org/) and TTD database (http://db.idrblab.net/ttd/). The used terms were as follows: lung cancer and lung carcinoma; gastric cancer, stomach cancer, gastric carcinoma and stomach carcinoma; liver cancer, hepatocellular carcinoma, hepatoma, hepatic carcinoma and hepatocarcinoma; breast cancer, breast carcinoma, mammary cancer and mammary carcinoma; ovarian cancer, ovarian carcinoma, ovary cancer and ovary carcinoma; cervical cancer, cervical carcinoma, cancer of cervix and uterine cervix cancer. In the same database, the potential targets were pooled and deduplicated. Meanwhile, the targets that appeared in two or more databases were taken as the final targets, for guaranteeing high correlation between targets and cancers.

Intersection of targets of bioactive compounds and targets of cancers

Overlap of targets of bioactive compounds and targets of cancers were analyzed using Venny2.1.0 database (https://bioinfogp.cnb.csic.es/tools/venny/). The intersected targets were considered as potential targets for lotus seedpod against above-mentioned cancers.

Construction of the compound-target-disease network

The aforementioned data were imported into software Cytoscape version 3.9.1 to construct the compound-target-disease network, which visualized the relationship between the bioactive compounds and intersected targets. Meanwhile, the network topology parameters including "Betweenness Centrality", "Closeness Centrality" and "Degree" were calculated to screen the core compounds [29].

Construction of the protein-protein interaction (PPI) network

The intersected targets were input into the STRING database (https://cn.string-db.org/) with "Homo sapiens" filter. The PPI network was constructed by setting the confidence level as "highest confidence (0.900)" [29], hiding the free targets and retaining other parameters as their defaults. PPI network image and data (tsv format files) were exported. Then, the tsv format files were opened using software Cytoscape version 3.9.1 to visualize the PPI network. At the same time, the parameters of "Betweenness Centrality", "Closeness Centrality" and "Degree" were analyzed to screen the core targets.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis

In order to explore the mechanism of lotus seedpod against cancers, the intersected targets were imported into Metascape (https://metascape.org/gp/index.html) for GO and KEGG pathways enrichment analysis. "Homo sapiens" were selected, and the enriched pathways met the criteria of $p < 0.01$ was extracted [30]. The pathways were ranked according to their significances by *p*-values, and the top 20 enriched pathways were visualized using the "ggplot2" package in Rstudio (v2022.02.1.461) software.

Molecular docking analysis

For investigating the interaction between the core compounds (as ligands) and core targets (as receptors) and verifying the accuracy of the network pharmacology prediction [29], molecular docking analysis was performed using AutoDock Vina software (v1.1.2) [31, 32]. Firstly, the three-dimensional (3D) structures of the target proteins were downloaded from Protein Data Bank (PDB) platform, and the ligand and water in the proteins were removed by PyMOL software (v2.5.3). Addition of hydrogen and calculation of charge were conducted by AutoDock Tools (v1.5.6), and "Macromolecule" was selected in the Grid module. The data was exported as pdbqt format file. Secondly, the two-dimensional (2D) structures of core compounds were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the 2D structures were transformed into 3D structures by OpenBabel software (v2.4.1) and saved as mol2 format files. The obtained mol2 format files were opened by AutoDock Tools (v1.5.6) for adding hydrogen, calculating charge and choosing torsions, and the pdbqt format files were exported. Thirdly, the size and orientation of docking box were adjusted to package the full protein, and files for parameters of the docking box were exported. Fourthly, information on parameters were written into config.txt file, and pdbqt format files for ligands and receptors were placed in the same folder of AutoDock Vina software (v1.1.2). Molecular docking was operated through AutoDock Vina software (v1.1.2) by invoking config.txt file using the CMD terminal command. Lastly, a heat map of the docking energy of each ligand and each receptor was developed by bioinformatics analysis, and molecular docking results were visualized using PyMOL software (v2.5.3).

Results and discussions

Bioactive compounds, potential targets and compound-target-disease networks for lotus seedpod against cancers

Based on SwissADME tool analysis and previous literatures, a total of 41 bioactive compounds were screened, as shown in Table 1. The compounds included 2 of polyphenols (procyanidins and catechol), 9 of organic acids (citric acid, chelidonic acid, isovanillic acid, phellibaumin A, sanleng acid, ricinoleic acid, linolenic acid, ρ-coumaric acid and palmitic acid), 9 of alkaloids (armepavine, liensinine, N-methylcoclaurine, pseudopurpurin, nuciferine, tetrandrine, lysicamine, futoamide and morphine), 12 of flavonoids (taxifolin, apigenin, luteolin, morin, tricin, gallocatechin, catechin, quercetin, hyperoside, isoquercitrin, kaempferol and isorhamnetin), 1 of alkynol (cireneol G), 2 of steroids (neotigogenin acetate and daucosterol), 3 of terpenoids (glycyrrhetinic acid, soyasapogenol B and ganoderiol F), 1 of ester (diisobutyl phthalate), 1 of sterol

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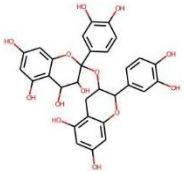
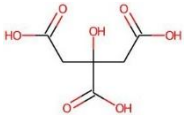
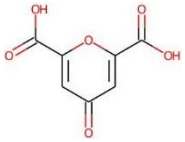
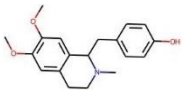
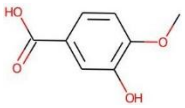
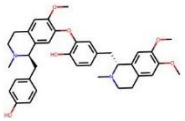
(β-sitosterol), and 1 of coumarin (5,7-dihydroxycoumarin). By using Swiss Target Prediction database, 595 targets were predicted after deleting duplicate targets.

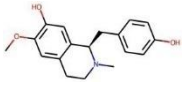
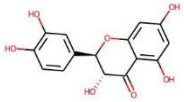
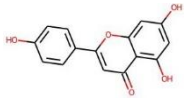
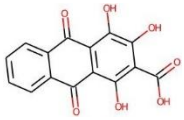
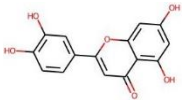
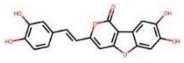
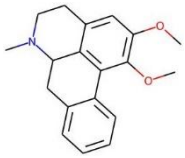
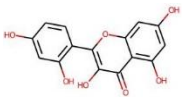
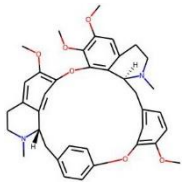
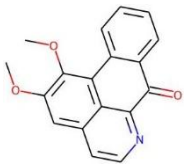
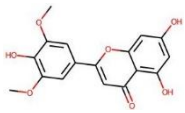
On the basis of GeneCards, OMIM, DisGeNET, PharmGKB and TTD databases, 1786, 1333, 3262, 1950, 1215 and 862 targets for lung cancer (Figure 2(A)), gastric cancer (Figure 3(A)), liver cancer (Figure 4(A)), breast cancer (Figure 5(A)), ovarian cancer (Figure 6(A)) and cervical cancer (Figure 7(A)) were collected, respectively. With further overlapping of above targets of bioactive compounds and targets of cancers using Venny analysis, 219 (10.1%), 192 (11.1%), 298 (8.4%), 235 (10.2%), 167 (10.2%) and 124 (9.3%) targets for lung cancer (Figure 2(B)), gastric cancer (Figure 3(B)), liver cancer (Figure 4(B)), breast cancer (Figure 5(B)), ovarian cancer (Figure 6(B)) and cervical cancer (Figure 7(B)) were intersected, respectively.

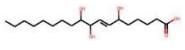
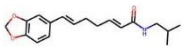
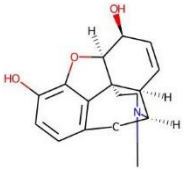
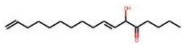
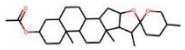
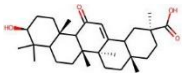
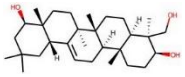
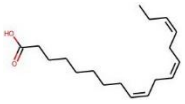
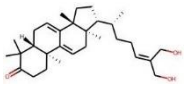
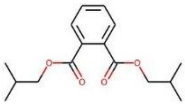
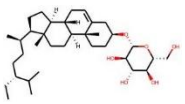
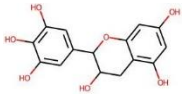
According to above-mentioned results, compound-target-disease networks for lotus seedpod against lung cancer (Figure 2(C)), gastric cancer (Figure 3(C)), liver cancer (Figure 4(C)), breast cancer (Figure 5(C)), ovarian cancer (Figure 6(C)) and cervical cancer (Figure7(C)) were constructed by Cytoscape software to display the relationship between the bioactive compounds and intersected targets. In the compound-target-disease networks, the bioactive compounds and intersected targets were used as the network nodes, and every compound was linked to its targets with edges. The figures reflect that one compound can act on different targets, and multiple compounds can also act on same targets. There are 259, 232, 338, 275, 207 and 164 nodes, along with 1249, 1114, 1654, 1288, 998 and 792 edges in Figure 2(C), Figure 3(C), Figure 4(C), Figure 5(C), Figure 6(C) and Figure7(C) respectively. Further topological analyses indicated that the four characteristic parameters (network density, network centralization, network heterogeneity, characteristic path length) of compound-target-disease networks for lotus seedpod against cancers were as follows: 0.037, 0.818, 1.788 and 2.183 (lung cancer); 0.041, 0.797, 1.670 and 2.198 (gastric cancer); 0.029, 0.861, 2.088 and 2.147 (liver cancer); 0.034, 0.830, 1.887 and 2.176 (breast cancer); 0.047, 0.772, 1.553 and 2.214 (ovarian cancer); 0.059, 0.710, 1.311 and 2.247 (cervical cancer).

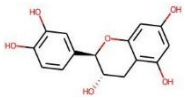
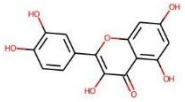
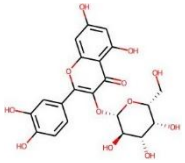
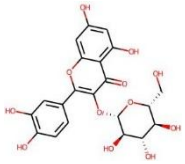
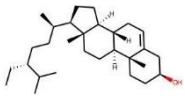
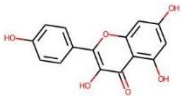
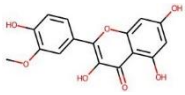
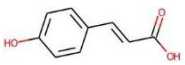
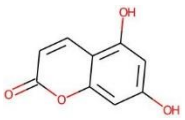
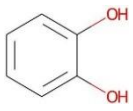
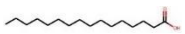
Meanwhile, three indexes of network node centrality included Betweenness Centrality, Closeness Centrality and Degree were calculated to screen the core compounds for lotus seedpod against cancers, as listed in Table 2. Different core compounds from lotus seedpod were identified for treating different cancers. For lung cancer, the core compounds were liensinine, tetrandrine, lysicamine, tricin, sanleng acid, cireneol G, ricinoleic acid, linolenic acid and 5,7-dihydroxycoumarin. In terms of gastric cancer, 8 of core compounds including luteolin, morin, tetrandrine, lysicamine, tricin, sanleng acid, quercetin and isorhamnetin were screened. Regarding to liver cancer, 3 of core compounds including tetrandrine, lysicamine and tricin were identified. As to breast cancer, 5 of core compounds including morin, tetrandrine, lysicamine, tricin and isorhamnetin were found. To ovarian cancer, the core compounds were morin, tetrandrine, lysicamine, tricin, quercetin and isorhamnetin. For cervical cancer, 6 of core compounds including liensinine, apigenin, luteolin, morin, tetrandrine and tricin were acquired.

Table 1 Properties of acquired bioactive compounds from lotus seedpod

ID	Compound name	Molecular type	Molecular structure	PubChem ID	GI absor-ption	Lipinski	Ghose	Veber	Egan	Muegge	References
LSC 01	Procyanidins	Polyphenol		107876	Low	No	No	No	No	No	[18]
LSC 02	Citric acid	Organic acid		311	Low	Yes	No	Yes	No	No	[19]
LSC 03	Chelidonic acid	Organic acid		7431	High	Yes	No	Yes	Yes	No	[19]
LSC 04	Armepavine	Alkaloid		98348	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 05	Isovanillic acid	Organic acid		12575	High	Yes	Yes	Yes	Yes	No	[19]
LSC 06	Liensinine	Alkaloid		160644	High	Yes	No	Yes	Yes	No	[19]

ID	Compound name	Molecular type	Molecular structure	PubChem ID	GI absor -ption	Lipinski	Ghose	Veber	Egan	Muegge	References
LSC 07	N-Methylcoclaurine	Alkaloid		440595	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 08	Taxifolin	Flavonoid		439533	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 09	Apigenin	Flavonoid		5280443	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 10	Pseudopurpurin	Alkaloid		442765	High	Yes	Yes	Yes	No	Yes	[19]
LSC 11	Luteolin	Flavonoid		5280445	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 12	Phellibaumin A	Organic acid		54581651	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 13	Nuciferine	Alkaloid		3108374	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 14	Morin	Flavonoid		5281670	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 15	Tetrandrine	Alkaloid		73078	High	Yes	No	Yes	Yes	No	[19]
LSC 16	Lysicamine	Alkaloid		122691	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 17	Tricin	Flavonoid		5281702	High	Yes	Yes	Yes	Yes	Yes	[19]

ID	Compound name	Molecular type	Molecular structure	PubChem ID	GI absor-ption	Lipinski	Ghose	Veber	Egan	Muegge	References
LSC 18	Sanleng acid	Organic acid		5321100	High	Yes	Yes	No	Yes	No	[19]
LSC 19	Futoamide	Alkaloid		15596445	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 20	Morphine	Alkaloid		5288826	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 21	Cireneol G	alkynol		Not found	High	Yes	Yes	No	Yes	No	[19]
LSC 22	Neotigogenin acetate	Steroid		313012	High	Yes	No	Yes	No	No	[19]
LSC 23	Glycyrrhetinic acid	Terpenoid		18526330	High	Yes	No	Yes	No	No	[19]
LSC 24	Ricinoleic acid	Organic acid		643684	High	Yes	Yes	No	Yes	No	[19]
LSC 25	Soyasapogenol B	Terpenoid		115012	High	Yes	No	Yes	No	No	[19]
LSC 26	Linolenic acid	Organic acid		5280934	High	Yes	No	No	Yes	No	[19]
LSC 27	Ganoderiol F	Terpenoid		471008	High	Yes	No	Yes	No	No	[19]
LSC 28	Diisobutyl phthalate	Ester		6782	High	Yes	Yes	Yes	Yes	Yes	[19]
LSC 29	Daucosterol	Steroid		5742590	Low	Yes	No	Yes	Yes	No	[19]
LSC 30	Gallocatechin	Flavonoid		1249	High	Yes	Yes	Yes	Yes	No	[20]

ID	Compound name	Molecular type	Molecular structure	PubChem ID	GI absor -ption	Lipinski	Ghose	Veber	Egan	Muegge	References
LSC 31	Catechin	Flavonoid		9064	High	Yes	Yes	Yes	Yes	Yes	[20]
LSC 32	Quercetin	Flavonoid		5280343	High	Yes	Yes	Yes	Yes	Yes	[20]
LSC 33	Hyperoside	Flavonoid		5281643	Low	No	No	No	No	No	[20]
LSC 34	Isoquercitrin	Flavonoid		5280804	Low	No	No	No	No	No	[20]
LSC 35	β-sitosterol	Sterol		222284	Low	Yes	No	Yes	No	No	[21]
LSC 36	Kaempferol	Flavonoid		5280863	High	Yes	Yes	Yes	Yes	Yes	[22]
LSC 37	Isorhamnetin	Flavonoid		5281654	High	Yes	Yes	Yes	Yes	Yes	[22]
LSC 38	ρ-Coumaric acid	Organic acid		637542	High	Yes	Yes	Yes	Yes	No	[23]
LSC 39	5,7-Dihydroxyco umarin	Coumarin		5324654	High	Yes	No	Yes	Yes	No	[24]
LSC 40	Catechol	Polyphenol		289	High	Yes	No	Yes	Yes	No	[24]
LSC 41	Palmitic acid	Organic acid		985	High	Yes	Yes	No	Yes	No	[24]

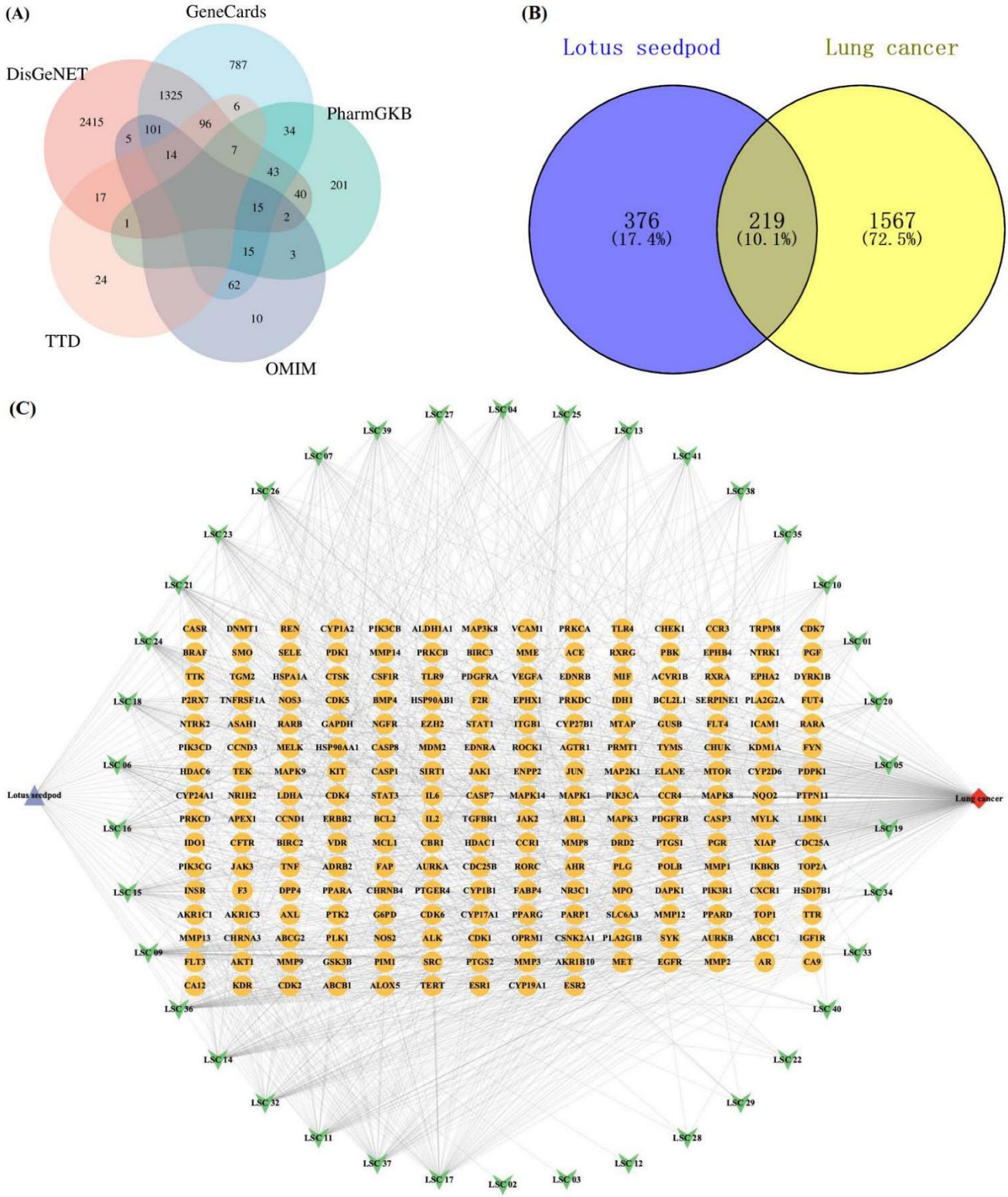


Figure 2 Venn diagram of the intersection of lung cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and lung cancer-related targets (B), and compound-target-disease network for lotus seedpod against lung cancer (C).

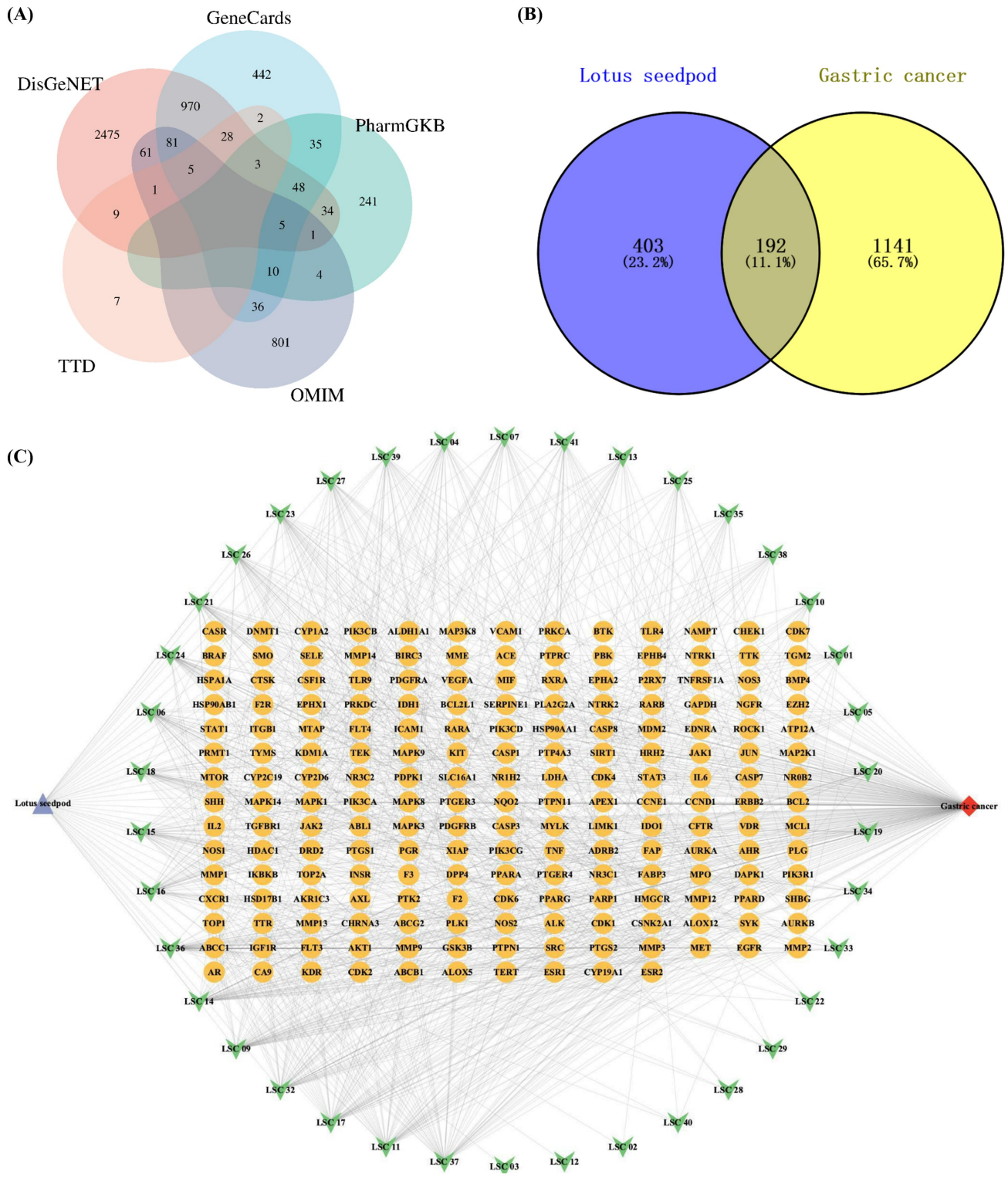


Figure 3 Venn diagram of the intersection of gastric cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and gastric cancer-related targets (B), and compound-target-disease network for lotus seedpod against gastric cancer (C).

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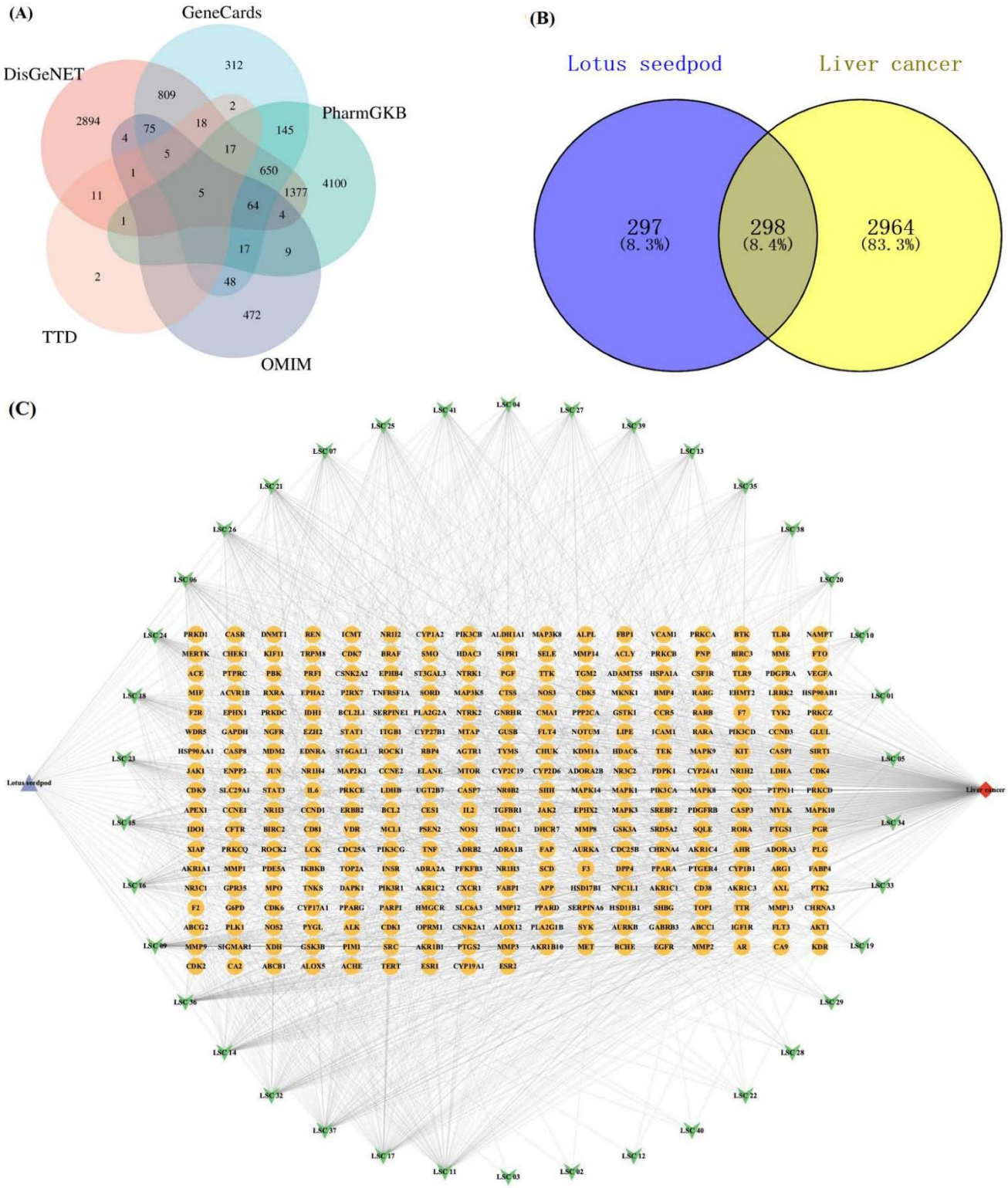

Figure 4 Venn diagram of the intersection of liver cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and liver cancer-related targets (B), and compound-target-disease network for lotus seedpod against liver cancer (C).

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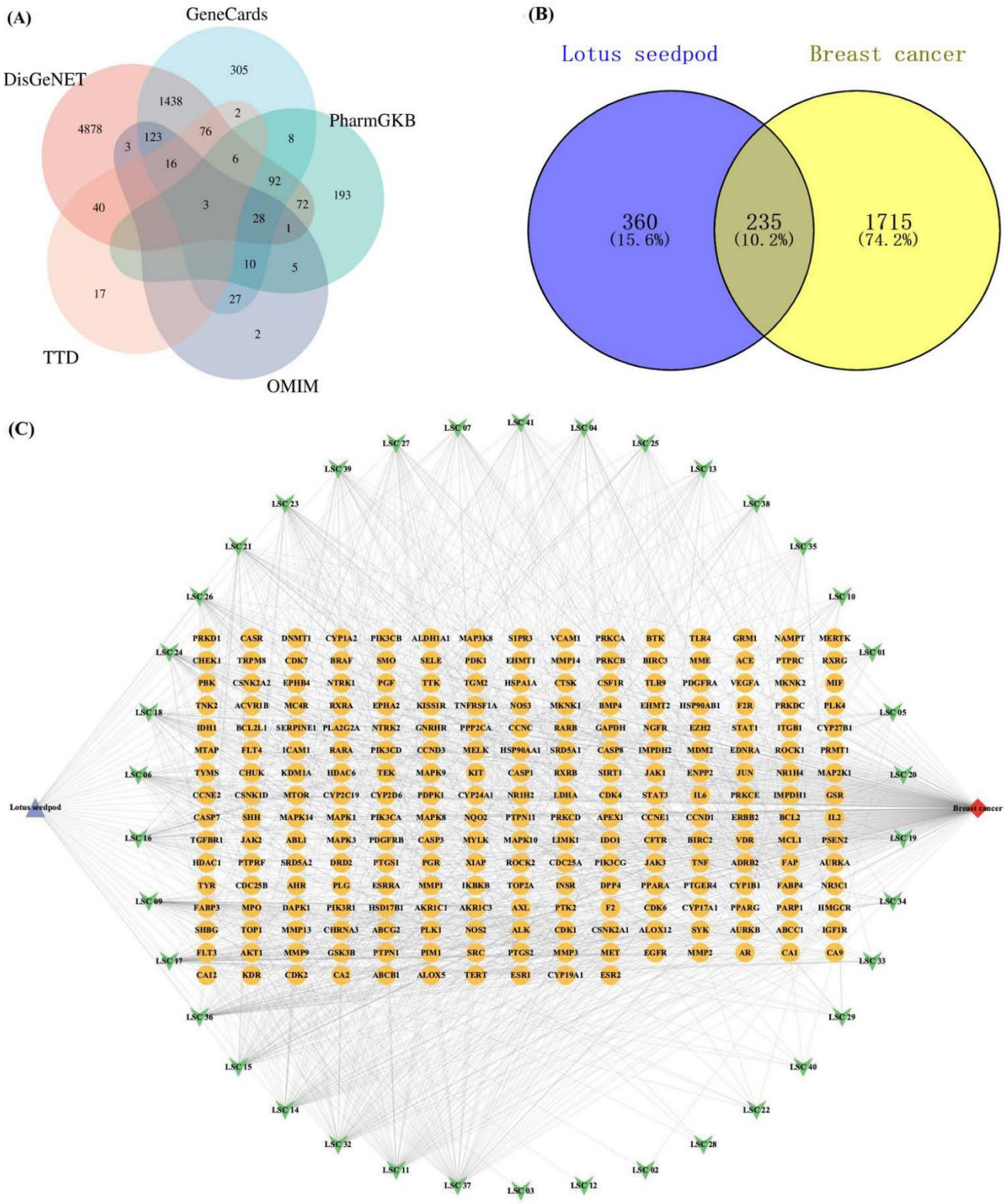

Figure 5 Venn diagram of the intersection of breast cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and breast cancer-related targets (B), and compound-target-disease network for lotus seedpod against breast cancer (C).

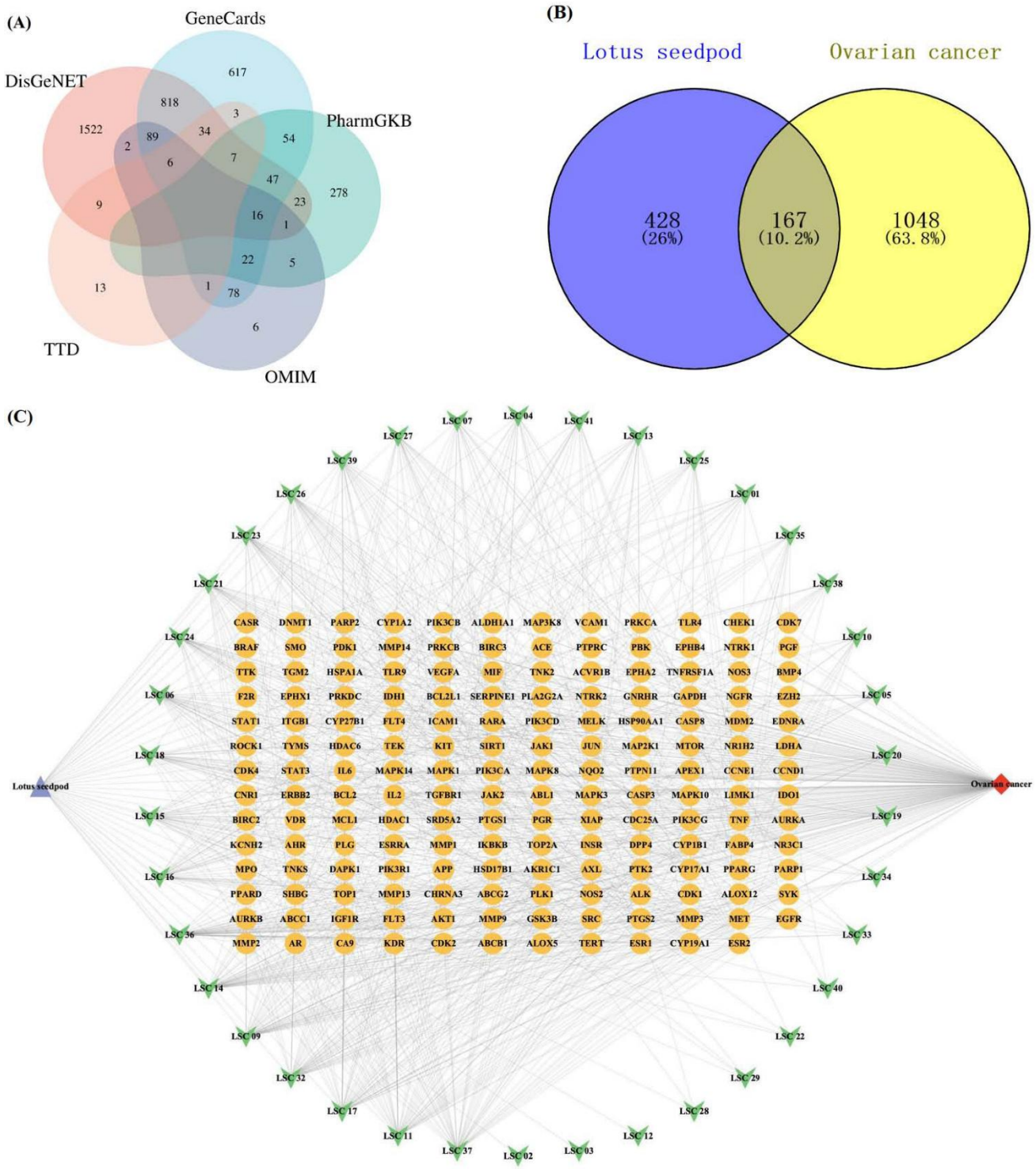

Figure 6 Venn diagram of the intersection of ovarian cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and ovarian cancer-related targets (B), and compound-target-disease network for lotus seedpod against ovarian cancer (C).

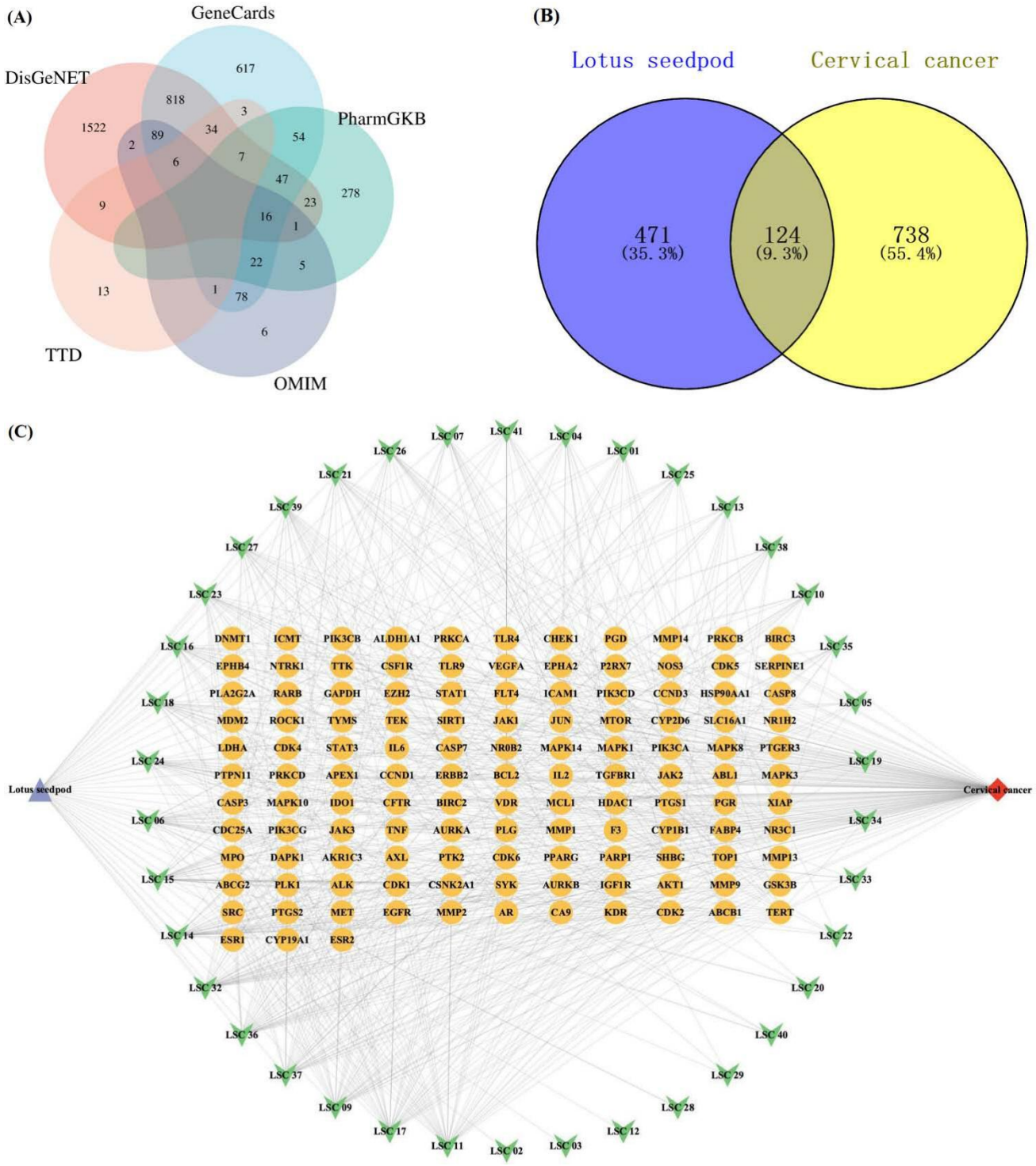

Figure 7 Venn diagram of the intersection of cervical cancer-related targets obtained from five databases (A), venn diagram of the intersection targets between lotus seedpod and cervical cancer-related targets (B), and compound-target-disease network for lotus seedpod against cervical cancer (C).

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Table 2 The core compounds and core targets of lotus seedpod against six cancers

Classification	Name of core compound or core target	Betweenness Centrality	Closeness Centrality	Degree
Lung cancer				
Core compounds	Liensinine	0.0896	0.3991	46
	Tetrandrine	0.1088	0.4041	51
	Lysicamine	0.1729	0.4016	47
	Tricin	0.0496	0.4119	55
	Sanleng acid	0.1106	0.3966	44
	Cireneol G	0.0824	0.3918	39
	Ricinoleic acid	0.0511	0.3942	41
	Linolenic acid	0.0547	0.3894	37
	5,7-Dihydroxycoumarin	0.0585	0.3813	30
Core targets	AKT1	0.0898	0.4974	45
	ESR1	0.0906	0.4774	31
	HSP90AA1	0.1049	0.4948	49
	MAPK1	0.0381	0.4762	44
	MAPK3	0.0490	0.4798	45
	PIK3CA	0.0299	0.4578	42
	PIK3R1	0.0426	0.4715	48
	SRC	0.1277	0.5163	56
	STAT3	0.1143	0.5040	54
Gastric cancer				
Core compounds	Luteolin	0.0109	0.4147	49
	Morin	0.0119	0.4132	48
	Tetrandrine	0.0182	0.4031	43
	Lysicamine	0.0246	0.4060	43
	Tricin	0.0128	0.4147	49
	Sanleng acid	0.0175	0.4003	40
	Quercetin	0.0112	0.4147	49
	Isorhamnetin	0.0121	0.4162	50
Core targets	AKT1	0.0971	0.5184	44
	ESR1	0.0633	0.4884	32
	HSP90AA1	0.1160	0.5168	48
	MAPK1	0.0418	0.4941	40
	MAPK3	0.0568	0.5015	42
	PIK3R1	0.0469	0.4941	46
	SRC	0.1309	0.5417	55
	STAT3	0.1207	0.5232	49
Liver cancer				
Core compounds	Tetrandrine	0.0164	0.3951	62
	Lysicamine	0.0221	0.3988	64
	Tricin	0.0102	0.4026	68
Core targets	AKT1	0.0805	0.4595	51
	ESR1	0.0712	0.4359	32
	HSP90AA1	0.0943	0.4529	53
	JUN	0.0520	0.4322	34
	MAPK1	0.0439	0.4482	51
	MAPK3	0.0521	0.4513	52
	PIK3R1	0.0328	0.4257	50
	SRC	0.1030	0.4662	63
	STAT3	0.1058	0.4603	58
Breast cancer				
Core compounds	Morin	0.0100	0.4053	54
	Tetrandrine	0.0202	0.4018	53
	Lysicamine	0.0217	0.3994	49
	Tricin	0.0097	0.4029	52
	Isorhamnetin	0.0098	0.4065	55
Core targets	AKT1	0.0872	0.5000	49
	ESR1	0.0972	0.4755	32
	HSP90AA1	0.0969	0.4988	49
	MAPK1	0.0499	0.4927	49
	MAPK3	0.0623	0.4988	51
	PIK3CA	0.0315	0.4574	43
	PIK3R1	0.0431	0.4722	49
	SRC	0.1309	0.5244	62
	STAT3	0.1064	0.5012	52

Classification	Name of core compound or core target	Betweenness Centrality	Closeness Centrality	Degree
Ovarian cancer				
Core compounds	Morin	0.0132	0.4204	45
	Tetrandrine	0.0191	0.4055	38
	Lysicamine	0.0255	0.4087	38
	Tricin	0.0150	0.4221	46
	Quercetin	0.0127	0.4221	46
	Isorhamnetin	0.0132	0.4221	46
Core targets	AKT1	0.0945	0.5139	37
	HSP90AA1	0.1284	0.5248	43
	MAPK3	0.0447	0.4950	36
	PIK3CA	0.0412	0.4774	36
	PIK3R1	0.0462	0.4933	41
	SRC	0.1266	0.5421	48
	STAT3	0.1230	0.5267	47
Cervical cancer				
Core compounds	Liensinine	0.0230	0.4190	31
	Apigenin	0.0145	0.4324	37
	Luteolin	0.0153	0.4347	38
	Morin	0.0144	0.4278	35
	Tetrandrine	0.0258	0.4212	33
	Tricin	0.0172	0.4347	38
Core targets	AKT1	0.0686	0.5340	29
	ESR1	0.1087	0.5314	28
	HSP90AA1	0.1359	0.5556	38
	SRC	0.1411	0.5820	43
	STAT3	0.0983	0.5699	41

PPI network and core targets

The intersected targets for lotus seedpod against the six cancers (lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer) were used to construct PPI networks by STRING platform, and the PPI networks were visualized by Cytoscape tool, as illustrated in Figure 8. In the PPI networks, the nodes represented the targets and the edges reflected the interactions between the targets. Similar to the aforementioned compound-target-disease networks, one target can link with multiple targets, and multiple targets can also link with one target. There are 197, 172, 262, 209, 151 and 113 nodes, accompanied by 1044, 899, 1296, 1123, 767 and 589 edges in Figure 8(A), 8(B), 8(C), 8(D), 8(E) and 8(F), respectively. Moreover, topological analysis implied that the network density, network centralization, network heterogeneity and characteristic path length parameters were identified to be 0.057, 0.240, 0.992 and 2.923 (lung cancer), 0.063, 0.266, 0.999 and 2.757 (gastric cancer), 0.040, 0.209, 1.084 and 3.211 (liver cancer), 0.054, 0.253, 1.000 and 2.879 (breast cancer), 0.069, 0.258, 0.951 and 2.763 (ovarian cancer), and 0.096, 0.300, 0.864 and 2.526 (cervical cancer).

With further calculation, three indexes that Betweenness Centrality, Closeness Centrality and Degree were applied to screen the core targets for lotus seedpod against the six cancers. Meanwhile, the sizes and colors of the nodes are proportional to the degree in the PPI networks (Figure 8), and the stronger is the interaction when the larger is the node and the deeper is the color [29]. Accordingly, the core targets are identified and displayed in Table 2. It can be seen that anti-cancer effects of lotus seedpod against different cancers had different targets. In terms of lung cancer and breast cancer, 9 of core targets, including AKT1, ESR1, HSP90AA1, MAPK1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3, were screened. Regarding to gastric cancer, the core targets were found to be AKT1, ESR1, HSP90AA1, MAPK1, MAPK3, PIK3R1, SRC and STAT3. For liver cancer, 9 of core targets that AKT1, ESR1, HSP90AA1, JUN, MAPK1, MAPK3, PIK3R1, SRC and STAT3 were gained. To ovarian cancer, 7 of core targets including AKT1, HSP90AA1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3 were identified. For cervical cancer, 5 of core targets including AKT1, ESR1, HSP90AA1, SRC and STAT3 were acquired.

GO and KEGG analyses

GO enrichment and KEGG pathway enrichment analyses were operated through Metascape platform to predict the potential biological roles and signaling pathways of the intersected targets. GO enrichment analyses suggested that a total of 2746, 2642, 3060, 2737, 2417 and 2016 items were acquired for lotus seedpod against lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer, respectively. The numbers of items belonging to biological process (BP), cell composition (CC) and molecular function (MF) were 2370, 123 and 253 (lung cancer), 2313, 117 and 212 (gastric cancer), 2618, 146 and 296 (liver cancer), 2375, 114 and 248 (breast cancer), 2111, 103 and 203 (ovarian cancer), and 1767, 94 and 155 (cervical cancer), in sequence. Among them, top 20 enriched items of BP, CC and MF visualized using Rstudio software, as shown in Figure 9, Figure 10, Figure 11, Figure 12, Figure 13 and Figure 14, respectively. Regarding to BP items, protein phosphorylation, response to hormone, cellular response to nitrogen compound, positive regulation of protein phosphorylation, and cellular response to organonitrogen compound were the same top five items for lotus seedpod against lung cancer (Figure 9(A)), gastric cancer (Figure 10(A)), and breast cancer (Figure 12(A)). While, protein phosphorylation, response to hormone, and positive regulation of protein phosphorylation were three common items in the top five items for lotus seedpod against liver cancer (Figure 11(A)) and ovarian cancer (Figure 13(A)). Additionally, protein phosphorylation, positive regulation of cell migration, positive regulation of transferase activity, enzyme-linked receptor protein signaling pathway, cellular response to organonitrogen compound were the top five items for lotus seedpod against cervical cancer (Figure 14(A)). In terms of CC results, membrane raft, membrane microdomain, receptor complex, and transferase complex, transferring phosphorus-containing groups were four uniform items in the top five items for lotus seedpod against lung cancer (Figure 9(B)), gastric cancer (Figure 10(B)), liver cancer (Figure 11(B)), breast cancer (Figure 12(B)), ovarian cancer (Figure 13(B)) and cervical cancer (Figure 14(B)). Similarly, network pharmacology analysis showed that CC items for *Cremastra Appendiculata* against breast cancer were comprised of membrane raft, membrane microdomain, receptor complex, etc [33]. As to MF items, protein kinase activity, phosphotransferase activity, alcohol group as

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acceptor, kinase activity, and protein serine/threonine kinase activity were four identical items in the top five items for lotus seedpod against lung cancer (Figure 9(C)), gastric cancer (Figure 10(C)), liver cancer (Figure 11(C)), breast cancer (Figure 12(C)), ovarian cancer (Figure 13(C)) and cervical cancer (Figure 14(C)). The study conducted by Zhang *et al* [33] has also implied that MF items for *Cremastra Appendiculata* against breast cancer consisted of protein kinase activity, phosphotransferase activity, alcohol group as acceptor, kinase activity, etc.

On the other hand, KEGG pathway enrichment analyses implied that there were 197, 192, 215, 197, 186 and 182 signaling pathways for lotus seedpod against lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer, respectively. The top 20 enriched pathways are displayed in Figure 9(D), Figure 10(D), Figure 11(D), Figure 12(D), Figure 13(D) and Figure 14(D). In terms of lung cancer (Figure 9(D)), the targets were mainly related to PI3K-Akt, TNF, Ras and MAPK signaling pathways. Regarding to gastric cancer (Figure 10(D)), liver cancer (Figure 11(D)), breast cancer (Figure 12(D)) and ovarian cancer (Figure 13(D)), the targets mainly correlated with PI3K-Akt, TNF, Ras, MAPK and HIF-1 signaling pathways. As to cervical cancer (Figure 14(D)), the targets were connected with PI3K-Akt, TNF, HIF-1 and C-type lectin receptor signaling pathways.

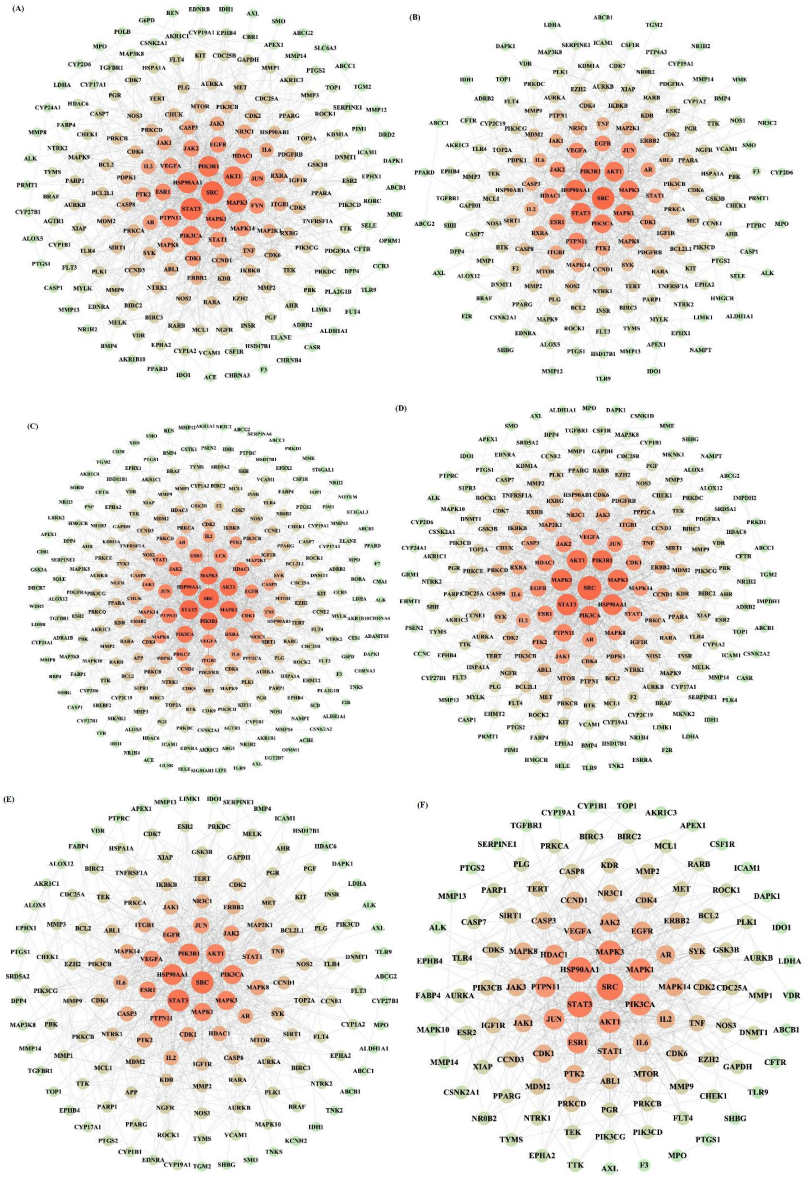

Figure 8 PPI networks of intersected targets for lotus seedpod aganist lung cancer (A), gastric cancer (B), liver cancer (C), breast cancer (D), ovarian cancer (E) and cervical cancer (F).

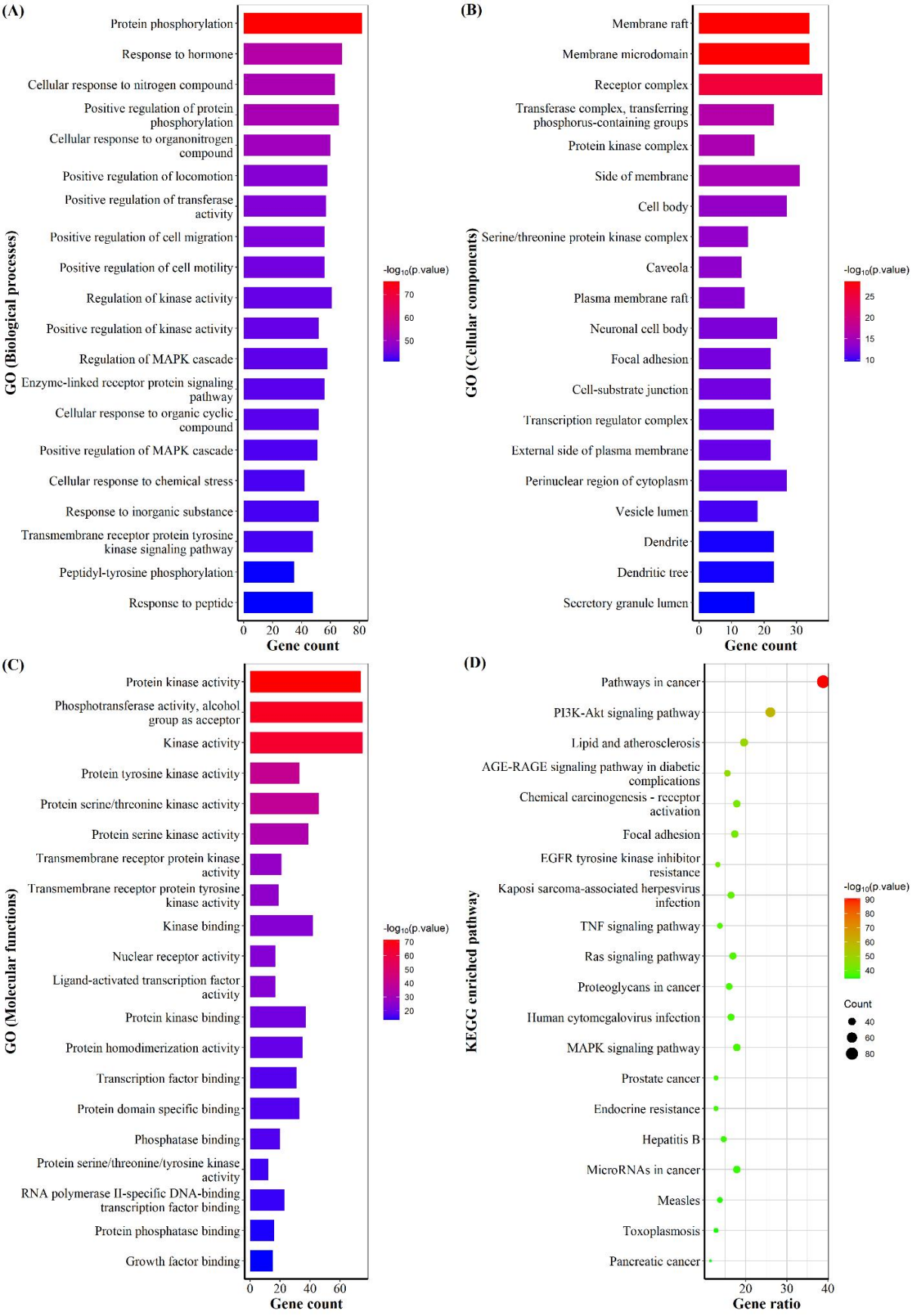

Figure 9 GO and KEGG analyses using intersected targets for lotus seedpod against lung cancer

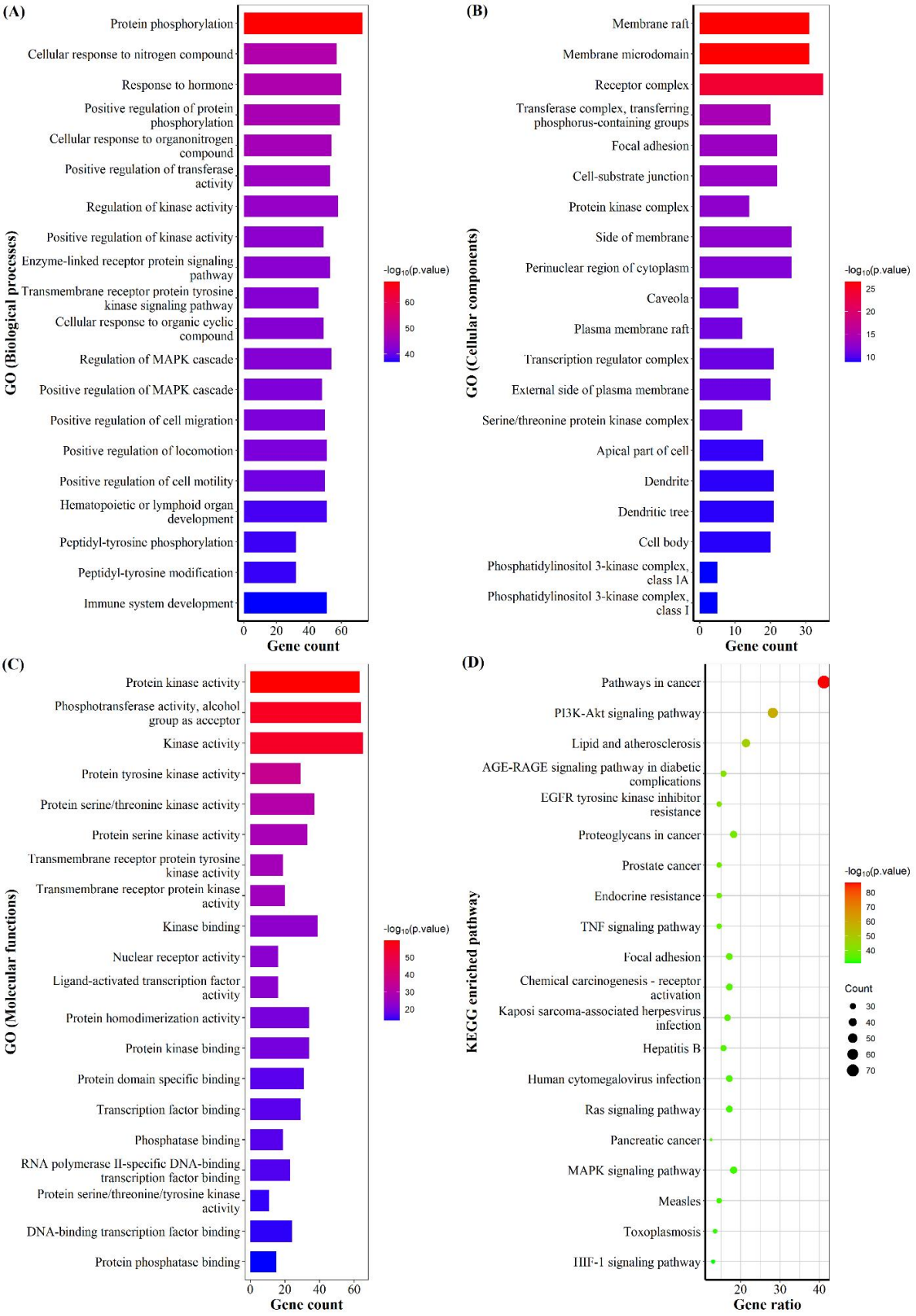

Figure 10 GO and KEGG analyses using intersected targets for lotus seedpod against gastric cancer

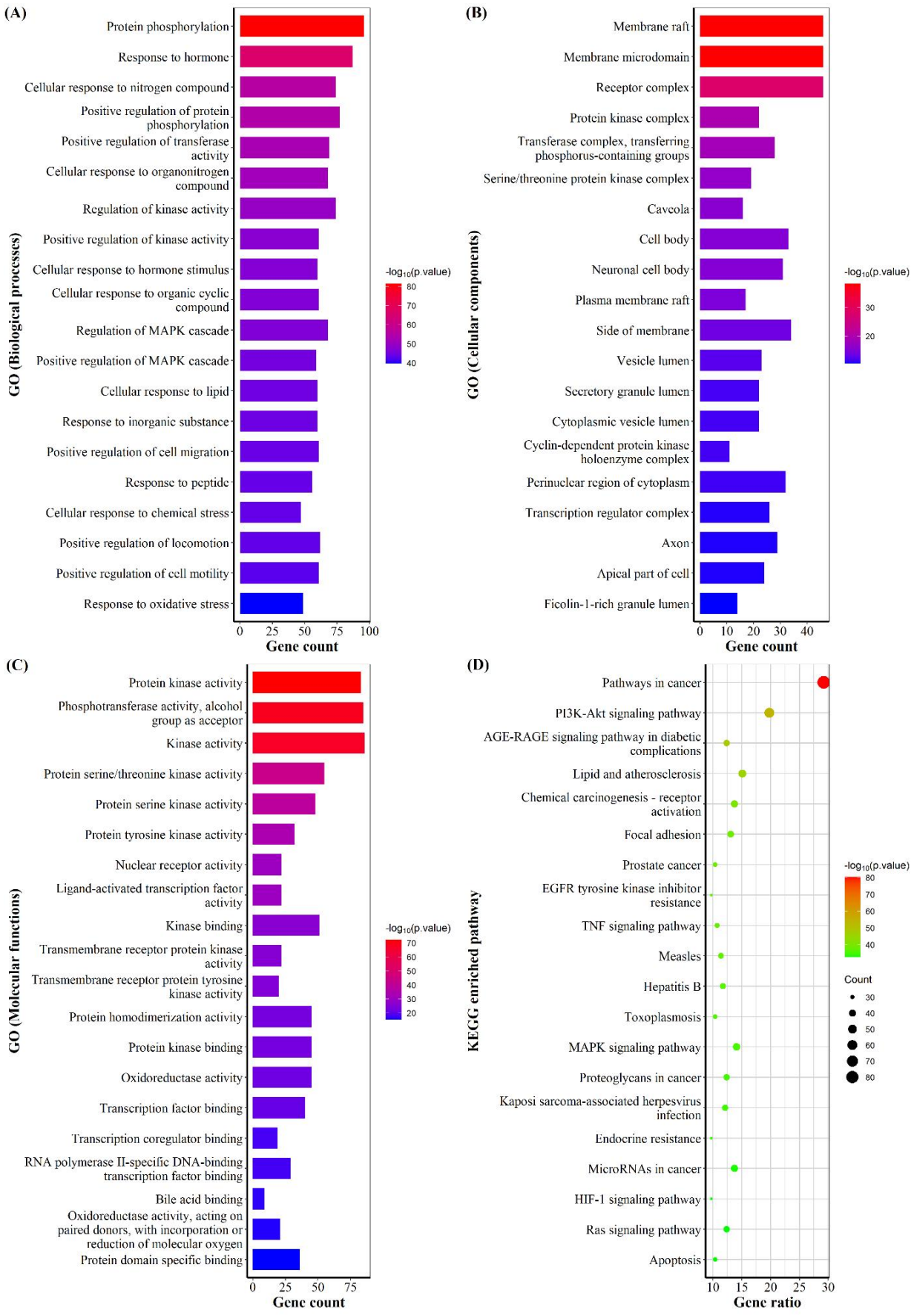

Figure 11 GO and KEGG analyses using intersected targets for lotus seedpod against liver cancer

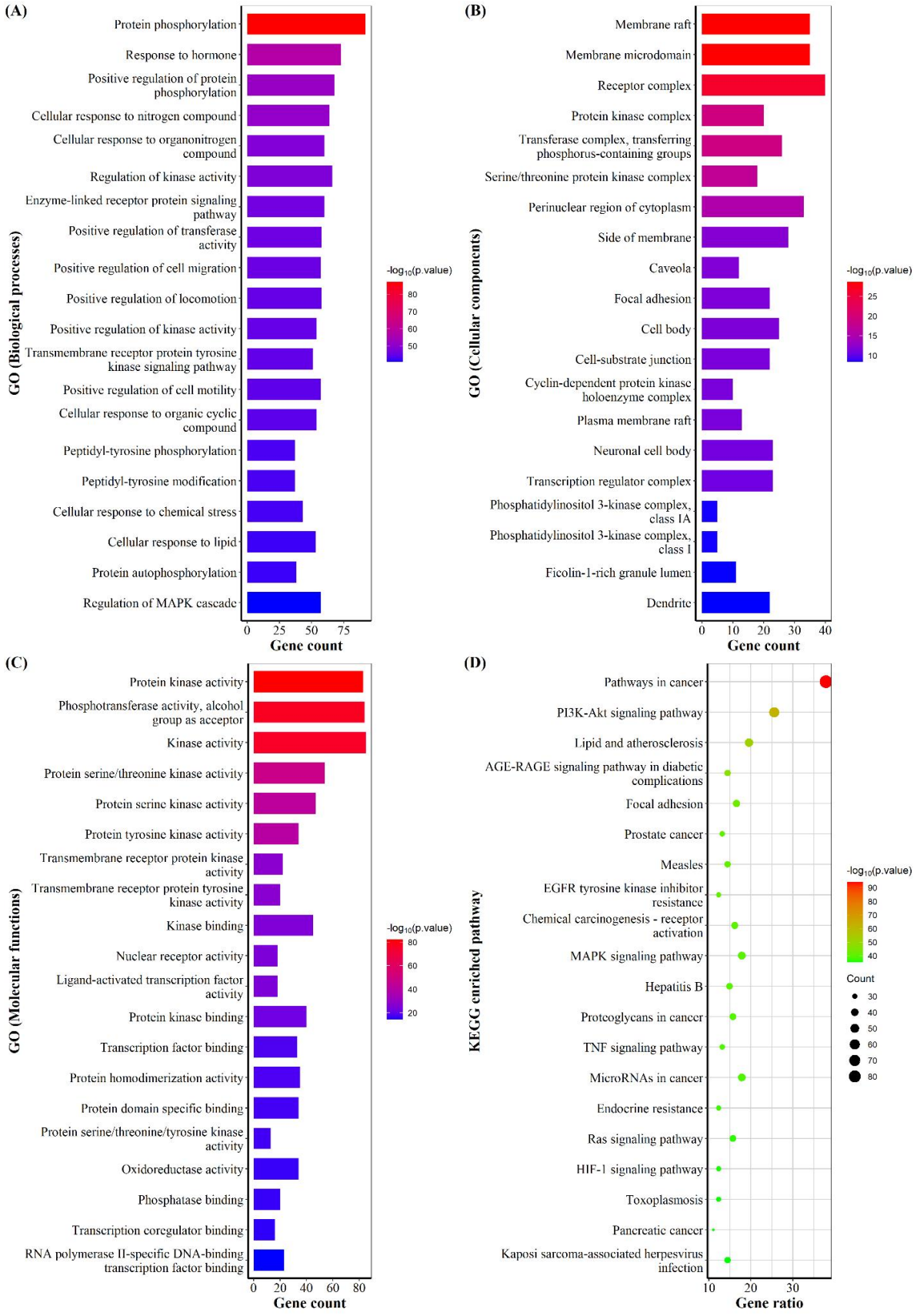

Figure 12 GO and KEGG analyses using intersected targets for lotus seedpod against breast cancer

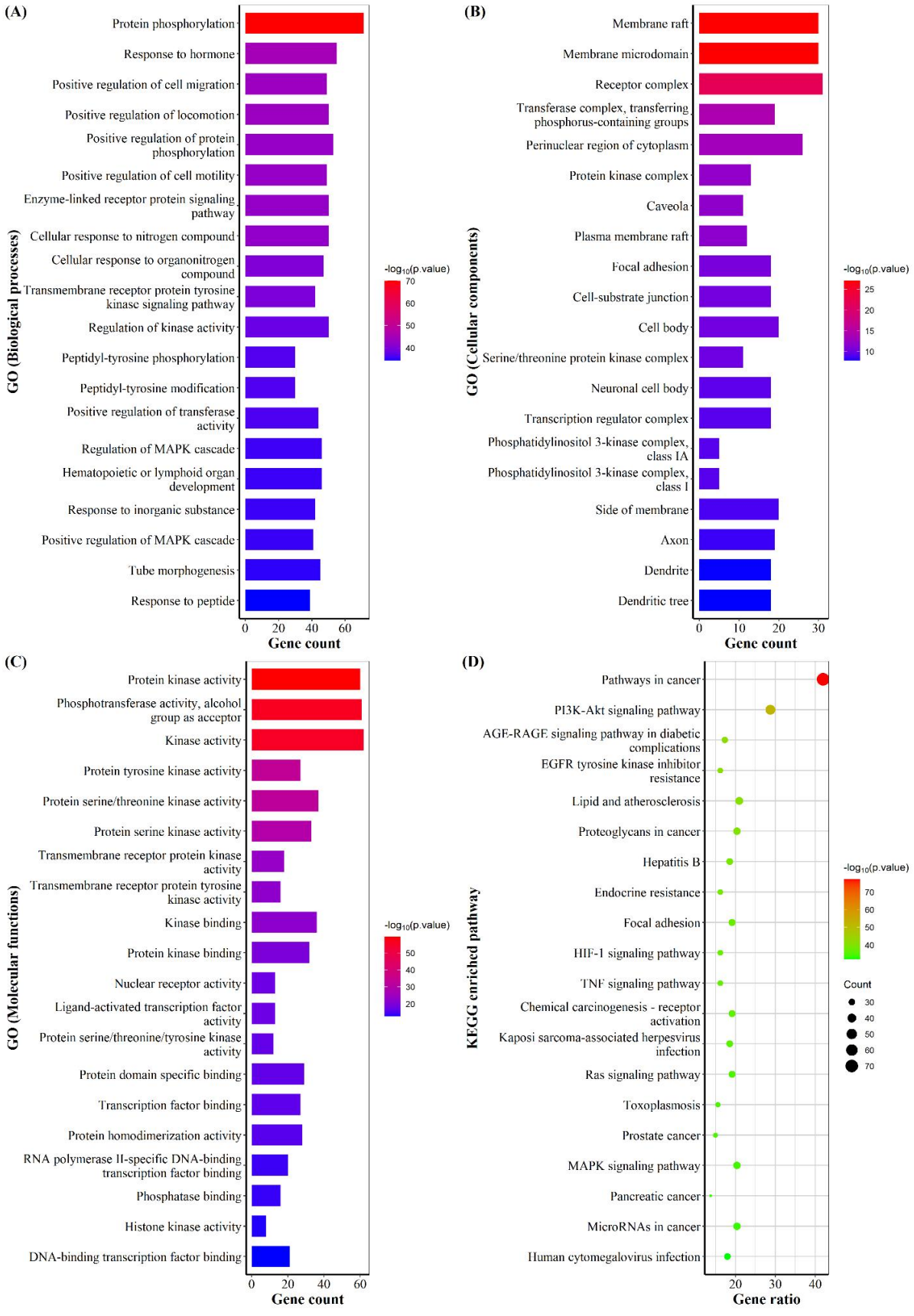

Figure 13 GO and KEGG analyses using intersected targets for lotus seedpod against ovarian cancer

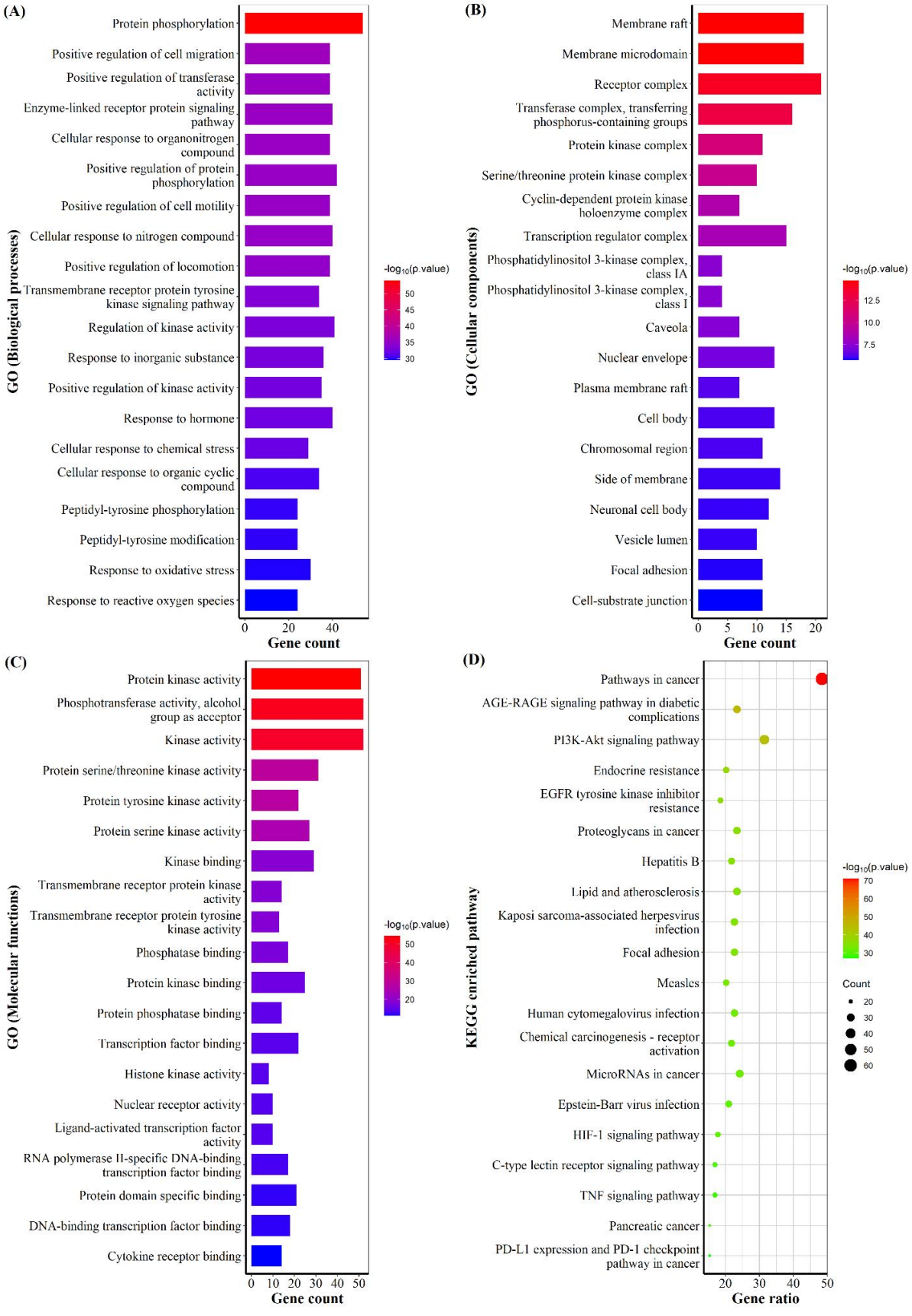

Figure 14 GO and KEGG analyses using intersected targets for lotus seedpod against cervical cancer

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Molecular docking

The screened core compounds and core targets for lotus seedpod against the six cancers were used for molecular docking analysis to validate the network pharmacology results and evaluate their binding capacities. After molecular docking, the heat maps for the docking energy of each ligand (core compound) and receptor (core target) were obtained (Figure 15). If the docking energy is lower than -5.0 kcal mol^{-1}, the binding activity between core compound and core target can be considered to be strong [34]. It could be seen that most of the molecular docking scores between every core compound and every core target of lotus seedpod against lung cancer (Figure 15(A)), gastric cancer (Figure 15(B)), liver cancer (Figure 15(C)), breast cancer (Figure 15(D)), ovarian cancer (Figure 15(E)) and cervical cancer (Figure 15(F)) were smaller than -5.0 kcal mol^{-1}, suggesting all of the bindings were strong and the network pharmacology results were credible. Meanwhile, the molecular docking scores were in the range of -10 kcal mol^{-1}~-4.6 kcal mol^{-1}, -10 kcal mol^{-1}~-5.1 kcal mol^{-1}, -10 kcal mol^{-1}~-6.5 kcal mol^{-1}, -10 kcal mol^{-1}~-6.5 kcal mol^{-1}, -10 kcal mol^{-1}~-6.8 kcal mol^{-1}, and -10 kcal mol^{-1}~-6.8 kcal mol^{-1}, respectively. Moreover, regarding to every cancer, each core compound possessed different docking scores with each core target. In addition, LSC 06 (liensinine) and lysicamine (LSC 16) exhibited the lowest docking scores (-10 kcal mol^{-1}) with AKT1, indicating the bindings between them were strongest. This might confirm the aforementioned results of KEGG analysis that PI3K-Akt signaling pathway plays important roles in the anti-cancer activities of lotus seedpod against the six cancers.

In order to further understanding the binding sites between core compounds and core targets, some representative 2D and 3D interaction diagrams are visualized in Figure 16. As implied in Figure 16(A), the PIK3R1-LSC 06 (liensinine) complex was connected to amino acid residues TYR-334, LYS-430 and VAL-437 by three hydrogen bonds. SRC-LSC 09 (apigenin) and SRC-LSC 32 (quercetin) complexes were stabilized at GLU-353, LYS-356, ARG-500 and THR-508 through four hydrogen bonds (Figure 16(B)), and at GLU-310, GLU-339, THR-338 and MET-341 by four hydrogen bonds (Figure 16(M)), respectively. ESR1 was bound with LSC 11 (luteolin) at LEU-346 by one hydrogen bond (Figure 16(C)), and at ARG-394 and LEU-387 via three hydrogen bonds (Figure 16(K)), respectively. LSC 14 (morin) and LSC 39 (5,7-dihydroxycoumarin) interacted with MAPK3 via seven hydrogen bonds on THR-80, GLN-83, ARG-84, ARG-87, ALA-188 and TYR-222 (Figure 16(D)), and via five hydrogen bonds on GLN-83, ARG-84, ARG-87 and ARG-165 (Figure 16(O)), respectively. The complexes of PIK3CA with LSC 17 (tricin) and LSC 21 (cireneol G) has connections at LYS-802, GLU-849, VAL-851 and ASP-933 through six hydrogen bonds (Figure 16(H)), at GLN-661 and HIS-701 by two hydrogen bonds (Figure 16(J)), respectively. Figure 16(I) reflects that AKT1-LSC 18 (sanleng acid) complex was connected to ASN-53, TRP-80, SER-205, THR-211 and ILE-290 via six hydrogen bonds. HSP90AA1 was bound with LSC26 (linolenic acid) at GLY-97 through one hydrogen bond (Figure 16(L)). In Figure 16(N), LSC 37 (isorhamnetin) interacted with STAT3 at ASP-369, LYS-370, ARG-379, SER-381 and LEU-438 by six hydrogen bonds. Otherwise, no hydrogen bonds were observed in MAPK1-LSC 15 (tetrandrine), JUN-LSC 15 (tetrandrine) and AKT1-LSC 16 (lysicamine) complexes (Figure 16(E), 16(F) and 16(G)), implying hydrophobic interactions might occurred between them. Overall, the aforementioned screened core compounds of lotus seedpod can be used for treatment of the six cancers.

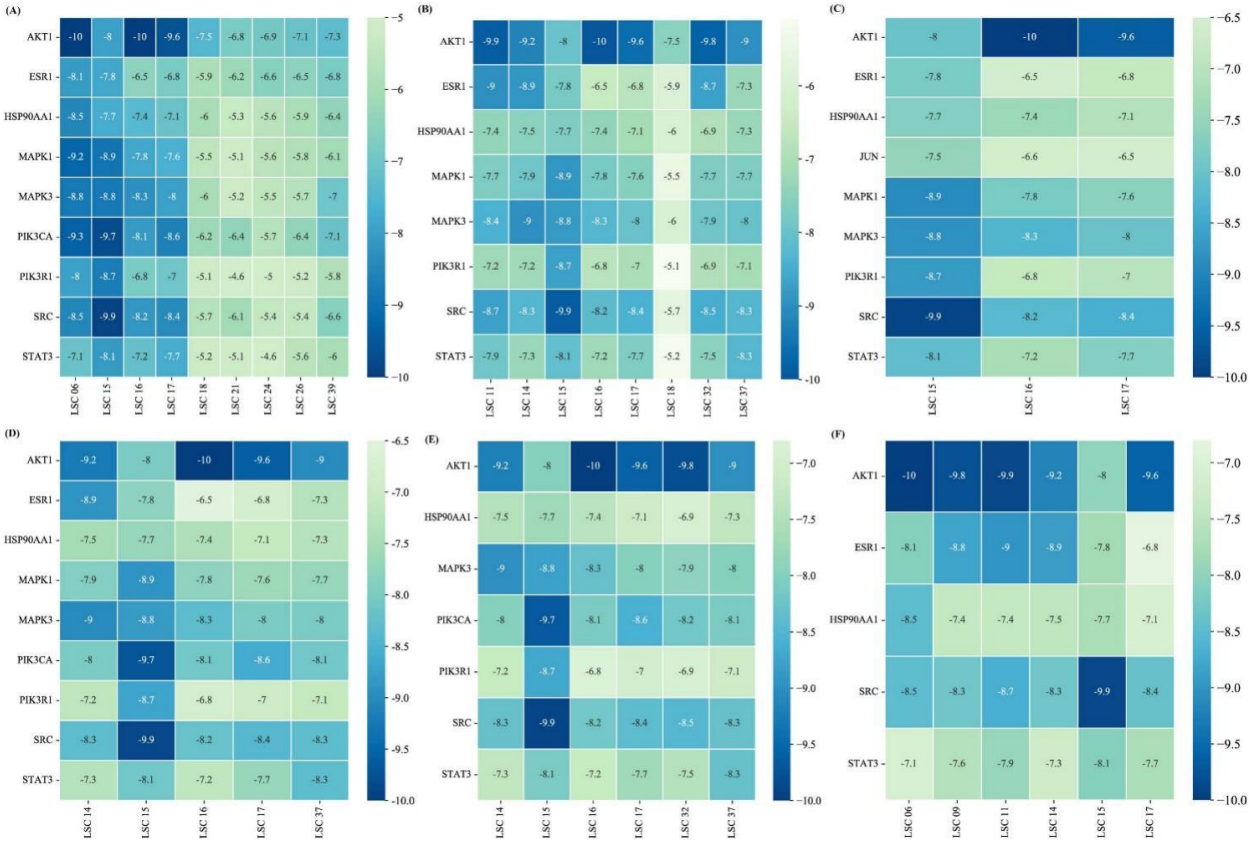

Figure 15 Heatmap of the molecular docking energy (kcal mol^{-1}) between core compounds and core targets for lotus seedpod against lung cancer (A), gastric cancer (B), liver cancer (C), breast cancer (D), ovarian cancer (E) and cervical cancer (F).

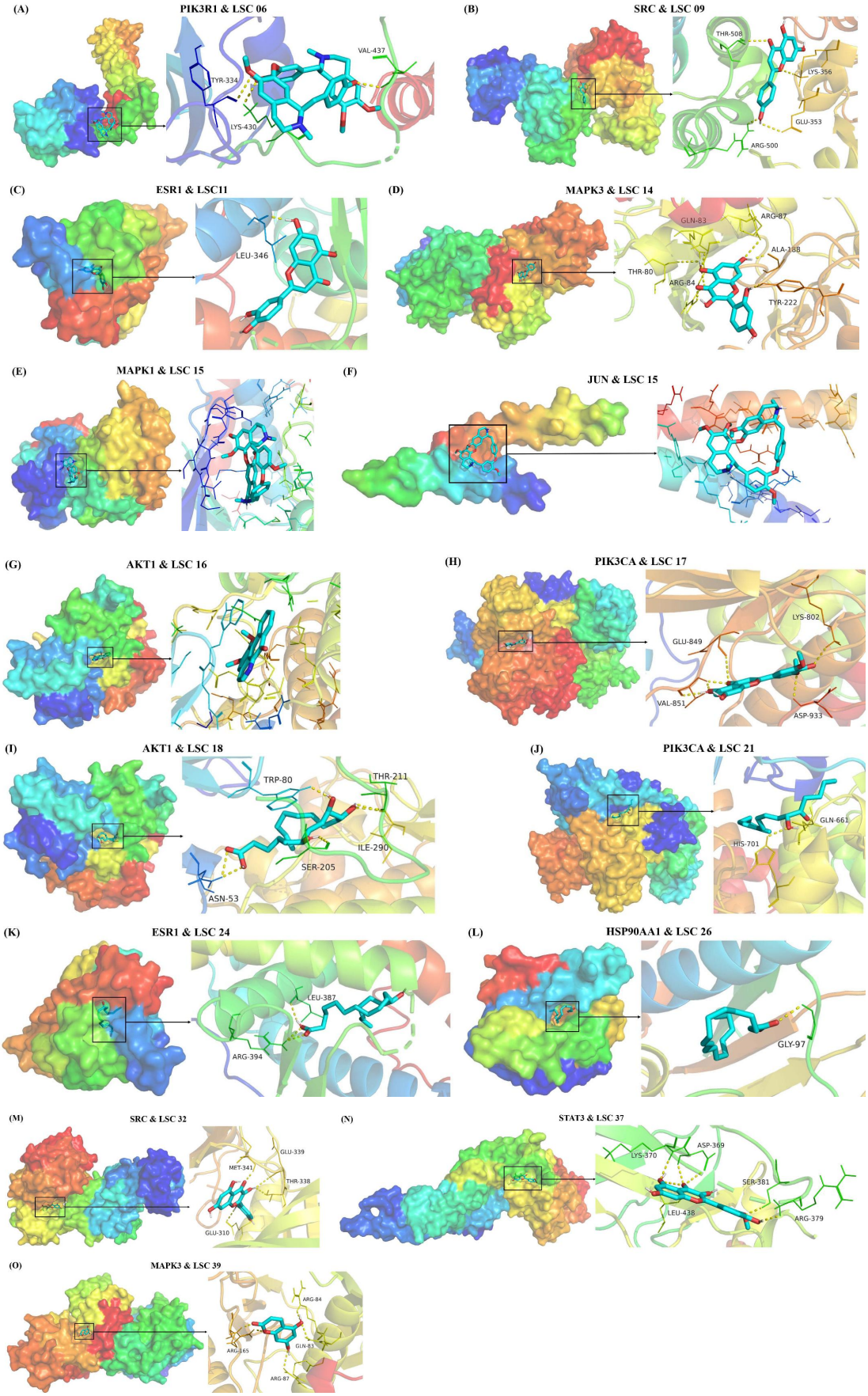

Figure 16 Partial interaction diagrams of core compounds and core targets obtained by molecular docking

Discussions

Seeking bioactive compounds and their cancer-related molecular targets for lotus seedpod is helpful to the treatment of cancers and the high-value utilization of lotus seedpod resources. In this study, a total of 14 core compounds, including liensinine, tetrandrine, lysicamine, tricin, sanleng acid, cireneol G, ricinoleic acid, linolenic acid, 5,7-dihydroxycoumarin, apigenin, luteolin, morin, quercetin and isorhamnetin, were screened for lotus seedpod against lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer. In previous studies, liensinine derived from Plumula Nelumbinis has been proven to have anti-tumor property on nonsmall-cell lung cancer *in vitro* and *in vivo* [35]. Tetrandrine has been demonstrated to possess anti-cancer effects on lung cancer [36], gastric cancer [37], liver cancer [38], breast cancer [39], ovarian cancer [40] and cervical cancer [41]. Lysicamine from leaves of *Nelumbo nucifera* Gaertn [42], leaves of *Phoebe grandis* [43] and *Goniothalamus elegans* [44] had anti-cancer activities against gastric cancer, liver cancer and breast cancer, respectively. Tricin is the active anti-cancer component in Weijing decoction [45], rice bran [46], Njavara [47] and *Echinochloa crus-galli* [48] for lung cancer, breast cancer, ovarian cancer and cervical cancer, successively. Sanleng acid has been identified as one of the active ingredients for *Sparganii rhizoma* on gastric cancer based on network pharmacology [27]. Apigenin has been determined to exert anti-cancer effects on cervical cancer cells (HeLa and C33A cells) and C33A tumor xenograft mice [49]. Luteolin has been indicated to suppress the proliferation of gastric adenocarcinoma cell line (SGC-7901 cells) [50], and inhibit invasion of cervical cancer [51]. Morin has been shown to have anti-cancer actions on lung cancer [52], breast cancer [53], ovarian cancer [54] and cervical cancer [55]. Quercetin has been found to exhibit anti-gastric cancer [56] and anti-ovarian cancer [57] effects. Isorhamnetin has been reported to reveal anti-tumor activities against gastric cancer [58], breast cancer [59] and ovarian cancer [60]. According to these existing references, the prediction of core compounds for lotus seedpod against the six cancers had good reliability. However, four core compounds including cireneol G, ricinoleic acid, linolenic acid and 5,7-dihydroxycoumarin might be the novel anti-cancer ingredients derived from lotus seedpod, as their anti-cancer activities have rarely or not yet been disclosed. Of course, this should be confirmed in future.

Meanwhile, there are 10 core targets for lotus seedpod against the six cancers, including AKT1, ESR1, HSP90AA1, JUN, MAPK1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3. AKT1 is one of isoforms of protein kinase B (AKT), it and its targeted genes play important roles in cell survival, proliferation, metabolism, growth, angiogenesis and metastasis of tumors [61]. Estrogen receptor alpha (ESR1) is a nuclear hormone receptor and oncoprotein, whose mutations are frequently considered as biomarkers in breast cancer [62]. HSP90AA1, a subtype of Hsp90, has high expression in most cancers and is a potential diagnostic and prognostic biomarker for cancers [63]. JUN, an oncogene, is frequently overexpressed in human cancers and can promote tumor cell progression by inhibiting apoptosis [64]. MAPK3 and MAPK1 are two predominant isoforms of extracellular signal-regulated kinase (ERK), whose upregulations contribute to invasion and progression of tumor [65]. PIK3CA is one of members of PI3K family and its mutation occurs at frequencies in endometrial, breast, bladder, cervical and colorectal cancers [66]. PIK3R1, the predominant regulatory isoform of PI3K, is a tumor-suppressor gene and has low expression in cancers [67]. SRC is one of members of the Src family kinases (SFKs), and its protein expression and/or activity is elevated in epithelial cancers like lung cancer, gastric cancer, breast cancer and ovarian cancer [68]. Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that can regulate the expression of genes related to cell cycle, cell survival and immune response for cancer progression and malignancy in diverse cancers [69]. In the past investigations, some of them have also been identified to be core targets for traditional Chinese medicines in treating cancers. For example, the findings of Hu *et al* [70] have shown that AKT1, ESR1, HSP90AA1, JUN, MAPK1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3 were included in the 62 hub targets of Hedysarum Multijugum Maxim-Curcumae Rhizoma herb pair for treating non-small cell lung cancer. In the research taken by Shi, Tian and Tian [71], AKT1, ESR1, JUN, MAPK1, MAPK3, SRC and STAT3 were within the 30 core targets for Fuzheng-Jiedu decoction against colorectal cancer. Moreover, molecular docking analysis suggested that the binding abilities between the core compounds and the core targets were mostly strong. Therefore, it could be inferred that lotus seedpod is a promising source for treating the six cancers, including lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer.

Further KEGG analyses indicated that PI3K-Akt, TNF, Ras, MAPK, HIF-1 and C-type lectin receptor signaling pathways were notably involved in the anti-cancer activities of lotus seedpod against the six cancers. In previous network pharmacology researches, PI3K-Akt, TNF, Ras, MAPK and HIF-1 signaling pathways have been identified in the KEGG enriched pathways of an herbal drug FDY003 against gastric cancer, liver cancer, breast cancer and/or ovarian cancer [72–75]. PI3K-Akt signaling pathway genes are frequently altered in human cancers, and the aberrant activation of this pathway was associated with cellular transformation, tumorigenesis, cancer progression, and drug resistance [76]. TNF involves in many diseases including cancer, and plays important roles in various cellular events such as septic shock, induction of other cytokines, cell proliferation, differentiation, necrosis and apoptosis [77]. Ras signaling pathway has attracted considerable attention as a target for anticancer therapy owing to its important role in carcinogenesis [78]. MAPK signaling pathway implicates in the pathogenesis of cancer and many mutations of components in this pathway can be found [79]. HIF-1 signaling pathway participates in ROS-mediated carcinogenesis to various human cancers such as ovarian, prostate and breast cancer [80]. C-type lectin receptor signaling pathway involves in the various steps for initiation of innate immune responses, thereby it may be potential therapeutic target for cancer immunotherapy [81]. In previous literatures, these signaling pathways have been determined to take part in the anti-cancer effects of some aforementioned core compounds for lotus seedpod. For instance, liensinine inhibited the cell proliferation of gastric cancer cells *in vitro* and *in vivo* partly through PI3K-Akt signaling pathway [82]. Luteolin induced apoptosis of gastric cancer cell line BGC-823 cells partially by MAPK signaling pathway [83]. HIF-1 signaling pathway involves in the autophagy inducing effect of quercetin to gastric cancer cell line AGS and MKN28 cells [84]. In view of this, the above-mentioned top signaling pathways might paly crucial roles in the anti-cancer actions of the screened core compounds from lotus seedpod.

Conclusions

Lotus seedpods are abundant resources produced during lotus seed processing. In the present study, Lotus seedpods were predicted to be potential candidate resources for treating six cancers (lung cancer, gastric cancer, liver cancer, breast cancer, ovarian cancer and cervical cancer). Combinedly, liensinine, tetrandrine, lysicamine, tricin, sanleng acid, cireneol G, ricinoleic acid, linolenic acid, 5,7-dihydroxycoumarin, apigenin, luteolin, morin, quercetin and isorhamnetin were screened to be the core compounds for lotus seedpod against the six cancers. AKT1, ESR1, HSP90AA1, JUN, MAPK1, MAPK3, PIK3CA, PIK3R1, SRC and STAT3 were identified to be the core targets. Meanwhile, the intersected targets between bioactive compounds of lotus seedpod and the six cancers were significantly related to biological process, cell composition and molecular function. PI3K-Akt, TNF, Ras, MAPK, HIF-1 and C-type lectin receptor signaling pathways were notably involved in the anti-cancer activities of lotus seedpod against the six cancers. Moreover, molecular docking analysis indicated that the binding abilities between the core compounds and the core targets were mostly strong. Our study supports the application of lotus seedpod in treating the six cancers, and provides theoretical basis for the further

active compounds screening and mechanism investigation of lotus seedpod in anti-cancers. Future work will focus on validation of obtained results by *in vitro* and *in vivo* experiments with more methods.

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