

Network Pharmacology-Based Investigation For Predicting Active Ingredients And Potential Targets Of Strychnos Nux-Vomica L. In Treating Multiple Myeloma

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Research

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Abstract

Background: Strychnos nux-vomica L. (SN) is a classic Chinese herb, have long been used for the treatment of cancer for many years, However, the pharmacological mechanisms of SN in treatment of Multiple myeloma L. remain vague. The aim of this study was to examine the network pharmacological potential effects of SN on Multiple myeloma using a systems pharmacology approach.

Methods: we collected putative targets of SN based on the Traditional Chinese Medicine System Pharmacology database and oral bioavailability and drug-likeness was screened using absorption, distribution, metabolism, and excretion (ADME) criteria. the network of the interactions among the putative targets of SN and known therapeutic targets of Multiple myeloma was built by using the STITCH database. Then, topological parameters, “Degree”, “Closeness” and “Betweenness” were calculated to identify the hub targets in the network. Furthermore, the hub targets were imported to the Database for Annotation, Visualization and Integrated Discovery to perform a pathway enrichment analysis.

Results: 60 of the identified potential targets of the SN were also Multiple Myeloma- related targets, including 14 putative targets of SN were observed to be major hubs in terms of topological importance. Additionally, the results of pathway enrichment analysis indicated that targets of SN in treating Multiple Myeloma were mainly clustered into multiple biological processes by activating on several signaling pathways (PI3K-Akt, p38-MAPK, Ras/Raf/MEK/ERK pathways), which implied that these were involved in the underlying mechanisms of SN on Multiple Myeloma.

Conclusions: Our works successfully explain the potential effects of SN for Multiple Myeloma treatment via the molecular mechanisms predicted by network pharmacology. Moreover, our present outcomes might shed light on the further clinical application of SN in treating Multiple Myeloma.

Background

Multiple myeloma (MM) is a clonal plasma cell hematologic malignancy that accounts for approximately more than 10% of all hematologic cancers. Advances in therapy over the last 2 decades have prolonged patients survival^[1-3]. These therapies via the ubiquitin proteasome pathway, a critical process for plasma cell survival to degrade target protein^[4]. Examples includes proteasome inhibitors (such as bortezomib) and the immunomodulatory drugs (such as lenalidomide and Thalidomide). However, these drugs have yielded desired responses while have also contribute to certain adverse reactions such as peripheral neuropathy, damage to liver and kidney function. Therefore, it is urgent to discover potential therapeutic targets and find novel and safe treatment strategies.

The use of traditional Chinese medicine (TCM) has obtained more and more acceptance all over the world due to its therapeutic efficacy and fewer side effects^[5-7]. Different from Western Medicine, TCM emphasizing on individualized diagnosis and treatment, maximizing the body's inherent healing ability^[8].

It's characterized by multiple ingredients while make it hard to distinguish the active ingredients and to identify the potential targets of the chemicals.

Strychnos nux-vomica L. (SN) belongs to the genus *Strychnos* of the family Loganiaceae, mainly contains alkaloids with the functions of anti-tumor, anti-inflammatory effects and effects on the nervous system, etc. In traditional Chinese medicine, SN have long been used for the treatment of liver cancer [9-12], The latest research also found SN can dramatically inhibit the growth of U266B1 and RPMI 8226 Multiple Myeloma cell line in vitro [13-14]. In spite of numerous therapeutic effects of SN, its effect on hematologic malignancies is still ill-defined. Besides, similar to other TCM formulas, SN is a multi-component and multi-target agent, it is hard to investigate the pharmacological mechanisms. With the rapid progress of bioinformatics, systems biology, and poly-pharmacology, network pharmacology based approaches have been proven to be a powerful way to decipher the complex mechanisms of action of effective substances of various herbs as well as TCM formulae [15-17]. For example, Yu et al. used a network pharmacology strategy to explore pharmacological mechanisms of Zuojinwan for treatment of gastritis [18]. He et al. also predicting active ingredients and potential targets of LiuWei DiHuang Pill in treating type 2 diabetes mellitus by the use of network pharmacology [19].

In this research, we exert a pharmacology network and experimental verification combination method to investigate how SN exerts the therapeutic effects on MM. The flowchart of the experimental procedures of our study is shown in Fig. 1.

Methods

Data preparation

Chemical ingredients database building and ADME Screening

To collect the ingredients in SN, we performed a search by Traditional Chinese Medicine Systems Pharmacology Database [20]. (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>), a specialized pharmacological platform for TCM. A total of 500 Chinese herbal medicines and 30069 ingredients from the Chinese Pharmacopoeia (2010 edition) were registered [21]. After that, we used absorption, distribution, metabolism, and excretion (ADME) to select bioactive components that contribute to its therapeutic effects. Based on previous studies, we regard the candidate components with Oral bioavailability (OB) $\geq 30\%$, Drug-likeness (DL) ≥ 0.18 as pharmacologically active [20, 22].

The prediction of targets acting on Multiple Myeloma

We collected Multiple myeloma targets from GeneCards (<https://www.genecards.org/>, ver.4.9.0) database, which is an online catalog of human genes and genetic diseases that combines detailed drug data with target genes information [23].

Target genes related to the identified compounds

SN active ingredient targets gene was collected by TCMSP and PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), we fished targets with a screening online tool called PharmMapper Prediction webserver (<http://www.lilab-ecust.cn/pharmmapper/>)²⁴ under the condition of “Homo sapiens” species setting. Gene information including name, gene ID and organism was confirmed using UniProt(<http://www.uniprot.org>)²⁵. The single universal gene names were further acquired. Finally, we matched the retrieved SN active ingredient targets to the known MM-related targets by the STRING (<https://string-db.org/>, ver. 11.0) database to draw the data of protein-protein Interactions (PPI)²⁶.

Network Construction And Analysis

The compound-target (C-T) network, Strychnos nux-vomica L. target-MM target interactional (T-T) network and the target pathway(T-P) network were all visualized by using Cytoscape ver. 3.5.1 (<http://www.cytoscape.org>)²⁷. In T-T network, the nodes represented compounds, targets, and the edges displayed the interactions between two nodes. Moreover, we selected “Degree” “Closeness” and “Betweenness” to evaluate the topological features of every node in the interaction network and screen SN candidate targets with topological importance²⁸⁻²⁹. A hub target in a network is regarded as a crucial node and used to measure the essence of the whole network. Based on previous study, a node was determined to be a hub if its “Degree” and “Betweenness” were greater than or equal to twice the median values while the “Closeness” were greater than median values of the network³⁰⁻³¹.

Functional Enrichment Analysis

We used DAVID (<https://david.ncifcrf.gov/v6.8,2019.8.14>) database for the T-P network, which includes GO-biological process (BP) and KEGG (<http://www.kegg.jp/>, 2019.8.14) enrichment analysis to further probe the vital biological process of achieved targets and to validate the reliability of the integrated results³².

Results

Active compounds in Strychnos nux-vomica L

A total of 113 compounds were identified in SN, all identified compounds were subjected to ADME screening, and 13 of the 113 had OB \geq 30% and DL \geq 0.18. The 13 active compounds that were obtained are listed in (Table 1).

To further understand the pharmacological mechanisms of SN, we constructed an compound-target network by linking the active compounds with their potential targets. There are totally 221 nodes and 390 edges composed the C-T network, including the 13 active compounds and 341 targets (Fig. 2). These active

compounds interacting with multiple targets, participated in the regulation of multiple targets, which signify that SN act synergistically on these targets, exerted pharmacological effects in Multiple myeloma.

Table 1
The list of 13 compounds of *Strychnos nux-vomica* L. and their OB and DL

NO.	Medicine	MOLNAME	compound	OB(%)	DL
1	<i>Strychnos nux-vomica</i> L.	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
2	<i>Strychnos nux-vomica</i> L.	MOL001476	(S)-Stylopine	51.15	0.85
3	<i>Strychnos nux-vomica</i> L.	MOL003410	Ziziphin_qt	66.95	0.62
4	<i>Strychnos nux-vomica</i> L.	MOL003411	Icaride A	48.74	0.43
5	<i>Strychnos nux-vomica</i> L.	MOL003413	Isostrychnine N-oxide (I)	35.45	0.80
6	<i>Strychnos nux-vomica</i> L.	MOL003414	Isostrychnine N-oxide (II)	37.33	0.80
7	<i>Strychnos nux-vomica</i> L.	MOL003418	Lokundjoxide_qt	32.82	0.76
8	<i>Strychnos nux-vomica</i> L.	MOL003432	vomicine	47.56	0.65
9	<i>Strychnos nux-vomica</i> L.	MOL003433	brucine-N-oxide	49.17	0.38
10	<i>Strychnos nux-vomica</i> L.	MOL003436	Isobrucine	33.58	0.80
11	<i>Strychnos nux-vomica</i> L.	MOL003440	Brucine N-oxide	52.63	0.38
12	<i>Strychnos nux-vomica</i> L.	MOL000449	Stigmasterol	43.83	0.76
13	<i>Strychnos nux-vomica</i> L.	MOL000492	(+)-catechin	54.83	0.24

Prediction of Hub Targets of *Strychnos nux-vomica* L. in treating Multiple Myeloma

The target data on MM from Drugbank was finally collected 1350 targets which the Relevance score is over 2.54 as Multiple Myeloma-related target. Notably, 60 of the identified potential targets of the SN were also be proved as Multiple Myeloma-related targets by the Venn diagram(Fig. 3a).Subsequently, to further select the hub targets of SN in treating Multiple Myeloma, we imported compound–disease co-targets

information into STRING database and set the upper limit of protein amount of “no more than 50 interactors”.The intersection network consisting of 109 nodes and 1530 edges (Fig. 3b).The topological feature analysis of the PPI selected targets used “degree”,“Betweenness” and “Closeness” to determine hub targets and finally 14 targets were screened based on the values of topological parameters.The details are shown in (Fig. 3c).

Nodes includes AKT1(degree = 76), EGFR(degree = 69), ALB(degree = 65), MAPK1(degree = 64), EGF(degree = 64), VEGFA(degree = 64), CCND1(degree = 63), SRC(degree = 61) and JUN (degree = 61) have higher degrees, the number of edges of each node is large as well,which suggesting that they play important roles in the treatment of Multiple Myeloma (Fig. 4).The topological parameters are shown in (Table 2).

Table 2
Topological feature values of all the hub targets for SN in treatment of MM.

Node name	Degree	Closeness	Betweenness
AKT1	76.00	92.00	660.44
EGFR	69.00	88.33	507.28
ALB	65.00	86.50	804.30
MAPK1	64.00	86.00	480.35
EGF	64.00	85.83	338.45
VEGFA	63.00	85.17	353.51
CCND1	63.00	85.50	328.68
SRC	61.00	84.33	375.28
JUN	61.00	84.50	273.06
CASP3	59.00	83.33	171.71
HSP90AA1	58.00	82.83	611.79
ESR1	58.00	83.00	324.07
MAPK8	53.00	80.33	270.90
FN1	52.00	79.67	303.70

GO biological process and KEGG pathway enrichment analysis

The biological processes were mainly involved in regulation of apoptotic apoptosis(GO:0043066), signal transduction(GO:0007165), regulation of transcription(GO:0045893) and cell proliferation (GO:0008284).

(Fig. 5). Moreover, according to the p-values of enriched pathways and their correlation with MM, we were most interested in the following signaling pathways including PI3K-Akt, p38 MAPK and Ras/Raf/MEK/ERK pathway (Fig. 6 and Table 3).

Table 3
Representative enriched KEGG pathway of the hub targets of SN in treating MM.

Pathway	Gene Count	P'Value	Pathway ID	Associated Genes
PI3K-Akt signaling pathway	8	4.79E-07	hsa04151	HSP90AA1, CCND1, EGF, FN1, MAPK1, AKT1, EGFR, VEGFA
MAPK signaling pathway	7	1.79E-06	hsa04010	JUN, MAPK8, EGF, CASP3, MAPK1, AKT1, EGFR
Ras signaling pathway	6	2.40E-05	hsa04014	MAPK8, EGF, MAPK1, AKT1, EGFR, VEGFA

Discussion

Network pharmacology analysis of traditional medicines is becoming a popular approach for researching the treatment of diseases manifested by complex and diverse factors such as multiple myeloma. We explored the potential mechanism by the network pharmacology approach to investigate the complex of ingredients, unknown targets, and pharmacological mechanisms in herb. Although a few studies have confirmed SN with the functions of anti-tumor^[9], but the therapeutic effects of SN against multiple myeloma has not yet been clearly elucidated. In this study, a total of 13 potential active ingredients of SN on Multiple Myeloma were screened through a series of network pharmacological methods, which corresponded to 14 hub targets by a topological method. Previous studies have reported the association between these targets and Multiple Myeloma. For instance, AKT1, EGFR, HSP90AA1 and ESR1 served as important regulators that overactive several myeloma growth cytokines^[33-36]. The over expression of MAPK8 and MAPK1 allows the p38 MAPK signaling pathway activated and inhibits osteoblast differentiation as well as bone formation in myeloma cells^[37-38]. VEGFA are recognized as key angiogenic factors in Multiple Myeloma, which stimulates vascular permeability, EC migration, proliferation and survival through the activation of the RAS/RAF/ERK/MAPK pathways^[39-40].

Enrichment analysis of GO-BP and KEGG on the hub targets further suggests that SN could exerts the therapeutic effects on Multiple Myeloma multiple biological processes by activating on several signaling pathways. As a result, we found that the targets have relatively large number of connections with the PI3K-Akt signaling pathway, MAPK signaling pathway and Ras signaling pathway. PI3K-Akt signaling pathway has been improved plays an integral role in MM disease biology, which is activated as a result of loss of function of tumor suppressor genes. Akt is a nodal regulator of cellular survival pathways, the activation of the Akt promote cell proliferation, anti-apoptosis, and metabolism signals leading to myeloma cells growth and survival^[41-43]. We demonstrate that p38 MAPK signaling pathway constitutively activated in human myeloma, which has been implicated in inhibiting osteoblast

differentiation and bone formation but also enhancing osteoclast maturation and bone resorption⁴⁴. The Ras/Raf/MEK/ERK pathway is critical for the proliferation of myeloma cells, implicated in the angiogenesis in multiple myeloma by the driven of vascular endothelial growth factor (VEGF)⁴⁵⁻⁴⁷. There are also diverse signaling pathways enhanced the expression of several growth factors and cytokines thus promote the accumulate clonal malignant plasma cells in the bone marrow⁴⁸.

Conclusion

Based on the results of this study, we investigate the potential mechanism of SN against multiple myeloma. 13 bioactive compounds of SN and 60 targets associated with multiple myeloma were selected. Furthermore, 14 core targets including AKT1, EGFR, MAPK1, MAPK8 might be the hub targets for SN on multiple myeloma treatment. Despite the valuable discoveries, Further in vitro and in vivo experimental validation is needed to support our research.

List Of Abbreviations

SN: Strychnos nux-vomica L.; MM: Multiple myeloma ; TCM: Traditional Chinese Medicine; ADME: Absorption, distribution, metabolism, and Excretion; OB: oral bioavailability; DL: drug-likeness; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; DAVID: Database for Annotation, Visualization and Integrated Discovery; C-T: compound-target; T-T: target-target; T-P: target pathway; AKT1: RAC-alpha serine/threonine-protein kinase; EGFR: Epidermal growth factor receptor; HSP90AA1: Heat shock protein 90 alpha family class A member 1; ESR1: Estrogen receptor; MAPK: Mitogen-activated protein kinase; VEGF: Vascular endothelial growth factor.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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Authors' contributions

YZ and KTJ collected data, performed the analyses, conducted the literature search, and Contributed to the writing of the manuscript. SD and JPS took part in the design of the study and made a revision of the manuscript. CJL and YRH conceptualised and designed the study, coordinated the study, helped interpret findings, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

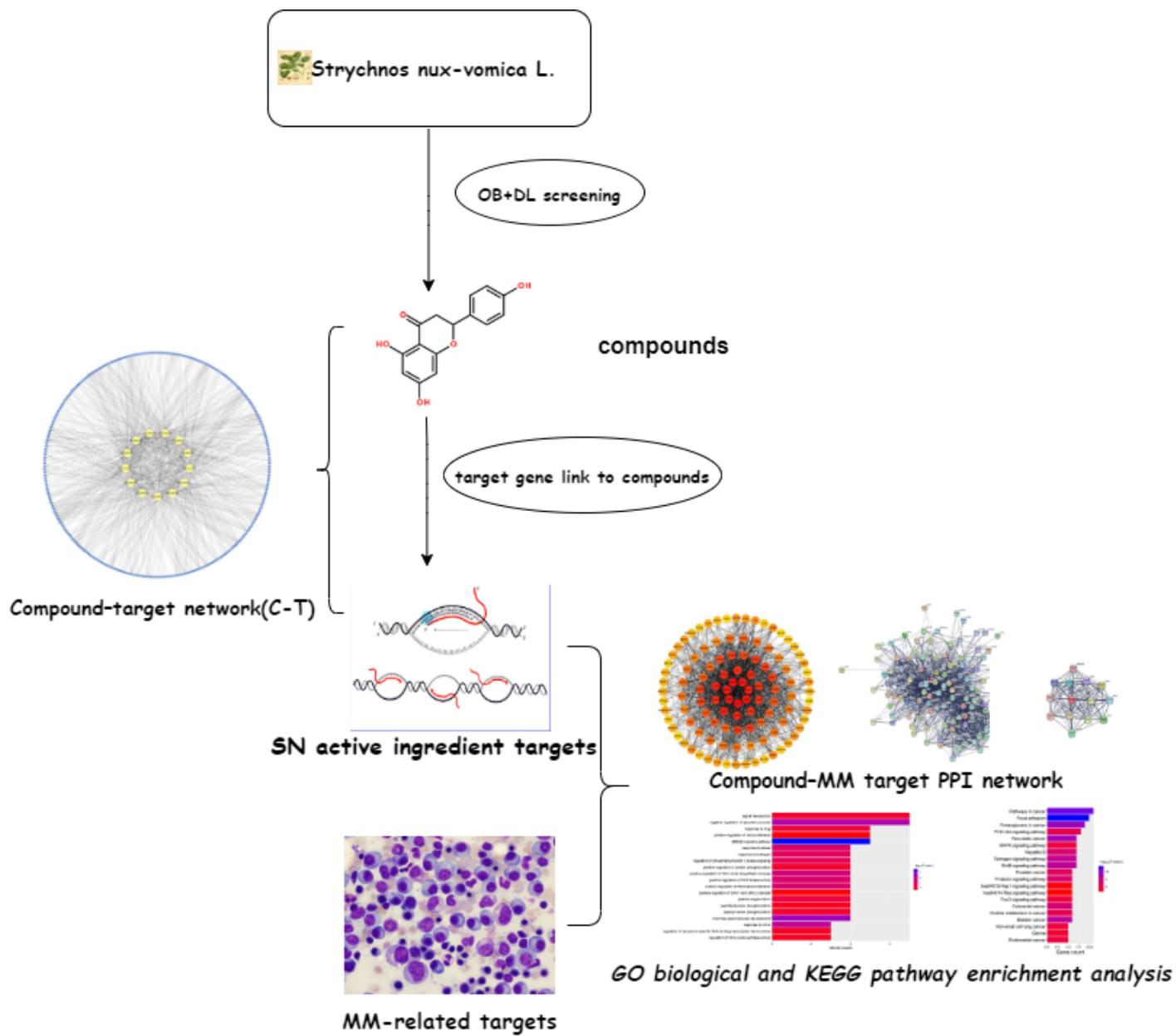


Figure 1

Workflow of network pharmacology analysis.

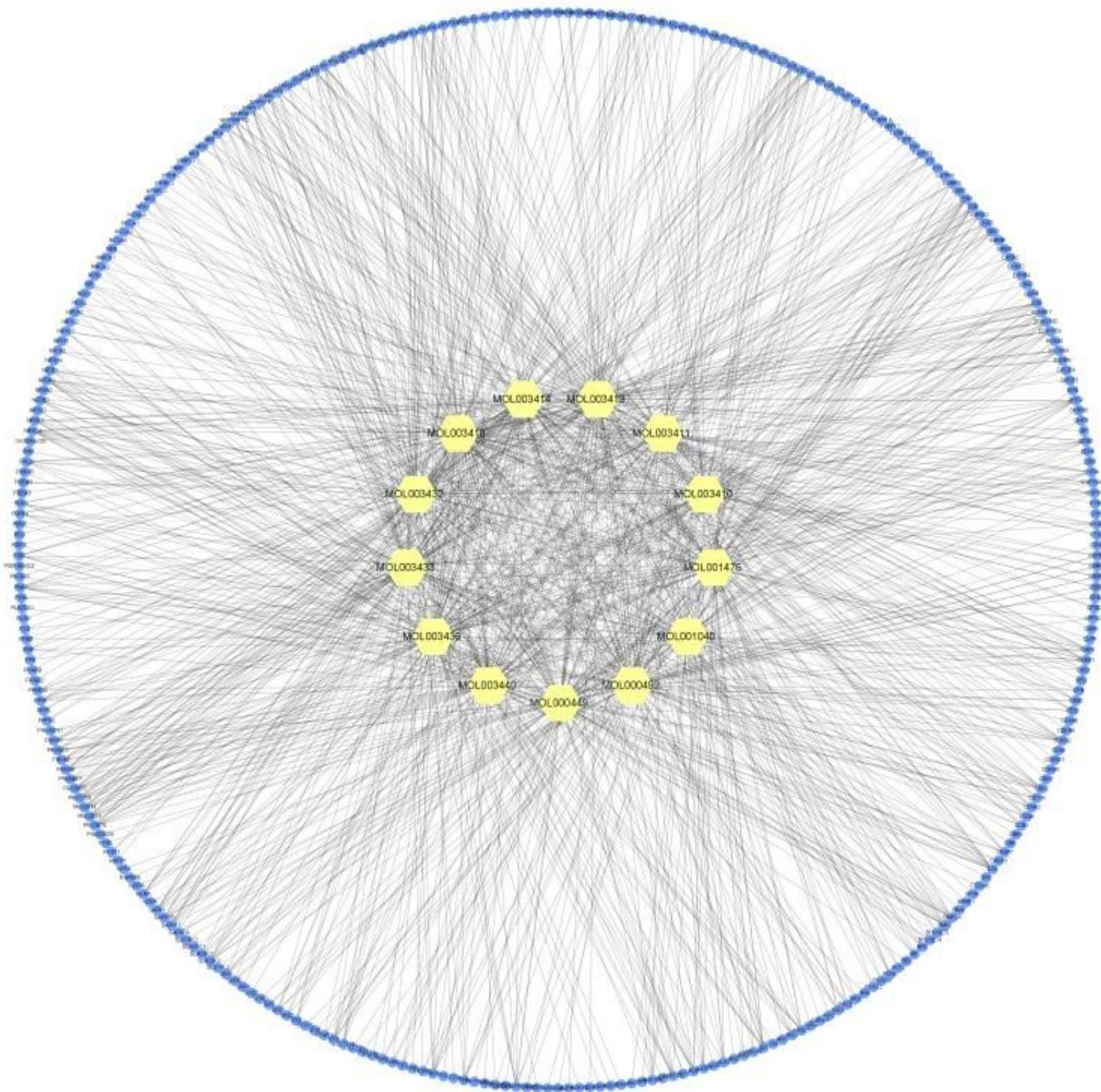


Figure 2

Compound–target network(C-T) of *Strychnos nux-vomica* L. The C-T network that consists of 221 nodes and 390 edges. Yellow and blue nodes denote the compounds and targets respectively.

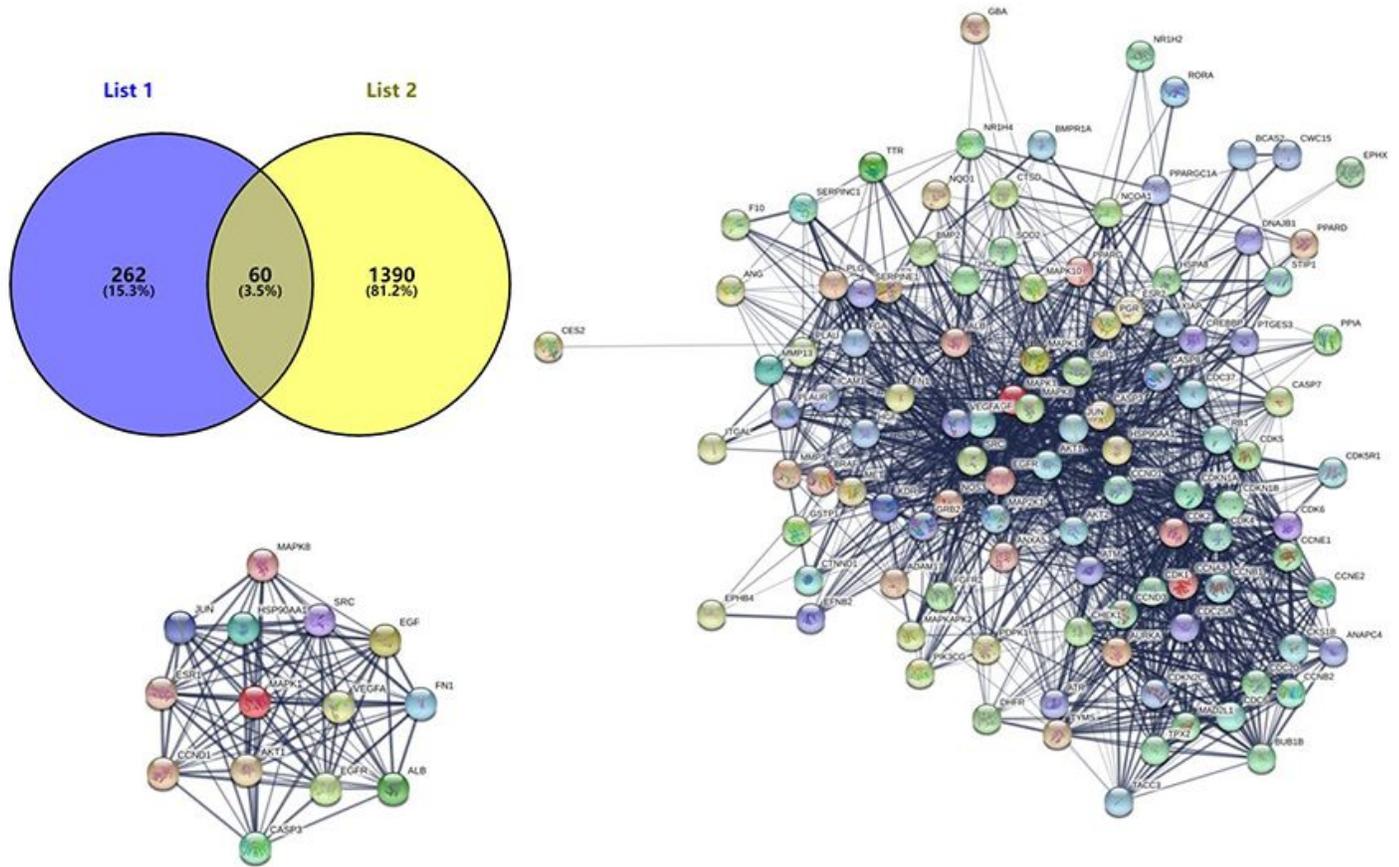


Figure 3

(a): 60 of the identified potential targets of the SN were also Multiple Myeloma- related targets by the Venn diagram.(b): Compound –Multiple Myeloma PPI network.(c):PPI network of the 14 hub targets consisted of 14 nodes and 91 edges.

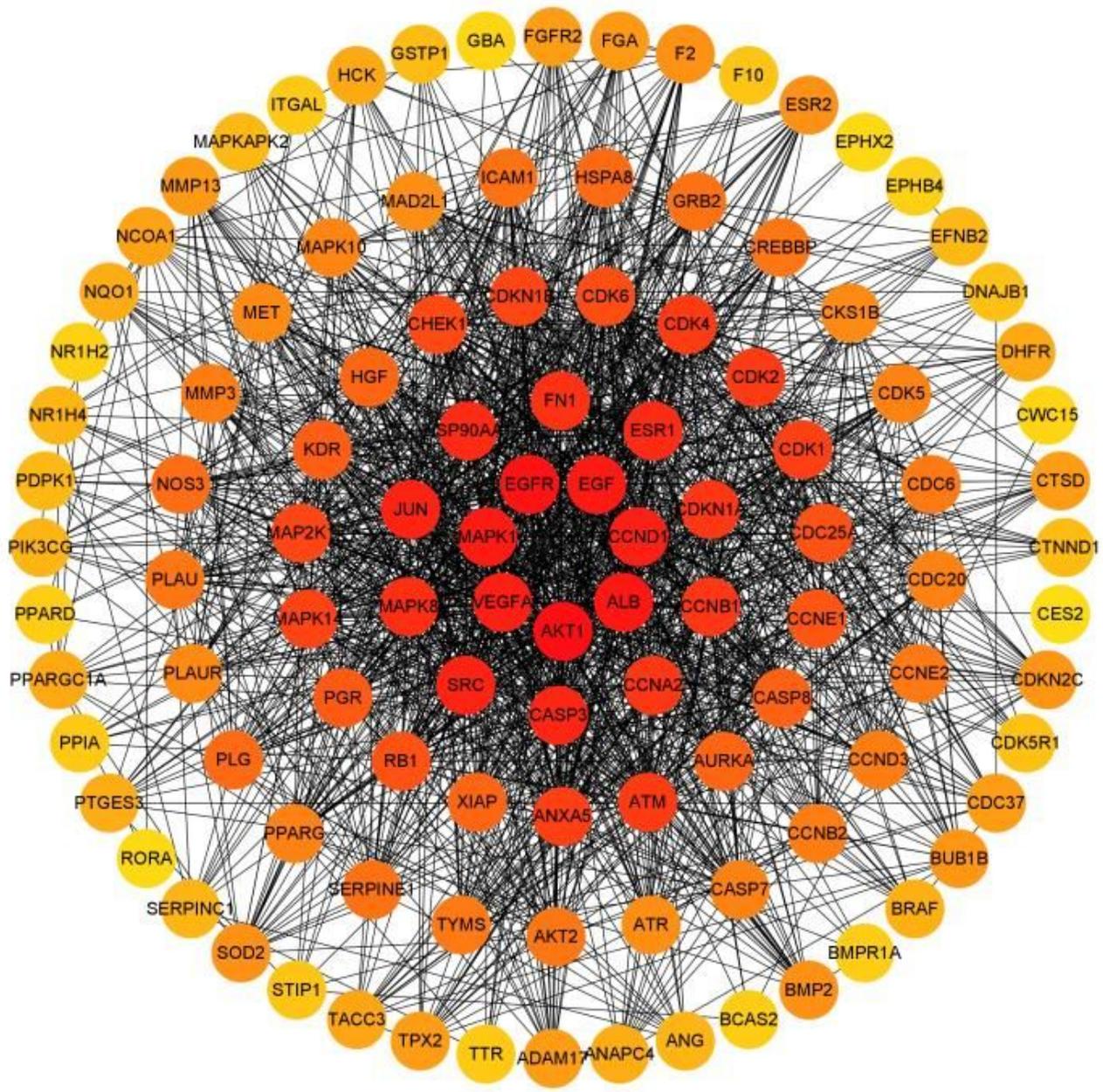


Figure 4

Construction of Compound–Multiple Myeloma network.the node with different depth of colour represented the different degree of nodes.

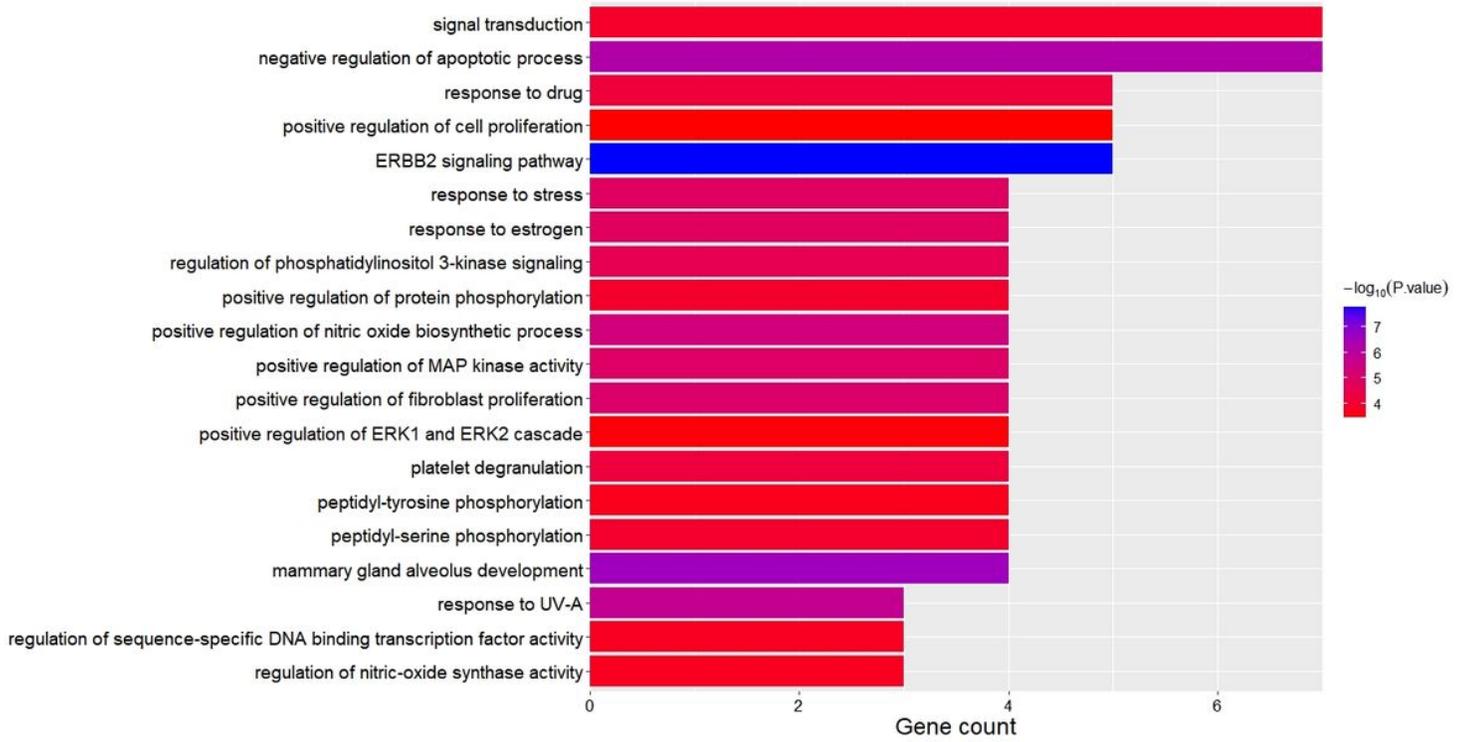


Figure 5

GO biological process by major hubs from the DAVID database.

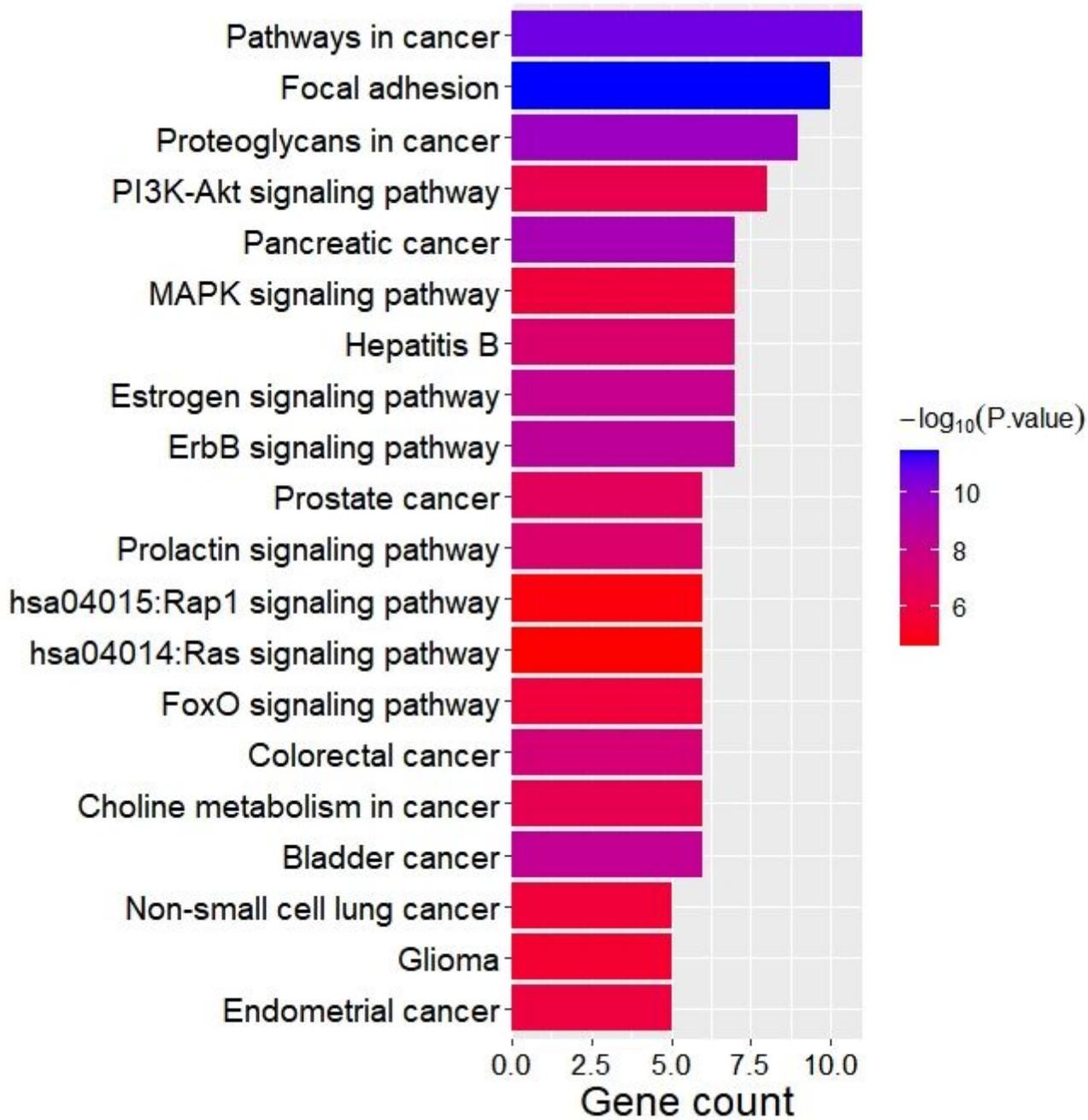


Figure 6

main pathways enriched by major hubs from the DAVID database.