ORIGINAL RESEARCH

Analysis of the Mechanism of Action of Kushen in the Treatment of Tuberculosis Based on Network Pharmacology

Min Lin, BM; Huan Zhou, BM; Rong Li, BM; Li-Li Quan, BM; Zhu Jin, MD; Xiao-Wei Tong, MD

ABSTRACT

Context • Drug-resistant tuberculosis (TB), especially multidrug-resistant TB, has continued to increase and pan-drug-resistant TB and even fully drug-resistant TB have emerged, bringing great challenges to the treatment of TB. Development of new, safe, and effective antituberculosis drugs is an urgent need.

Objective • The study intended to evaluate the use of the network pharmacology method to comprehensively and systematically analyze the network relationship of Kushen's main components, targets, and signaling pathways, aiming to provide new ideas and clues for an in-depth study of the mechanism of Kushen's main components in the treatment of pulmonary TB.

Design • The research team performed a Network pharmacology analysis.

Setting • The study took place in the Department of Respiratory and Critical Care Medicine at the Third People's Hospital of Yichang City in Yichang, Hubei, China.

Outcome Measures • The research team: (1) screened Kushen's active ingredients and related targets using the Traditional Chinese Medicine System Pharmacology (TCMSP) database and analysis platform; (2) used the GeneCards database and the Online Mendelian Inheritance in Man (OMIM) database to search for disease targets, (3) connected the active ingredient's targets to the disease targets to obtain predictive targets for Kushen to act against TB, (4) used the STRING database to construct a protein-protein interaction (PPI) network map, (5) used the Database for Annotation, Visualization and Integrated Discovery (DAVID) to subject the intersecting genes to gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and (6) used the TCMSP and Protein Data Bank (PDB) databases to dock the active ingredients with target-protein molecules.

Results • The research team found 45 active ingredients for Kushen and 177 target-protein genes related to active ingredients. The PPI network map of the Kushen-TB targets and found that the top 10 targets of Kushen were: (1) mitogen-activated protein kinase 8 (MAPK8); (2) protein kinase B (AKT1); (3) MAPK1, (4) estrogen receptor 1 (ESR1), (5) rel avian reticuloendotheliosis viral oncogene homolog A (RELA), (6) interleukin-6 (IL6), (7) MYC proto-oncogene, basic helix-loop-helix (bHLH) transcription factor MYC), (8) retinoid X receptor alpha (RXRA), (9) FOS proto-oncogene activator protein 1 (AP-1) transcription factor subunit (FOS), and (10) JUN proto-oncogene AP-1 transcription factor subunit (JUN). The KEGG analysis suggested that Kushen can intervene in TB through the hypoxia-inducible factor 1 (HIF-1) signaling pathway.

Conclusions • The network pharmacology analysis showed that Kushen's active ingredients can play a role in the treatment of TB through the HIF-1 signaling pathway. (*Altern Ther Health Med.* 2023;29(2):155-161)

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Tuberculosis (TB) is a chronic infectious disease that Mycobacterium tuberculosis (MBT) causes, and it's still a major, global, public-health problem. According to the World Health Organizations (WHO), in 2020 about 9.87-million new cases of TB and about 1.5 million deaths due to TB occurred worldwide. Although medications have controlled the TB epidemic globally in recent years, the continued increase in drug-resistant TB, especially multidrug-resistant TB, and the emergence of pan-drug-resistant TB and even fully drug-resistant TB have brought great challenges

to the treatment of TB.[1] Therefore, development of new, safe, and effective antituberculosis drugs is an urgent need.

Tuberculosis-related Genes

MBT is a facultative intracellular pathogen, and its main host cells in the body are macrophages. Researchers have found some genes that are related to TB, including the JUN proto-oncogene activator protein 1 (AP-1) transcription factor subunit JUN, mitogen-activated protein kinase (MAPK), and rel avian reticuloendotheliosis viral oncogene homolog A.

AKT1. AKT1 is a serine/threonine kinase that plays a key role in controlling the intracellular growth of Mycobacterium tuberculosis. Studies have shown that AKT1 polymorphisms are associated with susceptibility to tuberculosis[1].

FOS. The fos gene encodes a leucine zipper protein that dimerizes with the Jun family of proteins to form the transcription factor complex AP-1. Therefore, fos proteins are considered as regulators of cell proliferation, differentiation and transformation.

JUN. The JUN family of proteins is widely involved in the immune and inflammatory responses of TB and is associated with immune damage in patients with severe, secondary pulmonary TB[2].

MAPK. In infections from MBT, MAPK is involved in the immune response to pulmonary TB and is involved in the regulation of the cell cycle, environmental stress adaptation, and inflammatory response and other pathophysiological processes.[3,4] Lin et al found that MAPK1 has potential to be a new investigable marker during the latent TB infection.[5]

RELA. RELA is an important member of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) family. Its posttranslational modification can precisely regulate the transcriptional activity of NF-κB, which plays an important role in regulating inflammation, development of tumors, metabolism, and immune response. It plays an important role in the development of Physical activities and related diseases.[6] Geng et al found that RELA deletion can seriously impair the survival of MBT H37Rv in vivo.[7]

Kushen

Chinese medicinal materials have always been an important source for the discovery of drug compounds, including Kushen, Motherwort, Astragalus, and Paclitaxel. Records of antituberculosis treatments in Traditional Chinese Medicine (TCM) in China have existed since 2000 BCE. To treat scrofula and TB, the *Compendium of Materia Medica*[8] indicates that practitioners can use four taels of Sophora flavescens and add Achyranthes juice to make balls, using ingredients such as mung beans, to produce twenty supplements per serving, used with hot water.

Kushen is the dried root of the legume Sophora Radix. Matrine and oxymatrine are the most important alkaloid components in Kushen, and they have anti-inflammatory, analgesic, and antitumor effects.[9,10]Other studies have supported the findings that matrine has a variety of biological activities and pharmacological effects, including antibacterial, anti-inflammatory, antiviral, and antitumor effects.[11-13]

Li et al found that the minimum inhibitory concentration of matrine against MBT was 10 mg/L and that the proportion of growing MBT with use of 10 mg/L of matrine increased after 30 days from 23% to 53%.[14] That finding indicates that matrine had an inhibitory effect on the growth of MBT but that its bactericidal effect was weak.

Quercetin is an excellent natural antioxidant that can quench free radicals and protect cells from the damage that they cause.[15] Quercetin has chemopreventive and therapeutic effects on a variety of diseases. Quercetin's activities including antioxidant, anticancer, antihypertensive, antidiabetic, anti-inflammatory, antiviral, anti-Alzheimer's disease, cardiovascular protective, neuroprotective, anti-aging, and immunity enhancement.[16-18] Morikawa et al found that quercetin can inhibit the inflammatory response by regulating the synthesis of prostaglandins and the production of related cytokines.[19]

Current Study

The current study intended to evaluate the use of the network pharmacology method to comprehensively and systematically analyze the network relationship of Kushen's main components, targets, and signaling pathways, aiming to provide new ideas and clues for an in-depth study of the mechanism of Kushen's main components in the treatment of pulmonary TB.

METHODS

Procedures

The research team performed an analysis of traditional chinese medicine network pharmacology. The study took place in the Department of Respiratory and Critical Care Medicine at the Third People's Hospital of Yichang City in Yichang, Hubei, China.

Active ingredients and targets of Kushen drugs. The research team: (1) searched the Chinese Medicine System Pharmacology Database (TCMSP)[20] using the keyword Kushen; (2) initially screened Kushen's ingredients to find the active compounds; and (3) used the Uniprot database[21] to define the protein names and the species as Homo sapiens and corrected the target protein's name to the canonical gene name.

Kushen's predictive targets in treatment of pulmonary TB. To find potential targets for the treatment of TB, the research team used TB as the keyword to mine the GeneCards database[22], a database that provides genomic, proteomic, transcriptomic, genetic, and functional information on all known and predicted human genes, and the Online Mendelian Inheritance in Man (OMIM) database,[23] a comprehensive, authoritative compendium of human genes and genetic phenotypes.

Construction of the target disease network for Kushen's active ingredients. The research team: (1) put the active ingredients of Kushen and the potential targets for the treatment of TB into the bioinformatics analysis software Cytoscape (National Institute of General Medical

Sciences,U.S.) to construct a network of Kushen's effective active ingredients and disease targets.

Construction of protein-protein interaction (PPI) network. To clarify the interaction between Kushen-related targets and TB targets, the research team: (1) used the VENNY2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/) to find the intersection of the two targets and draw a Venn diagram; (2) transferred the intersection targets to the STRING database,[24] a database of known and predicted protein-protein interactions, to construct a PPI network mode[l25]; (3) searched by selecting multiple proteins and setting the biological species to Homo sapiens; (4) set the score at ≥0.9 to improve the credibility of the biological information; and (5) derived the PPI network graph through the processing of the above information.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analyses of targets. Using the Database for Annotation, Visualization and Integrated Discovery (DAVID),[26] a biological information annotation database, the research team performed the analyses on the targets of Kushen's potential treatment of TB.

Docking of active ingredients with target-protein molecules. The research team: (1) downloaded the three-dimensional (3D) structure files of the active ingredients from the TCMSP database; (2) downloaded the target protein file from the Protein Data Bank (PDB) database, a database for the structural data of large biological molecules, such as proteins and nucleic acids; (3) The small molecule model was performed to remove water molecules and add hydrogen bonds the model using the PyMOL software (Schrodinger, U.S.), an open source but proprietary molecular visualization system; and (4) performed molecular docking using the AutoDockTools, 1.5.6 (Scripps Institute, U.S.), software that allows prediction of how small molecules, such as substrates or drug candidates, will bind to a receptor of a known 3D structure.

Outcome Measures

Active ingredients and targets. The research team screened Kushen's ingredients to find the active compounds, using the following criteria: Oral bioavailability (OB) ≥ 30%, and drug-like index (DL) ≥ 0.18 were used as screening conditions for the preliminary screening of Kushen's active ingredients.

Predictive targets. Using "tuberculosis" as the keyword, the GeneCards database (https://www.genecards.org/) and the OMIM database (http://www.omim.org) were mined for potential targets for the treatment of tuberculosis. The higher the Score value in the GeneCards database, the more closely related the target is to the disease, and we screened on the condition that the Score value ≥ 1. The score value is provided by the Genecard database. The OMIM database has no score value, so it is all included, and finally the search results of the two databases are combined.

Construction of target disease network. Nodes in the network represent targets or compounds, and edges represent

Table 1. Characteristics of Kushen Compound

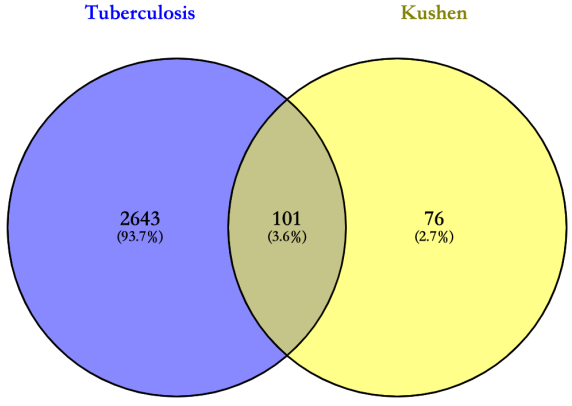
Number	Active Ingredient	OB (%)	DL
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	42.36	0.21
MOL001484	Inermine	75.18	0.54
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL003627	Sophocarpine	64.26	0.25
MOL003648	Inermin	65.83	0.54
MOL003673	Wighteone	42.8	0.36
MOL003676	Sophoramine	42.16	0.25
MOL003680	Sophoridine	60.07	0.25
MOL000392	Formononetin	69.67	0.21
MOL004580	cis-Dihydroquercetin	66.44	0.27
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one	71.12	0.18
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27
MOL005944	Matrine	63.77	0.25
MOL000006	Luteolin	36.16	0.25
MOL006561	(+)-14alpha-hydroxymatrine	35.73	0.29
MOL006562	(+)-7,11-dehydromatrine,(leontalbinine)	62.08	0.25
MOL006563	(+)-9alpha-hydroxymatrine	32.04	0.29
MOL006564	(+)-allomatrine	58.87	0.25
MOL006565	AIDS211310	68.68	0.25
MOL006566	(+)-lehmannine	58.34	0.25
MOL006568	isosophocarpine	61.57	0.25
MOL006569	(-)-14beta-hydroxymatrine	37.26	0.29
MOL006570	(-)-9alpha-hydroxysophoramine	35.23	0.29
MOL006571	Anagyrine	62.01	0.24
MOL006572	1,4-diazaindan-type,alkaloid,flavascensine	34.64	0.24
MOL006573	13,14-dehydrosophoridine	65.34	0.25
MOL006582	5α,9α-dihydroxymatrine	40.93	0.32
MOL006583	7,11-dehydromatrine	44.43	0.25
MOL006596	Glyceollin	97.27	0.76
MOL003347	Hyperforin	44.03	0.6
MOL006604	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-enyl) chroman-4-one	48.09	0.39
MOL006613	Kushenin	47.62	0.38
MOL006619	Kushenol J	51.39	0.74
MOL006620	Kushenol J_qt	50.86	0.24
MOL006622	Kushenol O	42.41	0.76
MOL006623	Kushenol, t	51.28	0.64
MOL006626	Leachianone, g	60.97	0.4
MOL006627	Lehmanine	62.23	0.25
MOL006628	(+)-Lupanine	52.71	0.24
MOL006630	Norartocarpetin	54.93	0.24
MOL000456	Phaseolin	78.2	0.73
MOL006649	Sophranol	55.42	0.28
MOL006650	(-)-Maackiain-3-O-glucosyl-6'-O-malonate	48.69	0.52
MOL006652	Trifolrhizin	48.53	0.74
MOL000098	Quercetin	46.43	0.28

Abbreviations: OB, oral bioavailability; DL, drug-like

Table 2. Potential Target Genes for Kushen

Targets	Uniprot ID	Targets	Uniprot ID	Targets	Uniprot ID
PTGS2	P35354	TOP2A	P11388	ALOX5	P09917
NR3C2	P08235	GSTP1	P09211	IL1A	P01583
NR3C1	P04150	INSR	P06213	MPO	P05164
HTR3A	P46098	CD40LG	P29965	NCF1	P14598
ADRB2	P07550	PTGES	O14684	ABCG2	Q9UNQ0
OPRM1	P35372	MET	P08581	HAS2	Q92819
PPARG	P37231	CYP3A4	P08684	NFE2L2	Q16236
KDR	P35968	CXCL8	P10145	NQO1	P15559
MAPK14	Q16539	NR1I2	O75469	PARP1	P09874
TNFSF15	O95150	RXRB	P28702	AHR	P35869
IL6	P05231	MMP3	P08254	CXCL11	O14625
NOS2	P35228	BCL2	P10415	CXCL2	P19875
JUN	P05412	FOS	P01100	NR1I3	Q14994
IL4	P05112	BAX	Q07812	PPARA	Q07869
ATP5F1B	P06576	PLAU	P00749	CRP	P02741
RELA	Q04206	EGF	P01133	CXCL10	P02778
MMP2	P08253	POR	P16435	SPP1	P10451
CASP3	P42574	CASP8	Q14790	CTSD	P07339
ICAM1	P05362	RAF1	P04049	IGF2	P01344
HPSE	Q9Y251	SOD1	P00441	IRF1	P10914
CD44	P16070	PRKCA	P17252	PON1	P27169
EGFR	P00533	HIF1A	Q16665	HK2	P52789
AKT1	P31749	STAT1	P42224	GSTM1	P09488
VEGFA	P15692	ACACA	Q13085		
CASP9	P55211	CYP1A2	P05177		
MMP9	P14780	CAV1	Q03135		
MAPK1	P28482	F3	P13726		
IL10	P22301	CYP1A1	P04798		
RB1	P06400	IL1B	P01584		
NFKBIA	P25963	CCL2	P13500		
TOP1	P11387	SELE	P16581		
MMP1	P03956	VCAM1	P19320		
PCNA	P12004	PRKCB	P05771		
ERBB2	P04626	DUOX2	Q9NRD8		
HMOX1	P09601	CYP1B1	Q16678		
MCL1	Q07820	PLAT	P00750		
BIRC5	O15392	THBD	P07204		
IL2	P60568	SERPINE1	P05121		
IFNG	P01579	COL1A1	P02452		

Figure 1. Venn Diagram of Common Target Genes



interactions between active ingredients and diseases. The node centrality of the complex network allows measurement of the influence ability of nodes in the network to judge the importance of nodes. The degree value represents the contribution of the node in the entire network, which can be calculated by Cytospace software.Among them, the degree value is an important parameter, and the higher the degree value, the more important the position of the component or target in the network.

Construction of PPI network. Since the network is too large, we set the minimum required interaction score to the highest confidence value (0.9).

GO and KEGG analyses. The GO analysis examined the biological processes, molecular functions, and cellular components of the potential target genes involved, with a threshold set at $P < .05$; ranked the targets by ascending P value; and created a bubble chart. The KEGG analysis examined the intersection of genes and screened the highly significant pathways according to p value, setting that value to $P < .05$ and mapping the 10 most significant pathways.

Molecular docking verification. Molecular docking is a process in which two or more molecules recognize each other through geometric matching and energy matching to find the best matching mode. Molecular docking is important application in enzymatic research and drug design. The smaller the binding energy of the molecule, the stronger the binding ability. The research team performed molecular docking verification for the top 5 hub genes (TOP5) and performed docking experiments according to the target molecule used to determine the docking components.

RESULTS

Kushen's active ingredients and their targets

The search of the TCMSP database found 45 active ingredients for Kushen and 177 target-protein genes related to active ingredients (Table 1).

Predictive targets of Kushen

The search of the GeneCards and OMIM databases found 2744 TB-related genes (Table 2 and Figure 1). The mapping of the TB-related genes with Kushen's component-gene targets found 101 target genes in common, which are the targets for Kushen's treatment of TB. Among Kushen's 45 active ingredients, 24 ingredients, including phaseolin, quercetin, matrine, luteolin, formononetin, and inermine were associated with tuberculosis (data not shown).

Network Topology

Figure 2 shows the network topology of Kushen's active ingredients and the disease targets, which includes 313 nodes. The orange node, a hexagon, represents Kushen; the green nodes, rectangles, represent Kushen's active ingredients; the blue nodes, ovals, represent the disease-related target genes; the red node, a diamond, represents TB; and the edges represent the interactions between Kushen's active ingredients and the disease. Among them, quercetin's edges were the

Figure 2. Component-target-signaling Pathway of Kushen's Treatment of Tuberculosis. The pathway has 313 nodes. The orange node, a hexagon, represents Kushen; the green nodes, rectangles, represent Kushen's active ingredients; the blue nodes, ovals, represent the disease-related target genes; the red node, a diamond represents tuberculosis; and the edges represent the interactions between Kushen's active ingredients and the disease. Among them, quercetin's edges were the largest, indicating that it plays a key role in Kushen's effects in the treatment of tuberculosis.

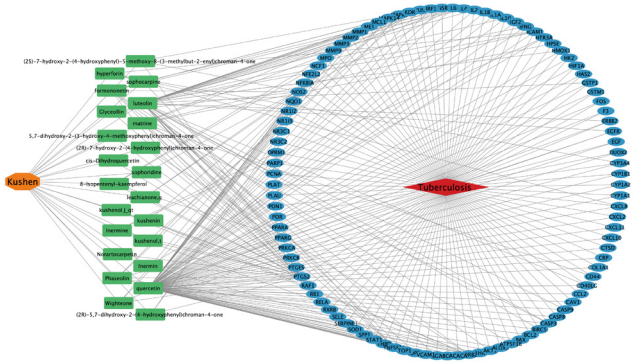
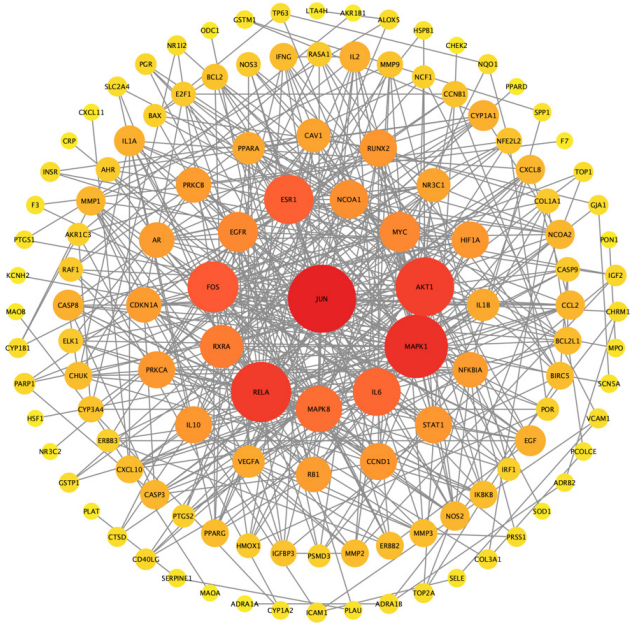


Figure 3. The Target PPI Network of Kushen Against Tuberculosis. The size and color of a shape represents the degree of the gene in the network. The larger the degree value, the larger the circle and the redder the color.



Abbreviations: PPI, protein-protein interaction.

largest, indicating that it plays a key role in Kushen's effects in the treatment of TB.

PPI Network Construction and Analysis

Figure 3 shows the PPI network map of the Kushen-TB targets. The network contains 101 nodes and 303 edges. The average degree of the nodes was 6, and the average local clustering coefficient was 0.39. The top 10 targets of Kushen in the treatment of TB were: (1) MAPK8; (2) protein kinase B (AKT1); (3) MAPK1; (4) estrogen receptor 1 (ESR1); (5) RELA; (6) interleukin-6 (IL6); (7) MYC proto-oncogene, basic helix-loop-helix (bHLH) transcription factor (MYC); (8) retinoid X receptor alpha (RXRA); (9) FOS proto-oncogene AP-1 transcription factor subunit (FOS); and (10) JUN. These genes play a relatively important role in the network and become key nodes that connect other nodes in the network.

GO and KEGG Analyses

The GO analysis found 10 GO entries. Figure 4 shows a bubble chart of the results. For the KEGG analysis, the study screened the top 10 pathways according to the *P* value. Kushen's signaling pathways include pathways related to diseases such as IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway, Lipid and atherosclerosis, Hepatitis B, etc.. (data not shown). The KEGG analysis suggested that Kushen can play a role in the treatment of TB through the hypoxia-inducible factor 1 (HIF-1) pathway, as Figure 5 shows. Figure 6 shows the pathway diagram.

Molecular Docking Verification

The top 5 hub genes (TOP5) were MAPK1, AKT1, JUN, FOS, and RELA, and 11 docking experiments occurred. The molecular binding energy: (1) of quercetin to FOS was -3.65 kcal/mol, (2) of luteolin to MAPK1 was -5.25 kcal/mol, (3) of luteolin to AKT1 was -5.93 kcal/mol, (4) of luteolin to JUN was -4.68 kcal/mol, and (5) of matrine to RELA was -6.26 kcal/mol (Figure 7).

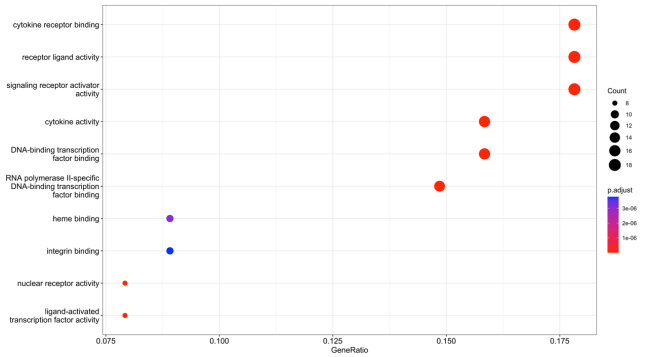
DISCUSSION

Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis infection. Allergy and inflammatory reactions are mainly participated by macrophages, T lymphocytes, cytokines (IFN-γ, TNF-α, interleukin), etc. Mycobacterium tuberculosis can manipulate macrophage activation through multiple pathways and establish a suitable proliferative environment within its cells to prevent elimination[27]. In this study, the main active ingredients of Kushen, Phaseolin, quercetin, matrine, luteolin, and formononetin, which were reported that related to anti-inflammatory, bacteriostatic, and anti-tumor effects.[28]

Regarding the molecular binding energy of quercetin, luteolin, and matrine, the current research team speculates that luteolin in Kushen may play an antitubercular role through MAPK1. This reflects the fact that Kushen plays a role in the treatment of TB through a combination of components.

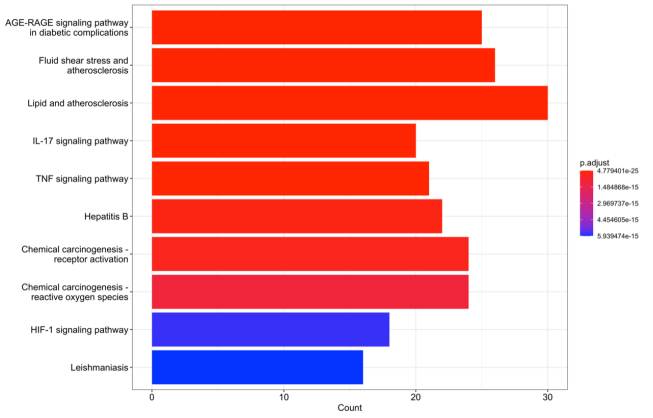
MAPK8, AKT1, MAPK1, ESR1, RELA, IL6, MYC, RXRA, FOS, and JUN were the top 10 target genes in the current study, which showed that they were related to the

Figure 4. Bubble Plot for GO Enrichment Analysis



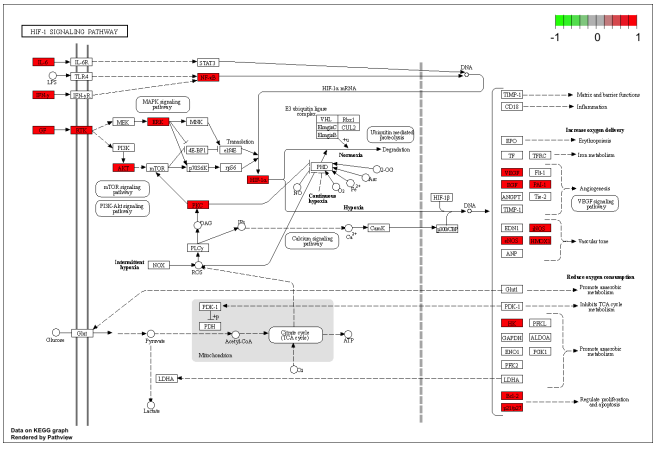
Abbreviations: GO, gene ontology.

Figure 5. KEGG Pathway Enrichment Analysis of Potential Target Genes



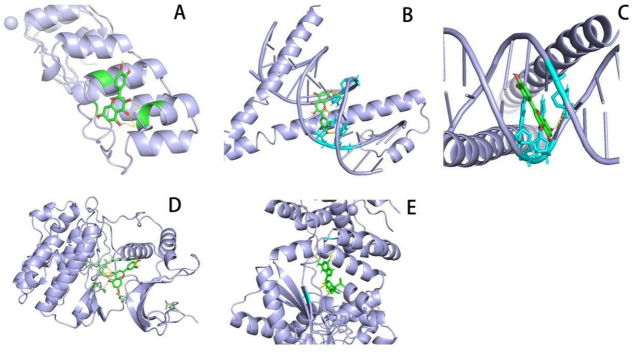
Abbreviations: AGE-RAGE, advanced glycation end products-receptor for advanced glycation end products; IL-17, interleukin-17; HIF-1, hypoxia-inducible factor 1; KEGG, Kyoto Encyclopedia of Genes and Genomes; TNF, tumor necrosis factor.

Figure 6. HIF-1 Signaling Pathway Map of Kushen's Treatment of Tuberculosis. The figure shows Kushen's targets in the HIF-1 signaling pathway. Red represents target proteins involved in the HIF-1 signaling pathway.



Abbreviations: HIF-1, hypoxia-inducible factor.

Figure 7. Molecular Docking of Active Ingredients to TOP5 Target Proteins. Figure 7A shows luteolin to AKT1, at -5.93 kcal/mol; Figure 7B shows quercetin to FOS, at -3.65 kcal/mol; Figure 7C shows luteolin to JUN, at -4.68 kcal/mol; Figure 7D shows luteolin to MAPK1, at -5.25 kcal/mol; and Figure 7E shows matrine to RELA, at -6.26 kcal/mol.



Abbreviations: AKT1, protein kinase B; FOS, Fos proto-oncogene, activator protein 1 (AP-1) transcription factor subunit; JUN, JUN proto-oncogene, AP-1 transcription factor subunit; MAPK1, mitogen-activated protein kinase 1; RELA, rel avian reticuloendotheliosis viral oncogene homolog A; TOP5, top 5 hub genes.

pathological process of pulmonary TB. The latest research results showed that MAPK8, AKT, IL6 and so on play a key role in the occurrence and development of tuberculosis[29,30]. This finding reflects that the selected drug components of Kushen were more reliable. The previously reported genes that are consistent with our results are considered to be more reliable and it shows that Kushen can play a role in the treatment of pulmonary TB through the combination of multiple components.

The results of the KEGG enrichment analysis showed that 158 pathways. Among them, IL-17 signaling pathway, TNF signaling pathway, HIF-1 signaling pathway are closely related to Kushen's role in the treatment of pulmonary TB. The most noteworthy was the HIF-1 signaling pathway.

HIF-1α my play different roles in the early and late stages of TB. Baay-Guzman et al[31] found that HIF-1α levels were elevated in a mouse model of progressive pulmonary TB in the early and late stages of TB. Inhibition of HIF-1α in the

early stages of infection led to an aggravation of the disease state in these mice, but inhibition of HIF-1α in the later stages of TB resulted in increased apoptosis of macrophages and decreased TB burden.

Under normoxic and hypoxic conditions, MTB infects macrophages, which leads to a metabolic switch to aerobic glycolysis, and HIF-1α plays an important role in the switch.[32] Upregulation of HIF-1α can result in an increase in the proinflammatory cytokine IL-1β and a decrease in bacterial survival.[33] Knight[34] et al also demonstrated that HIF-1α is a necessary condition for macrophages to generate lipid droplets as part of their immune response to MTB.

In recent years, with the continuous development and combination of biological information technology and computer network technology, researchers had widely used network pharmacology as a new technology. Based on the systematic interaction between TCM, ingredients, targets, and diseases, the rapid development of TCM has undoubtedly been accelerated. Through the network pharmacology method, the current study predicted that Kushen can act on key targets such as IL6, AKT1, MAPK8, MAPK1, JUN through its active ingredients

However, the research content of network pharmacology can predict the efficacy of drugs by using relevant databases, but some limitations still exist. First, the accuracy and comprehensiveness of target information in the database needs to improve. The current research predicted only the drug components and targets through network pharmacology technology, and animal experiments or even clinical trials still need to verify the definite pharmacological effects.

CONCLUSIONS

The network pharmacology analysis showed that Kushen's active ingredients can play a role in the treatment of TB through the HIF-1 signaling pathway.

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