TMR Pharmacology Research
doi: 10.12032/TMRPR20210429011

Exploration on antibacterial mechanism of Xiangsu Powder based on network pharmacology

Xu-Xuan-Hong Qian[1], Zhi-Yu Guan[1*]

[1] Jiangxi University of Traditional Chinese Medicine, Nanchang, China.

***Corresponding to**: Zhi-Yu Guan. School of Pharmacy, Jiangxi University of Traditional Chinese Medicine, No. 818 Meiling Road, Wanli District, Nanchang City, Jiangxi Province, 330004, China. E-mail: adlaiguan@126.com.

Abstract

Background: To explore the antibacterial mechanism of the active components of Xiangsu powder through the network pharmacological approach. **Methods:** TCMSP database was used to search and obtain the active components of Xiangsu powder and its corresponding action targets, and its network relationship was defined. Target points associated with antibacterial effect were searched in Genecards database, and core target genes of antibacterial effect were obtained by mapping the target points. Finally, the GO biological process and KEGG metabolic pathway were analyzed in the DAVID database. **Results:** There were 129 active components, 250 targets, and 66 biological processes (40 biological processes, 13 molecular functions, 13 cell compositions) and 35 related signaling pathways. Among them, quercetin may be the main substance that plays a role in the drugs contained in Xiangsu powder, followed by flavonol kaempferol and flavonoid luteolin. Antibacterial targets such as TNF, Casp3, PTGS2, ACTB, STAT1 and NFKB1 are mostly involved in antiviral, anti-inflammatory and immune pathways. It mainly plays a role in the hepatitis B pathway and tumor necrosis factor signaling pathway. **Conclusion:** Quercetin, kaempferol, luteolin and other active components in Xiangsu powder can participate in the action process of Toll-like receptor pathway, TNF signaling pathway and other pathways through acting on TNF, Casp3, PTGS2, etc., and finally achieve the purpose of antibacterial.

Keywords: network pharmacology; xiangsu powder; antibacterial; mechanism

***Acknowledgments*:**

This study was supported by Jiangxi Innovation and Entrepreneurship Training Program for College Students (S202010412079). Study on the design of mask for TCM epidemic prevention based on incense therapy.

***Competing interests*:**

The authors declare no conflicts of interest.

Data availability statement:

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

***Citation*:**

Qian XXH, Guan ZY. Exploration on antibacterial mechanism of Xiangsu Powder based on network pharmacology. *TMR Pharmacology Research.* 2021;1(2):11. doi: 10.12032/TMRPR20210429011.

***Executive editor*:** Lu Yang

***Submitted*:** 04 March 2021, ***Accepted*:** 16 April 2021, ***Online*:** 26 May 2021.

Background

Through the use of antibiotics, we can control the occurrence and development of many bacterial infectious diseases. However, with the large-scale use of antibiotics, bacterial drug resistance, a major worldwide problem, has emerged. At present, drug-resistant strains have appeared in the use of most antibiotics [1], indicating that human beings are facing the "antibiotic resistance crisis" [2]. In contrast, Chinese medicine is not easy to develop drug resistance and is called "natural antibiotics", but its bacteriostatic mechanism is complex and needs to be explored.

Yi Xue Xin Wu (an ancient Chinese medical book written by Zhong-Ling Cheng in the Qing Dynasty) said that "pestilence law..... Its syndrome headache and fever, Xiangsu Powder dispersed ". This paper intends to conduct a preliminary study on the antibacterial mechanism of Xiangsu Powder.

The composition of Xiangsu Powder mainly includes Xiangfu(Rhizoma Cyperi), Zisu(perilla leaf), Chenpi(Pericarpium Citri reticulatae) and Gancao(baked licorice), which has the functions of relieving exterior syndrome, regulating qi and blood, etc., and is a prescription for the Chinese medical doctors of all ages to treat pestilential diseases [3]. Through literature research, it is found that the composition of Xiangsu powder has good antibacterial effect.

The study of Kilani et al. [4] showed that the ethyl acetate extract of Rhizoma Cyperi could significantly inhibit the activity of a variety of bacteria. The study of Yu et al. [5] showed that the methanol extract of Rhizoma Cyperi could achieve a good killing effect on Streptococcus mutans. Yao MY et al. [6] found that single drug of Perilla leaf had good antiviral effect. The extract of Pericarpium Citri reticulatae can also inhibit the growth of common superficial mycosis [7]. As an antibacterial drug, Pericarpium Citri reticulatae is widely used in various prescriptions, and is often used in the treatment of acute and chronic bronchitis, asthma, gastritis and other diseases and synonyms [8]. The study of Zhang MF et al. [9] proved that licorice and glycyrrhizic acid components had significant effects on the anti-influenza virus, coronavirus and other respiratory viruses.

In recent years, network pharmacology has been applied in practice as a new model of drug research [10]. Network pharmacology is a research method to explain and explore the mechanism of action of drugs by establishing the network relationship between active components, targets and diseases, which is especially suitable for the study of the mechanism of Chinese herbal compounds with multi-component, multi-target and multi-pathway characteristics [11,12].

This paper aimed to investigate the network relationship among the active components, action targets and key pathways of Xiangsu powder by using network pharmacology research and analysis methods, and explain its antibacterial components and broad spectrum antibacterial and mechanism from the perspective of modern pharmacology, so as to provide theoretical guidance for clinical drug use.

Materials and methods

Software and Database

TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform，http://lsp.nwu.edu.cn/, Version 2.3), GeneCards database (http://www.genecards.org/, Version 4.5.0), Cytoscape software (Version 3.7.1), String database (https://string-db.org/, Version 10.5), DAVID (https://david.ncifcrf.gov, Version 6.8), ETCM database (http://www.tcmip.cn/ETCM/index.php)

Screening of active components and construction of active component-target network diagram

TCMSP database was used to screen and identify the active components with OB (oral bioavailability) ≥ 30% and DL (drug-likeness) ≥0.18 in Rhizoma Cyperi, perilla leaf, Pericarpium Citri reticulatae and baked licorice and their corresponding targets [13].

The active components and targets of Xiangsu Powder were imported into Cytoscape 3.7.1 to complete the construction of the network diagram of "drug-active component-target" of Xiangsu Powder [14].

Screening of antibacterial targets

The GeneCards database was used to find targets associated with antibacterial function. Potential targets of Rhizoma Cyperi, perilla leaf, Pericarpium Citri reticulatae and baked licorice were obtained by query in ETCM, and intersected with human antibacterial targets, that is, the shared genes of the two were selected as the intersection target.

Protein-protein interaction (PPI) network construction

The mapped intersection targets were imported into the STRING platform to complete the PPI network [15].

KEGG metabolic pathway and GO biological process

The GO biological pathway and KEGG metabolic pathway of related target proteins were analyzed by David 6.8 database [16].

Results

doi: 10.12032/TMRPR20210429011

Analysis of screening results of main active components and targets of Xiangsu Powder

Enter the name of traditional Chinese medicine in the TCMSP database to search for the active components in Rhizoma Cyperi, perilla leaf, Pericarpium Citri reticulatae and baked licorice. By selecting OB≥30% and DL≥0.18, a total of 129 active components were obtained, including 14 perilla leaf, 18 Rhizoma Cyperi, 92 baked licorice and 5 Pericarpium Citri reticulatae. See Table 1 for detailed information.

Drug - components- target network analysis

The 129 selected active components and their corresponding action targets were integrated and imported into Cytoscape 3.7.1, so as to complete the construction of the relationship network diagram of drug composition-active component-target of Xiangsu powder. As can be seen from Figure 1, it includes 383 nodes and 2562 lines, among which the degree value of MOL000098 is the largest, indicating that MOL000098(quercetin) is the main substance that plays a role in the drugs contained in Xiangsu powder.

Screening of antibacterial targets

Using GeneCards database, enter the key search word "Antibacterial activity" to find the genes related to Antibacterial effect. Then select the species is human and search for relevant genes. A total of 784 Antibacterial genes were found. All 582 potential targets composed of Xiangsu powder were obtained from ETCM databaseand, and 49 common target genes were obtained after mapping with the antibacterial genes found in GeneCards database,which were the potential antibacterial targets of the active components of Xiangsu powder.

PPI results of target

The mapped information of 49 potential antibacterial targets of Xiangsu powder was imported into the String database, the Multiple proteins function was used, and species “Homo Sapiens” were selected to obtain the PPI diagram of antibacterial targets of Xiangsu powder, as shown in Figure 2. As can be seen from Figure 2, the PPI network constructed by the 49 target genes mapped has 33 nodes and 124 lines representing interaction. Adjust and set the size and color of the target respectively by using the generate style from statistics function option in Cytoscape. And the thickness of the edge to represent the degree and combine score value of the target, finally a clear protein interaction network diagram is obtained, as shown in Figure 3.

By analysis and calculation, it is concluded that the mean values of network degree and medium of PPI were 7.52 and 0.703, respectively. There were 15 key target proteins (TNF、CASP3、PTGS2、ACTB、STAT1、NFKB1、IL17A、PPARG、HMOX1、CD40LG、IFNB1、NOS2 and SOD1、NLRP3、ANPEP) whose degree and medium were higher than the above mean value, which indicated that they were the key targets of Xiangsu powder in the process of its antibacterial action.

GO biological processes and KEGG pathway enrichment analysis results

Finally, for screened the active component of Xiangsu powder and antibacterial core targets , analyzed its metabolic pathways and biological processes on DAVID 6.8 platform , including the GO function analysis for a total of 66 GO items ($P < 0.01$, Figure 4), including 40 biological processes, in which it includes the virus into host cells, T cell aggregates, Blood pressure regulation, positive regulation of smooth muscle cell proliferation, etc. 13 molecular functions; 13 cell components;and 35 KEGG signaling pathways ($P < 0.01$, Figure 5).

Table 1 List of some active components of Xiangsu Powder

Traditional Chinese medicine (TCM)	active constituents
Perilla leaf	luteolin，gondoic acid，LAX，beta-carotene，cyanin，eugenyl-β-D-glucopyranoside(cirtrusinc)，beta-sitosterol，(+)-catechin，CLR，Supraene，ZINC03860434，poriferast-5-en-3beta-ol，Linolenic acid ethyl ester，methyl icosa-11,14-dienoate
Rhizoma Cyperi	Chryseriol，isorhamnetin，8-Isopentenyl-kaempferol，beta-sitosterol，sitosterol，1,4-Epoxy-16-hydroxyheneicos-1,3,12,14,18-pentaene，Isodalbergin，Khell，khellol glucoside，Resivit sitosterol，naringenin
Pericarpium Citri reticulatae	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one，Citromitin，nobiletin
Baked licorice	Inermine，DFV，Mairin，Glycyrol，Jaranol，Medicarpin isorhamnetin，sitosterol，Lupiwighteone，7-Methoxy-2-methyl isoflavone，formononetin，Calycosin，kaempferol，naringenin

Discussion

The antibacterial activity of traditional Chinese medicine is the common result of many effects on bacteria and organisms. The antibacterial pharmacodynamic mechanism of traditional Chinese medicine is mainly to stimulate potential antibacterial factors in organisms, enhance the immune response ability of the body to pathogenic bacteria, thereby reducing the damage of pathogenic bacteria to the body, and finally realize the antibacterial effect [17].

Through screening in the network pharmacological database, we can know that 129 effective active components in Xiangsu powder act on 250 targets, among which quercetin is the most active target, which may be the main substance that plays a role in the drug effect in Xiangsu powder, followed by flavonol kaempferol and flavonol luteolin. Modern studies have shown that quercetin can play antioxidant [18], antibacterial, anti-inflammatory [19], anti-tumor [20] and other effects. Li BB [21] found that quercetin can reduce the activity of Staphylococcus aureus. Teng N [22] found that quercetin can have certain effects on bacterial cell membrane and cell wall. Guo YR et al. found that [23] luteolin can kill arcanobacterium pyogenes. Kaempferol can also inhibit the activity of a variety of bacteria such as Staphylococcus aureus and shigella dysenteriae [24]. The above studies are basically consistent with the results of this experiment, which fully demonstrates the antibacterial mechanism of Xiangsu powder combined with multiple components.

The results of this study showed that TNF、CASP3、PTGS2、ACTB、STAT1、NFKB1、IL17A、PPARG、HMOX1、CD40LG、IFNB1、NOS2 and SOD1 、NLRP3 and ANPEP were the key antibacterial targets of Xiangsu Powder, and the antibacterial targets of Xiangsu Powder mainly involved proteins (cytoskeleton proteins, etc.), transcription factors, receptors, enzymes, etc., indicating that these proteins are involved in the antibacterial mechanism of Xiangsu Powder. Among them, TNF is an anti-tumor factor, which can kill tumor cells and make tumor cells apoptosis, and can improve the proliferation ability of T and B cells, and participate in the inflammatory reaction process, so as to effectively inhibit bacteria. PTGS2 is a dioxygenase that is associated with inflammation and promotes blood vessel formation. It is suggested that the antibacterial process of XiangSu powder may first act on bacterial surface proteins, and then indirectly inhibit bacterial proliferation by inhibiting bacterial protein synthesis, or inhibit the synthesis of various enzymes needed in the process of bacterial growth and reproduction, and finally achieve the antibacterial and bacteria-inhibiting effect.

Through KEGG pathway enrichment analysis, it was found that TNF signal pathway and Toll-like receptor pathway were the key antibacterial pathways of Xiangsu Powder. Toll-like receptors [25] can stimulate the production of various bactericidal substances and cytokines through receptor-associated kinases, and participate in the immune response process [26]. TNF can promote cell division, induce the occurrence of inflammatory response, participate in biological processes such as antibacterial and immune regulation, and enhance the killing effect of phagocytes [27]. The results indicated that Xiangsu Powder can realize its antibacterial action through many ways.

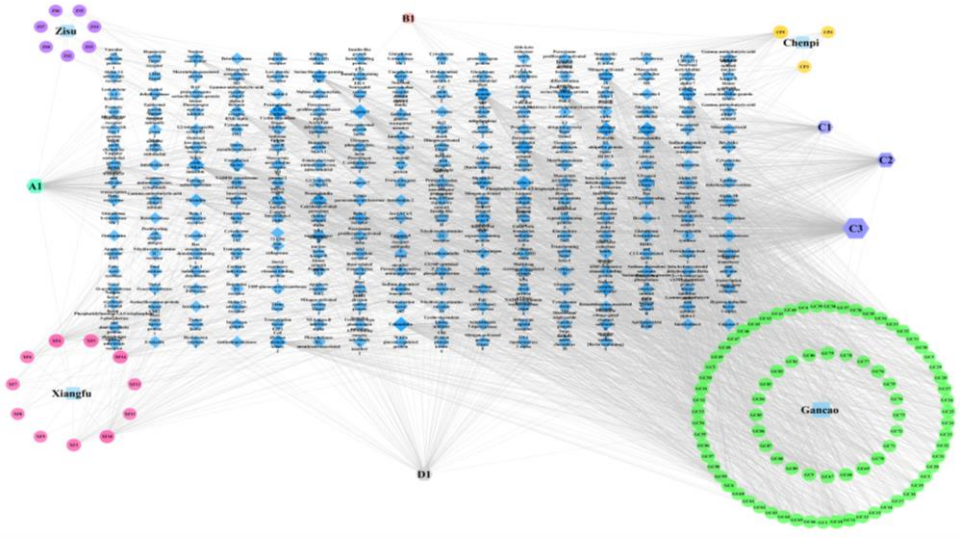


Figure 1 Network diagram of drug - component - target relationship of Xiangsu powder Square - Chinese medicine, circle - active component, diamond - target, hexagon - drug active component intersectio

doi: 10.12032/TMRPR20210429011

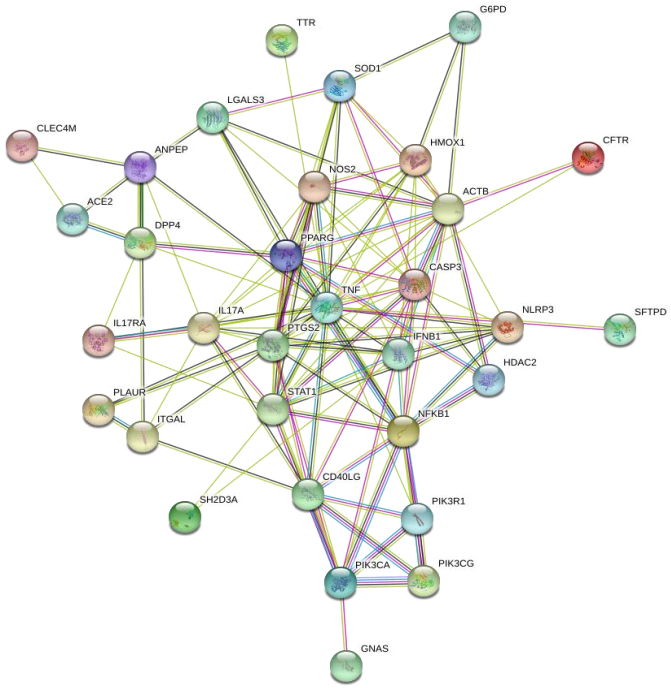


Figure 2 PPI network of Xiangsu powder targets

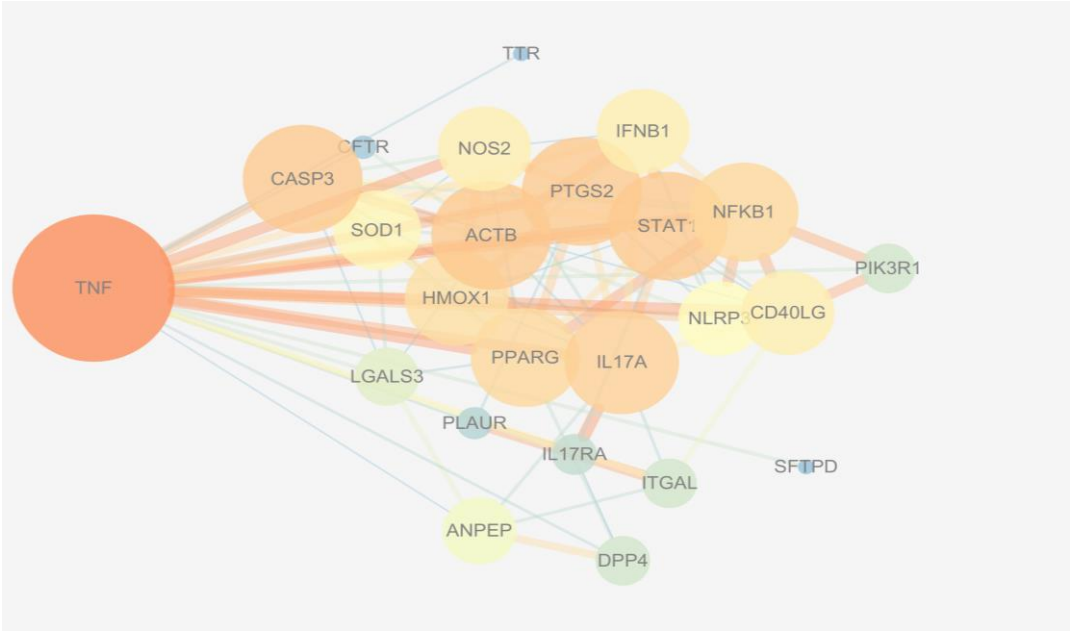
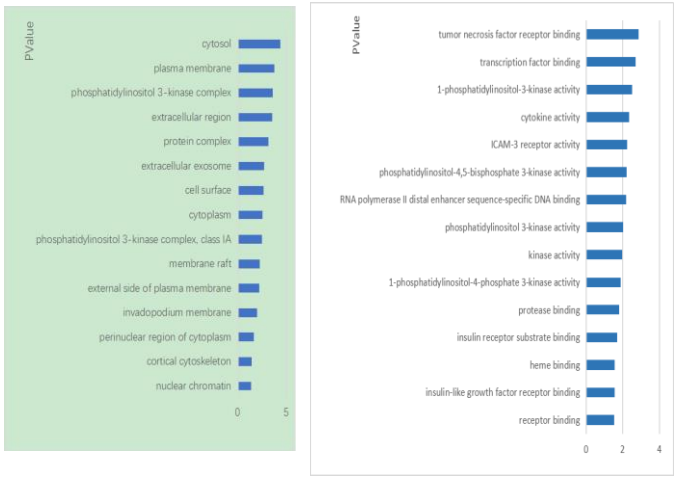


Figure 3 Target PPI Network Degree Graph

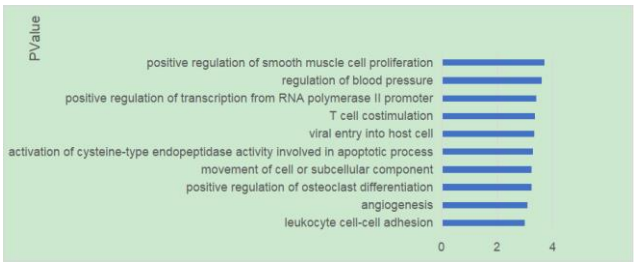
Conclusion

The results of this study showed that Xiangsu Powder can participate in the antibacterial process of the body through multi-components, multi-targets and multi-pathways, inhibit the growth of pathogenic bacteria or kill pathogens, and exert antibacterial effects. It further explained the mechanism of broad-spectrum antibacterial function of Xiangsu Powder, and provided theoretical guidance for clinical medication.



a. Cell composition

b. Molecular function



c. Biological processes

Figure 4 GO enrichment analysis of the intersection target of Xiangsu powder In the results of KEGG enrichment analysis, the first 25 signaling pathways were explored ($P < 0.05$), and a histogram was made, as shown in Figure 5, mainly including TNF signaling pathway, hepatitis B pathway, influenza A pathway, cancer pathway and other key pathways.

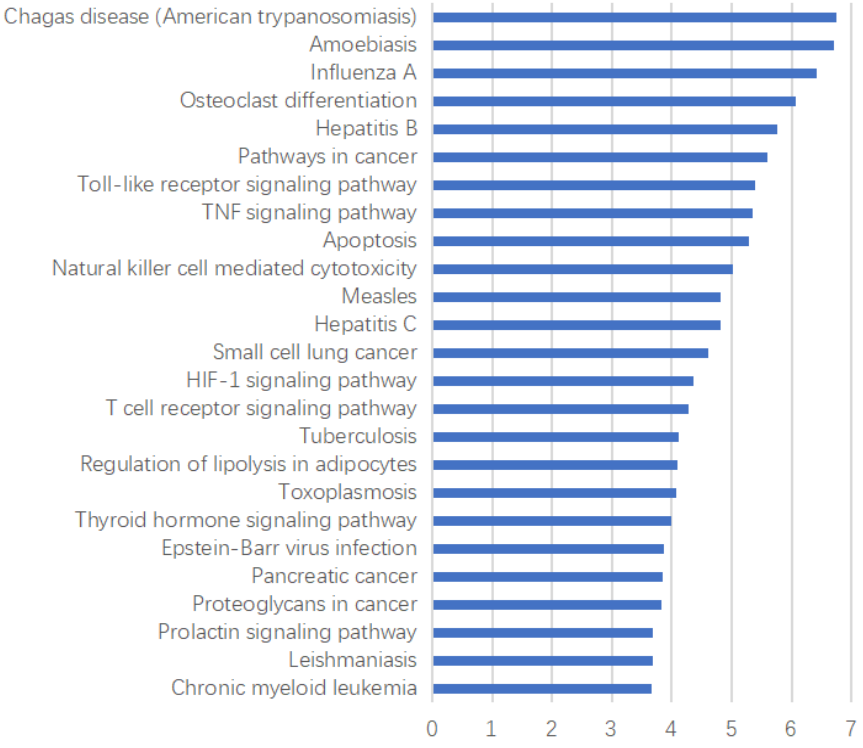


Figure 5 Top 25 pathways of KEGG enrichment analysis of Xiangsu powder targets

References

1. Li RM, Lei ZX. Bacterial resistance: a strategy for delaying and reversing bacterial resistance and treating drug-resistant bacterial infection with Chinese herbal medicine. Journal of Medicine and Philosophy, 2006,27(8):45-47.
2. Yin JX, Lin DR.Journal of Shaoxing University of Arts and Science, 2004,24(8):52-55.
3. Liang HY.New clinical use of Xiangsu Powder . Xinjiang Journal of Traditional Chinese Medicine,2011,29(06):84.
4. KILANI S, SGHAIER M B, LIMEM I, et al.In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus rotundus.Bioresource technology, 2008, 99 (18) : 9004-9008.
5. YU H H, LEE D H, SEO S J, et al. Anticariogenic properties of the extract of Cyperus rotundus. The american journal of chinese medicine, 2007,35 (3): 497-505.
6. Yao MY, Zhou CZ, Chen F, et al. World Journal of Integrated Traditional and Western Medicine,2015,10(06):782-784.
7. Zhang LP. New research progress of tangerine peel. Bright Chinese Medicine, 2005, 20 (1) :40.
8. Li X., Zhang J., Wang H., Ye F., Li H., Ye X. Progress in the study of tangerine peel. Jiangxi Journal of Traditional Chinese Medicine,2014,45(03):76-78.
9. Zhang MF, Shen YQ. Progress on the pharmacological effects of glycyrrhizic acid and glycyrrhizic acid components against viral pneumonia. Drug Evaluation Research,2020,43(07):1452-1468.
10. Liu ZH, Sun XB. Chinese Journal of Pharmaceutical Sciences,2012,47(6):696-703.
11. Li PL, Su WW. Latest application progress of network pharmacology in traditional Chinese medicine research. Chinese Journal of Herbal Medicine, 2016, 47 (16) :2938-2942.
12. Liu YF, Ai N, Keys A, et al.Network pharmacology bridges traditional application and modern development of traditional Chinese medicine.Chin Herb Med, 2015, 7 (1) :18-26.
13. Xu X, Zhang W, Huang C,et al.A Novel Chemometric Method for Prediction of Human Oral Systems . Journal of Molecular Sciences,2012,13(6):6964-6982.
14. Ru JL. Yang L, 2015. Formaldehyde and Utilization of Traditional Chinese Medicine Systems pharmacology database and analysis platform.
15. Tian HD, Guo LN, Wang SS, et al. Journal of Investigative Medicine, 2010, 38 (1): 78-83. Investigating the main components and mechanism of Panax notoginseng based on pharmacology network. Drug Evaluation Research, 2019,42(1):70-75.
16. Duan KX, Li YW, Liu HB, et al. Study on anti-inflammatory mechanism of couplet medicine of Notopterygiumincisum Angelica pubescens based on network pharmacology. China Pharmacy, 2019,30(9):1241-1246.
17. Xie CD, Wen Y, Zeng YW, et al.Antibacterial activity component of Coptis chinensis Franch.Hubei Agricultural Sciences, 2018, 57(23):85-88.
18. Ma L, Tao HL, Guan JJ,et al.Regulatory effect of quercetin on abnormal autophagy and oxidative stress in rats with middle cerebral artery occlusion.Pharmacology and Clinics of Chinese Materia Medica,2019,35(4):48-53.
19. Ren GY, Zhang BY, Huang JL. Protective effects of quercetin on the inflammation of mice RAW264.7 cells induced by LPS.Chinese Traditional Patent Medicine, 2019, 41(8):1795-1799.
20. Yang Y, Guo J. Research progress on active quercetin derivatives with antitumor effect Chinese traditional and herbal drugs.Chinese Traditional and Herbal Drugs, 2018, 49(6):1468-1475.
21. Li BB. Study on the inhibitory effect and mechanism of quercetin on Staphylococcus aureus Coa.Changchun:Jilin University,2018.
22. Teng N. Bacteriostasis of quercetin in vivo and in vitro. Harbin: Northeast Agricultural University,2015.
23. Guo YR, Wang RX, Qiu P, et al. Study on antibacterial mechanism of luteolin against Clostridium pyogenes [A]. Chinese Society of Animal Husbandry and Veterinary Medicine Veterinary Pharmacology Toxicology branch. Veterinary Pharmacology and Toxicology Branch of Chinese Society of Animal Science and Veterinary Medicine: Chinese Society of Animal Science and Veterinary Medicine,2019:1.
24. Zhang YW, Shao DY, Shi JL, et al. Advances in the biological function of kaanol. Life Science,2017,29(04):400-405.
25. KABEL A M, ESTFANOUS R S, ALROBAIAN M M.Targeting oxidative stress,proinflammatory cytokines,apoptosis and toll like receptor 4 by empagliflozin to ameliorate bleomycin-induced lung fibrosis.Respiratory Physiology & Neurobiology,2019,273:103316.
26. YIN Q Y, ZHAO B, QIU Y Y, et al. Research progress of mechanisms and drug therapy for atherosclerosis on Toll-like receptor pathway. Journal of Cardiovascular Pharmacology, 2019,74(5):379-388.

27. MONTFORT A, COLACIOS C, LEVADE T,et al.The TNF paradox in cancer progression and immunotherapy. Frontiers in Immunology, 2019,10:1818.