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Mechanism of Yixishu lotion in the treatment of vaginitis based on network pharmacology combined with experimental validation: an experimental research study

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Objective: Yixishu lotion (YXSL) originates from the summary of traditional Chinese medicine clinical experience and constantly improves in practice in clinical validation of the exact efficacy of traditional Chinese medicine prescription. To explore the mechanism of YXSL in treating vaginitis and the potential mechanisms based on network pharmacology and experimental verification.

Methods: The active components and drug-related targets of YXSL were retrieved from the TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform) database, and the target was predicted by the UniProt database. Searching for genes related to 'vaginitis' disease in the GeneCards database, a total of 2581 drug targets were obtained. The interaction between proteins (PPI – protein–protein interaction) relationship was obtained by STRING database and visualized by Cytoscape software. Finally, the 'Bioconductor' installation package in R software was used to analyze the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways of the target.

Results: In this study, by the method of network pharmacology, the key active components of YXSL were flavonoids such as quercetin, apigenin, kaempferol, luteolin, β-sitosterol; the main core proteins included MAPK14, TP53, FGF2, ESR1, MAPK3, MAPK1, VEGFA, JUN, IL-6, and the KEGG pathway was mainly involved in MAPK pathway, Th17 pathway, Malaria, TNF pathway, and other signaling pathways. Animal experiments showed that the clinical symptoms and vaginal tissue lesions of the YXSL group and the fluconazole group were improved, and the levels of TNF-α (tumor necrosis factor alpha), IL-6 (interleukin-6), MDA (malondialdehyde), SOD (superoxide dismutase), IL-4, and IFN-γ (interferon-γ) in vaginal tissue and serum were better than the model group.

Conclusion: YXSL may achieve its therapeutic effect on vaginitis by reducing the inflammatory response, improving oxidative stress response, and improving body immunity, and it provides a theoretical basis for further research on its pharmacodynamic material basis and mechanism of action.

Keywords: network pharmacology, target, vaginitis, Yixishu lotion

Introduction

Vaginitis is a gynecological disease characterized by genital itching, irritation, and increased vaginal secretions. In severe cases, it can lead to infertility and greatly reduce patients' quality of life. Bacterial and fungal vaginitis are common clinically. Epidemiological studies have found that the incidence of the disease is particularly prominent in some patients with weakened immunity[1]. Yixishu lotion (YXSL) comprises *Sophora flavescens*, *Cnidium*, *Xanthium*, *Epimedium*, and *Cortex Phellodendron*. In clinical practice, YXSL has a certain therapeutic effect on vaginitis. The application of various treatment methods and the lack of widely accepted scientific research methods hinder the development of traditional Chinese medicine. As an emerging interdisciplinary subject, network pharmacology

HIGHLIGHTS

- Yixishu lotion (YXSL) may achieve its therapeutic effect on vaginitis by reducing the inflammatory response, improving oxidative stress response, and improving body immunity.
- The key active components of YXSL were flavonoids such as quercetin, apigenin, kaempferol, luteolin, and β-sitosterol.
- The main core proteins of YXSL included MAPK14, TP53, FGF2, ESR1, MAPK3, MAPK1, VEGFA, JUN, and IL-6.

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has constructed a multilayered network of 'drug-targeting-disease', emphasizing multiple groups. According to the characteristics of the treatment of diseases, this is unified with the overall thinking of traditional Chinese medicine treatment of diseases and the idea of syndrome differentiation. Based on this, this study carried out relevant experimental verification based on network pharmacology prediction to explore the potential mechanism of YXSL in the treatment of vaginitis and to provide a reference for subsequent research.

Materials and methods

Common targets of YXSL in the treatment of vaginitis

The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform[2] (TCMSP, http://www.tcmspw.com/tcmsp.com/tcmsp.php) and the Traditional Chinese Medicines Integrated Database (TCMID, https://47.100.169.139:8000/tcmid) were used to collect the active compounds of YXSL, including *Sophora flavescens*, *Cnidium*, *Xanthium*, *Epimedium*, *Cortex Phellodendron*. The active compounds and their targets in the composition of YXSL were selected by ADME (absorption, distribution, metabolism, and excretion) evaluation systems[3], the main parameters of which were DL ≥ 0.18 and ALogP <5. Use the UniProt database (http://www.uniprot.org/) to search the potential target information corresponding to the screened active ingredients and standardize the protein target and gene information. GeneCards (https://genecards.org), OMIM (https://omim.org), PharmGKB (http://www.pharmgkb.org/), TTD (http://bidd.nus.edu.sg/group/cjttd/), DrugBank (http://www.drugbank.ca/), and CTD (http://www.ctdbase.org/) were utilized to collect genes, and target related with vaginitis and the species sources were humans ('*Homo sapiens*'). The common targets of vaginitis and active components were obtained by Draw Venn Diagram.

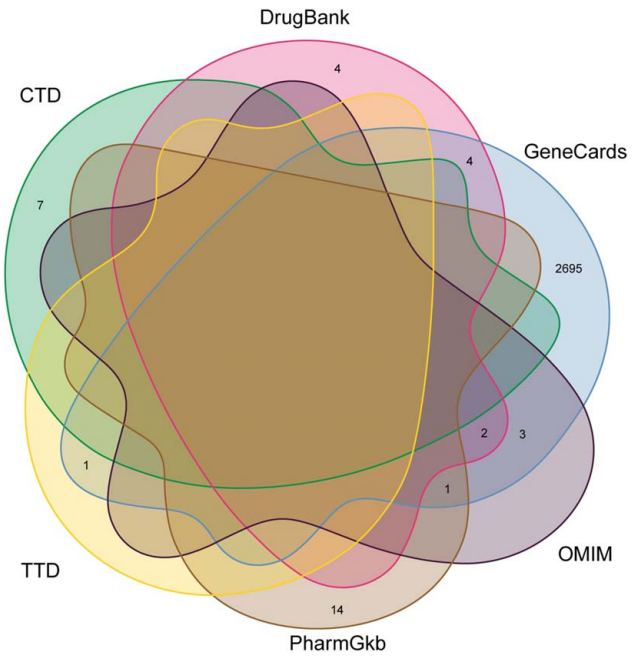

Figure 1. Intersection analysis of vaginitis-related genes obtained from disease databases.

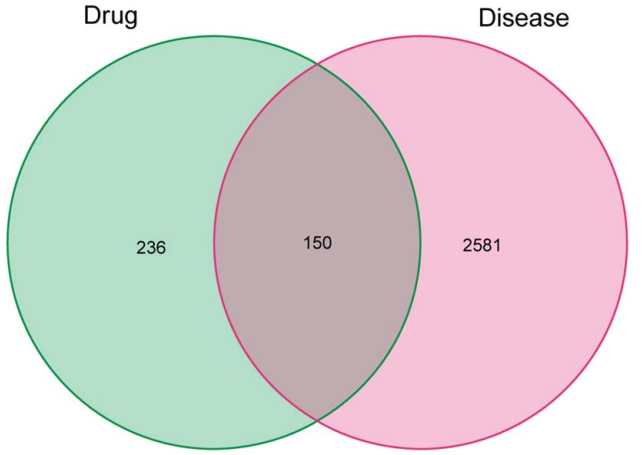

Figure 2. Intersection analysis of drug targets between Yixishu lotion and vaginitis.

Protein–protein interaction (PPI) network

The PPI network was obtained from the above common targets by STRING database (http://string-db.org/cgi/input.pl), and '*Homo sapiens*' was selected. The minimum interaction threshold was selected as 'medium confidence > 0.9.' The Cytoscape 3.8.0 software was used to analyze the core targets of the network, using the Network Analyzer tool for topology analysis.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)

Pathway enrichment analyses perform GO enrichment analysis and KEGG pathway analysis on common targets of drugs and diseases, using the STRING database to select biological processes (BPs), molecular functions (MFs), and cellular components (CCs) for enrichment analysis, $P < 0.05$ was significant.

Experimental validation

Candida albicans (CMCC 98001) was obtained from the National Institutes for Food and Drug Control, Beijing, China. The strains of *C. albicans* were cultured in Sabouraud Dextrose Broth Medium (SDBM; Qingdao Hope Bio-Technology Co., Ltd, Shangdong, China) for 24 h. After being centrifuged and washed with phosphate-buffered saline (PBS), the strain was diluted to a certain concentration (2×10^6 CFU/ml) for further experiments. Thirty-two female ICR (Institute of Cancer Research) mice with body weights of 18–22 g were provided by the Laboratory Animal Center of Xiamen University, Xiamen, Fujian, China (SCXK (Min) 2018-0003). The experimental animals were kept in Laboratory Animal Center, Xiamen University (SYXK (Min) 2018-0010). After 3 days of adaptive feeding, as previously described[4], to induce a pseudoestrus condition, all the mice received the subcutaneous injection with 0.1 ml of estradiol valerate (0.2 mg/ml) (Sichuan Jinke Pharmaceutical Co., Ltd, Sichuan, China) every 2 days throughout the experimental period. After pseudoestrus for 6 days, 10 μl suspension of 5.0×10^6 CFU/ml of *C. albicans* was inoculated intravaginally

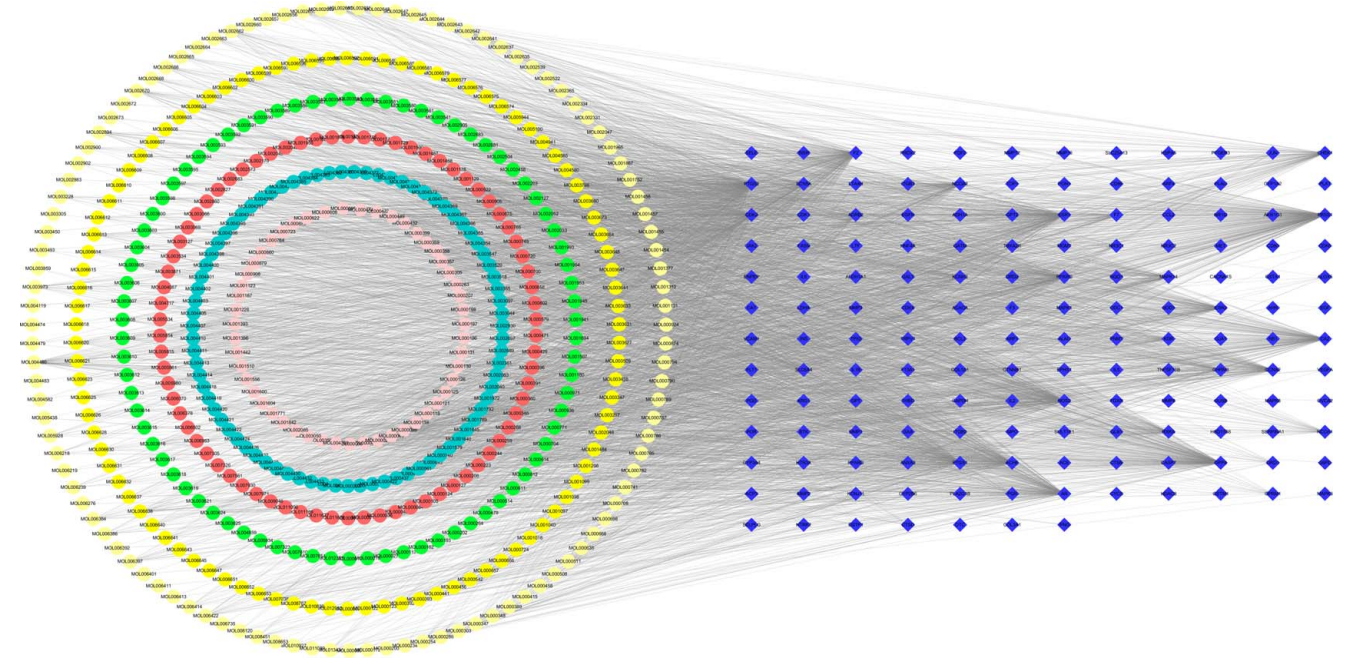

Figure 3. Network of active ingredients of Yixishu lotion.

continuously for 5 days ($n = 24$). After the inoculation, mice were played backward for 5 min and plugged the vaginal opening with a cotton swab to ensure the suspension stayed in the vagina without outflowing. Another eight mice as the control group was inoculated intravaginally continuously with 0.1 ml of 0.9% salt solution for 5 days. Forty-eight hours after the last injection, the mice were washed with vaginal secretions, and the vaginal secretions were inoculated on a YPD (Yeast–Peptone–Dextrose) medium. After culturing in a 37°C incubator for 24 h, the microscopic examination was performed to confirm whether the model was successful[5]. After successful modeling, continuous medication for 7 days.

The experimental group was described as follows:

Group 1 (control group): with 0.9% salt solution for 5 days, treated with 2.5% hydroxypropyl methylcellulose (HPMC) ($n = 8$);

Group 2 (model group): with fungal inoculation for 5 days, treated with 2.5% HPMC ($n = 8$);

Group 3 [miconazole nitrate (MN) group]: with fungal inoculation for 5 days, treated with 20 mg/kg MN (Sigma-Aldrich, USA) dissolving in 2.5% HPMC ($n = 8$);

Group 4 (YXSL group): with fungal inoculation for 5 days, treated with YXSL, continuously douche the vagina for 5 min ($n = 8$).

The last administration 2 days later, the mice's vagina was washed with 0.9% salt solution, and the whole vaginal canal was removed from the experimental mice for analysis. Collected vaginal canals were fixed with 10% formalin, embedded in paraffin, sectioned, and then stained with hematoxylin–eosin (H&E) to analyze the microstructural changes. After vaginal lavage, blood was sampled from the caudal vein of each mouse. The vaginal canal was quickly removed from the experimental mice. The collected vaginal canal was fixed with 10% formalin embedded in paraffin, sectioned, and then stained with H&E to analyze the microstructural changes. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) (Nanjing Jiancheng Bioengineering Institute), interleukin-4 (IL-4), interferon-γ (IFN-γ) (LMAT Bio) in serum were analyzed using enzyme-linked immunosorbent assay (ELISA) kits following the manual instruction. The work has been reported in line with the ARRIVE criteria[6].

Statistical analysis

All values are shown as the mean ± standard error of the mean. The statistical comparisons were measured by a one-way analysis of variance using SPSS18.0. A *P*-value of less than 0.05 was considered significant.

Results

Target screening of YXSL and vaginitis

In this study, they were set in the TCMSP database, and the active ingredients of YXSL were screened. Five traditional Chinese medicines were searched in the TC and Materia Medica databases base. Hundreds of chemical constituents were identified in YXSL, including 157 in *Sophora Radix* (Ku Shen), 134 in *Radix Cnidii* (She Chuang Zi), 135 in *Epimedium* (Yin Yang Huo), 155 in *Cortex Phellodendron* (Huang Bo), and 139 in *Xanthium* (Cang Er Zi). For a total of 1579 genes using 'vaginitis' as the keyword, we searched GeneCards, OMIM, PharmGKB, TTD, DrugBank, and CTD databases, respectively, and obtained a total of 2731 disease targets after deduplication (Fig. 1). Matching YXSL and vaginitis targets (Fig. 2).

Potential target genes and network analysis

The constructed herbal–chemical–protein networks were visualized using Cytoscape (Fig. 3). The circles on the left side of the

Table 1
Potential active ingredients of Yixishu lotion.

Degree	MolID	Molecule name	Herb
68	MOL000098	Quercetin	*Sophora flavescens*, *Cnidium*, *Xanthium*, *Epimedium*, *Cortex Phellodendri*
54	MOL004480	Acetic acid	*Cortex Phellodendri*
35	MOL000008	Apigenin	*S. flavescens*, *Cnidium*, *Epimedium*
34	MOL000422	Kaempferol	*Epimedium*
31	MOL000006	Luteolin	*S. flavescens*, *Cnidium*, *Epimedium*
29	MOL000358	Beta-sitosterol	*Xanthium*, *Cnidium*, *Cortex Phellodendri*
28	MOL007038	Dehydromiltirone	*S. flavescens*
27	MOL006625	Leachianone	*S. flavescens*, *Cnidium*
27	MOL000511	Ursolic acid	*Cortex Phellodendri*
26	MOL000922	(R)-p-Menth-1-en-4-ol	*Xanthium*
26	MOL000787	Fumarine	*Cortex Phellodendri*
26	MOL000790	Isocorypalmine	*Cortex Phellodendri*
25	MOL000472	Emodin	*Xanthium*, *Epimedium*
25	MOL004373	Anhydroicaritin	*Epimedium*
25	MOL002670	Cavidine	*Cortex Phellodendri*
24	MOL000325	(2R,3R)-2-(3,4-dimethoxyphenyl)-7-methoxy-3-methyl-5-[(E)-prop-1-enyl]-2,3-dihydrobenzofuran	*Epimedium*
24	MOL001457	Columbamine	*Epimedium*
24	MOL001455	(S)-Canadine	*Cortex Phellodendri*
24	MOL002642	Phellodendrine	*Cortex Phellodendri*
23	MOL002662	Rutaecarpine	*Cortex Phellodendri*
23	MOL000786	STOCK1N-14407	*Cortex Phellodendri*

circle in the figure, respectively, represent the first circle of the inner circle of common medicinal components; *Epimedium*, the second circle; *Xanthium*, the third circle; *Cnidium*, the fourth circle; *S. flavescens*, the fifth circle; *Cortex Phellodendri*, circle 6; a total of 483 active ingredients; the blue on the right represents 150 common drug-disease targets. The main active ingredients are quercetin, acetic acid, apigenin, kaempferol, luteolin, β-sitosterol, and dehydromiltirone (Table 1)

PPI network

The PPI network was obtained from the above common targets by STRING, and 'Homo species' was selected. The minimum interaction threshold was selected as 'medium confidence > 0.9' (Fig. 4).

Core target screening

Import the PPI network into Cystoscap 3.7.2, perform topology analysis through the Network Analyzer tool, take the four parameters of degree, betweenness centrality, average shortest path length, and closeness centrality as the reference standard, and sort by degree. Select genes with a score greater than the average score as core targets. The top 30 targets were plotted using R3.4.0 as a bar graph (Fig. 5). The core targets were screened. A total of eight core genes were obtained (Fig. 6). The core genes were MAPK14, TP53, FGF2, ESR1, MAPK3, MAPK1, VEGFA, JUN, and IL-6.

GO and KEGG enrichment analysis

GO and KEGG enrichment analysis of potential target genes GO enrichment and KEGG pathway enrichment analyses of the potential targets were performed to determine the underlying molecular mechanism of YXSL in vaginitis. GO enrichment analysis provided BP, MF, and CC results. The results revealed that the main BPs were 'response to nutrient levels', 'response to oxidative stress', and 'cellular response to oxidative stress'. The CCs were mainly associated with 'focal adhesion', 'plasma membrane raft', and 'membrane region'. The MFs were mainly associated with 'peroxidase activity', 'antioxidant activity', and 'endopeptidase activity'. Furthermore, GO analysis revealed the top 10 enrichment conditions in the BP, CC, and MF categories, and results are output as bar and bubble charts (Fig. 7). To examine the signaling pathways and functions of these target genes, KEGG pathway functional enrichment analysis was performed. The signaling pathways genes were found to primarily interact with the TNF, IL-17, MAPK, and Th17 signaling pathways. A KEGG bubble diagram was created using the top 30 signals. Results are output as bar and bubble charts (Fig. 8).

The protection of YXSL on the vagina of mice with vulvovaginal candidiasis (VVC)

Compared with healthy mice, a large number of neutrophils, eosinophils, and lymphocytes influx in the vaginal epithelium was noted in mice with VVC, while YXSL and MN-treated mice showed less influx of inflammatory cells in the vaginal epithelium than VVC mice (Fig. 9).

The protection of YXSL on the vagina of mice with VVC

Compared with healthy mice, a large number of neutrophil influxes in the vaginal epithelium were noted in mice with VVC, while YXSL and MN-treated mice showed less influx of neutral grains, eosinophils, and lymphocytes in the vaginal epithelium than VVC mice (Fig. 9). YXSL reduced the inflammatory cell infiltration of vagina of mice with VVC detecting by H&E staining ($n = 8$) ($50\times$, $200\times$)

The effect of YXSL on oxidative stress products in mice with VVC

In serum, compared with healthy mice, high levels of MDA and SOD were noted in mice with VVC, while YXSL resulted in the reduction of MDA and SOD, respectively (Table 2).

The effect of YXSL on inflammation in mice with VVC

In serum, compared with healthy mice, high TNF-α and IL-6 were noted in mice with VVC, while YXSL reduced TNF-α and IL-6, respectively (Table 2).

In serum, compared with healthy mice, high levels of IL-4 and IFN-γ were noted in mice with VVC, while YXSL resulted in the reduction of IL-4 and IFN-γ (Table 2).

Discussion

Vaginitis affects millions of women worldwide, negatively impacting patients' quality of life[7]. Traditional Chinese medicine plays a role in improving the clinical symptoms of vaginitis

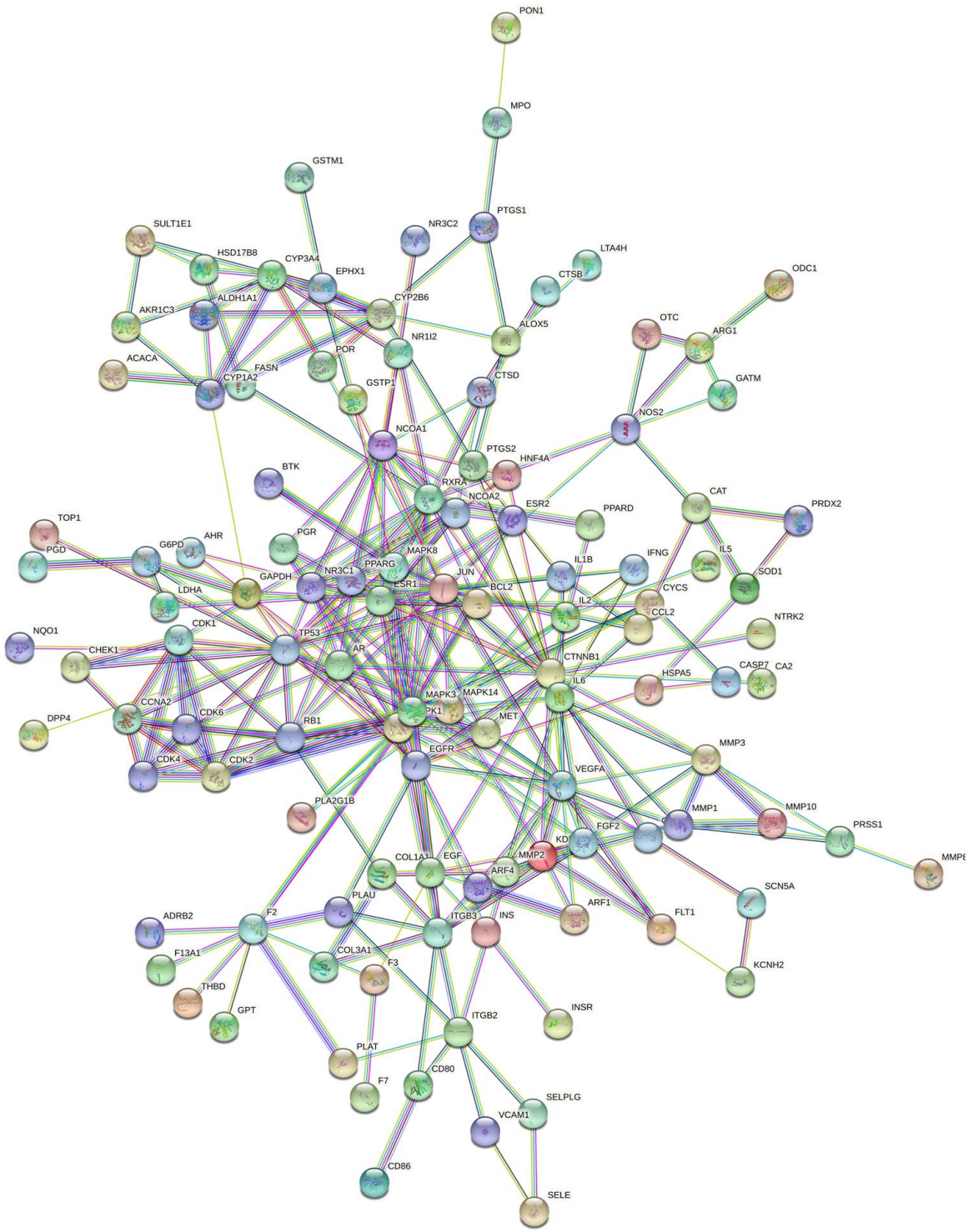

Figure 4. Protein–Protein Interaction network for intersection targets of Yixishu lotion and vaginitis.

and reducing the recurrence rate. In YXSL, *S. flavescens* clears heat and dries dampness, kills insects and diureses, and mainly contains a variety of alkaloids, flavonoids, quinones, and triterpenoid saponins[8]; *Cnidium* kills insects and relieves itching, dispels wind and dampness, warms kidney and strengthens yang, and mainly contains volatile oils, coumarin, and other ingredients[9]; *Epimedium* warms the kidneys and aphrodisiacs, strengthens muscles and bones, and dispels rheumatism, mainly contains flavonoid glycosides, sterols, polysaccharides and other chemical components[10]; *Cortex Phellodendri* can clear heat and dry dampness, purify fire and detoxify, and relieve asthenia heat, and it mainly contains various alkaloids such as berberine,

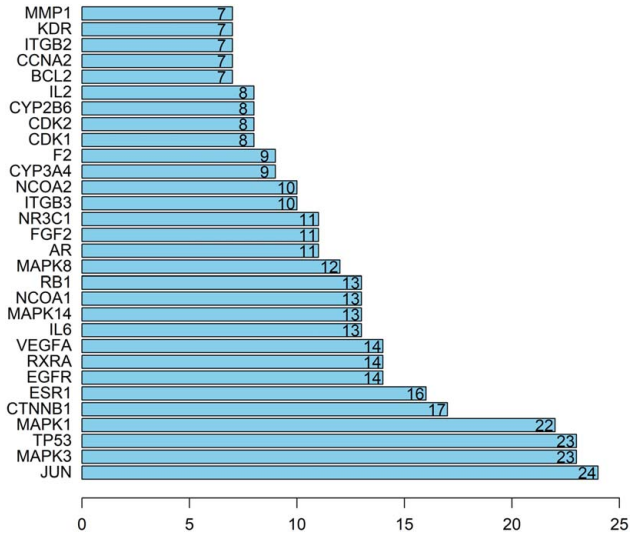

Figure 5. Ranking of core targets based on topological analysis of protein–protein interaction (top 30).

lactones, and other chemical components[11]; *Xanthium* expelling wind, removing dampness and relieving pain, mainly containing xanthin, xantholin, and amino acids[12]. The formula has good compatibility and functions of clearing away heat and dampness, killing insects, and relieving itching.

In this study, the mechanism of action of YXSL in the treatment of vaginitis was investigated based on the network pharmacology method and experimental verification. The topological analysis of the chemical component–target network revealed that the main active components were quercetin, β-sitosterol, kaempferol, apigenin, and luteolin.

Luteolin is a flavonoid with various pharmacological activities such as anticancer, anti-inflammatory, antioxidant, and immunomodulatory[13]. Studies have shown that low-dose luteolin can inhibit the activation of NLRP3 inflammasome and reduce M1 macrophages markers (expression of TNF-α, IL-6, and iNOS), enhance expression of M2 macrophage markers (Arg-1 and IL-10), confirming the anti-inflammatory activity of luteolin. Luteolin can effectively improve lipopolysaccharide-induced myocardial injury in mice, attenuating mitochondrial damage and inflammatory response, and reducing oxidative stress[14,15]. β-sitosterol has a variety of beneficial effects on the human body. Studies have shown that β-sitosterol has various physiological activities such as anti-inflammatory, antibacterial, antioxidant, and immune regulation. It is used in various clinical diseases, such as skin diseases and immune diseases[16,17]. Apigenin can downregulate the expression of cytokines such as tumor necrosis factor TNF-α and IL-6 ($P < 0.05$) by inhibiting

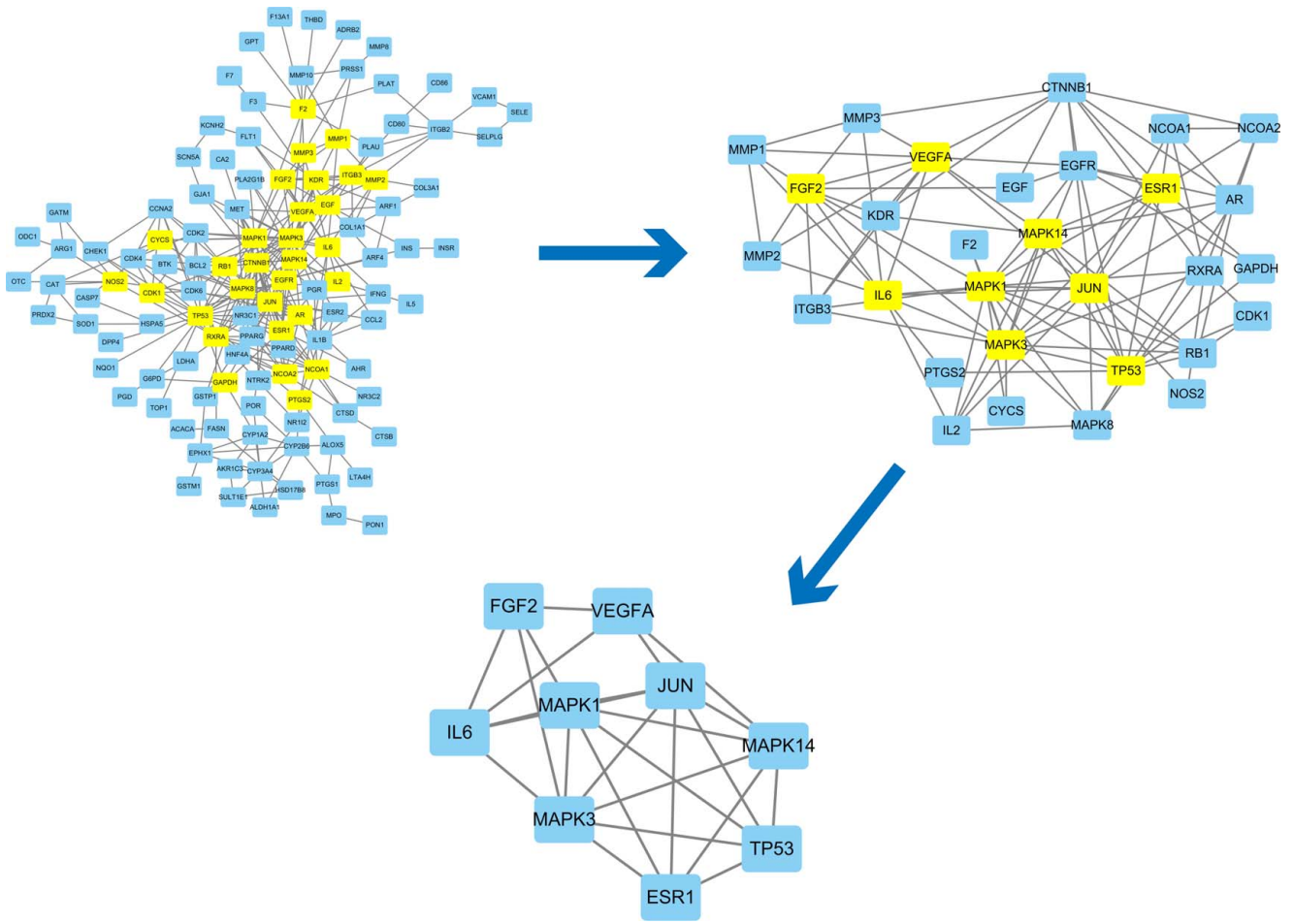

Figure 6. Topological analysis to screen core genes.

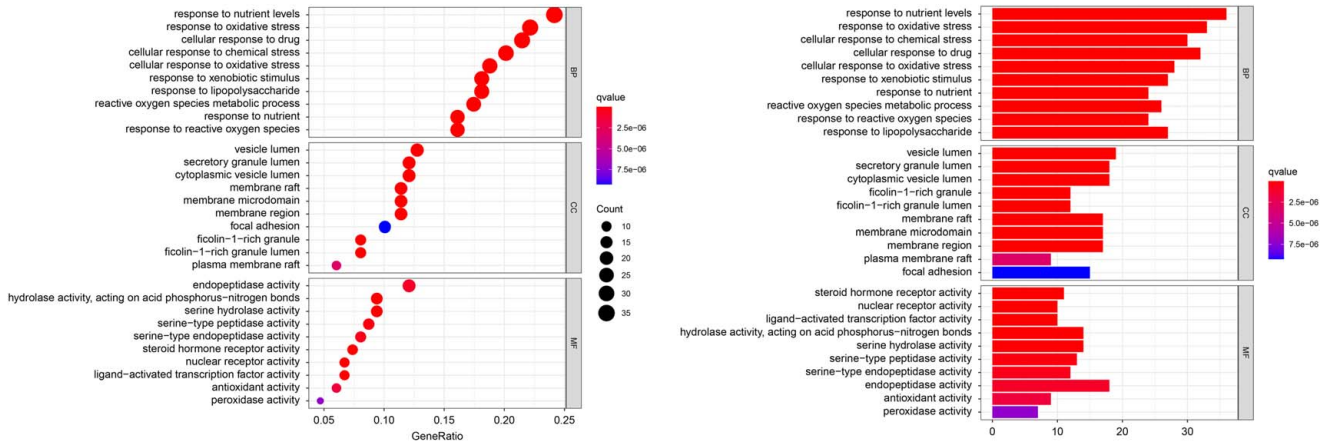

Figure 7. Enrichment analysis of Gene Ontology function of Yixishu lotion.

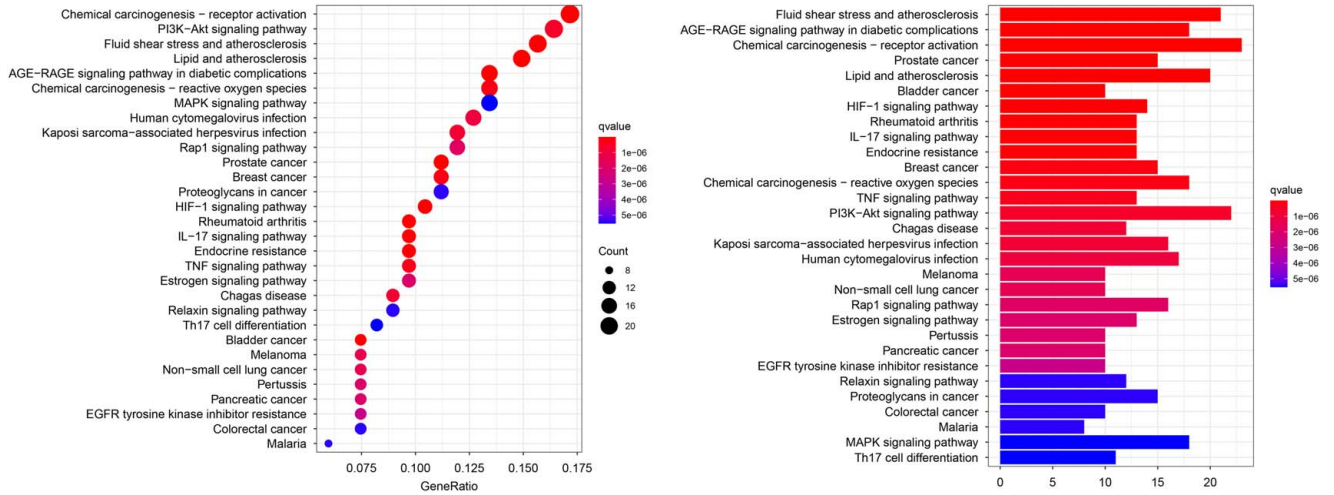

Figure 8. Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes pathway of Yixishu lotion.

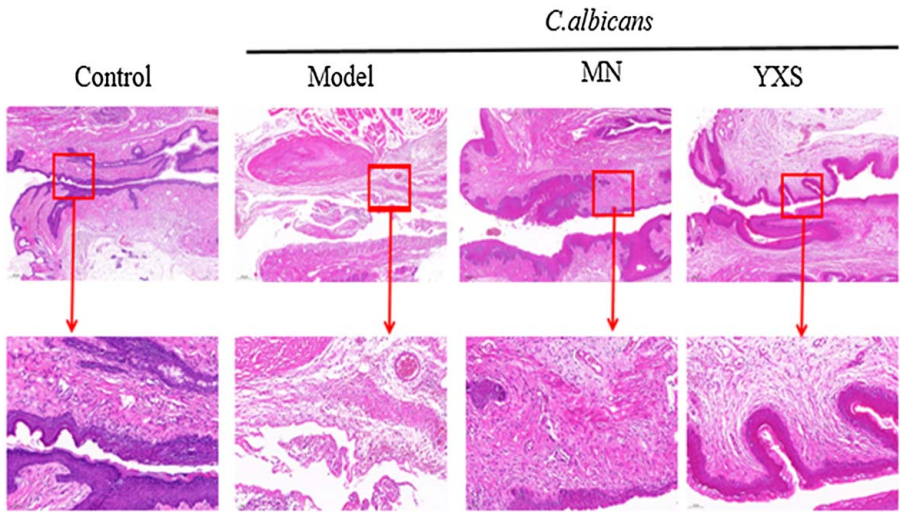

Figure 9. The protection of Yixishu lotion (YXSL) against inflammation in the vaginal. YXSL reduced the inflammatory cell infiltration of the vagina of mice with vulvovaginal candidiasis detected by H&E (hematoxylin–eosin) staining ($n = 8$) (50×, 200×).

Table 2

The effects of YXSL on relevant detection factors levels in serum mice with vulvovaginal candidiasis.

Sample	Control	Model	MN	YXSL
MDA (mmol/ml	004.28 ± 0.54	008.46 ± 0.828**	006.00 ± 0.62##	000.65 ± 0.57##
SOD (U/ml)	063.50 ± 2.64	050.89 ± 2.30**	057.51 ± 2.55##	056.24 ± 1.73##
TNF-α (pg/l	141.19 ± 4.83	163.76 ± 5.53**	151.00 ± 3.18##	156.85 ± 5. 75#
IL-6 (pg/l)	015.57 ± 1.42	029.63 ± 1.74**	023.75 ± 2.21##	024.72 ± 2.34##
IL-4 (pg/ml)	021.51 ± 1.18	118.54 ± 6.61**	070.69 ± 5.51##	074.62 ± 6.50##
INF-γ (pg/ml)	070.28 ± 5.30	135.20 ± 11.00**	092.32 ± 7.62##	096.42 ± 6.03##

Date were expressed as mean ± SEM ($n = 6$).
**$P < 0.01$ versus control mice.
#$P < 0.05$.
##$P < 0.01$ versus model mice.
MN, miconazole nitrate; YXSL, Yixishu lotion.

IKKβ–IκB–P65 signaling and exerting strong anti-inflammatory activity. Apigenin and flavonoids, which have the inhibitory effect of IKKβ, the upstream regulator of the NF-κB inflammatory signaling pathway, can also activate the Nrf2–ARE antioxidant signaling pathway and jointly exert anti-inflammatory/anti-oxidative activity[18]. The five traditional Chinese medicines in the prescription contain the main active ingredients separately or together, which reflects from the microscopic aspect that the combination of various traditional Chinese medicines can play a role in the treatment of vaginitis in various ways such as anti-oxidation, anti-inflammatory, immune regulation, and alleviating the toxicity of mycotoxins effect.

Through PPI network topology analysis, it can be seen that MAPK14, TP53, FGF2, ESR1, MAPK3, MAPK1, VEGFA, JUN, IL-6, and other targets are the relevant targets of YXSL in the treatment of vaginitis, which are closely related. Mitogen-activated protein kinase (MAPK) is a kind of intracellular serine/threonine protein kinase that plays a key role in cell proliferation, inflammation, immunity, oxidative stress, etc.[19,20]. As a key molecule of the p38MAPK signaling pathway, MAPK14 is closely related to the decline of immunity, inflammation, and oxidative stress caused by body dysfunction[20,21]. Fibroblast growth factor 2 (FGF2) plays an important role in regulating the inflammatory response in inflammatory diseases[22]. NTP53 gene mutation is closely related to cancer lesions. In addition, the expression of TP53 protein can promote the occurrence of the inflammatory response[23]. IL-6 is a cytokine of the innate immune system. It is an interleukin secreted by T cells and leukocytes. It plays an important role in tissue recovery, regulation of B cells, and participation in inflammatory responses. IL-6 is one of the main targets of therapeutic chronic inflammatory diseases[24]. JUN is mainly involved in cell proliferation and immune-related signaling pathways[25]. MAPK1 not only promotes the expression of inflammatory factors but also plays an important role in regulating the immune response[26]. In conclusion, the therapeutic effect of YXSL may be achieved by regulating inflammation, antioxidant and immune-related targets. The GO and KEGG analysis revealed that YXSL may affect signaling pathways such as inflammatory response and oxidative stress, so it may have side effects such as episodic allergic reactions.

KEGG signaling pathway mainly involves MAPK signaling pathway, Th17 cell differentiation signaling pathway, relaxin signaling pathway, TNF signaling pathway, etc. Th17 cell differentiation signaling pathway is a signaling pathway related to mediating cell proliferation, differentiation, inflammation development, and immune response[27]. The MAPK signaling pathway is involved in inflammation, oxidative stress, cell differentiation, and apoptosis and is a common pathway for cellular information transmission[20,28]. The TNF signaling pathway is involved in related pathways such as inflammation and immunity and can also promote the expression of proinflammatory factors and participate in inflammatory responses[29]. Relaxin can cause anti-inflammatory effects in a variety of conditions and also inhibit the influx of inflammatory cells, such as neutrophils and mast cells, into damaged organs[30]. It can be speculated that YXSL can restore the health of patients by inhibiting inflammatory responses, regulating immune function, and anti-oxidation.

Conclusion

This study applied a network pharmacology approach to explore the complex network relationship between YXSL and vaginitis. By constructing mice with VVC and measuring the levels of MDA, SOD, TNF-α, IL-6, IL-4, and IFN-γ, It is preliminarily verified that YXSL can relieve clinical symptoms and play a therapeutic role by enhancing antioxidant capacity, anti-inflammatory, and improving immunity. YXSL lotion is a topical preparation and should not be taken orally. It should not be used with soap and other detergents, and pregnant women and children need to follow medical advice. We recommend that the duration of treatment is approximately 10 days.

Ethical approval

The animal study was reviewed and approved by the Animal Ethics Committee of Shijiazhuang Fourth Hospital.

Consent

Not applicable.

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Author contribution

W.H. and M.F.: provided the experimental design; W.H. and J.Z.: performed the experiments; R.W. and S.T.: analyzed the data; Q.L. and S.G.: prepared all figures; W.H., Z.J., and M.F.: wrote the draft of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest disclosure

The authors declare that they have no conflicts of interest.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

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Provenance and peer review

Not commissioned, externally peer-reviewed.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

References

[1] Zhou R, Zhang YQ, *et al*. The pathogenic bacteria classification and age distribution of 445 female patients with vaginitis. Chin J Hum Sex 2016; 9:57–8.
[2] Ru J, Li P, Wang J, *et al*. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform 2014;6:13.
[3] Rebhan M, Prilusky J. Rapid access to biomedical knowledge with GeneCards and HotMolecBase: implications for the electrophoretic analysis of large sets of gene products. Electrophoresis 1997;18:2774–80.
[4] Cai X, Kong F, Wang R, *et al*. *Candida albicans* vaginitis in a murine model is reduced by polypeptide-enriched *Gastrodia elata* extracts. Future Microbiol 2019;14:839–46.
[5] Tan SP, Liao J, *et al*. The expression of IGFBP7 in Balb/c mouse model of candida vaginitis and its significance. Chin J Dermatovenereol 2010;24:203–6.
[6] Kilkenny C, Browne WJ, Cuthill IC, *et al*. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 2010;8:e1000412.
[7] de Santi MESO, Prates RA, França CM, *et al*. Antimicrobial photodynamic therapy as a new approach for the treatment of vulvovaginal candidiasis: preliminary results. Lasers Med Sci 2018;33:1925–31.
[8] Dong Y, Jia G, Hu J, *et al*. Determination of alkaloids and flavonoids in *Sophora flavescens* by UHPLC-Q-TOF/MS. J Anal Methods Chem 2021; 2021:9915027.
[9] Qiu Q, Cui Z, Liu T, *et al*. Determination of chemical constituents of the essential oil from *Cnidium monnieri* by GC–MS. Zhong Yao Cai 2002; 25:561–3.
[10] Li N, Xie L, Yang N, *et al*. Rapid classification and identification of chemical constituents in Epimedium koreanum Nakai by UPLC-Q–TOF–MS combined with data post-processing techniques. Phytochem Anal 2021;32:575–91.
[11] Li Y, Zhang T, Zhang X, *et al*. Chemical fingerprint analysis of Phellodendri Amurensis Cortex by ultra performance LC/Q–TOF–MS methods combined with chemometrics. J Sep Sci 2010;33:3347–53.
[12] Hu YM, Su GH, Sze SC, *et al*. Quality assessment of Cortex Phellodendri by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry. Biomed Chromatogr 2010;24:438–53.
[13] Gendrisch F, Esser PR, Schempp CM, *et al*. Luteolin as a modulator of skin aging and inflammation. Biofactors 2021;47:170–80.
[14] Zhang BC, Li Z, Xu W, *et al*. Luteolin alleviates NLRP3 inflammasome activation and directs macrophage polarization in lipopolysaccharide-stimulated RAW264.7 cells. Am J Transl Res 2018;10:265–73.
[15] Wu B, Song H, Fan M, *et al*. Luteolin attenuates sepsis-induced myocardial injury by enhancing autophagy in mice. Int J Mol Med 2020;45: 1477–87.
[16] Desai AJ, Dong M, Miller LJ. Beneficial effects of β-sitosterol on type 1 cholecystokinin receptor dysfunction induced by elevated membrane cholesterol. Clin Nutr 2016;35:1374–9.
[17] Le CF, Kailaivasan TH, Chow SC, *et al*. Phytosterols isolated from *Clinacanthus nutans* induce immunosuppressive activity in murine cells. Int Immunopharmacol 2017;44:203–10.
[18] Nile SH, Keum YS, Nile AS, *et al*. Antioxidant, anti-inflammatory, and enzyme inhibitory activity of natural plant flavonoids and their synthesized derivatives. J Biochem Mol Toxicol 2018;32.
[19] Bost F, Aouadi M, Caron L, *et al*. The role of MAPKs in adipocyte differentiation and obesity. Biochimie 2005;87:51–6.
[20] Tabaa MME, Aboalazm HM, Shaalan M, *et al*. Silymarin constrains diacetyl-prompted oxidative stress and neuroinflammation in rats: involvements of Dyn/GDNF and MAPK signaling pathway. Inflammopharmacology 2022;30:961–80.
[21] Shah NG, Tulapurkar ME, Ramarathnam A, *et al*. Novel noncatalytic substrate-selective p38α-specific MAPK inhibitors with endothelial-stabilizing and anti-inflammatory activity. J Immunol 2017;198: 3296–306.
[22] Schmidt MO, Garman KA, Lee YG, *et al*. German Mouse Clinic Consortium. The role of fibroblast growth factor-binding protein 1 in skin carcinogenesis and inflammation. J Invest Dermatol 2018;138: 179–88.
[23] Ham SW, Jeon HY, Jin X, *et al*. TP53 gain-of-function mutation promotes inflammation in glioblastoma. Cell Death Differ 2019;26: 409–25.
[24] Hirano T. IL-6 in inflammation, autoimmunity and cancer. Int Immunol 2021;33:127–48.
[25] Cui L, Chen SY, Lerbs T, *et al*. Activation of JUN in fibroblasts promotes pro-fibrotic programme and modulates protective immunity. Nat Commun 2020;11:2795.
[26] Aihaiti Y, Song Cai Y, Tuerhong X, *et al*. Therapeutic effects of naringin in rheumatoid arthritis: network pharmacology and experimental validation. Front Pharmacol 2021;12:672054.
[27] Gorczynski RM. IL-17 signaling in the tumor microenvironment. Adv Exp Med Biol 2020;1240:47–58.
[28] Koul HK, Pal M, Koul S. Role of p38 MAP kinase signal transduction in solid tumors. Genes Cancer 2013;4:342–59.
[29] Moura J, Sørensen A, Leal EC, *et al*. microRNA-155 inhibition restores Fibroblast Growth Factor 7 expression in diabetic skin and decreases wound inflammation. Sci Rep 2019;9:5836.
[30] Masini E, Nistri S, Vannacci A, *et al*. Relaxin inhibits the activation of human neutrophils: involvement of the nitric oxide pathway. Endocrinology 2004;145:1106–12.