Based on Network Pharmacology and Molecular Docking Technology, the Mechanism of Folium Sennae, Aloe Combined with Panax Quinquifolium in Treating Functional Constipation Was Investigated

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Abstract

Objective: To explore the mechanism of folium sennae, aloe combined with panax quinquefolium in treating functional constipation (FC) based on network pharmacology and molecular docking method. **Methods:** Retrieval of all chemical components and action targets of folium sennae, aloe combined with panax quinquefolium using Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (tcmsp). Relevant targets for functional constipation were accessed through Gencards, OMIM database, and first-line Western drug targets for the treatment of FC were searched through the drugbank database as a supplement. The STRING data platform was used to construct the interaction network of potential target proteins. Meanwhile, GO (gene ontology) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) signaling pathway data were obtained, and the mechanism of action was analyzed and predicted. The PPI network topology was analyzed by Cytoscape 3.8.0 software, and the network diagram of “component-target-pathway” of FC in the three-drug combination treatment was constructed. Autodock Vina was used for molecular docking to further verify the accuracy of network pharmacological analysis of folium sennae, aloe combined with panax quinquefolium in the treatment of functional constipation. **Results:** The core active compounds of folium sennae, aloe combined with panax quinquefolium for the treatment of functional constipation are quercetin, kaempferol, β -Carotene and ginsenoside F2, and the core targets include PTGS2, MAPK1, Jun, VEGFA, IL6, etc. KEGG pathway enrichment analysis showed that the key targets of folium sennae, aloe combined with panax quinquefolium in the treatment of FC were mainly enriched in TNF signaling pathway, IL-17 signaling pathway, VEGF signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, etc. Molecular docking results verified that the average value of binding energy for each compound docking to the core target was less than -5kcal/mol with a good binding activity. **Conclusion:** This study preliminarily revealed the synergistic effect of panax quinquefolium combined with folium sennae and aloe in the treatment of function-

al constipation, and also showed that the combination use of three drugs have the characteristics of multi-component, multi-target, and multi pathway, which laid a foundation for further study of its mechanism of action.

Keywords

Folium Sennae, Aloe, Panax Quinquifolium, Functional Constipation, Network Pharmacology

1. Introduction

Functional constipation (FC) is a kind of constipation caused by exclusion of secondary causes, such as neurogenesis, systemic diseases, intestinal organic lesions and drugs, etc. [1]. According to the diagnostic criteria of Rome IV functional constipation, the symptoms must include two or more of the following: (1) More than 1/4 of the stool or weeks of difficult defecation. (2) dry and hard defecation. (3) feeling of unfulfilled defecation. (4) feeling of anorectal blockage during defecation. (5) need manipulative assisted defecation. (6) defecation less than 3 times per week [2]. In recent years, epidemic epidemiology shows that the total prevalence of functional constipation in children and adults is about 9.5% [3], and the prevalence rate in adults is 14%, with more women than men [4]. FC is a serious threat to human physical and mental health. Long-term constipation can cause anorectal diseases, gastrointestinal nerve dysfunction, induce and aggravate various cardiovascular and cerebrovascular diseases, and even induce colorectal cancer, hepatic encephalopathy, etc. [5]. The pathogenesis of FC is mainly related to colorectal motility disorders, reduced pelvic floor function, psychosocial factors and abnormal gastrointestinal regulation function [6]. Currently, treatment methods for FC include drug therapy, traditional Chinese medicine technology therapy, enema therapy, biofeedback therapy and surgical therapy [7]. Drug therapy is the most commonly used method in clinical practice, including western medicine, Traditional Chinese medicine and Chinese patent medicine.

Traditional Chinese medicine plays an irreplaceable role in the treatment of functional constipation. Purgative drugs represented by folium sennae and aloe are frequently used in clinical practice. Both folium sennae and aloe are bitter and cold, and both belong to the large intestine meridian, which has laxative efficacy and can be used in the syndrome of heat accumulation, constipation and abdominal pain [8]. However, folium sennae and aloe are the products for relieving the bitter cold, so it is not suitable to be taken for a long time. Taking more than one dose will easily damage the healthy qi and Yin, and easily cause dependence. Panax quinquefolium replenishes qi and Yin, clearing heat and producing fluid [8], and can counteract the side effects of folium sennae and aloe, such as damaging the spleen and stomach due to the bitter cold. The combined use of the three drugs can improve intestinal peristalsis and promote the repair of colonic neurons [9], and at the same time can improve the ecological balance in the intestinal tract and restore the homeostasis between various bacterial communities [10]. Yet studies have reported the mechanism of action of three drugs used in combination treatment of functional constipation, this study based on the network pharmacology method [11] predict senna joint panax quinquefolium, aloe ingredient for FC and mechanism of action, and will receive compounds and key target protein, to verify that the network pharmacology method [12] the accuracy of the results, so as to provide clues for the research and development of new Chinese medicine.

2. Materials and methods

2.1 Collection of active components and target proteins from folium sennae, aloe and panax quinquefolium

Using Chinese medicine pharmacology system analysis platform (TCMSP, <https://tcmsp.com/tcmsp.php>) [13] retrieve senna, aloe and panax quinquefolium compounds. OB (Oral bioavailability) $\geq 30\%$ and DL (drug like) ≥ 0.18 were used as the screening conditions for active ingredients, and the effective compound targets were searched through TCMSP database. Use the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) [14] query has not been included in the TCMSP target compounds in 2D charts, and in SwissTargetPrediction online data platform (<http://www.swisstargetprediction.ch/>) using the 2D structure predict target compounds. All filtered targets were collected and standardized with the help of Uniprot data platform (<https://www.uniprot.org/>) [15].

2.2 Predictive targets of functional constipation

With “functional constipation” as keywords in GeneCards (<https://www.genecards.org>) and OMIM database (<https://omim.org>), retrieve the FC may target, The Drugbank database (<https://go.drugbank.com/>) was used to obtain first-line western drug targets for treatment FC as a supplement [16]. The total target of the disease was identified by combining all targets and removing duplicates.

2.3 Construction of protein interaction network

Folium sennae, aloe, panax quinquefolium and FC targets to upload Venny 2.1.0 platform (<https://bioinfo.gp.cnb.csic.es/tools/venny/>) [17], get targets the intersection of targets of drug targets and disease, three drug combination therapy is the potential targets for FC, the Venn diagram of the relationship between disease targets and drug targets was constructed. Then, the intersection target was uploaded to the String11.0 database (<https://string-db.org/>) [18], and the species was set as “human” to obtain the protein interaction network. The plug-in cytoNCA of CytoScape3.8.0 was used to analyze the PPI network topology, including degree centrality (DC), betweenness centrality (BC), and eigenvector centrality (EC) and closeness centrality (CC). Firstly, the median of DC nodes is calculated, and all nodes greater than twice the value are screened. Then, the nodes greater than the median value of several other index nodes are screened, which are the key nodes. Then, the String data platform was used to construct the protein interaction network model for the selected key nodes. Then, Cytoscape3.8.0 software was used to perform visual analysis on the protein protein interaction network (PPI network) obtained, and gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway data were obtained.

2.4 Target pathway analysis and visualization

Has a comprehensive comment function Metascape platform (<http://metascape.org/gp/index.html>), which is not only powerful and update monthly [19], using this data platform, enrichment analysis of GO biological process and enrichment analysis of KEGG pathway was performed for key targets of FC treatment of folium sennae, aloe combined with panax quinquefolium ($P < 0.01$). Finally using bioinformatics platform (<http://www.bioinformatics.com.cn/>) [20] with the result of data visualization.

2.5 Construct the network diagram of “component-target-pathway”

Cytoscape 3.8.0 software and its built-in tools were used to analyze network topology parameters such as Degree, Betweenness and Closeness, and to judge the core target and main active ingredients of drug action according to these parameters. At the same time, the network diagram of “component-target-pathway” was constructed.

2.6 Composition-target molecular docking

The top 4 targets ranked in degree in the network graph of “component-target-pathway” in the treatment of FC by folium sennae, aloe combined with panax quinquefolium were verified by molecular docking with all the compounds. The three-dimensional structures of target proteins and compounds were obtained from Protein Data Bank (PDB) database (<https://www1.rcsb.org/>) and TCMSP database. Then AutoDockTools 1.5.6 (<http://vina.scripps.edu/>) was used to convert it to PDBQT format [21], and finally Autodock Vina 1.1.2 was used for molecular docking. In order to increase the accuracy of calculation, the parameter exhaustiveness is set to 10, the number of connection modes is 20, and the binding energy range is 4. Retain the default values for other parameters. The docking binding energies of 5 proteins and 25 active ingredients were obtained, and the binding strength and activity of the target and compound were evaluated numerically according to the binding energies. The docking results were visually analyzed by pymol 2.4.0 [22].

3. Results

3.1 Collection and screening of components of folium sennae, aloe and panax quinquefolium and their corresponding targets

Preliminary extraction of chemical components in folium sennae, aloe and panax quinquefolium were 53, 53 and 153, respectively. After screening, 8 active components in folium sennae, 8 in aloe and 10 in Panax quinquefolium were obtained, including quercetin, kaempferol and ginsenoside, as shown in Table 1. Then the target proteins of the above active compounds were obtained through TCMSP database and SwissTargetPrediction online data platform. Finally, 95, 90 and 63 targets of the active components of folium sennae, aloe and panax quinquinus were

obtained, and 177 targets were obtained by deleting the duplicate value after combination.

Table 1. Main components of folium sennae, aloe and panax quinquefolium

ID	Mol ID	Molecule Name	OB (%)	DL	Source
MOL1	MOL000359	sitosterol	36.91	0.75	folium sennae and aloe
MOL2	MOL000422	kaempferol	41.88	0.24	folium sennae
MOL3	MOL000449	Stigmasterol	43.83	0.76	folium sennae
MOL4	MOL002259	Physciondiglucoside	41.65	0.63	folium sennae
MOL5	MOL002268	rhein	47.07	0.28	folium sennae
MOL6	MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.8	folium sennae
MOL7	MOL002293	Sennoside D_qt	61.06	0.61	folium sennae
MOL8	MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene	33.55	0.42	folium sennae
MOL9	MOL000098	quercetin	46.43	0.28	aloe
MOL10	MOL000471	aloe-emodin	83.38	0.24	aloe
MOL11	MOL000953	CLR	37.87	0.68	aloe
MOL12	MOL001439	arachidonic acid	45.57	0.2	aloe
MOL13	MOL002773	beta-carotene	37.18	0.58	aloe
MOL14	MOL005043	campest-5-en-3beta-ol	37.58	0.71	aloe
MOL15	MOL005051	Aloeresin C	34.99	0.5	aloe
MOL16	MOL000358	beta-sitosterol	36.91	0.75	panax quinquefolium
MOL17	MOL005344	ginsenoside rh2	36.32	0.56	panax quinquefolium
MOL18	MOL006774	stigmast-7-enol	37.42	0.75	panax quinquefolium
MOL19	MOL006980	papaverine	64.04	0.38	panax quinquefolium
MOL20	MOL008173	daucosterol_qt	36.91	0.75	panax quinquefolium
MOL21	MOL008397	Daturilin	50.37	0.77	panax quinquefolium
MOL22	MOL011394	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-[[[(3S,5R,8R,9R,10R,12R,13R,14R,17S)-12-hydroxy-4,4,8,10,14-pentamethyl-17-[(2S)-6-methyl-2-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyhept-5-en-2-yl]-2,3,5,6,7,9,11,12,13,15,16,17-dodecahydro-1H-c	36.43	0.25	panax quinquefolium
MOL23	MOL011435	PQ-2	36.74	0.19	panax quinquefolium
MOL24	MOL011442	(8S,9S,10R,13R,14S,17R)-17-[(1R,4R)-4-ethyl-1,5-dimethylhex-yl]-10,13-dimethyl-1,2,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthren-7-one	43.87	0.75	panax quinquefolium
MOL25	MOL011455	20-Hexadecanoylingenol	32.7	0.65	panax quinquefolium

3.2 Acquisition of FC related targets

A total of 5,248 functional constipation targets were obtained from Genecards, 25 from OMIM, and 27 from DrugBank database, respectively. Combined with the removal of repeated targets, a total of 5,280 FC related targets were obtained.

3.3 Establishment and analysis of PPI network

The screened targets and FC targets of senna, aloe and panax quinquinus were introduced into Venny2.1.0 for analysis, and the Venn diagram was drawn (see Figure 1). 133 intersecting targets of drug targets and disease targets were obtained, of which 12 were common targets of the three drugs, and also the similarities of FC mechanisms of

the three drugs. 34 were common targets of senna and aloe, which was consistent with the characteristics of compatibility and synergistic application of the two. 27 are the targets of senna leaf acting on FC alone, 38 are the targets of aloe vera acting on FC alone, and 21 are the targets of American ginseng acting on FC alone. The combined use of the three drugs expanded the range of targets for the treatment of the disease, and played a synergistic role.

The STRING 11.0 database was used to analyze the interactions between 133 intersecting targets, and topological analysis was performed using Cytoscape software plug-in cytoNCA. The median values of DC, BC, EC and CC were calculated to be 13, 59.137 21, 0.043 51 and 0.458 33, respectively. Based on the 1.3 screening criteria, 31 core targets were obtained, imported into the STRING database, and the results were clarified using Cytoscape software (Figure 2). The closer the target is to the center of concentric circles, the stronger the interaction and the deeper the color. The 14 innermost target proteins, PTGS2, MAPK1, JUN, VEGFA, IL6, TP53 and TNF, are the core action targets of senna leaf and aloe combined with American ginseng in the treatment of FC.

Figure 1. Venny analysis of targets of folium sennae, aloe and panax quinquefolium and functional constipation.

Figure 2. PPI network of folium sennae, aloe combined with panax quinquefolium for FC treatment.

3.4 Analysis of enrichment results of GO and KEGG pathways

Metascape data platform enrichment analysis results showed that 31 core targets of senna and aloe combined with American ginseng in FC treatment were enriched and 201 pathways were obtained, which were involved in 907 biological processes, 53 molecular functions and 29 cell components. The top biological processes in the screening list are: positive regulation of cell migration, cell response to oxidative stress, epithelial cell proliferation, blood vessel development, positive regulation of pri-miRNA transcription by RNA polymerase II, etc., as shown in Figure 3(A). In terms of molecular functions, it mainly involves nuclear receptor activity, cytokine activity, phosphatase binding, integrin binding, serine-type endopeptidase activity, protein domain specific binding, etc., as shown in Figure 3(B). In terms of cell components, it mainly involves: vesicle lumen, membrane raft, RNA polymerase II transcription factor complex, extracellular matrix, mitochondrial matrix, receptor complex, etc., as shown in Figure 3(C).

The enrichment results of KEGG pathway showed that tumors, blood vessels, inflammation, endocrine and other aspects were mainly involved, as shown in Figure 3(D). Including TNF signaling pathway, VEGF signaling pathway, IL-17 signaling pathway, Prolactin signaling Pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, NF- κ B signaling pathway, and the enrichment results of target pathways were shown in Table 2.

Figure 3. Enrichment analysis of core targets in folium sennae, aloe and panax quinquefolium.

Table 2. Enrichment results of target pathway of folium sennae, aloe combined with panax quinquefolium in the treatment of FC

GO	Description	Count	Log10(P)	Target
hsa05200	Pathways in cancer	20	-26.08	AR, BCL2L1, EGF, EGFR, ESR1, FGF2, HMOX1, HSP90AA1, IL2, IL6, JUN, MMP1, MMP2, PPARG, MAPK1, MAPK8, PTGS2, STAT3, TP53, VEGFA
ko05418	Fluid shear stress and atherosclerosis	12	-19.66	HMOX1, HSP90AA1, IL1B, JUN, KDR, MMP2, MAPK8, CCL2, TNF, TP53, VCAM1, VEGFA
ko04657	IL-17 signaling pathway	11	-19.65	HSP90AA1, IL1B, IL6, JUN, MMP1, MMP3, MAPK1, MAPK8, PTGS2, CCL2, TNF
hsa05205	Proteoglycans in cancer	12	-17.37	EGFR, ESR1, FGF2, IL6, KDR, MMP2, PLAUI, MAPK1, STAT3, TNF, TP53, VEGFA
hsa01521	EGFR tyrosine kinase inhibitor resistance	9	-15.89	BCL2L1, EGF, EGFR, FGF2, IL6, KDR, MAPK1, STAT3, VEGFA
ko04659	Th17 cell differentiation	8	-12.61	HSP90AA1, IL1B, IL2, IL6, JUN, MAPK1, MAPK8, STAT3
hsa05167	kaposi sarcoma-associated herpesvirus infection	9	-12.29	FGF2, IL6, JUN, MAPK1, MAPK8, PTGS2, STAT3, TP53, VEGFA
ko05202	Transcriptional misregulation in cancer	8	-10.78	BCL2L1, ELANE, IL6, MMP3, MPO, PLAUI, PPARG, TP53
ko04064	NF-kappa B signaling pathway	6	-9.06	BCL2L1, IL1B, PLAUI, PTGS2, TNF, VCAM1
hsa05225	hepatocellular carcinoma	6	-7.36	BCL2L1, EGFR, HMOX1, IL6, MAPK1, TP53
ko04370	VEGF signaling pathway	4	-6.28	KDR, MAPK1, PTGS2, VEGFA
ko04917	Prolactin signaling pathway	4	-5.98	ESR1, MAPK1, MAPK8, STAT3

3.5 Construction of the composite-target-pathway network diagram

CytoScape 3.8.0 software and its built-in tool Network Analyzer were used to analyze the network topology parameters of FC treated by folium sennae, aloe combined with panax quinquefolium, and a “component-target-pathway” network diagram was constructed, as shown in Figure 4. According to the results of network topology parameters, quercetin is predicted to be the primary active ingredient in the three-drug combination treatment of FC, its Degree is 22, the Betweenness Centrality is 0.2176, and the Closeness Centrality is 0.5377. Kaempferol, beta-carotene and ginsenoside F2 are the secondary active ingredients, as shown in Table 3.

The Degree of PTGS2 in the network was 16, the Betweenness Centrality was 0.1911, and the Closeness Centrality was 0.5089. PTGS2 was predicted to be the main target of folium sennae, aloe combined with panax quinquefolium in the treatment of FC, and MAPK1, JUN, VEGFA, IL6, TNF, MAPK8, STAT3, IL1B and so on were also relatively important targets, as shown in Table 2.

3.6 Molecular docking verification

The top 4 targets in FC “component-target-pathway” network of folium sennae, aloe combined with panax quinquefolium were selected: PTGS2 (PDB ID:5IKR), MAPK1 (PDB ID:6SLG), JUN (PDB ID:5FV8), VEGFA (PDB ID:4QAF), IL6 (PDB ID:1ALU) were validated by molecular docking with 25 selected active compounds. The results were shown in Table 4. The lower the binding energy of the ligand and the receptor is, the greater the possibility of interaction is, and the more stable the conformation is. When the binding energy is < -5kcal/mol, the

binding activity is good; when the binding energy is $< -7\text{kcal/mol}$, the binding activity is strong [23]. The average binding energies of PTGS2, MAPK1, JUN, VEGFA and IL6 with the selected 25 active compounds were less than -5kcal/mol , indicating good binding activity. The top 3 active ingredients with medium value in FC “component-target-pathway” network graph of folium sennae, aloe combined with panax quinquinae were selected: quercetin (MOL9), kaempferol (MOL2), beta-carotene (MOL13) and ginsenoside F2 (MOL22) for molecular interconnection image display and analysis, as shown in Figure 5.

Figure 4. Network diagram of “component-target-pathway” of FC treated by folium sennae, aloe combined with panax quinquifolium.

Table 3. Characteristic parameters of network nodes of main active components in folium sennae, aloe and panax quinquifolium

MOL ID	Molecule Name	Degree	Betweenness Centrality	Closeness Centrality
MOL000098	quercetin	22	0.217505349	0.537735849
MOL000422	kaempferol	9	0.024807572	0.413043478
MOL002773	beta-carotene	6	0.010748836	0.395833333
MOL011394	Ginsenoside F2	6	0.008696445	0.365384615
MOL000471	aloe-emodin	4	0.005073999	0.38
MOL001439	arachidonic acid	3	0.003556838	0.37012987

Figure Note: The compounds were shown in a stick model: quercetin in orange, kaempferol in purple, beta-carotene in green and ginsenoside F2 in pink; The protein complex is shown as a blue band model; The yellow dotted lines represent hydrogen bonds.

Figure 5. Schematic diagram of molecular docking.

Quercetin, kaempferol, beta-carotene and ginsenoside F2 had good binding activity with PTGS2, MAPK1, JUN, VEGFA and IL6, respectively. The average values of the binding energies were -7.4kcal/mol, -7.2kcal/mol, -8.8kcal/mol and -7.4kcal/mol, respectively. In its binding mode, quercetin, kaempferol and ginsenoside F2 had hydrogen bonding with amino acid residues in the five core target proteins, respectively, while beta-carotene did not have hydrogen bonding.

Table 4. The binding energy of active components from folium sennae, aloe and panax quinquefolium with key targets

ID	binding energy/(kcal·mol ⁻¹)					Average
	PTGS2	MAPK1	JUN	VEGFA	IL6	
MOL1	-8.0	-8.2	-6.9	-7.2	-7.5	-7.6
MOL2	-7.3	-7.9	-6.4	-7.4	-6.9	-7.2
MOL3	-7.5	-8.3	-6.8	-7.8	-6.6	-7.4
MOL4	-10.1	-9.5	-7.4	-8.4	-7.4	-8.6
MOL5	-7.8	-8.7	-6.4	-7.5	-6.8	-7.4
MOL6	-8.3	-9.4	-7.1	-8.0	-7.0	-8.3
MOL7	-8.5	-8.7	-8.1	-7.8	-7.7	-8.2
MOL8	-6.6	-7.2	-6.4	-6.6	-5.3	-6.4
MOL9	-8.1	-7.9	-6.4	-7.5	-6.9	-7.4
MOL10	-9.0	-8.3	-6.2	-6.9	-6.5	-7.0
MOL11	-7.2	-9.0	-6.6	-7.1	-6.9	-7.0
MOL12	-6.2	-5.6	-5.2	-6.6	-4.7	-5.5
MOL13	-10.0	-8.7	-8.3	-10.4	-7.7	-8.8
MOL14	-8.6	-9.6	-7.5	-8.0	-6.6	-8.1
MOL15	-8.0	-6.2	-6.7	-7.2	-6.8	-6.7
MOL16	-8.2	-9.0	-7.5	-7.8	-6.6	-7.5
MOL17	-8.3	-8.6	-7.5	-8.8	-7.3	-8.1
MOL18	-7.6	-8.2	-7.2	-7.4	-7.8	-7.6
MOL19	-8.4	-7.8	-6.1	-7.9	-6.0	-7.6
MOL20	-8.0	-8.0	-6.9	-7.0	-6.9	-6.9
MOL21	-8.7	-8.6	-8.0	-8.6	-7.5	-8.4
MOL22	-8.2	-7.6	-6.8	-7.3	-7.3	-7.4
MOL23	-5.0	-5.7	-5.0	-5.7	-4.4	-5.3
MOL24	-7.4	-9.2	-6.5	-6.5	-6.8	-7.3
MOL25	-7.4	-7.9	-6.4	-7.0	-6.8	-7.1

4. Discussion

The efficacy of senna and aloe combined with panax quinquefolium in the treatment of functional constipation has been widely recognized. Xu Zhenyuan et al. [24] found that panax quinquefolium could balance intestinal flora in mice with constipation model, promote intestinal peristalsis, improve intestinal motility, and improve intestinal slow transmission state. Panax quinquefolium has a good effect of clearing away heat and producing fluid. When used in proper amount with other purgative drugs, it can play a role of purging heat and defecating without hurting Yin fluid and assisting healthy qi to facilitate large intestine transmission, so it is suitable for patients with chronic constipation [25]. The combined treatment of three drugs for FC has a good laxative effect, its mechanism of action needs to be further explored.

In this study, 25 active components were screened based on the network pharmacology method, among which the core components were quercetin, kaempferol, beta-carotene, ginsenoside F2, etc. The gastrointestinal flora of

patients with constipation is in an unbalanced state, and various probiotics and strains may have beneficial effects on intestinal motility and constipation [26]. Zhang Jinwei et al. [27] found that quercetin can activate beneficial intestinal flora and inhibit harmful intestinal flora in mice, so as to protect intestinal mucosa and reduce the effects of cytokines on intestinal damage. AaLX et al. [28] showed that kaempferol can balance the intestinal flora and regulate microbial metabolism in mice by oral administration. Beta-carotene is A precursor of vitamin A [29]. Vitamin A-mediated regulation of intestinal epithelium and mucosal immune system jointly forms the intestinal microbiome to maintain the homeostasis of intestinal microbiome [30]. F2 for the original ginsenosides of ginseng glycol type saponins metabolites [31], yan-yan liu, et al. [32], the study found that ginseng saponins can increase mice intestinal fluorescent pseudomonas and butyric acid number of clostridium, in turn, promote the advantage bacterium proliferation, inhibit the growth of pathogenic bacteria in the gut of engraftment and thus improve the health level of mice. According to the literatures reviewed above, quercetin, kaempferol, beta-carotene, ginsenoside F2 and other active ingredients play a role in regulating constipation by regulating intestinal flora, promoting the growth of dominant flora and other mechanisms. Molecular docking validation results also showed that the above active ingredients had strong binding activities with the core targets (PTGS2, MAPK1, JUN, VEGFA, IL6). Therefore, the treatment of FC by folium sennae, aloe combined with panax quinquefolium may be mainly achieved by acting on quercetin, kaempferol, beta-carotene, ginsenoside F2 and other active ingredients.

The results showed that five core targets, PTGS2, MAPK1, JUN, VEGFA and IL6, played a major role in the network map. Prostaglandin (PG) H synthase exists as two subtypes, called PGH synthase -1 and -2, which are encoded by Ptgsl and Ptgsl2 genes and commonly known as cox-1 and COX-2 [33]. Studies have shown that COX-2 is involved in the occurrence of intestinal motor dysfunction after intestinal obstruction. When intestinal obstruction is proximal dilated, the contractility of periintestinal smooth muscle is significantly decreased, and the expression of COX-2 mRNA and protein is significantly up-regulated. The use of COX-2 inhibitor NS-398 can significantly improve the contractility of colonic smooth muscle cells in the model rats of colonic obstruction. Dilate the obstruction lumen [34]. MAPK is a complex pathway composed of multiple proteins [35], which can regulate the expression of AQP4 [36]. The expression of AQP3 and AQP4 in colon tissues of constipation model rats is significantly increased, which is also one of the mechanisms of STC. AQP3 and AQP4 can be down-regulated through MAPKs signaling pathway, thus reducing intestinal water reabsorption. Increase fecal water content [37]. C-jun is the main component of dimer transcription factor activator protein-1 (AP-1) [38], li Boqing et al. [39] demonstrated that the expression of AP-1 increased in the colon tissues of model mice with intestinal flora imbalance. VEGFA can relax the tight connections between intestinal cells, thus destroying the intestinal mucosal barrier and causing intestinal microorganisms to invade the intestinal wall [40]. IL-6 promotes intestinal mucosal inflammatory infiltration by affecting intestinal mucosal CD4 (+) T cells [41]. Exogenous IL-6 can directly promote colon contraction in rats, activate gastrointestinal submucosal neurons and participate in the regulation of gastrointestinal function, affect adrenal stress response and gastric peristalsis, and lead to gastrointestinal motility and secretion dysfunction [42]. These targets involve cell migration, oxidative stress, epithelial cell proliferation, vascular development, inflammation, reactive oxygen metabolism and other biological processes, including cytokines, proteins, enzymes, transcription factors, etc., and are the core substances of Senna, aloe vera combined with panax quinquefolium in the treatment of FC.

According to the enrichment analysis of KEGG pathway, senna and aloe combined with panax quinquefolium treated FC by regulating tumor necrosis factor, MAPK, PI3K-Akt, NF- κ B and other signaling pathways. Studies have shown that intrabdominal injection of TNF α in mice can significantly damage the cell network structure of Cajal mesenchymal cells [43], and Cajal mesenchymal cells are an important part of the gastrointestinal motility regulation unit and can promote intestinal transport [44]. Wan Ye min et al. [45] showed that regulating the expression of MPKA signaling pathway can increase intestinal water and promote intestinal peristalsis in model rats. PI3K/Akt signaling pathway plays an important role in signal transduction related to cell division, proliferation, movement, and metabolism [46], and participates in the pathogenesis of FC by affecting the function of distal colon tissue [47]. Jiang Z. C. and Huang S. X. [48] showed that the expression of NF- κ B protein and its mRNA in constipation model rats was significantly increased, indicating that NF- κ B signaling pathway is closely related to the pathogenesis of constipation. Other FC related pathways, such as interleukin-17 signaling pathway, vascular endothelial growth factor signaling pathway and prolactin signaling pathway, were enriched in KEGG analysis in this study, indicating that Senna and aloe combined with panax quinquefolium play a role in the treatment of functional constipation by regulating multiple signaling pathways.

The results show that panax quinquefolium joint senna, aloe synergies role in the treatment of functional constipation, panax quinquefolium tonifying qi, nourishing Yin, oneself clear heat, can make up for the senna, the aloe

vera in tianjin, the waste gas with tianjin disadvantage, at the same time, the main active ingredients of ginseng American ginseng saponin F2, saponins rh2 of ginseng has a regulation, and balance the role of the intestinal flora, other active components and core targets have high degree values in the network diagram. In addition, the enrichment analysis of KEGG pathway and molecular docking results also showed that panax quinquinosa plays an important role in the treatment of functional constipation in combination with Senna and aloe, which reflects that the combined use of the three drugs has the characteristics of multi-component, multi-target and multi-pathway, and the important targets and pathways are closely linked and unified. Through the analysis and verification of key components, key targets and important pathways in relevant literature, the specific mechanism of the treatment of FC by senna leaf and aloe combined with American ginseng was preliminaries understood, which provided ideas for clinical drug use and laid a foundation for further in-depth discussion of its mechanism of action. However, due to the limitation of network pharmacology methodology and the difference between TCM syndrome differentiation theory and modern medical thinking, animal experiments should be carried out to verify relevant targets and pathways.

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