

Exploring the material basis and mechanism of action of galangal in improving functional constipation based on network pharmacology

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Abstract

Research Objective Applying network pharmacology to explore the material basis of galangal and its mechanism of improving constipation. Method the effective components of galangal were collected, selected, and detected through the pharmacology database and analysis platform (TCMSP), and the corresponding target proteins were found in the "TCMSP" database. Match the corresponding genes of target proteins in the "Uniprot" and "Swiss Target Prediction" databases; The relevant results were visualized and analyzed for network topology using Cytoscape v3.9.0 software. Collect relevant targets related to constipation diseases based on keywords such as "Constipation", "Intrinsic peristalsis", "Interstitial cells of Cajal", and "Intrinsic inflammation" in databases such as "GeneCards" and "Drugbank"; Build a Venn diagram using the "Table" database and create a bubble chart of the enrichment results. Knowing the key targets of sorghum for improving constipation, and importing them into the "STRING" database to obtain the protein interaction network diagram of key proteins; Perform GO functional and KEGG pathway enrichment analysis on key targets using the Metascape database. Result Using the database, 30 effective active ingredients and 138 target genes of galangal were obtained. There were 2248 target genes related to constipation disease. After analysis, 167 network nodes and 515 edges were identified, with an average node value of 5.56. Seven active ingredients were screened, including quercetin, kaempferol, β -sitosterol, apigenin, emodin, oleic acid, and galangin. KEGG enrichment analysis revealed 102 signaling pathways. Analysis has found that substances such as quercetin, kaempferol, and β -sitosterol may be active ingredients in the laxative and laxative properties of ginger. Conclusion Galangal may improve constipation by regulating the calcium signaling pathway, phosphatidylinositol signaling pathway, PI3K Akt signaling pathway, and other pathways through active ingredients such as quercetin, kaempferol, β -sitosterol, apigenin, emodin, oleic acid, and galangal, as well as targets such as PTGS2, CHRM1, CHRM3, and ADRB2.

Keywords: Gaoliang ginger, Constipation, Network pharmacology, quercetin

Introduction

Introduction to Gaoliang Ginger

The herbaceous plant Gao Liangjiang belongs to the ginger family and can also be called Xiao Liangjiang, Liangjiang, etc. It is included in the historical edition of the Chinese Pharmacopoeia, but in fact, the earliest record of Gao Liangjiang is in the "Record of Famous Doctors" [1]. The subtropical regions of our country are the main growth areas for sorghum, which is not suitable for dry and cold climates, and should not be irrigated with excessive rainfall. It has no adaptability to cold air. The deep, fertile and loose acidic red soil is the favorite of sorghum, but it does not and does not affect its growth in the slightest. A place with sufficient rainfall and suitable temperature is the best environment for planting sorghum. A certain degree of shade is also essential for the growth of sorghum, but it can adapt during the mature stage of sorghum. Hainan, Guangdong, Guangxi, Yunnan and other provinces and regions are the main distribution areas of wild sorghum [2, 3]. The history of planting sorghum in China is very long, as recorded in the book "Guangdong Gazetteer": due to the unique aromatic aroma of sorghum, it has the functions of dispelling cold and keeping warm, relieving body pain, improving gastrointestinal vitality, and dispersing mosquitoes and insects. Therefore, it became an essential food for the court, and during the Northern Song Dynasty, Xuwen County was the place with the most tribute of sorghum. Starting from the Northern Song Dynasty, the Ming Dynasty flourished and continued until the Qing Dynasty. Official products always had a place for sorghum and were never allowed to be sold

by the public. Until 1949, the country had unified its acquisitions, with Southeast Asia, Central Asia, Africa, and other regions being the main export destinations. Gaoliang ginger has always been a traditional crop food with good economic benefits.

Effects of High-Quality Ginger

As an important medicinal and edible resource, there are increasing reports on the active ingredients and pharmacological effects of galangal. Flavonoids, diarylheptanes, volatile oils, sterols, etc. are its main active ingredients [4].

1. Antibacterial effect: As a traditional food, sorghum has significant antibacterial effects. Research shows that the antibacterial effect of sorghum mainly comes from flavonoids, diaryl compounds, volatile oils, and other substances in it. Moreover, when antibiotics are used together, they can enhance the antibacterial effect [6]. Flavonoids are the most important antibacterial substances in ginger, and the main flavonoid substance in ginger, galangin, can significantly increase potassium loss in *Staphylococcus aureus* cells, thereby achieving the effect of inhibiting bacteria. The main principle is to destroy the cytoplasmic membrane, damage the autolysis of the cell wall, thereby allowing the cell to undergo permeation and indirectly damaging the cell [7]. In recent years, there have been more and more scholars studying the antibacterial effects of diarylheptane compounds, which are also antibacterial components in ginger. Zhang BB [8] Experiments were conducted to

inhibit the growth of *Helicobacter pylori* Hp Sydney and HpF44 using diarylheptanes isolated from the ethanol extract of galangal, and it was found that galangal has pharmacological effects against the bacteria. Subramanian K^[9] The diarylheptane compounds in galangal have good antibacterial and bactericidal activity against 21 pathogenic *Escherichia coli* (EPEC) isolated from clinical samples, and effectively inhibit polysaccharide induced inflammatory responses in human peripheral blood mononuclear cells. Therefore, the decisive substance for the effect of ginger on *Helicobacter pylori* and *Escherichia coli* in the digestive system is derived from diarylheptanes, which confirms the application of ginger in improving intestinal diseases.

2. **Anti inflammatory effects:** Flavonoids and diarylheptanes in ginger have excellent anti-inflammatory effects on the substance. Galangin is one of the flavonoids that can reduce *Helicobacter pylori*, which causes an increase in inflammatory factors in human gastric cancer cells^[10] At the same time, it also has an inhibitory effect on the increase of inflammatory factors in human liver cancer cells and macrophages caused by *Helicobacter pylori*^[11, 12] Research has confirmed that the phosphatidylinositol signaling pathway (PI3K Akt signaling pathway) can also be inhibited by turmeric to achieve anti-inflammatory effects. Galangin can inhibit the induction of cellular inflammation in mice by suppressing the phosphatidylinositol signaling pathway. The production of NO, IL-1 β and TNF - α , as well as the expression of COX-2 gene, are also inseparable from the effect of diarylheptanes on the ERK1/2 MAPK pathway, thereby inhibiting induced macrophage inflammation^[14].
3. Antioxidant activity antioxidant activity is also one of the essential functions of galangal. N-hexane is one of the extracts of galangal, which can inhibit the production of NO in mononuclear macrophages in experimental mice^[15] To achieve antioxidant effect. The 70% methanol extract of galangal can demonstrate high DPPH free radical scavenging activity through daily increasing doses and is also effective in combating lipid peroxidation in hamster lung cells induced by H₂O₂ treatment^[17].

Application of Galangal

Gaoliang ginger has a wide range of biological activities and is often combined with multiple prescriptions in folk life^[19] - For example, equivalent to ginger and *Pinellia ternata*, it can warm the stomach, stop vomiting and diarrhea; Used in combination with Xixin, Zhicao Wu, Zhichuan Wu to treat insect teeth, wind and fire toothache, and more. At the same time, traditional Chinese patent medicines and simple preparations with galangal as the main raw material is also widely used in clinic, such as Tiantai Wuyao Powder and Jiangbai Tablets^[21, 22] Jiang Xiang'an Stomach Soft Capsules^[23] Wait. Gaoliang ginger is mainly concentrated in provinces such as Hainan, Guangdong, and Guangxi. Among them, Xuwen County in Guangdong is the most authentic production area of Gaoliang ginger in China, making it one of the famous "Top Ten Guangzhou Medicines"^[24, 25].

At present, besides its medicinal value, sorghum is also used as a food. Mainly used for making tea, brewing medicinal wine, enhancing the flavor of soup, brewing high-quality ginger vinegar, and as a spice in food^[26] At the same time, it can also be used as a fresh-keeping agent for fruits and vegetables, as well as a spice for food seasoning, driving oil, perfume and health products.

People have also begun to pay attention to the use of ginger as a natural and non-toxic material, because the components in ginger have antioxidant effects and inhibit microbial activity on the surface of fruits and vegetables from the extract of ginger. By reducing the influence of microbial activity in fruits and vegetables, the goal of lowering their respiration can be achieved, thereby maintaining their freshness. Volatile oils, diarylheptanes, flavonoids, phenylpropanoids, glycosides, and other compounds are the main chemical components of ginger, which can inhibit various bacteria^[27, 28].

Introduction to Constipation

Constipation is a type of chronic functional digestive disease, in which patients may experience symptoms such as dry stools, difficulty in defecation, and reduced frequency of bowel movements. In addition to factors such as dietary habits and medication use, intestinal peristalsis can be significantly inhibited in cases of dysbiosis, and with the accumulation of harmful bacteria, constipation symptoms in patients can further worsen. At present, Western medicine still mainly focuses on relieving constipation and improving gastrointestinal motility, with poor therapeutic effects, while traditional Chinese medicine treatment can improve organ function, alleviate symptoms, etc., while drug dependence is relatively low^[30, 31].

Constipation is mainly divided into two types, one is acute constipation, also known as organic constipation, such as acute peritonitis or intestinal paralysis.

Another type is chronic constipation, which differs from acute constipation in that the exact cause cannot be identified. Functional constipation is the most common subtype of chronic constipation, also known as narrow chronic constipation. Its pathophysiological mechanism is complex and has not been fully

elucidated^[32] Traditional Chinese Medicine believes that the occurrence of constipation is closely related to various organs in the body, but the large intestine is the main site of the disease, which may be caused by weak spleen and stomach leading to loss of activity, gastrointestinal fire, and downward displacement of lung fever, resulting in loss of intestinal conduction function. Traditional Chinese medicine mainly treats constipation by taking traditional Chinese medicine. Although it has a slow clinical effect, it causes less damage to the human body and has the advantages of multiple active ingredients and targets.

Pathological mechanism of constipation

Slow transit constipation (STC) is a chronic, primary, functional, colonic, and chronic constipation disease caused by problems with the large intestine leading to confusion and conduction disorders^[34] However, its pathogenic mechanism and causes are not yet clear, and its reasons may be related to disturbances in gastrointestinal hormone levels, insufficient gastrointestinal motility, changes in the number and morphology of interstitial cells (ICC), neurotransmitter content in the intestinal nervous system,

and dysbiosis of the intestinal microbiota [35] - [37] The incidence rate of STC is significantly increased due to people's eating habits, life pace, and psychological and psychological reasons. At present, the treatment of STC in clinical practice mainly includes laxatives, prokinetic agents, biofeedback, and surgical procedures. However, its efficacy is not ideal and there are significant adverse reactions, which seriously affect the quality of life of patients.

Constipation related signaling pathway studies have shown that PI3K is a second lipid messenger molecule that activates multiple intercellular signaling pathways. It plays an important role in enhancing T lymphocyte activity and promoting T cell proliferation [34, 39] This pathway affects the aggregation of acidic cells, enhances their activity, participates in the migration and detachment of giant cells, thereby inducing T cell juvenile activation and proliferation, inhibiting T cell apoptosis, promoting IL-2 secretion, and playing an important regulatory role in the inflammatory response of cells [40] Participated in the autophagy pathway of PI3K/Akt. Cytomycin protein kinase mTOR is a conserved molecule that plays an important role in regulating cellular autophagy. It can detect changes in intracellular ATP and amino acid composition, participate in and control autophagic cells. PI3K plays a decisive role in the mTOR signaling pathway, mainly regulating the mTOR signaling pathway to achieve the goal of regulating PI3K/Akt/mTOR, thereby regulating cellular autophagy [41] It is one of the two major pathways of autophagy. Huang Yebao [42] Through the application of network pharmacology, it was discovered that the effective active ingredients of Si mo Tang, including quercetin, kaempferol, β -sitosterol, stigmasterol, and 6,7-dimethoxy-2-(2-phenylethyl) chromone, improve intestinal peristalsis function by regulating the PI3K Akt signaling pathway to treat slow transit constipation (STC). The PI3K Akt signaling pathway is one of the pathways related to constipation. The CaM/MLCK pathway, also known as the coarse muscle filament related regulatory pathway, plays a key role in regulating smooth muscle cell contraction and signaling in colonic smooth muscle cell dynamics. CaM is an important transduction factor in the Ca²⁺-signaling pathway in cells, which participates in and regulates a series of biochemical processes caused by Ca²⁺, and regulates various activities such as cell proliferation and movement. After activation, 5-HT₄R acts on the smooth muscle cell membrane, activating the Ca²⁺-ion pathway, causing a rapid increase in the concentration of free calcium ions in the cell, and combining with calcium ions to form a complex, continuing to activate MLCK. MLCK is a Ca²⁺-CaM complex dependent kinase widely distributed in muscle tissues such as smooth muscle. On MLC20, the serine ester at position 19 is phosphorylated, and after MLC20 phosphorylation, ATP hydrolase is activated, ATP is hydrolyzed by hydrolase, and chemical energy is converted into mechanical energy, allowing myosin to slide on actin filaments and achieve muscle contraction [43] Wu Minna [43] By observing the use of Zhizhu Formula in the colon tissue of chronic transport constipation (HTC), it was found that Zhizhu Formula can significantly improve the symptoms of constipation in STC rats, and is related to the expression of 5-HT₄R in the colon tissue of rats and the activation of the CaM/MLCK signaling pathway.

Introduction to Network Pharmacology

Network pharmacology elucidates the etiology of diseases mainly by understanding the effects of drugs on the human body based on the balance between systems biology and biological networks, and then developing new drugs. The biggest difference between network pharmacology and traditional Western medicine is the construction of a biological network based on data analysis, computer virtual computing, and network database retrieval to study pharmacological effects. Network pharmacology revolves around systems biology to study the efficacy of drugs. The single target of biology, which focuses on drug effects, is not as important as biology and dynamics. Breaking the traditional model of "one disease, one drug, one target", it reflects the transformation of modern biomedical science [44] Currently, utilizing the structure and function of biological molecular networks to elucidate the pharmacological mechanisms of drugs is the future development direction, and referring to the research methods of pharmacology, exploring the chemical components corresponding to drug targets, and revealing the comprehensive overall effects of traditional Chinese medicine chemistry [45, 46].

Network Pharmacology Common Database Active Ingredient Collection Database: Traditional Chinese Medicine Systemic Pharmacology Analysis Platform (<https://tcmispw.com/tcmisp.php>) The Comprehensive Database of Traditional Chinese Medicine (<http://www.tcmidoc.cn/Default.xml>), a bioinformatics analysis tool for the molecular mechanism of traditional Chinese medicine (<http://bionet.ncpsb.org/batman-tcm/>) Target database: Pharmacology database and analysis platform (<https://tcmispw.com/tcmisp.pphp>) Integrate pharmacology platform (<http://www.tcmip.cn/TCMIP/index.php/Home/Login/login.html>) Target prediction online platform (<http://swisstargetprediction.ch/>). Uniprot database (<https://www.uniprot.org/>) Disease target construction database: Gene business card database (<https://www.genecards.org/>), Drugbankdatabase (<https://www.drugbank.org/>), Protein Analysis Database: Protein Action Database (<https://cn.string-db.org/>)

Network pharmacology and the composition and mechanism of action of traditional Chinese medicine for constipation are the achievements of network pharmacology from a systematic and holistic perspective, revealing the interactions between multiple components and targets of traditional Chinese medicine, and studying the relationship between drugs, targets, and diseases from another perspective, providing a new idea for the development and research of new drugs [47] It can provide a more systematic summary of the active ingredients, related targets, and mechanisms of action of drugs, reflecting the benign regulation of diseases by traditional Chinese medicine through multiple components and targets, which coincides with the holistic and dialectical views of traditional Chinese medicine theory. Different proteins or genes can jointly regulate the same disease, and a single protein or gene can also regulate multiple diseases, which is consistent with the theories of "same disease, different treatment" and "same treatment for different diseases" in traditional Chinese medicine [48] Therefore, the modernization development of

traditional Chinese medicine is closely related to network pharmacology research. Traditional Chinese Medicine External Treatment Therapy, as an effective treatment method in traditional Chinese medicine, can have multiple effects according to the different combinations of prescriptions, and is widely used in multiple disciplines such as internal medicine, external medicine, gynecology, and pediatrics. In recent years, some scholars have used network pharmacology research methods to predict the effective ingredients, targets, and mechanisms of action of traditional Chinese medicine topical drugs, and subsequent studies have confirmed the accuracy of their predictions, achieving fruitful results.

Chu Yifei and others^[49] By using network pharmacology to construct a chemical composition target metabolic signaling pathway network of Jichuan decoction, it was found that the treatment of constipation by Jichuan decoction mainly acts on PTGS2, PTGS1, NCOA2, SCN5A, ADRB2. These 5 key targets, along with cytochrome P450, regulate the metabolism of xenobiotics and retinol metabolism. Liu Yuanyuan and others^[50] Using network pharmacology, it was found that the key components of Bufei Tang and Maren Wan in treating constipation are flavonoids, β -sitosterol, kaempferol, etc. Key targets such as PPARG and ESR1 affect multiple biological processes related to constipation, including positive and negative regulation of RNA polymerase 2 promoter transcription, drug response, and apoptosis, as well as multiple signaling pathways such as cell apoptosis, phosphatidylinositol 3-kinase (PI3K-AKT), osteoclast differentiation, and T cell receptors. Shi Min and others^[51] Through network pharmacology, it was found that the active compounds in *Astragalus membranaceus* mainly exert therapeutic effects on slow transit constipation by acting on AKT1, IL6, CASP3 and other targets, such as folate, quercetin, and kaempferol, through regulating signaling pathways such as antibiotic response and oxidative stress response.

Research Purpose and Significance

So far, most of the drugs used to treat constipation are stimulating and lubricating drugs. Long term use may cause a decrease in intestinal stress and affect the absorption of vitamins. And it is easy to develop dependence on drugs. Given the basic theory of the same origin of medicine and food, traditional Chinese medicine therapy clearly has advantages such as significant therapeutic effects, low recurrence rates, and minimal toxic side effects. Given that the pharmacological activity of galangal is related to improving intestinal function, it is speculated that galangal contains potential active ingredients that can improve constipation. The research method of this study was proposed by a British pharmacologist in 2007^[52] By constructing a network of effective compounds and targets, as well as targets and diseases, this study aims to explore the potential active ingredients, targets, and signaling pathways of active ingredients in the treatment of constipation with ginger. The goal is to find the material basis and mechanism of action of ginger in improving constipation, and provide theoretical basis for further research on the function of ginger in improving constipation. In the hope that one day functional foods mainly composed of galangal can be launched, thereby increasing the added value of galangal, driving the development of the galangal industry, creating more employment opportunities, increasing farmers' income, and improving economic development.

Materials and Methods

Research Tools

Database Name	Database URL
Traditional Chinese	
Medicine System Pharmacology Database (TCMSP)	https://tcmssp.com/tcmssp.php and Analysis Platform
Uniprot database	https://www.uniprot.org/
Swiss Target Prediction database	http://www.swisstargetprediction.ch/
Pubchem database	https://pubchem.ncbi.nlm.nih.gov/
GeneCards database	https://www.genecards.org/
OMIM database	https://omim.org/
Drugbank database	https://www.drugbank.org/
Metascape database	https://metascape.org/gp/index.html#/main/step1
Cytoscape v3.9.0 software	

Research Methods

1. Collection of compounds related to galangal: Using the "TCMSP" database, a total of 553 chemical components were searched for in galangal, a traditional Chinese medicine. Drug similarity (DL) ≥ 0.1 and oral bioavailability (OB) $\geq 0\%$ were set, and effective compounds related to galangal were screened.
2. Target Prediction of Galangal Extract the targets of each active ingredient through the "TCMSP" database, input the relevant information of the targets into the "Uniprot" database, and supplement the targets through the "Swiss Target Prediction" database based on this. Introduced drugs and targets into Cytoscape v.9.0 software, established a network structure diagram of components targets, and achieved visualization of chemical components and targets.

Building a PPI network: Retrieve targets related to human constipation from the GeneCards and OMIM databases based on keywords such as "Constipation, Intrinsic peristalsis, interstitial cells of Cajal, and Intrinsic inflammation". The screened target of galangal and the relevant target related to human constipation will be imported into the "Table" database to obtain the common target between the two, which is the key target for galangal to improve constipation.

KEGG Enrichment Analysis: The key target KEGG analysis imported into the "Metascape" database is limited by the P-value, resulting in enrichment analysis (BP biological process), CC cell component analysis, and MF molecular function analysis.

Results and Analysis

Screening results of active ingredients in ginger

By searching the database, 553 active ingredients of sorghum were screened. Then, under the conditions of OB $\geq 20\%$ and DL ≥ 0.1 , 30 active ingredients of galangal were screened, as shown in Table 2 for specific information. Import the key targets into the "STRING" database and import the obtained results into Cytoscape 3.9.0 software to construct a PPI network diagram.

MOL ID	Molecule Name medicine	
MOL001748	methyl (E)-octadec-8-enoate	Gaoliang Jiang
MOL001771	poriferast-5-en-3beta-ol	Gaoliang Jiang
MOL000246	(2R)-5, 7-dihydroxy-2-phenylchroman-4-one	Gaoliang Jiang
MOL002523	(-)-Cyclosativene	Gaoliang Jiang
MOL002533	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	Gaoliang Jiang
MOL002536	Phenethyl octanoate	Gaoliang Jiang
MOL002543	(2S, 3R)-2-(3, 4-dimethoxyphenyl)-5	Gaoliang Jiang 7-dimethoxychroman-3-ol
MOL002544	1, 7-diphenyl-5-hydroxy-3-heptanone	Gaoliang Jiang
MOL002552	8-Hydroxybergapten	Gaoliang Jiang
MOL002554	5-methoxy-1, 7-diphenyl-3-heptanone	Gaoliang Jiang
MOL002555	7-Hydroxy-2-(2-hydroxy) propyl-5-methyl-benzopyran-gamma-o	Gaoliang Jiang
	Ne	
MOL002556	7-Methoxy-8-(2'-ethoxy-3'-hydroxy-3'-methybutyl) coumarin	Gaoliang Jiang
MOL002559	9-ethoxy-10-oxatricyclo [7.2.1.0(1, 6)] dodecan-11-one	Gaoliang Jiang
MOL002560	chrysin	Gaoliang Jiang
MOL002561	(2S, 3R)-2, 3-dihydroxy-1, 3-di(phenyl)propan-1-one	Gaoliang Jiang
MOL002563	galangin	Gaoliang Jiang
MOL002565	Medicarpin	Gaoliang Jiang
MOL002573	β-patchoulene	Gaoliang Jiang
MOL002575	butyl-2-ethylhexyl phthalate	Gaoliang Jiang
MOL000354	isorhamnetin	Gaoliang Jiang
MOL000357	Sitogluside	Gaoliang Jiang
MOL000358	beta-sitosterol	Gaoliang Jiang
MOL000359	sitosterol	Gaoliang Jiang
MOL000422	kaempferol	Gaoliang Jiang
MOL000472	emodin	Gaoliang Jiang
MOL000057	DIBP	Gaoliang Jiang Gaoliang Jiang
MOL000675	oleic acid	Gaoliang Jiang
MOL000676	DBP	Gaoliang Jiang
MOL000008	apigenin	Gaoliang Jiang
MOL000098	quercetin	Gaoliang Jiang

Construction of "Compound Target Protein" Network Galangal

30 active ingredients corresponding to gene cells were obtained from the "Uniport" and "Swiss Target Prediction" databases, and 138 duplicate targets were deleted. Import the compounds and targets into Cytoscape v3.9.0 software to obtain the "compound target" network construction diagram of sorghum. The network consists of 515 edges

(identified by edges for interactions between components and targets) and 167 nodes (identified by nodes for active components and drug targets of ginger), with an average node value of 5.56. By analyzing and calculating the degree, the core network of "active ingredients drug targets" in sorghum ginger was obtained, as shown in Fig 3. These active ingredients are closely related to their targets and may play an important role in improving constipation.

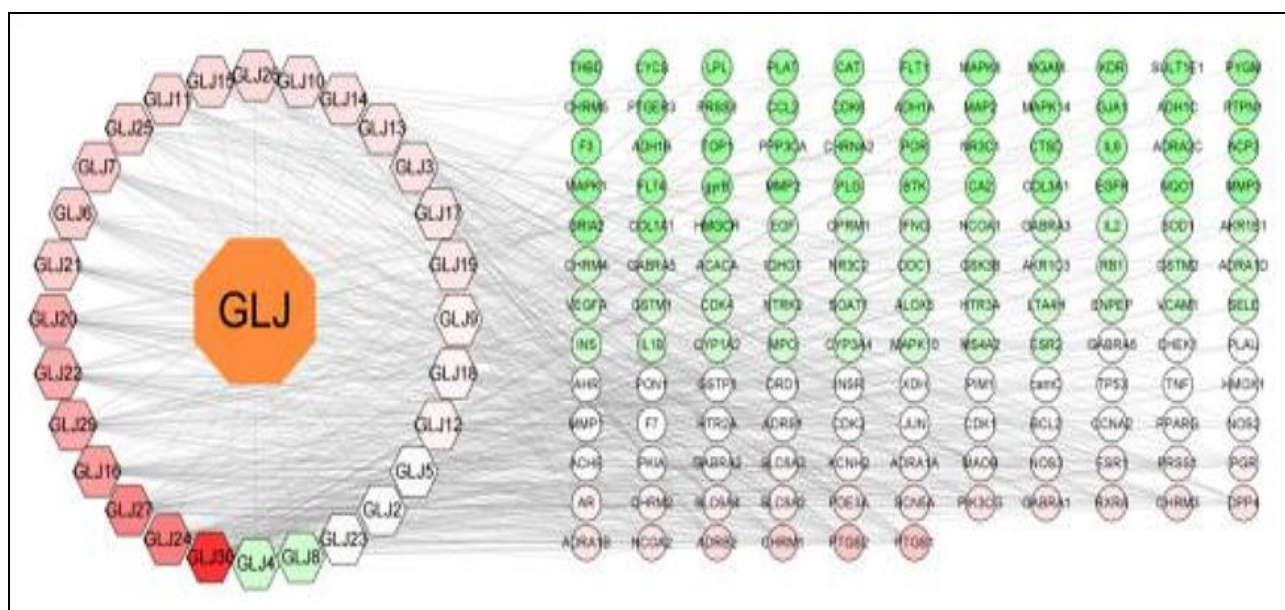


Fig 1: Compound Target"Network Diagram ofGalangal

Gaoliang ginger is octagonal, with hexagonal active ingredients and circular targets. The relationship between compounds and targets is represented by lines

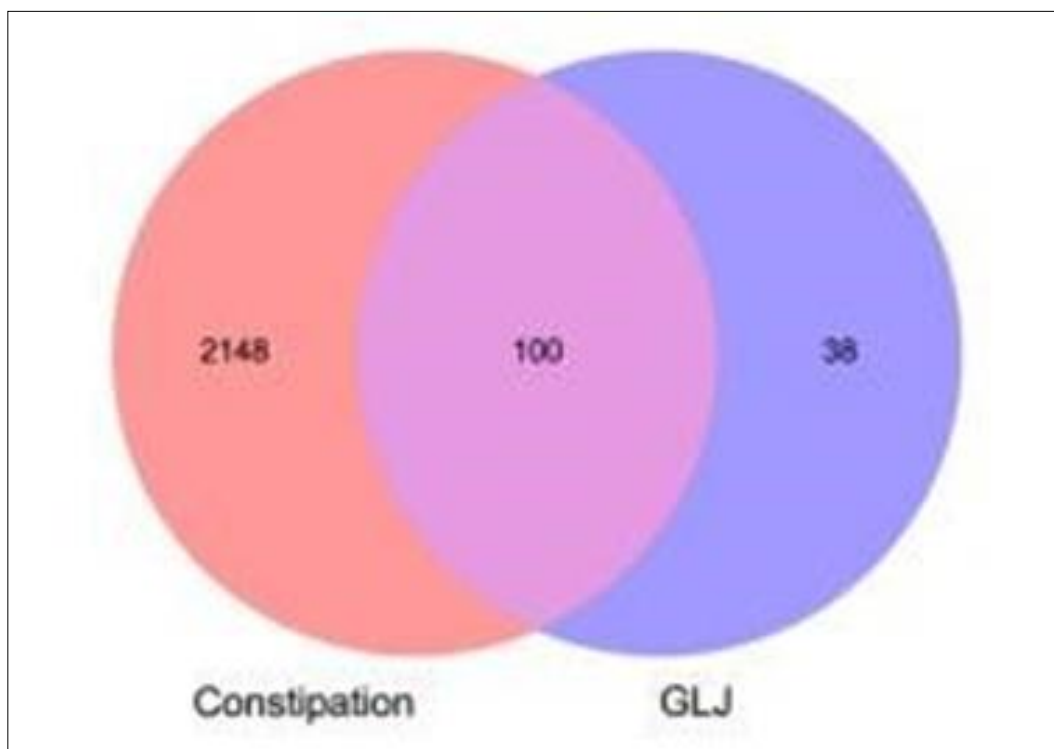


Fig 2: Venndiagram of common targets of galangaland constipation

Table 3: GaoLiangJiang and astriction Common target Serial Target Serial Target Serial Target Serial Target

Serial Number	Target	Serial Number	Target	Serial Number	Target
1	PGR	26	NOS2	51	HMOX1
2	PTGS1	27	PPARG	52	CYP3A4
3	ESR1	28	BCL2	53	CYP1A2
4	PTGS2	29	CDK4	54	SELE
5	ADRB2	30	CDK1	55	VCAM1
6	SLC6A4	31	GSTP1	56	ALOX5
7	GABRA1	32	HTR2A	57	INSR
8	PIK3CG	33	OPRM1	58	PPP3CA
9	MAOB	34	ESR2	59	GSTM1
10	CHRM2	35	MAPK10	60	KDR
11	CHRM1	36	PRSS1	61	FLT1
12	SLC6A2	37	MAPK14	62	EGF
13	KCNH2	38	GSK3B	63	TNF
14	SCN5A	39	AKR1B1	64	TP53
15	NOS3	40	F7	65	IL1B
16	CHRM3	41	ACHE	66	BTK
17	ADRB1	42	GRIA2	67	FLT4
18	ADRA1A	43	XDH	68	NR3C1
19	AR	44	HTR3A	69	ADH1C
20	CA2	45	JUN	70	PLAU
21	DPP4	46	PON1	71	SOD1
22	DRD1	47	MAP2	72	CAT
23	SLC6A3	48	NR3C2	73	LPL
24	CDK2	49	MAPK8	74	MPO
25	GABRA2	50	MMP1	75	INS

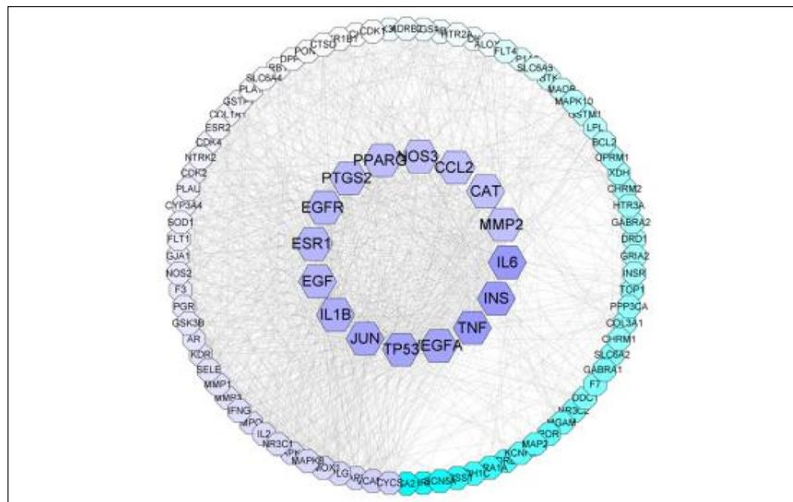


Fig 3: Key target PPI (protein-protein interaction analysis) network diagram

PPI protein interaction analysis

2248 retrieved targets and 138 constipation related targets from 30 effective components of ginger were imported into the "Table" database, resulting in 10 common constipation related targets, as shown in Table 3. This is an important target of galangal in constipation. Import 100 key targets into the STRING database and import the results into Cytoscape v3.9.0 software to establish a network of "active ingredients drug targets" for galangal.

The intersection targets of sorghum and constipation were systematically analyzed using the "String" database, and a component target network was constructed using Cytoscape v3.9.0 software, as shown in Fig 3. Through topology analysis using Cytoscape v3.9.0 software, with a Drgree value greater than or equal to 15 as the screening condition, it was found that EGFR, PTGS2, PPARO, NOS3, CCL2, CAT, IL6, INS, TNF, EGFA, TR53, JUN, IL1B, EGF, ESR 1, these targets may be the core targets of Gao Liangjiang for moistening intestines and promoting bowel movements.

Enrichment analysis results

Importing 100 key targets into the "Metascape" database, enrichment analysis was conducted under the condition of P less than 0.01 to obtain enrichment entry maps. Through enrichment analysis (BP biological process), CC cell component analysis, and MF molecular functional analysis, it was found that the response of cells to nitrogen-containing substances, organic nitrogen, and organic cyclic compounds to heterogeneous biological stimuli may be the mechanism of action of galangal in moistening intestines and promoting bowel movements. Among the top 15 paths with P-values, 15 are enriched in BP analysis, as shown in Fig 4. In the cellular component analysis of CC, 15 items were enriched, as shown in Fig 5. Including cytoplasm, extracellular space, and extracellular region. In the functional analysis of MF, there are 15 enriched elements, as shown in Fig 6. Involving protein binding, ATM binding, tyrosine kinase activity, protein kinase activity, etc. The results indicate that galangal participates in the regulation of various physiological functions and may promote the improvement of constipation.

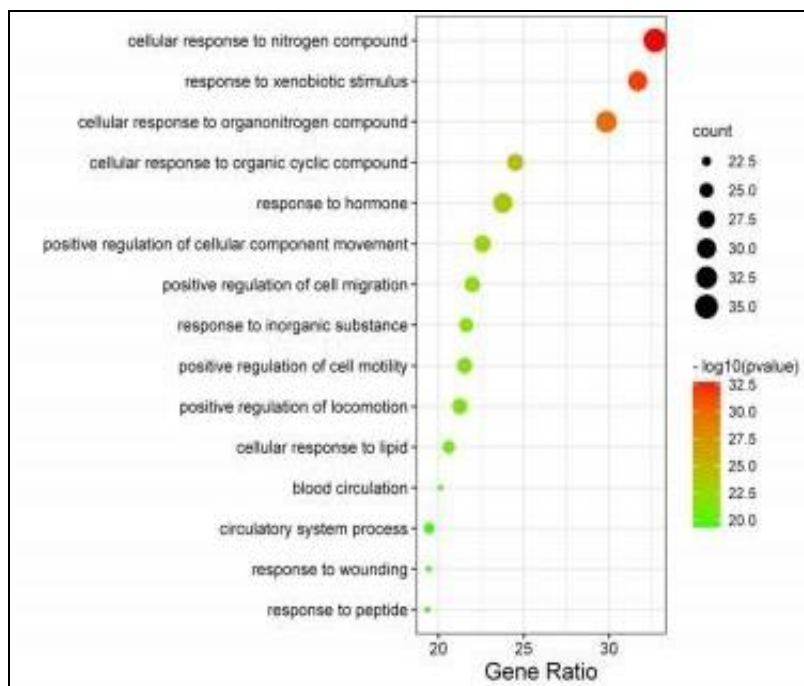


Fig 4: Enrichment analysis (BP biological process)

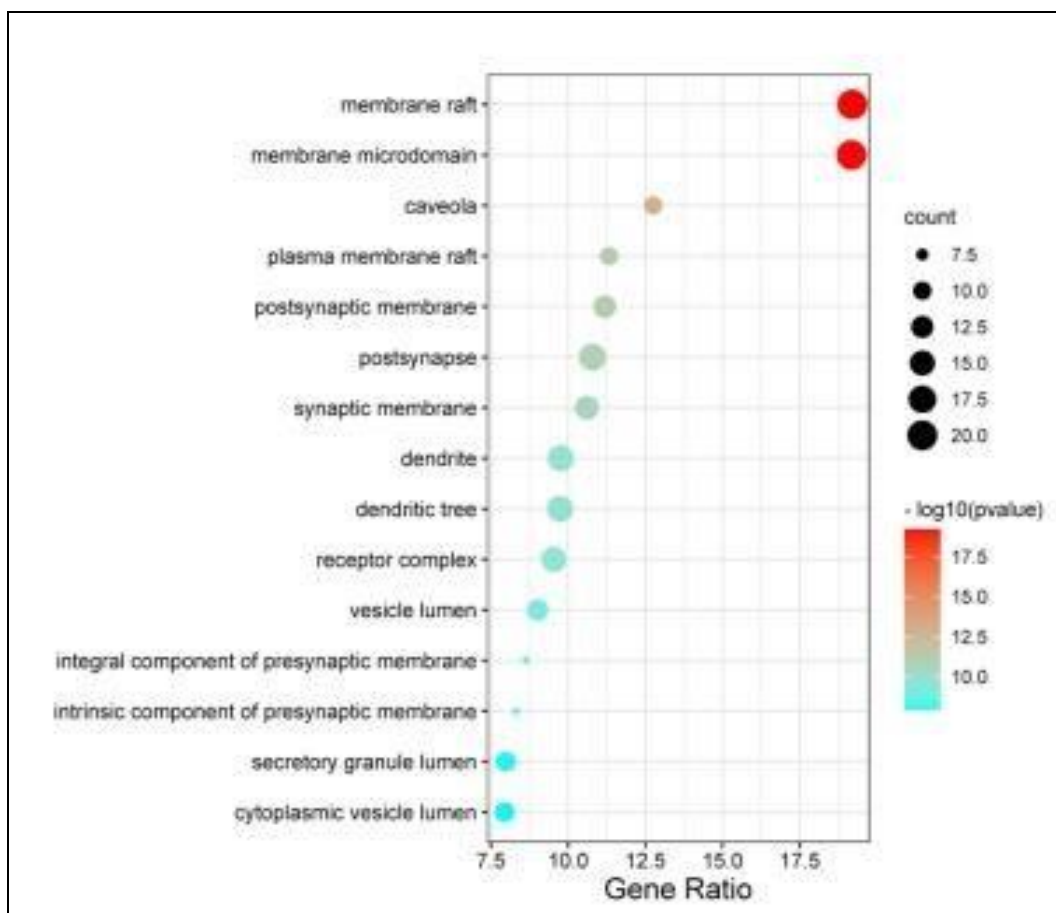


Fig 5: CC cell component analysis

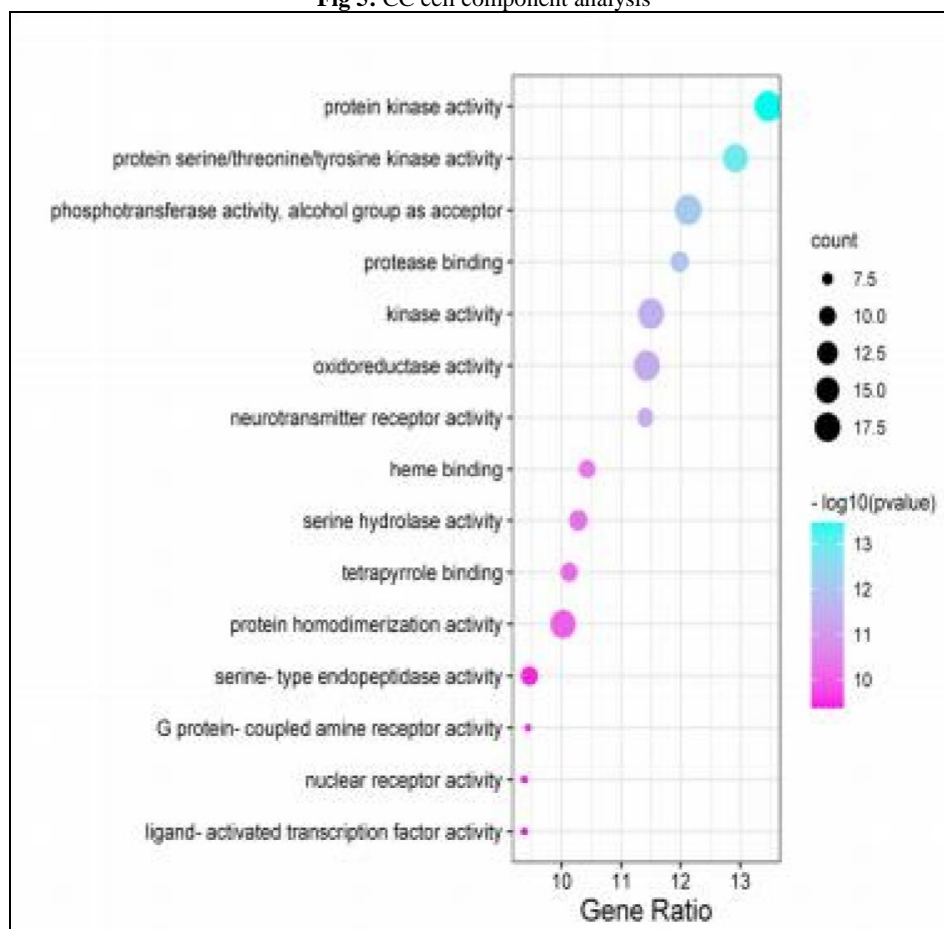


Fig 6: Functional analysis of MF molecule

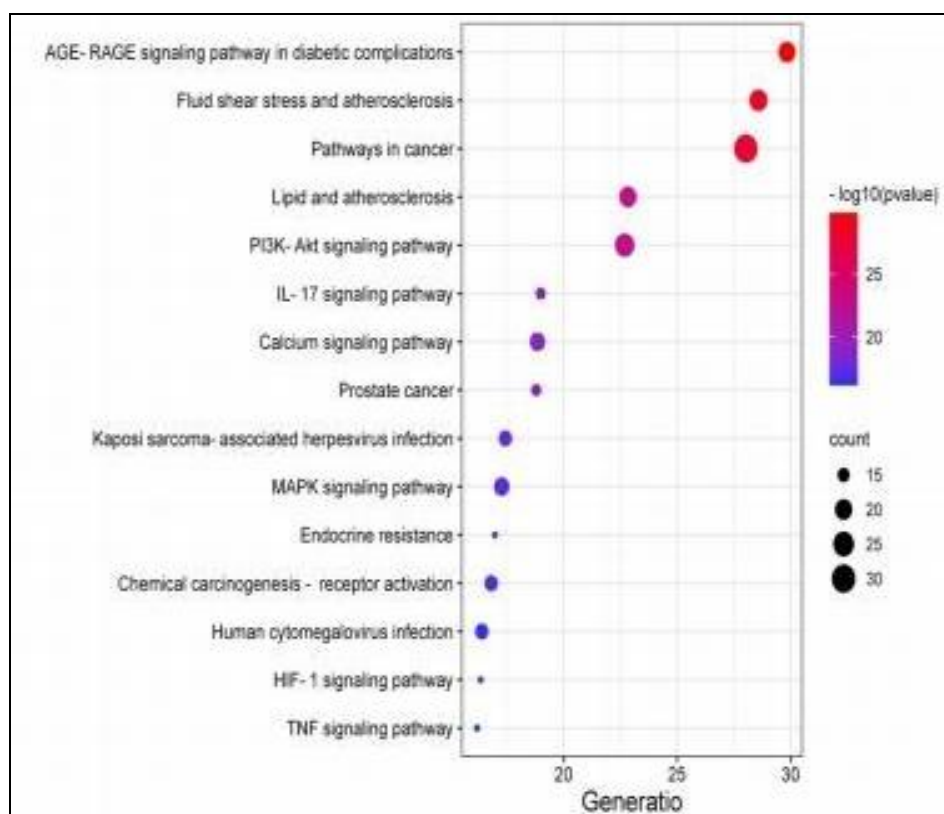


Fig 7: KEG analysis

KEGG Enrichment Analysis

Through KEGG pathway enrichment analysis, it was found that 100 keys targets were enriched in 102 pathways, ranking among the top 15 pathways in terms of P-value, as shown in Fig 7. Through network pharmacology enrichment analysis combined with literature related to constipation, it is suggested that the Calcium signaling pathway and the

PI3K Akt signaling pathway may be the mechanism by which Gaoliangjiangtong improves constipation.

Analysis of the enriched targets on the Calcium signaling pathway and PI 3K Akt signaling pathway revealed quercetin, β -sitosterol, emodin, galangin, and kaempferol, as shown in Table 4. These substances may be the basis for the moisturizing and laxative properties of galangal.

Table 4: Signal pathway “PI3K-Akt signaling pathway” enriches components-targets

Active ingredient	Number of targets	Targets
meletin	12	VEGFA, MAPK1, INSR, NOS3, IL2, IL6, EGFR, PIK3CG, PTGS2, TP53, EGF, INS
pelargidenon 1449	8	CDK4, CDK6, VEGFA, INS, TP53, INSR, EGF, IL2
campherol	5	CHRM2, PIK3CG, INSR, NOS3, INS
emodin	5	FLT1, FLT4, TP53, EGF, KDR
isorhamnetin	4	NOS3, MAPK1, GSK3B, CDK2
β -sitosterin	3	CHRM1, CHRM2, BCL2
cis-9-octadecenoic acid	3	CHRM2, GSK3B, NTRK2
2,3-dihydroxy-1,3-diphenyl-1-propanone	2	CDK4, BCL2
galangin	2	CHRM3, CHRM2
Diisobutyl phthalate	1	CHRM2
5,7-dihydroxy-2-phenyltryptamine-4-keto	1	PIK3CG
2-acetoxymethyl-3-methoxycarbonyl biphenyl	1	CHRM1
Syringol	1	NOS3
(2S, 3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol	1	CHRM1
8-hydroxy bergapten	1	CHRM1
5-methoxy-1,7-diphenyl-3-heptanone	1	CHRM1
7-hydroxy-2-(2-hydroxy) propyl-5-methylbenzopyran- γ -keto	1	CHRM1
coumarin	1	CHRM2
9-Ethoxyl-10-oxa-tricyclo [7.2.1.0(1,6)] dodecane-11-keto	1	PIK3CG
Chrysin	1	CHRM1
medicarpin	1	CHRM1
Butylphthalate-2-ethylhexyl ester	1	CHRM1
Sorbitol	1	CHRM1

Table 5: Signal pathway “Calcium signaling pathway” enriches components-targets

sActive ingredient	Number of targets	Target spot
meletin	Nine	ADRA1A, DRD1, EGF, EGFR, KDR, NOS2, NOS3, NTRK2, VEGFA
β-sitosterin	Eight	ADRA1A, CHRM3, ADRB2, DRD1, CHRM1, HTR2A, CHRM2, PTGS2
cis-9-octadecenoic acid	Seven	ADRA1A, CHRM3, ADRB2, HTR2A, CHRM1, CHRM2, NTRK2
galangin	Six	CDK2, PTGS2, CHRM2, MAPK1, CHRM1, PIK3CG
campherol	Five	CHRM2, CHRM3, NOS3, NTRK2, VEGFA
emodin	Four	EGF, FLT1, FLT4, KDR
Diisobutyl phthalate	Four	ADRB2, CHRM1, CHRM2, CHRM3
7-hydroxy-2-(2-hydroxy) propyl-5-methylbenzopyran-γ-keto	Four	ADRB2, CHRM1, CHRM3, DRD1
Butyl phthalate-2-ethylhexyl ester	Three	CHRM1, ADRA1A, CHRM3
isorhamnetin	Three	ADRB1, HTR2A, VEGFA
(2S, 3R)-2-(3,4-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol	Three	ADRA1A, ADRB1, CHRM3
2-acetoxy-methyl-3-methoxycarbonyl biphenyl	Two	ADRB2, CHRM1
Syringol	Two	ADRB2, NOS3
8-hydroxy bergapten	Two	ADRB2, CHRM1
Sorbitol	Two	CHRM1, CHRM3
pelargidenon 1449	Two	EGF, VEGFA
5,7-dihydroxy-2-phenyltryptamine-4-keto	One	ADRB2
Cycloserine	One	CHRM2
5-methoxy-1,7-diphenyl-3-heptanone	One	CHRM1
coumarin	One	CHRM2
Chrysin	One	CHRM1
2,3-dihydroxy-1,3-diphenyl-1-propanone	One	NOS2

Discussion

This study is based on network pharmacology technology and utilizes relevant databases and software to systematically explore the effects of galangal on constipation. Through network structure analysis, the main components of galangal are quercetin, β -sitosterol, galangin, kaempferol, oleic acid, apigenin, and seven other effective ingredients that have an impact on improving constipation.

Quercetin can improve lipid metabolism by reducing cholesterol synthesis, lowering triglycerides, and lowering LDL and HDL levels, thereby achieving the goal of preventing lipid peroxidation and accumulation.

β - sitosterol mainly regulates cell proliferation, apoptosis, and cell cycle by inhibiting the PI3K Akt signaling pathway [53] By activating this signaling path

way, it enhances cell proliferation ability and inhibits cell apoptosis. But its role in improving constipation is not clear and can be used as a future research direction.

A study has found that galangin (Gal) [54, 54] It has significant balancing effect on gastric emptying hyperactivity; Has a significant inhibitory effect on gastric smooth muscle caused by acetic acid; It can significantly inhibit the activity of the small intestine, shorten the forward distance, reduce the forward speed, and stimulate stomach acid to improve intestinal function.

Modern research has found that emodin can promote gastrointestinal motility, reduce inflammation complications, increase intestinal blood flow, and enhance the immune activity of the gastrointestinal tract against bacteria. It is an adjuvant drug for treating gastrointestinal dysfunction; Its active ingredients can enhance the immune function of the gastrointestinal tract, eliminate oxygen free radicals in the gastrointestinal tract, inhibit bacteria,

eliminate inflammatory reactions, resist virus invasion, and protect the liver and gallbladder [56].

Shanna phenol can be combined with TRPC5 to inhibit pathway opening in tumor cells, and the high expression level of TRPC5 in gastric cancer is related to its mediated calcium influx. By inhibiting the expression and function of TRPC5 gene, the metastatic ability of tumor cells is significantly reduced. Research on structural similarity has found that naringenin has a similar structure to TRPC5 inhibitors and can directly bind to TRPC5, exhibiting significant inhibitory effects both *in vivo* and *in vitro* [57].

Oleic acid has a significant inhibitory effect on cancer recurrence in clinical symptoms of colon cancer, and can improve its anti-cancer efficacy by being used in combination with chemotherapy drugs [58, 61] Oleic acid can inhibit esophageal cancer, thereby achieving regulatory purposes and increasing the activity of tumor suppressor genes such as p53, p21, and p27 [62] At the same time, oleic acid can increase the expression of NOX4 in the human body, activate caspase3 in the human body, and significantly increase reactive oxygen species, thereby causing cell apoptosis [63].

Some studies have shown that apigenine can reduce blood sugar in diabetes mice; Promote the occurrence of intestinal peristalsis; After medication, the expression level of MLCK significantly increased. It can be concluded that apigenine can promote the expression of MLCK in pancreatic tissue, promote the contraction of smooth muscle, thus promote the peristalsis of jejunum, and improve the intestinal motility of diabetes patients [64].

This study predicts that the key targets for improving constipation with so rghum are PTGS2, CHRM1, ADRB2, CHRM3, and four other effective targets. PTGS2, also known as cyclooxygenase-2, is a key enzyme in the prostaglandin biosynthesis pathway. It is overexpressed in

early and late stage colorectal cancer tissues, indicating poor prognosis [65] Inhibition of PTGS2 overexpression is beneficial for reducing the risk of colorectal cancer [66] This suggests that PTGS 2 may play an important role in improving constipation with ginger.

CHRM1 and CHRM3 (cholinergic receptors M1 and M3) are the most important muscarinic receptors in the central nervous system, highly expressed in neurons. Knocking out the CHRM1 and CHRM3 genes can reduce NREM sleep and suppress gene expression, weakening Ca²⁺-dependent neuronal signal transduction in stable cell lines expressing mutant Huntington's disease (HD) proteins [67] The expression of this receptor can achieve the effect of reducing patient s' anxiety and improving constipation. ADRB2 is a beta-adrenergic receptor, Magnon et al [68] In the early stages of prostate cancer, chemotherapy, surgical removal of sympathetic nervous system, and expression of ADRB2 gene can prevent tumor development. The stomach, like other organs in the human body, is controlled by nerve fibers of the sympathetic nervous system, mainly regulating various functions of the stomach, such as smooth muscle movement and glandular secretion.

KEGG pathway enrichment analysis found that the improvement of constipation by galangal may be related to two signaling pathways, PI3K Akt signaling pathway and Calcium signaling pathway. The PI3K Akt signaling pathway can maintain the cell cycle, promote cell proliferation, and inhibit cell apoptosis [69] , After activation of PI3K Akt, P110 can transform PIP2 phosphate into PIP3 and bind to the N-terminal PH region of AKT, thereby activating AKT. Improving constipation by regulating the expression of PI3K, AKT genes and proteins in gastric mucosa [70] The Calcium signaling pathway is one of the main pathways for Ca²⁺ to enter cells from the extracellular environment in non-excited cells, and also participates in the occurrence of TGF- β -induced CSCS [71] TGF- β is dependent on the transient receptor potential (TRP) TRPC6 on calcium ion channels, and can alleviate damage to podocytes by inhibiting the TRPC 6 pathway [72] TRPC6 is closely related to malignant tumors of the digestive system, especially in esophageal cancer [73] Gastric cancer [74] It plays an important regulatory role in the development of diseases. It can cause dysfunction of the digestive system, leading to constipation.

5 Conclusion

Through network pharmacology analysis, it was found that:

(1) Gaoliang ginger may have a potential improvement effect on constipation.

(2) The key active ingredients that improve constipation in galangal may be quercetin, β -sitosterol, galangin, emodin, kaempferol, oleic acid, and apigenin.

(3) The key targets for improving constipation with sorghum may be PTGS2, CHRM1, CHRM3, and ADRB2, which are four effective targets.

(4) The key signaling pathways for improving constipation with sorghum may be the calcium signaling pathway and the phosphatidylinositol signaling pathway (PI3K Akt signaling pathway).

2

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