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Original Article

A network pharmacology-based analysis of *Curcuma longa* L.

Yalan Zhang[1*], Ruijia Chen[2*], Wentan Xu[3]

[1]Department of Pharmacy, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian Province, China; [2]Department of Pharmacy, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou, Fujian Province, China; [3]Department of Pharmacy, Jinjiang Hospital, Quanzhou, Fujian Province, China. [*]Equal contributors and co-first authors.

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Abstract: Objective: To further analyze the pharmacological mechanism of *Curcuma longa* L. using network pharmacology. Methods: In this study, superior compounds were identified through the Traditional Chinese Medicine System Pharmacology Database. The Uniport database was used to determine the potential gene targets of *Curcuma longa* L. A compound-target network was constructed with Cytoscape 3.7.2. The String 11.0 database was used to construct the protein-protein interaction network of the targets and to screen out the core targets. In addition, the DAVID 6.8 database and ClueGo were used to analyze the target's gene ontology and enrich the pathway to analyze its potential harmful effects. Results: A total of 16 superior compounds, including curcumin, curcumol and procurcumadiol, were screened. The main target genes included AGT, NR3C1, PGR, HSPA8, AR, MAOA, ADRA1B, etc. and there were 53 corresponding target compounds. The adverse reactions mainly occurred through 21 related pathways, including the substance dependent pathway, the pathways related to endocrine metabolism, the pathways related to the digestive system, and the pathways related to nucleotide metabolism, which caused addictive reactions and the potential carcinogenicity of the substances in question. Conclusion: This study establishes a foundation for discovering more superior *Curcuma longa* L. compounds and for exploring their more valuable pharmacological effects.

Keywords: *Curcuma longa* L., network pharmacology, pharmacological effects

Introduction

Curcuma longa L. is a commonly-used Chinese medicines which is derived from the dried rhizome of *Curcuma longa* L. [1, 2]. With a pungent and bitter taste, *Curcuma longa* L. has a wide range of clinical applications, mainly unblock blood, promote menstruation, and relieve pain. Previous studies have confirmed that curcumol is the main active substance in *Curcuma longa* L., and it may have corresponding pharmacological effects on multiple systems [3, 4]. However, in addition to curcumol, *Curcuma longa* L. also contains other kinds of compounds that have multi-target effects, and there are synergistic interactions among the ingredients [5, 6]. Therefore, evaluating the pharmacological effects of *Curcuma longa* L. using only a single component is not comprehensive.

Network pharmacology is a newly-emerging field. It mainly promotes the process of understanding the mechanism of drug pharmacology through biology, informatics, and pharmacology [7, 8]. Through network pharmacology, complex interactions between compounds and targets can be reflected, new pharmacological drug effects can be predicted, and potentially toxic components can be screened. A comprehensive understanding of the pharmacological effects of traditional Chinese medicine provides a good guarantee for the wide application and safety of traditional Chinese medicine. Clinical pharmacists can gather biological networks based on network pharmacology to fully understand Chinese medicine and its potential related effects, which is conducive to the overall understanding of Chinese medicine and ultimately provides new ideas and perspectives for modern medical research on Chinese medicine

[9-11]. Based on the above, this study analyzes the traditional Chinese medicine component *Curcuma longa* L. and explains its modern pharmacological mechanism using network pharmacology.

Materials and methods

The database used in this study mainly included the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), String 11.0, DAVID 6.8 and the UniPort database.

Collection of the Curcuma longa L. compounds

We mainly searched in TCMSP, entering "curcuma longa L." into the search box, and the qualifier was "Herb name". In the end, we got a total of 52 compounds containing *Curcuma longa* L. [12].

Screening of the Curcuma longa L. superior compounds

According to the TCMSP database, the ADME properties of absorption, distribution, metabolism, and excretion associated with *Curcuma longa* L. were provided, and drugs with good drug-like properties and an oral utilization were statistically obtained. Pharmacokinetics is a qualitative concept used in drug design to help optimize pharmacokinetics and drug properties, such as solubility and chemical stability, with a set value of drug-like (DL) ≥ 0.1. Oral utilization is one of the most important pharmacokinetic properties of orally administered drugs, because it plays an important role in drug delivery to the body's circulation. Oral Bioavailability (OB) values ≥ 20%.

Construction of the compound-target network

Through the TCMSP database, we could not only query Chinese herbal medicines and their active ingredients, but we could also search for the targets of their active ingredients. We did a target search for the selected superior compounds of *Curcuma longa* L. in the TCMSP database, deleted the duplicate targets, and entered each target into the UniPort database limited to the species "human" and obtained the corresponding gene of the protein target. The compound-target data was imported into Cytoscape 3.7.2 software for visualization.

Construction of the protein-protein interaction (PPI) network

The target genes corresponding to the screened superior compounds were uploaded into the String 11.0 database with a combined score ≥ 0.4 as the screening criterion. The PPI information of the *Curcuma longa* L. superior compounds was imported into Cytoscape 3.7.2 software for visualization, and the core genes with a degree ≥ 15 were further analyzed to determine the role of the target at the gene level.

GO analysis

GO analyses are often used to annotate the biological functions of genes and gene products and mainly include the following three aspects: cell component (CC), molecular function (MF), and biological process (BP) [13]. The list of *Curcuma longa* L. compound targets was imported into the DAVID 6.8 database for the GO analysis to obtain a functional level analysis of the targets, and $P < 0.01$ was considered statistically significant.

KEGG analysis

KEGG analysis is a knowledge base of gene function system analysis used to analyze the relationship between genes and their biological pathways [14]. The plug-in ClueGO of Cytoscape 3.7.2 was used to perform a KEGG pathway analysis on the targets corresponding to the selected *Curcuma longa* L. compounds, with the threshold set to "human" and the analysis type limited to "KEGG" to analyze how the targets of the curcuma longa L. compounds function through the pathways.

Results

Screening the Curcuma longa L. superior compounds

According to the threshold of OB ≥ 20% and DL ≥ 0.1, this study screened relatively good compounds in *Curcuma longa* L., including curcumol, BRN 3094585, procurcumadiol and so on, as shown in **Table 1**.

Construction of the Curcuma longa L. compound-target interaction network

It was found in this study that the *Curcuma longa* L. compound-target network contained

Table 1. The active ingredients of *Curcuma longa* L

Number	MOLID	Compound	OB/%	DL
JH01	MOL000449	stigmasterol	43.83	0.76
JH02	MOL000493	campesterol	37.58	0.71
JH03	MOL000612	(-)-alpha-cedrene	55.56	0.13
JH04	MOL000900	(5R, 6R)-5-isopropenyl-3,6-dimethyl-6-vinyl-5,7-dihydrobenzofuran-4-one	57.05	0.11
JH05	MOL000901	BRN 3094585	87.82	0.13
JH06	MOL000902	curcumol (3S,3aS,8aR)	103.55	0.13
JH07	MOL000949	-3-hydroxy-5-isopropylidene-3-methyl-8-methylene-2,3a,4,8a-tetrahydro-1H-azulen-6-one	46.11	0.16
JH08	MOL000953	CLR	37.87	0.68
JH09	MOL000960	procurcumadiol (3S, 3aS, 8aR)	69.82	0.13
JH10	MOL000961	-3-hydroxy-5-isopropylidene-3,8-dimethyl-2,3a,4,8a-tetrahydro-1H-azulen-6-one	34.49	0.13
JH11	MOL000969	dicumene (1S, 6R, 7R)	38.08	0.11
JH12	MOL000898	-4-isopropylidene-1-methyl-7-(3-oxobutyl) norcaran-3-one (1S, 4E, 10S)	34.17	0.11
JH13	MOL000921	-8-isopropylidene-1,5-dimethyl-11-oxabicyclo (8.1.0) undec-4-en-7-one	27.26	0.11
JH14	MOL000948	4-methoxy-5-hydroxybis (3S, 3aS, 8aS)	53.67	0.11
JH15	MOL000952	-3-hydroxy-5-isopropylidene-3,8-dimethyl-2,3a,4,8a-tetrahydro-1H-azulen-6-one	25.01	0.13
JH16	MOL000959	zedoarondiol	59.37	0.12

64 nodes, of which 11 nodes (JH01-JH11) were compound nodes and 53 were target nodes. The edges represented the interaction relationships between the compounds and the target site. The higher the node degree, the more important the compound or target was in the network. And the highest degree value among the compounds was JH04, which interacted with 17 target proteins. On average, each active ingredient acted on 3.5 targets, and the average number of drugs linked to each target was 2.3 cases. In addition, when studying the targets, it was found that 13 targets can interact with 3 curcumin-compounds at the same time. Finally, the study also showed that different curcumin compounds act together on the same target. The above fully demonstrates that curcumin active compounds have multiple components and multiple targets, and there are synergistic features between the two, as shown in **Figure 1**.

PPI network construction of the Curcuma longa L. targets

In the present study, it was discovered that the *Curcuma longa* L. target PPI network graph contained 58 nodes and a total of 237 edges. The obtained PPI data were imported into Cytoscape 3.7 for visualization, and the results showed that the degree of AGT was the highest. A PPI analysis was carried out to screen out the core genes with degree ≥ 15, including AGT, NR3C1, PGR, HSPA8, AR, MAOA, and ADRA1B. **Figures 2** and **3** show the details.

GO analysis of the Curcuma longa L. targets

The biological processes of the *Curcuma longa* L. targets were obtained through a GO enrichment analysis of the David database, and 66 GO items (32 biological processes, 21 molecular functions, and 13 cell components) were determined according to the principle of significance $P < 0.01$, accounting for 48.48%, 31.82%, and 19.70%, respectively. The top 10 entries are shown in **Figure 4**.

KEGG analysis of the Curcuma longa L. targets

This study analyzed the signaling pathways involved in the *Curcuma longa* L. targets using ClueGo software, where the font represented the target, the circle represented the pathway, and the edge represented the interaction relationship between the target and the pathway or the pathway and the pathway. The size of the circle was proportional to the degree value. The larger the circle, the more important the pathway was in the target-pathway network, as shown in **Figure 5**. The KEGG pathway enrichment analysis indicated that the signal pathways involved in the *Curcuma longa* L. targets mainly included substance-dependent pathways, pathways related to endocrine metabolism, pathways related to the digestive system, and pathways related to nucleotide metabolism. The pathways related to substance dependence mainly included the morphine addiction pathway, the nicotine addiction pathway, the cocaine addiction pathway, and other path-

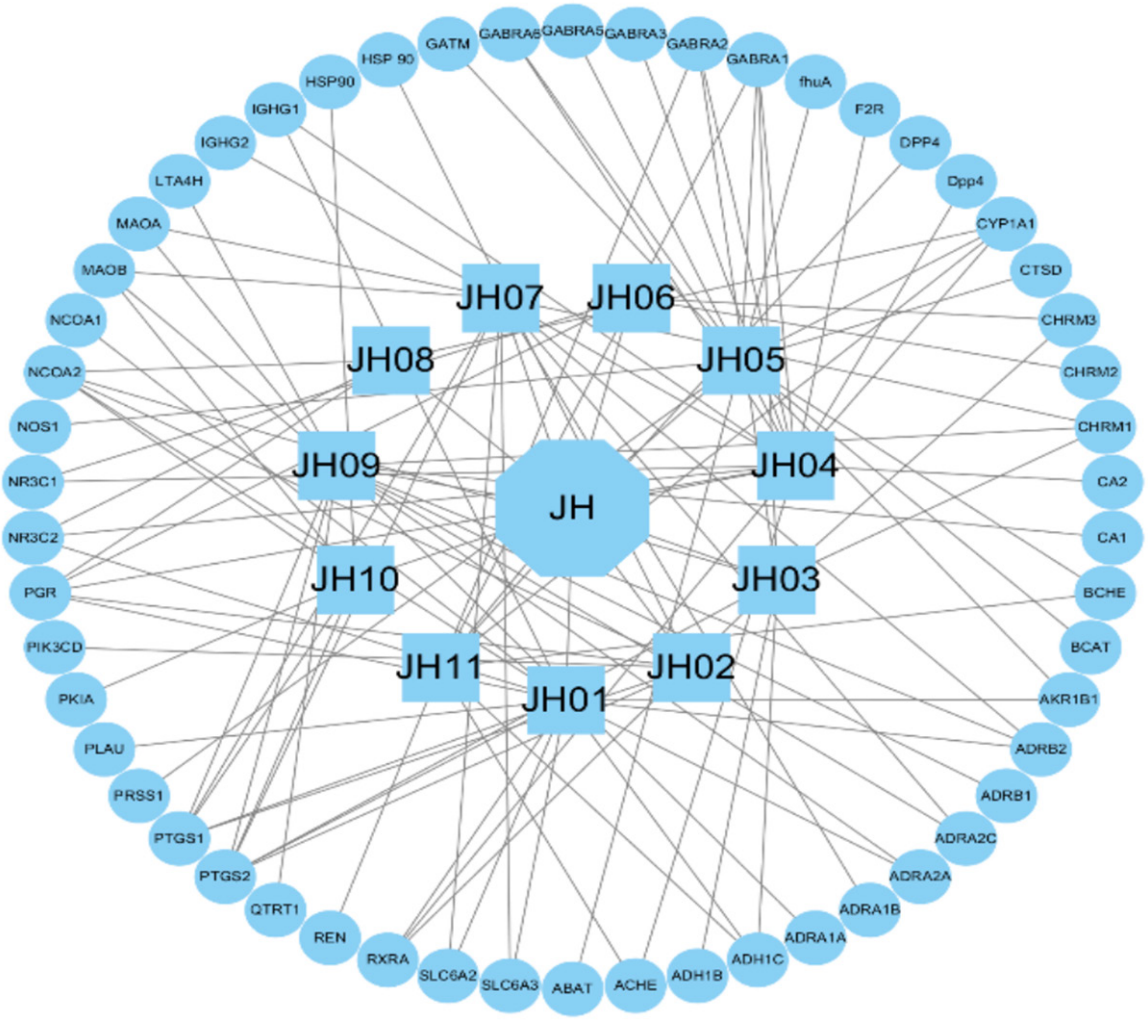

Figure 1. The *Curcuma longa* L. compound-target interaction network.

ways. The pathways related to endocrine metabolism mainly included the lipolysis regulation pathway in fat cells. See **Table 2**.

Discussion

Single Chinese medicine is composed of many components, and its chemical composition is complicated. Therefore, targeted and in-depth research on Chinese medicine is hindered. Network pharmacology can reflect to a large extent the complex interactions between biomolecules and chemical components. Screening out the more effective components of Chinese medicine through network pharmacology can provide a basis for more targeted Chinese medicine research and can clarify the characteristics of Chinese medicine. Superior compounds are also consistent with the modernization needs of traditional Chinese medicine. In order to further clarify the pharmacological mechanism of *Curcuma longa* L., the TCMSP platform was adopted in this study, and the ADME parameters OB and DL were set as thresholds to screen out the superior compounds of *Curcuma longa* L. with OB and DL. Moreover, a compound-target network was constructed to explore the effects of *Curcuma longa* L. The relationship between the superior compound and the target and the target is annotated at the gene level. It provides a research foundation for the multi-target and multi-path pharmacological action mechanism corresponding to the superior compound of *Curcuma longa* L. [15].

There are many compounds in *Curcuma longa* L., and the molecular targets obtained through

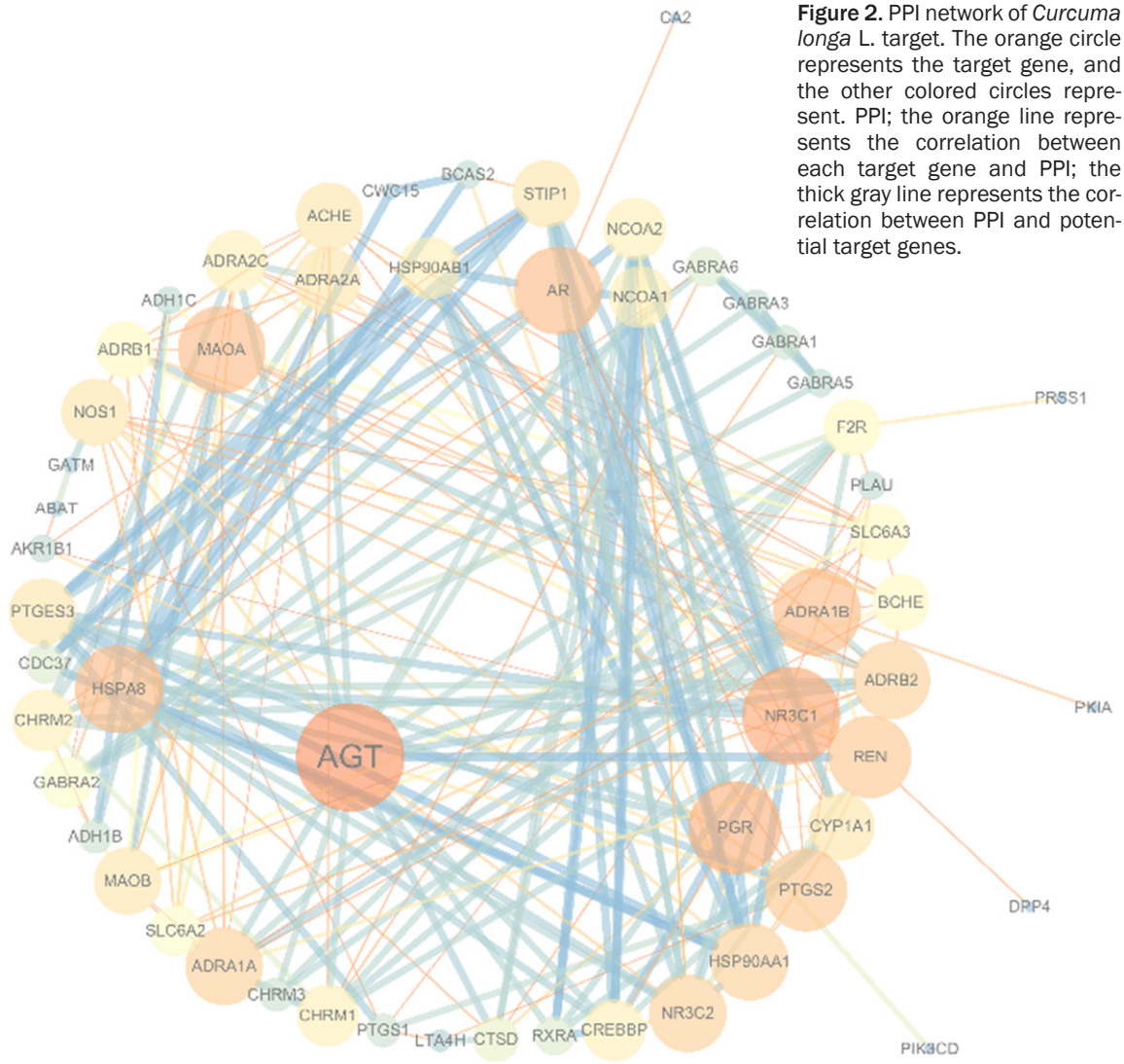

Figure 2. PPI network of *Curcuma longa* L. target. The orange circle represents the target gene, and the other colored circles represent. PPI; the orange line represents the correlation between each target gene and PPI; the thick gray line represents the correlation between PPI and potential target genes.

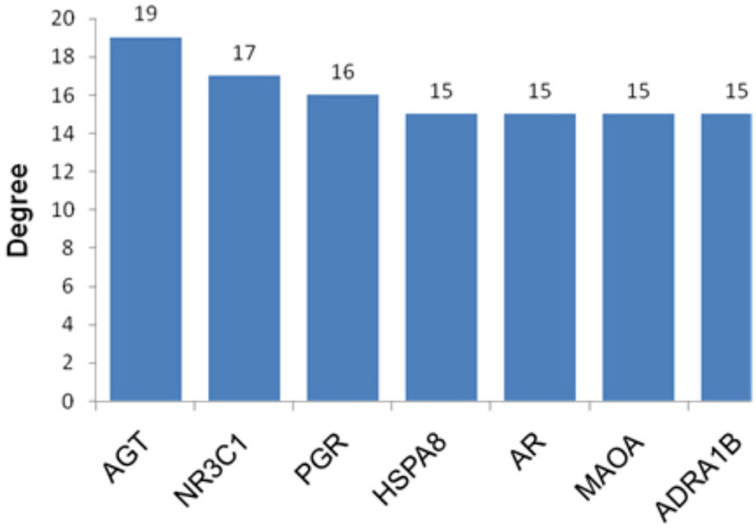

Figure 3. The core targets in the PPI network.

the screening may be the key material basis for *Curcuma longa* L. to exert its pharmacological mechanism. Studies have shown that curcumin is the main component of *Curcuma longa* L., a finding that is basically consistent with the molecular targets screened out by the TCMSP database, indicating that the accuracy of predicting the main molecular targets contained in curcumin through network pharmacology is relatively high [16]. Previous KEGG studies have shown that *Curcuma longa* L. can regulate a vari-

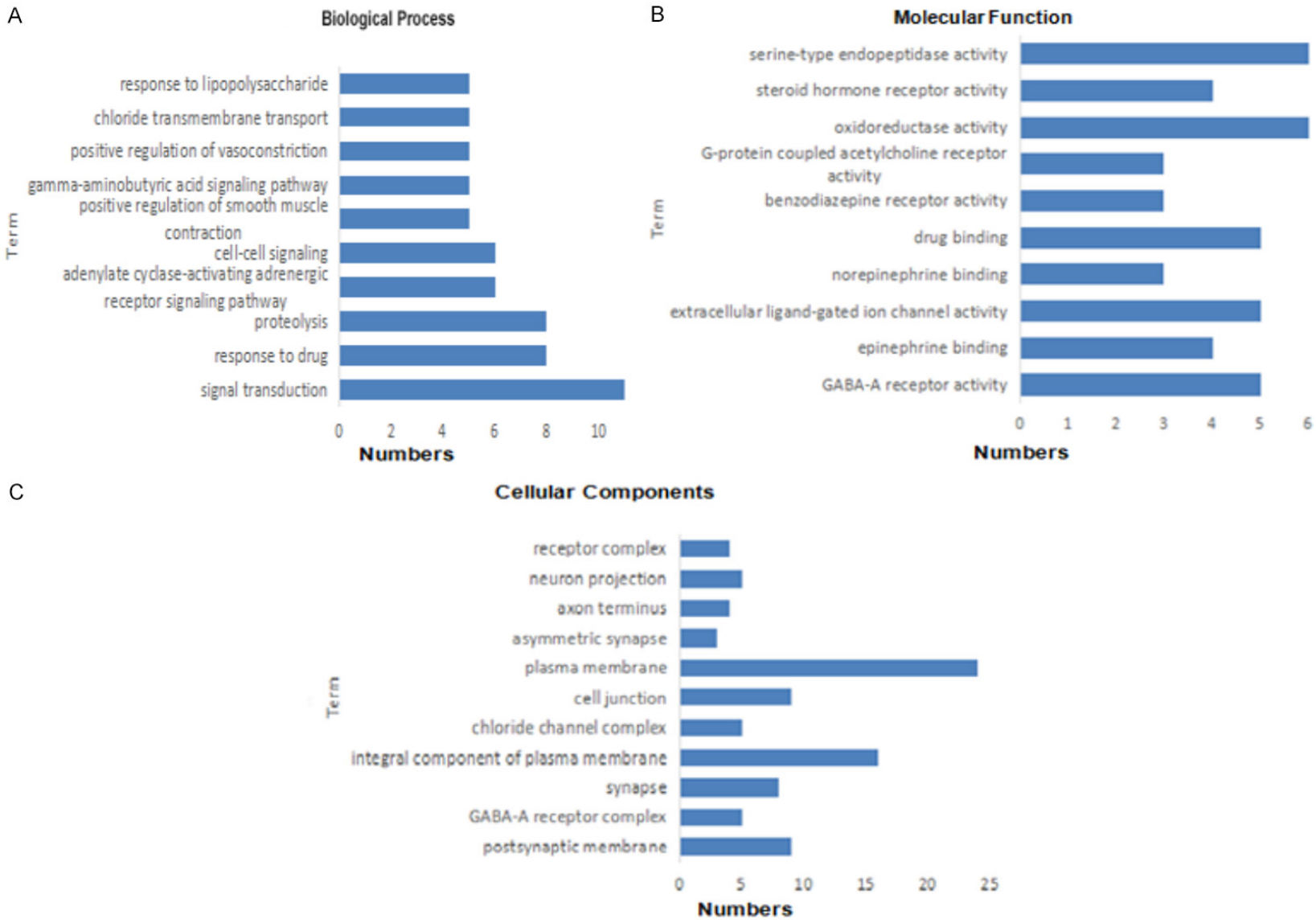

Figure 4. An analysis of the GO function of *Curcuma longa* L.'s compound target. A. The biological process of the *Curcuma longa* L. target; B. The molecular function of the *Curcuma longa* L. target; C. The cellular component of the *Curcuma longa* L. target.

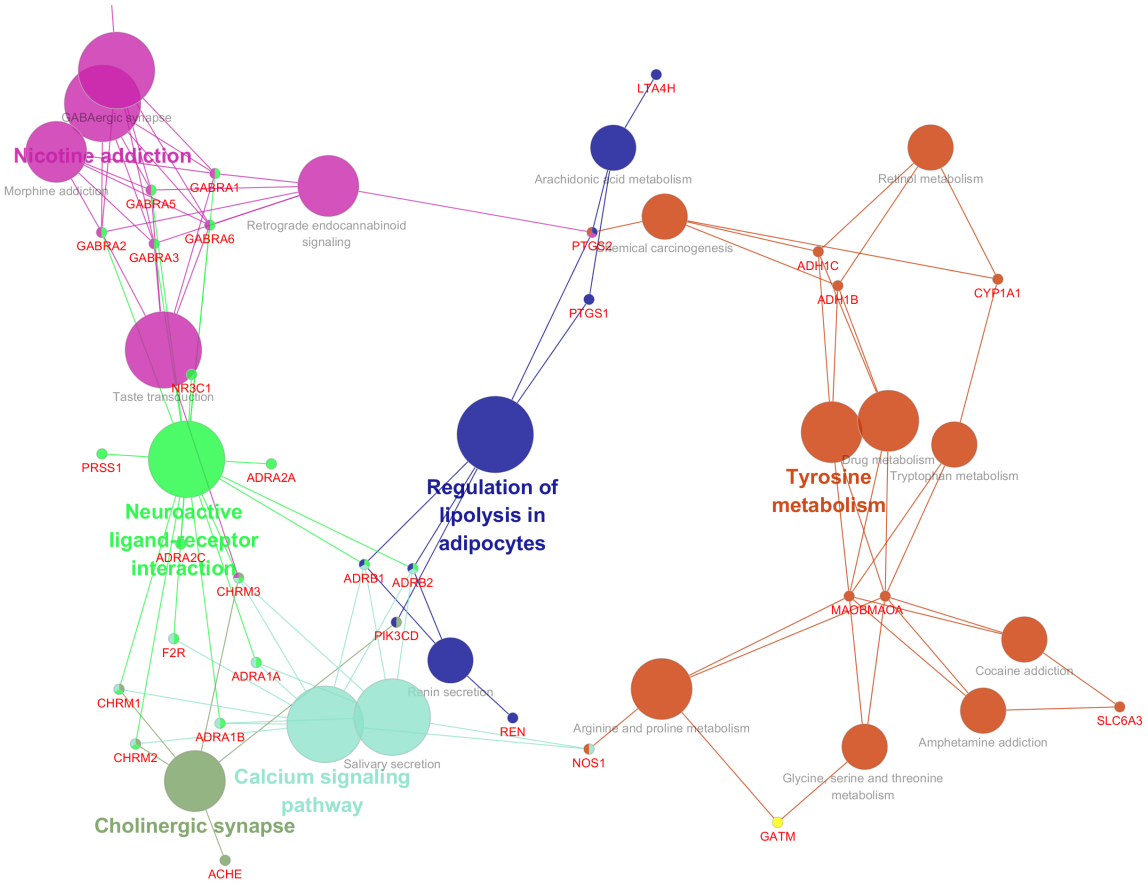

Figure 5. Target-signal pathway network diagram. The font represents the target, the circle represents the pathway, and the edge represents the common regulatory target existing between the pathways.

ety of diseases, such as tumors, reproduction, blood pressure, and so on. At the same time, our GO analysis indicated that the biological functions of *Curcuma longa* L. are mainly enriched in terms of their drug response, drug binding, signal transduction, oxidoreductase activity, cell membrane function, and other processes. The pathways are mainly enriched in the substance dependent pathways, the pathways related to endocrine metabolism, the pathways related to the digestive system, and the pathways related to nucleotide metabolism. The results of this study show that *Curcuma longa* L. participates in many of the body's physiological and pathological processes through material metabolism and endocrine metabolism, results which are consistent with the conclusions of previous studies [17].

The *Curcuma longa* L.-target PPI network map contains 58 targets, and a further analysis of the *Curcuma longa* L. targets using PPI screened seven core genes, including AGT, NR3C1, PGR, HSPA8, AR, MAOA, and ADRA1B. This suggests that *Curcuma longa* L. may play a therapeutic role in disease through the interactions of its potent compounds with selected key targets. AGT encodes angiotensinogen. Studies have shown that AGT polymorphisms are associated with the development of primary hypertension [18]. NR3C1 mainly encodes glucocorticoid receptors, which are involved in related pathophysiological processes such as inflammation and tumor cell proliferation [19]. Others, such as PGR, HSPA8, and AR, are involved in pregnancy, tumors, and male reproductive processes, respectively. MAOA is related to Bruner syndrome and antisocial personality disorder. ADRA1B has been confirmed by in vitro cell experiments to induce tumor transformation. It was further confirmed that *Curcuma longa* L. might play a role in tumors, reproduction, hormone metabolism, and other diseases by regulating these core genes or other genes in the network, a confirmation that supports the conclusions of previous studies [20, 21].

Table 2. KEGG of target

Enrichment pathway	Percentage (%)	Enriched gene
Cholinergic synapse	4.42	(ACHE, CHRM1, CHRM2, CHRM3, PIK3CD)
Neuroactive ligand-receptor interaction	5.00	(ADRA1A, ADRA1B, ADRA2A, ADRA2C, ADRB1, ADRB2, CHRM1, CHRM2, CHRM3, F2R, GABRA1, GABRA2, GABRA3, GABRA5, GABRA6, NR3C1, PRSS1)
Calcium signaling pathway	4.66	(ADRA1A, ADRA1B, ADRB1, ADRB2, CHRM1, CHRM2, CHRM3, F2R, NOS1)
Salivation	6.59	(ADRA1A, ADRA1B, ADRB1, ADRB2, CHRM3, NOS1)
Arachidonic acid metabolism	4.76	(LTA4H, PTGS1, PTGS2)
Regulation of lipolysis in fat cells	8.93	(ADRB1, ADRB2, PIK3CD, PTGS1, PTGS2)
Renin secretion	4.35	(ADRB1, ADRB2, REN)
Retrograde endocannabinoid signaling	4.05	(GABRA1, GABRA2, GABRA3, GABRA5, GABRA6, PTGS2)
GABAergic synapse	6.74	(ABAT, GABRA1, GABRA2, GABRA3, GABRA5, GABRA6)
Taste transduction	7.23	(CHRM3, GABRA1, GABRA2, GABRA3, GABRA5, GABRA6)
Morphine addiction	5.49	(GABRA1, GABRA2, GABRA3, GABRA5, GABRA6)
Nicotine addiction	12.50	(GABRA1, GABRA2, GABRA3, GABRA5, GABRA6)
Metabolism of glycine, serine and threonine	7.50	(GATM, MAOA, MAOB)
Arginine and proline metabolism	8.00	(GATM, MAOA, MAOB, NOS1)
Tyrosine metabolism	11.11	(ADH1B, ADH1C, MAOA, MAOB)
Tryptophan metabolism	7.14	(CYP1A1, MAOA, MAOB)
Retinol metabolism	4.48	(ADH1B, ADH1C, CYP1A1)
Drug Metabolism-Cytochrome P450	5.56	(ADH1B, ADH1C, MAOA, MAOB)
Cocaine addiction	6.12	(MAOA, MAOB, SLC6A3)
Amphetamine addiction	4.35	(MAOA, MAOB, SLC6A3)
Chemical carcinogenesis	4.82	(ADH1B, ADH1C, CYP1A1, PTGS2)

In summary, this study has made a preliminary exploration of the pharmacological compounds, targets, and pathways of *Curcuma longa* L. with the help of network pharmacology, which provides a theoretical basis for the potential pharmacological effects of *Curcuma longa* L., its targets and pathways, but further experiments are still needed to verify the pharmacological effects of *Curcuma longa* L. In addition, we also found that *Curcuma longa* L. may be able to regulate substance addiction, such as cocaine addiction and amphetamine addiction, a finding that needs to be confirmed by further experiments.

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Disclosure of conflict of interest

None.

Address correspondence to: Wentan Xu, Department of Pharmacy, Jinjiang Hospital, No. 392 Xinhua Road, Quanzhou 362200, Fujian Province, China. Tel: +86-13505903720; E-mail: xuwentan-17qz@163.com

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