A network pharmacology-based analysis to distinguish the differences between Lonicerae Japonicae Flos and Lonicerae Flos

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Research

Abstract

Multiple basal plants are commonly used as materia medica in the traditional medicine of various nationalities and ethnicities worldwide. We call this practice "multibasal-plant materia medica" (MBPMM). So we proposed the application of network pharmacological method that it can provide a new way of distinguishing the differences among the different basal plants used in traditional medicines. We apply the method in investigating the differences and similarities in the material bases and mechanisms of anti-inflammatory activities of Lonicerae Japonicae Flos and Lonicerae Flos. Lonicerae Japonicae Flos and Lonicerae Flos share plenty of similarities in terms of anti-inflammatory mechanisms and material bases. Both of them mainly act on airway inflammation and tumour inflammation via the NF-κB signalling pathway and immune response, oxidation and signal transduction. However, Lonicerae Flos acts on inflammation with greater intensity than Lonicerae Japonicae Flos. We argue that they can be used interchangeably for the prevention and treatment of tumours and airway inflammation at a proper dosage. Otherwise, Lonicerae Flos may be more appropriate for treating neurological and metabolism-related inflammation, whereas Lonicerae Japonicae Flos is more suitable for the treatment of inflammation of systemic organs, such as intestines.

1. Introduction

Traditional medicine is a valuable experience that people have gained in their struggle against nature and diseases. People derive benefits from traditional medicines, such as artemisnin and reserpine [1, 2]. As one of the three systems of traditional medicine, traditional Chinese medicine (TCM) has been an important part of health care systems in many countries, especially in Asia. TCM has good clinical treatment and disease-prevention effects; thus, it has gained widespread recognition worldwide[3].

The use of "multibasal-plant materia medica" (MBPMM) is a widely accepted practice in TCM. MBPMM is recognised in various pharmacy books and health literature, such as the Pharmacopoeia of the People's Republic of China[4]. However, different species have different chemical compositions that exert different pharmacodynamics. For example, Asari Eadix Et Rhizoma is a typical TCM that includes three basal plants, two of which are *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag. and *A. sieboldii* Miq. Experiments demonstrated that *A. heterotropoides* var. *mandshuricum* has a stronger anti-inflammatory activity than *A. sieboldii* Miq[5].

What are the differences in the pharmacological properties of different basal herbs, and under what conditions can they be used interchangeably? This scientific question is crucial in TCM. In the past, the chemical composition of the original herbal plant or the corresponding pharmacological experiment is used for comparison. The difference between Lonicerae Japonicae Flos (LJF) and Lonicerae Flos (LF) is a typical representative of such issues. For hundreds of years, these herbs have been considered just one drug until the 2005 edition of the Pharmacopoeia of the People's Republic of China were separated them as two materia medicas. Thereafter, the two herbs can no longer be used interchangeably. This change has sparked heated debates among researchers concerning the problems with MBPMM.

The base plant of LJF is *Lonicera japonica* Thunb., which is mainly found in northern Chinese provinces, such as Shandong, Shaanxi and Henan. The main source plant of LF is *L. macranthoides* Hand.-Mazz. In addition, LF also include *L. hypoglauca* Miq., L. *confuse* DC. and *L. fulvotomentosa* Hsu et S.C. Cheng. These plants are mainly found in Sichuan, Hunan and Guangdong Provinces in southern China[6, 7]. These basal plants belong to the same genus. The two herbs are identical in nature and flavour xiong, meridian tropism, directions and dosage. They can treat sores, rooted sores, wind−heat common cold and weakness (Chinese Pharmacopoeia Commission, 2015). In modern pharmacology, both LJF and LF have anti-inflammatory, antibacterial, antiviral, antioxidant and hepatoprotective pharmacological effects[8−11]. However, several studies have indicated that LJF and LF have different pharmacological effects. LJF has a wider range of antimicrobial activity than LF. The LD_{50} values of the two herbs are not considerably different, but the potential toxicity of LF may be greater than that of LFJ because of the former's hypersensitivity reaction[12]. At present, comparative studies on LJF and LF are reductionist and mostly conducted in terms of the content of their characteristic components or specific pharmacological activities.

The network pharmacology approach is based on systems biology and multidirectional pharmacology. This approach constructs the relationship between active compounds and the organism from the perspective of component−target−pathway−disease. This approach is mostly used to explain the characteristics and mechanisms of multicomponent, multitarget and multipathway actions in TCM[13]. This approach has been successfully adopted in TCM research[14]. In this study, the network pharmacology method was employed to investigate the differences between LJF and LF.

We adopted the network pharmacology method to compare the differences and similarities in the pharmacological effects of LJF and LF. The results provided a reference for the combined use of these herbs. Specifically, we examined the anti-inflammatory pharmacological activities of LJF and LF and evaluated whether they can be used as substitutes for each other. This study provides new perspectives for research and addressing similar problems in TCM.

2. Method

2.1. Establishment of Chemical Composition Database

We retrieved information on the chemical compositions of LJF and LF from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform and The Encyclopaedia of Traditional Chinese Medicine[15, 16]. These databases are a repository of chemical information of traditional Chinese herbs. Moreover, we collected additional information about these herbs from the literature[17, 18]. Then the free chemical information online website Chemspider[19] and PubChem has been used to translate and check the file type database[20]. Finally, MOE 2019 software was been choosen to merge the data and establish a database.

2.2. Target Fishing

Most TCM compounds exert corresponding biological functions by acting on protein targets, which simultaneously induce a series of physiological change. Identifying the targets is meaningful to understand the mechanism of a compounds' action. In this study, we employed the SEAware and Swiss Target Prediction webserver for the target fishing of the compounds we collected[21]. The target fishing methods we performed were based on 'similarity hypothesis' of similarity among small molecules. This hypothesis states that compounds with similar structures have similar physical and chemical properties and biological activities. The activity and targets of unknown molecules can be predicted by comparing the small molecules with activity data[22]. We calibrated the names of the targets to the official gene name through the Uniprot database[23].

2.3. Construction of Disease Target Database

We consulted several databases for information on inflammation-related targets. Genecard database assembles data from 150 websites and provides comprehensive genomic, proteomics, genetic, clinical and functional information[24]. We used the keywords 'inflammation' and 'inflammatory' in the retrieval, and we collected the genes with high scores (≥ 10) to build a database of targets related to inflammatory diseases. Finally, we integrated the targets from target fishing with the disease target database, and then the overlapping parts as the inflammation-related targets of LJF and LF were selected.

STRING[25] was used to obtain PPI network data of LJF and LF with high credibility score (≥ 0.7), and then these data were putted into Cytoscape[26]. We used Degree to indicate the number of connections between a node and other nodes and Betweenness Centrality to reflect the value of the bridge centrality of the node. These parameters are crucial indicators to screen, obtain and compare the core target relationship network of two Chinese medicines[27].

2.4. Enrichment Analysis

We used the Database for Annotation, Visualisation and Integrated Discovery[28] to analyse the Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) of inflammation-related genes. GO enrichment analysis included biological process (BP), molecular function (MF) and cellular component (CC). We determined and compared the anti-inflammatory mechanisms of LJF and LF.

2.5. Comparison of Key Compounds

Using degree as reference values, we compared the key compounds that act on the anti-inflammatory targets of LJF and LF on the basis of their types and related features.

3. Results

3.1. Collection of Compounds and Targets

The databases of LJF and LF contained 243 and 200 compounds, respectively, 66 of which were common to both herbs (Supplementary Table 1). A total of 207 targets for LJF and 198 targets for LF were obtained as potential targets after screening and weight reduction, of which 176 were the same. A

total of 1,006 genes related to inflammation were collected in GeneCards. We integrated the compound and inflammation targets to obtain the common targets with inflammation. Fifty-seven targets were identified from both LJF and LF, 49 of which were shared by them (Fig. 3).

3.2. Analysis of Key Target Network

We introduced the inflammation targets of the two herbs to STRING. To illustrate the strength of the correlation between proteins, we acquired two PPI maps after setting the confidence level higher than 0.7. After filtering using the cut-off value, the PPI of LJF consisted of 44 nodes with 90 edges, whereas that of LF contained 48 nodes with 105 edges. The key targets were screened by analysing the values of Degree and Betweeness Centrality of each target in the respective networks and by using their medians as thresholds (Fig. 4). Results showed that LJF and LF had nine core targets. Six common targets, namely, APP, VEGFA, MMP9, EGFR, PPARG and ALOX5, were found. Information on the 12 target genes is listed in Table 1.

Table 1
Information on core targets.

Gene ID	Gene Name	Source	Degree	Betweenness Centrality
APP	Amyloid-beta precursor protein	LJF	13	0.445
		LF	12	0.347
VEGFA	Vascular endothelial growth factor A	LJF	13	0.268
		LF	11	0.190
MMP9	Matrix metalloproteinase-9	LJF	11	0.245
		LF	11	0.142
ALOX5	Arachidonate 5-lipoxygenase	LJF	6	0.185
		LF	9	0.057
EGFR	Epidermal growth factor receptor	LJF	9	0.116
		LF	8	0.119
PPARG	Peroxisome proliferator-activated receptor gamma	LJF	6	0.097
		LF	8	0.075
CASP3	Caspase-3	LJF	8	0.123
CNR1	Cannabinoid receptor 1	LJF	6	0.095
MPO	Myeloperoxidase	LJF	5	0.051
RELA	Transcription factor p65	LF	8	0.100
PTGS2	Prostaglandin G/H synthase 2	LF	16	0.294
ESR1	Estrogen receptor	LF	7	0.068

3.3. Enrichment Analysis Results

GO and KEGG data were obtained via David database enrichment analysis, with P-value ≤ 0.05 and Benjiamini ≤ 0.5 as the screening conditions. The P-value in the statistical analyses represented the probability of getting GO/pathway term error. Benjiamini value was used to globally correct the enrichment P-value of individual term members. Therefore, we were able to outcrop more accurate LJF and LF terms through the two condition pairs. A total of 135 GO terms were obtained from LJF, 16 of which were for CC, 83 for BP and 36 for MF. By comparison, 163 GO terms were obtained for LF, 20 of which were for CC, 70 for BP and 30 for MF. A total of 114 terms were common for both herbs, of which 14 were for CC, 70 for BP and 30 for MF. A comparison of the top 20 terms between LJF and LF in each GO term is shown in Fig. 5.

LJF and LF mainly act in extracellular space, including lysosomes and other cell components, for inflammation. The targets of LJF were located on the external side of plasma membrane, including pivotal proteins with anti-inflammatory properties, such as ApoA-I[29]. The I-κB/NF-κB complex was found to be more relevant to LF than to LJF.

In MF, the two drugs specifically have RNA polymerase II transcription factor activity and combine various molecular functions, including enzyme, chromatin binding, transcription factor and other molecular functions. Furthermore, LJF has special functions related to chemokine receptor activity, whereas LF has peroxidase activity. We analysed the involved biological processes and divided the co-participatory processes into five categories: apoptosis and proliferation (GO: 0022617, GO: 0043066), direct inflammation (GO: 0006954, GO: 0019369), immunity (GO: 0019372), oxidation (GO: 0055114) and signal transduction (GO: 0070374). These processes are directly or indirectly related to the development of inflammation. A total of 11 and 18 pathways of LJF and LF, respectively, were screened by KEGG pathway, of which nine terms were the same for both herbs. Two 'target-path' combination networks of LJF and LF were constructed (Fig. 6). The circle represents the corresponding anti-inflammatory target, whereas the triangle denotes the enrichment pathway. Nodes depicted in blue indicate the unique targets/pathways of LJF and LF. LF showed more characteristic pathways than LJF. The targets of LJF and LF in the same pathway were not exactly the same. The pathways common to both herbs included arachidonic acid metabolism, inflammatory mediator regulation of TRP channels and tumour-related pathways, which are closely related to inflammation. In addition, LF has a unique TNF signalling pathway, and HIF-1 signalling pathway is related to inflammation.

3.4. Comparison of Compounds

We analysed the compounds of LJF and LF and their relevant anti-inflammatory targets. After that, eight and seven key compounds from LJF and LF has been obtained, respectively. These compounds could be divided into flavonoids or organic acid. Three groups of LJF and LF compounds, namely, J185 and S144, J179 and S141 and J180 and S142, have the same structures (Fig. 7).

Due to technical limitations, Table 2 is provided in the Supplementary Files section.

The compounds corresponding to anti-inflammatory targets were classified according to the main types of these herbs. As shown in Fig. 8, the class of each compound (organic acids, triterpenes and others) was compared, showing that the structures of these compounds only slightly differ. The quantity and types of flavonoids in LJF were more plentiful than those in LF.

4. Discussion

Researchers debate the differences in pharmacological effects of LJF and LF. Both claims are supported by experimental data. Existing comparative studies are incomplete and not systematically designed. In the present study, we adopted a network pharmacology approach to compare the differences in

pharmacological mechanisms of two basal plants with anti-inflammatory activity. We hope to provide a reference for the comparative study of LFJ and LF.

We employed a common screening criterion to compare the protein–protein interaction network of LJF and LF. Among the nine core genes obtained, six were common in both herbs, accounting for 66.7%. We investigated each gene separately (Table 3). Results showed that both LJF and LF mainly act on airway inflammation and tumour-induced inflammation. Both herbs were mainly involved in the NF-κB signalling pathway for anti-inflammatory effects. The extract of LF blocks the activation of the NF-κB inflammatory signalling pathway by inhibiting IκBα phosphorylation with NF-κB p65 and IκBα degradation[30]. The extract of LJF substantially reduces p50 and IKK expression levels on the NF-κB pathway[31]. Therefore, LJF and LF act synergistically on the NF-κB signalling pathway but under different mechanisms. LJF has a considerable effect on ovalbumin-induced asthma in a rat model[32]. By contrast, research on LF is few and there is no study has directly demonstrated that whether the LF have an proportional effect on asthma or not. According to the results of the analysis of specific genes, LF is more correlated with the NF-κB pathway and has an effect on neuro-inflammation, whereas LJF is more biased towards systemic inflammation, such as enteritis, pneumonia and inflammation by microbial infection.

Table 3
Information on core targets in relation to anti-inflammatory genes.

Attributes Of The Gene Target	Gene Name	Inflammatory-Related Mechanisms/Etiology/Disease
Common	APP	Activates individual nuclear phagocytes in the brain and causes an inflammatory response Activated TLR4 signaling;
Common	VEGFA	Airway inflammation such as asthma[33]; Tumor-induced inflammation;
Common	MMP9	Airway inflammation[34] Suppression of malignancy through inflammation[35];
Common	EGFR	Possible activation of NF-kappa-B signal; Airway inflammation[36];
Common	PPARG	Enteritis Tumor-induced inflammation[37] Inhibits NF-κ-B-mediated proinflammatory response;
Common	ALOX5	Asthma Inhibits NF-κ-B-mediated proinflammatory response;
LF	PTGS2	Regulates inflammatory prostaglandins NK-kβ-related[38] neuro-inflammation[39];
LF	RELA	Activated TLR4 signalling; NF-κB Signaling[40]
LF	ESR1	Insulin resistance [41]; Inhibition of NF-kapa-B-mediated IL6; promoter transcription
LJF	CASP3	Inflammation of tumors[42] Indirectly related to downstream NF-kβ;
LJF	CNR1	inflammation of the intestines[43];
LJF	MPO	Inflammation caused bymicroorganisms[44] inflammation of the lungs[45];

GO and KEGG enrichment analyses of the gene targets revealed that both LJF and LF exert anti-inflammatory biological activity in a multitargeted multipathway manner. The targets of both herbs are mostly distributed in the extracellular gap and involve five types of biological processes and associated inflammatory pathways. Various substances, such as cyclophilin A and exosomes, are associated with inflammation in the extracellular gap. Exosomes play an important regulatory role in the development of chronic inflammatory airway disease[46]. Among the five major biological processes, apoptosis and aberrant proliferation are the most important processes and features of tumorigenesis, such as extracellular matrix decomposition (GO: 0022617), which breaks down extracellular matrix and multiple cytokines that affect cell survival and regulate cell proliferation, thereby avoiding acute or chronic inflammatory damage caused by tumours. Thus, LJF and LF are closely associated with tumours and inflammation, a conclusion that is consistent with the results of core target analysis. Two main aspects are involved in the immune process, namely, the inflammation caused by immune abnormalities and the immune response that accompanies the inflammatory response. The main pathological response of the former is autoimmune disease and tumour-associated inflammatory response. LJF and LF can exert anti-inflammatory effects by regulating the root cause of inflammation, that is, immunity. In the latter, LJF and LF assist in decreasing the inflammatory response by activating or enhancing the immune response by releasing anti-inflammatory factors. Both oxidation and signal transduction are also directly related to the occurrence and regulation of inflammation. These features are the similarities in anti-inflammatory mechanisms found in the GO and KEGG enrichment analyses of LJF and LF. These similarities can be visualised from the bubble and network diagrams. Their GO entries are mostly the same, although the mechanism of LJF and LF is different.

When it comes to the pathway of LJF and LF, here are some similarities. Figure 8 is one of the representatives. But more often, the GO results shows LJF and LF are not involved in exactly the same targets, such as inflammatory response (GO: 0006954) and Fig. 6. Hence, LJF and LF probably act in different ways on the same pathway and end up acting at different intensities because of the different correlations between the targets. In addition, given that LF contains more anti-inflammatory-related entries, we analysed the characteristic targets of the source of this phenomenon. LF has more characteristic targets of action in the immune pathways than LJF, and both are neurologically involved or related to fat metabolism[47, 48]. We inferred that the anti-inflammatory effects of LF are more intense than those of LJF, as well as targeted more to neurological and metabolic aspects of inflammation. However, no relevant studies are available to support this conjecture, and pharmacological experiments should validate these results.

We further analysed the material basis of the anti-inflammatory activity of LJF and LF. We found many similarities and differences. The types of central compounds of both LJF and LF are flavonoids and organic acids, which are the main compounds that exert anti-inflammatory effects. Flavonoids are recognised for their anti-inflammatory activity, and the organic acids in LJF and LF have been shown to be the main anti-inflammatory components[49–51]. Moreover, three other flavonoids, namely, luteolin, quercetin and kaempferol, were identical in the central compound. These compounds are reportedly the major components of LJF (in large amounts) and have clear anti-inflammatory activity[52, 53]. The

results revealed the similarity in substance bases of the two herbs and demonstrated the effectiveness of the screening method. The pharmacological activities of the other central compounds of LJF and LF are not reported in the literature. Hence, future studies should investigate these aspects. Although the compounds are different, their biological activities are similar because their structures are similar. This condition leads to a large target repetitive rate. With regard to compounds that correspond to common targets associated with inflammation, LJF has more flavonoids than LF. The other chemical types were not substantially different. The content of chlorogenic acid analogues in LF is substantially higher than that in LJF[54]. We hypothesise that the material differences between LJF and LF are mainly due to considerably different flavonoids and organic acids.

This study is a new application of the network pharmacology method in the field of traditional medicine. This method provides a new avenue for solving the problem of variability between different basal plants used in traditional Chinese medicine and offers a theoretical basis for their clinical use. Traditional methods for comparing medicinal plants are mainly based on chemical compositions or specific pharmacological experiments. A comparison of medicinal plants via a network pharmacology approach is more comprehensive and can delve deeper into target groups, pathways, target diseases and drug efficacy. Compared with traditional chemical methods, this method is more efficient and instructive. Explicit pharmacological experiments based on reliable theoretical analysis can greatly improve the efficiency of the research. This method of comparison can also be extended to investigations of the efficacy of prescriptions or other nonsingle compound preparations.

5. Conclusion

In this study, the mechanism of action of two Chinese herbal medicines and the material basis of their anti-inflammatory effects were investigated via a network pharmacology approach. The targets of LJF and LF were predicted. LJF and LF were found to have many similarities in terms of mechanisms and material bases. Both them mainly act on airway inflammation and tumour inflammation and influence the NF-κB signalling pathway. Their mechanisms involve five types of biological processes, such as apoptosis and proliferation, direct inflammation-related, immune response, oxidation and signal transduction. Given that LF has a richer material base, it may act on inflammation with greater intensity than LJF. Therefore, we suggest that LJF and LF can be substituted for each other for the prevention and treatment of tumours and airway inflammation at an appropriate dosage. LF is likely more appropriately used in neurological and metabolism-related inflammation, whereas LJF is more suitable for the treatment of inflammation of systemic organs, such as intestines and lungs. The results provide a reference for addressing the problems with MBPMM. Network pharmacology may be adopted to study similar issues.

Abbreviations

MBPMM
Multi-basal-plants materia medica

TCM
Traditional Chinese medicine
LJF
Lonicerae Japonicae Flos
LF
Lonicerae Flos
GO
Gene ontology,
BP
Biological process
MF
Molecular function
CC
Cellular component

Declarations

Availability of data and material: The datasets used during the current study are available from the corresponding author upon reasonable request.

Patient consent for publication: Not applicable.

Ethics approval and consent to participate: Not applicable.

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Competing interests: The authors declare that they have no competing interests.

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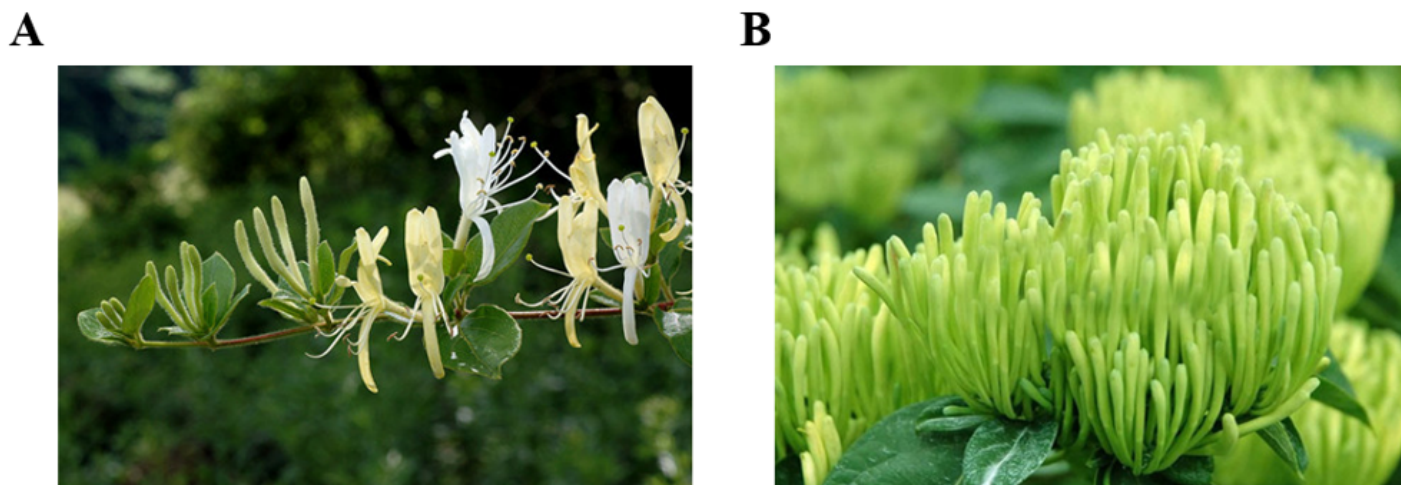
Figures

Figure 1

Images of LJF and LF plants A. Lonicera japonica Thunb.; B. L. macranthoides Hand.-Mazz.
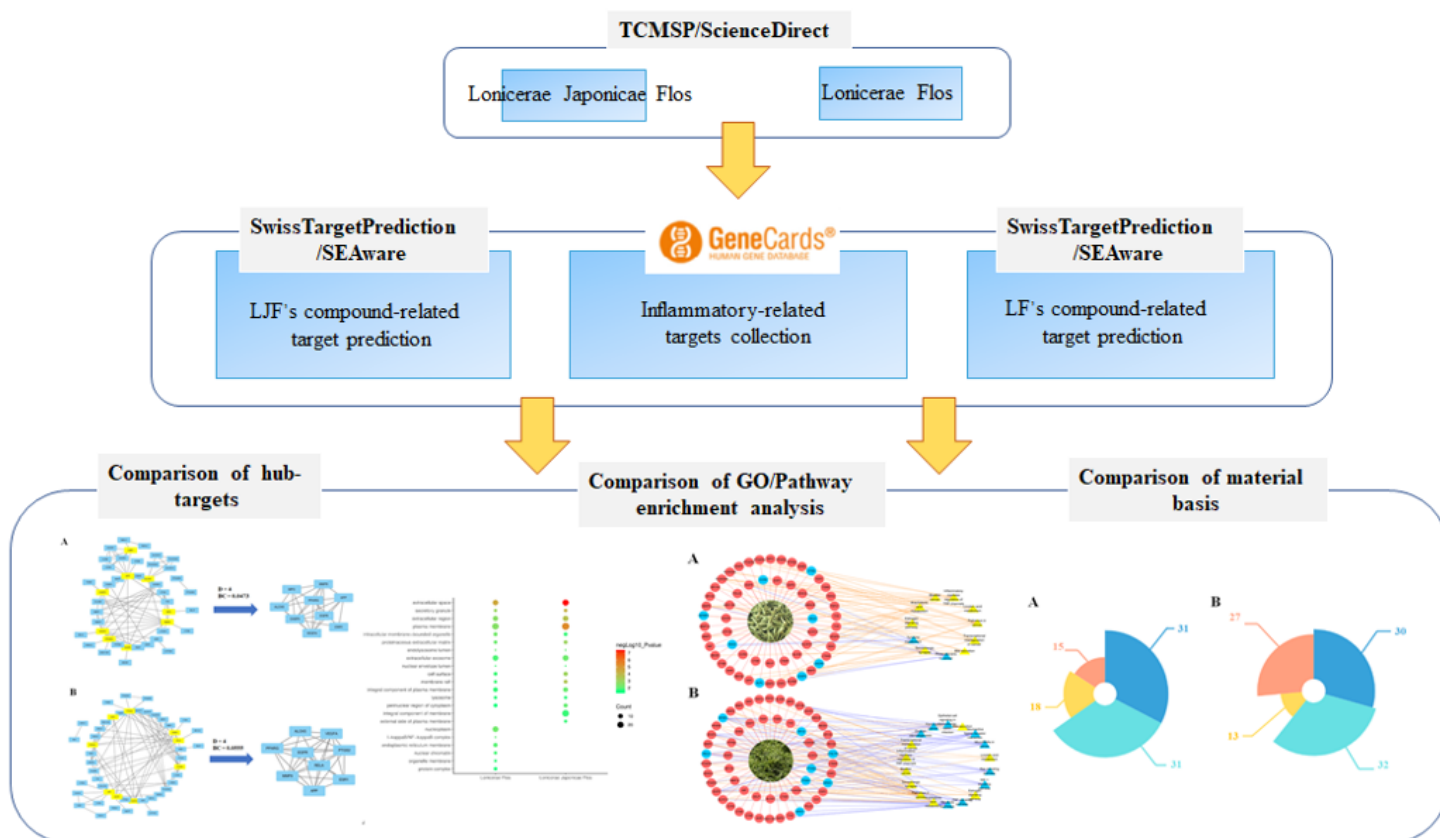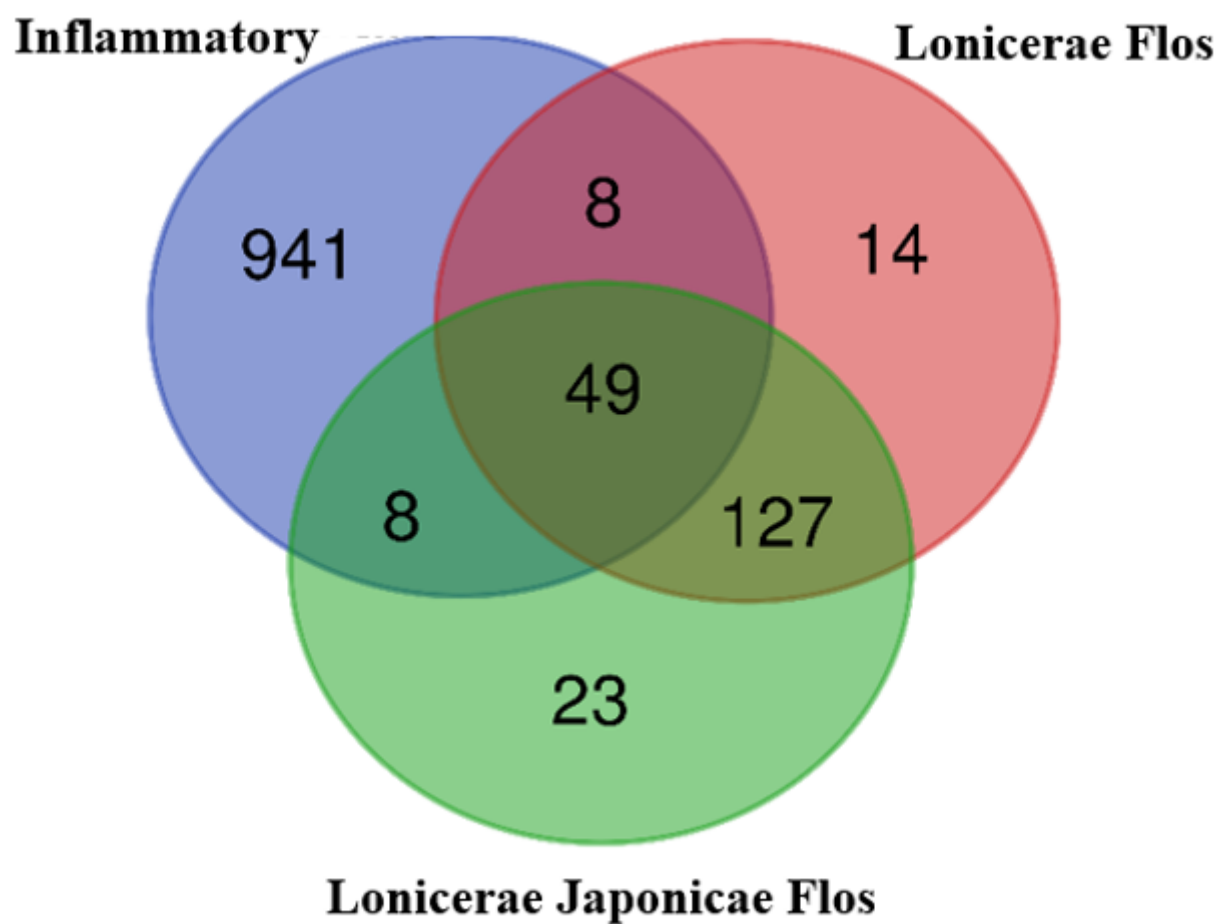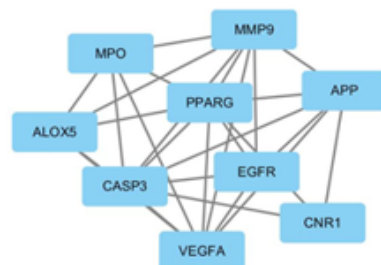

Figure 2

Research flow chart of the study

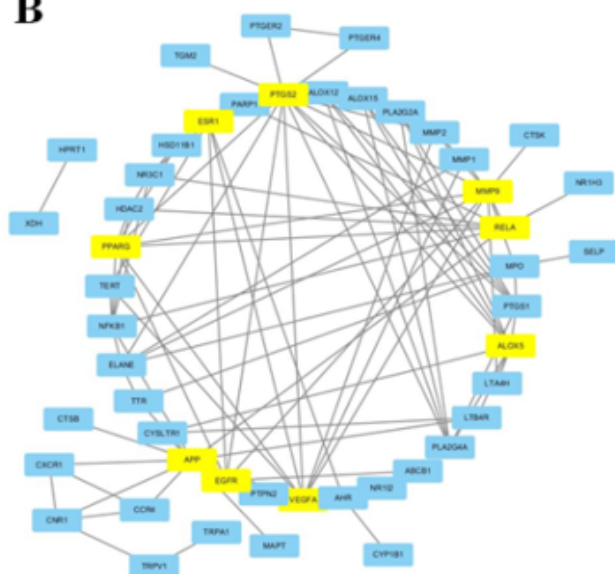**Figure 3**

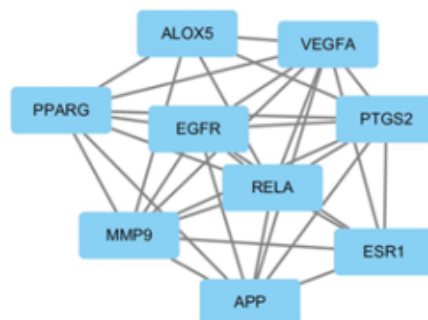Venn diagram of the targets relationship.

Figure 4
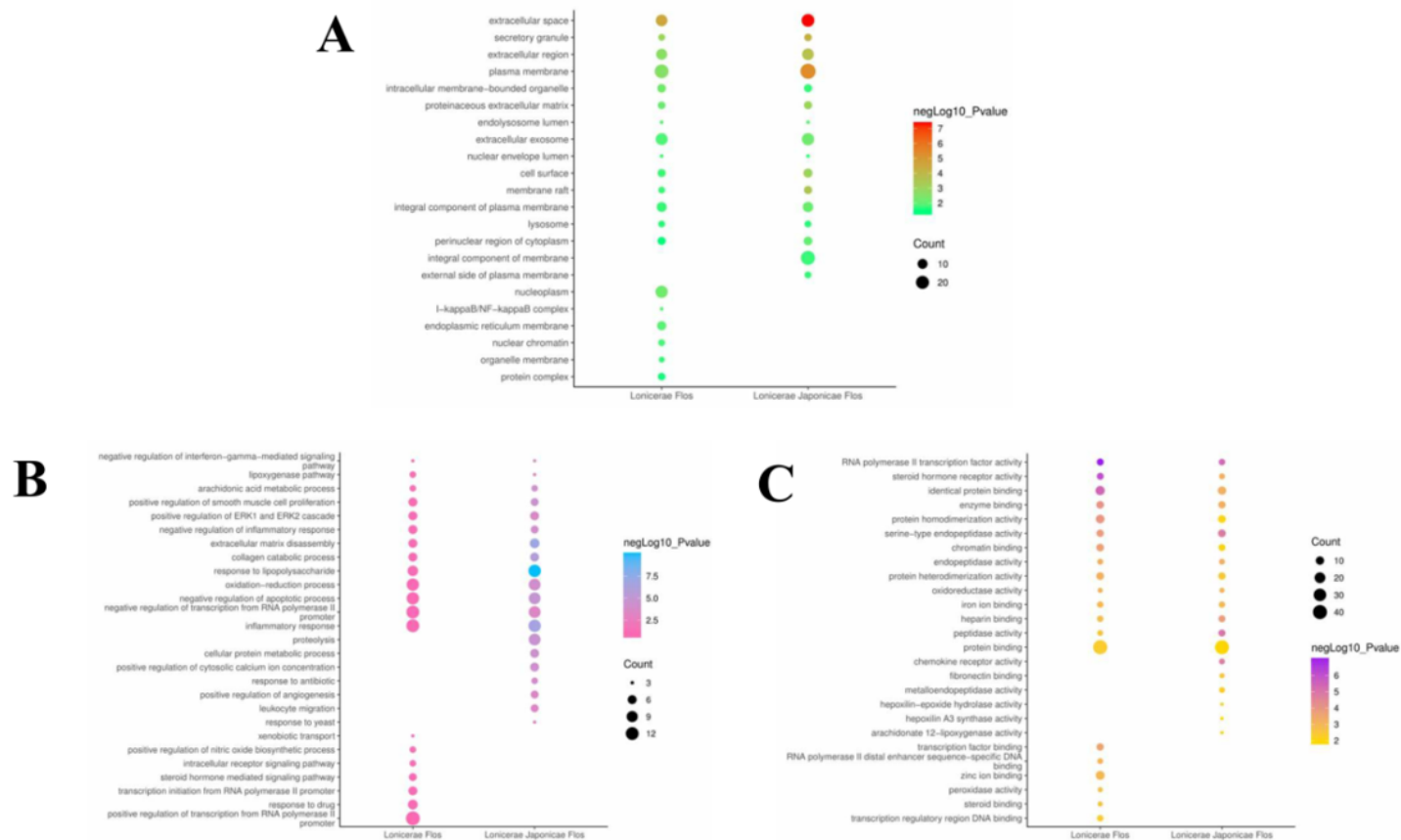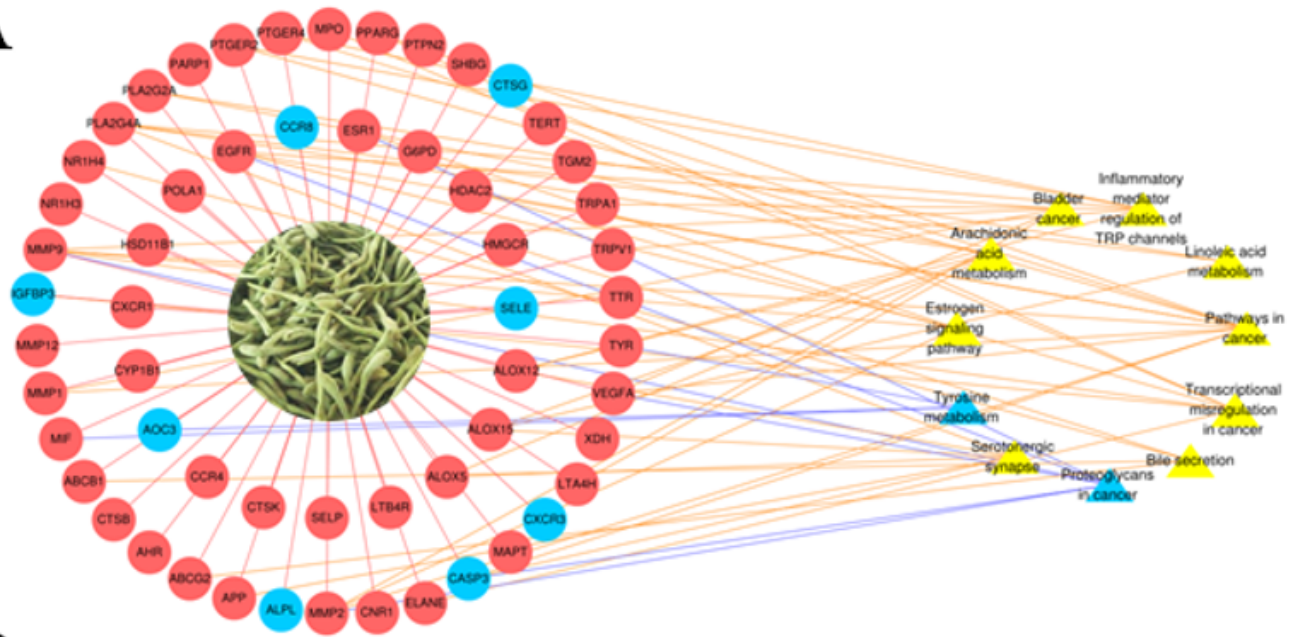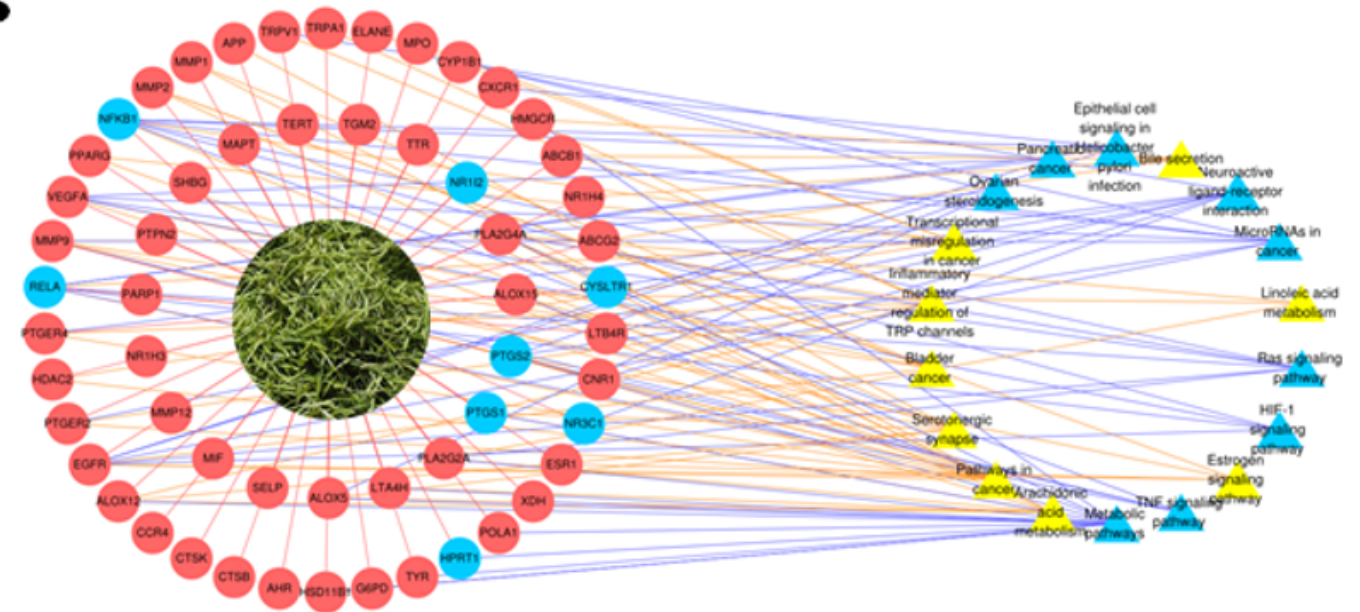
Network maps of targets and core targets. A. LJF; B. LF.

Figure 5

LJF and LF's comparison of GO terms. A. CC; B. BP; C. MF.

Figure 6

'Target-pathway' network diagram combination of LJF and LF. Blue nodes indicate the unique targets/pathways of LJF and LF. Red and yellow nodes denote the same targets/pathways.
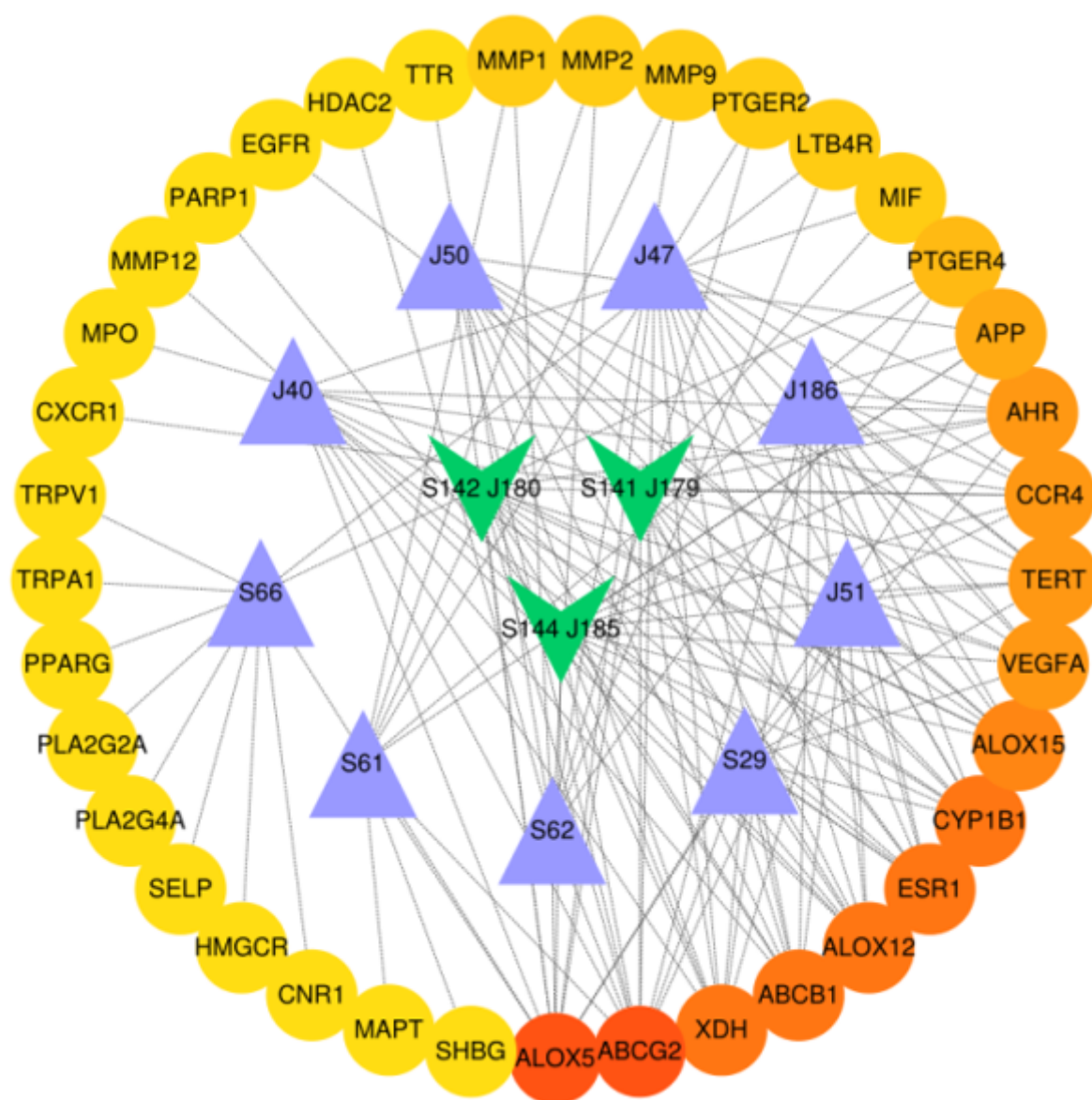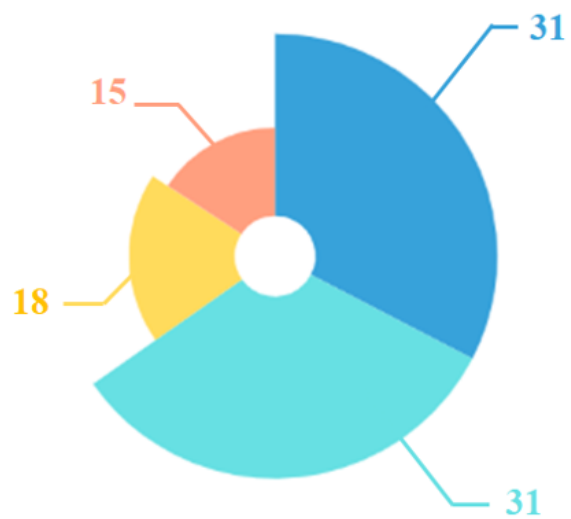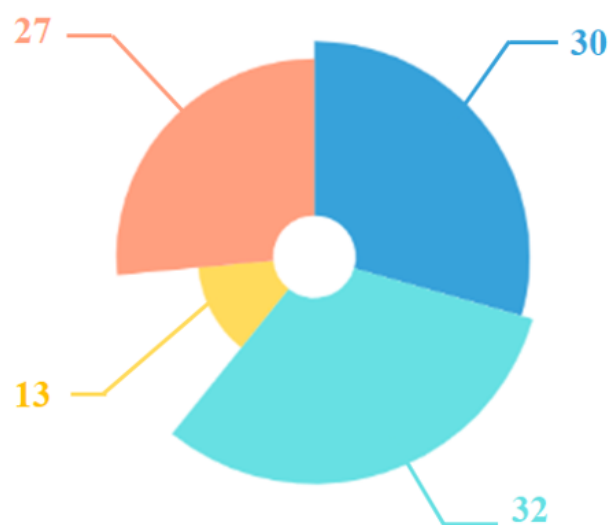
Figure 7

Diagram of the target network of core compounds. Round nodes represent gene protein. Green nodes denote the same compounds from the two herbs. purple nodes respectively indicate LJF and LF compounds.

Figure 8

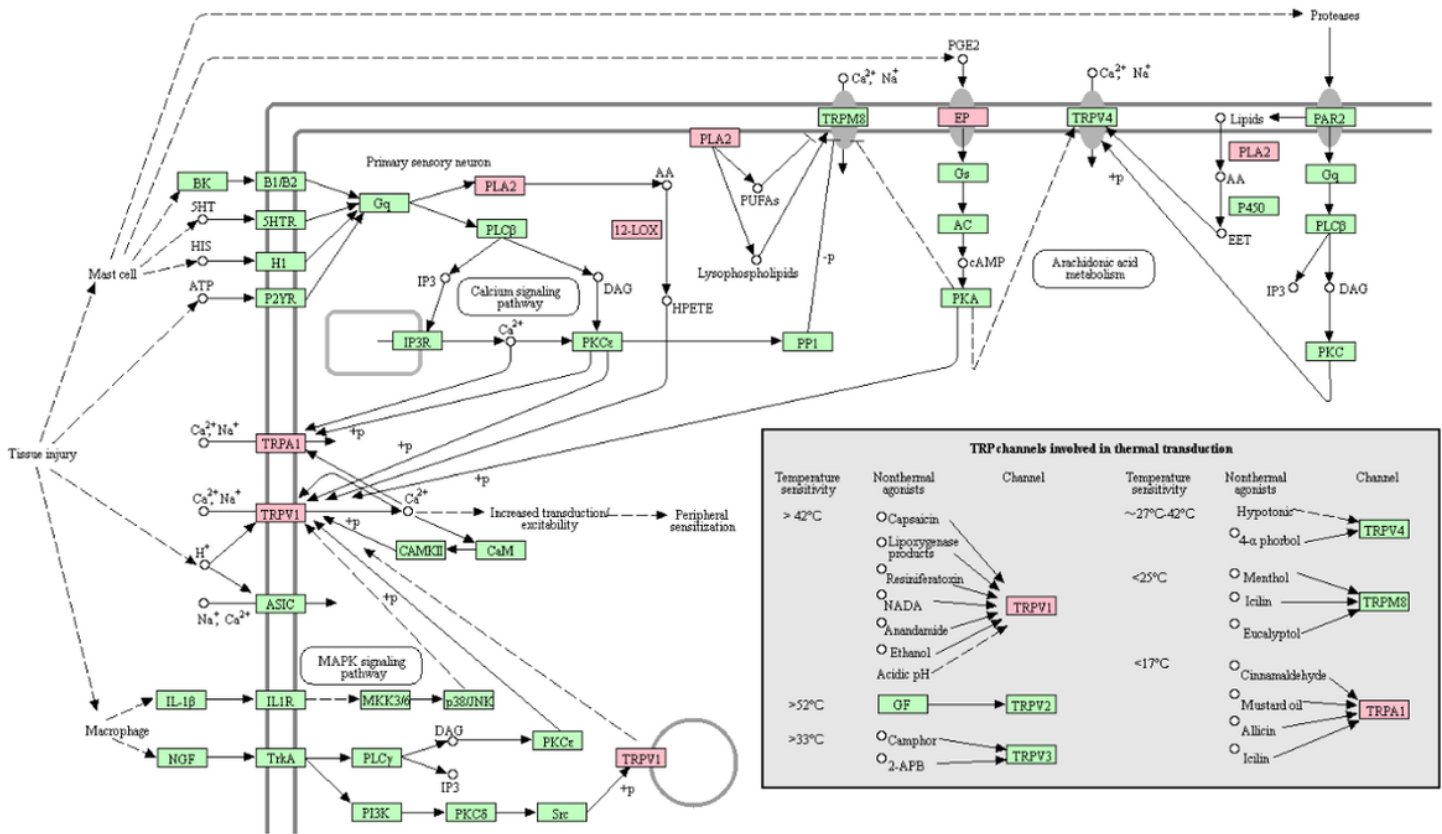Comparison of compounds. A. LF ; B. LJF.

Figure 9

The inflammatory mediator regulation of TRP channels of LJF and LF. The pink nodes represent the targets of two herbs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx
- SupplementaryTable1.xlsx