

Extraction and antioxidant activity of phenolic compound from *Lonicera japonica* Thunb. and network pharmacology study on their multi-target mechanism against acne

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Abstract Acne is a highly prevalent chronic inflammatory skin disease. The overuse of antibiotics in current clinical treatment tends to induce drug resistance and skin irritation, highlighting the great significance of developing safe and effective natural anti-acne agents. *Lonicera japonica* Thunb. is rich in polyphenols with prominent anti-inflammatory and antioxidant activities, and has been widely used in the treatment of skin diseases. In this study, polyphenols from *Lonicera japonica* Thunb. were extracted by ultrasonic-assisted extraction and purified by macroporous resin, and their antioxidant activities were evaluated *in vitro*, including DPPH, ABTS, FRAP, hydroxyl radical and superoxide anion scavenging assays. Combined with network pharmacology and molecular docking, the anti-acne active components, targets and pathways were screened. The results showed that polyphenols from *Lonicera japonica* Thunb. exhibited significant antioxidant capacity. A total of 7 core polyphenol components and 121 intersecting targets associated with acne were obtained, among which IL-6 and AKT1 were identified as key targets. These components mainly functioned through TNF, IL-17, MAPK and PI3K-Akt signaling pathways. Moreover, luteolin, kaempferol and quercetin could stably bind to core targets. This study revealed the multi-component, multi-target and multi-pathway mechanism of polyphenols from *Lonicera japonica* Thunb. against acne, providing a theoretical basis for their development as natural anti-acne drugs.

Keywords: *Lonicera japonica* Thunb. Polyphenols; acne; antioxidant; network pharmacology

1 Introduction

Acne is a chronic inflammatory skin disorder involving the pilosebaceous unit, affecting an estimated 650 million people worldwide. As the second most prevalent skin disease globally, it primarily manifests on the face, chest, upper back, and neck regions, exerting a significant impact on the quality of life and mental health particularly among adolescents and Previous studies have clearly identified

Propionibacterium acnes (*P. acnes*), excessive sebum secretion, follicular hyperkeratosis, and inflammation as the primary drivers of acne development. Current research indicates that *P. acnes* hydrolyzes sebum into free fatty acids; once released, these fatty acids trigger an inflammatory cascade. Clinically, the treatment of acne currently relies heavily on antibiotics. However, it has been demonstrated that topical application of most of these drugs is insufficient to address all underlying factors comprehensively. Additionally, they exhibit skin irritancy and may induce the emergence of drug-resistant bacterial strains in acne patients ^[2]. Presently, most commercially available drugs have shown reduced efficacy due to the development of

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professional women [1].

resistance in *P. acnes*^[3]. Therefore, the development of innovative, safe, and effective anti-acne formulations is of crucial importance.

Compared with synthetic drugs, active compounds extracted from medicinal plants for acne treatment offer unique advantages, including higher biocompatibility and fewer minimal adverse effects^[4]. *Lonicera japonica* Thunb. is rich in organic acids, polyphenols, volatile oils, and iridoid glycosides^[5]. It exerts effects of clearing heat, detoxifying, antiviral, and anti-inflammation. Clinically, it is frequently used to treat heat-toxin-induced carbuncles, sores, erysipelas, and burns/scalds, and can be administered both internal and external use. Su et al. further confirmed that the ethanol extract of *Lonicera japonica* Thunb. exhibits potent anti-inflammatory activity^[6-7]. Notably, its phenolic acid and flavonoid components can reduce lipopolysaccharide (LPS)-induced inflammation and possess excellent antioxidant properties^[8-9]. Among these components, polyphenols are the key material basis for *Lonicera japonica* Thunb. to exert its anti-inflammatory and antioxidant effects. They can alleviate skin inflammatory injury by scavenging free radicals, reducing oxidative stress, and inhibiting the release of inflammatory factors.

To clarify the actual efficacy of polyphenols from *Lonicera japonica* Thunb., this study adopted ultrasound-assisted extraction combined with

macroporous resin purification to prepare honeysuckle polyphenols. A series of *in vitro* antioxidant assays, including DPPH, ABTS, hydroxyl radical, superoxide anion scavenging assays, and ferric reducing antioxidant power (FRAP) assay, were performed to systematically evaluate their antioxidant activity. On this basis, network pharmacology and molecular docking were integrated to screen the potential active components, core targets, and key signaling pathways of *Lonicera japonica* Thunb. polyphenols in the treatment of acne, and to preliminarily reveal their synergistic anti-acne mechanism in a “multi-component, multi-target” manner.

This study was carried out from multiple aspects: extraction and purification, *in vitro* antioxidant evaluation, target prediction, and molecular verification. It comprehensively elucidates the material basis and mechanism of *Lonicera japonica* Thunb. polyphenols against acne, providing experimental evidence for the efficient utilization of *Lonicera japonica* Thunb. resources, and laying a theoretical and technical foundation for the development of safe, natural, and highly effective novel anti-acne products.

2 Materials and methods

2.1 Materials and reagents

Table 1 Materials and reagents

Materials / Reagents	Batch No. / CAS No.
<i>Lonicera japonica</i> Thunb.	25070102
PBS, 1×(Phosphate Buffered Saline)	G4202-500ML
Potassium ferricyanide (K ₃ [Fe(CN) ₆])	GB/T 644-2011
Trichloroacetic acid (CCl ₃ COOH)	76-03-9
Ferric chloride	H/T 3474-2000
Inhibition and produce superoxide anion assay kit	A052-1-1
Hydroxyl Free Radical assay kit	A018-1-1
1,1-Diphenyl-2-picrylhydrazyl Free Radical	1898-66-4
ABTS diammonium salt	30931-67-0

2.2 Preparation of polyphenols from *Lonicera japonica* Thunb.

Polyphenols were extracted by ultrasound-assisted extraction: Firstly, dried *Lonicera japonica* Thunb. was ground into powder using a grinder. Then, the flower buds and pollen of *Lonicera japonica* Thunb. were added into 70% methanol at a solid-liquid ratio of 1:20 and soaked for 6 h^[10]. The mixture was treated in an ultrasonic device at 50 °C for 50 min. After ultrasonic treatment, the mixture was filtered to obtain the filtrate, and methanol was removed by rotary evaporation at 50 °C.

The crude extract was further purified with AB-8 macroporous resin as follows^[11]. Firstly, the crude polyphenol solution (5.0 mg/mL) was loaded onto an AB-8 resin column at a resin-to-eluent ratio of 1:3, and adsorbed for 2 h. Then, the column was eluted with distilled water until the eluate became colorless, followed by elution of polyphenols with 95% ethanol. The organic solvent was removed by rotary evaporation. Finally, purified polyphenols were obtained by vacuum freeze-drying.

2.3 DPPH radical scavenging activity

200 µL of *Lonicera japonica* Thunb. extract solutions with different mass concentrations (6.125, 12.5, 25, 50, 100, 200, 400, 800 µg/mL) were mixed with 200 µL of DPPH absolute ethanol solution (0.2 mmol/L). After thorough mixing, the mixture was incubated at room temperature in the dark for 30 min. A microplate reader was used to measure the absorbance of the sample at 517 nm, denoted as A. Distilled water was used as a blank control (absorbance denoted as Ax), while absolute ethanol was used to replace the DPPH solution to set the background controls for the *Lonicera japonica* Thunb. extract solution and vitamin C (VC)^[12]. All other conditions remained unchanged, and the above operations were repeated. Each sample was measured in triplicate, and the average value was calculated^[13]. The DPPH radical scavenging rate was calculated using the following formula: DPPH radical scavenging rate (S)

$$= [Ax - (Ay - Az)] / Ax \times 100\%^{[14]}.$$

Where S represents the DPPH radical scavenging rate (%); Ax represents the absorbance of the blank group; Az represents the absorbance of the background group; and Ay represents the absorbance of the sample group.

2.4 ABTS⁺radical scavenging activity

A 7.4 mmol/L ABTS solution and a 2.6 mmol/L potassium persulfate solution were mixed at a volume ratio of 1:1. After standing in the dark for 12 h, the mixture was diluted with distilled water to adjust its absorbance at 734 nm to 0.70 ± 0.02 . 200 µL of *Lonicera japonica* Thunb. extract solutions with different mass concentrations (12.5, 25, 50, 100, 200, 400, 800 µg/mL) were mixed with 200 µL of the diluted ABTS solution. After thorough mixing, the mixture was incubated at room temperature in the dark for 6 min. A microplate reader was used to measure the absorbance of the sample at 734 nm. Distilled water was used to replace the sample as the blank group, while potassium persulfate solution was used to replace the ABTS solution to determine the absorbance of the control group. VC was used as the positive control. The ABTS⁺scavenging rate (%) was calculated using the formula: ABTS⁺ scavenging rate (S) = $[Ax - (Ay - Az)] / Ax \times 100\%$.

2.5 Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing ability was determined according to a previously reported method with minor modifications. Briefly, 0.2 mol/L phosphate buffer (pH 6.6) and 1% potassium ferricyanide solution were prepared. Then, 1 mL of polyphenol solutions at various mass concentrations was mixed with 2.5 mL of phosphate buffer and 2.5 mL of 1% potassium ferricyanide solution. After thorough mixing, the mixture was incubated in a water bath at 50 °C for 20 min. Subsequently, 10% trichloroacetic acid (TCA) solution was added, and the mixture was mixed well. The absorbance was measured at 700 nm, and all tests were performed in triplicate, with the average value

calculated. For comparison, the absorbance of vitamin C (Vc) was determined using the same procedure. The reducing power of polyphenols was evaluated by comparing the absorbance values.

2.6 $\cdot OH$ scavenging activity

Prepare a solution of polyphenols at the above-mentioned concentration, and follow the instructions in the kit manual for operation.

2.7 $O_2^{\cdot -}$ scavenging activity

Prepare a solution of polyphenols at the above-mentioned concentration, and follow the instructions in the kit manual for operation.

2.8 Acquisition of active pharmaceutical components and targets

The active components of *Lonicera japonica* Thunb. were systematically retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). The screening criteria were set as oral bioavailability (OB) $\geq 30\%$ and drug-likeness index (DL) ≥ 0.18 . Subsequently, polyphenolic substances were screened out based on the conditions that yield favorable activity in *Lonicera japonica* Thunb. The screened *Lonicera japonica* Thunb. polyphenol compounds were then input into the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), through which the Simplified Molecular-Input Line-Entry System (SMILES) structures of the active components were obtained. The SMILES structures were then imported into the SwissTargetPrediction database (<http://www.swisstargetprediction.ch/>) to predict the corresponding targets of each component, with targets having a prediction probability > 0 retained. Finally, after merging and removing duplicates, a target database for *Lonicera japonica* Thunb. polyphenols was established.

2.9 Acquisition of disease targets

Acne-related disease targets were retrieved from

the GeneCards database (<https://www.genecards.org/>), Therapeutic Target Database (TTD, <https://db.idrblab.net/ttd/>), and Online Mendelian Inheritance in Man (OMIM) platform (<https://www.omim.org/>) using “Acne” as the keyword. After deduplication, the relevant target information for acne was summarized and organized.

2.10 Acquisition of common targets between active pharmaceutical components and disease

The intersection operation was performed on the targets of *Lonicera japonica* Thunb. polyphenols and acne to determine the potential therapeutic targets of *Lonicera japonica* Thunb. polyphenols for acne. Venn diagram was generated using the Wei Sheng Xin platform for visual representation of these common targets.

2.11 Construction and analysis of protein-protein interaction (PPI) network

To construct the PPI network and perform topological analysis, the common targets of *Lonicera japonica* Thunb. polyphenols and acne were imported into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. The species was set to “Homo sapiens”, and the minimum interaction score was set to 0.400 to construct the target PPI network. Subsequently, the TSV format file of this PPI network was imported into Cytoscape 3.9.1 software for topological structure analysis. The color and size of nodes were adjusted according to their degree values, while the thickness and color of edges were adjusted based on their degree values, resulting in a PPI network of key targets.

2.12 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

For KEGG pathway enrichment analysis and Gene Ontology (GO) enrichment analysis, the intersecting targets of *Lonicera japonica* Thunb. polyphenols and acne were uploaded to the Database

for Annotation, Visualization and Integrated Discovery (DAVID). The species was set to “Homo sapiens”, and KEGG pathway enrichment and GO biological process enrichment analyses were performed with a screening criterion of $P < 0.01$. The GO enrichment bar chart and the KEGG enrichment bubble chart were generated using the Wei Sheng Xin platform.

2.13 Construction of “Drug-Component-Target-Pathway” Network

The *Lonicera japonica* Thunb. polyphenols, their corresponding targets, and the most significant pathways were mapped to the obtained intersecting targets. This dataset was then imported into Cytoscape 3.9.1 software to construct a “drug-component-target-pathway” network. Network parameters (e.g. degree, closeness and betweenness) were analyzed and calculated using the software tools to illustrate the interaction relationships among active components, intersecting targets, and related pathways.

2.14 Verification by molecular docking

Core targets identified from the PPI network were selected as receptors, while active components were designated as ligands for molecular docking verification. First, the 3D structure files (in SDF format) of the active components of *Lonicera japonica* Thunb. were obtained from the PubChem database, and their format was converted to PDB using the

OpenBabel GUI tool. Next, hydrogen atoms were added to these components using AutoDockTools software, and the files were exported in PDBQT format. Meanwhile, the core target protein files were downloaded from the Protein Data Bank (PDB) database; water molecules and original ligands were removed using PyMOL software before the proteins were imported into AutoDockTools for hydrogenation and final export in PDBQT format. The prepared receptor macromolecules and ligand small molecules were imported into AutoDockTools software, and the docking process was repeated 3 times to calculate the binding energy. Finally, PyMOL software was used to generate docking mode diagrams of the key active components of *Lonicera japonica* Thunb. with core target proteins for visual analysis.

3 Results

3.1 In vitro bioactivity evaluation of *Lonicera japonica* Thunb. polyphenols against antioxidant

The results showed a positive correlation between the concentration of *Lonicera japonica* Thunb. extract and the DPPH radical scavenging rate as shown in Fig. 1. When the concentration of the extract increased from 6.125 $\mu\text{g/mL}$ to 800 $\mu\text{g/mL}$, the DPPH radical scavenging rate significantly improved. At a concentration of 50 $\mu\text{g/mL}$, the scavenging rate reached 94.59%, indicating excellent antioxidant activity of the *Lonicera japonica* Thunb. Extract^[16].

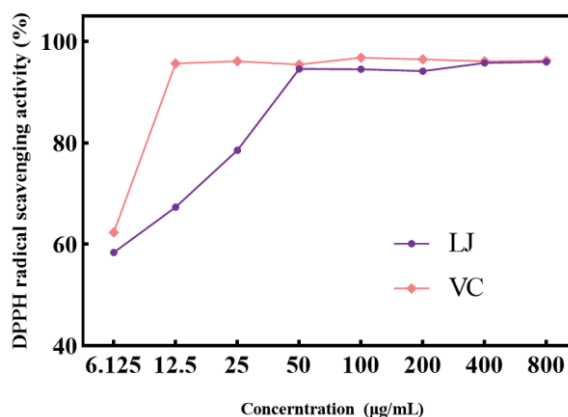


Fig. 1 Line graph of DPPH radical scavenging activity

Similarly, a significant positive correlation was observed between the extract concentration and the ABTS radical scavenging rate shown in Fig. 2. As the concentration of the *Lonicera japonica* Thunb. extract increased from 6.125 µg/mL to 800 µg/mL, the

ABTS radical scavenging rate increased significantly. At a concentration of 200 µg/mL, the scavenging rate reached 97.92%, further confirming the strong antioxidant activity of the *Lonicera japonica* Thunb. Extract^[17].

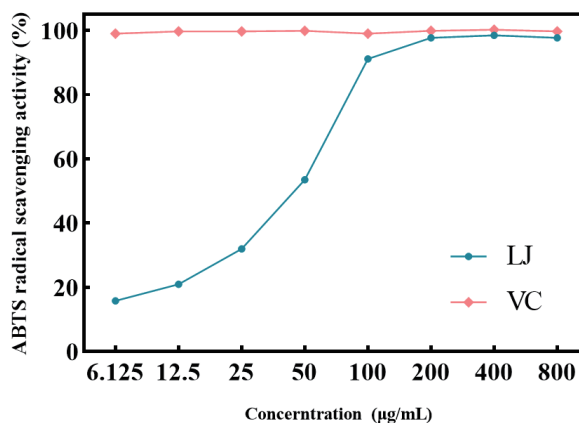


Fig. 2 Line graph of ABTS radical scavenging activity

Metal ions are necessary for the functioning of cellular biochemical and physiological processes, but in some cases and when their mechanism of action is not well controlled, these same ions can cause lipid peroxidation, oxidative stress or tissue injury. For the FRAP method, the revelation of the reducing power is based on the shift from the yellow colour of potassium ferrocyanide to a greenish blue colour whose intensity

depends on the reducing power of each sample. As shown in Fig. 3, when the concentration of polyphenols from *Lonicera japonica* Thunb. reached 200 µg/mL, its FRAP value was almost equivalent to that of vitamin C at 50 µg/mL, indicating that polyphenols from *Lonicera japonica* Thunb. exhibited strong ferric reducing ability.

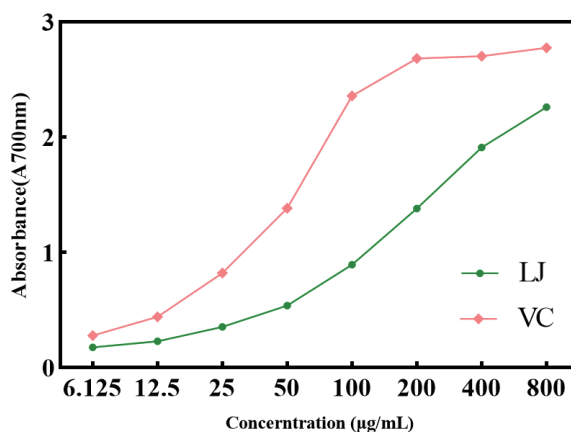


Fig. 3 Line graph of FRAP value scavenging activity

The results are shown in Fig. 4. When the mass concentration of polyphenols from *Lonicera japonica* Thunb. reached 200 µg/mL, its superoxide anion ($\cdot\text{O}_2^-$) scavenging activity was basically equivalent to that of

vitamin C at 100 µg/mL, indicating that polyphenols from *Lonicera japonica* Thunb. exhibited strong scavenging ability against superoxide anion ($\cdot\text{O}_2^-$).

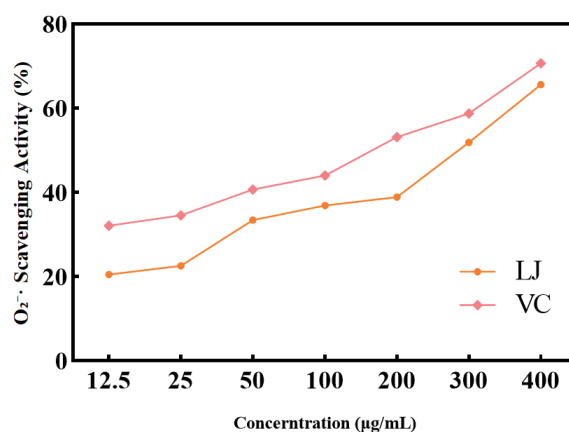


Fig. 4 Line graph of $\cdot\text{O}_2^-$ radical scavenging activity

The results are shown in Fig. 5. When the mass concentration of polyphenols from *Lonicera japonica* Thunb. reached 50 µg/mL, its hydroxyl radical ($\cdot\text{OH}$) scavenging activity was almost identical to that of

vitamin C at 50 µg/mL, indicating that polyphenols from *Lonicera japonica* Thunb. exhibited strong scavenging ability against hydroxyl radicals ($\cdot\text{OH}$).

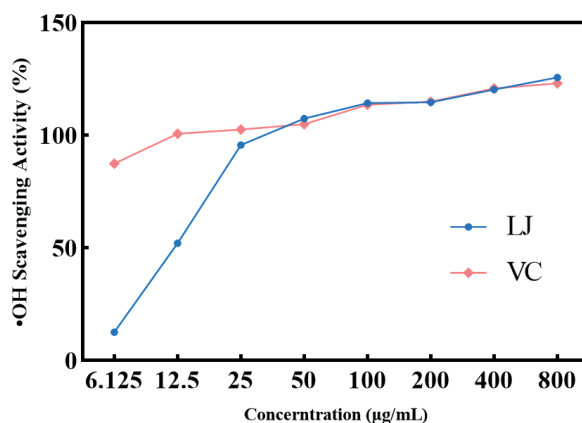


Fig. 5 Line graph of $\cdot\text{OH}$ radical scavenging activity

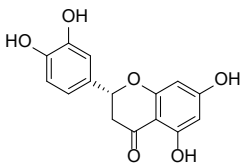
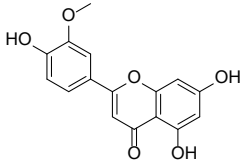
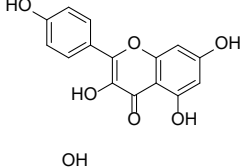
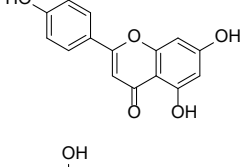
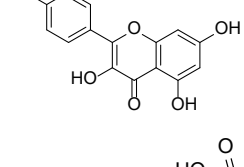
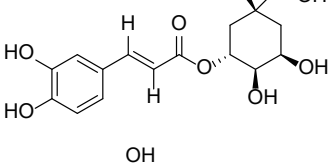
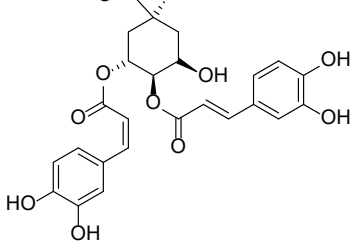
3.2 Main polyphenolic components of *Lonicera japonica* Thunb. and their corresponding acne targets

First, *Lonicera japonica* Thunb. was searched in the TCMSP database with the criteria of oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 , leading to the screening of 5 polyphenolic components. However, subsequent literature review revealed that chlorogenic acid and isochlorogenic acid have high contents in *Lonicera japonica* Thunb. and exhibit significant anti-inflammatory and antioxidant activities^[15]. Therefore, chlorogenic acid and isochlorogenic acid were supplemented as active

components, and a total of 7 polyphenols were finally selected from the active components of *Lonicera japonica* Thunb. for further study, namely Eriodyctiol (flavanone), Chryseriol, kaempferol, luteolin, quercetin, chlorogenic acid, and isochlorogenic acid C. These details are listed in Table 2.

Subsequently, the Simplified Molecular-Input Line-Entry System (SMILES) structures of each component were obtained via the PubChem database and imported into the SwissTargetPrediction platform for screening. After retaining targets with a prediction probability > 0 and removing duplicate targets, a total of 320 potential targets related to *Lonicera japonica* Thunb. polyphenols were finally obtained.

Table 2 Main active components of polyphenols in *Lonicera japonica* Thunb.

No.	MOLID	Compound	Structure
JYH1	MOL002914	Eriodyctiol (flavanone)	
JYH2	MOL003044	Chryseriol	
JYH3	MOL000422	kaempferol	
JYH4	MOL000006	luteolin	
JYH5	MOL000098	quercetin	
JYH6	MOL001955	Chlorogenic acid	
JYH7	MOL003118	Isochlorogenic acid C	

3.3 Disease targets of acne and common targets of *Lonicera japonica* Thunb. polyphenols

First, “Acne” was used as the keyword to retrieve all targets from the GeneCards, OMIM, and TTD databases. After removing duplicate targets, 2083

acne-related targets were finally obtained. Intersection analysis of these disease targets with the 320 targets of *Lonicera japonica* Thunb. polyphenols yielded 121 common targets as shown in Fig. 6. Finally, a Venn diagram was generated using the Wei Sheng Xin website.

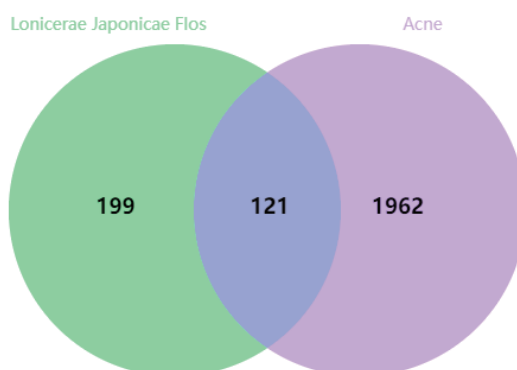


Fig. 5 Line graph of $\cdot\text{OH}$ radical scavenging activity

3.4 Construction and analysis of the “Active Component-Target” network for *Lonicera japonica* Thunb. polyphenols against acne

Using the Cytoscape 3.9.1 bioinformatics analysis software, a “component-target-disease” network and a “drug-component-target” interaction network were

constructed based on degree values, as shown in Fig. 7. In the Figure, purple arrows represent the common targets of *Lonicera japonica* Thunb. polyphenols and acne, while yellow circles represent the 7 polyphenolic active components of *Lonicera japonica* Thunb. This diagram reveals the interaction relationship between *Lonicera japonica* Thunb. polyphenols and acne.

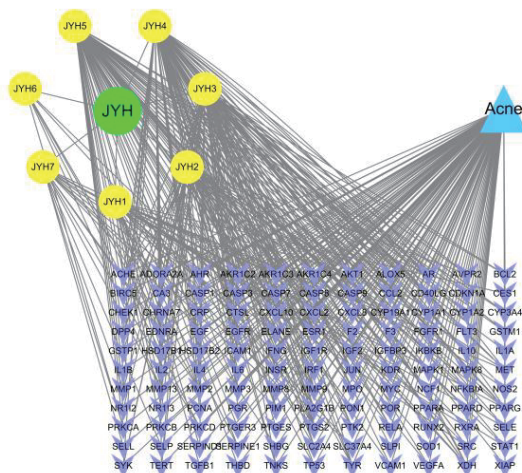


Fig. 7 Network diagram of “drug components - targets - diseases”

3.5 Construction and analysis of the Protein-Protein Interaction (PPI) network of *Lonicera japonica* Thunb. polyphenol targets

The 121 common targets were input into the STRING database to obtain the PPI network of acne-related targets associated with *Lonicera japonica* Thunb. polyphenols. In this network, a higher number of interactive nodes for a target indicates a higher degree value, signifying a more critical core position in the network; the results are presented in

Fig. 8. Based on the degree values, the top 10 targets were IL-6, AKT1, VEGFA, IL-1 β , TP53, CASP3, PTGS2, JUN, MMP9, and CXCL8 (Table 3). This result confirms that *Lonicera japonica* Thunb. exerts a therapeutic effect on acne through a “multi-component, multi-target” mode. Among these targets, IL-6 and AKT1 exhibited relatively higher degree values, suggesting that these two targets may be the core targets of *Lonicera japonica* Thunb. polyphenols in acne treatment and play a crucial role in the therapeutic effect.

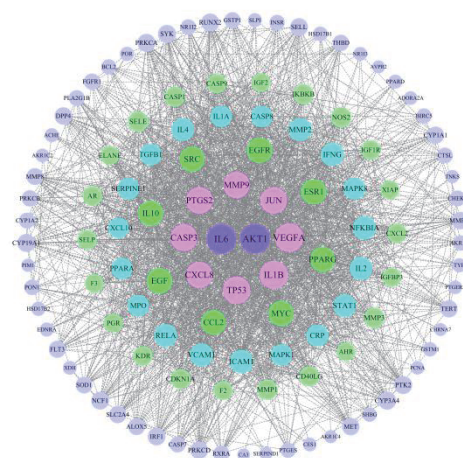


Fig. 8 PPI network of identified major targets

Table 3 Information of 120 core targets

Degree	Targets	Degree	Targets	Degree	Targets	Degree	Targets	Degree	Targets
91	AKT1	53	IL4	35	IGF1R	25	TERT	10	SHBG
85	VEGFA	53	IL2	35	SELP	25	CYP1A1	10	GSTM1
82	IL1B	49	MAPK8	33	PGR	24	FLT3	9	POR
79	TP53	49	CRP	32	ELANE	23	ALOX5	9	HSD17B2
76	CASP3	48	SERPINE1	32	XIAP	23	MMP8	9	TYR
75	PTGS2	47	CASP8	32	F2	22	SOD1	8	SLPI
74	JUN	47	MPO	31	IGF2	20	THBD	8	EDNRA
73	MMP9	47	TGFB1	31	IGFBP3	20	RXRA	8	PPARD
73	CXCL8	46	IL1A	31	F3	20	FGFR1	8	ACHE
69	EGF	46	MAPK1	31	AHR	20	PRKCB	7	XDH
68	EGFR	45	PPARA	29	PRKCA	19	GSTP1	7	NR1I3
67	MYC	42	CXCL10	29	SYK	18	CHEK1	6	CES1
66	ESR1	40	SELE	29	RUNX2	18	CASP7	6	PTGER3
66	SRC	40	NOS2	29	PTK2	18	PLA2G1B	6	AKR1C2
66	PPARG	40	MMP1	29	CYP3A4	18	CYP1A2	6	ADORA2A
66	IL10	40	MMP3	29	CYP19A1	17	CTSL	5	PCNA
64	CCL2	39	CASP1	28	PRKCD	16	NR1I2	4	TNKS
56	ICAM1	39	IKBKB	28	NCF1	16	PON1	4	SERPIND1
56	NFKBIA	39	CD40LG	28	SELL	15	INSR	4	PIM1
55	VCAM1	38	CDKN1A	27	IRF1	15	PTGES	3	AKR1C4
55	MMP2	37	AR	26	MET	15	AKR1C3	2	AVPR2
55	IFNG	36	KDR	26	DPP4	14	BCL2	2	CHRNA7
54	RELA	35	CXCL2	26	MMP13	14	BIRC5	1	CA3

3.6 KEGG pathway and GO enrichment analysis of core targets of *Lonicera japonica* Thunb. polyphenols against acne

KEGG pathway enrichment analysis was performed on the potential targets of *Lonicera japonica* Thunb. and acne, resulting in a total of 162 pathways. These pathways were ranked by the number of genes in descending order, and the top 25 pathways were selected. A bubble chart for KEGG enrichment analysis was generated using the Wei Sheng Xin website, with the results presented in Fig. 9. In the Figure, the area of each scatter point reflects the number of genes involved—i.e., a larger area indicates more genes—while the color of the scatter points represents the significance level, gradually transitioning from green to purple with increasing correlation strength.

The KEGG enrichment analysis results revealed a series of key pathways involved in the therapeutic effect of *Lonicera japonica* Thunb. polyphenols on acne, including the TNF signaling pathway, IL-17 signaling pathway, MAPK signaling pathway, and PI3K-Akt signaling pathway. Additionally, GO enrichment analysis was conducted on the 121 common targets, covering biological processes (BP), molecular functions (MF), and cellular components

(CC), with a screening criterion of $P < 0.01$. This analysis yielded 571 BP terms, 167 MF terms, and 41 CC terms. The pathways were ranked by the number of genes in descending order, and the top 10 pathways were selected to generate a GO enrichment bar chart (Fig. 10).

Specifically, in the BP category, the targets were involved in “cellular response to lipopolysaccharide”, “positive regulation of cell population proliferation”, “positive regulation of phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signal transduction”, “positive regulation of MAPK cascade”, “positive regulation of transcription by RNA polymerase II”, “positive regulation of gene expression”, “negative regulation of apoptotic process”, “apoptotic process”, “response to lipopolysaccharide”, and “positive regulation of miRNA transcription”.

In the CC category, the targets were localized to “extracellular space”, “protein-containing complex”, “perinuclear region of cytoplasm”, “collagen-containing extracellular matrix”, and so on.

In the MF category, the targets were associated with “enzyme binding”, “transcription coactivator binding”, “histone H2AX Y142 kinase activity”, “histone H3 Y41 kinase activity”, “protein tyrosine kinase activity”, and “peptidase activity”.

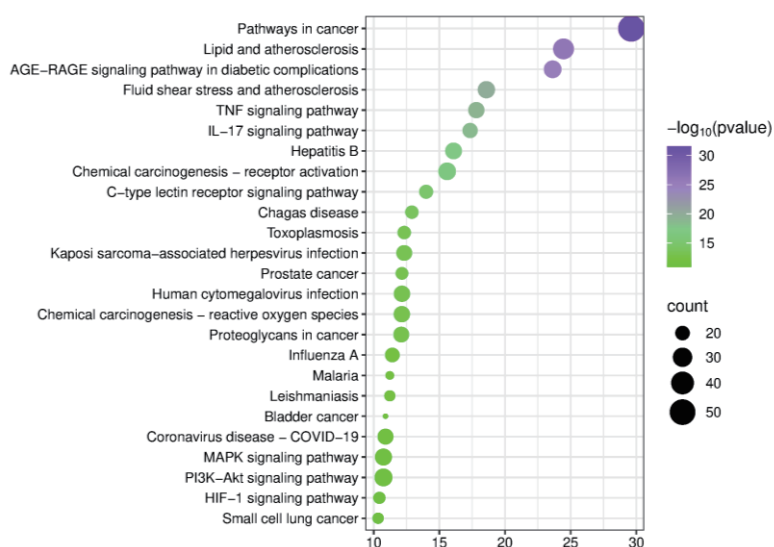


Fig. 9 KEGG pathway enrichment analysis

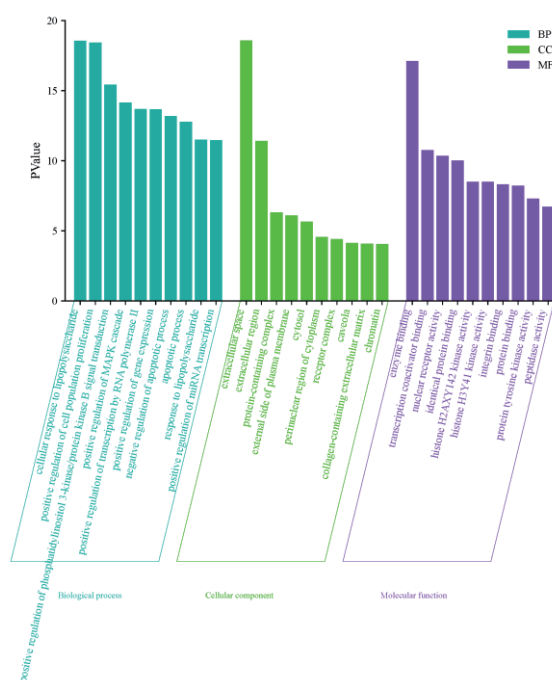


Fig. 10 GO pathway enrichment analysis

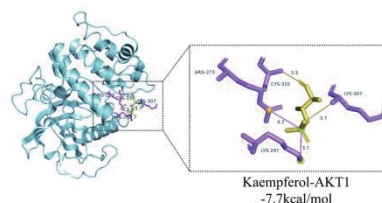
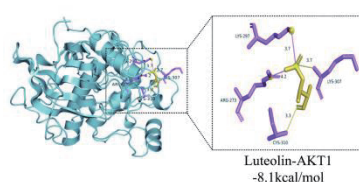
3.7 Molecular docking analysis of *Lonicera japonica* Thunb. polyphenols with core anti-acne targets

Based on the aforementioned results, among the active components of *Lonicera japonica* Thunb., kaempferol, luteolin, and quercetin were found to interact with a relatively large number of targets. These three components were selected for molecular docking with AKT1 and IL-6, where the active components of *Lonicera japonica* Thunb. served as ligands and AKT1/IL-6 served as receptors.

When the binding energy is below -5 kcal/mol ($1 \text{ cal} = 4.2 \text{ J}$), the ligand and receptor exhibit good binding ability. The results are presented in Fig. 11 and Table 4. The molecular docking results showed that the binding energies of luteolin-AKT1, kaempferol-AKT1, and quercetin-AKT1 pairs were -8.1 kcal/mol, -7.7 kcal/mol, and -8.0 kcal/mol, respectively. For the IL-6-ligand pairs, the binding energies of luteolin-IL-6, kaempferol-IL-6, and quercetin-IL-6 were -7.2 kcal/mol, -6.5 kcal/mol, and -7.1 kcal/mol, respectively—all indicating good binding ability.

Table 4 Molecular docking results

Gene	kcal/mol		
	Luteolin	Kaempferol	Quercetin
AKT1	-8.1	-7.7	-8.0
IL-6	-7.2	-6.5	-7.1



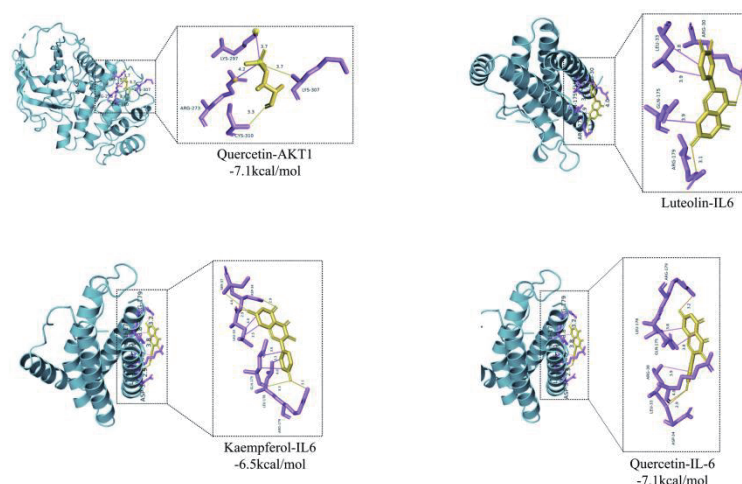


Fig. 11 Molecular docking and visualization of docking results

4 Discussion

IL-17 is located in the pilosebaceous unit during acne pathogenesis and promotes redness and pustules induced by Cutibacterium acnes infection [18]. As a key molecule in the PI3K-Akt signaling pathway, AKT1 regulates cell proliferation and apoptosis [19], thus affecting follicular hyperkeratosis and abnormal sebum secretion, two major pathological processes of acne [20–21]. These results indicate that polyphenols from *Lonicera japonica* Thunb. may improve acne through simultaneous inhibition of inflammation and regulation of keratinization [22].

In vitro antioxidant assays confirmed that *Lonicera japonica* Thunb. polyphenols exhibit strong free radical-scavenging activities against DPPH, ABTS, hydroxyl radicals, and superoxide anions, as well as high ferric reducing antioxidant power (FRAP). By reducing oxidative stress and lipid peroxidation, these polyphenols alleviate skin injury and suppress inflammatory responses, which is critical for delaying acne progression.

KEGG enrichment analysis showed that TNF, IL-17, MAPK, and PI3K-Akt signaling pathways were significantly enriched [23]. Among them, TNF and IL-17 pathways mediate inflammatory responses by promoting the release of IL-6, IL-1 β and other cytokines [24]. MAPK and PI3K-Akt pathways are

involved in sebocyte proliferation and keratinocyte differentiation. Therefore, *Lonicera japonica* Thunb. polyphenols may interrupt acne development by inhibiting inflammation, regulating keratinization, and reducing excessive sebum secretion [25–26], which is consistent with its traditional effects of clearing heat, detoxification, detumescence and resolving masses.

Kaempferol, luteolin, and quercetin can stably bind to AKT1 and IL-6, supporting their target regulatory effects [27]. Combined with antioxidant activity, it is suggested that *Lonicera japonica* Thunb. polyphenols exert anti-acne effects through a synergistic mechanism of antioxidant, anti-inflammation and target modulation [19, 28].

This study has certain limitations. Target prediction is based on existing databases, and in-depth verification in cellular and animal models is needed [29]. Future studies will focus on experimental validation to clarify the *in vivo* mechanism.

In conclusion, polyphenols from *Lonicera japonica* Thunb. exert significant antioxidant and anti-inflammatory effects, and regulate multiple signaling pathways and core targets related to acne. This study provides a scientific basis for the application of *Lonicera japonica* Thunb. in acne treatment and supports the development of safe and effective natural anti-acne products.

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