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Mechanism of Rosae Rugosae Flos flavonoids in the treatment of hyperlipidemia and optimization of extraction process based on network pharmacology

Yunxiao Xia [†], Aijinxiu Ma [†], Zihan Hou, Xu Zhao[*]

Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract This study aims to identify a natural plant chemical with hypolipidemic effects that can be used to treat high cholesterol without adverse reactions. Through network pharmacology screening, it was found that Rosae Rugosae Flos (RF) flavonoids had potential therapeutic effects on hyperlipidemia and its mechanism of action was discussed. TCMSP and GeneCards databases were used to obtain active ingredients and disease targets. Venn diagrams were drawn to illustrate the findings. The interaction network diagram was created using Cytoscape 3.8.0 software. The PPI protein network was constructed using String. GO and KEGG enrichment analysis was performed using Metascape. The results revealed 2 active flavonoid ingredients and 60 potential targets in RF. The key targets, including CCL2, PPARG, and PPARA, were found to play a role in multiple pathways such as the AGE-RAGE signaling pathway, lipid and atherosclerosis, and cancer pathway in diabetic complications. The solvent extraction method was optimized for efficient flavonoid extraction based on network pharmacology prediction results. This was achieved through a single factor and orthogonal test, resulting in an optimum process with a reflux time of 1.5 h, a solid-liquid ratio of 1:13 g/mL, and an ethanol concentration of 50%.

Keywords: Rosae Rugosae Flos; flavonoids; extraction; process optimization; network pharmacology; hyperlipidemia

1 Introduction

Hyperlipidemia is a systemic disorder of lipid metabolism [1], which seriously endangers people's health. Its incidence in China is increasing year by year. Therefore, the prevention and treatment of hyperlipidemia is particularly important. Currently, lipid-lowering chemical drugs such as statins and fibrates are commonly used in the treatment of hyperlipidemia [2]. However, they have potential adverse reactions or drug dependence. Therefore, seeking substances with hypolipidemic effects from natural plant chemicals has been put on the agenda [3].

Rosae Rugosae Flos (RF) is the dried flower bud of *Rosa rugosa* Thunb. from the Rosaceae family, it is widely distributed in Europe, the Middle East, Asia, and North America.

In addition to its good ornamental value in the field of horticulture, RF has high development and utilization value in the perfume, cosmetics, pharmaceutical, and food industries [4]. RF has long been used in traditional medicine to treat excessive stomach acid, constipation, abdominal pain, dysmenorrhea, depression, and various other diseases [5]. And because it is rich in carbohydrates

* Corresponding author: Xu Zhao (zhaoxu_1010@163.com).

† These authors contributed equally to this work;

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and antioxidant-rich compounds such as polyphenols, carotenoids, and anthocyanins [6], it has good biological activity and helps improve people's health. Among them, flavonoids, as one of the main components of RF, have good pharmacological effects such as anti-cardiovascular and other inflammatory diseases [7], antioxidants, anti-tumor [8], and anti-hyperlipidemia [9].

Network pharmacology is a new discipline based on the theory of system biology, which can investigate the complex network relationship between "drug-target-disease" from an overall perspective [10], analyze the biological system network, and select specific signal nodes for multi-target drug molecular design. Currently, articles are studying the potential pharmacological effects of RF, but there are few studies on the mechanism of action of RF in hyperlipidemia treatment. As one of the main active components of rose, the pharmacological effect of flavonoids has been paid attention to.

There are many methods for extracting flavonoids, including solvent extraction [11], ultrasonic extraction [12], microwave extraction [13], enzymatic hydrolysis [14], and double-aqueous phase extraction separation [15]. Due to the diverse structure of flavonoids, there are also great differences in the extraction of carrying agents and extraction methods. In addition, flavonoids are unstable to heat and easily decomposed by heat, and too high a temperature will make the structure of flavonoids unstable and affect their biological activity [16].

In this study, flavonoids, one of the main components of RF, were selected as the research object to explore the main active components and disease targets of RF flavonoids in the treatment of hyperlipidemia by network pharmacology. To extract RF flavonoids efficiently, based on the solvent extraction method, the process parameters were optimized by single factor experiment and orthogonal experiment to obtain the best process. This study provided a theoretical basis for the development and utilization of RF flavonoids and provided a new perspective for the development and clinical treatment of natural hypolipidemic drugs.

2 Experimental materials and methods

2.1 Experimental drugs and reagents

RF powder was purchased from Shanghai Jinliang Food Technology Co., Ltd., rutin reference substance was purchased from Dalian Meilunbio Co., Ltd., and purified water was purchased from Hangzhou Wahaha Beverage Co., Ltd. The reagents used are anhydrous ethanol (Rionlon Bohua (Tianjin) Pharmaceutical & Chemical Co., Ltd.); Sodium hydroxide (Tianjin Ruijinte Chemicals Co., Ltd.); Anhydrous aluminum trichloride (Tianjin Fuchen Chemical Reagent Co., Ltd.); Sodium nitrite (Tianjin Hengxing Chemical Reagent Co., Ltd.), are analytical grade chemicals.

2.2 Experimental methods

2.2.1 Network pharmacology prediction

(1) Acquisition of active ingredients of RF and target prediction. With "Rosae Rugosae Flos" as the keyword, oral bioavailability (OB) ≥ 30%, and druglike property (DL) ≥ 0.18 as the standard, the active ingredients of RF were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php).

(2) Acquisition of potential targets and construction of target protein interaction network (PPI). The relevant disease targets were obtained by retrieving "hyperlipidemia" from the Gene Cards database (https://www.genecards.org) and OMIM database (https://www.omim.org/) respectively. The obtained drug targets and disease targets were converted into standardized gene names by using the UniProt database, and then a Venn diagram was drawn by using Venny 2.1 to obtain the intersection of drug targets and disease targets, which was the potential target. The active "component-disease-target" interaction network was constructed by Cytoscape 3.8.0.

The target was imported into the String database

(https://string-db.org/), multiple proteins were selected, and the species was limited to homo sapiens. The PPI relationship map was constructed and sorted according to the association degree between proteins.

(3) Enrichment analysis. The above-mentioned data of drug targets and disease targets with interaction were input into the Metascape analysis platform (https://metascape.org/gp/index.htmL#/main/step1) for gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) signaling pathway enrichment analysis.

2.2.2 Preparation of rutin standard solution

40 mg rutin standard was weighed precisely, a small amount of 60% ethanol solution was added, heated in a water bath to dissolve, and the solution was transferred to a 100 mL volumetric flask. The remaining solids were dissolved in a 60% ethanol solution and transferred to a volumetric flask. The above steps were repeated until all rutin standards were transferred to the volumetric flask. The rutin standard solution with a concentration of 400 mg/L was obtained.

2.2.3 Methodological investigation

(1) Linear range. 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL to 10 mL measuring flasks of 0.2 mg/mL rutin standard solution were taken, 60% absolute ethanol was added to 5 mL, 0.3 mL of 5% $NaNO_2$ solution was added, shaken well, and allowed to stand for 8 min, 0.3 mL of 10% $Al(NO_3)_3$ solution was added, shaken well, and allowed to stand for 10 min, 4 mL of 4% NaOH solution was added, and distilled water was added to 10 mL, shaken, and let stand for 10 min to obtain a blank solution and a rutin standard solution at concentrations of 0, 10, 20, 30, 40, 50 and 60 μg/mL.

The absorbance was determined at a wavelength of 508 nm and zeroed with a blank solution.

(2) Instrument precision. Low, medium, and high concentrations of 20, 40, and 60 μg/mL rutin standard solution to be tested were taken, and the absorbance was measured 6 times at the wavelength of 508 nm, and zero with a blank solution.

(3) Solution stability. The sample solution to be measured was placed for 0, 1, 2, 3, 4, 5, and 6 h, and the absorbance was measured at a wavelength of 508 nm, and the blank solution was adjusted to zero.

(4) Repeatability of the method. Six samples of the sample solution were precisely weighed and configured, the absorbance was measured at the wavelength of 508 nm, and the blank solution was zeroed.

(5) Standardized recovery rate. The sample solution to be measured was taken and the rutin standard solution was added respectively, and then prepared according to the above chromogenic method, and the absorbance was determined at a wavelength of 508 nm with a blank solution.

2.2.4 Determination of samples

0.2 mL RF sample solution was taken into a 10 mL colorimetric tube, 60% ethanol solution was added to 5.0 mL, and 5% $NaNO_2$ solution was added to 0.3 mL, mixed, and stood for 8 min. Then 0.3 mL of 10% $AlCl_3$ solution was added and shaken well. After standing for 10 min, 4.0 mL of 4% NaOH solution was added. The solution was diluted to the scale with 60% ethanol solution, shaken well, and standing for 10 min. The absorbance value of the solution without sodium hydroxide reagent was used as a blank, and the concentration of total flavonoids in the sample was calculated according to the standard curve.

The solution preparation method is the same as 2.2.3.

2.2.5 Extraction of effective components of RF

The coarse powder of crushed RF was accurately weighed, and a certain concentration of ethanol solution was used to heat and reflux extracted in a water bath, several factors with large total flavonoid content were extracted by alcohol extraction: solid-liquid ratio, extraction time, ethanol concentration, and reflux time were tested to determine the optimal extraction conditions. The single-factor

test was carried out to determine the best extraction conditions.

The solid-liquid ratio was selected 1/5, 1/10, 1/15, 1/20, and 1/25 (g/mL) for investigation; the extraction time was selected 0.5, 1, 1.5, 2, and 2.5 h for investigation; The reflux times were selected as 1, 2 and 3 times for investigation; the ethanol concentrations were selected as 0%, 40%, 60%, 80% and 100% for investigation.

2.2.6 Orthogonal test

According to the results of the orthogonal test, the L_9 (3^4) orthogonal test was performed to optimize the extraction conditions of RF according to the three factors of reflux time (A), solid-liquid ratio (B), and ethanol concentration (C). The total flavonoid content was used as the evaluation index. The factor level table is shown in Table 1.

Table 1 Factor level of RF orthogonal test

Level	Factor		
	A reflux time (h)	B solid-liquid ratio (g/mL)	C ethanol concentration (%)
1	1.25	1/7	30
2	1.50	1/10	40
3	1.75	1/13	50

2.2.7 Verification of the best extraction process

1.00 g of RF was accurately weighed. Three groups were set up in parallel. The samples were prepared according to the best extraction conditions: reflux time 1.5 h, solid-liquid ratio 1/13 (g/mL), and ethanol concentration 50%. The total flavonoid content was calculated, and the RSD value was calculated.

2.2.8 Determination of total flavonoid content

(1) Preparation of solution. 1 g of RF solid drink was weighed, and the solid drink sample solution was prepared by adding distilled water to 50 mL, centrifuging at 2 800 r/min for 10 min, and then filtered through a 0.45 μm membrane to be used later.

Preparation of 0.2 mg/mL rutin standard solution: as described in “2.2.1”.

Preparation of 5% $NaNO_2$ solution, 10% $AlCl_3$ solution, and 4% NaOH solution: as described in “2.2.3”.

(2) Determination method of total flavonoid content in samples. 2 mL to 10 mL volumetric flasks were used for the sample solution of RF, which was prepared according to the color developing method described in “2.1.3”, and the sample solution of RF was prepared for testing. The absorbance of the sample solution to be tested was measured at the wavelength of 508 nm, and zero was set with a blank solution. The results were expressed as rutin equivalent (mg RE/g).

2.2.9 Determination of total flavonoids purity

D101, AB-8, and HPD400 macroporous resins were soaked in anhydrous ethanol for 24 h, and then rinsed with distilled water until no alcohol taste was found, and were set aside.

According to the optimal extraction process, the crude extracts of total flavonoids from RF were poured into the pretreated macroporous resins and eluted with 30%, 60%, and 90% ethanol of different concentrations. After combining the concentrated total flavonoids eluent, the content of total flavonoids was calculated according to the above method.

The formula for calculating the total flavonoid purity was: Total flavonoids purity: Total flavonoid

$$\text{purity} = \frac{\text{Total flavonoid content after elution}}{\text{Total flavonoid content before elution}} \times 100\%$$

3 Experiental results and discussion

3.1 Network pharmacological prediction results

3.1.1 Active components and their targets of flavonoids in RF

Ten active components were obtained by searching “Rosae Rugosae Flos” as the keyword in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php). After screening by the Swiss ADME platform, two active components were finally obtained: demethoxycapillarisin and quercetin (Table 2) , which were based on the condition that GI absorption scored “high” and druglike-ness passed at least two “Yes”.

Table 2 Information on the active ingredients of flavonoids in Rosae Rugosae Flos

MOL ID	Compound	MF	OB (%)	DL	Structure
MOL008046	Demethoxycapillarisin	$C_{15}H_{10}O_6$	52.33	0.25	HO, O, O, OH, OH, O
MOL000098	Quercetin	$C_{15}H_{10}O_7$	46.43	0.28	HO, O, OH, OH, OH, OH, O

3.1.2 Collection of disease targets

After screening and deduplication based on GeneCards and OMIM platforms, 1 328 and 6 targets were obtained respectively. After combining the targets of the two databases and removing duplicates, 1 331 potential targets were obtained.

3.1.3 “Component-disease-targe” network analysis

A Wayne diagram was drawn by intersecting the screened RF active ingredient targets and disease targets, and 60 common targets were obtained, as shown in Fig. 1. The active “component – disease – target” interaction network diagram was constructed from Cytoscape 3.8.0, as shown in Fig. 2. The diamond shape is the flavonoid active ingredient in RF, and the square shape is the hyperlipidemia-related target corresponding to the active ingredient.

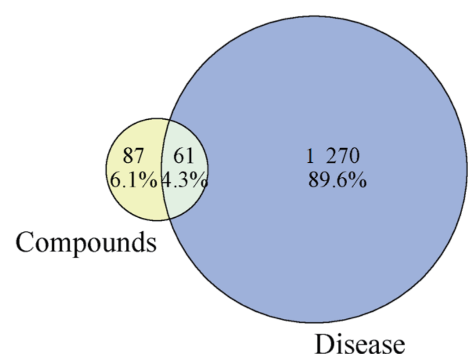

Fig. 1 RF flavonoids active ingredient target vs disease target Venn diagram

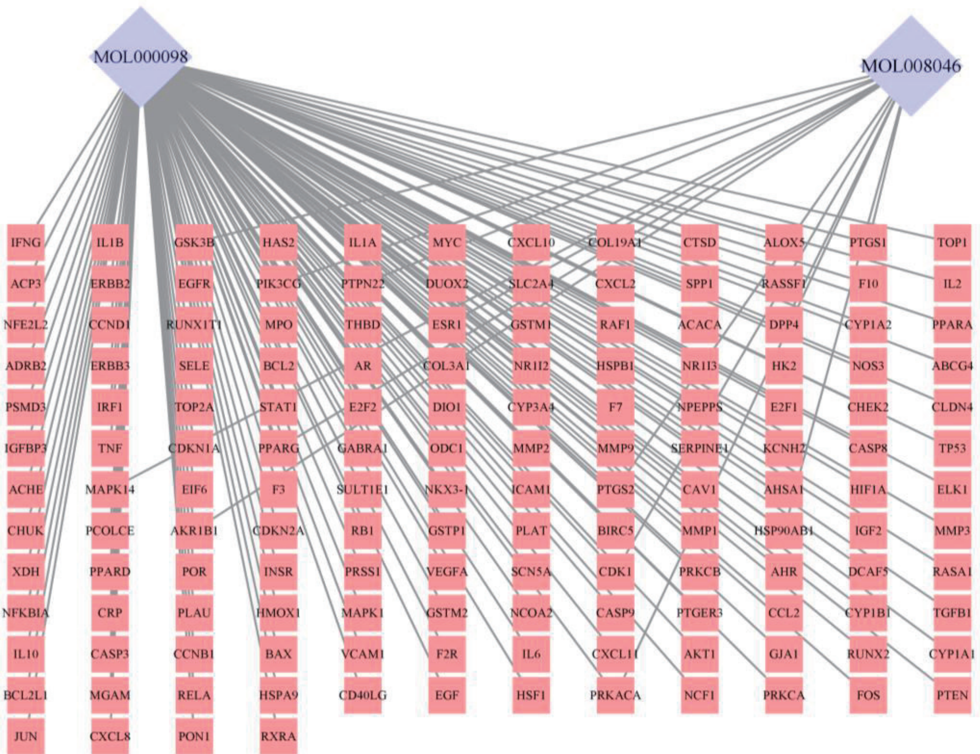

Fig. 2 “Component-disease-target” interaction network diagram

3.1.4 Target protein interaction network (PPI) network

Sixty intersecting targets were entered into the STRING database for analysis and visualized using Cytoscape software to plot the PPI network (Fig. 3).

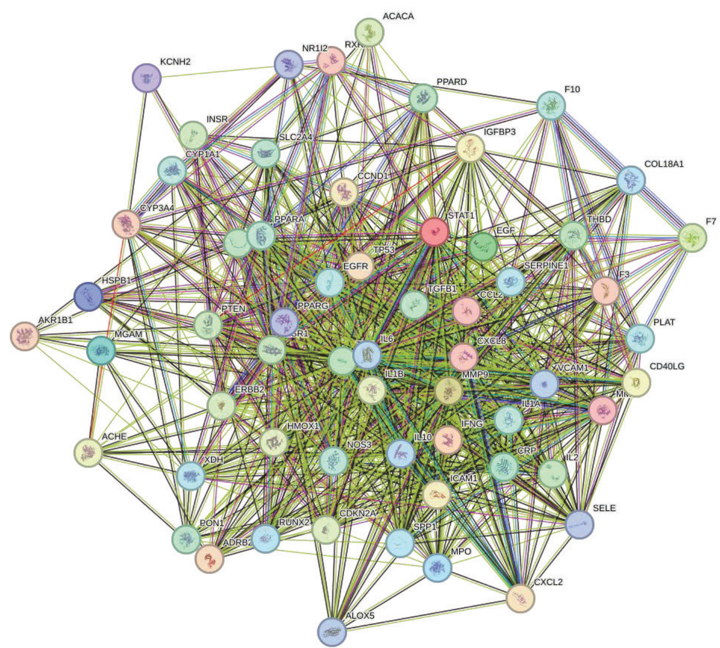

Fig. 3 Network diagram of RF flavonoids in the treatment of hyperlipidemia target protein

There are 60 nodes and 870 edges in the PPI network, with an average node degree of 29 and an average local clustering coefficient of 0.761. A total of 18 core targets were screened by the Centiscape 2.2 plug-in, a built-in plug-in of Cytoscape 3.8.0 software, including Transforming growth factor beta-1 (TGFB1), C-C motif chemokine 2 (CCL2), Cellular tumor antigen p53 (TP53), and Peroxisome proliferator-activated receptor gamma (PPARG), Peroxisome proliferator-activated receptor alpha (PPARA). The network diagram of the core target of RF flavonoids in the treatment of hyperlipidemia after screening is shown in Fig. 4.

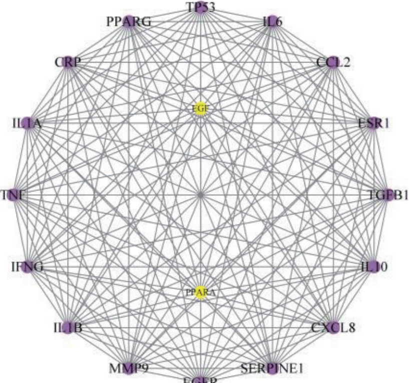

Fig. 4 Network diagram of the core targets of RF flavonoids in the treatment of hyperlipidemia

3.1.5 Enrichment analysis

A total of 1 112 GO entries were enriched, including 995 Biological process entries, 78 Molecular function entries, and 39 Cellular component entries. The biological process is mainly involved in the response of cells to nitrogenous compounds, hormones, peptide hormones, and insulin stimulation; Molecular function mainly involves the catalytic activity of enzymes such as protein kinase, phosphotransferase, protein tyrosine kinase, protein serine kinase, etc.; Cellular component mainly involves cell bodies, organelles, neurons, etc. With the help of platform for visualization, the specific entries are shown in Fig. 5. Using $P < 0.05$ as the criterion, the top 20 pathways in the KEGG analysis were screened out for visualization, as shown in Fig. 6.

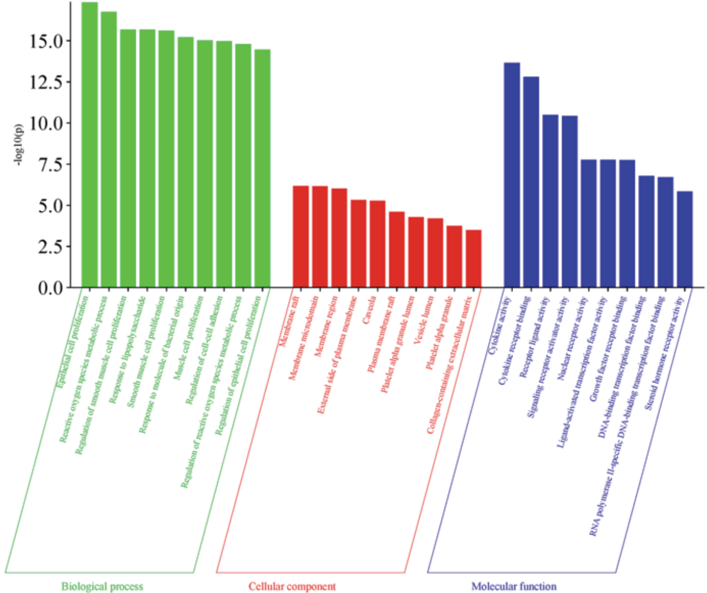

Fig. 5 GO enrichment histogram of RF flavonoids in the treatment of hyperlipidemia

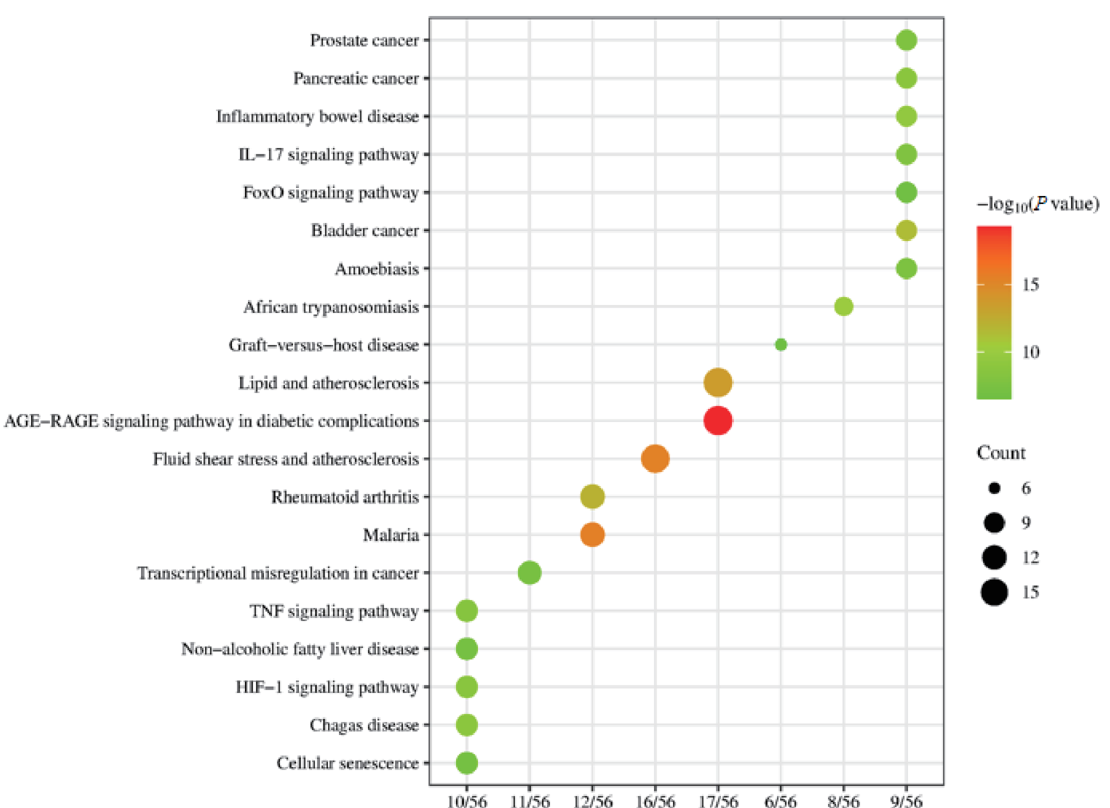

Fig. 6 KEGG signaling pathway enrichment map of RF flavonoids in the treatment of hyperlipidemia

3.2 Flavonoids extraction and process optimization results

3.2.1 Methodological validation

(1) Linear range. The concentration of rutin standard solution was used as the abscissa and the absorbance was used as the ordinate, and the standard curve was plotted. The results showed that the regression equation of the rutin standard curve is $y = 0.012\ 9x + 0.005\ 32$, and the correlation coefficient r is 0.999 8, which shows that the linear relationship between rutin concentration in 10 − 60 μg/mL is good.

(2) Instrument precision. The absorbance of 20, 40, 60 μg/mL rutin standard solution was measured at 508 nm for 6 times, and the absorbance of 20 and 40 μg/mL rutin standard solution was the same for 6 times, which was 0.21 and 0.43, respectively. The determination results of 60 μg/mL rutin standard solution were 0.66, 0.65, 0.65, 0.66, 0.65, and 0.66, respectively. The RSD values of the standard solution of the three concentrations were 0.72%, 0.67%, and 0.82%, respectively, which were all less than 2%, indicating that the instrument had good precision.

(3) Solution stability. The results of the stability of the solution were 26.50, 26.35, 26.18, 25.84, 25.49, and 25.21 mg/g, respectively. The RSD value of the sample solution was 1.95%, less than 2%, indicating that the solution remained stable for 6 h.

(4) Method repeatability. The repeatability of the method for the determination of total flavonoids was 26.01, 26.53, 25.49, 26.68, 25.66, and 25.84 mg/g, respectively. The RSD value was 1.83%, less than 2%, indicating that the method had good repeatability.

(5) Spike recovery. The recovery results of the spiked rutin standard are shown in Table 3, from which it can be seen that the spiked recoveries of the three samples are between 95%–105%, and the RSD values are less than 2%, indicating that the recovery of the method is good.

Table 3 Recovery test result

Sample	Original amount (μg)	Scalar (μg)	Measured rutin content (μg)	Recovery (%)	Average recovery (%)	RSD (%)
RF powder	24.38	19.50	43.95	100.3	99.3	1.05
	24.38	19.50	43.60	98.6		
	24.38	19.50	43.43	97.7		
	24.38	24.38	48.79	100.1		
	24.38	24.38	48.77	100.0		
	24.38	24.38	48.38	98.5		
	24.38	29.26	53.09	98.1		
	24.38	29.26	53.51	99.6		
	24.38	29.26	53.75	100.4		

3.2.2 Results of one-factor experiments

RF powder was refluxed in a 60% ethanol solution three times, for 1.5 h each time, according to a solid-liquid ratio of 1:15 g/mL. The effect of different reflux times on the total flavonoid content was investigated, as shown in Fig. 7 (A). The reflux time was set at 0.5, 1, 1.5, 2, and 2.5 h (solid-liquid ratio 1:15 g/mL, extraction temperature 85 °C), respectively, and the optimal extraction time was 1.5 h as can be seen from Fig. 7 (B). Fig. 7 (C) shows the effect of the solid-liquid ratio on the total flavonoid content after reflux extraction at 85 °C in a 60% ethanol solution of 1:5, 1:10, 1:15, 1:20, and 1:25 g/mL for 1.5 h. The highest content was found at a solid-liquid ratio of 1:10 g/mL. The fixed reflux time was 1.5 h, the solid-liquid ratio was 1:10 g/mL, the water bath temperature was 85 °C, and the reflux extraction was carried out under the conditions of ethanol concentration of 0%, 40%, 60%, 80%, and 100%, respectively. The results of total flavonoid content are shown in Fig. 7 (D). The results showed that with the increase of ethanol concentration, the total flavonoid content of RF first increased and then decreased, which may be due to the calcification of the cell wall caused by high concentration of ethanol, which decreased the extraction rate of total flavonoids. The total flavonoid content reached the highest when the ethanol concentration was 40%. Therefore, the number of reflux was 1 time, the reflux time was 1.5 h, the solid-liquid ratio was 1:10 g/mL, and the ethanol concentration was 40% as the optimal extraction conditions for RF for subsequent experiments.

3.2.3 Orthogonal test results

According to the orthogonal test design, the total flavonoid content of each test group was determined, and the experimental data were analyzed by visual analysis and variance analysis. The R values of the three factors were 9.99, 11.75, and 9.89, respectively, $R_b > R_a > R_c$, so the order of influence of each factor on the content of total flavonoids was as follows: solid-liquid ratio (B) > reflux time (A) > ethanol concentration (C). The results of variance analysis showed that the P values of the three were all less than 0.5, so ethanol concentration (C), reflux time (A), and solid-liquid ratio (B) had significant effects on the content of total flavonoids. Based on the results of visual analysis and variance analysis, the optimum extraction process was $A_2B_3C_3$, that is, the optimum extraction conditions were a reflux time of 1.5 h, solid-liquid ratio of 1:13 g/mL, ethanol concentration of 50%, and a total flavonoid content was the highest.

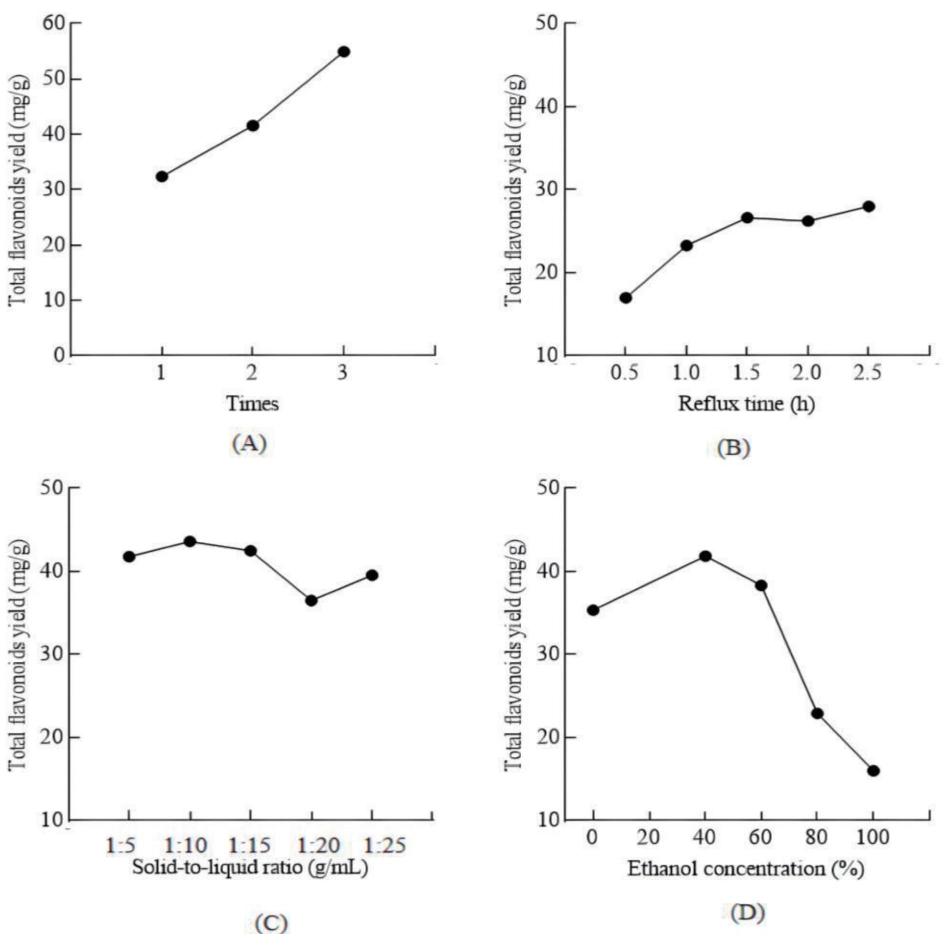

Table 4 Verify test results

Number	Total flavonoid content (mg/g)	Average value	Standard deviation	RSD (%)
RF 1	40.83			
RF 2	41.65	41.30	0.42	1.02
RF 3	41.41			

3.2.4 Process validation results

The best process verification for the orthogonal test of RF is shown in Table 4. The RSD value was 1.02%, less than 2%, indicating that the extraction process had good repeatability and the extraction process was stable.

3.2.5 Purification results of total flavonoids

The purities of total flavonoids in D101, AB-8, and HPD400 macroporous resin RF extracts were 65.85 ± 0.69%, 62.29 ± 0.13%, and 60.50 ± 0.05%, respectively. After purification, the purified products of the total flavonoids extracted from RF were of high purity and could be used for follow-up experiments.

The experimental results showed that the purification effect of D101 macroporous resin was better than that of the other two macroporous resins. This may be because D101 macroporous resin is a non-polar macroporous resin, which generally has a strong adsorption capacity for non-polar organic

compounds. In the experiment, the ethanol solution with a concentration of 30%, 60%, and 90% was selected for elution, and the ethanol solution with a concentration of 60% was found to have the best elution effect. This may be related to the polarity of the eluent. The concentration of ethanol solution is inversely proportional to polarity, and when the concentration of ethanol is low, the polarity is larger. Vice versa. Therefore, it may be that the ethanol concentration at 60% is moderately polar and the elution effect is better [17].

3.3 Discussion

Hyperlipidemia is the most important risk factor for the occurrence of other cardiovascular and cerebrovascular diseases, which increases the risk of atherosclerosis. Based on hyperlipidemia, the mortality rate and cardiovascular and cerebrovascular morbidity will increase accordingly. Several studies have explored the treatment of hyperlipidemia with botanical drugs and found that traditional folk medicines contain a variety of active ingredients with anti-hyperlipidemic activity, such as triterpenoids, flavonoids, polyphenols, and others [18, 19]. Chinese herbal medicine has the distinctive characteristics of multi-component, multi-target, and multi-pathway, which may play an important role in the regulation of long-term blood lipids in patients with hyperlipidemia.

In this study, the interaction network of “drug-component-target-disease” was constructed through network pharmacology, and the anti-hyperlipidemic substances of RF flavonoids were screened out: demethoxycapillarisin and quercetin. Studies have shown that quercetin can improve lipid profile and liver damage through antioxidant activity, as well as anti-atherosclerotic effects [20]. Therefore, it is speculated that the main active ingredient in RF flavonoids against hyperlipidemia may be quercetin.

The PPI network found that RF flavonoids played a role in combating hyperlipidemia through multiple targets, and the top three key targets were CCL2, PPARG, and PPARA. CCL2 can be produced by a variety of cells and is thought to be a molecular link between oxidized lipoproteins and foam cell recruitment to the vessel wall. A link has been shown between CCL2 levels and dyslipidemia [21]. Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent nuclear transcription factors with 3 isoforms expressed in humans, including PPARG and PPARA. PPARG is a transcription factor involved in atherosclerosis and related diseases, and studies have shown that the PPARG gene is mainly expressed in adipose tissue, controls fat storage and release, maintains energy homeostasis, regulates insulin resistance and blood glucose levels, and reduces vascular inflammation and atherosclerosis [22]. PPARA-regulated genes are involved in the regulation of extracellular lipid metabolism, fatty acid oxidation, lipoprotein and glucose homeostasis, and key proteins in inflammation, and their expression or activity may affect coronary heart disease risk through multiple pathways, including alterations in lipid or glucose concentrations, obesity, insulin resistance, or inflammatory responses [23].

KEGG pathway enrichment found that RF flavonoids can play a role through multiple pathways such as the AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, and pathways in cancer in diabetic complications. The results showed that the flavonoids in RF had the characteristics of multi-component, multi-target, and multi-pathway in the treatment of hyperlipidemia, which fully reflected the holistic and systematic therapeutic mechanism of traditional Chinese medicine. In the pathway, RAGE can be expressed in endothelial cells and macrophages in pure hyperlipidemia-dependent atherosclerosis animal models independent of diabetes mellitus or by oxidative stress [24]. The lipid and atherosclerosis pathways are involved in atherosclerosis by regulating low-density lipoprotein (LDL) cholesterol levels. These pathways have the potential to prove that RF flavonoids have potential pharmacological effects in the treatment of hyperlipidemia.

As a traditional method for extracting flavonoids from Chinese herbal medicine, solvent extraction may lead to changes in the extraction rate of flavonoids according to the different conditions such as reflux

time, solid-liquid ratio, polarity, and concentration of the extraction solvent. Similarly, the selection of macroporous resins of different polarities for enrichment can have an impact on the purification of flavonoids. Therefore, the extraction conditions and polarity of macroporous resin were screened in this study, and the optimal process verification was carried out, which provided a theoretical basis for the full extraction and application of RF flavonoids.

In summary, this study combined network pharmacology and orthogonal test process optimization to study the therapeutic mechanism and extraction process of RF flavonoids against hyperlipidemia, which fully proved the potential role of RF flavonoids in pharmacology and provided a reference for the further development and utilization of RF flavonoids.

4 Conclusion

This study used network pharmacology to predict that RF flavonoids could regulate hyperlipidemia through AGE-RAGE, lipid, and atherosclerosis signaling pathways based on CCL2, PPARG, PPARA, and other targets. The results suggest that RF flavonoids have potential therapeutic effects on hyperlipidemia. The optimum process was obtained as follows: Reflux time 1.5 h, solid-liquid ratio 1:13 g/mL, ethanol concentration 50%. Further *in vivo* and *in vitro* experiments are required to fully understand the mechanism of action of RF flavonoids in reducing blood lipids. These experimental results offer new insights into the treatment of hyperlipidemia with Chinese herbal medicines and provide a theoretical basis for the further development and utilization of natural medicines.

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