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Screening and quantification of TNF-α ligand from *Angelicae Pubescentis Radix* by biosensor and UPLC-MS/MS

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ABSTRACT

The aim of this study is to establish a method for rapid screening of active ingredients targeting TNF-α from Chinese herbal medicines. Take *Angelicae Pubescentis Radix* (APR) as an example, surface plasma resonance technique was used to establish for screening small molecule inhibitors of TNF-α from APR extract. Then UPLC-MS/MS coupled with chemometric was used for quantitative and evaluate the differences of the candidate compounds bound to TNF- in APR from different sources. In the experiment, TNF-α protein was fixed on the CM5 chip surface of biacore T200 biosensor by amino coupling. A series of small molecular compounds in APR were screened and six phenolic acid compounds had a strong affinity for TNF-α protein and could be used as TNF-α antagonists. In summary, the targeted drug screening method for TNF-α protein based on SPR technology established in this study can be used to screen anti-TNF-α small molecule inhibitors. UPLC-MS/MS can accurately quantify 15 active ingredients, which provides reliable experimental data and new research ideas for targeted drug research on TNF-α protein.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with symmetrical polyarthritis as the main clinical manifestation [1]. In the process of disease development, arthrosis are slowly and gradually damaged, if not timely and effective treatment, which seriously affects patients' health and quality of life, and brings heavy economic burden to the society [2]. Studies have shown that TNF-α concentrations in synovial membrane and synovial fluid of the arthrosis in RA patients were significantly higher than those in the normal group [3]. Thus some researchers chose TNF-α as the target for RA treatment, because more and more evidences show that TNF-α is the main molecular regulator of RA [4–8].

Currently, TNF-α targeting therapy has greatly improved the therapeutic effect of RA. There are many TNF-α targeted inhibitors include Inliximab, Adamuzumab, Cetolizumab, Etanercept and P38 mitogen activator protein kinase [6]. And they have been widely used in clinical treatment and have achieved excellent results, such as strong targeting, rapid onset, long half-life, good tolerance and significant efficacy, but the problems also emerge, the main disadvantages are that it is extremely expensive and difficult for general patients to bear [9,10]. Therefore, it is imperative to develop a reasonable price, significant curative effect and good stability of TNF-α inhibitor.

Traditional Chinese medicines (TCMs) have many characteristics such as: abundant resources, suitable price, low toxicity and side effects, so more and more scholars turn to TCMs to search for affordable and effective TNF-α inhibitors. Some studies indicate that *Angelicae Pubescentis Radix* (APR) had protective effect on the treatment of knee osteoarthritis and had effective in treating adjuvant arthritis. It is the first choice for the treatment of RA [9,11]. However, as an essential medicine for the treatment of RA, the composition of APR is complicated [12], only a small number of chemical components are considered to have biological activity, modern pharmacological studies have failed to elucidate the key components of RA therapy [13,14]. Therefore, it is very important to establish a method for screening the active ingredients of anti-RA.

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Surface plasmon resonance (SPR) technology is a biosensing analysis technology for real-time observation of interactions between biomoleculars [15]. With the improvement of the technology, it has quickly penetrated into all aspects of TCM research [16]. Cao et al. have developed a SPR-UPLC-MS system for screening TNF-α receptor from Chinese herbs and Physcion-8-*O*-β-D-monoglucoside was screened [17]. Zhang et al. have developed a platform to screen human serum albumin binders from *Radix Astragali*, and 20 compounds was considered as active compounds which having an affinity with human serum albumin [17,18]. Compared to traditional methods, the mainly advantage of SPR in term of screening effective compounds from TCMs is that it can directly detect active compounds in complex ingredients, and the sample can be detected without labeling, saving time and cost; and the detection process is fast and convenient, the result is intuitive [19]. So we also established a SPR method to screen active compounds from APR. It can provide an important evidence for further research on the potential active substances in APR.

The aim of this study was to screen TNF-α inhibitors from herbal extracts of APR by SPR. And the SPR assay was used to observe whether the candidate compounds had a high response signal with TNF-α and could be used as TNF-α ligand. Then UPLC-MS/MS method was used to control the quality of candidate compounds. As far as we know, this is the first time to screen out a small molecule inhibitor of TNF-α from APR. The results indicate that the proposed SPR method is an effective method for screening target compounds for specific proteins from complex matrices. It also provides guidance for SPR screening of active ingredients in TCM and other complex pharmaceutical systems.

2. Materials and methods

2.1. Chemicals, reagents and materials

Acetonitrile was HPLC grade and supplied from Fisher Scientific (Pittsburgh, PA, USA). Formic acid was HPLC grade and supplied from Dikma Co. (USA). CM5 chip, phosphate buffer solution, sodium acetate solution, N-hydroxysuccinyl (NHS) and ethyl [3-(dimethylaminyl) propyl] carbimide (EDC), ethanolamine hydrochloride (ETH) were supplied from GE Healthcare (Shanghai, China). Reference standards of neochlorogenic acid (5-CQA), chlorogenic acid (3-CQA), cryptochlorogenic acid (4-CQA), umbelliferone, scopoletin, 3,4-dicaffeoylquinic acids (3,4-diCQA), 3,5-dicaffeoylquinic acids (3,5-diCQA), 4,5-dicaffeoylquinic acids (4,5-diCQA), 5-methoxypsoralen, columbianetin acetate, imperatorin, osthole were supplied from Chengdu Must Biotechnology (Chengdu, China), and columbianetin, 8-methoxypsoralen, psoralen, kaempferol-3-*O*-rutinoside (internal standard, IS1) and chloramphenicol (internal standard, IS2) were supplied from Shanghai Nature Standard Biotechnology (Shanghai, China). The structure of 15 compounds and 2 ISs were shown in Fig. 1.

APR samples were identified as the dry root of Angelica pubescens Maxim. f. biserrata Shan et Yuan by Prof Lianjie Su from Heilongjiang University of Chinese Medicine, Harbin, China. All APR samples were placed in a constant temperature condition before use.

2.2. Preparation of herbal extracts solutions

APR powder (20 g) was accurately weighed and extracted by ultrasound with 200 ml 90% ethyl alcohol/water for 60 min at room temperature. And centrifuge at 5000 rpm for 15min. Then all clear supernatant was concentrated under vacuum with heat, keeping the temperature below 40 °C, and the residue was dissolved with 40 ml purified water, and through 0.22 μm filter membrane before analysis.

2.3. TNF-α activation, coupling and sealing

The Biacore T200 system (GE Healthcare, Uppsala, Sweden) was used for SPR analysis. 200 μl NHS and EDC were centrifuged to remove bubbles and then activating the dextran on CM5 sensor chip surface. Dissolve 10 μg TNF-α with sodium acetate solution pH 4 and immobilized the TNF-α protein on the CM5 sensor chip by amino coupling method according to the manufacturer's agreement and the contact time was 600s with a flow rate was 10 μl/ml. Finally, 200 μl ETH was used to seal dextran that did not bind to the protein on the chip surface.

2.4. Recovery of compounds bound to TNF-α

Recover the components which bound to TNF-α on the sensor surface by Biacore T200 system. First, APR extract solutions through the sensor chip surface to bound to TNF-α. Due to the specificity of protein binding to small molecular compounds, the components that bound to TNF-α remain on the chip surface, while other components that do not bound to TNF-α elute out of the system with the mobile phase. Second, the mobile phase of PBS flowed over the protein surface to wash off substances not bound to TNF-α. Third, 0.5% formic acid was used as the dissociation solution to inject into sensor chip surface to dissociation the bound ingredients into recovery solution (As shown in Fig. 2). Then the recovery solution was dried with nitrogen at 35°C, and the residue was dissolved with 100 μl 50% methanol/water (v/v). Subsequently, the recovery solution was analyzed by UPLC-MS/MS.

2.5. Affinity test

The reference candidate compounds were diluted with PBS to a series of concentration. And the affinity of candidate compounds to TNF- α was assessed using a Biacore T200 Evaluation Software 3.0. The contact time and dissociation time were 120s and 300s, respectively. And the flow rate was 30 μl/min. The flow cells without the conjugated protein was used as blank controls. The system error was corrected by using a blank solution without analytes. The bound ability of small molecules to TNF-α protein was evaluated by equilibrium dissociation constant (K_D). The experimental data were analyzed by Biacore T200 instrument.

2.6. Chromatographic and mass spectrometric conditions

The ultra-high performance liquid chromatography (Thermo Scientific TM, Vanquish TM, (Waltham, MA, USA)) was used for the chromatographic analysis. Chromatographic separation was carried out on a Thermo Hypersil GOLD C18 column (100 mm × 2.1 mm, 1.9 μm). The column temperature was kept at 35°C. Acetonitrile (solvent A) and 0.1% (v/v) formic acid aqueous (solvent B) was selected as mobile phase with a gradient elution of 7–8% A at 0–4 min, 8–19% A at 4–5 min, 19-19% A at 5–12 min, 19–60% A at 12–15 min, 60–100% A at 15–18 min. The flow rate was maintained 0.3 ml/min. Mass spectrometry detection was carried out using a Thermo TSQ QUANTIS Triple Quadrupole with an electrospray ion source and was analyzed in the multi-reaction monitoring mode and the positive and negative ion exchange mode. And the optimization parameters of the 15 compounds were shown in Supporting material 1.

2.7. Preparation of quantitative standards solution

Reference of 15 compounds was individually dissolved by methanol to obtain the stock solutions at a concentration of 1.0000 mg/ml, respectively. Then the stock solutions were diluted by 50% methanol/water (v/v) to a range of concentrations to prepare the working

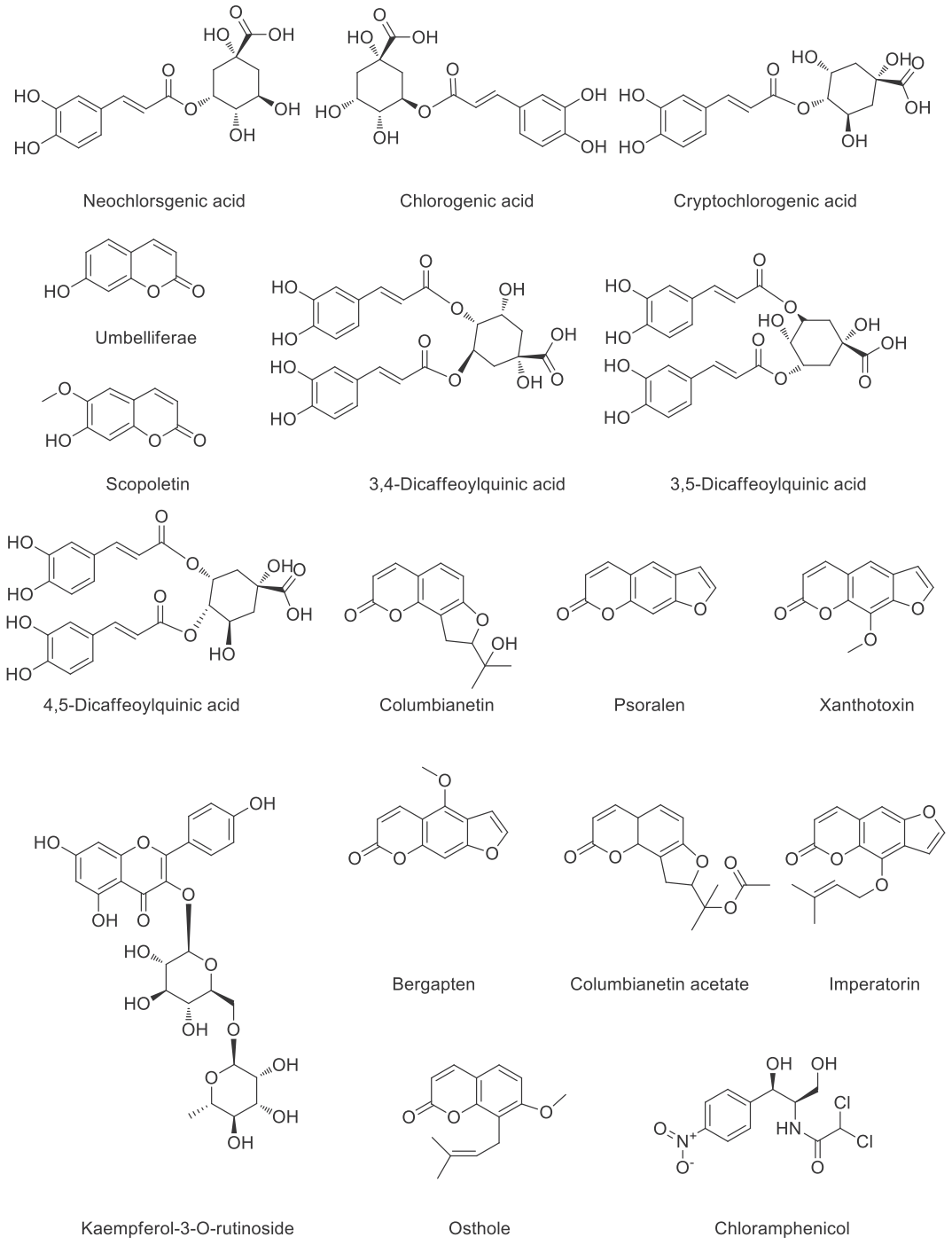

Fig. 1. The structure of 15 compounds and 2 ISs.

standard solutions. All the working standard solutions were stored at 4 ˚C before analysis.

2.8. Preparation of quantitative sample solutions

The APR samples powder (0.05 g) was accurately weighed and extracted by ultrasound with 10 ml 90% ethyl alcohol/water for 60 min at room temperature. After being centrifuged (5000 rpm, 15min), the supernatant was filtered through a membrane filter (0.22 μm) and all the sample solutions were stored at 4 ˚C before using.

2.9. Method validation

To verify the constructed method, the linearity, stability, repeatability, LOD, LOQ, precision, and accuracy of the 15 candidate compounds were tested. The reference standard solution of 15 candidate compounds was diluted to a series of the concentrations, and each concentration was injected six times. Calibration curves of 15 candidate

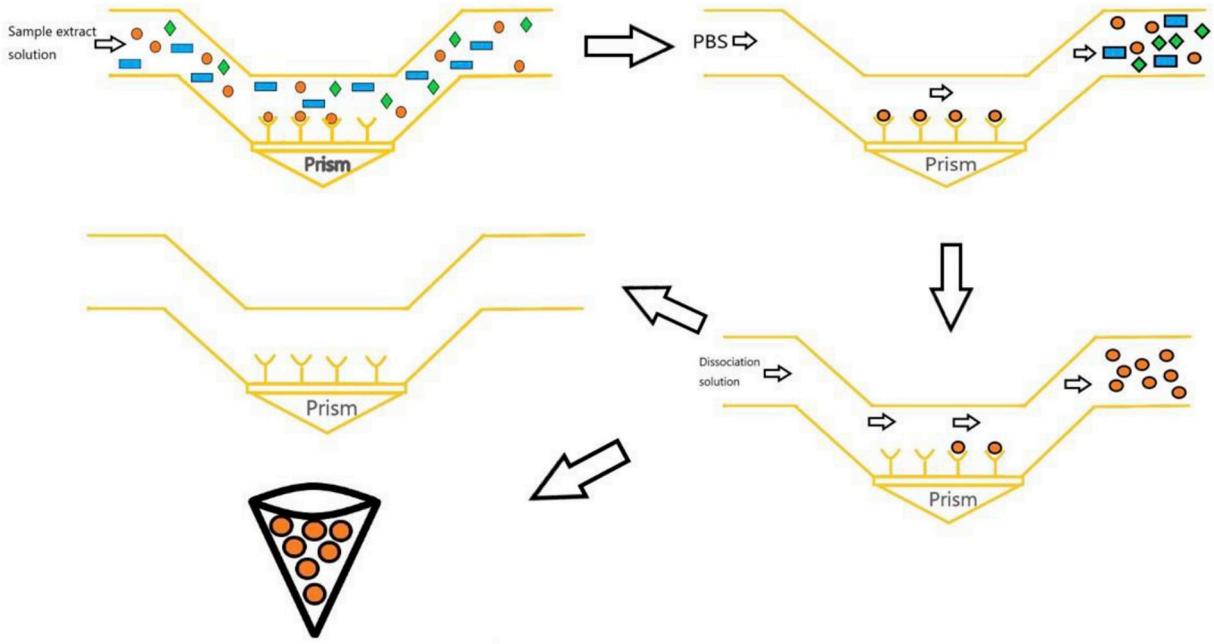

Fig. 2. The process of screening of small molecular compounds bound to TNF-α

compounds were established by plotting the peak area versus of each candidate compound to IS against the corresponding concentration. The lowest quantitative limit (LOQ) and lowest detection limit (LOD) of 15 candidate compounds were calculated based on S/N = 3 and S/N = 10, respectively. The stability of 15 candidate compounds was measured at 0, 2, 4, 8, 16 and 24 h by 6 replicate injections. To investigate the repeatability, six replicates of the samples were analyzed. The stability and repeatability were expressed by relative standard deviation (RSD). The intra-day precision was determined during one day, while the inter-day precision was determined during three consecutive days. And the RSD were used to evaluate the method precision. The accuracy of the method was evaluated by combining three level concentrations (80%, 100% and 120%) of the 15 candidate compounds standard solutions with 0.05 g APR sample extracted solutions in triplicate for recovery test. The recovery formula is:

$$\text{Recovery } (\%) = \frac{(\text{amount found} - \text{sample amout})}{\text{amount spiked}} \times 100\%$$

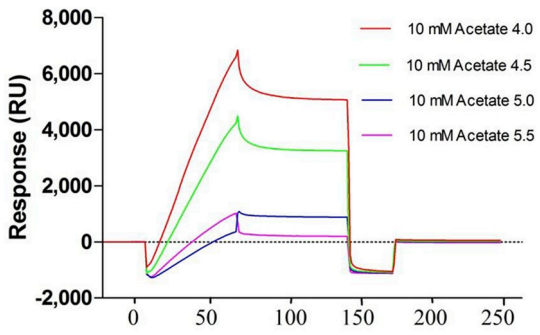

Fig. 3. Response of immobilization test of sodium acetate with TNF-α in different PH.

3. Result

3.1. Protein pre-coupling

In order to found the optimal equivalent point of protein, more proteins could be fixed on the chip surface, and different PH values of sodium acetate were optimized. TNF-α was dissolved in sodium acetate of different PH (PH = 4.0, 4.5, 5.0, 5.5) and analyzed by Biacore T200 system. And the results showed that sodium acetate (pH of 4.0) was selected as the dilution buffer to dilute TNF- α in the immobilization assay can obtain the highest bound response (Fig. 3). Therefore, sodium acetate PH 4 was selected as the optimal condition to dilution TNF-α and the bound response of immobilization level was 6837.88 RU (response unites, RU).

3.2. Affinity test

The recovery solution was detected by UPLC-MS/MS, and 15 candidate compounds include 5-CQA, 3-CQA, 4-CQA, umbelliferone, scopoletin, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, columbianetin, 5-methoxypsoralen, 8-methoxypsoralen, psoralen, columbianetin acetate, imperatorin, osthole were identified, kaempferol-3-*O*-rutinoside and chloramphenicol were used as internal standard compounds, and the chromatographic diagram is shown in Fig. 4. To verify the candidate compounds if have a strong affinity with TNF-α, the affinity assays were assessed using the Biacore T200 Evaluation Software 3.0. The sensor diagram and fitting curve were shown in Fig. 5. The results indicated that the K_D value for the interaction between 6 phenolic acids compounds and TNF-α were more than 2.232×10^{-4}, representing a good affinity between 6 phenolic acids and TNF-α. This may be the main reason why APR has anti-inflammatory effects.

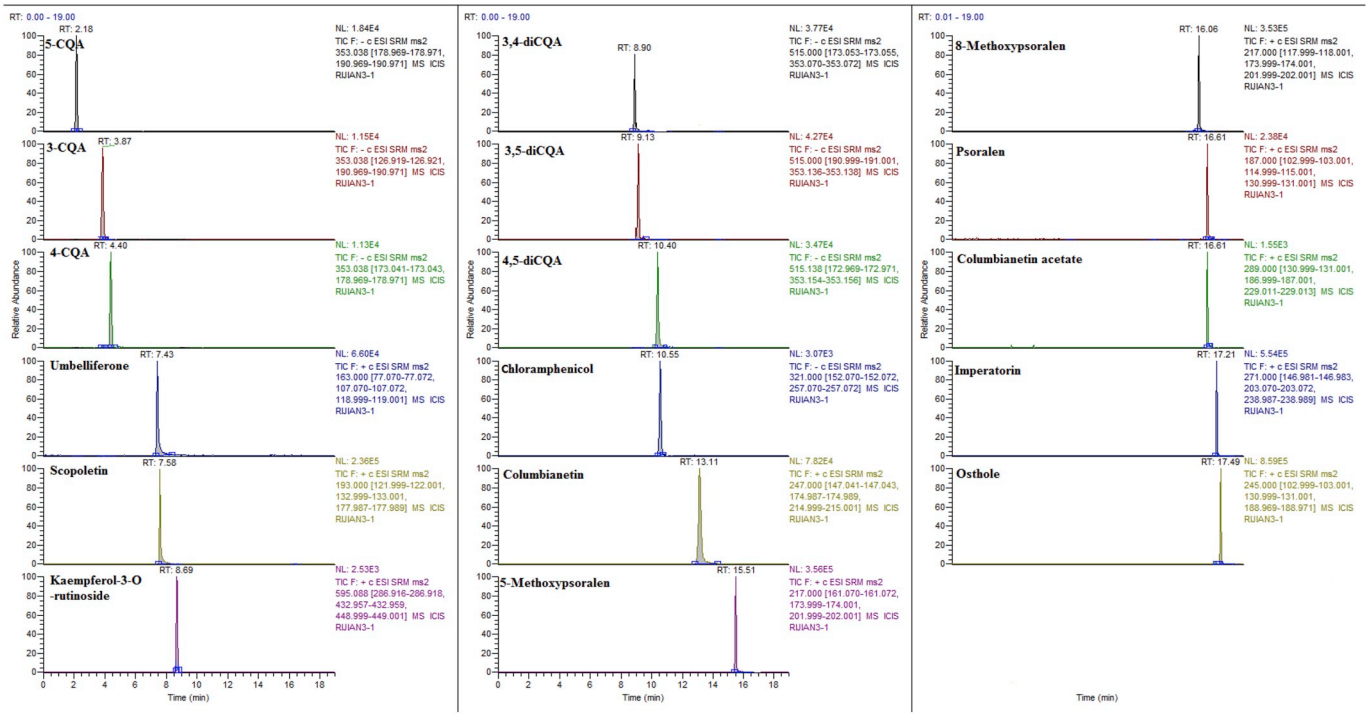

Fig. 4. Chromatogram of the 15 candidate compounds of APR and 2 internal standard compounds (Top to bottom and left to right are: 5-CQA, 3-CQA, 4-CQA, umbelliferone, scopoletin, kaempferol-3-O-rutinoside (IS1), 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, chloramphenicol (IS2), columbianetin, 5-methoxypsoralen, 8-methoxypsoralen, psoralen, columbianetin acetate, imperatorin and osthole, respectively).

3.3. Method validation

A satisfactory linear relationship was obtained within the corresponding concentration range and the correlation coefficients of 15 candidate compounds were all greater than 0.9990. And the LOD and LOQ of 15 candidate compounds were ranging from 0.0001 to 0.0067 μg/ml and 0.0003–0.0226 μg/ml, respectively. The RSD% of stability was between 1.00% and 4.94%, which manifest that the constructed method was stable enough during the experiment. The repeatability was tested, and the RSD% was less than 2.78%. The results indicated that the method has good repeatability. Intra-day and inter-day RSD% were within 2.54% and 4.06%, respectively. The results clarified that the method had good precision. The linear, correlation coefficients, LOD, LOQ, stability and repeatability results were shown in Table 1. The recovery rate of 15 candidate compounds was 95.71–103.99%, RSD% was between 0.16% and 4.28%. The result reflected that this method has remarkable accuracy for the assessment of 15 candidate compounds. The results can be seen in Table 2.

4. Discussion

4.1. Screening the small molecular ligands of TNF-α

The recovery solution was analyzed by UPLC-MS/MS and compared with the standard substance, and the components of the 15 compounds were determined as follows: neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, umbelliferone, scopoletin, 3,4-dicaffeoylquinic acids, 3,5-dicaffeoylquinic acids, 4,5-dicaffeoylquinic acids, 5-methoxypsoralen, columbianetin, 8-methoxypsoralen, psoralen, columbianetin acetate, imperatorin, osthole. Since these 15 compounds can bind to TNF-α as small molecule inhibitors, and they can block the conduction of the downstream signaling pathway of TNF-α, which may improve the development of RA, they were selected as candidate compounds. As for which compound is the most important TNF-α ligand, in this experimental, the affinity of 15 candidate compounds to TNF-α was used as an indicator of which substance was the most important compound as TNF-α ligand. It can be seen from the results that the affinity order of the six phenolic acids to TNF-α is: 3-CQA > 5-CQA > 4,5-diCQA > 3,5-diCQA > 3,4-diCQA > 4-CQA. Therefore, we believe that 3-CQA and 5-CQA are the most important ligands for TNF-α. We speculate that 3-CQA and 5-CQA have a strong affinity with TNF-α might be related to the combination of 3-CQA and 5-CQA with multi-site on TNF-α protein, which would increase the protein loading and bound response. We will explore and study the specific mechanism of the anti-inflammatory activity of how does the binding of 3-CQA and 5-CQA to TNF-α play an inhibitory role in the development of arthritis in future experiments.

4.2. Quantitative analysis

The contents of 15 compounds were calculated by regression equation. As shown in Table 3, import the data into ChemPtn 2017 Pro (Umetrics, Umeå, Sweden) and OriginLab (Northampton, MA, USA) software to observe the associations and differences between batches of 15 samples. The box plot charts showed the level of the total content of 15 compounds in 15 batches (Fig. 6), among which the total content of 15 compounds in S8 is the highest (28506.43±2094.47, μg/g). The total contents of 15 compounds were higher in Anhui, Hubei, Hunan, Sichuan provinces and lower in Gansu and Hebei province. In conclusion, there is a great difference in the content of compounds in APR from different sources, so the quality control of APR in this study is very significant.

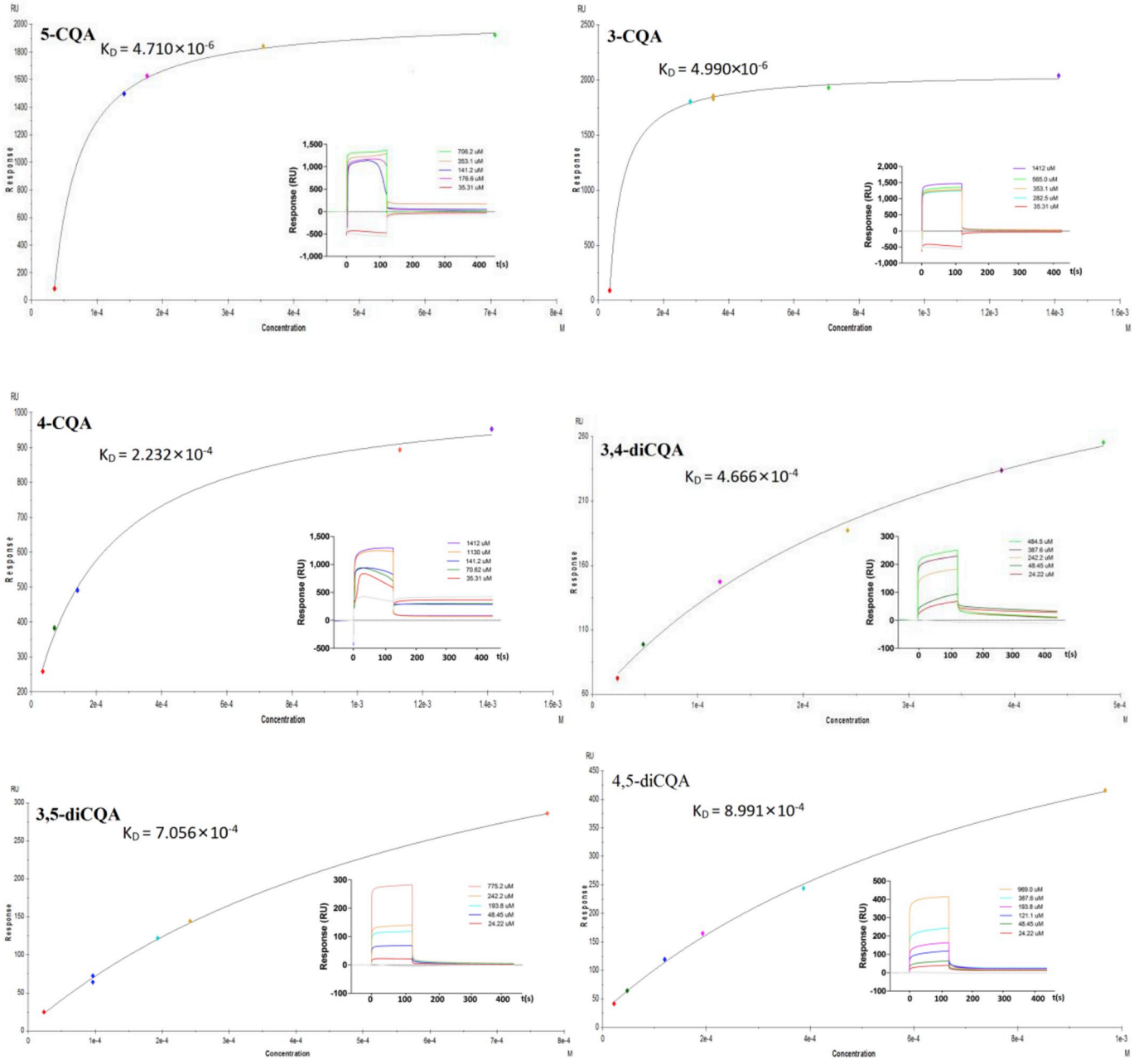

Fig. 5. Affinity sensing diagram and fitting curve of a series of concentrations of phenolic acid compounds with TNF-α

4.3. Chemometrics analysis

4.3.1. Principal component analysis

Principal Component Analysis (PCA) is one of the multivariate statistical dimension reduction technique and the method of feature extraction, with most of the original data information can be represented by a small amount of data. Therefore, PCA was adopted in this study to analyze the 15 batches of APR from different sources. From Fig. 7(A) and (B), we can see the 15 batches of APR samples were clearly separated. The green point represents the samples from Gansu province, the pink point represents the samples from Hubei province, the purple point represents the samples from Sichuan province, and the other three points respectively represents the samples come from Anhui, Hebei and Hunan province. The PCA figure directly reflects the differences between APR from different sources. However, S10 (yellow point) has a significant deviation, and we conducted a statistical analysis of its chemical composition and found that the content of osthole in S10 only accounts for 0.19% of the total content (<0.5%), which is inconformity with the provisions of Chinese Pharmacopoeia (2015 Edition). This unqualified sample was probably a fake product similar to the appearance of APR. Therefore, it is necessary to monitor the quality of APR for its authenticity.

4.3.2. Hierarchical clustering analysis

In this study, the data obtained from 15 batches of APR from different sources were analyzed by Hierarchical Clustering Analysis

Table 1
The calibration curves, correlation coefficient, linear range, LOD, LOQ, precision, repeatability and stability for the 15 candidate compounds.

Compound	Calibration curves	R^2	Linear range	LOD	LOQ	Precision (RSD, %)		Repeatability	Stability
			(μg/ml)	(μg/ml)	(μg/ml)	intra-day (n = 6)	inter-day (n = 3)	(RSD, %)	(RSD, %)
5-CQA	y = 7.9783x-0.0013	0.9997	0.0054-1.0770	0.0016	0.0054	1.04	3.66	1.53	2.63
3-CQA	y = 6.1188x-0.0358	0.9993	0.0114-39.6145	0.0034	0.0114	0.28	2.86	2.33	1.92
4-CQA	y = 6.8952x-0.048	0.9999	0.0109-2.9369	0.0032	0.0109	0.55	1.76	1.07	2.21
Umbelliferone	y = 43.153x+0.423	0.9992	0.0091-2.3750	0.0027	0.0091	2.54	3.00	2.58	3.94
Scopoletin	y = 204.46x-0.3555	0.9997	0.0016-1.0500	0.0004	0.0016	1.68	2.91	1.73	1.63
3,4-DiCQA	y = 21.192x-0.0963	0.9996	0.0055-2.3433	0.0016	0.0055	1.08	2.97	2.78	1.15
3,5-DiCQA	y = 25.451x-0.346	0.9997	0.0090-4.9012	0.0027	0.0090	1.06	3.22	2.47	2.97
4,5-DiCQA	y = 23.232x-0.3522	0.9997	0.0160-1.1000	0.0048	0.0160	1.20	2.64	1.34	1.00
Columbianetin	y = 107.29x-0.4031	0.9993	0.0025-4.7701	0.0007	0.0025	1.16	3.36	1.18	2.78
Psoralen	y = 16.768x-0.0466	0.9993	0.0049-24.2989	0.0014	0.0049	0.35	3.16	1.98	4.94
8-Methoxypsoralen	y = 211x+1.7133	0.9992	0.0016-11.2244	0.0005	0.0016	1.56	3.72	0.87	1.56
5-Methoxypsoralen	y = 187.54x+2.298	0.9990	0.0013–23.8353	0.0004	0.0013	1.45	4.06	1.80	4.43
Columbianetin acetate	y = 1.2291x-0.0208	0.9996	0.0226-73.1300	0.0067	0.0226	2.44	3.71	1.95	1.38
Imperatorin	y = 267.55x+0.0212	0.9994	0.0014-2.4300	0.0004	0.0014	2.29	3.69	2.54	1.27
Osthole	y = 411.57x+5.8415	0.9990	0.0003–46.0772	0.0001	0.0003	1.78	3.64	2.41	1.05

Table 2
Recovery rates and the RSD of the 15 candidate compounds with three levels concentrations.

Compound	Sample(μg/ml)	Spiked (μg/ml)	Found (μg/ml)	Recovery (%)	RSD (%, n = 3)
5-CQA	0.0156	0.0125	0.0277	96.53	1.48
		0.0157	0.0306	95.76	1.17
		0.1879	0.2066	101.65	2.49
3-CQA	1.7836	1.4280	3.1504	95.71	1.68
		1.7850	3.5344	98.08	2.52
		2.1420	3.9717	102.14	1.72
4-CQA	0.0353	0.0261	0.0610	97.89	2.38
		0.0353	0.0719	103.67	1.44
		0.0424	0.0780	100.35	1.08
Umbelliferone	0.1740	0.1392	0.3160	101.96	1.19
		0.1741	0.3497	100.90	0.19
		0.2089	0.3868	101.81	1.64
Scopoletin	0.0521	0.0416	0.0942	100.97	0.16
		0.0520	0.1037	99.19	0.20
		0.0625	0.1167	103.31	2.54
3,4-DiCQA	0.0587	0.0469	0.1066	102.08	0.42
		0.0590	0.1194	102.78	2.05
		0.0704	0.1315	103.40	1.41
3,5-DiCQA	0.4035	0.3186	0.7194	99.17	0.58
		0.4036	0.8162	102.27	1.57
		0.4842	0.8923	100.94	0.89
4,5-DiCQA	0.0712	0.5687	0.1271	98.35	1.20
		0.0711	0.1441	102.47	0.73
		0.0854	0.1577	101.18	0.44
Columbianetin	0.2688	0.2196	0.4877	99.69	0.44
		0.2687	0.5444	102.55	1.79
		0.3225	0.6057	104.43	1.10
Psoralen	2.0298	1.6490	3.7447	103.99	4.28
		2.0273	4.0389	99.10	1.03
		2.4347	4.5380	103.01	1.17
8-Methoxypsoralen	1.0728	0.8568	1.9165	98.46	0.51
		1.0710	2.1651	101.98	1.86
		1.2852	2.3565	99.88	0.39
5-Methoxypsoralen	0.4690	0.3808	0.8483	99.62	1.36
		0.4689	0.9407	100.58	0.68
		0.5627	1.0431	102.01	0.28
Columbianetin acetate	4.1165	3.2960	7.4545	101.27	0.53
		4.1200	8.3139	101.87	2.09
		4.9440	8.9875	98.52	2.07
Imperatorin	0.0171	0.0136	0.0307	99.40	2.10
		0.0172	0.0349	103.52	3.21
		0.0205	0.0381	102.22	1.50
Osthole	5.4159	4.9795	10.3373	98.83	1.13
		5.4165	10.7594	98.65	2.34
		6.4975	11.9142	100.01	1.41

Table 3
The contents of the 15 candidate compounds of APR from different sources (μg/g, mean ± SD).

NO.	Source	1	2	3	4	5	6	7
S1	Anhui	28.69±2.32	3.65±0.41	7.80±0.43	43.95±5.45	11.75±0.07	37.61±1.53	60.73±2.64
S2	Gansu	1432.29±70.32	368.53±21.05	701.04±102.63	4574.4±7414.05	1133.04±94.68	2623.70±156.68	2827.61±109.20
S3	Gansu	33.18±1.76	6.43±0.31	7.96±0.75	70.05±8.30	15.04±0.47	45.45±3.40	76.38±4.96
S4	Gansu	147.14±4.50	35.29±1.73	50.89±4.39	71.42±5.23	100.52±1.07	160.69±9.49	221.35±17.02
S5	Hebei	33.45±1.52	10.24±0.93	13.96±1.59	15.71±1.06	18.52±0.78	22.01±1.05	46.40±2.65
S6	Hubei	50.83±1.49	8.09±0.57	11.27±1.10	96.94±9.15	23.21±1.33	66.47±4.02	74.83±3.03
S7	Hubei	178.07±6.99	81.15±6.31	175.58±21.90	352.98±34.66	217.97±0.38	278.45±17.11	330.97±9.60
S8	Hubei	30.87±1.31	14.62±0.66	20.45±0.63	58.43±5.17	43.24±3.16	51.58±3.30	109.18±3.15
S9	Hubei	476.22±17.77	53.45±4.72	87.08±13.06	252.79±12.96	68.37±3.66	359.47±18.37	369.08±15.56
S10	Hunan	3690.14±146.11	345.32±25.99	438.68±56.70	2521.39±201.98	1015.73±20.51	3153.26±185.08	3679.01±259.76
S11	Sichuan	550.40±39.79	208.31±16.78	291.54±44.95	281.7±11.94	619.08±4.81	627.62±33.15	354.92±20.48
S12	Sichuan	718.98±22.30	94.45±6.78	166.44±15.50	175.83±12.71	206.24±4.03	393.67±11.36	239.56±14.15
S13	Sichuan	8518.91±240.41	807.45±67.46	1080.51±98.55	6607.80±228.93	3627.59±89.35	10787.99±502.28	10184.15±680.23
S14	Sichuan	72.46±4.51	3.50±0.25	5.17±0.72	21.89±0.84	9.12±0.39	40.53±2.58	23.02±0.82
S15	Sichuan	7983.75±256.65	889.39±82.80	1111.06±127.28	5923.43±440.30	3484.71±114.00	8801.57±435.40	8031.21±442.83

NO.	8	9	10	11	12	13	14	15
S1	43.66±7.25	39.37±2.90	3.47±0.41	22.59±1.19	27.00±2.32	40.93±1.35	43.07±5.28	52.61±1.91
S2	3318.98±290.70	3769.10±336.33	39.35±15.20	1947.61±73.51	2314.49±128.62	2709.31±89.08	2319.12±241.10	2556.23±95.60
S3	52.89±9.16	46.30±2.10	7.25±0.37	22.92±2.06	40.65±2.19	43.85±4.22	39.14±2.23	87.25±4.08
S4	166.46±12.62	72.89±5.96	32.57±4.45	56.45±3.80	64.66±6.77	92.18±8.81	102.83±3.86	314.97±7.97
S5	22.01±0.53	16.97±1.28	11.61±1.71	7.22±0.48	13.28±0.41	26.29±1.87	27.53±1.58	25.53±1.21
S6	77.10±4.80	66.40±3.35	9.55±4.33	39.90±0.83	49.23±1.57	64.10±1.48	68.74±6.17	68.46±2.20
S7	344.65±47.27	187.48±16.10	19.56±7.81	160.96±2.71	140.72±5.87	198.17±7.00	218.26±20.78	185.43±7.46
S8	53.01±5.87	31.80±0.82	22.84±5.69	21.66±0.94	30.33±1.50	53.38±1.65	66.81±5.37	55.53±1.57
S9	369.32±31.02	252.05±19.44	13.25±0.86	158.33±9.83	217.86±7.36	508.03±34.12	564.92±13.96	909.15±33.64
S10	2639.23±167.35	3973.68±220.20	22.41±0.40	3124.55±227.28	2302.62±143.27	2546.58±182.05	2362.99±82.53	2725.59±177.89
S11	670.27±70.75	222.03±0.59	1431.52±185.15	248.39±19.63	220.14±14.42	565.84±30.57	630.04±31.42	896.01±26.23
S12	399.41±43.38	131.76±11.49	514.44±67.48	190.42±15.99	145.29±4.79	383.80±18.48	412.74±27.45	415.94±19.03
S13	11281.36±905.42	10300.34±809.13	467.86±76.68	8764.90±67.82	7486.39±1417.06	7669.07±535.48	7302.64±275.29	7052.96±202.56
S14	43.45±1.78	19.09±1.44	49.66±7.82	20.49±1.91	12.33±0.91	16.49±1.02	17.12±0.19	37.72±0.74
S15	9024.55±842.96	7616.47±529.63	9.48±0.47	5659.50±387.54	5175.49±208.60	5620.35±121.59	5092.15±199.28	6310.02±174.90

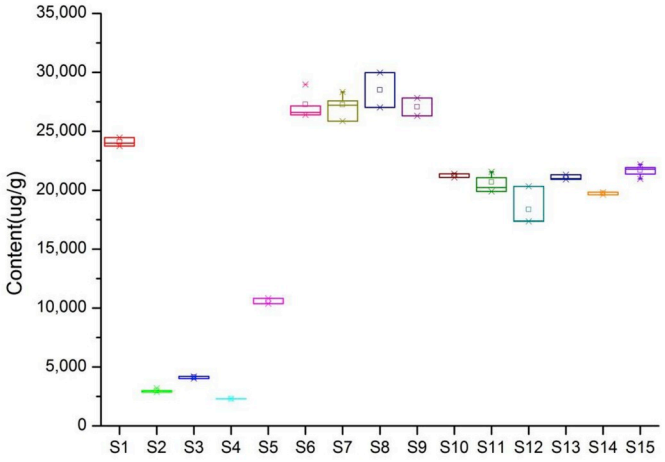

Fig. 6. Box plot charts of the total content of 15 candidate compounds in 15 batches of APR from different sources.

(HCA), as shown in Fig. 8. The 15 batches of samples were divided into Group I and Group II, Since the content of S2–S5 were significantly lower than that of other groups, they were divided into a cluster separately, which was consistent with the results of box plot charts. Group II was further divided into two groups: II1 and II2. We can see that APR samples from Sichuan province (S11–S15) and Hubei province (S6–S9) were divided into Groups II1 and II2, respectively, and the results were corresponded with the results of PCA. From the results of HCA, we know that the main reason for the significant difference in samples from different sources was caused by the four compounds of psoralen, chlorogenic acid, osthole and columbianetin acetate. S6–S9 from Hubei province contain the highest content of osthole and columbianetin acetate, which were obviously different from other samples. Previous studies have reported that they have anti-inflammatory activity. Therefore, the APR can be widely planted in Hubei province to improve the quality of APR in the market.

5. Conclusion

The purpose of this study was to establish a biosensor technology based on surface plasma resonance (SPR) for screening small molecules

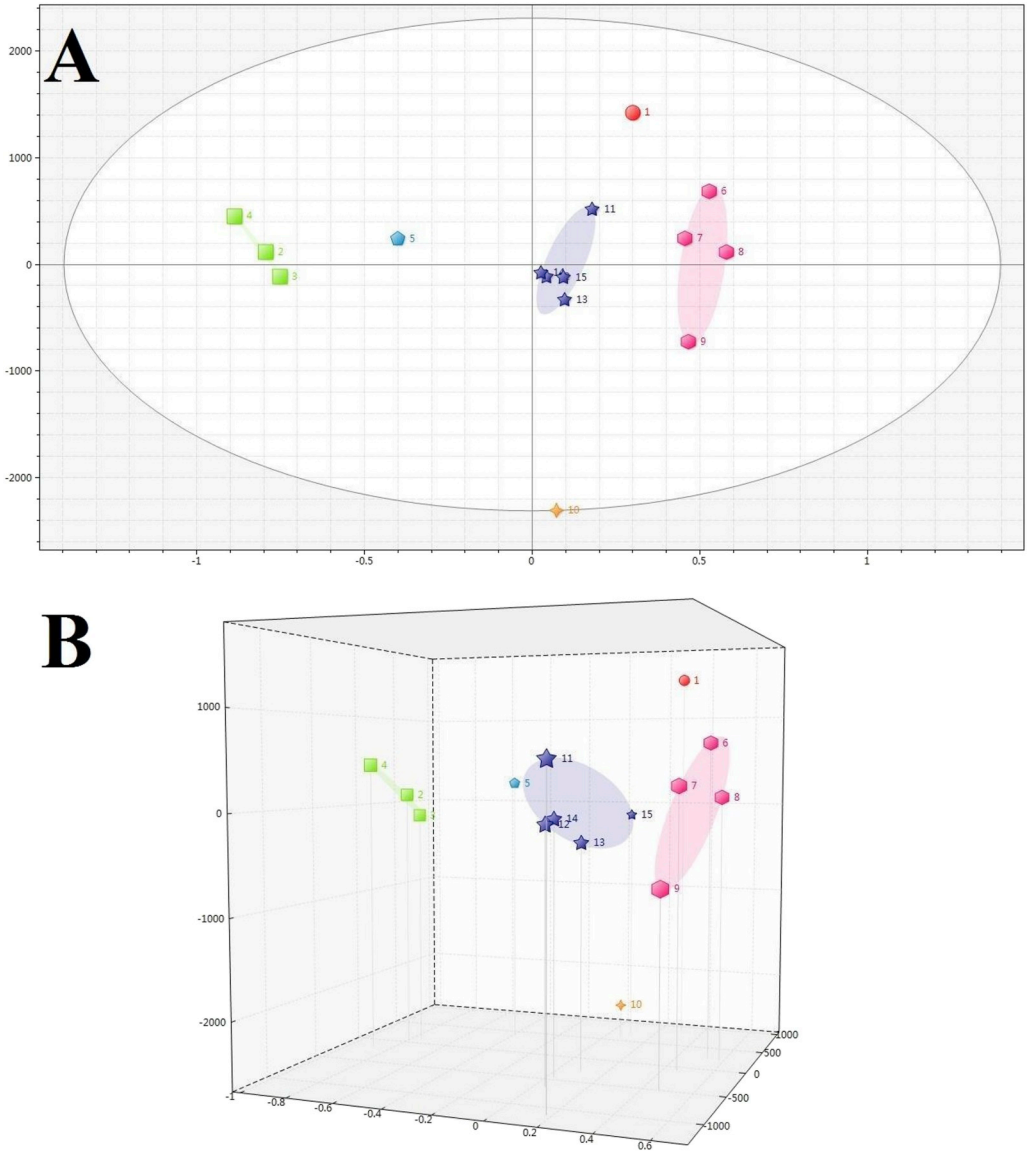

Fig. 7. The PCA plot and three-dimensional of the 15 batches of APR.

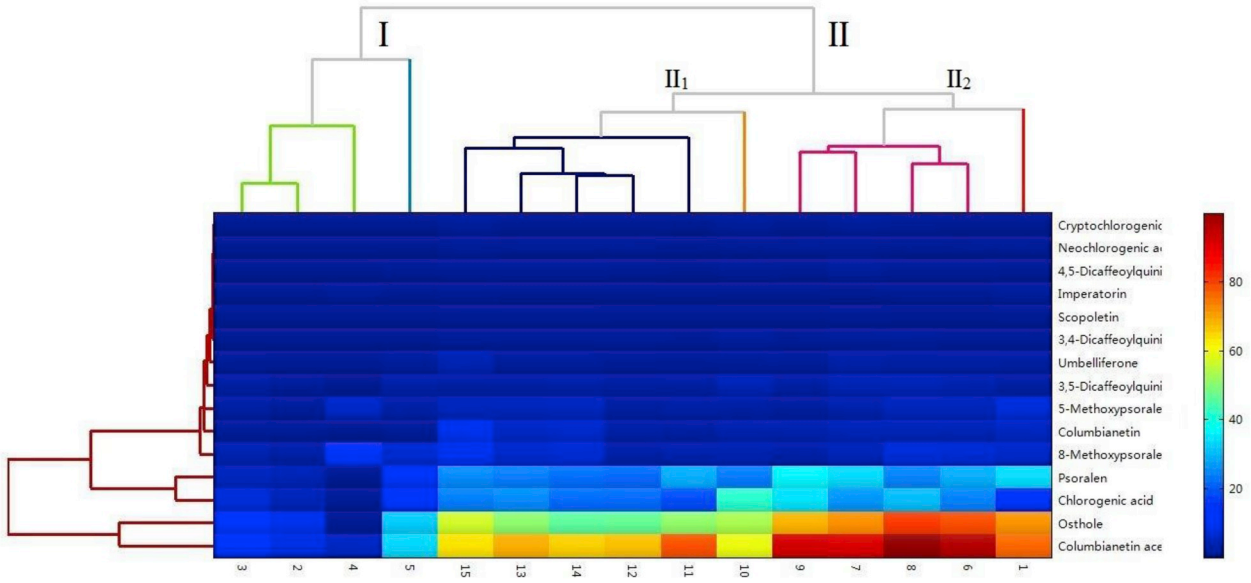

Fig. 8. HCA diagram and heat diagram of the 15 batches of APR.

bound to TNF-α in APR. Then UPLC-MS/MS was used to quantify the selected small molecules, and chemometrics were used to analyze APR from 15 batches of different origins. The results show that the established SPR method can quickly, effectively and reliably screen the active components bound to TNF-α in APR. The affinity test results showed that the selected small molecules had a good affinity with TNF-α in a range of concentration. Methodological studies show that 15 compounds have good linearity, precision and accuracy in the linear range. In conclusion, this study is the first successful application of SPR to screen TNF-α small molecule inhibitors from APR, which can provide important indicators and basis for in-depth study of potential drugs with anti-RA effect in TCMs, and plays an increasingly important role in drug research and development.

Data availability

All data generated or analyzed during this study are included in this article.

CRediT authorship contribution statement

Liu Yang: Writing - original draft. **Ajiao Hou:** Writing - original draft. **Song Wang:** Writing - original draft. **Jiaxu Zhang:** Writing - original draft. **Wenjing Man:** Writing - original draft. **Xinyue Guo:** Writing - original draft. **Bingyou Yang:** Writing - original draft. **Qiuhong Wang:** Writing - original draft. **Hai Jiang:** Writing - original draft. **Haixue Kuang:** Writing - original draft.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2020.113643.

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