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Original Article

A new strategy for choosing "Q-markers" via network pharmacology, application to the quality control of a Chinese medical preparation

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ARTICLE INFO

Article history:
Received 4 July 2017
Received in revised form
3 September 2017
Accepted 14 October 2017
Available online 10 November 2017

Keywords:
Quality marker
Network pharmacology
Quality control
Shenxian Shengmai oral liquid

ABSTRACT

Due to its chemical complexity, proper quality control for a Chinese medical preparation (CMP) has been a great challenge. Choosing the appropriate quality markers (Q-markers) for quality control of CMP is an important work. Best of all, the chosen Q-markers are the main chemical compounds from the herbals as well as the active constituents of this CMP. Only in this way the established quality control system can really achieve the purpose of controlling the quality of CMP and ensuring the safely and effectively use of CMP. To achieve the purpose, network pharmacology combined with the contents of chemical compounds in the CMP has been used in this research. We took an anti-arrhythmic CMP, Shenxian-Shengmai oral liquid (SSOL), as an example. Firstly, UPLC-QTOF-MS/MS method was used to analyze the main components of SSOL. A total of 64 compounds were unambiguously or tentatively identified and 32 of them were further validated by reference compounds. Secondly, the network was constructed based on the identified compounds to predict the effective compounds related to cardiac arrhythmias. Based on the existing database and the operation method of topology, a method of double network analysis (DNAA) was proposed, from which 10 important targets in the pathway of arrhythmia were screened out, and 26 compounds had good antiarrhythmic activity. Based on the prediction results of network pharmacology along with the contents of the compounds in this CMP, ten representative compounds were chosen as the Q-markers for the quality control of SSOL. We find that five of these ten compounds, including danshensu, rosmarinic acid,

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https://doi.org/10.1016/j.jfda.2017.10.003

salvianolic acid A, epimedin A and icariin, have antiarrhythmic activity. Then, the UPLC-DAD method was established as the control method for SSOL.

1. Introduction

The characteristic of Chinese medical preparation (CMP), which contains most complicated chemical constituents, is embodied in the concept of multi-components and multi-targets [1]. Because of a large amount of natural metabolites in it, it is very difficult to control the quality of CMP. Currently, to ensure the safety and efficacy of CMP, fingerprinting combined with chemometrics and representative components determination are two common strategies [2]. To promote the healthy development of pharmaceutical industry and improve the quality standard system, core of national pharmacopoeia standards and product quality standards of CMP, the concept of quality marker (Q-marker), which is for the quality control for Chinese medicinal products, was proposed by Liu et al. [3,4]. Consequently, how to scientifically choose the appropriate Q-markers has been a great challenge for quality management.

Network pharmacology, which was first proposed by Hopkins [5,6], offers an ideal paradigm to deal with multi-target combination drugs and has recently been successfully adopted to investigate the formulae in traditional Chinese medicine (TCM) [7,8]. In our previous research, we have analyzed and predicted the active chemical constituents in Lianhua-Qingwen capsule [9,10] and Dangui-Jianzhong fomula [11] using the network analysis, and successfully validated the predicted results. Hence, the network analysis method was used to predict the active compounds to be the Q-markers in this work. In this case, we use the network pharmacology method combined with drug contents to select Q-markers, which is defined as the chemical constituents from the herb medicine or generated compounds during the processing preparation, to achieve control of the quality of CMP [12].

Finally, Shenxian-Shengmai oral liquid (SSOL) was used as a model to illustrate the confirmation process of Q-markers selection and application. SSOL is composed of 8 traditional Chinese herbs, including Gingseng Radix et Rhizoma Rubra (Hongshen), Epimedh Folium (Yinyanghuo), Psoralea Fructus (Buguzhi), Lych Fructus (Gouqi), Ephedrae Herba (Mahuang), Asari Radix et Rhizoma(Xixin), Salviae Miltiorrhizae Radix et Rhizoma(Danshen), Hirudo(Shuizhi), which has the efficacy of warming and invigorating heart and kidney, as well as promoting blood circulation to dissipate blood stasis. In this study, ultra performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) [13–17] was used to analyze the major chemical constituents of SSOL. A total of 64 compounds were unambiguously or tentatively identified by comparing primary and secondary MS/MS spectral data with reference compounds or literature data and reference standards [13–22]. On the basis of qualitative analysis, the network pharmacology method was introduced to choose the Q-markers, 26 predicted effective ingredients were successfully identified. Based on the research results of qualitative analysis and network pharmacology, combined with the source of compounds which was contained in the medicinal materials, 10 representation compounds including danshensu, protocatechuic aldehyde, psoralenoside, isopsoralenoside, rosmarinic acid, salvianolic acid A, epimedin A_1, epimedin A, epimedin C and icariin were selected as the Q-markers of SSOL. The contents of the 10 compounds were determined by UPLC-DAD [22,23] method, which was accurate, sensitive and reliable and laid a good foundation for the quality control of SSOL.

2. Experimental

2.1. Chemicals and reagents

Epimedin A was purchased from Beijing LEYBOLD Cable Technology Co. Ltd. Standard compounds of citric acid, succinic acid, magnoflorine, rosmarinic acid, lithospermic acid, salvianolic acid C, p-hydroxybenzaldehyde, epimedin B and icariin, baohuoside I, ginsenoside Re, ginsenoside Rg_1, ginsenoside R_f, ginsenoside Rb_1, ginsenoside Rb_2, ginsenoside Rb_3, ginsenoside Ro, ginsenoside Rc and ginsenoside Rd were purchased from Shanghai Source Leaf Biotechnology Co. Ltd. Danshensu, protocatechuic acid, protocatechuic aldehyde, salvianolic acid B, salvianolic acid A, epimedin A_1, epimedin C and neobavaisoflavone were purchased from Nanjing Biological Engineering Co. Ltd. Psoralen glycosides, isopsoralen glycosides, psoralen and isopsoralen were obtained by our research team. The purity of each chemical was equal to or greater than 97%. SSOL was generously provided by Shandong Heze Buchang Pharma. All solvents, including methanol and acetonitrile with purity of 98% were of HPLC grade purchased from Sigma (U.S.A.), except formic acid which was purchased from Tedia (U.S.A.). Water was obtained from a Elix/RiO water purification system (Millipore, Bedford, MA, U.S.A.). All other reagents and chemicals were of analytical grade.

2.2. Preparation of standard and sample

2.2.1. The first group of standard and sample

Citric acid, succinic acid, danshensu, protocatechuic acid, protocatechuic aldehyde, magnoflorine, p-hydroxybenzaldehyde, psoralen and isopsoralen glycosides, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid A, psoralen, isopsoralen, epimedin A_1, epimedin A, salvianolic acid C, epimedin B, epimedin C, icariin, baohuoside I,

neobavaisoflavone, ginsenoside Re, ginsenoside Rg_1, ginsenoside R_f, ginsenoside Rb_1, ginsenoside Rb_2, ginsenoside Rb_3, ginsenoside Ro, ginsenoside Rc and ginsenoside Rd were separately dissolved in 50% methanol–water (v/v) to obtain 32 reference compound stock solutions (at 200 μg/mL), and stored at 4 °C in a refrigerator. All solutions were filtered through a 0.22 μm organic microporous membrane filter before subjecting to UPLC-QTOF-MS analysis.

Five mL of SSOL were transferred to a 10 mL volumetric flask, mixed with a suitable volume of methanol and then sonicated for 30 min. Then the sample was cooled to the room temperature to complement the weight and kept for shaking on centrifuge at 14,000 r/min for about 10 min, and the supernatant was diluted by 100 times with 50% methanol–water solution. In the end, the solution was filtered through a 0.22 μm organic microporous membrane filter before subjecting to UHPLC-QTOF-MS analysis.

2.2.2. *The second group of standard and sample*

Appropriate amounts of reference standards of danshensu, protocatechuic aldehyde, psoralenoside, isopsoralenoside, rosmarinic acid, salvianolic acid A, epimedin A_1, epimedin A and icariin and epimedin C were weighed, and each of these standards was prepared by adding a suitable volume of each stock solution to a 10 mL flask and diluted with 50% methanol–water solution at the concentration of 111 g/mL for danshensu, 16.4 g/mL for protocatechuic aldehyde, 404 g/mL for psoralenoside, 451.4 g/mL for isopsoralenoside, 9.1 g/mL for rosmarinic acid, 46.9 g/mL for salvianolic acid A, 54.5 g/mL for epimedin A_1, 7.0 g/mL epimedin A, 17.8 g/mL for epimedin C and 23.9 g/mL for icariin. A mixed solution containing all of the 10 reference compounds were then prepared and serially diluted with 50% methanol–water to obtain five reference solutions with different concentrations. The stock solution was stored at −80 °C until needed for fresh dilution.

The procedure for preparing SSOL sample solutions was similar to that of 2.2.1, except that 1 mL SSOL were taken and transferred to a 50 mL volumetric flask.

2.3. *Qualitative analysis of the major constituents in SSOL by UPLC-QTOF-MS/MS*

2.3.1. *UPLC-QTOF-MS/MS conditions*

UPLC experiments were performed on an ACQUITY UPLC BEH C_{18} column (100 × 2.1 mm, 1.7 μm; Waters Corp, Milford, MA, USA). The mobile phase consisting of 0.1% formic acid in water (phase A) and methanol (phase B) was delivered at a flow rate of 0.3 mL/min using a gradient program as follows: 5% (B) from 0 to 5 min, 5–32% (B) from 5 to 23 min, and 32–98% (B) from 23 to 33 min, and 98% (B) from 33 to 35 min. The column temperature was maintained at 30 °C. The autosampler temperature was maintained at 4 °C, and 2 μL of sample solution was injected. The detection wavelengths of DAD were set at 203 nm, 225 nm, 254 nm and 280 nm.

The mass spectrometer was operated in positive/negative ionization mode with the capillary voltage set at 3.0/2.5 kV, respectively. The ion source and desolvation temperatures were set to 100 and 400 °C, respectively. Nitrogen was used as the desolvation gas with flow rate of 600 L/h; the first range scan was set at *m/z* 50–1500 Da.

2.3.2. *Establishment of the database of chemical constituents of SSOL*

According to Pubmed, ChemSpider and related literature, the chemical names, molecular formulas, molecular weight and about 900 fragmentation information were collected from various chemical components of eight medicinal materials in SSOL. Furthermore, the Waters MassLynx V4.1 SCN901 software (including the accurate molecular mass of each element) was adopted for information, on the basis of exact molecular masses, the possible molecular formulas could be calculated with a less than or equal to 5 ppm error.

2.4. *Network pharmacology analysis of SSOL*

2.4.1. *Network construction*

As shown in Table S1, 64 compounds were identified in SSOL by UPLC-QTOF-MS/MS. With their names and/or chemical

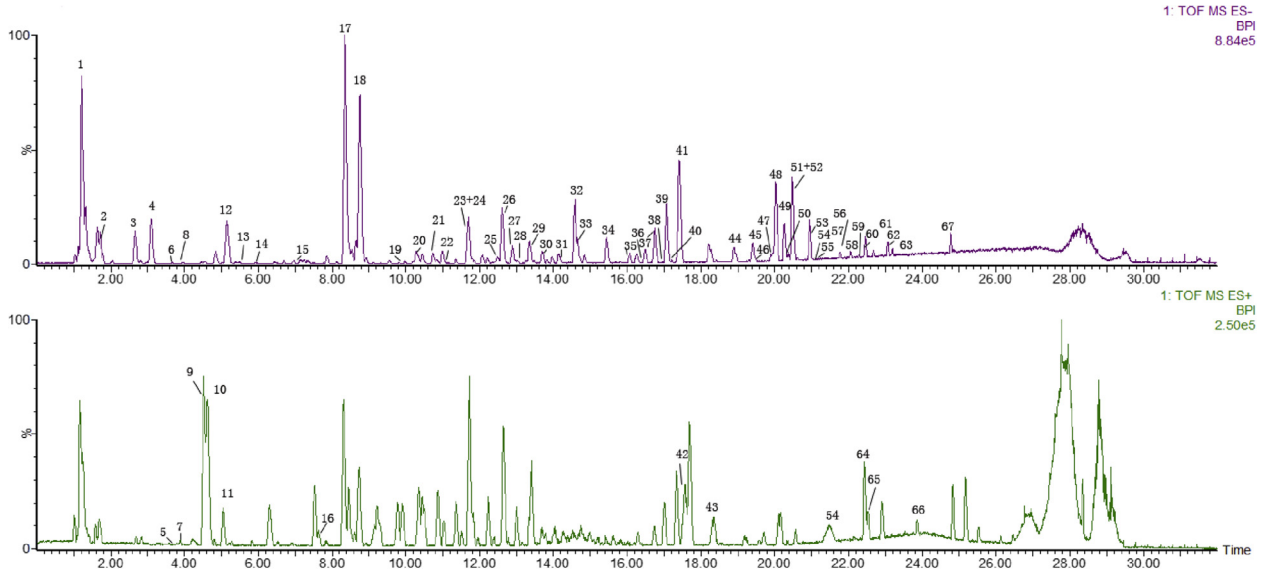

Fig. 1 – The representative BPI total ion chromatogram from a prepared sample of SSOL based on UPLC-QTOF-MS.

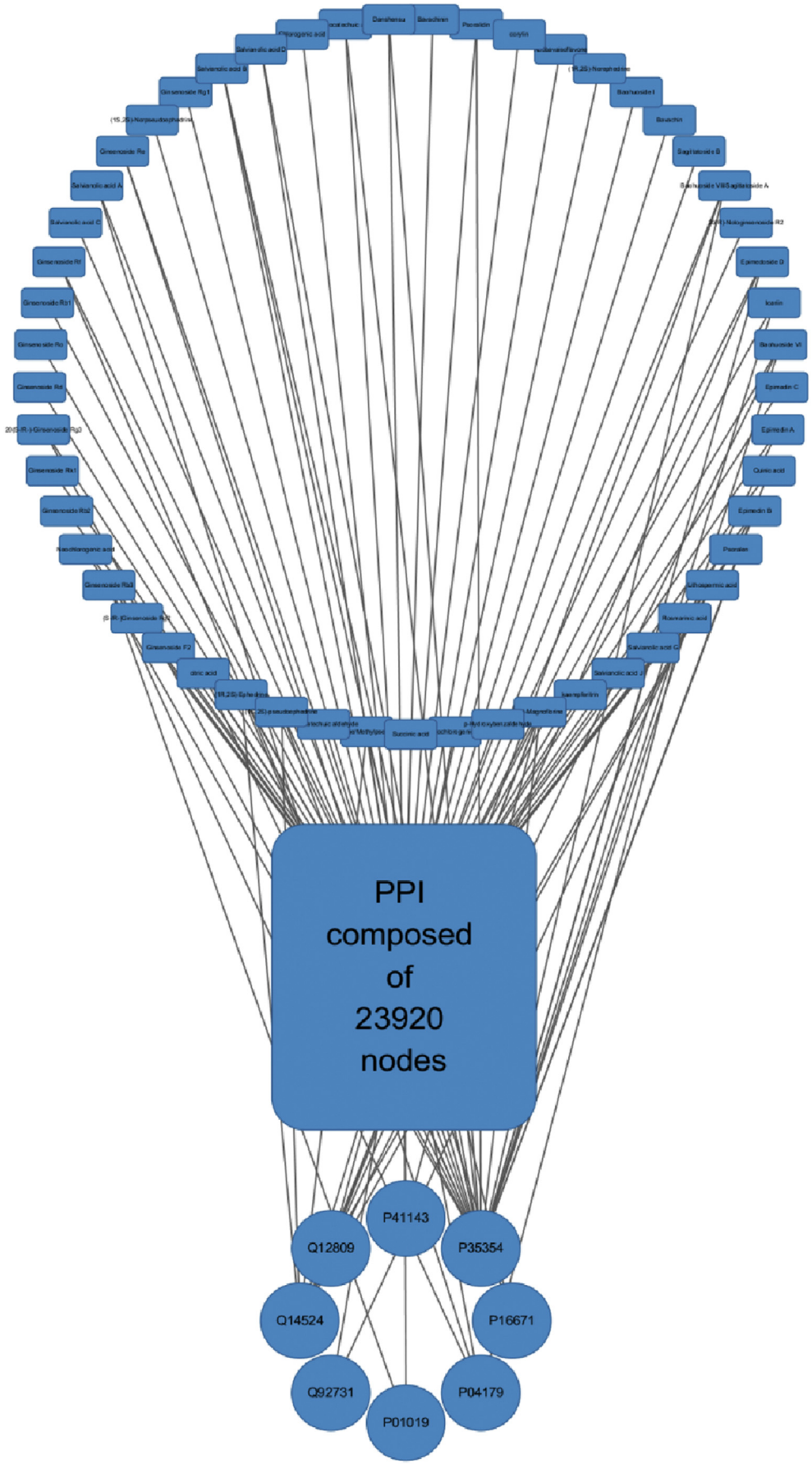

Fig. 2 – The DT network of the main components in SSOL which composed of the nodes of the 53 candidate ingredients (rectangles) and the 8 directly related targets (ellipses).

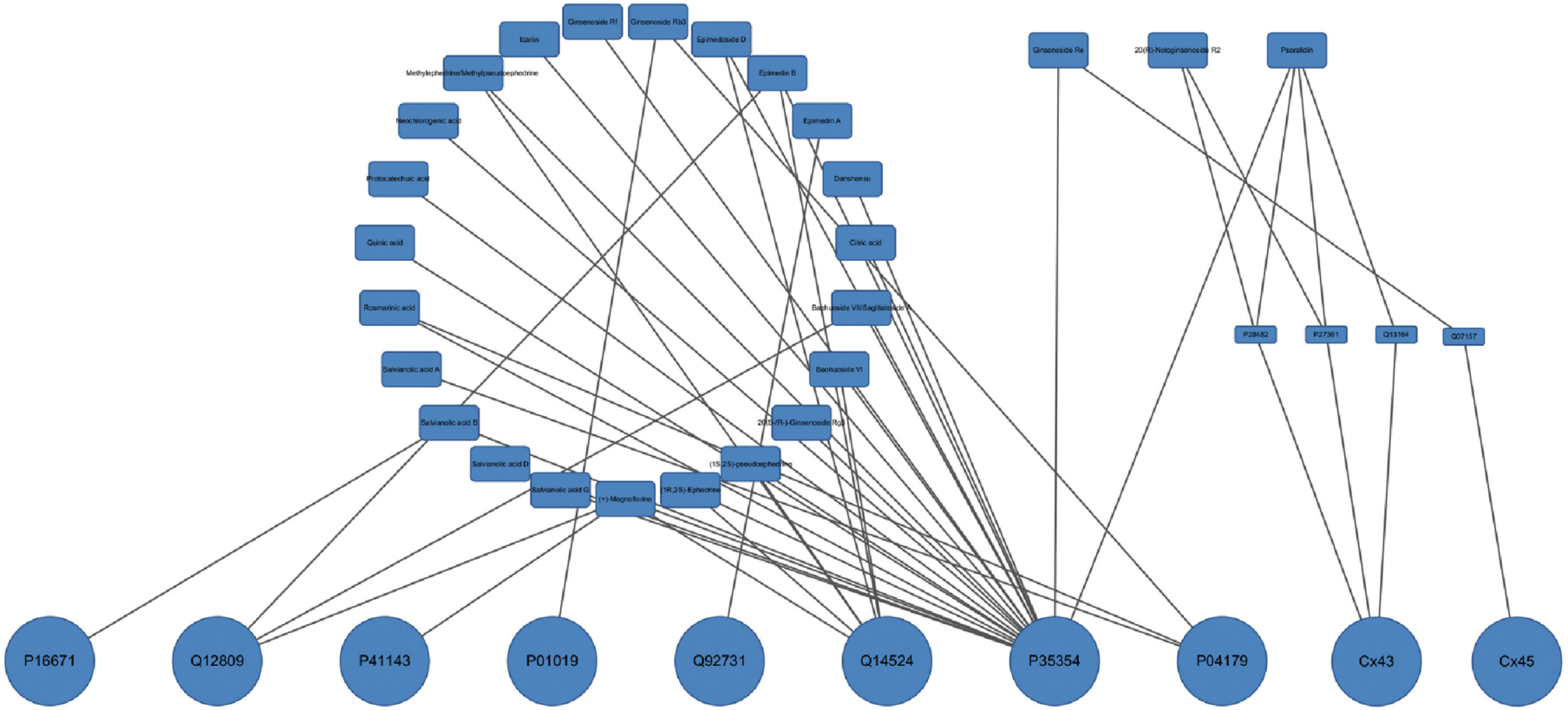

Fig. 3 – The DT network of the effective components in SSOL which composed of the nodes of the 26 effective ingredients (rectangles) and the 8 directly related targets (ellipses) and the 2 indirectly related targets (ellipses).

Table 1 – Regression equation, R^2, linear ranges, LODs and LOQs, intraday and interday precisions, stability, repeatability and recovery for 10 compounds.

Compounds	Regression equation[a] ($n = 3$)	R^2	Linear range (μg/mL)	LOD[b] (μg/mL)	LOQ[c] (μg/mL)	Intraday (RSD, %) ($n = 6$)	Interday (RSD, %) ($n = 3$)	Stability (RSD, %)	Repeatability ($n = 6$)		Recovery ($n = 6$)				
									Mean (μg/mL)	RSD (%)	Original (μg)	Spiked (μg)	Detected (μg)	Recovery (%)	RSD (%)
Danshensu	$y = 11139x - 6818.2$	0.9999	6.94–111.00	0.69	3.08	0.71	0.81	0.68	1337.44 ± 5.65	0.42	668.72	666.00	1332.06	99.60	2.53
Protocatechuic aldehyde	$y = 75449x - 4117.7$	1.0000	1.03–16.40	0.10	0.46	0.57	0.64	0.26	258.17 ± 1.70	0.66	129.08	131.20	251.15	93.04	1.89
Psoralenoside	$y = 15175x + 29849$	0.9999	25.25–404.00	0.17	0.68	0.06	0.46	0.18	5180.42 ± 17.58	0.34	2590.21	2828.00	5173.96	91.36	1.51
Isopsoralenoside	$y = 18199x + 72833$	0.9995	28.21–451.40	0.13	0.50	0.09	0.64	0.13	3852.62 ± 13.77	0.36	1926.31	1805.60	3897.14	109.15	0.77
Rosmarinic acid	$y = 21616x + 311.6$	0.9998	0.57–9.10	0.25	0.58	0.67	0.78	1.16	141.42 ± 1.65	1.16	70.71	72.80	146.13	103.60	4.04
Salvianolic acid A	$y = 18887x + 1583.4$	0.9993	2.93–46.90	0.43	1.37	0.88	2.83	1.19	627.83 ± 20.75	3.30	313.91	281.40	580.38	94.69	4.60
Epimedin A_1	$y = 17512x + 440.33$	1.0000	3.41–54.5	0.27	1.36	0.44	2.74	0.41	701.70 ± 12.70	1.81	350.85	327.00	669.77	97.53	4.66
Epimedin A	$y = 16271x + 465.79$	0.9993	0.0.44–7.00	0.17	0.44	0.58	1.25	1.28	69.04 ± 1.00	1.45	34.52	42.00	75.66	97.96	3.38
Epimedin C	$y = 16609x + 621.1$	0.9999	1.11–17.80	0.22	0.56	0.17	0.35	0.21	220.17 ± 2.55	1.16	110.08	106.80	212.12	95.54	3.92
Icariin	$y = 20888x + 1526.4$	0.9998	1.49–23.90	0.30	0.99	0.23	0.23	0.14	355.19 ± 1.19	0.33	177.60	191.2	360.16	95.48	1.51

[a] The regressive equations are presented as $y = ax + b$. y is the peak area, x is the concentration of compound.
[b] LOD, limit of detection, S/N = 3.
[c] LOQ, limit of quantification, S/N = 10.

Table 2 – Contents of the 10 compounds in 10 batches.

No.	Contents (μg/mL)									
	Danshensu	Protocatechuic aldehyde	Psoralenoside	Isopsoralenoside	Rosmarinic acid	Salvianolic acid A	Epimedin A_1	Epimedin A	Epimedin C	Icariin
Lot.1	1329.48	259.21	5184.24	3940.92	144.79	605.88	654.02	69.73	220.77	353.14
Lot.2	1047.99	186.36	4288.40	3422.60	120.45	477.39	559.41	60.54	196.19	313.92
Lot.3	1135.04	208.18	4725.56	3839.58	127.01	586.37	582.91	57.83	211.89	303.81
Lot.4	1200.33	209.22	4686.14	3600.00	122.31	482.11	639.02	62.83	204.42	336.68
Lot.5	1245.06	231.65	4873.31	3818.90	135.73	559.34	615.73	63.32	214.62	339.43
Lot.6	1189.02	218.76	4602.29	3635.48	118.90	506.44	594.88	59.87	240.05	342.99
Lot.7	1234.93	248.48	4946.83	3907.56	129.34	558.82	621.27	65.45	243.56	356.30
Lot.8	1306.64	243.83	4729.74	3720.76	131.08	551.87	598.12	53.28	231.11	304.97
Lot.9	1179.56	185.98	4360.10	3449.39	112.36	527.08	580.58	61.91	190.01	302.62
Lot.10	1139.49	175.75	4626.03	3531.57	124.16	701.04	693.34	66.46	311.16	371.03
Average	1200.75 ± 83.61	216.74 ± 28.77	4702.26 ± 263.29	3686.68 ± 187.29	126.61 ± 9.23	555.63 ± 66.18	613.93 ± 39.81	62.12 ± 4.64	226.38 ± 34.62	332.49 ± 24.66
RSD%	6.96	13.27	5.61	5.08	7.29	11.91	6.48	7.47	15.29	7.42

structures (denoted by the simplified molecular-input line-entry system (SMILES) strings) as key words, ingredient-related proteins were collected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) [24] and the Search Tool for Interactions of Chemicals (STITCH) [25]. Eight of 53 compounds were further validated to have the directly related effect on protein, and these active ingredients of the SSOL connect to their targets to achieve therapeutic effects against cardiac arrhythmias.

In another analysis, disease-related targets were collected from DisGeNET [26] with "Cardiac Arrhythmias" as the keyword. Furthermore, relevant PPI network was extracted from Human Protein Reference Database (HPRD) [27] and iRefIndex [28]. This along with the active ingredients, ingredient-related proteins and disease-related proteins were input into Cytoscape to build a complete DT network for SSOL.

2.4.2. *Analyzing the effectiveness of active ingredient*

In order to quantify the effectiveness of compounds, we assumed that the information was transferred from one node to the other node through the shortest paths connecting them in the DT network, it was worthwhile to note that each node only once equivalently affect its neighbors. In response to we referred to the topology of the concept of degree, here the pertinent algorithm formulas were as follows:

$$I_i(m \to n) = \frac{1}{k_m} \prod_{j \in \nu(i)} \frac{1}{k_j - 1} \tag{1}$$

$$I(m \to n) = \sum_i I_i(m \to n) \tag{2}$$

$$I(m) = \sum_n I(m \to n) \tag{3}$$

2.5. *Quantitative analysis of the major constituents in SSOL by UPLC-DAD*

2.5.1. *UPLC-DAD conditions*

The scheme had been revised in accordance with UPLC-QTOF-MS/MS conditions. Especially, 5 μL of sample solution was injected and the detection wavelengths of DAD were set at 280 nm.

2.5.2. *Method validation*

2.5.2.1. *Linearity and LOD and LOQ*. The linearity was determined by the average peak areas of 5 standard solutions at 5 concentrations. Afterwards, the linearity curve was drawn with the concentration of standard solution (x) as abscissa and the average peak area (y) as ordinate, and the equation of linear regression was calculated. The lowest concentration of mixed standard solution was diluted, then the results showed that signal to noise ratio (S/N) equal to 3 for the limit of detection (LOD), while S/N = 10 as the limit of quantification (LOQ).

2.5.2.2. *Precision*. The test solution was prepared as described for the second group of sample solution, and successively injected 6 times under the condition of the UPLC-DAD conditions to measure the peak area of each compound, after which we detected intra-day precision and calculated RSD%. Moreover, the test solution was continuously injected for 3 days, and the peak area was measured by sampling 6 times a day, after which we detected inter-day precision and calculated RSD%.

2.5.2.3. *Stability*. Test solution was prepared as above, and 5 μL samples were successively injected at 0, 2, 4, 6, 8, 10, 12, and 24 h under the UPLC-DAD conditions to measure the peak area of each compound. RSD% was calculated for the stability of the test solution.

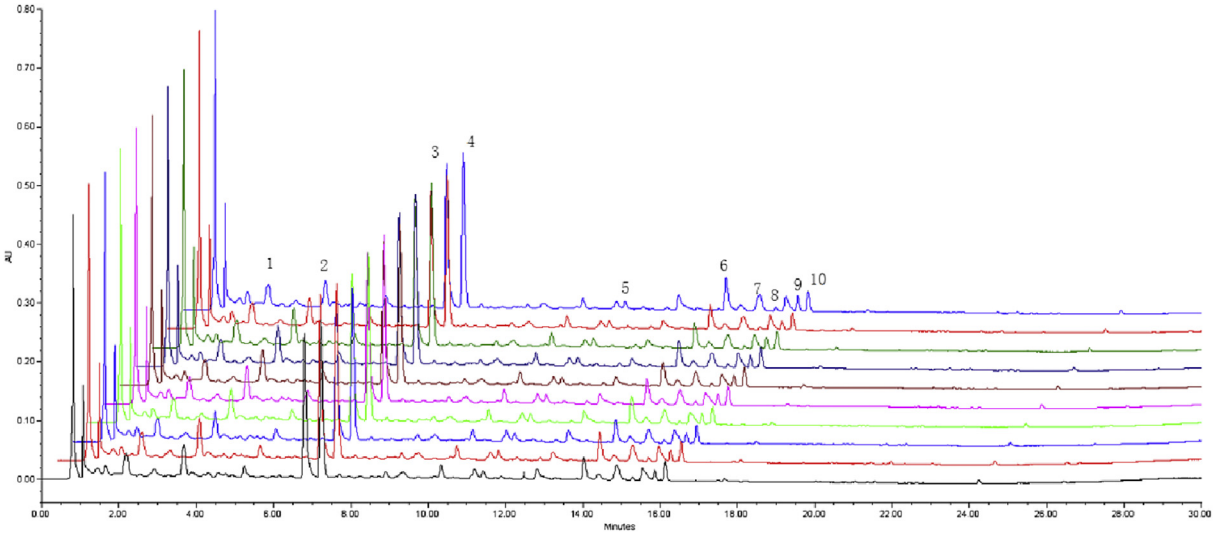

Fig. 4 – The fingerprint of ten batches of SSOL (280 nm).

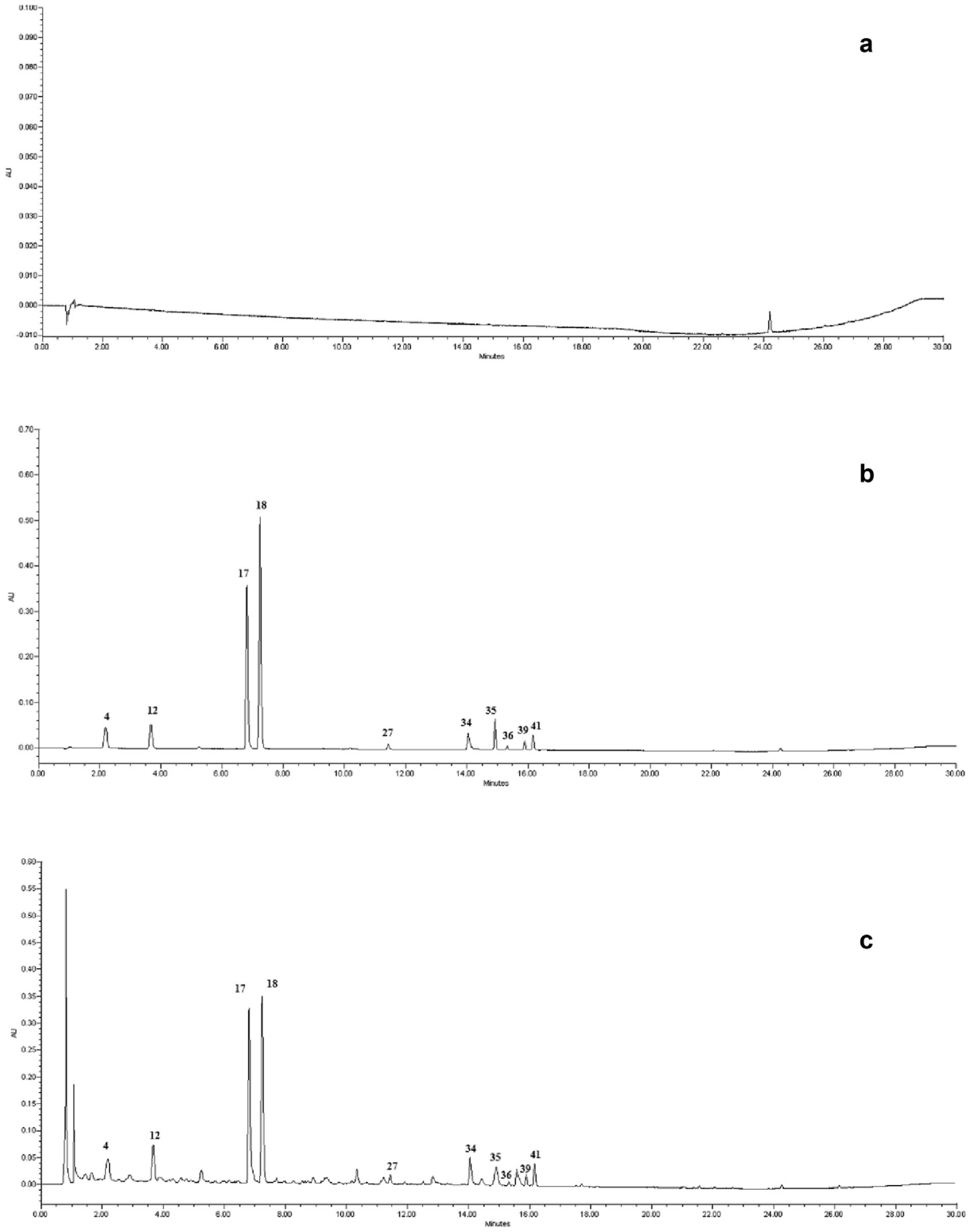

Fig. 5 – UPLC-DAD chromatograms of (a) the blank reference solution, (b) the mixed reference solution and (c) the SSOL sample solution of 280 nm (4: danshensu, 12: protocatechuic aldehyde, 17: psoralenoside, 18: isopsoralenoside, 27: rosmarinic acid, 34: salvianolic acid A, 35: epimedin A_1, 36: epimedin A, 39: epimedin C, 41: icarin).

2.5.2.4. *Repeatability*. Six test solutions were prepared as described for the second group of sample solution in parallel, and successively injected 5 μL under the UPLC-DAD conditions to measure the peak area of each compound. The content of each compound and RSD% were calculated afterwards.

2.5.2.5. *Average recovery*. According to the method of the UPLC-DAD conditions, the average recovery of analytes were determined by analyzing replicates ($n = 6$) of samples at several concentration levels for each of the analytes, which were added to the precise amount of 0.5 mL of SSOL with the

aid of existing method. To evaluate the average recovery of analytes, the corresponding peak areas were measured.

2.5.3. *Content determination*

Sample solutions were in parallel prepared in accordance with the above method for 10 representative compounds in the ten batches of SSOL, and injected 5 μL under the UPLC-DAD conditions to measure the peak area of each compound, as well as the drug contents were calculated.

3. Results and discussion

3.1. *Qualitative analysis of the major constituents in SSOL by UPLC-QTOF-MS/MS*

A total of 64 compounds in SSOL were identified by comparing the retention times, accurate relative molecular masses, fragmentation information and MS-splitting-decomposition law with references or literature data in SSOL (structures of 3 compounds were not identified), Most of these compounds, including phenylpropanoids, flavonoids, alkaloids, triterpenoids and triterpenoid saponins, could find their origin in herbs in SSOL such as Hongshen, Danshen, Yinyanghuo, Buguzhi, and Mahuang, whereas the experimental method failed to identify ingredients of Gouqi, Shuizhi, and Xixin. Possible reasons for why the ingredients of these three herbs were not detected are as follows: 1) our extraction protocol for SSOL favors small and polar organic compounds while Gouqi mainly contains polysaccharides, Shuizhi mainly contains amino acids and peptides, and Xixin mainly contains volatile oils, all of which are nonpolar and/or much larger in size. 2) The procedures and conditions of our UPLC-QTOF-MS method we used to analyze the major chemical constituents of SSOL might not be optimized for a highly sensitive detection of less abundant constituents. The character and structure of components in SSOL were distinguished by BPI total ion chromatography (TIC), even the method provided for identification and quality control of herbal material. The results of TIC are shown in Fig. 1, the structures of all components are presented in Fig. S1, and the identified chemical constituents of SSOL are summarized in Table S1.

3.2. *Network pharmacology analysis of SSOL*

As described in method Section 2.4, the interaction relationship between the 53 SSOL compounds and their corresponding 8 direct targets, P41143, P35354, P16671, P04179, P01019, Q92731, Q14524, and Q12809, are illustrated in Fig. 2. We subsequently calculated the effectiveness scores (i.e., $I(m)$ values) and the specificity scores (i.e., the maximal value of $I(m \rightarrow n)$) for these 53 ingredients via a simple algorithm, selected the threshold of the effectiveness scores and the specificity scores, and finally found 23 more effective compounds which are associated with the direct target, including icariin, ginsenoside R_f, ginsenoside Rb_3, epimedoside D, epimedin B, epimedin A, danshensu, citric acid, baohuoside VII/sagittatoside A, baohuoside VI, 20(S/R)-ginsenoside Rg_3, (1S, 2S)-pseudoephedrine, (1R, 2S)-ephedrine, (+)-magnoflorine, salvianolic acid G, salvianolic acid D, salvianolic acid B, salvianolic acid A, quinic acid, protocatechuic acid, rosmarinic acid, neochlorogenic acid and methylephedrine/methylpseudoephedrine as displayed in Fig. 3. Three compounds, ginsenoside Re, 20(R)-notoginsenoside R_2 and psoralidin, indirectly interacting with targets Cx43, Cx45 and P35354 via P28482, P27361, Q13164, and Q07157, are displayed in Fig. 3. The results of Q-marker screening are presented in Table S2.

3.3. *Quantitative analysis of the major constituents in SSOL by UPLC-DAD*

As shown in Table 1, all the 10 standards showed good linear regression ($R^2 > 0.9993$) within the test ranges, and the LODs of the 10 compounds were estimated to be 0.10–0.69 μg/mL, and the LOQs were 0.44–3.08 μg/mL. The 10 analytes were stable in sample solution at room temperature within 24 h, with a RSD less than 1.28%. The RSD values of intraday and interday precisions were less than 0.88% and 2.83%, respectively. The repeatability of RSD was less than 3.30%. The average recovery was 91.36–109.15% with a RSD less than 4.66%. These results indicated that the method was satisfactory with high accuracy, good reproducibility, and high sensitivity which would be beneficial to the analytical investigation and quality control for SSOL.

The analytical results, summarized in Table 2 and shown in Fig. 4, showed that slightly larger differences were found in average contents of the constituents, analyzed in triplicates in 10 collected batches, with RSD of 5.08%–15.29%. Main reasons leading to the differences might be the source of plant materials, the production processes and/or the samples storage conditions. Among the 10 constituents, salvianolic acid A showed the biggest differences (477.39–701.04 μg/mL), possibly due to its easy degradable nature. While psoralenoside showed the highest amount (4288.40–5184.24, μg/mL) followed by isopsoralenoside (3422.60–3940.92 μg/mL), epimedin A had the lowest amount (53.28–69.73 μg/mL).

3.3.1. *Optimization of chromatographic conditions*

In order to improve the sensitivity and resolution of the separation and shorten the analysis time, the composition of mobile phase, column temperature and detection wavelength were investigated. Due to the existence of acidic components in the test solution, 0.1% formic acid was added in water phase to suppress compound ionization and improve the peak-shapes.

Test results indicated that 1) a better chromatographic peak shape could be achieved by using acetonitrile–0.1% formic acid aqueous solution as mobile phase than that using methanol–0.1% formic acid aqueous solution; 2) no significant difference was detected by comparing column oven temperatures of 30 °C, 40 °C and 50 °C; 3) We found that the absorption of the 10 representative compounds was relatively high at the wavelength of 280 nm by full wavelength scan mode. The UPLC-DAD chromatograms of the blank reference solution, the mixed reference solution and the SSOL sample solution are presented in Fig. 5.

3.3.2. *Selection of Q-markers*

SSOL is composed of 8 traditional Chinese herbs, including Hongshen, Yinyanghuo, Buguzhi, Gouqi, Mahuang, Xixin, Danshen, Shuizhi. According to the Chinese Pharmacopoeia (2015 edition), the content standards of SSOL are determined using Hongshen as calculated by ginsenoside Rg_1, Re and Rb_1; Yinyanghuo as calculated by icariin; Buguzhi as calculated by psoralen and isopsoralen; Gouqi as calculated by Lycium barbarum polysaccharide (LBP); Mahuang as calculated by ephedrine hydrochloride; Xixin as calculated by asarinin; Danshen as calculated by tanshinone IIA, cryptotanshinones and tanshinone I, or salvianolic acids was calculated by salvianolic acid B in Danshen; And Shuizhi as calculated by antithrombin.

Our prediction results of the network pharmacology showed that, danshensu, rosmarinic acid, salvianolic acid A, epimedin A and icariin had a good antiarrhythmic activity. In addition, our qualitative analysis of SSOL by UPLC-Q-TOF-MS/MS also included danshensu, protocatechuic aldehyde, psoralenoside, isopsoralenoside, rosmarinic acid, salvianolic acid A, epimedin A_1, epimedin A, epimedin C and icariin. Meanwhile, the National Drug Standards WS3-065 (Z-010)-2003 (Z) stipulates that ginsenoside Rg_1, Re and Rb_1, ephedrine hydrochloride, psoralen and isopsoralen, icariin and protocatechualdehyde should be detected in SSOL by TLC. What's more, these 10 components meet with the experimental conditions of UPLC-DAD method and actual situations. To summarize, based on active ingredients, and guided by the Pharmacopoeia and previous experimental results, we selected 10 representative compounds and established a quality control method of SSOL by UPLC-DAD method. This method is simple, rapid and high accuracy. However, limitations exist in this method. 1) In all cases, the absorption of triterpenoid saponins was low by UPLC-DAD at 280 nm, while the content of psoralenoside and isopsoralenoside were significantly higher than that of psoralen and isopsoralen. 2) Likewise, salvianolic acid A was higher than salvianolic acid B with good chromatographic peaks profile, and the experimental data were more accurate and representative. 3) In addition, since Gouqi mainly contains polysaccharides, alkaloids are the main component in Mahuang, and volatile oils exist widely in Xixin, the current method did not detect components of Gouqi, Mahuang and Xixin possibly due to the polarity and content of these chemical components and the extraction process of SSOL.

3.3.3. *Content determination of psoralenoside, isopsoralenoside and salvianolic acid A*

Since the self-prepared psoralenoside and isopsoralenoside were easily degraded in aqueous solution at room temperature and the degradation rate of psoralenoside was quicker than that of isopsoralenoside, it may explain why the recovery of psoralenoside was low. Salvianolic acid A was dissolved in 50%-methanol aqueous solution, nevertheless, easily degraded when preserved in −80 °C refrigerator, so we should use it rightly after it had been prepared. Meanwhile, the stability experiments showed that salvianolic acid A was stable when stored in 4 °C refrigerator up to 24 h, while unstable with a higher degradation rate after 48 h.

4. Conclusions

Although a few ideas and methods have been tried to choose the suitable Q-markers for a CMP, it remains a difficult task and the selection criteria is yet to be established. Guided by the TCM theory and composed of multiple herbal components, the quality, safety, efficacy and cost-effectiveness are the main elements to a CMP. Using SSOL, an anti-arrhythmic CMP as a model, we applied network pharmacology to predict the effective constituents and combined with the contents of these compounds to choose the Q-markers. Firstly, the chemical composition of SSOL was analyzed by UPLC-QTOF-MS/MS and 64 compounds were successfully identified, including triterpenoids and triterpenoid saponins, flavonoids, phenylpropanoids, alkaloids and others. Then, we put forward a double network analysis method to predict the effective components of SSOL and identified 26 compounds related to antiarrhythmic activity. Based on the results of network pharmacology analysis, 10 representative compounds were selected as the Q-markers of SSOL, and a quantitative UPLC-DAD analysis method was established for it.

In brief, we combined the chemical profiling and network pharmacology to select the Q-markers for CMP, a new attempt for CMP quality control. With other key factors such as efficacy, toxicity, content, and origin to be considered and prioritized for Q-markers, a balanced and effective quality control method for CMP still has a long way to go and requires intense future investigations.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgments

This work was supported by the Tianjin Science and Technology Commissioner Project (No. 16JCTPJC49000), Science and Technology Program of Tianjin (Nos. 15PTCYSY00030 and 14TXZYJC00440), the National Natural Science Foundation (Nos. 81403059 and 81573826) and Natural Science Foundation of Tianjin, China (No. 16JCYBJC43900).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfda.2017.10.003.

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