Research Article

Identification of “Multiple Components-Multiple Targets-Multiple Pathways” Associated with Naoxintong Capsule in the Treatment of Heart Diseases Using UPLC/Q-TOF-MS and Network Pharmacology

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Naoxintong capsule (NXT) is a commercial medicinal product approved by the China Food and Drug Administration which is used in the treatment of stroke and coronary heart disease. However, the research on the composition and mechanism of NXT is still lacking. Our research aimed to identify the absorbable components, potential targets, and associated pathways of NXT with network pharmacology method. We explored the chemical compositions of NXT based on UPLC/Q-TOF-MS. Then, we used the five principles of drug absorption to identify absorbable ingredients. The databases of PharmMapper, Universal Protein, and the Molecule Annotation System were used to predict the main targets and related pathways. By the five principles of drug absorption as a judgment rule, we identified 63 compositions that could be absorbed in the blood in all 81 chemical compositions. Based on the constructed networks by the significant regulated 123 targets and 77 pathways, the main components that mediated the efficacy of NXT were organic acids, saponins, and tanshinones. Radix Astragali was the critical herbal medicine in NXT, which contained more active components than other herbs and regulated more targets and pathways. Our results showed that NXT had a therapeutic effect on heart diseases through the pattern “multiple components-multiple targets-multiple pathways.”

1. Introduction

Naoxintong capsule (NXT) is a commercial medicinal product approved by the China Food and Drug Administration which is widely used in the treatment of stroke and coronary heart disease. NXT contains 16 Chinese herbal medicines (Table 1). NXT exerts significant therapeutic effects and has high safety for stroke recovery in the clinical setting [1]. Recent studies showed that NXT could reduce the infarct size of acute myocardial infarction (AMI) patients by improving vascular endothelial function [2]. Long-term administration of NXT was also reported to alleviate inflammation, reduce the recurrence of angina pectoris, and decrease the incidence of ACS attack in borderline lesion coronary heart disease patients [3]. Some studies investigated the mechanisms of NXT in vitro or in vivo. NXT was reported to protect against atherosclerosis through its lipid-lowering activity [4] and to reduce the expression of iNOS mRNA and the NO level in the vessel wall to benefit the treatment of atherosclerosis [5]. NXT also protected cardiomyoblasts against H2O2-induced oxidative injury [6]. Although some mechanisms of NXT have been reported, existing studies on unilateral factors and single targets could not demonstrate the complex mechanisms of NXT, a herbal prescription with 16 medicines which is prescribed for the treatment of complex diseases like cardiovascular and cerebrovascular diseases.

With the prominence of network pharmacology in system biology, this distinct and novel approach to the study of complicated analytical systems is becoming more widely known and more frequently used in the field of drug research.
Table 1: Sixteen Chinese traditional medical herbs of NXT.

| Abbreviation | Medicinal herbs                  | Original plants                                                   | Content (g)* |
|--------------|----------------------------------|-------------------------------------------------------------------|--------------|
| RA           | Radix Astragali                  | Astragalus membranaceus (Fisch.) Beg. var. mongholicus (Bge.) Hsiao or A. membranaceus (Fisch.) Bge. | 66           |
| RPR          | Radix Paeoniae Rubra             | Paeonia lactiflora Pall. or P. veitchii Lynch                     | 27           |
| RSM          | Radix Salviae Miltiorrhizae      | Salvia miltiorrhiza Bge.                                          | 27           |
| RAS          | Radix Angelicae Sinensis         | Angelica sinensis (Oliv) Diels.                                   | 27           |
| RCX          | Rhizoma Chuanxiong               | Ligusticum chuanxiong Hort.                                       | 27           |
| SP           | Semen Persicae                   | Prunus persica (L.) Batsch or Prunus davidiana (Carr.) Franch.    | 27           |
| FC           | Flos Carthami                    | Carthamus tinctorius L.                                            | 13           |
| FK           | Frankincense                     | Boswellia cartterii Birdw.                                        | 13           |
| MRH          | Myrrha                           | Commiphora myrrha Engl.                                           | 13           |
| CS           | Caulis Spatholobi                | Spatholobus suberecctus Dunn                                      | 20           |
| RAB          | Radix Achyranthis Bidentatae     | Achyranthes bidentata BL or Cymathula officinalis Kuan            | 27           |
| RC           | Ramulus Cinnamomi                | Cinnamomum cassia Presl                                           | 20           |
| RM           | Ramulus Mori                     | Morus alba L.                                                     | 27           |
| PT           | Pheretima                        | Pheretima asperillum (E. Perrier) or Pheretima vulgaris Chen. or Pheretima guilelmi (Michaelsen) or Pheretima pectinifera Michaelsen | 27           |
| SCP          | Scorpio                          | Buthus martensii Karsch                                           | 13           |
| HRD          | Hirudo                           | Whitmania nigra Whitman or Hirudo nipponica Whitman or Whitmania acranulata Whitman | 27           |

* Note: The content of 16 Chinese traditional medical herbs of NXT came from Chinese Pharmacopoeia 2015.

The functions of network pharmacology include uncovering the functions of traditional Chinese medicines (TCMs), providing deeper insights into and scientific evidence for TCMs, and identifying TCMs as scientifically proven. Here, we attempt to explore the mechanism of NXT using this method.

In the current study, based on the use of UPLC/Q-TOF-MS to investigate the involved components, we aimed to analyse the absorbable components of NXT, to identify potential targets and associated pathways using the network pharmacology method, and to systematically discuss the mechanism of NXT in the treatment of heart diseases.

2. Material and Methods

2.1. Prediction of Components

2.1.1. Sample Preparation. NXT was obtained from Heze-Buchang Pharmaceutical Co., Ltd. (Heze, China). Deionized water was prepared from aqua distillate using a Milli-Q system (Millipore, Bedford, MA, USA). Analytical grade methanol was purchased from Merck (Darmstadt, Germany). We dissolved 1 g of NXT powder in 10 mL of 75% analytical grade methanol and subjected the mixture to ultrasonic extraction for 30 min. We then brought the solution to room temperature and obtained the supernatant as a capture reagent. The sample was filtered using a 0.22 μm microporous membrane before UPLC analysis.

2.1.2. UPLC/Q-TOF-MS. We used a Waters Acquity UPLC System (Waters Co., USA) furnished with a photodiode array detector for the analysis. The sample was diluted on a Waters Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm). UV detection was achieved at 190–400 nm. The system was controlled using the MassLynx version 4.1 software (Waters Co.). The gradient duration program for A (UPLC-grade acetonitrile) and B (water with 0.1% formic acid) was performed as follows: 2% A from 0 min to 3 min, 10% to 50% A from 3 min to 12 min, 50% to 63% A from 12 min to 18 min, 63% to 83% A from 18 min to 21 min, 83% to 84% A from 21 min to 22 min, 84% to 87% A from 22 min to 26 min, 87% to 90% A from 26 min to 28 min, 90% to 95% A from 28 min to 31 min, 95% to 100% A from 31 min to 33 min, 100% to 100% A from 33 min to 35 min, and 100% to 2% A from 35 min to 37 min. The flow rate was maintained at 0.4 mL/min, and the column temperature was maintained at 30°C.

The components of NXT were identified using a Waters Q-TOF Premier with an electrospray ionization (ESI) system (Waters MS Technologies, Manchester, UK). The ESI-MS spectra were acquired at both negative and positive ion voltages. The capillary voltage was set to 2.5 kV for the negative mode and to 3 kV for the positive mode. The sample cone voltage was set to 30 V, and the source temperature was 110°C. High-purity nitrogen was used as the nebulization and auxiliary gas. The nebulization gas was set to 600 L/h, the cone gas was set to 50 L/h, and the desolation temperature was 350°C. The Q-TOF Premier acquisition rate was 0.1 s, and there was a 0.02 s interscan delay. Argon, which was
used as the collision gas, was maintained at a pressure of $5.3 \times 10^{-5}$ Torr. The instrument was operated with the first resolving quadruple in a wide pass mode (100 Da–1500 Da). Leucine enkephalinamide acetate was used as the lock mass ([M – H]$^-$ = 553.2775, [M + H]$^+$ = 555.2931).

2.2. Calculation and Prediction of Absorbable Components. First, we determined the structural formulas of the chemical components that were identified in compound NXT from the Chemical Book website and used the Chemdraw software to draw these formulas. Then, we imported these structural formulas into the Online SMILES Translator and Structure File Generator (http://cactus.nci.nih.gov/translate/) to obtain the smiles format. Finally, we input the smiles format of the chemical components into the Molispiration Smiles website (http://www.molinspiration.com/cgi-bin/properties) to calculate the prediction parameters of drug absorption. According to the five principles of drug absorption, if a component was subject to the following provisions of the corresponding parameters, it could be identified as an absorbable component: hydrogen bond donor (the number of hydrogen atoms attached to the O and N) $n_{OHNH} \leq 5$; relative molecular mass $MW \leq 500$; fat water partition coefficient $mi\log P \leq 5$; and hydrogen bond acceptor (the number of O and N) $n_{ON} \leq 10$.

2.3. Prediction and Screening of Targets. Using the software of Chembio3D Ultra12.0, we transformed the structure of the absorbed components into the sdf structure format. Then, to predict the possible targets, we imported the components into the public network server of the target database of the efficacy group PharmaMapper website (http://59.78.96.61/pharmmapper/) to perform reverse docking. We selected the top 10 targets for subsequent study.

2.4. Prediction and Screening of Pathways. We imported the obtained targets into the Bio database (http://bioinfo.capitalbio.com/mas3/) and then screened for pathways that met the criterion of $P < 0.01$.

2.5. Construction of Network. According to the screening pathways with their corresponding targets and components, we created a component-target-pathway illustration using Cytoscape. Then, according to the main selected targets, we drew a target-composition diagram.

3. Results

3.1. UPLC/Q-TOF-MS Analysis. We analysed the chemical components of NXT using ultra performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry. Because different chemical components had better responses in different modes, MS data were obtained in both positive ion mode (Figure 1(a)) and negative ion mode (Figure 1(b)). MS data in (+/−) ESI modes and the identification results for the constituents in NXT were presented in Table 2. In all 16 herbs from NXT, no related component in Myrrha and Hirudo was found.

3.2. Absorption Parameters of Components. Using a computer prediction method to calculate the identified compounds of NXT, we obtained absorption parameters that could determine whether the chemical compositions could be absorbed.
| Peak number | RT (min) | Identification          | Mode | MS (m/z) | Composition       | Herbal source |
|-------------|----------|-------------------------|------|----------|-------------------|---------------|
| 1           | 0.647    | Arginine                | Pos/Neg | 174.2024 | C_{6}H_{14}N_{2}O_{3} | PT            |
| 2           | 0.702    | Valine                  | Pos   | 117.1478 | C_{6}H_{11}NO_{2}  | PT            |
| 3           | 0.721    | Proline                 | Pos   | 115.1331 | C_{6}H_{14}NO_{2}  | PT            |
| 4           | 0.776    | Malic acid              | Neg   | 134.0911 | C_{6}H_{12}O_{5}   | RA            |
| 5           | 1.053    | Citric acid             | Neg   | 192.1286 | C_{6}H_{12}O_{7}   | RA            |
| 6           | 1.201    | D-5-oxoproline          | Neg   | 129.1174 | C_{6}H_{12}NO_{3}  | RAS           |
| 7           | 1.201    | L-5-oxoproline          | Neg   | 129.1174 | C_{6}H_{12}NO_{3}  | RAS           |
| 8           | 1.275    | Succinic acid           | Neg   | 118.0910 | C_{6}H_{12}O_{4}   | RAS, RAB, PT  |
| 9           | 1.294    | \(\rho\)-Coumaric acid | Pos   | 164.1601 | C_{6}H_{12}O_{3}   | RAS           |
| 10          | 1.310    | o-Phthalic acid         | Pos   | 166.1294 | C_{6}H_{12}O_{4}   | RAS           |
| 11          | 1.312    | Adenosine               | Pos   | 267.2403 | C_{10}H_{17}N_{4}O_{4} | RAS, PT, RCX |
| 12          | 1.331    | Leucine                 | Pos   | 131.1688 | C_{6}H_{13}NO_{2}  | PT            |
| 13          | 1.460    | Isoleucine              | Pos   | 131.1688 | C_{6}H_{13}NO_{2}  | RAB           |
| 14          | 1.589    | Gallic acid\(^a\)       | Neg   | 170.1207 | C_{6}H_{12}O_{3}   | RPR           |
| 15          | 2.199    | Phenylalanine           | Pos   | 165.1874 | C_{6}H_{11}NO_{2}  | FC            |
| 16          | 2.459    | Danshensu              | Neg   | 198.1701 | C_{6}H_{10}O_{4}   | RSM           |
| 17          | 2.606    | Palmitic acid           | Neg   | 256.3380 | C_{16}H_{32}O_{2}  | RAS, FC, RA, SCP |
| 18          | 3.438    | Senkyunolide B          | Neg   | 204.2374 | C_{12}H_{12}O_{3}  | RCX           |
| 19          | 3.456    | Senkyunolide C          | Neg   | 204.2374 | C_{12}H_{12}O_{3}  | RCX           |
| 20          | 3.600    | Protocatechuic aldehyde | Neg   | 138.1185 | C_{6}H_{14}O_{3}   | RSM, RC       |
| 21          | 3.974    | Mulberroside A\(^a\)    | Neg   | 568.5277 | C_{26}H_{12}O_{14} | RM            |
| 22          | 4.122    | Gallicin                | Neg   | 184.1453 | C_{6}H_{14}O_{3}   | RPR           |
| 23          | 4.230    | Hydroxysafflor yellow A | Pos/Neg | 612.5364 | C_{27}H_{13}O_{16} | FC            |
| 24          | 4.232    | 7-Hydroxycoumarin       | Pos   | 162.1457 | C_{6}H_{12}O_{4}   | RM            |
| 25          | 4.565    | Vanillic acid           | Neg   | 168.1459 | C_{6}H_{12}O_{4}   | RCM, RPR      |
| 26          | 4.694    | Benzoic acid            | Neg   | 122.1209 | C_{6}H_{12}O_{2}   | RPR           |
| 27          | 4.935    | Epicatechin             | Neg   | 290.2674 | C_{12}H_{14}O_{6}  | CS            |
| 28          | 5.157    | Catechin                | Neg   | 290.2674 | C_{12}H_{14}O_{6}  | RPR           |
| 29          | 5.212    | Albiflorin              | Pos   | 480.4653 | C_{23}H_{20}O_{11} | RPR           |
| 30          | 5.730    | Quercetin-7-O-glucoside | Neg   | 464.3754 | C_{21}H_{16}O_{12} | FC            |
| 31          | 5.952    | Rutin                   | Neg   | 610.5203 | C_{27}H_{30}O_{16} | RA            |
| 32          | 5.970    | Calycosin\(^a\)         | Neg   | 284.2679 | C_{16}H_{12}O_{5}  | RA            |
| 33          | 5.988    | Calycosin-7-O-glucoside | Pos   | 446.4075 | C_{22}H_{12}O_{10} | RA            |
| 34          | 5.989    | Ferulic acid\(^a\)      | Neg   | 194.1815 | C_{16}H_{12}O_{4}  | RA, RCX, RAS, RAB |
| 35          | 6.321    | Paenoflorin\(^a\)       | Pos   | 480.466  | C_{23}H_{20}O_{11} | RPR           |
| 36          | 6.358    | Pentagalloylgucose\(^a\) | Neg   | 940.68   | C_{41}H_{12}O_{26} | RPR           |
| 37          | 6.413    | Kaempferol-3-O-rutinoside\(^a\) | Pos/Neg | 594.5179 | C_{27}H_{30}O_{15} | FC            |
| 38          | 6.654    | 3,5-Di-O-caffeoylquinic acid\(^a\) | Pos/Neg | 516.4573 | C_{25}H_{34}O_{12} | CS            |
| 39          | 6.987    | Dicafeoylquinic acid    | Neg   | 116.1275 | C_{25}H_{34}O_{12} | RCX           |
| 40          | 7.042    | Z-Butyldenelephthalide\(^a\) | Neg   | 188.2259 | C_{12}H_{12}O_{2}  | RCX           |
| 41          | 7.210    | Salvianolic acid A      | Neg   | 494.4578 | C_{26}H_{22}O_{10} | RSM           |
| 42          | 7.449    | 4-Hydroxyl-3-butyphthalide | Pos   | 206.2346 | C_{12}H_{12}O_{3}  | RCX           |
| 43          | 7.540    | Salvianolic acid B      | Neg   | 718.6220 | C_{36}H_{16}O_{16} | RSM           |
| 44          | 7.688    | Ononin                  | Pos   | 430.4107 | C_{22}H_{12}O_{3}  | CS            |
| 45          | 7.763    | Senkyunolide F          | Pos   | 206.1017 | C_{12}H_{12}O_{3}  | RCX, RAS      |
| 46          | 7.855    | Salvianolic acid E      | Neg   | 718.1512 | C_{36}H_{16}O_{16} | RSM           |
| 47          | 8.243    | Biochanin A             | Pos/Neg | 284.2689 | C_{16}H_{12}O_{5}  | CS            |
| 48          | 8.262    | (6aR,11aR)-3-Hydroxy-9,10-dimethoxy pterocarpan | Pos   | 300.3107 | C_{12}H_{12}O_{3}  | RA            |
| 49          | 8.594    | N1-N5-(Z)-N10-(E)-tri-p-coumaroylspermidine | Pos   | 583.2703 | C_{14}H_{32}N_{3}O_{6} | FC            |
3.3. Potential Targets and Pathways. By importing 63 chemical compositions that were predicted to be absorbable into the PharmMapper database for directional docking, we obtained a total of 123 targets. We then imported these targets into the Molecule Annotation System and obtained 77 pathways regulated by NXT with highly significant differences, from which we chose the top 40 pathways that met the criterion of \( P < 0.01 \) (Table 4). A total of 34 targets were related to these top 40 pathways, and HRAS, MAP2K1, and MAPK14 were associated with most of these pathways, so we considered these factors to be the main targets. As shown in Table 4, NFAT and hypertrophy of the heart (transcription in the broken heart) ranked first among these pathways.

In Table 5, these top 40 pathways were classified into 5 categories, which included pathways associated with heart diseases and blood vessels, metabolism, cell cycle (with proliferation and apoptosis), immunity, and other pathways. By classifying these pathways, we accessed and marked the corresponding medicinal materials of NXT (Table 5). In the pathways associated with heart diseases and blood vessels,
Table 3: Absorption parameters of the components.

| Number | Compounds                      | MW    | nON | nOHNH | miLogP | Results |
|--------|--------------------------------|-------|-----|-------|--------|---------|
| 1      | Arginine                       | 174.204 | 6   | 7     | −3.632 | ×       |
| 2      | Valine                         | 117.15 | 3   | 3     | −1.91  | ✓       |
| 3      | Proline                        | 115.132 | 3   | 2     | −1.723 | ✓       |
| 4      | Malic acid                     | 134.087 | 5   | 3     | −1.57  | ✓       |
| 5      | Citric acid                    | 192.123 | 7   | 4     | −1.983 | ✓       |
| 6      | D-5-oxoproline                 | 129.115 | 4   | 2     | −2.402 | ✓       |
| 7      | L-5-oxoproline                 | 129.115 | 4   | 2     | −2.402 | ✓       |
| 8      | Succinic acid                  | 118.088 | 4   | 2     | −0.655 | ✓       |
| 9      | ρ-Coumaric acid                | 164.160 | 3   | 2     | 1.43   | ✓       |
| 10     | o-Phthalic acid                | 166.132 | 4   | 2     | 1.034  | ✓       |
| 11     | Adenosine                      | 267.245 | 9   | 5     | −0.854 | ✓       |
| 12     | Leucine                        | 131.175 | 3   | 3     | −1.382 | ✓       |
| 13     | Isoleucine                     | 131.175 | 3   | 3     | −1.41  | ✓       |
| 14     | Gallic acid\(^a\)              | 170.120 | 5   | 4     | 0.589  | ✓       |
| 15     | Phenylalanine                  | 165.192 | 3   | 3     | −1.231 | ✓       |
| 16     | Danshensu                      | 198.174 | 5   | 4     | −0.251 | ✓       |
| 17     | Palmitic acid                  | 256.43 | 2   | 1     | 7.059  | √       |
| 18     | Senkyunolide B                 | 204.225 | 3   | 1     | 2.81   | ✓       |
| 19     | Senkyunolide C                 | 204.225 | 3   | 1     | 2.574  | ✓       |
| 20     | Protocatechuic aldehyde        | 138.122 | 3   | 2     | 0.759  | ✓       |
| 21     | Mulberroside A\(^a\)           | 568.528 | 14  | 10    | −0.852 | ✓       |
| 22     | Gallicin                       | 184.147 | 5   | 3     | 0.848  | ✓       |
| 23     | Hydroxysafflor yellow A        | 612.54 | 16  | 12    | −4.12  | ✓       |
| 24     | 7-Hydroxycoumarin              | 162.144 | 3   | 1     | 1.511  | ✓       |
| 25     | Vanillic acid                  | 168.148 | 4   | 2     | 1.187  | ✓       |
| 26     | Benzoic acid                   | 122.123 | 2   | 1     | 1.848  | ✓       |
| 27     | Epicatechin                    | 290.271 | 6   | 5     | 1.369  | ✓       |
| 28     | Catechin                       | 290.271 | 6   | 5     | 1.369  | ✓       |
| 29     | Albizlorin                     | 480.466 | 11  | 5     | −1.636 | ✗       |
| 30     | Quercetin-7-O-glucoside        | 464.379 | 12  | 8     | −0.104 | ✗       |
| 31     | Rutin                          | 610.521 | 16  | 10    | −1.063 | ✓       |
| 32     | Calycosin\(^a\)                | 284.267 | 5   | 2     | 2.377  | ✓       |
| 33     | Calycosin-7-O-glucoside        | 446.408 | 10  | 5     | 0.59   | ✓       |
| 34     | Ferulic acid\(^a\)             | 194.186 | 4   | 2     | 1.249  | ✓       |
| 35     | Paoniflorin\(^a\)              | 480.466 | 11  | 5     | 0.044  | ✗       |
| 36     | Pentagalloylgucose\(^a\)       | 940.681 | 26  | 15    | 2.761  | ✓       |
| 37     | Kaempferol-3-O-rutinoside\(^a\)| 594.522 | 15  | 9     | −0.574 | ✓       |
| 38     | 3,5-Di-O-caffeylquinic acid\(^a\)| 516.455 | 12  | 7     | 1.424  | ✗       |
| 39     | Dicaffeoylquinic acid           | 516.46 | 12  | 7     | 1.21   | ✗       |
| 40     | Z-Butylidenephenolide\(^a\)    | 188.226 | 2   | 0     | 3.077  | ✓       |
| 41     | Salvianolic acid A             | 494.452 | 10  | 7     | 3.014  | ✗       |
| 42     | 4-Hydroxy-3-butylphthalalide    | 206.241 | 3   | 1     | 3.42   | ✓       |
| 43     | Salvianolic acid B             | 718.620 | 16  | 9     | 1.615  | ✗       |
| 44     | Ononin                         | 430.409 | 9   | 4     | 1.307  | ✓       |
| 45     | Senkyunolide F                 | 206.24 | 3   | 1     | 1.72   | ✓       |
| 46     | Salvianolic acid E             | 718.62 | 16  | 10    | 2.83   | ✗       |
| 47     | Biochanin A                    | 284.267 | 5   | 2     | 2.804  | ✓       |
| 48     | (6aR,11aR)-3-Hydroxy-9,10-dimethoxy pterocarpan | 300.31 | 5 | 1 | 2.546 | ✓       |
| 49     | N1-N5-(Z)-N10-(E)-tri-p-coumaroyspermidine | 538.68 | 9 | 5 | 4.3 | ✗       |
| 50     | Benzoylpaeoniflorin            | 584.574 | 12  | 4     | 2.472  | ✗       |
| 51     | Pratensein                     | 300.27 | 6   | 3     | 2.09   | ✓       |
| Number | Compounds                  | MW     | nON | nOHNH | miLogP | Results |
|--------|----------------------------|--------|-----|-------|--------|---------|
| 52     | Hydroxyl calendic acid     | 294.435| 3   | 2     | 4.93   | √       |
| 53     | Trans-Oxyresveratrol       | 244.246| 4   | 4     | 2.723  | √       |
| 54     | Formononetin               | 268.268| 4   | 1     | 3.095  | √       |
| 55     | Astragaloside IV           | 784.98 | 14  | 9     | 1.21   | √       |
| 56     | Senkyunolide H             | 220.224| 4   | 2     | 2.314  | √       |
| 57     | Astragaloside II           | 827.02 | 15  | 8     | 1.91   | √       |
| 58     | Soyasaponin I             | 943.13 | 18  | 11    | 1.7    | ×       |
| 59     | Methyl tanshinonate        | 338.36 | 5   | 0     | 0.93   | √       |
| 60     | Carnosic acid             | 332.440| 4   | 3     | 4.603  | √       |
| 61     | Kaempferol-3-O-glucoside  | 448.380| 11  | 7     | 0.125  | ×       |
| 62     | Hydroxytanshinone IIA     | 310.35 | 4   | 1     | 3.24   | √       |
| 63     | 3-Butylidene-7-hydroxyphthalide | 204.225 | 3 | 1 | 2.81 | √ |
| 64     | Tanshinone II-B           | 310.35 | 4   | 1     | 2.97   | √       |
| 65     | Senkyunolide A            | 192.258| 2   | 0     | 3.521  | √       |
| 66     | Salvianolic acid F         | 314.29 | 6   | 5     | 2.33   | √       |
| 67     | Kumatakenin                | 314.29 | 6   | 2     | 2.98   | √       |
| 68     | 3-n-Butylphthalide        | 190.242| 2   | 0     | 3.483  | √       |
| 69     | (Z)-Ligustilide           | 190.242| 2   | 0     | 2.927  | √       |
| 70     | (E)-Ligustilide           | 190.242| 2   | 0     | 2.927  | √       |
| 71     | Trijuganone B             | 280.32 | 3   | 1     | 3.9    | √       |
| 72     | Cryptotanshinone           | 296.366| 3   | 0     | 3.83   | √       |
| 73     | Senkyunolide M            | 278.35 | 4   | 1     | 2.55   | √       |
| 74     | O-Phthalic anhydride       | 148.12 | 3   | 0     | 0.93   | √       |
| 75     | Chlorogenic acid           | 354.311| 9   | 6     | −0.453 | ×       |
| 76     | Tanshinone IIA            | 294.350| 3   | 0     | 4.158  | ×       |
| 77     | Angelic acid               | 380.48 | 4   | 0     | 5.73   | √       |
| 78     | Carthamidin                | 288.255| 6   | 4     | 1.649  | √       |
| 79     | Linoleic acid             | 280.45 | 2   | 1     | 6.86   | ×       |
| 80     | Acetyl-11-keto-β-boswellic acid | 512.73 | 5 | 1 | 6.39 | × |
| 81     | Oleanolic acid            | 456.71 | 3   | 2     | 6.72   | ×       |

Note. “√” means that component could be absorbed; “×” means that component could not be absorbed. “a” refers to the component has been verified by standard substance.

RCX, RSM, and FC were the most important. In the regulation of metabolism, RA, RSM, and RCX showed diametrical effect. All the herbs except Semen Persicae (SP) were related metabolism pathways due to the current research. RA, RSM, RCX, and FC could regulate the pathways about cell cycle, proliferation, and apoptosis. Some other important pathways were also affected by some herbs like RA, RSM, and RCX, for example, Insulin Signaling Pathway and p38 MAPK Signaling Pathway.

3.4. Pharmacology Network of NXT. Using the Cytoscape software, we constructed a pharmacology network of NXT (Figure 3), which showed us the relationships of the top 40 pathways, targets, and chemical components. We obtained preliminary understanding of the mechanism of NXT through this network.

In this research, we found three major targets of NXT: HRAS, MAP2K1, and MAPK14, which were involved in most regulated pathways. By Figure 4, based on illustration of the main targets with their corresponding compounds, we found the most effective ingredients of NXT were organic acids, saponins, and tanshinones. The main sources of organic acids were RA, RCX, RAS, and RAB. The saponins were mainly derived from RA. Meanwhile, tanshinones were mainly concentrated in RSM.

4. Discussion

The burden of cardiovascular and circulatory disease is becoming more and more serious, with cerebrovascular disease (CBD) and ischemic heart disease being the most serious [7]. As the causes of cardiovascular disease (CVD) and CBD are complicated, the symptoms of these diseases are also very diverse. NXT is commonly used during clinical treatment of CVD and CBD, and the effect of this drug is remarkable. Although complex traditional Chinese medicine has great significance for the treatment of complex diseases, some questions such as the material basis and the potential mechanisms remain unanswered.
Table 4: Top 40 Biocarta pathways regulated by NXT ($P < 0.01$).

| Rank | Pathway                                                                 | Count | $P$-value  | $q$-value  | Gene                                      |
|------|-------------------------------------------------------------------------|-------|------------|------------|-------------------------------------------|
| 1    | NFAT and hypertrophy of the heart (transcription in the broken heart)   | 6     | $5.75E-10$ | $3.58E-09$ | HRAS; GSK3B; MAPK14; FKBP1A; F2; MAP2K1   |
| 2    | Phosphoinositides and their downstream targets                         | 5     | $1.39E-09$ | $8.47E-09$ | GSK3B; PDPK1; BTK; RAB5A; EEAI           |
| 3    | Intrinsic Prothrombin Activation Pathway                               | 4     | $8.50E-08$ | $2.82E-07$ | F10; FGG; F1; F2                        |
| 4    | Bioactive Peptide Induced Signaling Pathway                            | 4     | $4.08E-07$ | $9.30E-07$ | HRAS; MAPK14; F2; MAP2K1                 |
| 5    | BCR Signaling Pathway                                                  | 4     | $4.88E-07$ | $1.08E-06$ | HRAS; MAPK14; BTK; MAP2K1                |
| 6    | Estrogen-responsive protein Efp controls cell cycle and breast tumors  | 3     | $6.40E-07$ | $1.34E-06$ | CDK2; ESR1; CDK6                         |
| 7    | Nuclear receptors in lipid metabolism and toxicity                     | 4     | $8.02E-07$ | $1.58E-06$ | CYP2C9; VDR; NR1H3; PPARA                |
| 8    | Map kinase inactivation of SMRT corepressor                           | 3     | $1.53E-06$ | $2.48E-06$ | THR8; MAPK14; MAP2K1                     |
| 9    | MAP Kinase Signaling Pathway                                           | 5     | $2.09E-06$ | $3.05E-06$ | HRAS; MAPK10; MAPK14; TGFBR1; MAP2K1    |
| 10   | Extrinsic Prothrombin Activation Pathway                               | 3     | $2.99E-06$ | $4.05E-06$ | F10; FGG; F2                             |
| 11   | Lipid metabolism and toxicity                                         | 3     | $5.17E-06$ | $6.40E-06$ | F10; FGG; F2                             |
| 12   | Roles of $\beta$-arrestin-dependent recruitment of Src kinases         | 3     | $6.57E-06$ | $7.86E-06$ | HRAS; HCK; MAP2K1                        |
| 13   | Aspirin blocks signaling pathway involved in platelet activation        | 3     | $8.19E-06$ | $9.49E-06$ | HRAS; CDK2; CDK6                         |
| 14   | Insulin Signaling Pathway                                              | 3     | $2.03E-05$ | $2.03E-05$ | HRAS; INSR; MAP2K1                       |
| 15   | IL-2 Signaling Pathway                                                 | 3     | $2.37E-05$ | $2.29E-05$ | HRAS; MAP2K1; LCK                       |
| 16   | Role of ERBB2 in signal transduction and oncology                      | 3     | $2.37E-05$ | $2.29E-05$ | HRAS; ESR1; MAP2K1                       |
| 17   | Links between Pyk2 and MAP kinases                                     | 3     | $2.74E-05$ | $2.45E-05$ | HRAS; MAPK14; MAP2K1                     |
| 18   | NF-κB activation by nontypeable Hemophilus influenzae                   | 3     | $2.74E-05$ | $2.45E-05$ | MAPK14; TGFBR1; NR3CI                    |
| 19   | Influence of Ras and Rho proteins on G1 to S transition and fMLP induced chemokine gene expression in HMC-1 cells | 3     | $3.14E-05$ | $2.82E-05$ | HRAS; CDK2; CDK6                         |
| 20   | Growth Hormone Signaling Pathway                                       | 3     | $3.14E-05$ | $2.82E-05$ | HRAS; MAPK14; MAP2K1                     |
| 21   | Cell cycle: G1/S checkpoint                                            | 3     | $4.06E-05$ | $3.37E-05$ | CDK2; GSK3B; CDK6                       |
| 22   | Fc epsilon receptor I signaling in mast cells                          | 3     | $4.58E-05$ | $3.70E-05$ | HRAS; BTK; MAP2K1                        |
| 23   | Signaling of hepatocyte growth factor receptor                         | 3     | $6.40E-05$ | $4.89E-05$ | HRAS; MET; MAP2K1                        |
| 24   | p38 MAPK signaling pathway                                             | 3     | $7.85E-05$ | $5.76E-05$ | HRAS; MAPK14; TGFBR1                    |
| 25   | Keratinocyte differentiation                                           | 3     | $1.13E-04$ | $7.81E-05$ | HRAS; MAPK14; MAP2K1                     |
| 26   | T cell receptor signaling pathway                                      | 3     | $1.13E-04$ | $7.81E-05$ | HRAS; MAP2K1; LCK                       |
| 27   | TSP-1 induced apoptosis in microvascular endothelial cell              | 2     | $1.46E-04$ | $9.59E-05$ | CASP3; MAPK14                            |
| 28   | The role of FYVE-finger proteins in vesicle transport                  | 2     | $1.46E-04$ | $9.59E-05$ | RAB5A; EEAI                             |
| 29   | Mechanism of gene regulation by peroxisome proliferators via PPARa(alpha) | 3     | $1.82E-04$ | $1.15E-04$ | HSP90AA1; NR1H3; PPARA                   |
| 30   | Visceral fat deposits and the metabolic syndrome                       | 2     | $1.95E-04$ | $1.21E-04$ | HSD1IB1; NR3C1                           |
| 31   | RB tumor suppressor/checkpoint signaling in response to DNA damage    | 2     | $2.50E-04$ | $1.44E-04$ | CDK2; CHEK1                              |
| 32   | Platelet Amyloid Precursor Protein Pathway                             | 2     | $2.50E-04$ | $1.44E-04$ | F1; F2                                  |
| 33   | Fibrinolysis Pathway                                                  | 2     | $3.12E-04$ | $1.77E-04$ | FGG; F2                                 |
| 34   | Corticosteroids and cardioprotection                                   | 2     | $3.12E-04$ | $1.77E-04$ | HSP90AA1; NR3C1                         |
| 35   | Phosphorylation of MEK1 by cdk5/p35 downregulates the MAP kinase pathway | 2     | $3.81E-04$ | $2.09E-04$ | HRAS; MAP2K1                            |
Table 4: Continued.

| Rank | Pathway                                      | Count | P-value   | q-value   | Gene                  |
|------|----------------------------------------------|-------|-----------|-----------|-----------------------|
| 37   | VEGF, hypoxia, and angiogenesis             | 2     | 5.38E-04  | 2.79E-04  | HRAS; KDR            |
| 38   | How progesterone initiates oocyte membrane  | 2     | 6.27E-04  | 3.17E-04  | HRAS; PGR            |
| 39   | IL-3 Signaling Pathway                      | 2     | 6.27E-04  | 3.17E-04  | HRAS; MAP2K1         |
| 40   | Sprouty regulation of tyrosine kinase signals | 2     | 6.27E-04  | 3.17E-04  | HRAS; MAP2K1         |

**Figure 2:** Structures of 63 absorbable components.
Table 5: The herbs of NXT involved in the top 40 pathways.

| Pathway associated with heart diseases and blood vessels | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
|--------------------------------------------------------|----------|---------|-----|----|-----|-----|-----|-----|----|----|----|-----|----|----|----|
| Pathway associated with heart diseases and blood vessels | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
| Pathway associated with metabolism | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
| Pathway associated with metabolism | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
| Pathway associated with immunity | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
| Pathway associated with cell cycle, proliferation, and apoptosis | Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
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Table 5: Continued.

| Category | Pathway | NXT | RA | RPR | RSM | RAS | RCX | SP | FC | CS | RAB | RC | RM | PT |
|----------|---------|-----|----|-----|-----|-----|-----|----|----|----|-----|----|----|----|
| Influence of Ras and Rho proteins on G1 to S transition | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Cell cycle: G1/S checkpoint | 1 1 1 1 1 1 0 0 1 0 0 0 0 |
| Fc epsilon receptor I signaling in mast cells | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Signaling of hepatocyte growth factor receptor | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Keratinocyte differentiation | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| RB tumor suppressor/checkpoint signaling in response to DNA damage | 1 1 1 1 1 1 0 0 1 0 0 0 0 |
| IL-3 Signaling Pathway | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Sprouty regulation of tyrosine kinase signals | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Bioactive Peptide Induced Signaling Pathway | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| amiPathway | 1 0 1 1 1 1 1 0 1 0 0 0 0 |
| Insulin Signaling Pathway | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| p38 MAPK Signaling Pathway | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| The role of FYVE-finger proteins in vesicle transport | 1 0 0 0 0 0 0 1 0 0 0 0 0 |
| Other pathways | 1 1 1 1 1 1 0 1 0 0 0 0 0 |
| Mechanism of gene regulation by peroxisome proliferators via PPAR | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| Phosphorylation of MEK1 by cdk5/p35 downregulates the MAP kinase pathway | 1 1 1 1 1 1 0 1 1 1 1 1 1 |
| How progesterone initiates oocyte membrane | 1 1 1 1 1 1 0 1 1 1 1 1 1 |

Note. “1” means that the Chinese herbal medicine acts on the pathway while “0” means it does not. The pathways in each category are sorted by the significant differences in $P$ value.

Our study successfully predicted absorbable chemical compositions of NXT. These constituents primarily included ferulic acid, succinic acid, astagaloside IV, and tanshinone IIA. Ferulic acid, which is derived primarily from RA, RCX, RAS, and RAB, is reported to act as an angiogenic agent that augments angiogenesis, which is critical in ischemic diseases, such as myocardial infarction and stroke [8]. Succinic acid has been demonstrated to activate Akt phosphorylation to inhibit apoptosis and necrosis caused by cardiomyocyte hypoxia/reoxygenation [9]. Previous studies demonstrated that astagaloside IV could protect the heart through NO-dependent mechanism [10]. NO has been confirmed to prevent the mitochondrial permeability transition pore from opening [11]. During early reperfusion, it can prevent the heart from reperfusion injury by inhibiting the opening of the mitochondrial permeability transition pore [12]. Tanshinone IIA also has cardioprotective effects, such as protection of cardiomyocytes from oxidative stress-triggered damage [13]. These reports were consistent with our results.

In addition to active ingredients, we also successfully predicted drug targets of NXT. The major targets were HRAS, MAP2KI, and MAPK14. The HRAS gene encodes the GTPase HRas, which is an enzyme known as transforming protein p21 [14]. With the ability to increase the effects of growth factor, HRas plays an important role in regulating the growth, differentiation, and death of endothelial cells [15]. The MAP2KI gene encodes an enzyme named dual specificity mitogen-activated protein kinase kinase 1, and MAPK14 encodes p38-α. Both of these factors are closely related to inflammation and p38-α is also associated with cardiac hypertrophy via p38 MAPK activity in the heart. In addition, p38-α has been recognized as an isoenzyme of cardiovascular importance [16].

Among the numerous identified pathways, NFAT and hypertrophy of the heart (transcription in the broken heart) were ranked first. Nuclear factor of activated T-cells (NFAT) transcription factors, which have four different isoforms, plays crucial roles in the regulation of gene expression during
heart development [17]. The isoforms NFATc3 and NFATc4 are involved in hypertrophic development, while NFATc1 plays a key role in cardiac development [18]. The dephosphorylation of NFATs can promote calcineurin regulating immune response genes [19]. Via compensatory hypertrophy, the heart adapts to persistent stress conditions, but, over time, dysfunction and myocardial failure evolve [20]. Like NFAT and hypertrophy of the heart (transcription in the broken heart), most of these pathways are involved in the formation and regulation of cardiovascular disease, such as nuclear receptors in lipid metabolism and toxicity. Nuclear receptors include a superfamily of ligand-dependent transcription factors that regulate genetic networks that control cell growth, development, and metabolism. Regulating nuclear receptors is beneficial for patients with metabolic diseases, such as cardiovascular disease, due to the requirement for balance among a number of pathways for normal metabolic control [21]. These studies confirmed the validity of our study.

From the above results, we also found the different significances of the total of 16 herbs in NXT. According to Chinese Pharmacopoeia 2015, the content of RA in NXT is 66 g, which is 2-3 times the content of any other herb in the whole prescription. It was reported that RA was the monarch drug of NXT and played a key role in improving the immune system, invigorating blood circulation, and the condition of myocardial ischemia and hypoxia [22]. Our study found that RA contained a lot of effective components, organic acids, and saponins and was critical source of the main active components of NXT. Through the comparison of the herbs involved in the top 40 pathways, RA was also proved to be the most important. In the top 40 pathways regulated by NXT, RA was involved in 33 pathways. Some other herbs, such as...
RSM, RCX, FC, and RAS, were also the important contents in the whole prescription of NXT.

The network pharmacology method used in this study is a novel methodology based on the construction of multilayer networks of disease phenotype-gene-drug to predict drug targets in a holistic manner and promote efficient drug discovery [23]. This method represents a breakthrough in comparison to the traditional herbal medicine research pattern "gene-target-disease" and initiates the new pattern "multiple genes-multiple targets-complex diseases" [24]. By this method, we proved that RA was the critical ingredient mainly involved in the regulation of metabolism and immunity in NXT. RAS was a major herb that regulated cell growth. RSM, RCX, and FC also played important roles in regulation of heart disease, blood vessels, and others. The results indicated that NXT, a complex prescription in the treatment of complex diseases, played a therapeutic effect through multiple targets and multiple pathways. This was the first study to investigate the mechanism of NXT using this method, and we successfully predicted the main targets and pathways, providing a foundation for further research. This method has important value for the study of complex drugs and should be applied in future studies.

5. Conclusion

The main components that mediated the efficacy of NXT were organic acids, saponins, and tanshinones. Radix Astragali was the critical herbal medicine in NXT, which contained more active components than others and regulated more targets.
and pathways. NXT had a therapeutic effect on the treatment of heart diseases through the pattern "multiple components-multiple targets-multiple pathways."

**Abbreviations**

| ACS | Acute coronary syndrome |
| AMI | Acute myocardial infarction |
| CBD | Cerebrovascular disease |
| CVD | Cardiovascular disease |
| ESI | Electrospray ionization |
| HRAS | Harvey rat sarcoma viral oncogene homolog |
| MAP2KI | Mitogen-activated protein kinase kinase 1 |
| MAPK14 | Mitogen-activated protein kinase 14 |
| NFAT | Nuclear factor of activated T cells |
| NXT | Naoxintong capsule |
| TCMs | Traditional Chinese medicines |
| UPLC/Q-TOF-MS | Ultrasound liquid chromatography/quadrupole time-of-flight mass spectrometry |

**Competing Interests**
The authors have no conflicting financial interests.

**Authors’ Contributions**
Xianghui Ma and Bin Lv contributed equally to this work.

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