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Research article

Compounds identification and mechanism prediction of YuXueBi capsule in the treatment of arthritis by integrating UPLC/IM-QTOF-MS and network pharmacology

Xiaoyu Zhang [a,1], Xueyuan Dong [a,e,1], Ruihu Zhang [a], Shufan Zhou [d], Wei Wang [d], Yu Yang [d], Yuefei Wang [a,b], Huijuan Yu [a,b], Jing Ma [c,**], Xin Chai [a,b,*]

[a] National Key Laboratory of Chinese Medicine Modernization, State Key Laboratory of Component-based Chinese Medicine, Tianjin Key Laboratory of TCM Chemistry and Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, China
[b] Haihe Laboratory of Modern Chinese Medicine, Tianjin, 301617, China
[c] First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, 300381, China
[d] Liaoning Good Nurse Pharmaceutical (Group) Co., Ltd., Liaoning, 117201, China
[e] Clinical Trials Center, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that seriously affects the life quality of patients. As a patent medicine of Chinese traditional medicine, YuXueBi capsule (YXBC) is widely used for treating RA with significant effects. However, its active compounds and therapeutic mechanisms are not fully illuminated, encumbering the satisfactory clinical application. In this study, we developed a method for identifying the chemical compounds of YXBC and the absorbed compounds into blood of rats using ultra performance liquid chromatography/ion mobility-quadrupole time-of-flight mass spectrometry (UPLC/IM-QTOF-MS) combined with UNIFI analysis software. A total of 58 compounds in YXBC were unambiguously or tentatively identified, 16 compounds from which were found in serum of rats after administration of YXBC. By network pharmacology, these prototype compounds identified in serum were predicted to regulate 30 main pathways (including HIF-1 signaling pathway, neuroactive ligand-receptor interaction, IL-17 signaling pathway, and so on) through 146 targets, resulting in promoting blood circulation and removing blood stasis, analgesia, and anti-inflammatory activities. This study provides a scientific basis for the clinical efficacy of YXBC in the treatment of RA.

Abbreviations: RA, rheumatoid arthritis; YXBC, YuXueBi capsule; CHMs, Chinese herbal medicines; UPLC, ultra performance liquid chromatography; IM-QTOF-MS, ion mobility-quadrupole time-of-flight mass spectrometry; PBCRBS, promoting blood circulation and removing blood stasis; AI, anti-inflammation; AKBA, 3-acetyl-11-keto-beta-boswellic acid; BPI, base peak intensity.

* Corresponding author. National Key Laboratory of Chinese Medicine Modernization, State Key Laboratory of Component-based Chinese Medicine, Tianjin Key Laboratory of TCM Chemistry and Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, China.
** Corresponding author.
E-mail addresses: majing2609@163.com (J. Ma), chaix0622@tjutcm.edu.cn (X. Chai).
[1] These authors contributed equally to this work.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by joint swelling and progressive damage to articular cartilage and some vital organs, such as the heart, kidney, lung, and so on [1–3]. Current research has documented that 0.5%–1.0% of adults are suffering from RA, which poses a significant challenge to quality of life, leading to a great burden of health care [4]. At present, long-term treatment with glucocorticoids and nonsteroidal anti-inflammatory drugs is the primary means to alleviate RA symptoms [5]. However, prolonged use of them is linked to the manifestation of several adverse side effects such as gastrointestinal distress, high blood pressure, and cardiovascular complications [6,7]. Traditional Chinese medicine (TCM) is employed as a credible alternative for treating RA. It is believed that the meridian paralysis and invasion of wind, dampness, or cold patterns into the human body and blood vessels are the primary reasons for the occurrence and development of RA [8].

As a kind of arthralgia syndrome, RA is usually treated with TCM to promote blood circulation, remove blood stasis, dredge collaterals, and relieve pain [9–11]. YuXueBi capsule (YXBC), as a patent medicine for treating RA, has been widely employed to alleviate and treat arthralgia syndrome, which is mainly composed of eleven Chinese herbal medicines (CHMs), including Radix et Rhizoma Clematidis (Weilingxian, WLX), Flos Carthami (Honghua, HH), Radix et Rhizoma Salviae Miltiorrhizae (Danshen, DS), Olibanum (Ruxiang, RX), Myrrha (Moyao, MY), Rhizoma Chuanxiong (Chuanxiong, CX), Rhizoma Curcumae Longae (Jianghuang, JH), Radix Cyathulae (Chuanniuxi, CNX), Radix Angelicae Sinensis (Danggui, DG), Rhizoma Cyperi (Xiangfu, XF), and Radix Astragali Praeparata Cum Melle (Zhihuangqi, HQ). Pharmacological studies have shown that YXBC can restrain angiogenesis by inhibition of LOX/Ras/Raf-1 signaling, which decreases the disease severity of RA and reduces bone erosion [12]. YXBC has the effect of analgesic *via* inhibiting the migration of macrophage to the spinal cord mediated by CCL3 [13] and plays anti-inflammatory (AI) role by regulating the phosphorylation of NF-κB p65, JNK, and p38 [14]. However, few systematic studies have been reported for unveiling the compounds in YXBC and its potential therapeutic mechanism for RA.

In this study, we employed ultra-high performance liquid chromatography/ion mobility-quadrupole time-of-flight mass spectrometry (UPLC/IM-QTOF-MS) to comprehensively analyze and identify the chemical compounds in YXBC and the absorbed compounds in blood. Then, focusing on promoting blood circulation and removing blood stasis (PBCRBS), analgesia, and AI, we employed the network pharmacology approach to explore its active compounds and clarify potential therapeutic mechanisms for treating RA by YXBC. This strategy shows promising perspectives in illumination on active compounds and potential therapeutic mechanisms for complex prescriptions of TCM.

2. Materials and methods

2.1. Materials and reagents

YXBCs were provided by Liaoning Good Nurse Pharmaceutical (Group) Co., Ltd. (Liaoning, China). Acetonitrile and methanol (HPLC grade) were purchased from Sigma-Aldrich Crop. (St. Louis, MO, USA). Formic acid (HPLC grade) was obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). The used water was purified by a Milli-Q water purification system (Millipore, Billerica, MA, USA). As reference compounds, danshensu, chlorogenic acid, ferulic acid, isochlorogenic acid B, rosmarinic acid, salvianolic acid B, salvianolic acid A, dihydrotanshinone I, tanshinone IIA, and 3-acetyl-11-keto-β-boswellic acid (AKBA) were supplied by the Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China), whose purities were all above 98%. Cryptotanshinone (>98%) was obtained from National Institutes for Food and Drug Control (Beijing, China).

2.2. Preparation of standard solutions

Accurately weighed compounds were respectively dissolved in 50% methanol aqueous solution (*v/v*) to obtain 0.5020 mg/mL danshensu, 0.1006 mg/mL chlorogenic acid, 0.05020 mg/mL ferulic acid, 0.05020 mg/mL isochlorogenic acid B, 0.2510 mg/mL rosmarinic acid, and 2.004 mg/mL salvianolic acid B. Moreover, the other compounds were separately dissolved in methanol to prepare 0.2008 mg/mL salvianolic acid A, 0.04980 mg/mL dihydrotanshinone I, 0.1992 mg/mL cryptotanshinone, 0.2000 mg/mL tanshinone IIA, and 0.4980 mg/mL AKBA. By using the prepared stock solutions, the mixed solution of the tested compounds was obtained at 4.204 μg/mL for danshensu, 0.7294 μg/mL for chlorogenic acid, 0.3891 μg/mL for ferulic acid, 0.7279 μg/mL for isochlorogenic acid B, 2.698 μg/mL for rosmarinic acid, 44.59 μg/mL for salvianolic acid B, 2.510 μg/mL for salvianolic acid A, 0.4358 μg/mL for dihydrotanshinone I, 1.370 μg/mL for cryptotanshinone, 1.250 μg/mL for tanshinone IIA, and 5.042 μg/mL for AKBA, respectively.

2.3. Sample preparation of YXBC

The accurately weighed YXBC powder (0.2 g) was transferred into a 50 mL conical flask and ultrasonically extracted with 25 mL methanol at 60 °C for 30 min, which was centrifugated at 12700 rpm for 10 min. The supernatant was obtained for analysis.

The accurately weighed YXBC powder (6.3 g) was dissolved in 36 mL normal saline and ultrasonically mixed to obtain the suspension at 0.175 g/mL for intragastric administration.

2.4. Serum sample preparation

Male Sprague-Dawley (SD) rats (200 ± 10 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were fed in a satisfactory environment (12 h light-dark cycle, temperature 20–22 °C, and relative humidity 40%–60%) for a week, which were fasted overnight with free access to distilled water for 12 h before the experiment. Then they were randomly divided into blank and YXBC groups ($n = 3$ per group). Rats in the YXBC and blank groups were respectively given at the dose of 2.625 g/kg YXBC and the same volume of normal saline for consecutive 7 days. After the last administration, the blood was taken from the fundus venous plexus at 15, 30, 60, and 90 min, which was placed at room temperature for 1 h and then centrifuged at 5000 rpm for 10 min at 4 °C. The serum was collected and frozen immediately at −80 °C until analysis. Blank serum samples were collected in the same way. Animal studies were conducted according to protocols approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2022105).

The serum samples were thawed and homogenized at room temperature in advance. Equal amounts (50 μL) of serum collected at different times were mixed and 1 mL methanol was added, which was vortex-mixed for 5 min and centrifuged at 5000 rpm for 10 min at 4 °C. The obtained supernatant was evaporated by nitrogen at room temperature. Then, the residues were dissolved in methanol (150 μL) and vortexed for 1 min, which was followed by centrifugation at 12700 rpm for 10 min at 4 °C. The supernatant was employed for analysis.

2.5. UPLC/IM-QTOF-MS analytical conditions

By employing ACQUITY™ UPLC system (Waters, Milford, USA), chromatographic separation was carried out on an ACQUITY™ UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) held at 40 °C. The mobile phase consisted of 0.2% formic acid aqueous solution (A) and acetonitrile (B) with a flow rate of 0.3 mL/min using a gradient elution of 3%–10% B between 0 and 2 min, 10%–20% B between 2 and 3 min, 20%–28% B between 3 and 7 min, 28%–45% B between 7 and 7.5 min, 45%–90% B between 7.5 and 20 min, and 90%–3% B between 20 and 21 min. The injection volume was 5 μL.

The MS analysis was performed on a Vion IM-Q TOF mass spectrometer (Waters Corporation, Milford, USA) using electrospray ionization (ESI) in both positive and negative ion modes. MS conditions were as follows: the capillary voltage at 3.0 and −2.5 kV in positive and negative ion modes, respectively; the source temperature at 120 °C; the temperature of the desolvation gas at 400 °C; the flow rate of the desolvation gas (N_2) at 700 L/h; the flow rate of the cone gas (N_2) and collision gas (Ar) at 50 L/h and 0.20 mL/min, respectively; mass range: m/z 150–1500; collision energy, 6 eV and 20–60 eV ramping. Data acquisition was controlled with UNIFI 1.8.0 informatics platform (Waters Corporation, Milford, USA).

2.6. UNIFI data processing method

The systematical information about compounds from eleven herbs of YXBC was collected by searching China National Knowledge Infrastructure (CNKI), PubMed, PubChem, ChemSpider, and other databases. The self-built database of YXBC was constructed by importing information, such as the compound name, molecular formula, chemical structure, and accurate molecular weight, into the UNIFI system combining the Chinese medicine component database. The collected UPLC-Q-TOF-MS information was imported into the UNIFI self-built database for matching compounds by the automatic matching function of UNIFI software. The acceptable error of molecular weight was ±10 ppm. The adduct ions include +H, +Na, +K, and $+NH_4$ in the positive ion mode, and the adduct ions contain +HCOO, +Cl, $+CH_3COO$, and +Br in the negative ion mode. The identified compounds were checked by standards or literature reports.

2.7. Network pharmacology analysis

The structures of the focused compounds absorbed into blood from YXBC were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and imported into SwissTargetPrediction database (http://www.swisstargetprediction.ch/) to acquire potential targets. PBCRBS [15], Analgesic [16], and AI [17] were selected as the main bioactivities of YXBC and RA was used as the disease treated by YXBC to search for relevant targets from the GeneCards database (https://www.genecards.org/). The targets with a relevance score ≥1 were collected as main targets related to bioactivities and disease. The intersection of the collected RA-related targets and compounds-related targets was considered as the common targets, which were further overlapped with the biological targets to obtain the shared targets of compounds-bioactivities-RA. A CHMs-compounds-targets-bioactivities-RA network was constructed to display relationships among them by Cytoscape (version 3.7.2). The overlapped targets of PBCRBS-compounds-RA, Analgesic-compounds-RA, and AI-compounds-RA were imported into DAVID database (https://david.ncifcrf.gov/) for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, respectively.

3. Results and discussion

3.1. Identification of compounds in YXBC by UPLC/IM-QTOF-MS combined with UNIFI software

To ensure the comprehensiveness and accuracy of compounds identification, we established a self-built database with the aid of UNIFI software. 381 compounds from eleven CHMs of YXBC were retrieved into the self-built database, including compound name,

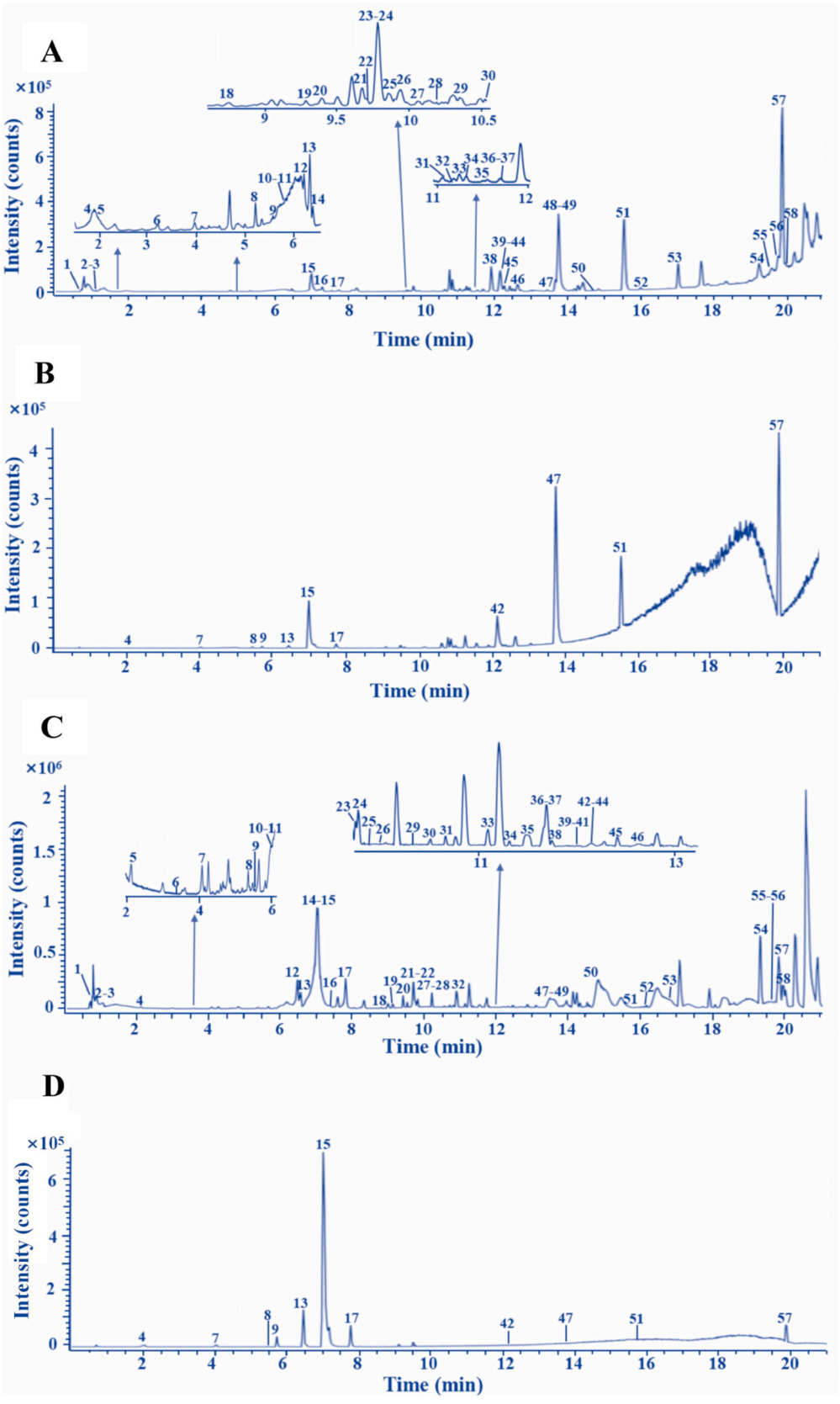

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Fig. 1. BPI chromatograms of YXBC in the positive (A) and negative (C) ion modes and mixed standards in the positive (B) and negative (D) ion modes.

molecular formulae, chemical structure, accurate molecular mass, and so on. Importantly, the comprehensive information on the analyzed compounds was acquired in both positive and negative ion modes by UPLC/IM-QTOF-MS. The base peak intensity (BPI) chromatograms of YXBC and the mixed standard solution are shown in Fig. 1. The acquired MS data were matched with the data from the self-built database in UNIFI software to deduce the structures of compounds. 58 compounds in YXBC (Fig. 1 A and C), including 16 organic acids ("a"), 12 quinones ("q"), 7 terpenoids ("t"), 5 flavonoids ("f"), 2 saponins ("s"), and 16 others ("o"), were tentatively characterized. Among these, eleven compounds (peaks 4, 7, 8, 9, 13, 15, 17, 42, 47, 51, and 57) were unambiguously identified by comparing with the retention time, quasi-molecular ions, and fragment ions of the reference standards (Fig. 1 B and D). Detailed information on the chemical compounds was summarized in Table 1. Additionally, 58 compounds in YXBC were also traced to their sources of CHMs, as shown in Fig. S1 and Table 1. It was found that the identified compounds were mainly originated from DS (22 compounds), HQ (eleven compounds), DG (ten compounds), CX (seven compounds), HH (three compounds), XF (three compounds), JH (two compounds), RX (two compounds), and WLX (one compound).

According to the quasi-molecular ions, fragment ions, and adduct ions of the standard compounds, the chemical structures of identified compounds and their fragmentation pathways were proposed. Compound 7 was identified as chlorogenic acid derived from Honghua, which showed $[M-H]^-$ at *m/z* 353.0873 and fragment ions at *m/z* 191.0754, 179.0483, 135.0554, corresponded with $[M–H–Caffeoyl]^-$, $[M–H–QAr]^-$, and $[M–H–QAr–CO_2]^-$ [18]. The mass spectrum of compound 15 exhibited $[M-H]^-$ ion at *m/z* 717.1231, fragment ions at *m/z* 519.1432, 339.0831, 321.0713, 295.0894, and 293.0360 assigned to $[M–H–Danshensu]^-$, $[M–H–Danshensu–CA]^-$, $[M–H–2Danshensu]^-$, $[M–H–Danshensu–CA–CO_2]^-$, and $[M–H–2Danshensu–CO]^-$, respectively, which was identified as salvianolic acid B originated from Danshen [19]. Tanshinone IIA (compound 51) from Danshen, a quinone compound, yielded $[M+H]^+$ ion at *m/z* 295.1230, whose product ions at 277.1157 $[M + H–H_2O]^+$ and 249.1204 $[M + H–H_2O–CO]^+$ were also observed [20].

3.2. Identification of prototype compounds from YXBC in serum of rats

The identification of prototype compounds *in vivo* is helpful to clarify the material basis of the efficacy of Chinese Materia medica (CMM) and its compound prescription [21]. To identify candidate therapeutic substances of YXBC, chemical profiling of the rat serum collected after administration of YXBC was conducted. On the basis of the identified compounds in YXBC, UPLC/IM-QTOF-MS combined with UNIFI software was also employed to identify the prototype compounds absorbed into the blood from YXBC by analyzing the dosed and blank serum. The extracted ion chromatograms of prototype compounds in the serum are displayed in Fig. 2 (A-D). 16 prototype compounds were obviously detected in rat serum, including six quinones (danshenxinkun A, danshenxinkun D, dihydrotanshinone I, hydroxytanshinone IIA, isocryptotanshinone, and tanshinone IIA), four terpenoids (8-hydroxy-ar-turmerone, α-cyperone, β-boswellic acid, and AKBA), two flavonoids (methylnissolin and formononetin), two phthalide (senkyunolide D and *Z*-butylidenephthalide), one organic acid (9,12,13-trihydroxy-10-octadecenoic acid), and one lactone (brefeldin A), from which three compounds were unambiguously identified by comparison with standard references.

The prototype compounds of YXBC *in vivo* play a key role in the prevention and treatment of arthritis. For example, tanshinone IIA exerts AI, anticoagulant, antithrombotic, and neuroprotective effects by regulating the TLR/NF-κB and MAPKs/NF-κB pathways [22]. β-Boswellic acid and AKBA demonstrate synergistic effects in exerting AI and anti-arthritic activities by inhibiting the activity of the 5-lipoxygenase (5-LOX) pathway, resulting in improved physical and functional capacity, as well as reduced pain and stiffness [23]. Studies have shown that *Z*-butylidenephthalide, a phthalide compound, exhibits analgesic, AI, anti-proliferative, and antifungal properties [24] by significantly reducing the content of PGE2 in inflammatory tissues [25]. Formononetin can protect articular cartilage by inhibiting the expression and activation of pro-inflammatory cytokine-induced cartilage degrading enzymes. Also, it can antagonize the catabolism of proteoglycan in chondrocytes induced by IL-1β and prevent the degradation of articular cartilage under pro-inflammatory action [26]. α-Cyperone was reported to ameliorate osteoarthritis by down-regulating NF-κB and MAPKs signaling pathways, attenuating chondrocyte inflammation, and reducing extracellular matrix degradation [27]. In summary, analgesia, PBCRBS, and AI were considered as the characteristic activities of the 16 bioactive compounds in YXBC.

3.3. Construction of network for CHMs-preparation-compounds-targets-bioactivities-RA

In recent years, network pharmacology analysis has been successfully applied to prediction of therapeutic mechanisms in various CMM [28]. As a particularly effective web-based tool, SwissTargetPrediction can accurately predict potential targets for the reported bioactive compounds and novel synthetic analogs, thereby providing reliable predictions and identifying more targets [29,30]. Potential targets of the prototype compounds in serum from YXBC were retrieved by SwissTargetPrediction. After removing duplicate targets, a total of 695 targets were acquired, in which hydroxytanshinone IIA, 8-hydroxyarturmerone, *Z*-butylidenephthalide, danshenxinkun D, and brefeldin A were found to target the most proteins, and the number of their targets was 274, 148, 130, 124, and 122, respectively. By GeneCards, 2875, 97, 375, and 1560 targets associated with RA and the bioactivities of analgesia, PBCRBS, and AI were obtained, respectively. Using the venny (2.1.0) website, 26 targets from the intersection of compounds-RA-analgesia, 85 targets from compounds-RA-PBCRBS, and 230 targets from compounds-RA-AI were acquired after merging and removing the duplicate targets, respectively (Fig. S2). The intricate relationship among eleven CHMs, 16 absorbed compounds in blood, 3 bioactivities, and

Table 1
Analysis of chemical compounds from YXBC by UPLC/IM-QTOF-MS.

Serial No.	Identification (compounds category[#])	t_R/ min	Formula	Quasi-molecular ion/adduct ion	Observed value (*m/z*)	Mass error **(ppm)**	Fragment ions (*m/z*)	Absorbed compounds in serum	Source[&]
1	arginyl-fructose (o)	0.75	$C_{12}H_{24}N_4O_7$	$[M+H]^+$	337.1716	–0.5	319.1516, 175.1198	–	DG
2	guanosine (o)	1.11	$C_{10}H_{13}N_5O_5$	$[M-H]^-$	282.0843	–0.3	150.0426	–	HQ, DG
3	citric acid (a)	1.12	$C_6H_8O_7$	$[M-H]^-$	191.0194	–1.6	173.0090	–	DS
4*	danshensu (a)	2.05	$C_9H_{10}O_5$	$[M-H]^-$	197.0451	–2.2	179.0346, 151.0393	–	DS
5	succinyladenosine (o)	2.06	$C_{14}H_{17}N_5O_8$	$[M+H]^+$	384.1164	3.6	252.0746, 162.0788, 188.0580	–	DG, HQ
6	indole-3-acrylic acid (a)	3.48	$C_{11}H_9NO_2$	$[M+H]^+$	188.0713	3.6	170.0613	–	DG, HQ
7*	chlorogenic acid (a)	4.04	$C_{16}H_{18}O_9$	$[M-H]^-$	353.0873	–1.4	191.0754, 179.0483, 135.0554	–	HH
8*	ferulic acid (a)	5.45	$C_{10}H_{10}O_4$	$[M-H]^-$	193.0502	–2.4	177.0917, 162.8387	–	HQ
9*	isochlorogenic acid B (a)	5.72	$C_{25}H_{24}O_{12}$	$[M-H]^-$	515.1171	–4.7	191.0551, 173.0450	–	WLX
10	lithospermic acid (a)	5.80	$C_{27}H_{22}O_{12}$	$[M-H]^-$	537.1020	–3.5	339.0493, 295.0596, 185.0236	–	DS
11	salvianolic acid D (a)	5.83	$C_{20}H_{18}O_{10}$	$[M-H]^-$	417.0808	–4.6	339.0493, 197.0454, 179.0344, 157.0289	–	DS
12	cartormin (f)	6.20	$C_{27}H_{29}NO_{13}$	$[M+H]^+$	576.1729	2.9	414.1197, 249.0553	–	HH
13*	rosmarinic acid (a)	6.45	$C_{18}H_{16}O_8$	$[M-H]^-$	359.0764	–2.5	197.0455, 179.0350, 161.0242, 151.0398	–	DS
14	salvianolic acid G (a)	6.53	$C_{18}H_{12}O_7$	$[M+H]^+$	341.0658	0.6	295.0608, 279.0660, 187.0399	–	DS
15*	salvianolic acid B (a)	7.01	$C_{36}H_{30}O_{16}$	$[M-H]^-$	717.1231	–3.8	519.1432, 339.0831, 321.0713, 295.0894, 293.0360	–	DS
16	baicalin (f)	7.43	$C_{21}H_{18}O_{11}$	$[M-H]^-$	445.0786	2.3	269.0458, 241.1204, 223.0393	–	HQ
17*	salvianolic acid A (a)	7.77	$C_{26}H_{22}O_{10}$	$[M-H]^-$	493.111	–6.2	295.0595, 267.0647, 185.0237	–	DS
18	isosalvianolic acid C (a)	8.71	$C_{26}H_{20}O_{10}$	$[M-H]^-$	491.0984	0.1	311.0556, 293.0449	–	DS
19	senkyunolide D (o)	9.23	$C_{12}H_{14}O_4$	$[M-H]^-$	221.0816	–1.5	203.0712, 177.0919, 173.0227	+	CX
20	calycosin (f)	9.34	$C_{16}H_{12}O_5$	$[M-H]^-$	283.0608	–1.5	161.0242	–	HQ
21	methylnissolin (f)	9.67	$C_{17}H_{16}O_5$	$[M-H]^-$	299.0921	–1.4	167.0718	+	HQ
22	9,12,13-trihydroxy-10-octadecenoic acid (a)	9.68	$C_{18}H_{34}O_5$	$[M-H]^-$	329.2319	–4.3	311.2222, 229.1442, 171.1021	+	HH
23	β -rotunol (t)	9.79	$C_{15}H_{22}O_2$	$[M+H]^+$	235.1699	2.9	197.0606	–	XF
24	*Z*-butylidenephthalide (o)	9.86	$C_{12}H_{12}O_2$	$[M+H]^+$	189.0917	3.5	159.0810	+	DG
25	3-butylphthalide (o)	9.94	$C_{12}H_{14}O_2$	$[M+H]^+$	191.1074	3.7	161.0609	–	DG
26	formononetin (f)	9.98	$C_{16}H_{12}O_4$	$[M-H]^-$	267.0652	–4.0	252.0416	+	HQ

(*continued on next page*)

Table 1 (*continued*)

Serial No.	Identification (compounds category[#])	t_R/ min	Formula	Quasi-molecular ion/ adduct ion	Observed value (*m/z*)	Mass error **(ppm)**	Fragment ions (*m/z*)	Absorbed compounds in serum	Source[&]
27	3- butyl-4-hydroxyphthalide (o)	10.06	$C_{12}H_{14}O_3$	$[M-H]^-$	205.0867	–1.6	161.0692	–	CX
28	astragaloside II (s)	10.20	$C_{43}H_{70}O_{15}$	$[M+H]^+$	827.4793	0.7	571.2917, 419.2423, 353.2316	–	HQ
29	salvianolic acid F (a)	10.36	$C_{17}H_{14}O_6$	$[M+H]^+$	315.0870	2.0	295.1343, 243.0680, 203.1074	–	DS
30	β -cyperone (t)	10.54	$C_{15}H_{22}O$	$[M+H]^+$	219.1747	6.4	159.0816	–	XF
31	curcumin (o)	11.10	$C_{21}H_{20}O_6$	$[M-H]^-$	367.1178	–2.4	285.1129, 245.0833, 175.0763	–	JH
32	senkyunolide (o)	11.18	$C_{12}H_{16}O_2$	$[M+H]^+$	193.1227	1.8	177.1285, 175.1127, 163.1137	–	CX
33	8-(3-((3-pentyloxiran-2-yl) methyl)oxiran-2-yl)octanoic acid (a)	11.21	$C_{18}H_{32}O_4$	$[M-H]^-$	311.2219	–2.7	293.2119, 223.1702	–	HQ
34	danshenxinkun A (q)	11.36	$C_{18}H_{16}O_4$	$[M-H]^-$	295.0970	–1.8	277.0859, 262.0641	+	DS
35	1,2,5,6-tetrahydrotanshinone I (q)	11.52	$C_{18}H_{16}O_3$	$[M+NH_4]^+$	298.1449	3.7	253.1237, 215.1081	–	DS
Serial No.	Identification (compounds category[#])	t_R/ min	Formula	Quasi-molecular ion/ adduct ion	Observed value (*m/z*)	Mass error **(ppm)**	Fragment ions (*m/z*)	Absorbed compounds in serum	Source[&]
36	isoastragaloside I (s)	11.68	$C_{45}H_{72}O_{16}$	$[M+H]^+$	869.4903	1.1	851.4818, 689.4253, 671.4164, 473.3637	–	HQ
37	danshenxinkun D (q)	11.69	$C_{21}H_{20}O_4$	$[M+H]^+$	337.1417	–5.2	309.1145, 279.1028	+	DS
38	dehydromiltirone (q)	12.00	$C_{19}H_{20}O_2$	$[M+H]^+$	281.1547	4.1	266.1315, 251.1090, 239.1081	–	DS
39	8-hydroxyarturmerone (t)	12.01	$C_{15}H_{20}O_2$	$[M+H]^+$	233.1531	–2.4	191.1081, 173.0976	+	JH
40	α-cyperone (t)	12.07	$C_{15}H_{22}O$	$[M+H]^+$	219.1752	3.8	203.1444, 187.1122	+	XF
41	2-methoxy-5-acetoxy-fruranogermacr-1(10)-en-6-one (o)	12.09	$C_{18}H_{24}O_5$	$[M+H]^+$	321.1709	3.8	292.1344, 229.1242	–	DG
42*	dihydrotanshinone I (q)	12.15	$C_{18}H_{14}O_3$	$[M+H]^+$	279.1017	0.5	261.0914, 233.0963, 205.1015	+	DS
43	hydroxytanshinone IIA (q)	12.18	$C_{19}H_{18}O_4$	$[M+H]^+$	311.1288	3.3	293.1183, 278.0946	+	DS
44	sedanolide (o)	12.20	$C_{12}H_{18}O_2$	$[M+H]^+$	195.1390	5.3	161.0975	–	CX
45	*Z*-ligustilide (o)	12.26	$C_{12}H_{14}O_2$	$[M+H]^+$	191.1070	1.5	173.0972, 163.1129	–	CX
46	danshenxinkun B (q)	12.62	$C_{18}H_{16}O_3$	$[M+H]^+$	281.1172	1.6	253.0876, 235.1123	–	DS
Serial No.	Identification (compounds category[#])	t_R/ min	Formula	Quasi-molecular ion/adduct ion	Observed value (*m/z*)	Mass error **(ppm)**	Fragment ions (*m/z*)	Absorbed compounds in serum	Source[&]
47*	cryptotanshinone (q)	13.73	$C_{19}H_{20}O_3$	$[M+H]^+$	297.1485	2.9	279.1298, 282.1185, 254.0855, 251.1362	–	DS
48	brefeldin A (o)	13.74	$C_{16}H_{24}O_4$	$[M+K]^+$	319.1311	1.4	178.0580	+	DG
49	isocryptotanshinone (q)	13.75	$C_{19}H_{20}O_3$	$[M+H]^+$	297.1494	2.9	279.1386, 251.1436	+	DS
50	dan shen spiroketal lactone (o)	14.82	$C_{18}H_{22}O_3$	$[M+H]^+$	287.1648	2.1	269.1546, 241.1605, 199.0768	–	DS

(*continued on next page*)

Table 1 (*continued*)

Serial No.	Identification (compounds category[#])	t_R/ min	Formula	Quasi-molecular ion/adduct ion	Observed value (*m/z*)	Mass error **(ppm)**	Fragment ions (*m/z*)	Absorbed compounds in serum	Source[&]
51*	tanshinone IIA (q)	15.55	$C_{19}H_{18}O_3$	$[M+H]^+$	295.1230	1.7	277.1157, 249.1204, 262.0922, 262.0988	+	DS
52	rosmariquinone (q)	16.13	$C_{19}H_{22}O_2$	$[M+H]^+$	283.1697	1.5	267.1408, 241.1227, 225.0920	–	DS
53	senkyunone (q)	16.80	$C_{22}H_{30}O_2$	$[M-H]^-$	325.2189	5.0	241.1223, 297.1873	–	CX
54	stigmasterol (o)	19.28	$C_{29}H_{48}O$	$[M+H]^+$	413.3782	1.0	233.1929, 226.1700	–	DG
55	ethyl linoleate (o)	19.63	$C_{20}H_{36}O_2$	$[M+H]^+$	309.2791	1.0	263.2388	–	CX
56	*β*-boswellic acid (t)	19.74	$C_{30}H_{48}O_3$	$[M-H]^-$	455.3521	–2.2	385.2735, 373.2739, 339.2687	+	RX
57*	AKBA (t)	19.86	$C_{32}H_{48}O_5$	$[M+H]^+$	513.3567	–1.5	407.3297, 173.1329	+	RX
58	ursolic acid (t)	19.97	$C_{30}H_{48}O_3$	$[M+H]^+$	457.3668	–1.7	413.2691, 259.2429, 203.1803	–	DG

"*" Compared with the reference standard; "#" Compounds category; "–" Not detected; "+" Detected.
"&" Source of compounds; "a" organic acids; "q" quinones; "t" terpenoids; "f" flavonoids; "s" saponins; "o"others.
"DG" Danggui; "HQ" Zhihuangqi; "DS" Danshen; "HH" Honghua; "WLX" Weilingxian; "CX" Chuanxiong; "JH" Jianghuang; "XF" Xiangfu; "RX" Ruxiang.

RA-related targets was clearly illustrated by Cytoscape 3.7.2 in Fig. 3. The results indicated that the absorbed compounds in blood primarily exhibited anti-RA biological activity by targeting proteins such as ALB, ILB, PTGS2, and MMP9. Specifically, 8-hydroxyarturmerone demonstrated AI effect by targeting HMOX1, OPRM1, CYP19A1, CASR, and LTA4H proteins. *Z*-Butylidenephthalide played analgesic role by targeting P2RX7, PTGS1, PTGS2, FAAH, and ABCB1 proteins. Formononetin was found to mainly affect PBCRBS through its interaction with IL2, ALOX12, EGFR, XDH, and ESR1 proteins. Especially, dihydrotanshinone I was found to play a key role in PBCRBS, AI, and analgesia through ACHE, HTR3A, TSPO, KDR, ERBB2, EGFR, ADAM17, and PLAUR targets. These findings suggest that YXBC contains multiple active compounds with different biological activities, which might be the effective compounds for YXBC in treating RA.

3.4. KEGG enrichment analysis of core targets

The intersected targets for the absorbed compounds into blood-RA-bioactivities were imported into DAVID for KEGG enrichment analysis to respectively obtain the 10 shared signaling pathways associated with analgesic, PBCRBS, and AI. As shown in Fig. 4, the signaling pathways related to analgesic mainly include neuroactive ligand-receptor interaction, serotonergic synapse, retrograde endocannabinoid signaling, and calcium signaling pathway. The signaling pathways related to PBCRBS primarily involve rapl signaling pathway, lipid and atherosclerosis, and HIF-1 signaling pathway. Furthermore, the signaling pathways associated with AI mainly consist of virus infection-related pathways, immune regulation-related pathways, and phospholipase D signaling pathway. To further predict the potential therapeutic mechanisms of the absorbed compounds from YXBC for the treatment of RA, the enriched representative pathways were analyzed in detail. In the neuroactive ligand-receptor interaction signaling pathway, the compounds absorbed in the blood can regulate the pathway by interacting with targets such as P2RX7, OPRD1, MC1R, CNR1, HTR1A, and TSPO, leading to analgesic effects. Similarly, in the HIF-1 signaling pathway, the active compounds from YXBC can target IL6, FLT1, PIK3CA, NOS2, NOS3, and ERBB2 to regulate the pathway and achieve the purpose of PBCRBS. In the IL-17 signaling pathway, the prototype compounds from YXBC play a regulatory role by acting on GSK3B, MMP1, MMP3, PTGS2, and MAPK1 targets to achieve the effect of AI. For example, dihydrotanshinone I achieves analgesic by regulating the target HTR1A in neuroactive ligand-receptor interaction, which is associated with the balance of neuro function [31] (Fig. 4A). HIF-1 signaling pathway is involved in angiogenesis and erythropoiesis [32], dihydrotanshinone I may perform PBCRBS by exerting impacts on the ERBB2 and EGFR in HIF-1 signaling pathway (Fig. 4B). Recent research has revealed that IL-17 and its related cytokines lead to the activation of the transcription factors NF-*κ*B, AP1, and C-EBP, which induce transcription of multiple genes in a tissue-specific fashion, including major inflammatory cytokines (TNF and IL-6) [33]. For IL-17 signaling pathway, dihydrotanshinone I probably plays an important role in AI *via* MAPK8 (Fig. 4C). Therefore, YXBC may play an anti-RA role by regulating signaling pathways related to cardiovascular, inflammatory responses, viral infections, cancer, immunity, and central nervous system. These results indicated that 16 prototype compounds *in vivo* exert pharmacological activity in the treatment of RA, which provided evidence for the clinical application of YXBC.

However, the use of network pharmacology approach has certain limitations in identification of active ingredients and clarification of potential mechanisms, which overlooks the impact of the compounds' content in TCM on the activity. Therefore, it is crucial to conduct further experimental analysis and verification to validate the active ingredients and related targets regarding the mechanism

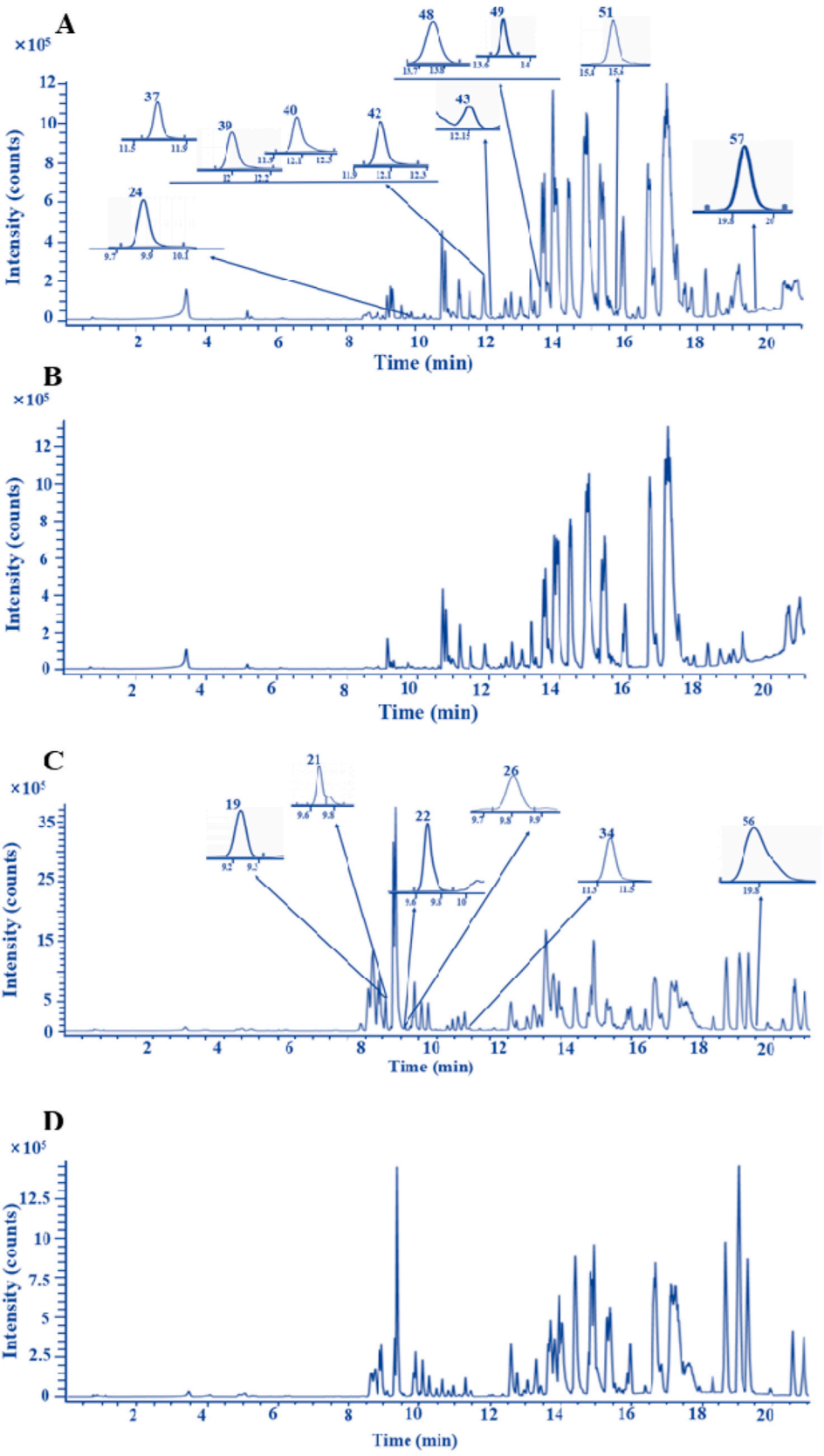

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Fig. 2. The extracted ion chromatograms of prototype compounds from the dosed serum in the positive (A) and negative (C) ion modes and blank serum in the positive (B) and negative (D) ion modes.

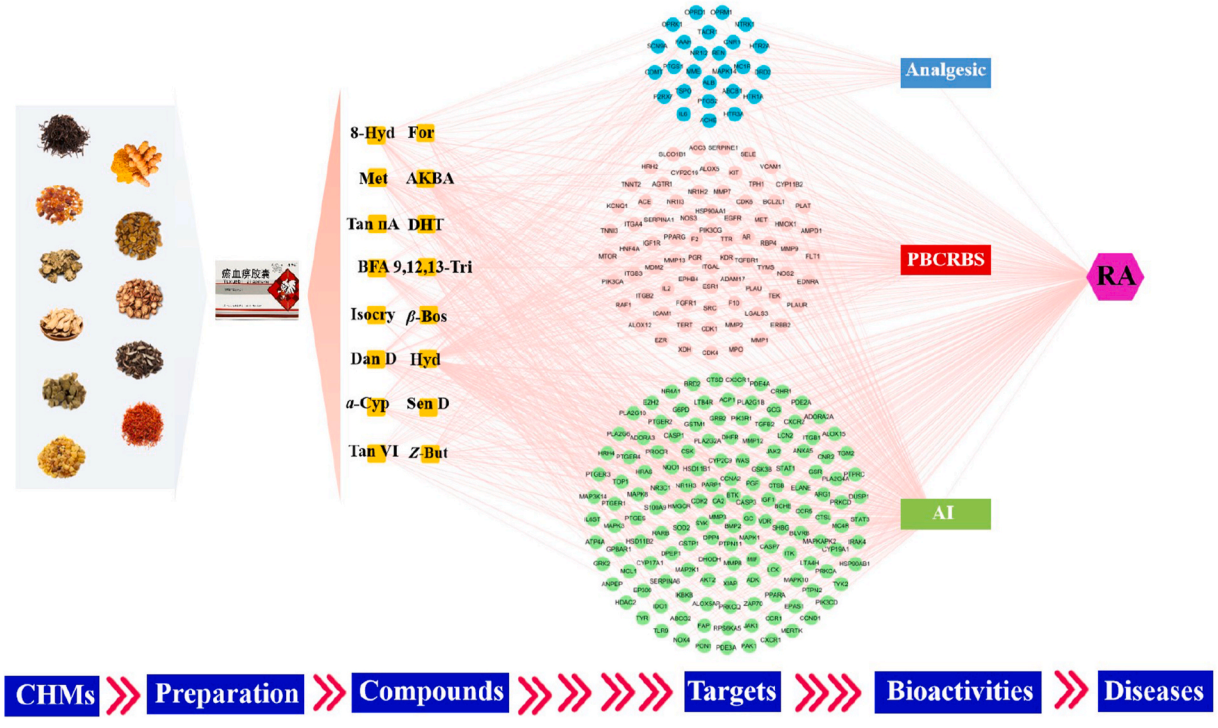

Fig. 3. The network of "CHMs-compounds-targets-bioactivities-RA".

of YXBC in the treatment of RA.

4. Conclusions

This research developed an integrative approach integrated with UPLC/IM-QTOF-MS, serum pharmacochemistry, and network pharmacology to investigate the active compounds and action mechanisms of YXBC in the treatment of RA. A total of 58 compounds in YXBC were unambiguously or tentatively identified, and 16 compounds were found in rat serum after administration of YXBC, suggesting their potential biological activity. The network pharmacology analysis revealed that the therapeutic effects of YXBC against RA may be attributed to analgesia, PBCRBS, and AI bioactivities of the active compounds that could exert regulatory effects on significant signaling pathways including the IL-17 signaling pathway, neuroactive ligand-receptor interaction, and HIF-1 signaling pathway. This study aims to elucidate the potential molecular mechanisms of YXBC in the treatment of RA, thereby enhancing the effectiveness and specificity for YXBC clinical treatment.

Ethics statement

The animal study protocol was approved by the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2022105).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xiaoyu Zhang: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Xueyuan Dong:** Writing – original draft, Validation, Methodology, Conceptualization. **Ruihu Zhang:** Visualization, Investigation, Formal analysis. **Shufan Zhou:** Visualization, Investigation, Formal analysis. **Wei Wang:** Visualization, Investigation, Formal analysis, Data curation. **Yu Yang:** Visualization, Resources, Formal analysis, Data curation. **Yuefei Wang:** Project administration, Funding acquisition, Formal analysis. **Huijuan Yu:** Resources, Project administration, Funding acquisition, Formal analysis. **Jing Ma:** Writing –

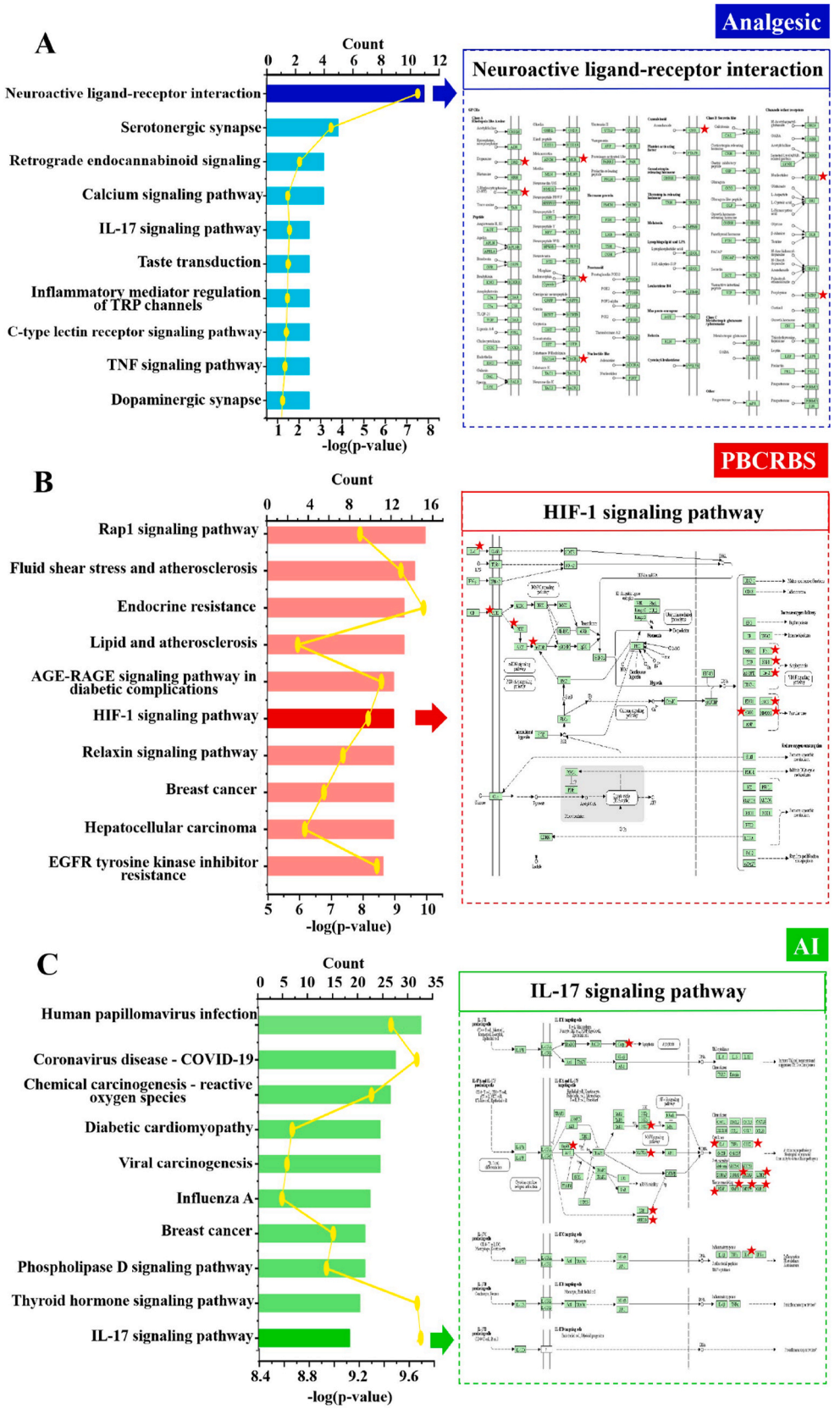

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Fig. 4. The pathways and representative signaling pathway of Analgesia (A), PBCRBS (B), and AI (C) associated with the absorbed compounds into blood.

review & editing, Supervision. **Xin Chai:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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