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Investigating the pharmacological basis and mechanisms of the anti-thrombotic effects of *Paeoniae Radix Rubra*, both pre- and post-stir-frying with rice wine

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ABSTRACT

Paeoniae Radix Rubra (PRR) is a commonly used traditional Chinese medicine known for promoting blood circulation and alleviating pain. Stir-frying PRR with rice wine enhances its effectiveness in improving blood flow and resolving stasis. To explore the pharmacodynamic basis and mechanisms behind PRR's anti-thrombotic effects—both pre- and post-stir-frying—a comprehensive approach combining pharmacological research, metabolomics, and chemistry was employed. Fraction inter-cross validation experiments, pharmacological assessments, and metabolomic analyses confirm the efficacy of the fraction separation process. Wine-stir-fried PRR (WPRR) shows superior blood activation compared to PRR. PRR mainly affects the TCA cycle and sphingolipid metabolism pathways through its small molecules, enhancing coagulation and fibrinolysis systems' functionality. All the subdivided components of WPRR have anti-thrombotic effects, especially polysaccharides and oligosaccharides. WPRR improves thrombotic conditions in rat blood stasis by modulating arachidonic acid metabolism, TCA cycle, lipid status enhancement, and angiogenesis stimulation while regulating platelet adhesion and aggregation. In conclusion, compared with PRR, the main material basis for the blood-activating efficacy of WPRR has transformed from small molecule compounds to polysaccharide and oligosaccharide components. The action pathways of WPRR in exerting anti-thrombotic efficacy have increased. The polysaccharide components in WPRR might possess considerable potential for being developed into natural anti-thrombotic drugs.

1. Introduction

Thrombotic diseases are serious circulatory system disorders caused by the deposition of abnormal substances in the inner walls of blood vessels, resulting in vessel narrowing or even occlusion[1]. The pathogenesis of thrombosis involves genetic and environmental factors, and the cardiovascular diseases (CVD) caused by it have become one of the main causes of death in humans, with an increasing incidence rate[2-3]. In China, when it comes to the proportion of deaths from diseases among urban and rural residents, CVD still ranks first. In 2020, CVD accounted for 48.00% and 45.86% of the causes of death among rural and urban residents, respectively. Every five deaths is caused by CVD. It is estimated that there are currently around 330 million CVD patients in China[4]. Interventional procedures, such as angioplasty, are not suitable for all types of vascular diseases because they require specialized skills and equipment, and are expensive[5]. At present, several mainstream antithrombotic drugs in the market all have their own shortcomings, such as the limitation of administration methods, side effects like hemolysis and gastrointestinal reactions. Thus, the discovery and development of antithrombotic drugs is an important research field.

In recent years, an increasing number of studies have confirmed that traditional Chinese medicine (TCM) has advantages such as fewer or no side effects, low production costs, and easy access, which are effective in treating thrombosis[6-7]. In TCM, the formation

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of blood clots is believed to be related to the patient's poor blood circulation and blood stasis. Therefore, in clinical practice, TCM often uses blood-activating and blood-dispersing herbs to treat blood clot-related diseases. Representative drugs include *Salviae Miltiorrhizae Radix Et Rhizoma*, *Notoginseng Radix Et Rhizoma*, *Chuanxiong Rhizoma*, and *Paeoniae Radix Rubra* (PRR)[8-9]. These drugs not only inhibit platelet activation and inflammatory responses, but also improve the fibrinolytic system, coagulation system, etc. They exert anti-thrombotic effects through multiple pathways. PRR, a member of the Ranunculaceae family, is the dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch. Its taste is bitter and slightly cold, and it belongs to the liver meridian. It has the functions of clearing heat and cooling blood, dispersing stasis and relieving pain, and is a commonly used TCM in clinical practice[10]. TCM requires that herbs be processed (Paozhi) before they can be used in treatment, which is one of the unique features of TCM[11]. Rice wine (Huangjiu) has a sweet and spicy taste, and it is very warm in nature. Its fragrant aroma can promote and disperse, enhance the efficacy of medicines, and has the functions of promoting blood circulation, relieving wind and cold, and removing bad odors and fishy smells[12]. Wine has a sweet, spicy, and very hot nature, and it can both ascend and disperse. Therefore, TCM often uses wine to process medicinal herbs to enhance the efficacy of the medicine and promote blood circulation to remove meridian obstruction. It is widely accepted that the blood-activating and stasis-dispersing effects of PRR are significantly enhanced following stir-frying with rice wine[13]. However, there have been no systematic research reports on the anti-thrombotic substance basis and mechanism of PRR before and after stir-frying with rice wine. Therefore, it is necessary to clarify the changes in the anti-thrombotic substance basis of the raw PRR and wine-stir-fried PRR (WPRR), so as to provide scientific basis for fully developing and utilizing the pharmaceutical resources of PRR, expanding the clinical uses of drugs, and guiding clinical symptomatic treatment.

Professor Kang, based on his many years of experience in TCM research, has made a profound and innovative summary of the theory of drug properties, and has proposed for the first time a research method for dissecting and combining drug flavors[14]. The taste and properties of TCM affect its efficacy, so studying the pharmacological basis and mechanism of action of PRR before and after stir-frying with rice wine as an anti-thrombotic agent can also be elucidated using component fractionation methods. The systematic features of metabolomics are consistent with the holistic theory of TCM, and have become a powerful technical tool for exploring the mechanisms of action of Chinese herbal medicine[15]. A comprehensive strategy that combines various methods, including chemical analysis, pharmacodynamics, and metabolomics, has a certain persuasive power. Therefore, in this study, we used a comprehensive approach of fractionation of TCM components, pharmacological analysis, and non-targeted metabolomics to elucidate the pharmacological basis and mechanism of PRR's anti-thrombotic effects before and after stir-frying with rice wine. The experimental flowchart is shown in Fig. 1.

2. Material and methods

2.1 Reagents and materials

Methanol (chromatography grade) and acetonitrile (chromatography grade) were purchased from Thermo Fisher Scientific in the United States; formic acid (chromatography grade) was produced by Dikma in the United States. Distilled water was purchased from the A.S. Watson Company. PRR is produced in Yichun City (Heilongjiang, China). The rice wine was purchased from Qimo Rice wine Factory Co., Ltd. The ELISA kits for tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PA1-1), fibronectin (FN), von Wilebrand factor (vWF), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), thromboxane B2 (TXB2), platelet factor 4 (PF4), β-thrombodlobulin (β-TG) rats were purchased from Beijing Boao Tuoda Technology Co., Ltd. (Beijing, China).

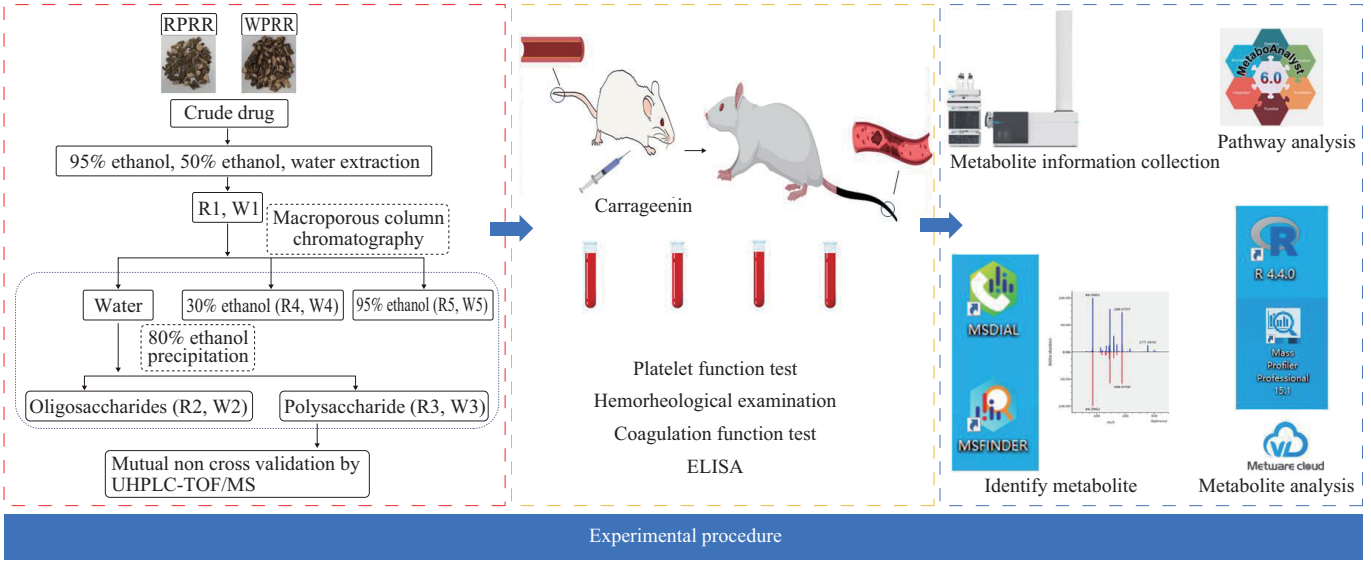

Fig. 1 A comprehensive approach is proposed to discover the pharmacological substance basis and mechanism of action for PRR and WPRR in anti-thrombotic effects.

2.2 Preparation of PRR, WPRR, and their subdivided fractions extracts

WPRR was processed according to the method specified in the 2020 edition of the Chinese Pharmacopoeia. Using a continuous heat reflux extraction method, PRR and WPRR were extracted successively with 95% ethanol water, 50% ethanol water, and pure water, each for 2 h, a total of 3 times. The filtrate was combined, and the solvent was evaporated under reduced pressure to obtain the full-component extracts of PRR and WPRR (R1 and W1, respectively). The separation was performed on D101 macroporous adsorption resin column using column chromatography. The eluents used were water, 30% ethanol water (R4 and W4, respectively), and 95% ethanol water (R5 and W5, respectively), in that order. The process flow is shown in Fig. 1. The aqueous elution was reduced in pressure and concentrated. Then 95% ethanol water was added and stirred continuously. The final ethanol concentration was above 80%. The mixture was left at room temperature for 24 h, and the precipitate and supernatant were collected. Obtain solutions of polysaccharide components (R2 and W2, respectively) and oligosaccharide (R3 and W3, respectively) fractions.

2.3 Study of the non-overlapping chemical compositions of the fractions

Take each component solution, dilute it, and filter it through a 0.22 μm microporous filter. The filtrate is placed in a sample bottle for analysis. Ultra HPLC coupled with quadrupole time-of-flight mass spectrometer (UHPLC-QTOF-MS/MS) was applied to detect compound information from these fractions. According to the information obtained from the detection, the information of each fractions is verified without crossing. The UHPLC instrument used is the Agilent 1290, and the QTOF mass spectrometer used is the Agilent 6546 (Agilent Technologies, Inc., United States). The chromatographic column of ChromCore™ AQ C_{18} (2.1 mm × 100 mm, 1.8 μm, Suzhou, China) performed chromatographic separation. The mobile phase was 0.1% formic acid-water (Phase A) and acetonitrile (Phase B). Besides, the column temperature was set as 35°C. The washing process is set as follows: 0–4 min, 0% B; 4–6 min, 0–8% B; 6–15 min, 8%–25% B; 15–20 min, 25%–35% B; 20–30 min, 35%–45% B; 30–35 min, 45%–60% B; 35–37 min, 60%–75% B; 37–43 min, 75%–100% B; 43–45 min, 100% B. The flow rate is set to 0.3 mL/min, and the injection volume is set to 2 μL.

Samples were analyzed using an Agilent Jet Stream source in both positive and negative ionization modes. The parameter settings were as follows: Gas Temp is set to 320 °C, Dying Gas is set to 8 L/min, Nebuizer is set to 35 psi, Sheath Gas Temp is set to 350 °C, Sheath Gas Flow is set to 11 L/min. VCap is set to 3 500 V and the Fragmentor is set to 175 V. The collected information relies on the auto MS/MS scanning mode for research. The full scan range of *m/z* was set between 100 and 1 200 Da.

2.4 Animal experiment

Sprague Dawley male rats were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China). During the experiment, all rats were allowed to eat and drink freely and were housed in a sterile, professional animal laboratory with a temperature maintained at room temperature, a relative humidity controlled between 55% and 65%, a 12-h light/dark cycle, good ventilation, and bedding changed every 3 days. The experimental process strictly adheres to the ethical guidelines for laboratory animals. The animal study was approved by Heilongjiang University of Chinese Medicine. The study was conducted inaccordance with the local legislation and institutional requirements. After 7 days of adaptive feeding, the rats were randomly divided into 13 groups of 20 each, named Control (Con), Thrombus (Th), Aspirin, R1 to R5, and W1 to W5. The Con and Th groups received physiological saline *via* gastric administration, while the experimental groups were given corresponding drug solutions in the same manner[16]. A total of 24 h after establishing the model, rat tails were observed and measured for total length and thrombotic black tail length using a caliper. Calculate the relative average tail length (RATL) and the inhibition rate of thrombus relative length (IR) of the rat tail[17]. Blood was collected from the abdominal aorta using heparin, EDTA, and sodium heparin tubes for platelet, hemorheology, and coagulation tests. Blood was collected from the abdominal aorta using non-anticoagulant tubes, and serum was obtained by centrifuging at 3 000 r/min for 10 min at 4 °C. The serum was then divided and stored at –80 °C for ELISA and untargeted metabolomics experiments. In the formula (1), L_1 represents the length of the black tail of the rat (in mm), and L_2 represents the total length of the rat's tail (in mm). In the formula (2), A_1 represents the average RATL value in the treatment group, and A_2 represents the average RATL value in the Th group.

$$\text{RATL}\ (\%) = \frac{L_1}{L_2} \times 100 \tag{1}$$

$$\text{IR}\ (\%) = (1 - \frac{A_1}{A_2}) \times 100 \tag{2}$$

2.5 Blood test

Use EDTA-anticoagulated blood to determine key platelet parameters, including platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV), platelet crit (PCT), and platelet-large cell ratio (PLCR) with an automated blood cell analyzer. Use blood rheometer to measure whole blood viscosity (WBV), plasma specific viscosity (PSV), hematocrit (HCT), erythrocyte sedimentation rate (ESR), and other related indicators. Coagulation function indicators activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (FIB), and thrombin time (TT) are measured with a coagulation analyzer. The levels of t-PA, PAI-1, FN, vWF, cAMP, cGMP, 6-keto-$PGF_{1\alpha}$, TXB2, PF4, and β-TG were determined by ELISA kits in each group.

2.6 Metabolomics analysis

2.6.1 Sample preparation

Before analysis, samples were thawed in an ice bath and mixed with 4 times the volume of cold methanol. After vortexing for 5 min, the supernatant was obtained by centrifugation at 12 000 × *g* for 10 min. A 2 μL aliquot of serum supernatant was injected into the UHPLC-QTOF-MS/MS system. Inject QC samples after every 10 samples. QC samples were injected every ten samples, with each contributing 2 μL to form a combined QC sample. A total of 2 μL from each sample is combined to create a QC sample.

2.6.2 UHPLC- QTOF-MS/MS conditions of serum samples

The machine, column, separation temperature, mobile phase, and the above should all be consistent. The mobile phase flow rate is 0.3 mL/min, with an elution gradient of 0–10 min at 10%–80% B and 10–20 min at 80%–100% B. They are sequentially analyzed in MS, with the first level scan having a mass-to-charge ratio (*m/z*) of 100–1 200 Da and the second level scan ranging from 50–1 200 Da. The parameter settings were as follows: gas temp is set to 250 ℃, dying gas is set to 8 L/min, nebuizer is set to 35 psi, sheath gas temp is set to 350 ℃, sheath gas flow is set to 11 L/min, and VCap is set to 3 500 V, fragmentor is 120 V. The information was collected using the auto MS/MS scan mode, with collision energy set to 30 and 50 eV.

2.6.3 Data processing

Origin is used to process and draw the mass spectrum, and SIMCA is used to non-cross-verify the mass spectrum data. Use GraphPad Prism for pharmacological data analysis and MassHunter software for mass spectrometry data processing. Combine MS-DAIL and MS-FINDER to align peaks, remove background noise, normalize, and calibrate raw data. The filtered data was then imported into Mass Profiler Professional and Metware Cloud for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Visualize metabolite pathways using MetaboAnalyst 6.0. The images were created using MedPeer.

3. Results

3.1 Results of component fractionation studies

Extract 2 kg of PRR to obtain R1 fraction of 595.20 g. Extract 1.94 kg of WPRR (equivalent to 2 kg of PRR) to obtain W1 fraction of 476.00 g. Fig. 1 illustrates the fractionation process applied to R1 and W1 for component separation, with quality and yield details in Table 1. Polysaccharide and oligosaccharide fractions showed significant changes in outpouring rate before and after stir-frying with rice wine. The components were measured by UHPLC- QTOF-MS/MS, with the PRR and chromatograms of each component shown in Figs. 2A and B. Figs. 2C and D display the chromatograms of WPRR and its fractions. Data from each group was imported into SIMCA 14.0 for non-cross-validation, with results shown in Figs. 2E-G. Fig. 2E indicates good separation between R1 and W1, with no cross-contamination. The mass spectra peaks of polysaccharides and oligosaccharides are similar, so the samples are close in the PCA graph. Other fractions were effectively separated without cross-contamination, demonstrating the feasibility of the separation process and good results for each fraction.

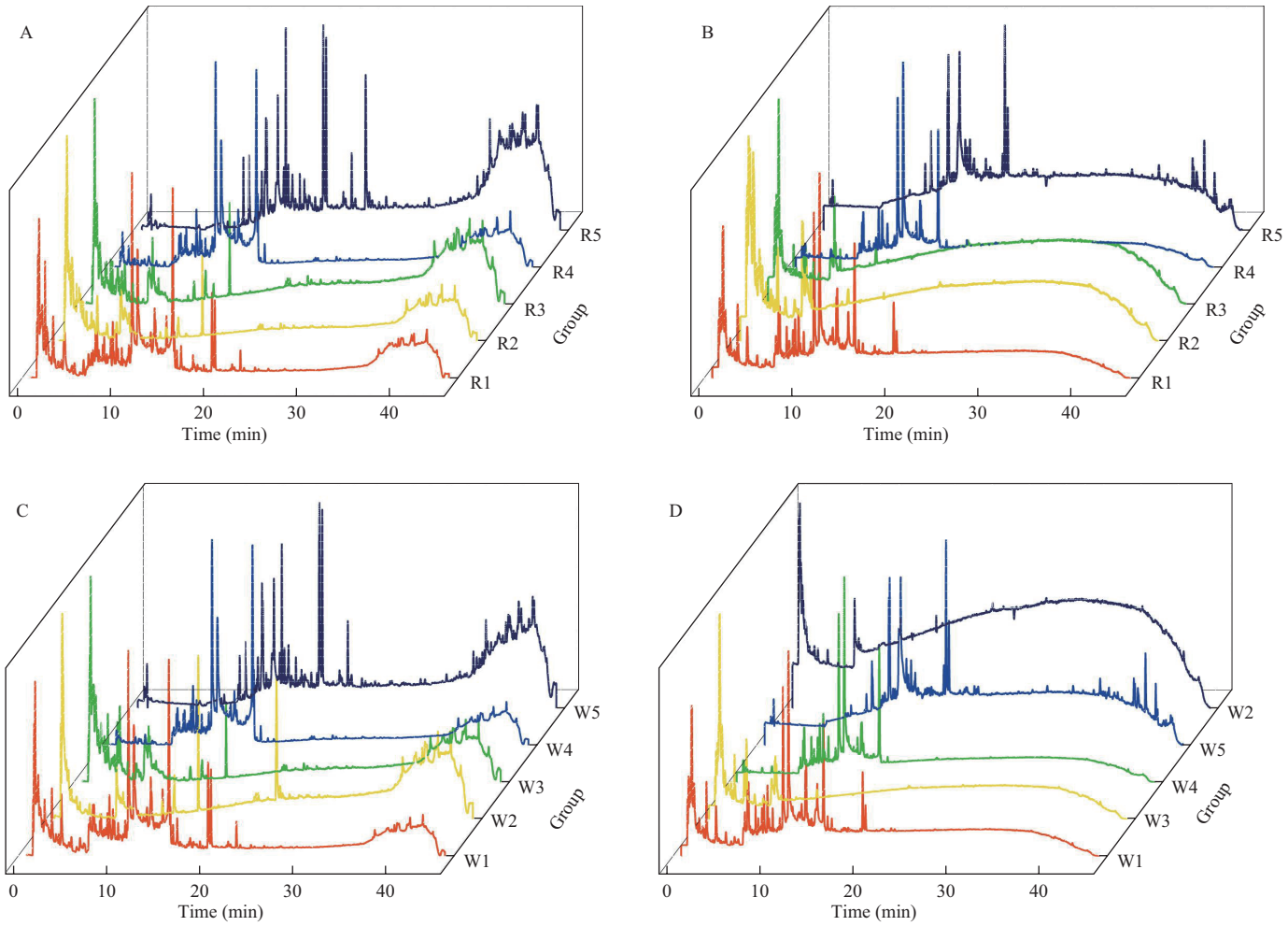

Fig. 2 Results of fractions separation for PRR and WPRR. (A-D) Fraction separation results of PRR and WPRR in both positive and negative ion modes. A and C are positive ion modes. B and D are negative ion modes. (E) PCA results for R1 and W1, (F and G) PCA results for R2-R5 and W2-W5, respectively.

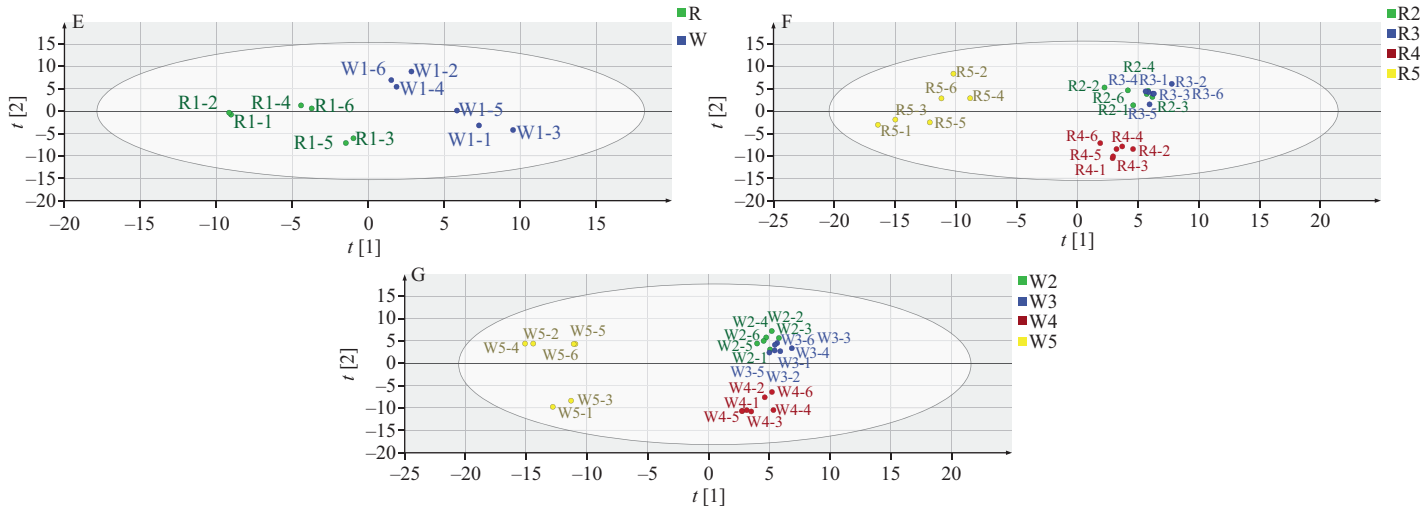

Fig. 2 (*Continued*)

Table 1
The mass and yield of each fractions.

Fraction	Quality (g)	Extraction amount (%)
R1	595.20	29.76
W1	476.00	23.80
R2	262.40	13.12
W2	128.00	6.40
R3	72.00	3.60
W3	177.40	8.87
R4	97.40	4.87
W4	96.00	8.87
R5	38.60	1.93
W5	32.60	1.63

3.2 Pharmacodynamic evaluation

3.2.1 Impact of individual components on black tail suppression in rats

Crageenin injection induces blood clots in rat tails. A total of 2 h after the last dose, it was injected into the lower abdomen to create the model. All rat groups except the Con group exhibited varying degrees of dark red thrombi in their tails, as shown in Fig. 3A. RATL and IR are displayed in Figs. 3B and C. In the Th group, the black tail rate was 100%, and thrombosis accounted for about 70% of total tail length, showing a significant difference compared to the Con group ($P < 0.01$), indicating successful model establishment. Compared to group Th, groups R2 and R3 showed no significant improvement in caudal thrombi length and RATL, indicating a lack of anti-thrombotic

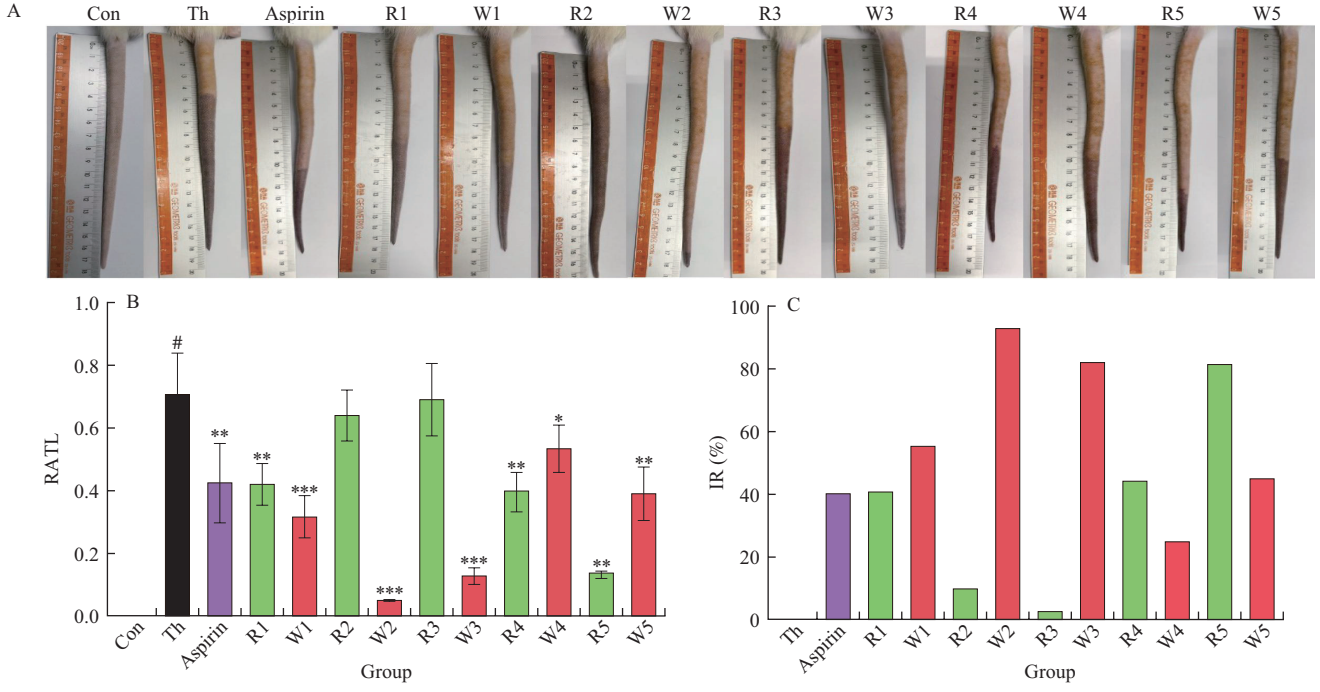

Fig. 3 Results of carrageenin-induced thrombosis in rats by tail length. (A) Length of thrombus tails in each rat group, (B) RATL results, and (C) IR results.

ability in these fractions. The lengths of thrombi in the remaining components and RATL were significantly reduced ($P < 0.05$). R1 has a blood clot prevention ability that is basically on par with Aspirin, and W1 has a stronger anti-thrombotic effect than R1 and Aspirin, indicating that PRR stir-frying with rice wine enhances its anti-thrombotic ability. Compared to R2, R3, W2, and W3, the anti-thrombotic ability is significantly stronger than that of the positive drug Aspirin. W2 had an IR of 93.06%, while R4 and R5 exhibited a stronger anti-thrombotic effect than W4 and W5.

3.2.2 Impact of fractions on coagulation and fibrinolysis in thrombosis rats

The results of coagulation and fibrinolysis function tests for each group in the experiment are shown in Fig. 4. APTT, PT and TT are common coagulation indicators. In the common coagulation pathway, the generated thrombin converts FIB into fibrinogen degradation products (FDP), which can be reflected by TT[18-20]. The APTT, PT, and TT values in Th group were significantly different from those of the Con group ($P < 0.05$), thereby confirming the successful establishment of the thrombosis model. In comparison to Th group, both Aspirin and R4 significantly prolonged APTT, PT, and TT, suggesting that they exert anticoagulant effects by inhibiting both endogenous and exogenous pathways. W4 extends PT and TT, showing that it achieves its anti-coagulant effect by inhibiting the exogenous pathway. Compared to Th group, R1 prolonged PT, while W1 prolonged APTT. R2 and R3 not only had no anticoagulant effects but also shortened APTT and TT. The fractions W2 and W3 that had the side effect of stir-frying with rice wine disappeared. R5 and W5 both extend PT and TT. Since the FIB degradation product FDP prolongs TT, some use TT as a screening test for the fibrinolytic system.

FIB is a glycoprotein (α2β2γ2) synthesized and secreted by liver cells, which is an important protein in the coagulation and hemostasis process[21]. Elevated FIB levels are a key risk factor for thrombotic diseases and serve as a disease state marker in clinical practice[22]. Fig. 4D shows that the FIB value in the Th group is significantly higher than in the Con group, indicating a marked increase in FIB content during thrombus formation after establishing the tail thrombosis model, thus confirming its validity. The remaining treatment groups were able to lower the FIB content in the blood of thrombosis rats, thereby reducing the speed of thrombosis formation. Among them, W2 reduced the FIB content in thrombosis rats to normal. The fibrinolysis system, also known as the fibrinolytic system, refers to the system in which plasminogen is converted into plasmin by plasminogen activator, and then plasmin degrades FIB and other proteins[23]. The fibrinolytic system is similar to the coagulation system, also including the activation of proenzymes, feedback enhancement and inhibition of enzyme activity, and ultimately achieving a balance with inhibitors to reduce fibrinolytic activity, which can lead to the formation of blood clots[24]. Plasminogen is the core component of the fibrinolytic system, and its level directly influences fibrinolytic ability, making it a potential enhancer of fibrinolysis. The balance between coagulation and fibrinolysis is crucial for normal blood circulation and tissue repair. t-PA and PAI-1 are key regulators of this system[24]. The balance between t-PA and PAI-1 is crucial for the stability of the fibrinolytic system in blood plasma. Figs. 4E and F show the blood concentrations of t-PA and PAI-1 in each group. Compared to the Con group, t-PA content in the Th group was significantly lower ($P < 0.05$), while PAI-1 content was significantly higher ($P < 0.05$). After drug administration, t-PA levels increased significantly in all groups except for the polysaccharide group (R2, W2) compared to the Th group ($P < 0.05$), and PAI-1 levels decreased significantly ($P < 0.05$).

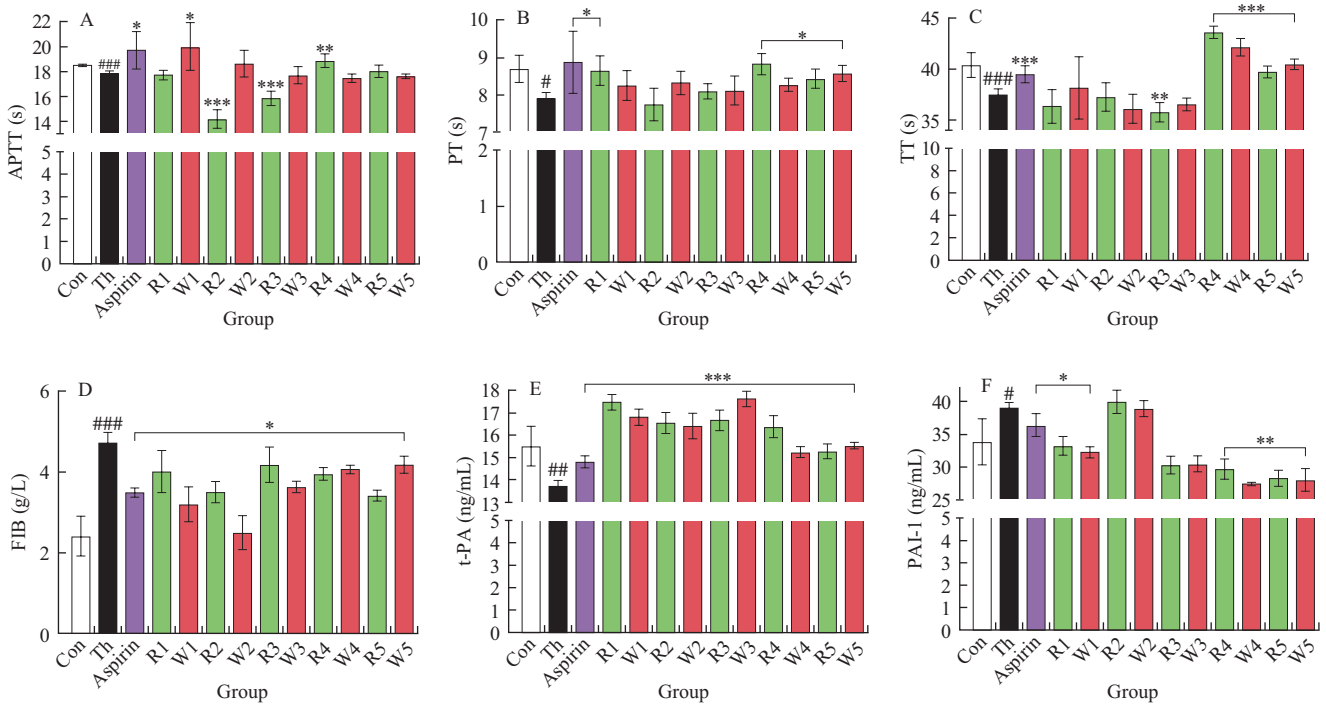

Fig. 4 Coagulation and fibrinolysis results in each group. (A-F) Experimental results for APTT, PT, TT, FIB, t-PA, and PAI-1.

3.2.3 *Impact of individual fractions on platelets and their functions in thrombosis in rats*

Blood platelet parameters related indicators are shown in Figs. 5A-E. Compared to the Con group, PLT and PCT levels were significantly lower in the Th group ($P < 0.05$). After treatment, PLT and PCT levels increased significantly in the W2 and W3 groups ($P < 0.05$), with R3 also regulating PLT levels. In the Th group, PDW, MPV, and PLCR were significantly higher than in the Con group ($P < 0.05$). After treatment, PDW levels in all groups were lower than those in the Th group ($P < 0.05$). Aspirin, R1, R2, W2, R3, R5, W5 showed a decrease in MPV compared to the Th group ($P < 0.05$), while all other drug groups except R4 and W4 could affect PLCR ($P < 0.05$). The influence of the W2 component on all parameters associated with platelet indices is evident.

Blood platelets remain in a resting state during normal circulation but can be activated through membrane signal transduction by regulating proteins and second messengers in specific physiological or pathological conditions. This activation causes stress-induced shape changes, pseudopodia formation, adhesion, aggregation, and release reactions, ultimately contributing to the blood clot along with fibrin formed by thrombin's enzymatic action on FIB[25]. VWF in plasma binds to platelet glycoprotein receptors, forming a complex that promotes platelet adhesion and serves as an early marker of activation[26-27]. FN interacts with integrins to enhance platelet adhesion, resulting in the formation of a platelet thrombus at the site of injury[28]. Figs. 5F and G show the changes in vWF and FN levels in the plasma of each group. In the Th group, vWF and FN levels were significantly higher than in the Con group ($P < 0.01$). After treatment, Aspirin and W2 notably reduced vWF and FN levels in thrombosis rat plasma compared to the Th group ($P < 0.01$), while R3, W3, R4, and R5 not only failed to counteract but also worsened platelet adhesion. Platelet aggregation is the process where platelets stick together *via* adhesion receptors, forming clumps. TXA2 strongly

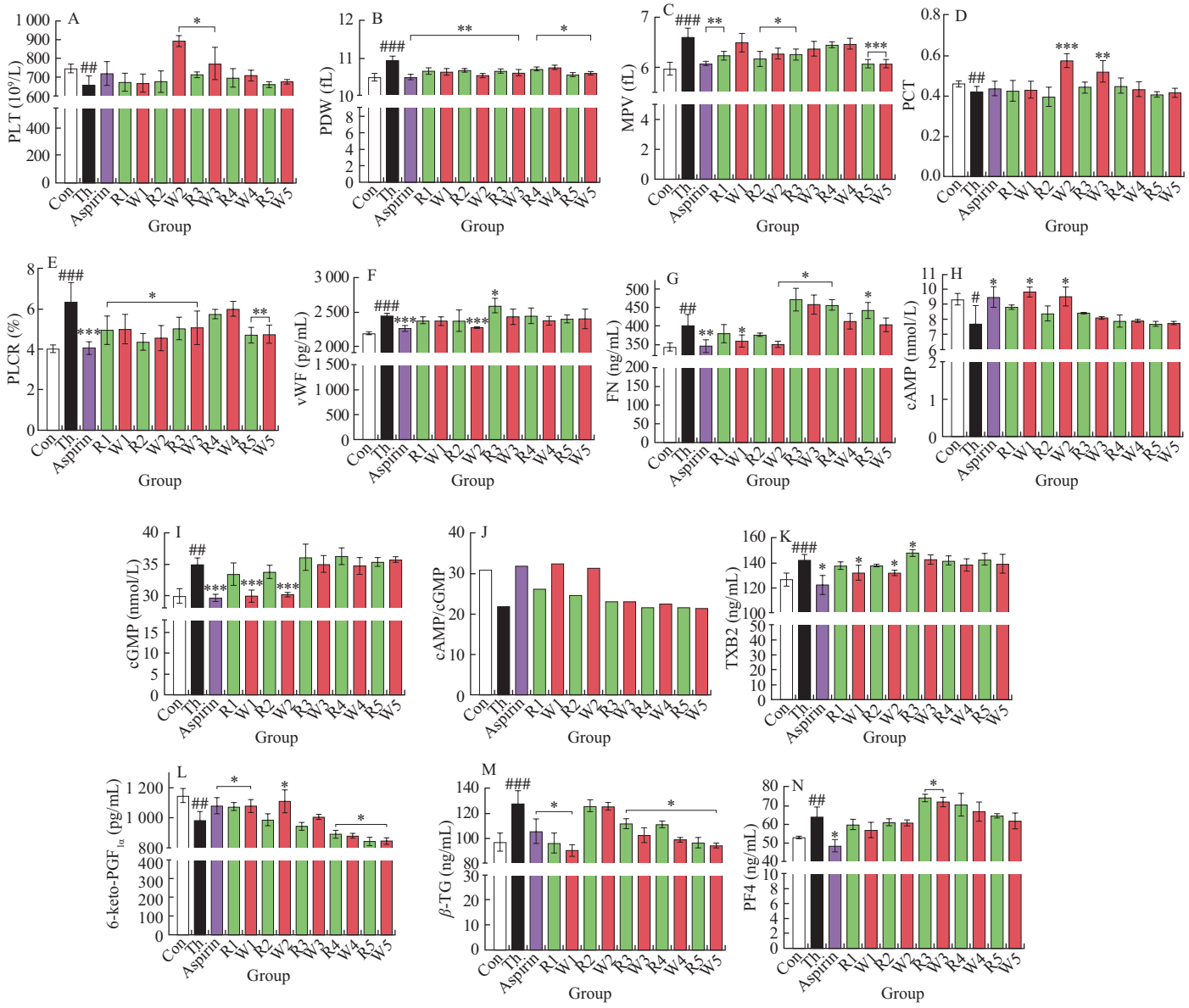

Fig. 5 Effects on platelets and their functions. (A-E) Platelet parameters, (F-G) platelet activation and adhesion results, (H-L) platelet aggregation data, (M-N) platelet release finding.

promotes vascular contraction and platelet aggregation, while PGI2, secreted by endothelial cells, inhibits platelet aggregation and dilates blood vessels[29]. CAMP also inhibits platelet aggregation, whereas cGMP has the opposite effect[23]. Because TXA2 and PGI2 spontaneously convert into more stable products TXB2 and 6-keto-$PGF_{1\alpha}$, the levels of TXB2, 6-keto-$PGF_{1\alpha}$, cAMP, and cGMP can be used to reflect the platelet aggregation in the body[17]. Figs. 5H-L present the results of platelet aggregation indicators. In the Th group, compared to the Con group, TXB2 and cGMP levels significantly increased ($P < 0.05$), while 6-keto-$PGF_{1\alpha}$ and cAMP levels significantly decreased ($P < 0.01$), indicating enhanced platelet aggregation in blood clot rats. Compared with Th group, the cAMP content in the blood clot rats after administration of Aspirin, W1, and W2 was significantly increased ($P < 0.05$), while the cGMP content was significantly decreased ($P < 0.05$), resulting in a marked increase in the cAMP/cGMP ratio, which was close to that of normal rats. Aspirin, W1, and W2 can also significantly reduce the content of TXB2 in thrombosis rats and increase the content of 6-keto-$PGF_{1\alpha}$ ($P < 0.05$). R1 can increase the content of 6-keto-$PGF_{1\alpha}$, but it cannot reduce the content of TXB2 in rats. The R4, W4, R5, and W5 components inhibited the feedback of 6-keto-$PGF_{1\alpha}$, potentially worsening platelet aggregation. After platelet activation, an important process is the contraction of the cytoskeleton and the release of granule contents. PF4 prompts platelets to release cytokines and growth factors with a pro-thrombotic effect[30]. β-TG is a specific protein produced and released by platelet alpha granules, promoting platelet aggregation and thrombosis[26]. Figs. 5M and N show the changes in β-TG and PF4 levels in the plasma of each group. In the Th group, β-TG and PF4 levels were significantly higher than in the Con group ($P < 0.05$). Aspirin treatment notably reduced the release of both factors. R1, W1, R4, W4, R5, and W5 decreased β-TG levels ($P < 0.05$) but had minimal impact on PF4. R3 and W3 decreased β-TG levels while increasing PF4 levels.

In summary, the polysaccharide component W2 mainly exerts an anti-thrombotic effect by affecting platelet adhesion and aggregation, while the small molecule components (R4, W4, R5, W5) have minimal impact on platelets in thrombotic rats and may even cause side effects.

3.2.4 *Impact of fractions on hemorheology in thrombosis rats*

Blood rheology testing predominantly assesses alterations in the fluidity, stasis, and viscosity of blood resulting from variations in its constituents[31-32]. Fig. 6 shows the effects of the components on blood rheology in thrombosis rats. In comparison to the Con group, the Th group exhibited a significant increase in WBV, PSV, HCT, erythrocyte sedimentation rate equation K value (ESRK), red blood cell aggregation index (AI), whole blood low shear rate viscosity (WBLSR), whole blood medium shear rate viscosity (WBMSR), whole blood high shear rate viscosity (WBHSR), and Kason viscosity (KS) with statistical significance noted at $P < 0.05$. This suggests that the Th group rats have poor blood flow and thick, sticky blood. After administration, Aspirin, R1, and W1 were able to significantly

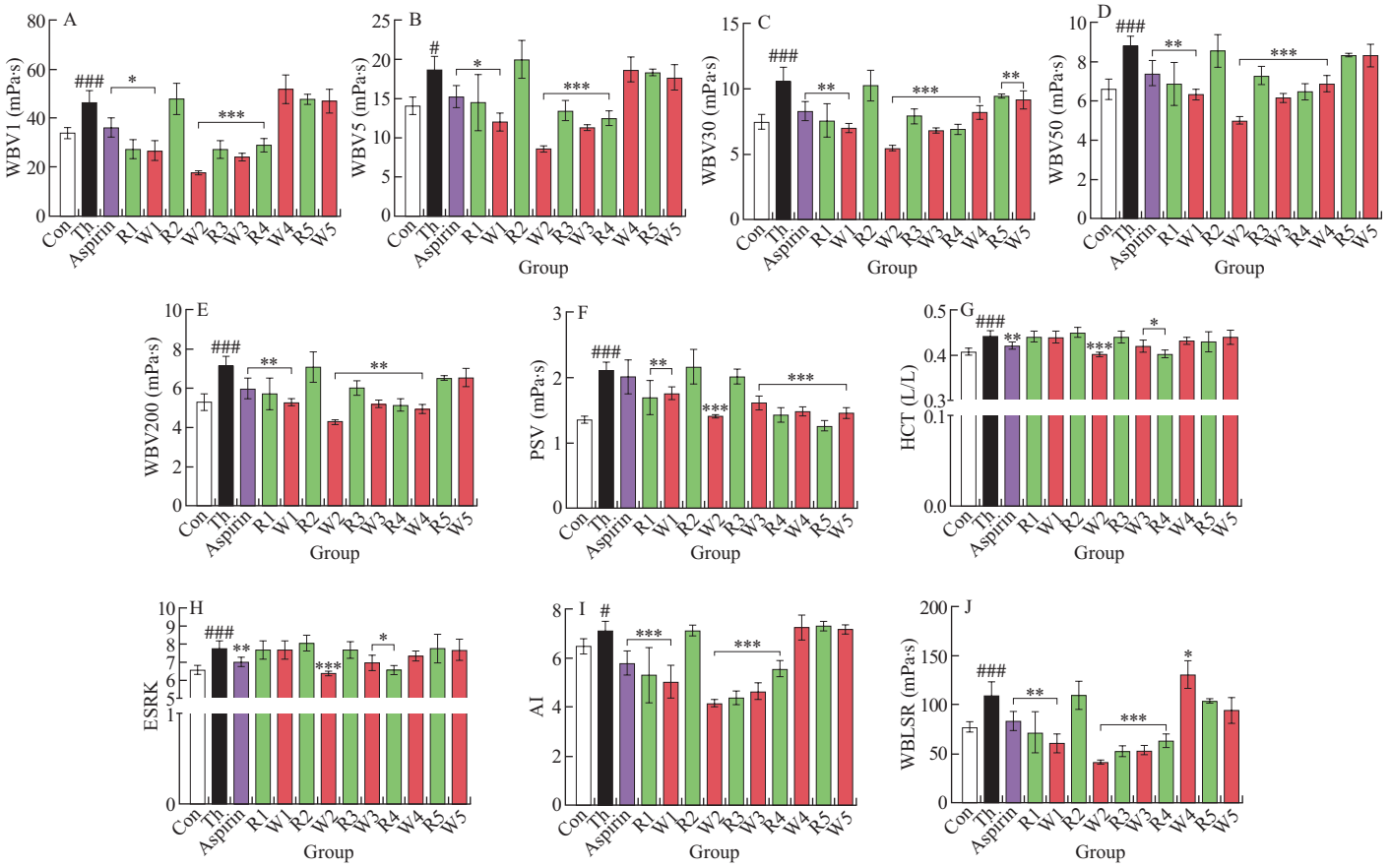

Fig. 6 Effects of thrombosis on the hemorheology of rat blood. (A-M) Tests for WBV1, WBV5, WBV30, WBV50, WBV200, PSV, HCT, ESRK, AI, WBLSR, WBMSR, WBHSR, and KS.

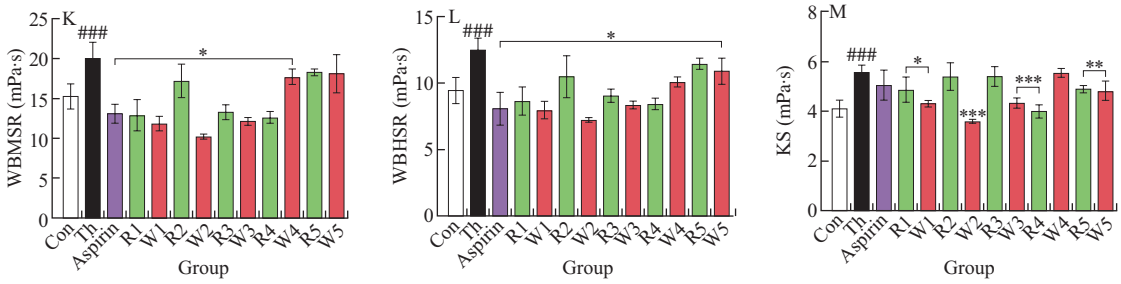

Fig 6 (*Continued*)

reduce WBV, WBLSR, WBMSR, and WBHSR compared to the Th group ($P < 0.05$). R1 and W1 can lower PSV and KS, with W1 having a stronger effect than R1. The W2 group is significantly influenced by all hemorheological tests compared to the Th group, while R2 only affects WBMSR and WBHSR ($P < 0.05$). The hemorheological performance of the W2 group differs significantly from that of R2. R3 and W3 both reduce blood viscosity and enhance its rheological state, but W3 also significantly lowers PSV, KS, and affects the HCT and ESRK of red blood cells. W3 has a stronger anti-thrombosis activity than R3. Additionally, R4 is significantly more effective than W4 in improving blood rheology. R5, W5 compared to the Th group, significantly reduced WBV30, PSV, WBHSR, and KS. The erythrocyte electrophoresis index, Erythrocyte flow coefficient TK value, Erythrocyet index of rigidity, erythrocyte sedimentation rate, these indicators were not significantly different between Th group and Con group.

3.2.5 Chemometrics analysis

The blood system is a complex cascading network, making it challenging for drugs to target only one pathway. However, from a structure-activity perspective, different structural compounds should primarily affect distinct anti-thrombotic pathways. Chemometrics can assist in analyzing the correlation between fractions and their effects.

The coagulation and fibrinolysis data from each rat group were analyzed using SIMCA software for PCA. With 2 principal components extracted, $R^2_X1 = 0.387$, $R^2_X2 = 0.242$, and their cumulative contribution rate reached 60%, confirming the reliability of the results. Fig. 7A shows that the Th group is on the left side of the *Y* axis, while the Con group is on the right, indicating a significant difference between them. R2 and R3's position to the left of Th suggests they may have side effects on coagulation and fibrinolysis function. Conversely, W1, R4, W4, R5, and W5 are positioned on the right side of the *X* axis, highlighting their important role in restoring coagulation and fibrinolysis function. From the perspective of TCM, small molecule components R4, W4, R5, and W5 primarily exert an anti-thrombotic effect by influencing coagulation and fibrinolysis systems.

The data concerning platelets and their associated functions across the various groups of rats were imported into SIMCA software for principal component analysis (PCA). Upon extraction of 2 principal components, R^2_X1 was found to be 0.549, R^2_X2 was 0.175, and the cumulative contribution rate of these two components reached 60%, thereby indicating that the results are reliable. The results presented in Fig. 7B demonstrate that the Th group is positioned on the left side of the *Y* axis, whereas the Con group is situated on the right side, indicating a significant disparity between these 2 groups. R3 and R4 are nearly superimposed upon Th, suggesting that they exert minimal influence on platelet recovery and their associated functions. Conversely, R1, W1, R2, and W2 are located on the right side of the *X* axis, signifying that these 4 components play a crucial role in enhancing platelet recovery and functionality. From the standpoint of analyzing TCM, it is evident that R2 and W2, which are polysaccharide constituents, primarily exert an anti-thrombotic effect by modulating platelet behavior, including their adhesion and aggregation.

The blood rheology-related data from each group of rats were imported into SIMCA software for PCA. Upon extraction of 2 principal components, R^2_X1 was found to be 0.737 and R^2_X2 was 0.154, resulting in a cumulative contribution rate of 60% for these components, thereby confirming the reliability of the findings. Fig. 7C shows that the Th group is on the right side of the *Y* axis, while the Con group is on the left, indicating a significant difference between them. R2, R5, and W5 are positioned on the right side of the *X* axis, suggesting they have a weak influence on blood viscosity and red blood cell aggregation in the thrombosis rat model. In contrast, W1, W3, R1, R3, and R4 are found on the left with the Con group, indicating their potential to improve hemorheological ability; among these components, W1, W2, W3, and R4 exhibit stronger effects on blood viscosity. From the perspective of fractionating TCM, small molecule and oligosaccharide components in PRR can improve blood rheology, while polysaccharides and oligosaccharides in WPRR also enhance it.

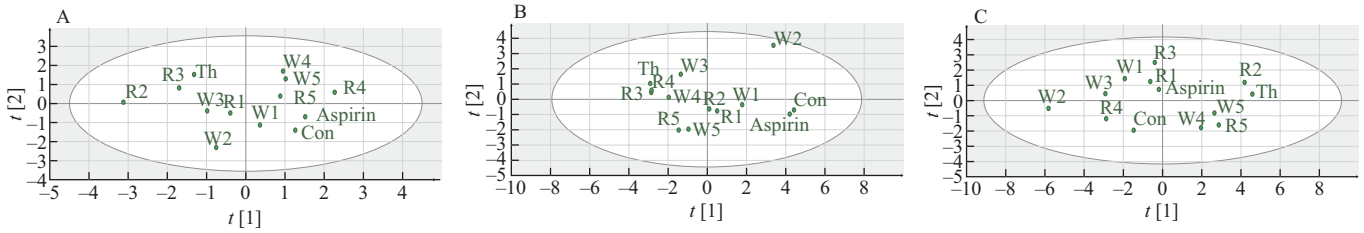

Fig. 7 Results of chemometrics analysis. (A) PCA results of coagulation and fibrinolysis, (B) PCA results of platelets and their functions, and (C) PCA results of blood rheology in each group of thrombosis rats.

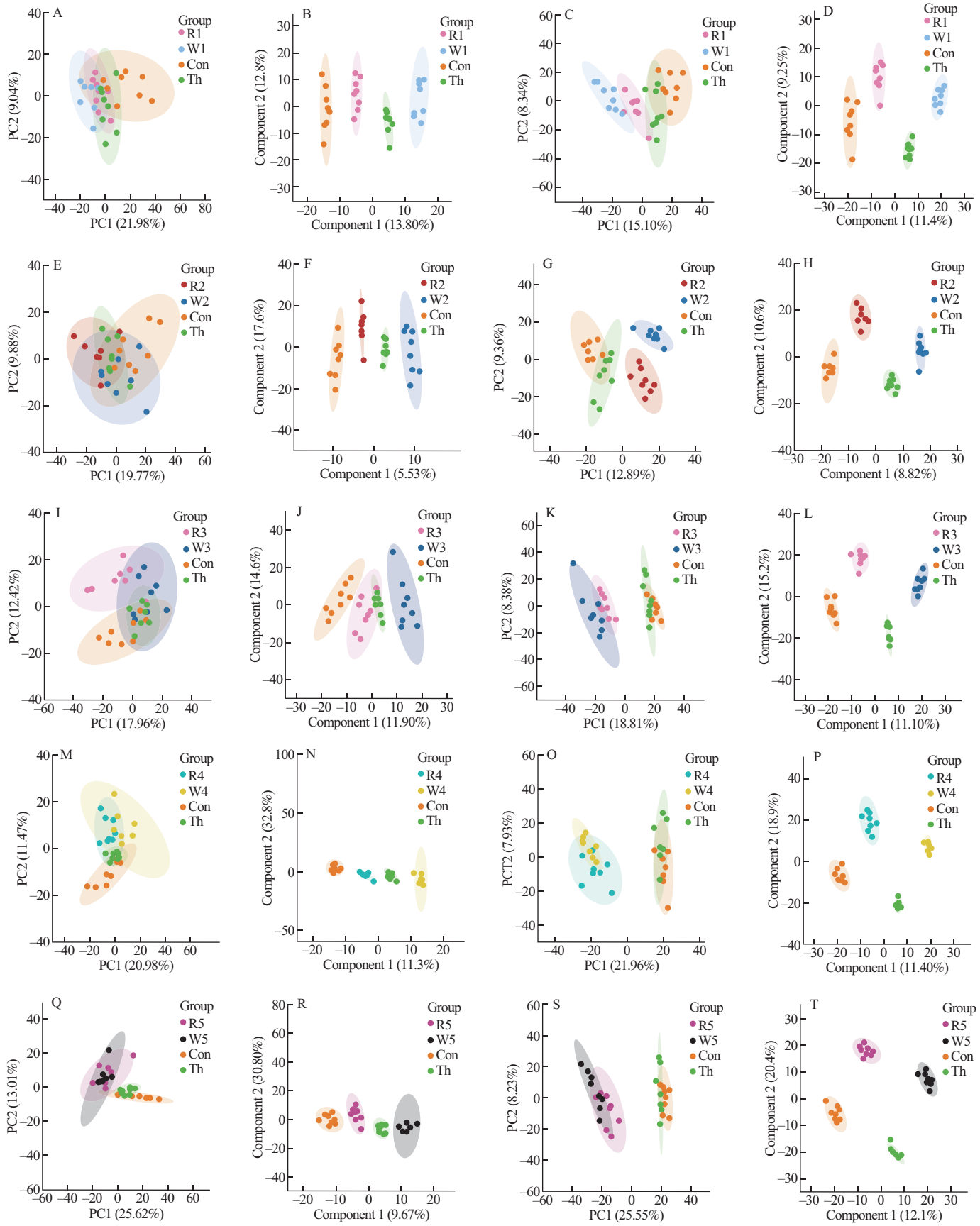

Fig. 8 Effects of individual components on the metabolic profile of thrombosis rat model result 1. Multivariate statistical analysis results for (A-D) Con, Th, R1, and W1; (E-H) Con, Th, R2, and W2; (I-L) Con, Th, R3, and W3; (M-P) Con, Th, R4, and W4; and (Q-T) Con, Th, R5, and W5. A, B, E, F, I, J, M, N, Q, R are results from negative ion mode analysis; C, D, G, H, K, L, O, P, S, T are from positive ion mode analysis.

The results indicate that disassembling TCM led to some improvement in hemorheology for R2 and R3 in the PRR, but their negative effects on the coagulation system resulted in an overall lack of anti-thrombotic efficacy. R4 and R5 primarily prevent blood clots by influencing the coagulation system, fibrinolysis system, and blood viscosity. In WPRR, W2 enhanced platelet adhesion and aggregation while reducing blood viscosity in thrombosis rats, demonstrating strong anti-thrombosis effects. W3 also exhibited an anti-thrombosis effect by improving the hemorheological function of these rats. W4 and W5 have weaker anti-thrombotic abilities than R4 and R5, but their modes of action are identical. The biochemical indicator results align with the macroscopic findings of RATL in rats, suggesting that the fraction separation process is effective. The pharmacodynamic material basis for anti-thrombosis altered following PRR stir-frying with rice wine; specifically, the raw PRR's anti-thrombotic properties are primarily attributed to small molecular substances, whereas WPRR's anti-thrombotic effects are predominantly associated with polysaccharides, oligosaccharides, and other relatively high molecular weight compounds.

3.3 Metabolomics results

Multivariate statistical analysis methods are commonly used in metabolomics, including PCA and OPLS-DA[33]. This study used PCA and OPLS-DA to analyze the differences in serum metabolic profiles of thrombosis rats treated with various components. The score plots indicate sample differences based on their relative positions. Fig. 8 shows that PCA and OPLS-DA analyses of serum metabolic profiles in both positive and negative ion modes reveal a clear separation between the different rat groups. This aligns with the anti-thrombotic activity and pathway changes observed in the PRR stir-fried with rice wine in both pre- and post-treatment groups. Fig. 9 shows that the metabolic pathways affected by different fractions of PRR and WPRR have significant differences, and the metabolic profile analysis is consistent with the pharmacological results.

Using variable importance in projection (VIP) values reflects the contribution of each metabolite to inter-group differences in the OPLS-DA score map, and combined with the *P*-value and fold change (FC) values from univariate analysis, further metabolite markers for

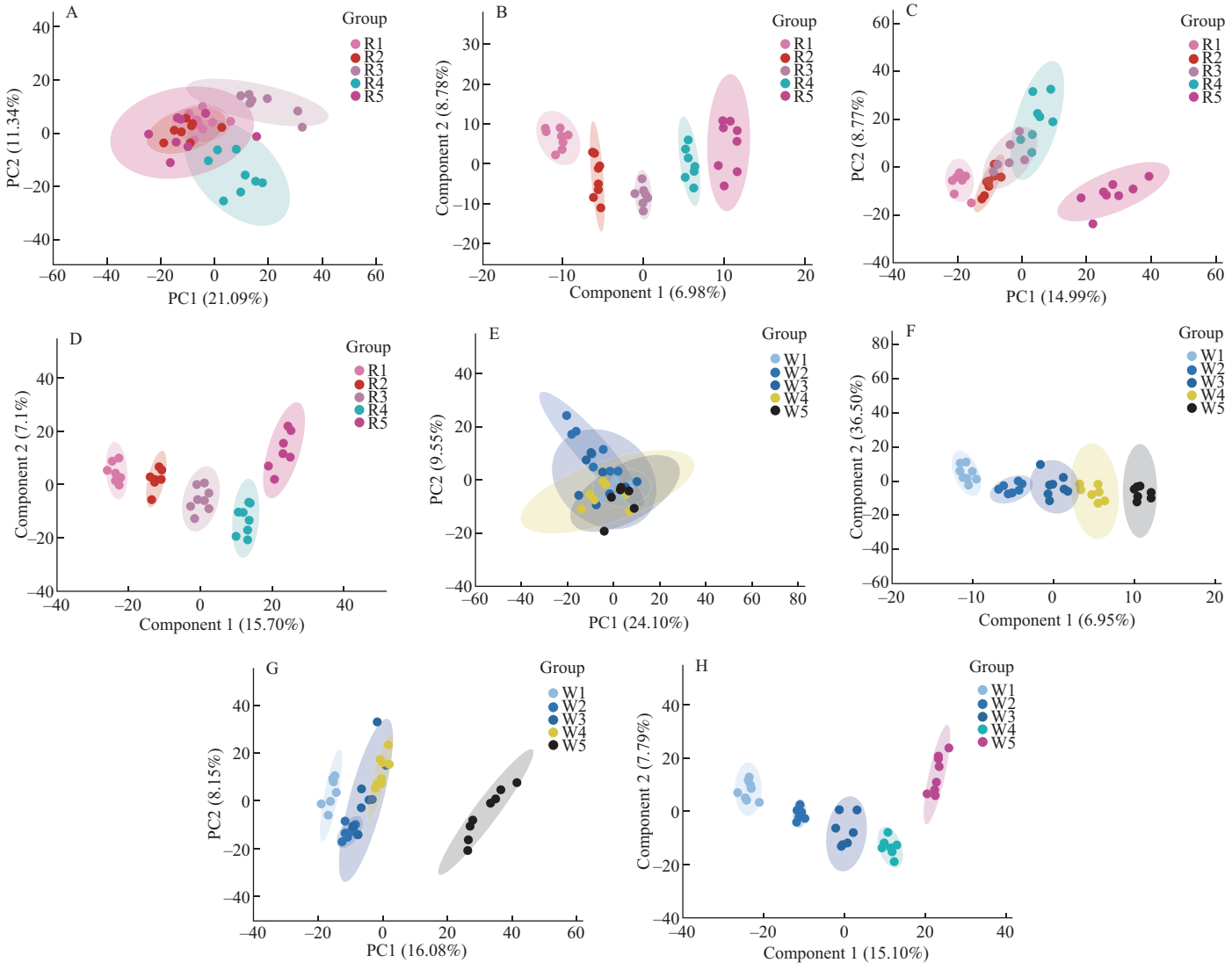

Fig. 9 Effects of individual components on the metabolic profile of thrombosis rat model result 2. Multivariate statistical analysis results for (A-D) R1-R5, and (E-H) W1-W5. A, B, E, and F are the results from negative ion mode analysis, while C, D, G, and H come from positive ion mode analysis.

anti-thrombosis are screened. For metabolites with VIP > 1.0, $P < 0.05$, FC > 2.0 or FC < 0.5, they were selected as candidate metabolic biomarkers. The candidate metabolic biomarkers were identified using precise molecular mass (with an error of 10×10^{-6} or less) and MS/MS mass spectrometry fragmentation data that provided the required structural information. The identified candidate differential metabolites were searched and compared with the HMDB online database to further obtain the endogenous differential metabolites that were significantly affected by intervention, as shown in Tables S1-12. In the serum samples, 6 overlapping differential metabolites were identified in the Th/Con and R1/Th groups, while 19 were found in the Th/Con and W1/Th groups, as shown in Figs. 10A-B. W1 has stronger anti-thrombotic activity than R1, possibly due to its effect on more differential metabolites. In the serum samples, 19 overlapping

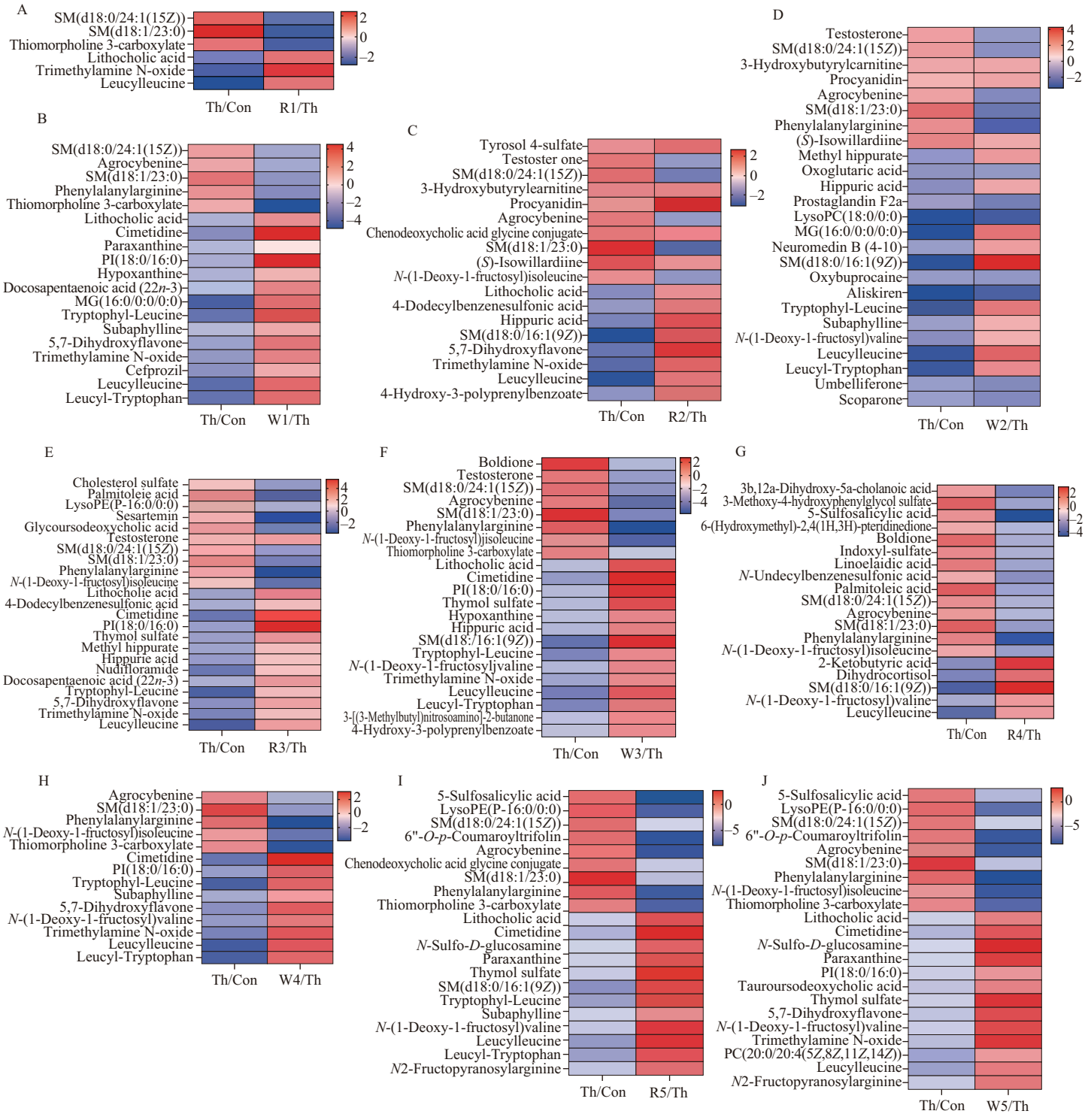

Fig. 10 Differentially identified metabolites in each serum sample. Red indicates an increase, blue indicates a decrease, and the color change indicates the size of the $\log_2$ FC value.

differential metabolites were identified between the Th/Con and R2/Th groups, 25 between Th/Con and W2/Th, 23 between Th/Con and R3/Th, and another 23 between Th/Con and W3/Th, as shown in Figs. 10C-F. Although the number of differentially metabolized compounds in the Th/Con with W3/Th and R3/Th groups is equal, the specific affected compounds differ. In the serum samples, 19 overlapping differential metabolites were identified between the Th/Con and R4/Th groups, 14 between Th/Con and W4/Th, 21 between Th/Con and R5/Th, and 22 between Th/Con and W5/Th. Results are shown in Figs. 10G-J, with specific details listed in Table S12.

Pathway enrichment analysis of differential metabolites in each group was conducted using Metaboanalyst 5.0, HMDB, and KEGG with $P < 0.05$ and Impact > 0. The PRR fractions R2 and R3 showed no significant anti-thrombotic activities, so they were excluded from the analysis. Fig. S1 shows the results of metabolic pathway analysis. Results for R1, R4, and R5 are in Table 2, while results for W1-W5 are in Table 3. PRR, WPRR and their fractions mainly affect arachidonic acid metabolism, sphingolipid metabolism and TCA cycle, arginine biosynthesis, steroid hormone biosynthesis, biosynthesis of unsaturated fatty acids, ether lipid metabolism, linoleic acid metabolism, tryptophan metabolism, caffeine metabolism, primary bile acid biosynthesis and pyrimidine metabolism12 metabolic pathways. The detailed pathway effects are shown in Figs. S2-34. Upon reviewing the relevant literature on these metabolic pathways, steroid hormone biosynthesis, linoleic acid metabolism, tryptophan metabolism, and caffeine metabolism are not significantly associated with CVD. R5 and W5 influence the biosynthesis of unsaturated fatty acids and ether lipid metabolism, potentially improving lipid status in thrombosis rats[34-36]. W4 and W5 respectively influence primary bile acid biosynthesis and pyrimidine metabolism. While these pathways relate to CVD, their specific connection requires further study[37-38]. The arginine biosynthesis pathway promotes angiogenesis, enhances vascular repair, and boosts the effectiveness of anti-thrombotic drugs[39]. However, there has been relatively little research on the arginine biosynthesis metabolic pathway in CVD. arachidonic acid metabolism, sphingolipid metabolism, and TCA cycle are metabolic pathways that have been studied extensively and are closely related to the development of thrombosis and CVD[40-42]. Besides R1, the other fractions also exert anti-thrombotic effects by regulating arachidonic acid metabolism. R1, W1, and W3 influence the TCA cycle, which

Table 2
Pathway analysis results of the main effects of PRR.

Group	Pathway name	Total	Hits	*P*	–lg *P*	Impact
R1/Th	Citrate cycle (TCA cycle)	20	3	0.002 181	2.661 5	0.193 92
	Sphingolipid metabolism	32	3	0.008 531	2.0690	0.231 39
	Arginine biosynthesis	14	2	0.014 753	1.831 1	0.076 14
R4/Th	Arachidonic acid metabolism	44	8	0.000 600	3.221 7	0.190 67
	Sphingolipid metabolism	32	6	0.002 588	2.587 1	0.460 28
	Steroid hormone biosynthesis	80	8	0.026 073	1.583 8	0.160 36
R5/Th	Biosynthesis of unsaturated fatty acids	36	10	3.81×10^{-5}	4.418 9	0.090 91
	Arachidonic acid metabolism	44	9	0.001 159	2.935 9	0.479 97
	Sphingolipid metabolism	32	7	0.002 808	2.551 7	0.348 96
	Ether lipid metabolism	20	4	0.032 287	1.4910	0.081 76
	Linoleic acid metabolism	5	2	0.034 320	1.464 5	1
	Tryptophan metabolism	41	6	0.039 012	1.408 8	0.174 88

Table 3
Pathway analysis results of the main effects of WPRR.

Group	Pathway name	Total	Hits	*P*	–lg *P*	Impact
W1/Th	Arachidonic acid metabolism	44	8	0.000 357	3.446 7	0.479 97
	Citrate cycle (TCA cycle)	20	4	0.008 566	2.067 2	0.226 65
W2/Th	Arachidonic acid metabolism	44	6	0.000 635	3.197 3	0.088 78
	Caffeine metabolism	12	2	0.035 488	1.449 9	0.692 31
	Sphingolipid metabolism	32	3	0.045 455	1.342 4	0.313 32
	Arginine biosynthesis	14	2	0.047 392	1.324 3	0.076 14
	Steroid hormone biosynthesis	80	5	0.048 785	1.311 7	0.118 65
W3/Th	Arachidonic acid metabolism	44	7	0.001 345	2.871 2	0.132 45
	Arginine biosynthesis	14	3	0.015 983	1.796 3	0.076 14
	Citrate cycle (TCA cycle)	20	3	0.042 252	1.374 2	0.181 67
W4/Th	Arachidonic acid metabolism	44	7	0.003 578	2.446 3	0.132 45
	Tryptophan metabolism	41	6	0.010 609	1.974 3	0.188 33
	Sphingolipid metabolism	32	5	0.014 873	1.827 6	0.314 57
	Primary bile acid biosynthesis	46	6	0.018 347	1.736 4	0.122 34
	Arginine biosynthesis	14	3	0.024 943	1.6030	0.076 14
	Biosynthesis of unsaturated fatty acids	36	10	9.92×10^{-5}	4.003 4	0.090 91
W5/Th	Arachidonic acid metabolism	44	9	0.002 528	2.597 2	0.479 97
	Sphingolipid metabolism	32	7	0.005 215	2.282 7	0.348 96
	Caffeine metabolism	12	4	0.007 193	2.143 1	0.692 31
	Ether lipid metabolism	20	4	0.045 669	1.340 4	0.081 76
	Pyrimidine metabolism	39	6	0.049 628	1.304 3	0.254 53

may be a mechanism for their anti-thrombotic effects. Sphingolipids are key lipids in eukaryotic cells, believed to be part of the plasma membrane and involved in cell-cell interactions and recognition[43]. Recent years have seen an increased focus on biologically active lipids, especially sphingolipids. R1, W2, R4, R5, W4, and W5 may inhibit thrombosis *via* sphingolipid metabolism.

4. Discussion

Thrombus is an abnormal blood clot that forms in the body's blood vessels[25]. The formation of a blood clot is a complex physiological and pathological process involving various factors, including blood cells, coagulation factors in plasma, vessel wall components, interactions between blood cells and the vessel wall, vascular contraction and dilation, coagulation and anticoagulation mechanisms, fibrinolysis activation and inhibition, as well as plasma viscosity[44]. In clinical practice, TCM commonly employs blood-activating and stasis-dispelling drugs to prevent clots, relieve pain and symptoms, and enhance quality of life. PRR is a commonly used blood-activating and stasis-dispelling medicine in clinical practice, known for its anti-thrombotic properties. Stir-frying with rice wine enhances the blood-activating ability of PRR and improves its anti-thrombotic activity[45]. However, the pharmacological substance basis and mechanism of anti-thrombosis before and after PRR stir-frying with rice wine have not yet been systematically studied. Therefore, this study aims to explore the effects of PRR and WPRR on various thrombosis-related physicochemical parameters from the perspective of fractionation of herbal fractions, to determine the pharmacological basis and mechanism of anti-thrombotic effects of PRR and WPRR.

Based on the structural features and properties of the chemical components in PRR and WPRR, along with reference literature and clinical drug characteristics, the herbs were extracted into polysaccharides, oligosaccharides, 30% ethanol water elution components, and 95% ethanol water elution components using methods like water decoction, ethanol precipitation from water extraction, and column chromatography. The fractions of TCM were verified by UHPLC-QTOF-MS/MS without interference, demonstrating a feasible separation process and good separation for each fraction. The primary method to evaluate the efficacy of anti-thrombotic fractions and their anticoagulation pathways is to establish a thrombosis model in experimental animals. Blood clot formation in experimental animals can be categorized into arterial, venous, microvascular, and mixed thrombi based on their location[46]. In experiments assessing anti-thrombotic effects, arteries or veins are typically used as models. This experiment used carrageenan to induce a thrombosis model in rats, evaluating it 24 h post-establishment. Compared to chemical, mechanical, and physical methods for creating blood clots, the rat caudal vein thrombosis model is easy to perform and allows direct observation of clot length. It avoids mechanical injury and minimizes harm to experimental animals, making it suitable for initial drug activity screening.

The degree of tail color change in rats serves as a key indicator of thrombosis severity. In this study, both PRR and WPRR reduced the black tail coloring induced by carrageenan, with WPRR showing significantly stronger anti-thrombotic activity than PRR. After separating the PRR and WPRR, each fraction showed a significant change in anti-thrombotic efficacy. The small molecule fractions R4 and R5 from PRR are responsible for its anti-thrombotic effect, while all fractions from WPRR exhibit anti-thrombotic properties, with the W2 fraction demonstrating the strongest effect. Hematoxylin-eosin (HE) staining is commonly used for tissue pathology, but in this experiment, the uneven tail lengths of rats across groups made it challenging to obtain suitable pathological sections; thus, no HE staining was performed. Blood clots and coagulation result from an imbalance between the body's coagulation and anti-coagulation systems. Virchow identified three factors contributing to thrombosis: abnormal blood flow, activation of coagulation factors, and vessel wall abnormalities[47]. Approved anti-thrombotic drugs are categorized into 3 groups: anticoagulants, platelet inhibitors, and fibrinolytic agents, which inhibit the anticoagulation pathway, block platelet activation, and promote fibrinolysis to prevent thrombosis[44]. The chemical structures of compounds vary, leading to different pharmacological effects and mechanisms. Therefore, we assessed coagulation function, fibrinolysis, platelet activity, and related functions in each group of rats to identify the primary pharmacological pathway for each fraction. The small molecule fractions (R4, R5, W4, W5) primarily inhibit blood clot formation by affecting coagulation and fibrinolysis while improving blood rheology. The polysaccharides in WPRR, called W2 fraction, mainly prevent blood clots by inhibiting platelet activation and lowering blood viscosity in rat thrombosis model. The low-polysaccharides, particularly the W3 fraction in WPRR, primarily exert anti-thrombotic effects by enhancing blood rheology; however, the exact mechanism remains unclear. It may involve improved nutritional status and reduced endothelial dysfunction in blood vessels. Further exploration of this pharmacological function is needed.

Metabolomics, a key aspect of systems biology, builds on the research methods of genomics and proteomics to study how external environmental disturbances affect the types and quantities of endogenous metabolites in the body, thereby providing a holistic view of an organism's response mechanisms to stimuli[48]. Metabolomics sits at the base of the systems biology pyramid, amplifying subtle functional changes in the genome and proteome at the metabolic level for easier detection[49]. Using a UHPLC-QTOF-MS/MS metabolomics approach, we aim to investigate the effects of PRR and WPRR, along with their fractions, on endogenous metabolites to identify specific biomarkers for each fraction's anti-thrombotic action and clarify their mechanisms, providing references for disease diagnosis and treatment. The experimental results indicate that both R1 and W1 can prevent thrombosis by regulating the TCA cycle, with R1 also influencing sphingolipid metabolism and W1 affecting arachidonic acid metabolism. The mechanisms of blood circulation activation by PRR and WPR differ significantly. Sphingolipids are structural components of cell membranes, derived from the amino alcohol sphingosine. The sphingolipid base undergoes various enzymatic transformations to produce bioactive compounds. Sphingolipid metabolism plays a crucial role in many aspects of cell biology, including growth, the cell cycle, apoptosis, aging, inflammation, immune response, adhesion and migration, angiogenesis, nutrient uptake and metabolism, stress response, and autophagy[42]. Sphingolipid metabolism influences membrane organization, dynamics, and vesicle transport. Numerous experimental and clinical studies highlight the crucial role of sphingolipids in CVD pathophysiology, especially in ischemic heart disease, hypertension, heart failure, and stroke. Research indicates that modifying sphingolipid metabolism can have a cardioprotective

effect in conditions like myocardial infarction[43,50-51]. Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid with 4 double bonds, essential for biological cell membranes due to its fluidity and flexibility. It can be converted into various metabolic products in the body, most of which have significant physiological effects and play key roles in cell regulation, making it vital for cellular function[52-53]. Platelets are crucial to thrombi, facilitating the recruitment of inflammatory cells to the lesion and releasing significant amounts of inflammatory mediators, thereby enhancing the inflammatory environment[54]. Blood platelets can be activated by various stimuli, with AA (20:4) being one of the compounds responsible for this activation[55-56]. In the cytoplasm of platelets, AA is metabolized, and for this, the activity of phospholipase A2 is needed to release AA from the platelet membrane. Unsaturated fatty acids are the precursors of biologically active compounds called prostaglandins; after release, AA can be oxidized through the cyclooxygenase, lipoxygenase, or lipoxygenase pathways[57-59]. The oxidative modification of arachidonic acid is being studied as a novel therapy for thrombosis[60]. The TCA cycle, also known as the citric acid cycle or the Krebs cycle, is a series of reactions that forms the metabolic engine within the cell in a closed loop[61]. The TCA cycle is the central hub of cellular metabolism, integrating the metabolism of sugars, lipids, proteins, and nucleic acids[41]. Recent studies indicate that mitochondria have a fifth mechanism: releasing TCA cycle metabolites to regulate cell fate and function, thus influencing thrombosis progression[62]. R4, R5, W4, and W5 influence the arachidonic acid and sphingolipid metabolism pathways. W2 and W3 regulate the arachidonic acid pathway, while W2 also regulates the sphingolipid pathway, and W3 regulates the TCA cycle. The impact of each fraction on these pathways varies. The relationships among the differentially metabolized compounds in each treatment group compared to the Th group, those in the Th/Con group, and the mechanism by which carrageenin induces thrombosis in the blood clot model appear somewhat random. This may stem from differences between how carrageenin causes thrombosis and human disease mechanisms in normal individuals. Future experiments might benefit from a surgical modeling approach for studying blood clots.

While traditional anti-thrombotic drugs are effective for thrombotic diseases, their use can be limited by various factors. Recently developed thrombolytic drugs outperform traditional options, like biomimetic drugs, in dissolving blood clots and restoring flow. These biomimetic drugs release medication under specific conditions at targeted locations[44]. High doses of non-specific fibrinolytic drugs can impair coagulation and cause life-threatening bleeding, making it crucial to develop targeted, selective, biocompatible fibrinolytics with reduced bleeding risk. In this study, W2 and W3 are anticipated to offer valuable insights for the advancement of novel therapeutics targeting CVD.

5. Conclusion

This study clarifies the pharmacological basis and mechanism of anti-thrombosis for PRR and WPRR using component fractionation, pharmacological analysis, and non-targeted metabolomics. Fraction-wise cross-validation, pharmacodynamic, and metabolomics results show that Fraction separation is effective. PRR enhances the hemorheological state in thrombosis rats both before and after processing, with WPRR showing a significantly stronger effect than PRR. PRR mainly influences the TCA cycle and sphingolipid metabolism by R4 and R5 fractions, thus enhancing coagulation and fibrinolysis functions. WPRR, except for W4, has weak anti-thrombotic activity, while the other components, especially W2 and W3, exhibit strong effects. WPRR enhances the thrombotic state in rat blood stasis by influencing arachidonic acid metabolism, the TCA cycle, and other pathways that improve lipid levels and promote angiogenesis. It also regulates platelet adhesion and aggregation while enhancing coagulation and fibrinolysis functions. Based on the aforementioned results, in contrast to PRR, the principal material basis for the blood-activating efficacy of WPRR has transformed from small molecule compounds to polysaccharides and oligosaccharide components. Moreover, the action pathways of WPRR in exerting anti-thrombotic efficacy have augmented. The polysaccharide components of WPRR might even possess considerable potential for being developed into natural anti-thrombotic drugs.

Conflict of interest

All the authors have not submitted the manuscript to another journal and all authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250453.

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