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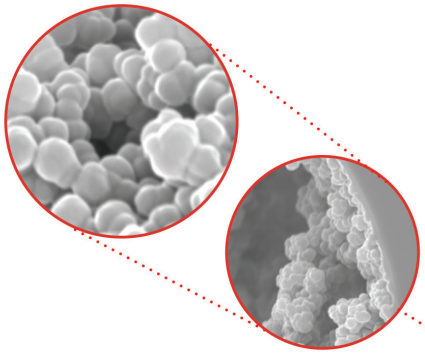

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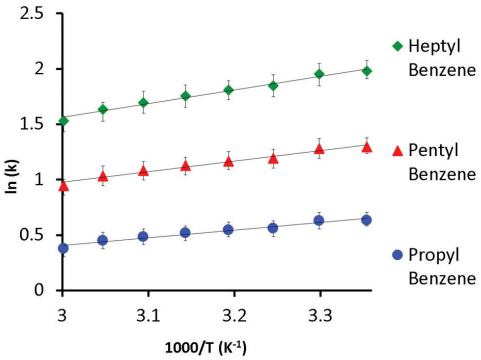

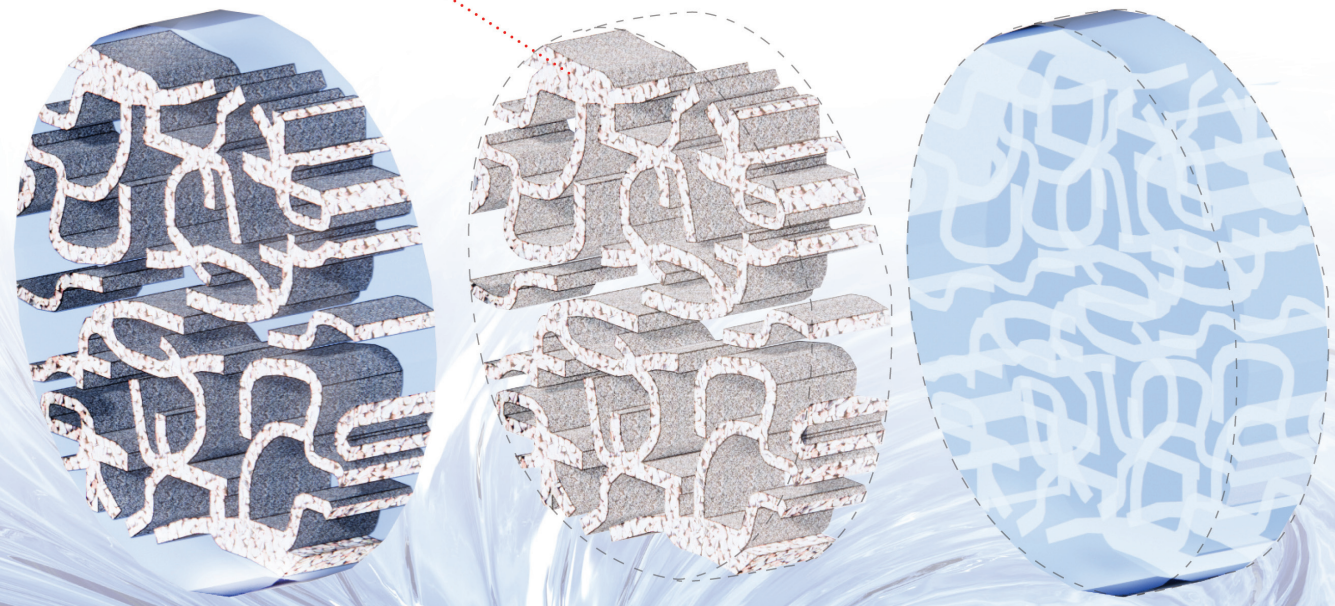

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RESEARCH ARTICLE

Rapid screening and identification of anticoagulation component from carthami flos by two-dimensional thrombin affinity chromatography combined with HPLC-MS/MS

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Carthami flos, commonly known as Honghua in China, is the dried floret of safflower and widely acknowledged as a blood stasis promoting herb. The study aimed at investigating the relationship between thrombin and carthami flos through a high-performance thrombin affinity chromatography combined with a high-performance liquid chromatography-tandem mass spectrometry system. First, thrombin was immobilized on the glutaraldehyde-modified amino silica gel to prepare the thrombin affinity stationary phase, which was packed into a small column (1.0 × 2.0 mm, id) for recognizing the anticoagulant active components of carthami flos. The target component was enriched and analyzed by the high-performance liquid chromatography-tandem mass spectrometry system. Finally, hydroxysafflor yellow A was screened out and identified as the active component. The anticoagulant effects of hydroxysafflor yellow A were analyzed by anticoagulant experiments in vitro, and the interaction of hydroxysafflor yellow A with thrombin was investigated by the molecular docking method. The results proved that hydroxysafflor yellow A (30 μg/mL, 0.05 mM) and carthami flos extract (30 μg/mL) could prolong activated partial thrombin time and thrombin time by 50 and 11%, respectively. Moreover, hydroxysafflor yellow A exhibits a good hydrogen bond field and stereo field matching with thrombin. Overall, it was concluded that hydroxysafflor yellow A might exert an anticoagulation effect by interacting with thrombin and thus could be potential anticoagulant drugs for the prevention and treatment of venous thrombosis.

KEYWORDS
anticoagulant component, carthami flos, high performance liquid chromatography, hydroxysafflor yellow A, thrombin affinity chromatography

Article Related Abbreviations: APTT, activated partial thrombin time; CF, carthami flos; CFE, CF extract; GA, glutaraldehyde; HSYA, hydroxysafflor yellow A; HSYB, hydroxysafflor yellow B; HSYC, hydroxysafflor yellow C; PT, prothrombin time; TAC, thrombin affinity chromatography; TAC, thrombin affinity chromatography; TT, thrombin time

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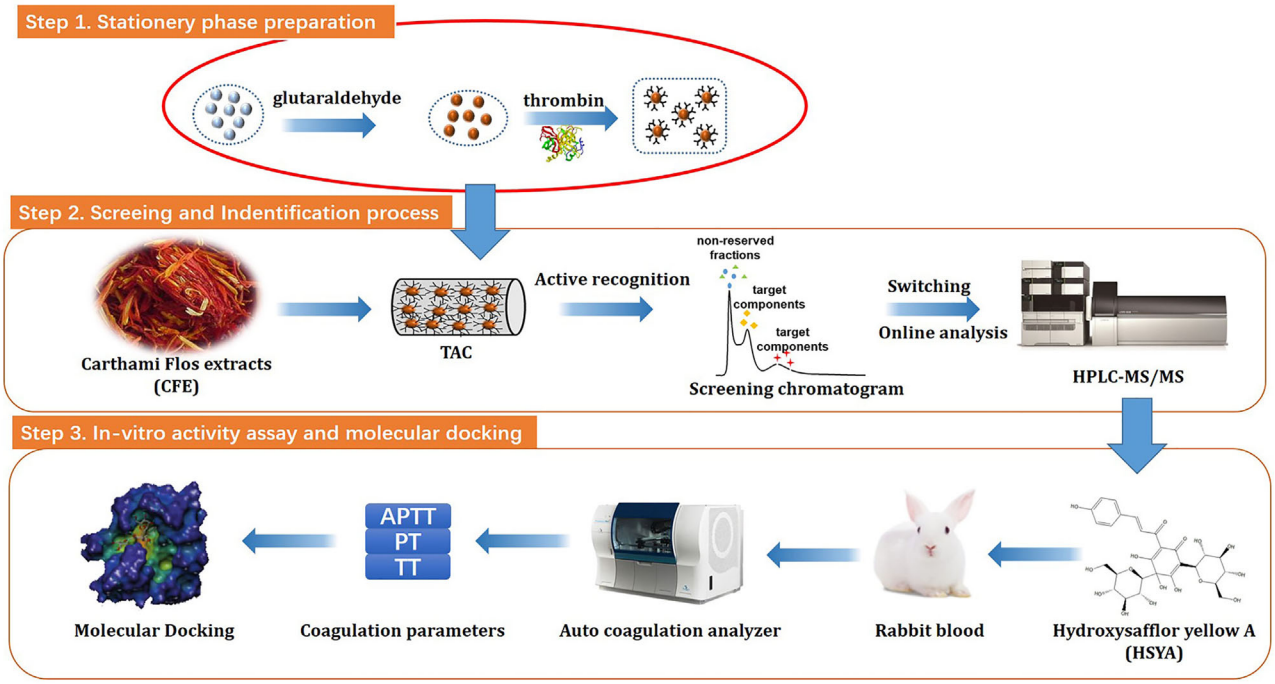

FIGURE 1 Scheme of the screening process using the thrombin affinity chromatography (TAC) column

1 | INTRODUCTION

Carthami flos (CF), commonly known as Honghua in China, is the dried floret of safflower and widely acknowledged as a blood stasis promoting herb [1]. According to modern pharmacological investigations, CF extract (CFE) or pure isolated components exert protective effects on brain tissue, myocardial tissue, as well as antithrombotic, anti-inflammatory, and antitumor effects, are associated with the anticoagulation activity, blood circulation promotion, blood stasis removal, and pain relief [2]. Therefore, the study aimed to investigate the relationship between thrombin and CF.

Thrombin is an essential serine enzyme in hemostasis, thrombosis, inflammation, and vascular remodeling [3]. Further extending its application, thrombin generation inhibition could minimize intravascular thrombosis by preserving physiologic hemostasis [4]. Hence, the screening of active anticoagulant components of CF and the development of anticoagulant drugs could be beneficial for the prevention and treatment of venous thrombosis.

CF is a very complex system constituting glycerides, linoleic acid, and oleic acid, etc. [5, 6]. Therefore, establishing a method for rapid screening and identification of the primary anticoagulant components of the complex system is necessitate. The current screening methods are divided into two categories: the maximum separation techniques [7–10] and enzyme immobilized techniques [11–14]. The maximum separation techniques utilize organic solvent, material, and time consuming, while the enzyme immobilization technique is the more efficient method because it is based on the enzyme activity screening, such as ultrafiltration and magnetic immobilization methods. However, several disadvantages, such as a large amount of enzyme consumption and nonspecific absorption, limit their further applications [15–17]. The development of an immobilized capillary enzyme reactor could improve the screening efficiency, but the interface technology of CE and MS limits its application.

In recent years, more and more studies have proved that affinity chromatography plays an important role in the screening of active ingredients in complex systems [18–22]. Based on the previous 2D chromatography studies [23–26], a high-performance thrombin affinity chromatography combined with HPLC-MS/MS (TAC-HPLC-MS/MS) system was developed to screen the anticoagulant components of CF. And the relationship between thrombin and the anticoagulant components was studied. The schematic representation is shown in Figure 1. Briefly, thrombin was immobilized on the surface of amino-modified silica gel by glutaraldehyde (GA) to prepare a thrombin stationary phase, which was wetly packed into a short chromatographic column (2.0 × 1.0 mm, id). This high-performance thrombin affinity column was used to screen anticoagulant components from complex Traditional Chinese medicine (TCMs). Furthermore, the anticoagulant components were identified online by MS.

2 | MATERIALS AND METHODS

2.1 | Materials

Thrombin freeze-dried powder (1000 U/bottle) was supplied by Hunan Yige Pharmaceutical. Heparin sodium (2 mL: 12 500 units) was purchased from Tianjin Biochemical Pharmaceutical. Hydroxysafflower yellow A (HSYA) (purity ≥ 98%) and argatroban (Arg, purity ≥98%) were supplied by the Chengdu EFA Biotechnology. Carthami flos was supplied by the Chinese Medicine Store (Xi'an, China) and certified by the pharmacology department of Xi'an Jiaotong University. SiliaSphere spherical silica gel (S10005M; 5 μm, 100 Å) was obtained from SiliCycle (Quebec, Canada). Pure sodium chloride, sodium carbonate, and sodium bicarbonate were purchased from Tianjin Komo Chemical Reagent. HPLC formic acid and methanol (chromatographic purity) were purchased from Tedia. All the aqueous solutions were prepared using ultrapure water supplied by Millipore Milli-Q Plus ultrapure water system (MK-459 Millipore Milli-Q Plus).

2.2 | Animals

The healthy male New Zealand white rabbits weighing 1.5–2.8 kg were supplied by the United Nations Quality Detection, Xi'an, China. The rabbits were housed in a conventional animal facility with free access to food and water where the environmental temperature (16–26°C) and relative humidity (40–70%) were monitored and controlled.

2.3 | Standard solutions and sample preparation

HSYA and Arg standard stock solutions (1 mg/mL) were prepared in methanol and stored at 4°C in the dark until further use. Standard stock solutions were diluted to suitable concentrations using the mobile phase before use.

The CFE was obtained as follows: About 0.4 g of CF powder was refluxed with 25 mL of methanol for 1 h at 65°C. The filtrate was transferred to a clean round bottom flask, and the residue was refluxed at 65°C for 0.5 h in 50 mL 25% methanol. The combined filtrate and residual reflux were concentrated in a rotary evaporator to obtain CFE. Then, approximately 2 mg of CFE was dissolved in 2 mL of 25% methanol and stored in a dark environment. The sample was filtered with a Millipore filter (0.45 m) before use.

2.4 | Preparation of thrombin affinity stationary phase

The thrombin affinity stationary phase was prepared according to the method reported by Shi et al. [27]. Briefly, the macroporous silica gel (model ZEX-II; 5 μm, 100 Å) was mixed with 200 mL of 1 mol/L HCl and refluxed for 2 h under 105°C. The reaction mixture was filtered and then washed with ultra-pure water until neutral pH. The silica gel was activated by heating at 105°C before use.

Amino-silica gel (NH_2-SiO_2) was prepared as follows: 10 g of silica gel, 5 g of 3-aminopropyldimethylchlorosilane, and 100 ml of toluene were mixed and refluxed for 12 h at 110°C. After the reaction was completed, it was washed with toluene and methanol using vacuum filtration. The filtrate was collected and dried under vacuum drying conditions to obtain an isopropyl side chain modified NH_2-SiO_2.

Note that 1 g of NH_2-SiO_2 and 20 mL of 2.5% GA were placed in a 100-mL beaker. The mixture was stirred by a magnetic force at room temperature for 2 h and then washed several times with ultra-pure water for removing the free GA to obtain GA-SiO_2.

The freeze-dried thrombin powder was dissolved in sterile water and diluted to 10 U/mL. Later, the prepared GA-SiO_2 powder was mixed with thrombin at 4°C and stirred for 4 h to react completely. The reaction product (THR-SiO_2) was thoroughly rinsed with distilled water to remove the unbound thrombin. Finally, THR-SiO_2 was fixed into a column using an rPL-ZD10 pump (Dalian Rept Technology, Dalian, China) for 5 min at 10 Mpa to obtain a high-performance thrombin affinity column (10 mm × 2.0 mm diameter, 5 μm).

2.5 | Application of the 2D TAC system

The 2D TAC-HPLC-MS/MS was constructed and employed to screen the active components of CFE. The chromatographic conditions and ESI-MS/MS conditions were as follows:

The first-dimension part, used for screening of the active components, consisted of six parts, including the TAC column, LC-20AD pump, the DGU-20A3 degasser, the SIL-20AXR automatic sampler, the CTO 20AC column thermostat, and the SPD-20A UV/visible detector (Shashimu, Kyoto, Japan). The second-dimension part was provided

by HPLC-MS/MS (Shimadzu Production House, Kyoto, Japan) and primarily used for identification. The two parts were connected via VICIAG 10G-0911V six-way two-position switching valve (VICI Valco Instruments, Houston, Texas, USA). Particularly, One Shim-pack VP-ODS pre-column (10 mm × 2.0 mm id, 5 μm; Shimadzu Corporation) was used to enrich the active ingredient collected from the first dimension.

Na_2HPO_4 solution (5 mM, pH 7.4) was selected as the mobile phase for the first-dimension part with a flow rate of 0.1 mL/min. Meanwhile, the 0.1% formic acid water (A) and acetonitrile (B) were used as the mobile phase for HPLC-MS/MS system. The flow rate was set at 1.0 mL/min, and the gradient elution ratio was set as 0–5 min, 5—10% B, 5–30 min, 10—25% B; 30–60 min, 25—90% B. The MS was set as: nebulizer gas, N_2, purity > 99.999%, pressure at 109 kPa; drying gas, N_2, purity > 99.999%, and flow rate 1.5 L/min; interface was ESI source. The curve desolvation line temperature was set at 200°C. Subsequently, the heating block temperature was set at 200°C; the interface voltage was set at 4.5 kV; the detector voltage was set to 1.57 kV; the purity of CID gas and He gas, purity > 99.999%; the CID energy was set to 50; ion accumulation: 30.0 ms; the scanning mode was positive ionization; automatic precursor ion selection; scan range was 100 to 1000 *m/z*.

2.6 | In vitro activity assay

Blood was collected from the ear marginal arteries of three healthy male rabbits through a polyethylene cannula using a 10-mL plastic flask containing 3.8% sodium citrate (blood: sodium citrate = 9:1, v/v). This was then mixed and centrifuged at 3500 rpm for 15 min to obtain platelet-poor plasma for determining activated partial thrombin time (APTT), prothrombin time (PT), and thrombin time (TT).

In brief, the plasma samples (495 μL) were incubated with 5 μL of Arg (0.05 μg/mL), CFE (30 μg/mL), and HSYA (3, 10, 30 μg/mL), respectively, for 3 min at 37°C. The anticoagulant effect of the drug was then evaluated using the results of a stA-Compact Max coagulation analyzer (Diagnostica Stago, France), which was evaluated by APTT, PT, and TT. Note that 1% DMSO was used as the control for the analyzer.

APTT index setting: 50 μL plasma samples were mixed with 50 μL APTT assay reagent (STA-C.K. PREST 5 kit; Diagnostica Stago) for 4 min, and 50 μL 0.025 M $CaCl_2$ was added to determine the coagulation time. TT index setting: 100 μL treated plasma samples were added to 100 μL TT assay reagent (STA-Thrombin 10 kit; Diagnostica Stago). The coagulation time was recorded after 10 min of preincubation at 37°C. PT index setting: 50 μL of treated plasma samples were mixed with 100 μL of PT analysis reagents (STA-Neoplastine Cl Plus 10 kit; Diagnostica Stago) to determine the coagulation time.

2.7 | Molecular docking assay

Molecular modeling was used to verify the binding force of HSYA and thrombin, and the docking operation steps were as follows: First, the connected molecules were drawn, and the energy was minimized using Tripos force field and Gasteiger-Huckel charge with the convergence criterion of 0.005 kcal/(Å mol) and repeated for 1000 times. Then, the target protein's structural file was downloaded from PDB.org (PDB ID = 1DWC). The water molecules were then replaced with hydrogen, and the AMBER7 FF99 Force field and the Gasteiger-Huckel charge were used to minimize the protein molecules. The 5.0 Å residue around the original ligand was defined as the active capsule. Finally, the constructed molecules were docked into the active sites of the protein with other parameters as default. The above operation was implemented on the Surflex-Dock module on SYBYL X 2.0 (Tripos).

3 | RESULTS AND DISCUSSION

3.1 | Preparation of the thrombin affinity column

The thrombin affinity column stationary phase and column were prepared according to the methods reported in our previous work [27]. Briefly, three factors, including immobilization time, glutaraldehyde concentration, and thrombin amount were investigated to prepare the thrombin affinity column stationary phase. The 4 h incubation time, 2.5% GA, 3.36 mg thrombin were selected as the optimum conditions. About 1.21 mg of THR was fixed on 10 mg of GA-SiO_2. The immobilized thrombin activity decreased moderately within 7 days at room temperature compared with the free status of thrombin. This might have happened due to the availability of a more stable stereo structure in immobilized thrombin than the free status. The immobilization efficiency was assessed by thrombin substrate S-2238, about 30–35% as a ratio of immobilized THR activity to that of initial total THR.

Further, the morphological characterization and elemental analysis of the stationary phase particles were investigated. The SEM results demonstrated that the stationary phase surface coated with thrombin (THR-SiO_2) had a rougher surface than the amino-silica gel (NH_2-SiO_2) and glutaraldehyde-modified amino-silica gel (GA-SiO_2). The FT-IR analysis revealed C—N, C═O, C—H bonds on THR-SiO_2 particle, and the content of N in THR-SiO_2 was

much higher than in NH_2-SiO_2 and GA-SiO_2. These results indicated that thrombin had been successfully immobilized on the stationary phase.

3.2 | Selectivity and repeatability of the thrombin affinity column

Arg is a small, reversible molecule with a direct thrombin inhibitor selective for the catalytic site of the thrombin molecule, while heparin sodium is an indirect thrombin inhibitor. Therefore, these two anti-thrombus drugs were used to investigate the thrombin affinity column's selectivity. The detailed results have been reported in our previous study [27]. Briefly, Arg exhibited a retention characteristic (Rt: 15.4 min) on the thrombin affinity column, while sodium heparin did not. This result proved that the thrombin affinity column could selectively screen the drugs acting on thrombin. The repeatability of the thrombin affinity column was assessed by injecting Arg (5 μL, 0.1 mg/mL) at 1 day ($n = 6$) and 3 days ($n = 3$), respectively. The intra- and interday precisions were 1.5 ($n = 6$) and 6.9 % ($n = 3$), and the RSD for the three batches of thrombin affinity was 2.9%. The variation of the retention times of Arg within 3 days was observed to evaluate the thrombin affinity column's activity. This result confirmed that the thrombin affinity column's activity decreased by 30% within 3 days.

Because enzymes are greatly affected by temperature and pH. In the preparation and experiment processes, every operation step should be kept as low as possible. Because pH 8.3 shows the maximum enzyme activity, Na_2HPO_4 solution (5 mM, pH 7.4) was selected as the mobile phase with a flow rate of 0.1 mL/min (column pressure <3 MPa) to keep a low activity of thrombin. All these conditions helped to maintain the activity and structure of thrombin on the column.

3.3 | Validation of the 2D TAC system

Arg was employed to validate the 2D TAC-HPLC-MS/MS system. As depicted in Figure 2A, a retained broad peak of Arg was observed on the thrombin affinity column. The retained components were enriched and transferred to the capture column through a switching valve for HPLC-MS/MS analysis. Figure 2B shows the HPLC chromatogram and mass spectrum of the reserved component (MW: 509.25). This result proved that the substance was Arg. Figure 2C shows the HPLC chromatogram of Arg standard solution. The retention times in Figure 2B and 2C confirm the substance to be the same. Therefore, the 2D TAC-HPLC-MS/MS system can be used to screen and identify the active components.

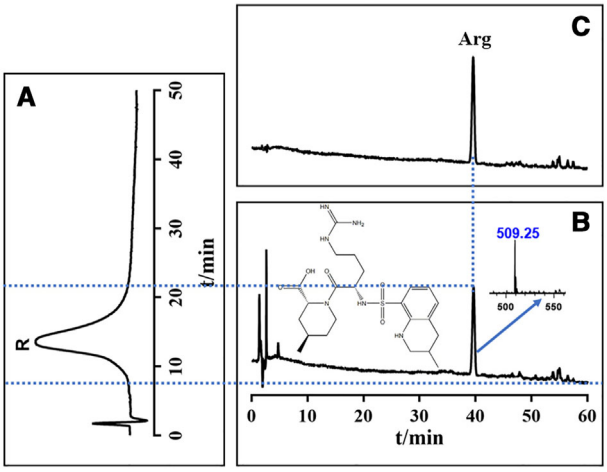

FIGURE 2 Validation of thrombin affinity chromatography combined with HPLC-MS/MS (TAC-HPLC-MS/MS) system with argatroban (Arg). (A) Chromatogram of Arg on the thrombin affinity column; (B) HPLC chromatogram and mass spectrum of the retained component; (C) HPLC chromatogram of Arg standard solution. Arg: argatroban; R: retained fraction; TAC: thrombin affinity chromatography

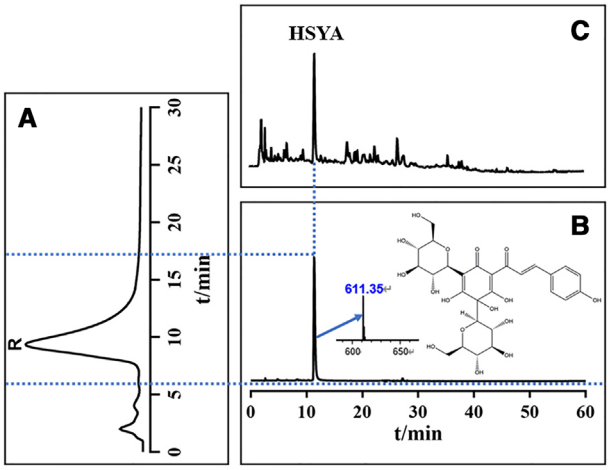

FIGURE 3 Screening active components from carthami flos extract (CFE) with thrombin affinity chromatography combined with HPLC-MS/MS (TAC-HPLC-MS/MS) system. (A) Chromatogram of CFE on the TAC column; (B) HPLC chromatogram and mass spectra of the retained component; (C) CFE standard solution chromatogram. CFE: carthami flos extract; HSYA: hydroxysafflor yellow A; R: retention fraction; TAC: thrombin affinity chromatography

3.4 | Practical application

The TAC-HPLC-MS/MS system was employed to screen the potential thrombin inhibitors of CFE interacting with thrombin. The results are shown in Figure 3. As depicted in Figure 3A, CFE exhibited a retention peak at 9.5 min on the thrombin affinity column, and the retained component

TABLE 1 Anticoagulant test in vitro

Sample	Concentration (μg/mL)	APTT (s)	PT (s)	TT (s)
Blank	/	35.43 ± 2.061	8.250 ± 0.050	14.20 ± 0.000
HSYA	3	48.55 ± 0.850*	8.400 ± 0.100	15.40 ± 0.100**
HSYA	10	52.87 ± 2.027**	8.600 ± 0.058*	15.77 ± 0.088**
HSYA	30	53.75 ± 0.450**	8.650 ± 0.150	15.90 ± 0.100**
CFE	30	53.95 ± 0.750**	8.550 ± 0.050	15.90 ± 0.100**
Arg	0.05	53.77 ± 1.710**	8.70 ± 0.000**	23.53 ± 0.150**

Abbreviations: APTT, activated partial thromboplastin time; Arg, argatroban; CFE, carthami flos extract; HSYA, hydroxysafflor yellow A; PT, prothrombin time; TT, thrombin time.

The results were expressed as mean ± SEM. ($n = 3$). Compared with the blank group, *$P < 0.05$, **$P < 0.01$.

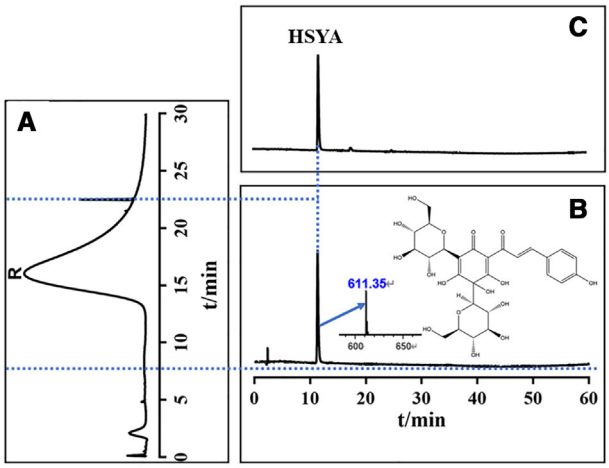

FIGURE 4 Validation of hydroxysafflor yellow A (HSYA) with thrombin affinity chromatography combined with HPLC-MS/MS (TAC-HPLC-MS/MS) system. (A) Chromatogram of HSYA on the TAC column; (B) HPLC chromatograms and mass spectra of the retained component; (C) HPLC chromatogram of HSYA standard solution. HSYA: hydroxysafflor yellow A; R: retention fraction; TAC: thrombin affinity chromatography

was labeled as R. This component (R) was enriched and identified by HPLC-MS/MS. As depicted in Figure 3B, the molecular weight of R was 611.35, which was identified as HSYA. Compared with the HPLC chromatogram of CFE (Figure 3C), the retention times of these two components were almost the same, about 11.8 min.

3.5 | Validation of active components using the 2D TAC system

HYSA was reinjected into the TAC-HPLC-MS/MS system to verify the results further. As depicted in Figure 4, a comparison between the retention behavior on the thrombin affinity column (Figure 4A), retention time in Figure 4B, and MS data in Figure 4C showed HSYA to be a potential anticoagulant component of thrombin.

3.6 | In-vitro assays of active components

The anticoagulation parameters, such as TT, PT, and APTT, were investigated to verify the anticoagulant activity of HYSA. PT was the main screening indexes of the exogenous blood coagulation system and a prolonged PT indicated a deficiency in coagulation factors V, VII, and X. In contrast, APTT and TT reflected intrinsic coagulation system, and a prolonged APTT showed a deficiency in factors VIII, IX, XI, XII, and Von Willebrand's factor.

These results are summarized in Table 1 and Figure 5. Compared with the blank, HYSA and CFE showed better anticoagulant activity. Further HYSA (30 μg/mL, 0.05 mM) and CFE (30 μg/mL) prolonged the APTT and TT by 50 and 11%. The positive drug Arg (0.1 μM, 0.05 μg/mL) prolonged APTT and TT by 50 and 66%. The above results confirmed that HYSA (30 μg/mL, 0.05 mM) and Arg (0.1 μM, 0.05 μg/mL) had the same extension effect on APTT, suggesting anticoagulant effects of both HYSA (3–30 μg/mL) and CFE (30 μg/mL).

Wang et al. found 0.15 mM (91.88, μg/mL), 0.30 mM (183.76 μg/mL), and 0.60 mM (367.52 μg/mL) of HYSA could prolong the APTT by 5–10%, PT by 2–32%, and TT by 5–13% [10]. Yao et al. found that 0.5 and 0.7 mg/mL of HYSA could significantly prolong the APTT without any effect on PT and TT [28]. Liu et al. found that HYSA (SD rat oral administration 60 mg/kg) could prolong the APTT and PT by 10 and 15% in vivo without any effect on TT [29]. Sun et al. proved that HSYA could inhibit the expression of prothrombin mRNA by detecting the levels of plasma prothrombin activation fragment 1+2 (F1+2) and thrombin–antithrombin complex [30]. All the above results confirmed the anticoagulant effect of HYSA.

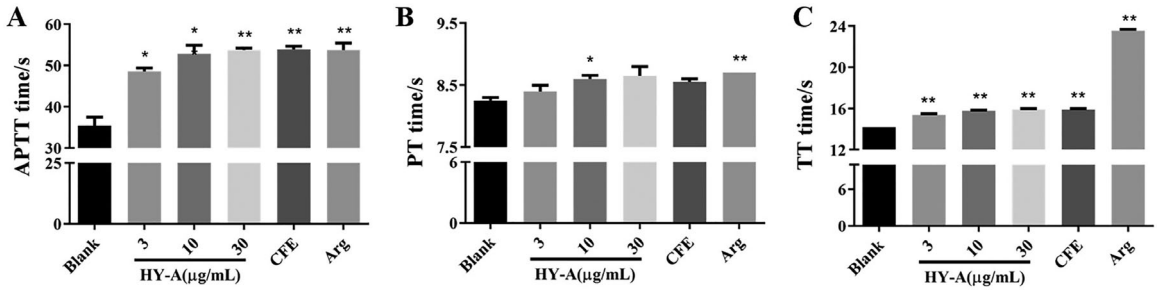

FIGURE 5 Effect of hydroxysafflor yellow A (HSYA) on platelet-poor (PPP) plasma coagulation parameters. (A) Effect of HSYA on activated partial thrombin time (APTT) index; (B) Effect of HSYA on PT index; (C) Effect of HSYA on thrombin time (TT) index. HSYA: hydroxysafflor yellow A; CFE: carthami flos extract; Arg: argatroban. The results were expressed as mean ± SEM. ($n = 3$). Compared with the blank group, $^{*}P < 0.05$, $^{**}P < 0.01$. APTT: activated partial thromboplastin time; PT: prothrombin time, TT: thrombin time

3.7 | Molecular docking assay

The interaction between small-molecule ligands and receptor proteins was detected by molecular docking through the Surflex-dock module of Sybyl-X 2.0. HSYA was used to evaluate its binding mode with thrombin (PDB ID: 1DWC). The results are summarized in Figure 6A and B. The hydroxyl group of the β-D-glucopyranose group in HSYA was embedded in the hydrophobic pocket S2 and formed three hydrogen bonds with the amino group of the active site Lys 60F with bond lengths of 2.18, 2.15, and 2.73 Å. The hydroxymethyl and Leu 41 formed a hydrogen bond with a bond length of 1.83 Å. The phenolic hydroxyl group in HY-A formed a hydrogen bond with the carbonyl group of Asp 189 (bond length: 1.94 Å) embedded in the specific pocket S1. Additionally, the hydroxyl group in another β-D-glucopyranosyl group formed a hydrogen bond with the carbonyl group of Glu 146 (bond length: 1.99 Å) and the amino group of Glu 192 (bond length: 2.73 Å) at the S1 site. The carbonyl group of Glu 192 formed a hydrogen bond with the hydroxyl group of cyclohexadien-1-one (bond length: 2.73 Å). The 1-oxo in HSYA formed a hydrogen bond with Gly 216 (bond length: 2.29 Å) located at the S1 site. The carbonyl group in cyclohexadien-1-one and the pyran ring in β-D-glucopyranosyl formed two hydrogen bonds with Ser 195 (bond lengths: 2.47and 2.72 Å) located in the catalytic three-membered ring of thrombin. The molecular docking results showed that HSYA had an excellent hydrogen bond field and stereo field matching with thrombin, which might explain its inhibitory effect during the anticoagulation test.

HSYA belongs to flavonoids. Actually, there are also structurally similar flavonoids such as hydroxysafflor yellow B (HSYB) and hydroxysafflor yellow C (HSYC) in the CFE. As shown in Supporting Information Figure S1: (A1, B1), (A2, B2), and (A3, B3) represent the (2D, 3D) structure of HSYA, HSYB, and HSYC, respectively. Although the general structure is similar, there are subtle differences among these molecular. For example, the C4 in the structure of quinoid chalcone is a chiral carbon, and

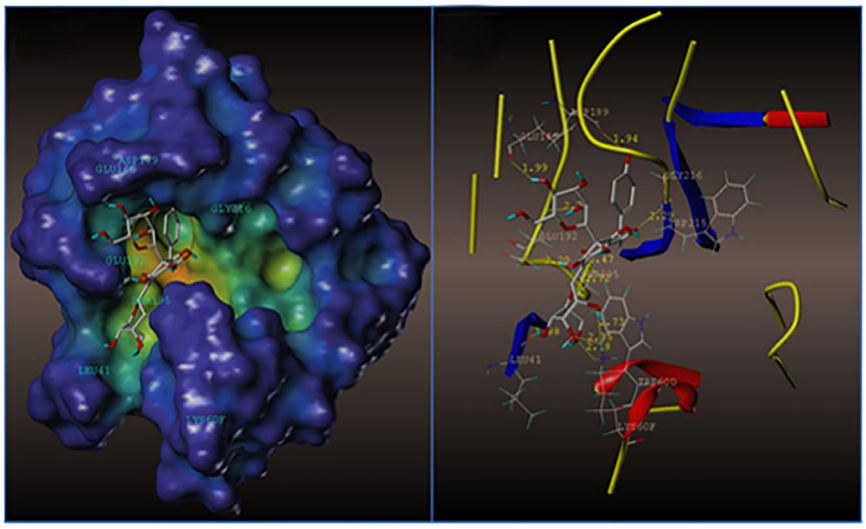

FIGURE 6 Molecular docking results of hydroxysafflor yellow A (HSYA) with thrombin (PDB ID = 1DWC). HSYA: hydroxysafflor yellow A

the hydroxyl group positions in HSYB and HSYC are the same and form a larger steric hindrance with hydroxyl in quinoid chalcone (red circle). The position of the hydroxyl group in HSYA is different and has no steric hindrance on the formation of hydrogen bond between the hydroxyl group of cyclohexadien-1-one and Glu 192 (bond length: 2.73 Å). In addition, the hydroxymethyl in HSYA (yellow circle) and Leu 41 formed a hydrogen bond with a bond length of 1.83 Å, while HSYB and HSYC do not have the hydroxymethyl group. Therefore, only HSYA was screened out and identified as an active component from CFE in our study.

4 | CONCLUDING REMARKS

In conclusion, HSYA was screened and identified from CFE by TAC-HPLC-MS/MS system within 30 min. HSYA (30 μg/mL, 0.05 mM) and CFE (30 μg/mL) could prolong APTT and TT by 50 and 11%, respectively. The docking results showed that HSYA exhibits a good hydrogen bond field and stereo field matching with thrombin. Moreover, HSYA may exert an anticoagulation effect by interacting with thrombin. As a natural pigment, HSYA has gained extensive attention due to its broad and effective pharmacological activities, such as neuroprotection, anticancer properties, metabolism regulation, liver, and lung, and endothelium cell protection. Therefore, HSYA could be a potential anticoagulant drug for the prevention and treatment of venous thrombosis.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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