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Mon-Pu (*Glochidion perakense* Hook.f.) as a Promising Novel Natural Source of Antithrombotic and Antioxidant Benefits

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ABSTRACT

Current evidence suggests that regular consumption of food-functioned fruits and vegetables could reduce the risk of thrombosis. While some indigenous vegetables from Southern Thailand have been reported to exhibit anticoagulant and antiplatelet activities, their fibrinolytic and thrombolytic properties remain unexplored. This study investigated the fibrinolytic, thrombolytic, and antioxidant potential of six indigenous vegetables from Southern Thailand, including *Champereia manillana* (Blume) Merr. (Phak-phum), *Citrus reticulata* Blanco. (Bai-som-paen), *Alpinia conchigera* Griff. (Kha-ling), *Anacardium occidentale* L. (Mamuang himmaphan), *Curcuma longa* L. (Kha-min-oan), and *Glochidion perakense* Hook.f. (Mon-Pu). Their aqueous and methanolic extracts were screened using the fibrin-agar-plate method for in vitro fibrinolytic activity. Effective extracts were further evaluated through whole blood clot lysis assays for thrombolytic potential. Antioxidant activity was assessed using the DPPH radical scavenging assay, and phytochemical profiling of active extracts was performed using liquid chromatography–electrospray ionization mass spectrometry (ESI-MS). Among the vegetables tested, the methanolic extracts of young Mon-Pu leaves exhibited significant antithrombotic effects, showing the highest fibrinolytic activity, indicative of antithrombotic potential, with a lysis area of $20.72 \pm 1.54 \text{ mm}^2$ at a concentration of $10 \mu\text{g}/\mu\text{L}$. In thrombolytic assays, Mon-Pu extract achieved $36.52 \pm 1.62\%$ clot lysis, compared to $65.37 \pm 2.18\%$ for standard streptokinase, demonstrating a dose-dependent effect. In addition, Mon-Pu extract exhibited strong antioxidant activity against DPPH radicals, with an IC_{50} of $3.14 \pm 0.05 \mu\text{g}/\text{mL}$, comparable to ascorbic acid (IC_{50} : $2.50 \pm 0.05 \mu\text{g}/\text{mL}$) used as the positive control. LC-ESI-MS analysis identified gallic acid and quercetin as major compounds in the extract, likely contributing to its antioxidant and antithrombotic effects. These findings first report the fibrinolytic and thrombolytic activities of Mon-Pu extract and highlight Mon-Pu's potential as a nutraceutical source for antithrombotic agents, offering a natural alternative for reducing thromboembolic risk.

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1 | Introduction

Atherothrombosis, characterized by blood clot formation within arteries, is a major global health concern and a leading cause of mortality. This condition arises from the gradual accumulation of plaque beneath the vessel walls, which can progressively narrow the arteries and impede blood flow to vital organs [1]. The development of an obstructive thrombus may result in acute coronary syndromes, ischemic strokes, and peripheral arterial diseases [2]. Thrombolytic therapy, the preferred treatment for life-threatening thrombosis, enhances blood circulation and helps prevent tissue and organ damage. This therapy works by promoting fibrinolysis through the intravenous administration of plasminogen activator (PA) analogs, such as streptokinase (SK), tissue-type PA (t-PA), urokinase (u-PA), and alteplase, which dissolve harmful clots [3]. Despite their efficacy, these thrombolytic agents often exhibit low specificity for fibrin and can have various adverse effects, in addition to being costly [4]. Thus, exploring plant-based therapies may provide safer and more effective alternatives.

Fruits and vegetables play a crucial role in health and wellness, with epidemiological studies suggesting that foods with antithrombotic properties can reduce the risk of cardiovascular diseases and thrombosis [1, 5]. Certain fruits and vegetables, including raspberry [6], kiwi [7], pineapple [8], onion [9], garlic [10], ginger [10], capsicum [11], and mushroom [12], have shown enhanced fibrinolytic and thrombolytic activities. For instance, protease-rich extracts from green kiwifruit (*Actinidia deliciosa*) have demonstrated over 70% in vitro clot lysis within 6 hours via fibrin degradation, and their fibrinolytic enzyme, actinidin, has been proposed as a natural thrombolytic candidate with potential therapeutic applications [7]. Therefore, the development of plant-derived therapeutics may provide safer and more sustainable alternatives for thrombosis prevention.

The southern region of Thailand has a wide abundance of indigenous vegetables that are consumed as daily food, providing diverse bioactive phytochemicals such as macronutrition, vitamins, and minerals [13]. Some of these vegetables have been used in traditional medicine for treating conditions such as arthritis, diabetes, and hypertension in Thailand [14]. However, studies on the antithrombotic activity of Southern Thai indigenous vegetables remain limited. Therefore, this study aims to evaluate the fibrinolytic and thrombolytic activities, antioxidant properties, and chemical compositions of selected indigenous vegetables from Southern Thailand.

2 | Materials and Methods

2.1 | Chemicals and Reagents

Dimethyl sulfoxide (DMSO) (Cat. No. D5879), boric acid (Cat. No. B0252), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Cat. No. D9132), formic acid (Cat. No. F0507), human plasma fibrinogen (Cat. No. F3879), plasmin (Cat. No. P1867), thrombin (Cat. No. T6884), and skim milk powder (Cat. No. 70166) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Acetonitrile (Cat. No. A998-1) and methanol (ACS-grade, Cat. No. M4000/17) were obtained from Thermo Fisher Scientific, Waltham, MA, USA. Thromboflux infusion (SK, 1,500,000 IU) was obtained from Cosma Medical Co. Ltd., Thailand.

2.2 | Vegetable Collection

Glochidion perakense Hook.f. (Euphorbiaceae) (accession ID: WU number 1477-1482), *Citrus reticulata* Blanco (Rutaceae) (accession ID: WU number 1483-1487), *Champereia manillana* (Blume) Merr. (Opiliaceae) (accession ID: WU number 1490-1496), *Alpinia conchigera* Griff. (Zingiberaceae) (accession ID: WU number 1472-1476), *Anacardium occidentale* L. (Anacardiaceae) (accession ID: WU number 1468-1471), and *Curcuma longa* L. (Zingiberaceae) (accession ID: BKF number 194769) were purchased as fresh products from three markets in Hua It Market (8°28'15.0"N 99°57'30.7"E), Mueang Nakhon Si Thammarat District, and Nap Anusorn Fresh Market, Thasala District (8°39'58.6"N 99°55'48.0"E), Nakhon Si Thammarat province and Po Wai Market, Mueang Surat Thani District (GP: 9°09'10.2"N 99°20'08.6"E) Surat Thani province, Southern Thailand. The six indigenous vegetables used in this study are listed in Table 1 and shown in Figure 1. The voucher specimens (Table 1) were botanically identified by the taxonomist and associate professor Dr. Chatchai Kanlayanapaphon of the Department of Biology, School of Sciences, Walailak University. These specimens have been deposited at the Forest Herbarium of the Royal Forest Department in Bangkok and the Walailak University Herbarium in Nakhon Si Thammarat, Thailand, for future reference.

2.3 | Extract Preparation

All samples were washed with water, soaked in distilled water, and sliced into small pieces of approximately 1 cm². We selected 80% (v/v) methanol to extract polar and moderately nonpolar phytochemicals (e.g., flavonoids and phenolic acids) and water to recover highly polar, water-soluble constituents [15]. Methanolic extraction was then performed as previously described, with slight modifications [6]. In brief, 50 g of each fresh, sliced vegetable was homogenized with 150 mL of 80% (v/v) methanol (1:3, w/v) for 1 min at maximum speed using a homogenizer (Tefal, Blendforce, France). The suspensions were stirred at room temperature (30°C) for 15 h. A parallel aqueous extraction was carried out by homogenizing 50 g of sliced vegetable in 150 mL of distilled water (1:3 w/v). The extraction was carried out by heat-assisted extraction and in a water bath at 50°C for 15 h. Both suspensions were sedimented by centrifugation at 700 × g for 10 min at room temperature and then filtered by using Whatman filter paper No. 1 (GE Healthcare, Buckinghamshire, UK). The solvent was removed from the extract using a rotary evaporator (Yamato, NY, USA) at 40°C. Aqueous extracts were lyophilized using a lyophilizer (Christ, Osterode, Germany) at −45°C under < 0.1 mbar vacuum for 48 h. Both dried extracts were stored at −70°C until use. Stock solutions of the extracts were prepared by mixing with 100% DMSO to a final concentration of 10 µg/µL in 1% DMSO. The suspension was then filtered with a sterilized 0.22 µm syringe filter (Merck Millipore, MS, Germany) before being used in the assay.

2.4 | Fibrinolytic Activity (FBA) Assay

FBA assay was assessed using a modified fibrin-agar-plate method [16]. Human fibrinogen (Sigma-Aldrich, MO, USA) was dissolved in a sodium borate buffer (50 mM H₃BO₃, pH 7.9) to give a final concentration of 2 mg/mL. A 2% agarose solution in sodium borate buffer (5 mL) was mixed with 5 mL of the

TABLE 1 | Selected Southern Thai indigenous vegetables and their fibrinolytic activity (FBA).

Scientific name	Common name	Local Thai name	Plant part	Accession ID	FBA (mm ²)	
					Aqueous extract	Methanolic extract
<i>Champerita manillana</i> (Blume) Merr.	False olive young	Phak-phum	Young leaves	WU No. 1490-1496	0	0
<i>Citrus reticulata</i> Blanco	Tangerine	Bai-som-paen	Young leaves	WU No. 1483-1487	0	0
<i>Alpinia conchigera</i> Griff.	Joint-whip ginger	Kha-ling	Young fruits	WU No. 1472-1476	0	0
<i>Anacardium occidentale</i> L.	Cashew	Mamuang himmaphan	Young leaves	WU No. 1468-1471	13.83 ± 2.10	12.59 ± 0.00
<i>Curcuma longa</i> L.	Turmeric	Kha-min-oan	Rhizomes	BKF No. 194769	0	0
<i>Glochidion perakense</i> Hook.f.	No English name	Mon-Pu	Young leaves	WU No. 1477-1482	12.72 ± 1.82	20.72 ± 1.54*
Positive control	Human plasmin (0.0125 mg/mL in PBS, pH 7.4)	—	—	—	56.97 ± 3.86	—
Negative control	Normal saline solution (NSS)	—	—	—	0	0
Vehicle control	1% Dimethyl sulfoxide (DMSO)	—	—	—	0	0

Note: Values are expressed as mean ± SD. FBA, fibrinolytic activity. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Human plasmin, prepared in aqueous PBS (pH 7.4), was used as the positive control and is reported only in the aqueous column. —, not applicable for methanolic. NSS and 1% DMSO served as negative and vehicle controls, respectively, and showed no fibrinolytic activity; therefore, values are reported as 0 in both aqueous and methanolic columns.

*p < 0.05 indicates a significant difference compared to other extracts showing fibrinolytic activity.

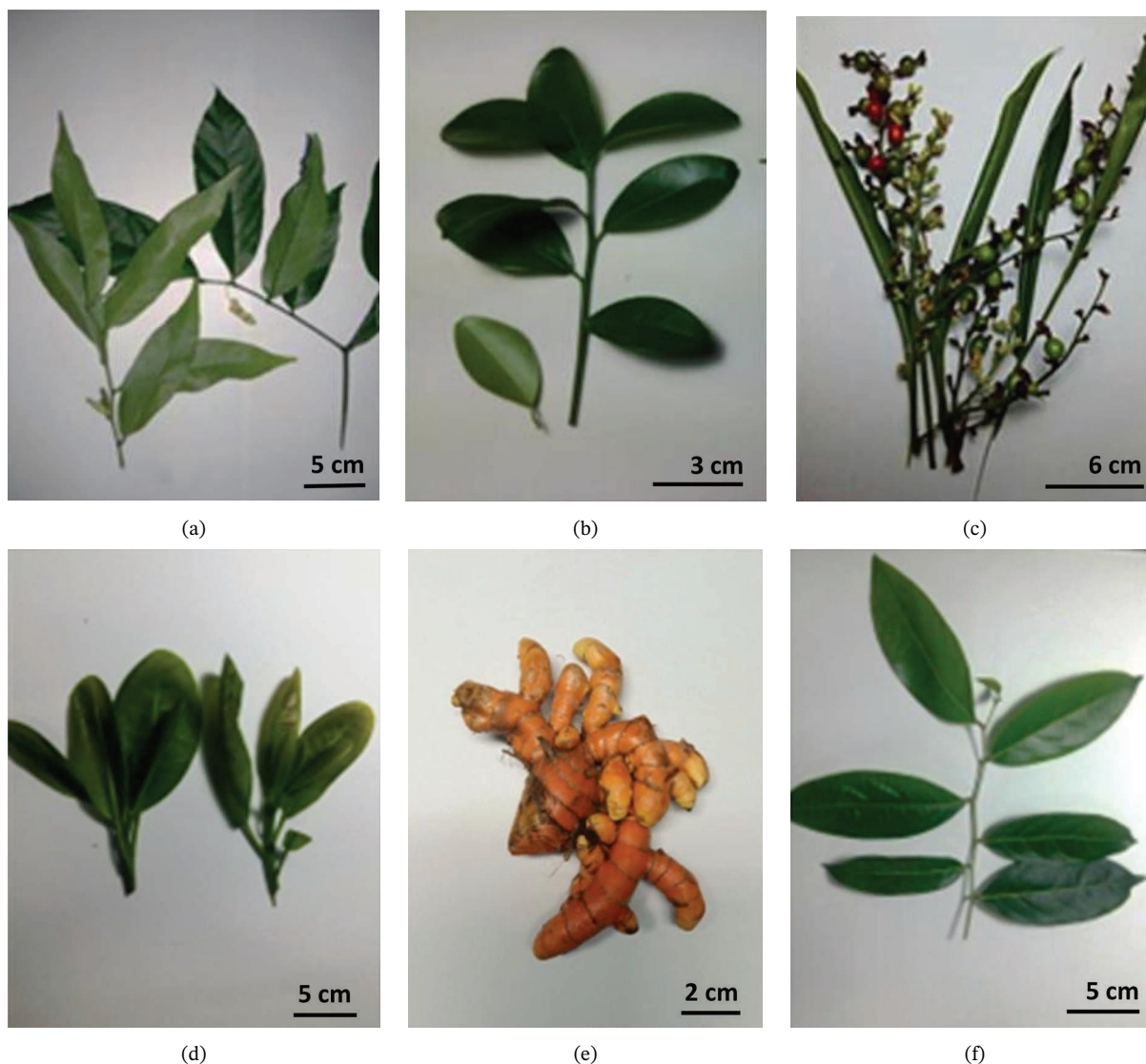


FIGURE 1 | Sample specimen of locally used six Thai vegetables. Sample specimens are identified as (a) *Champereia manillana* (Blume) Merr. (accession ID: WU number 1490-1496), (b) *Citrus reticulata* Blanco (accession ID: WU number 1483-1487), (c) *Alpinia conchigera* Griff. (accession ID: WU number 1472-1476), (d) *Anacardium occidentale* L. (accession ID: WU number 1468-1471), (e) *Curcuma longa* L. (accession ID: BKF number 194769), and (f) *Glochidion perakense* Hook.f. (accession ID: WU number 1477-1482).

fibrinogen solution at 45°C. The suspension was poured into a 9-cm Petri dish, and 200 μ L of thrombin (10 units/mL, Sigma-Aldrich, MO, USA) was added, followed by gentle mixing, inducing fibrin formation. Once the agar–fibrinogen mixture solidified and became turbid at room temperature, a hole with a diameter of 2 mm was punched using a capillary tube. A 2 μ L sample (10 μ g/ μ L) of each extract was then added to the hole, and the plates were incubated at 37°C for 18 h. Positive, vehicle, and negative controls included 0.0125 mg/mL of human plasmin (Sigma-Aldrich, MO, USA), 1% DMSO, and normal saline solution (NSS), respectively. All samples and controls were tested in triplicate. The clear zone around the sample indicates fibrin breakdown by fibrinolytic substances in the extract. The diameter of the clear zone was measured, and FBA was expressed as the area of the clear zone in mm².

2.5 | Proteolytic Activity Assay

The specificity of the FBA of vegetable extracts was investigated using an agar plate containing 1% skimmed milk [17]. To prepare the assay, skim milk powder and agarose gel were dissolved in a 50 mM sodium borate buffer (pH 7.9) to achieve a final concentration of 1% (v/w). This suspension was poured into a 9-cm Petri dish. Proteolytic activity was assessed at a concentration of 10 μ g/ μ L of the vegetable extracts. 2 μ L of each extract was applied into holes with a diameter of 2 mm, and the plates were incubated at 37°C for 18 h. Each sample was tested in triplicate. The diameter of the clear zone formed on the skim milk plate (indicating proteolytic activity, P) was measured and compared with the fibrin plate (indicating FBA, F). An F/P ratio greater than 1.0 indicates the occurrence of fibrinolysis rather than proteolysis.

2.6 | Determination of DPPH Radical Scavenging Activities

The DPPH assay was carried out as described by Rumpf et al., with slight modifications [18]. In brief, a 0.1 mL portion of extract (5, 10, 20, 40, 60, 80, and 100 $\mu\text{g/mL}$) was mixed with 3.90 mL of a DPPH-methanol solution at a concentration of 36 mg/L. After a 1-h incubation in darkness at room temperature, the absorbance was measured at 515 nm relative to a blank sample. The percentage suppression of the free radical DPPH was calculated using the formula $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$, where A_{blank} represents the absorbance of the control reaction and A_{sample} represents the absorbance in the presence of the plant extract. The IC_{50} value, indicating the concentration of extract required for 50% inhibition, was determined using the regression equation that correlates the extract concentration to the percentage inhibition. Ascorbic acid was used as a positive control.

2.7 | Collection of Human Blood Samples and Ethical Approval

Ten milliliters of venous blood were collected from ten healthy volunteers through a vein in the arm, after obtaining written informed consent from all participants. This study is part of the research titled “In Vitro Antithrombotic Effect of Indigenous Vegetable Extracts from Southern Thailand,” which was reviewed and approved by the Human Research Ethics Committee of Walailak University in accordance with the Declaration of Helsinki. The ethics approval number is WUEC-18-024-01. The study on in vitro clot lysis using human blood clots was conducted during this approved period at Walailak University, Nakhon Si Thammarat, 80,160, Thailand. Subsequently, phytochemical analysis was performed to further support and expand on the research findings.

2.8 | Volunteer Recruitment, Eligibility, and Informed Consent

The volunteers were recruited through local community announcements and underwent a screening process that included an interview about their medical history as well as recent consumption history that could significantly affect the hemostatic systems, ensuring they were in satisfactory health. The inclusion criteria required participants to be healthy adults aged 18–40 years. Exclusion criteria included individuals using medications associated with clotting abnormalities, such as antiplatelet drugs, anticoagulants, or thrombolytic agents; those with platelet disorders, including Glanzmann thrombasthenia, storage pool disease, gray platelet syndrome, or Bernard-Soulier syndrome; and those with coagulation factor disorders, such as liver disease, hemophilia, or Von Willebrand disease. Their eligibility was confirmed based on the study protocol approved by the Ethics Committee. The collection of venous blood samples was solely for research purposes and was not part of any routine medical or diagnostic procedure. Informed consent was obtained in writing from all participants prior to their involvement, ensuring they fully understood the study procedures, objectives, and potential risks. This process ensured that the study was conducted in accordance with the protocol approved by the Ethics Committee (approval number: WUEC-18-024-01).

2.9 | Thrombolytic Activity Assay

The thrombolytic activity of all vegetable extracts was evaluated by in vitro thrombolytic assay [19]. A total of 10 mL of whole blood was drawn. The first 1 mL was discarded to avoid tissue-factor contamination, and the remaining 9 mL was dispensed as 500 μL aliquots into eighteen preweighed sterile microcentrifuge tubes. To promote clot formation, the blood-containing tubes were incubated at 37°C for 45 min. Following clot formation, the serum was carefully removed using a Pasteur pipette (Thermo Fisher Scientific, MA, USA). Each tube was reweighed, and the clot weight was calculated by subtracting the weight of the empty tube from the tube containing the clot. To each clot-containing microcentrifuge tube, 100 μL of vegetable extract (10 $\mu\text{g}/\mu\text{L}$) was added. For the positive control, 100 μL of SK was added to the control tubes, while 100 μL of NSS was used as the negative control. The blood clot lysis assay was performed after incubating the tubes at 37°C for 90 min. The experiment was conducted in triplicate with blood samples from ten independent volunteers. Variability was minimized by running all treatments in parallel within each donor and normalizing each extract's lysis to that donor's control. After incubation, the fluid released from the lysed clot was separated, and the tubes were weighed again to determine the residual clot. The thrombolytic activity was expressed as a percentage of clot lysis (1).

$$\% \text{ of Clot lysis} = \frac{\text{weight of released clot}}{\text{clot weight}} \times 100. \quad (1)$$

2.10 | Phytochemical Analysis by Liquid Chromatography–Electrospray Ionization Mass Spectrometry (LC-ESI-MS)

The study was conducted with minor modifications based on a previous study [20], which utilized an inverse stage C18 column to separate phenolic acids from a methanolic extract. The sample solution (100 $\mu\text{g/mL}$) was produced using HPLC-grade MeOH solvent and analyzed by LC-ESI-MS (Agilent, Santa Clara, CA, USA). Ten microliters of the sample were put into the LC-ESI-MS equipment. Gradient elution of the mobile phase was performed at a flow rate of 500 $\mu\text{L}/\text{min}$ utilizing two eluents: H_2O acidified with 0.1% formic acid and acetonitrile. In negative ion mode, the settings employed were a source temperature of 150°C and a desolvation temperature of 450°C, with mass spectra identified utilizing the ESI negative ion mode. The Thermo Scientific Surveyor MSQ Plus software was utilized to examine spectra, which were tentatively identified by comparing retention time (RT) with mass range and international data. Gallic acid, chlorogenic acid, epicatechin, vanillic acid, p -coumaric acid, kaempferol, and quercetin were utilized as reference standards.

2.11 | Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare mean values among groups. The analyses were performed by GraphPad Prism software, Version 9.0 (San Diego, California, USA). A p value of < 0.05 was considered statistically significant.

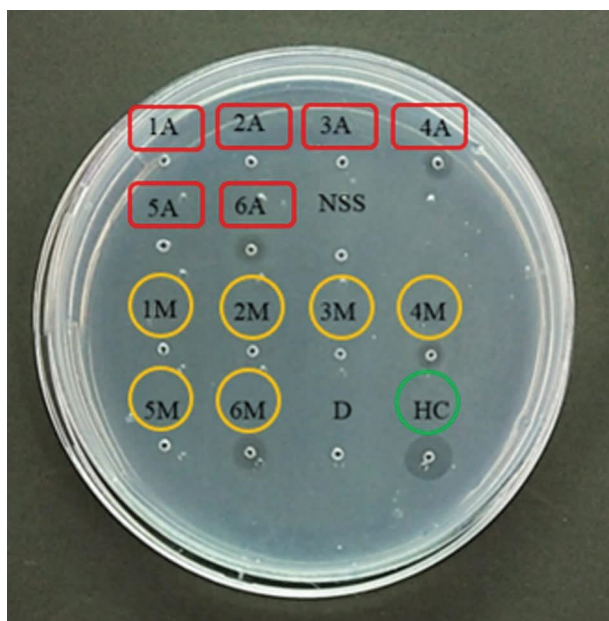


FIGURE 2 | Fibrinolytic activity of vegetable extracts by using the fibrin-agar-plate method. A, aqueous extract; 1A, *C. manillana*; 2A, *C. reticulata*; 3A, *A. conchigera*; 4A, *A. occidentale*; 5A, *C. longa*; 6A, *G. perakense*, M-methanolic extract; 1M, *C. manillana*; 2M, *C. reticulata*; 3M, *A. conchigera*; 4M, *A. occidentale*; 5M, *C. longa*; 6M, *G. perakense*; NSS, normal saline solution; D, 1% DMSO; HC, 0.0125 mg/mL human plasmin control.

3 | Results

3.1 | Effect of Vegetable Extract on the FBA

In the fibrin-agar-plate assay of the six selected vegetables, only the young leaves of Mon-Pu (*Glochidion perakense* Hook.f) and young cashew leaves (*Anacardium occidentale* L.) exhibited FBA. Human plasmin (0.0125 mg/mL) served as the positive control and displayed FBA with a clear zone of $56.97 \pm 3.86 \text{ mm}^2$. The vehicle control (1% DMSO) and the negative control (NSS) did not show any FBA. Among the extracts, the methanolic extract of Mon-Pu demonstrated the highest FBA, producing a clear zone of $20.72 \pm 1.54 \text{ mm}^2$, which was superior to its aqueous extract. However, no significant difference in FBA was observed between the aqueous and methanolic extracts of cashew (Figure 2 and Table 1).

The FBA of different concentrations (1, 2, 3, 4, 5, and $10 \mu\text{g}/\mu\text{L}$) of Mon-Pu and cashew extracts is shown in Figure 3. Both the aqueous and methanolic extracts of Mon-Pu and cashew showed dose-dependent FBA, with detectable activity even at the lowest concentration of $2 \mu\text{g}/\mu\text{L}$. Specific FBA, as determined on agar plates containing 1% skimmed milk, is presented in Table 2. The methanolic extract of Mon-Pu exhibited a high F/P ratio, suggesting that fibrinolysis was more prominent than nonspecific proteolysis.

3.2 | Thrombolytic Activity of Mon-Pu and Cashew Extracts

The thrombolytic effects of Mon-Pu and cashew were evaluated using an in vitro clot lysis assay, with the results shown in Figure 4. Both the methanolic and aqueous extracts of Mon-Pu

and cashew demonstrated significant thrombolytic activity. The highest clot lysis percentages were observed with the methanolic extract of Mon-Pu, which achieved $36.52 \pm 1.62\%$ clot lysis, followed by its aqueous extract at $27.55 \pm 1.86\%$. Similarly, the methanolic and aqueous extracts of cashew also displayed thrombolytic activity, with clot lysis values of $27.47 \pm 1.62\%$ and $24.35 \pm 1.02\%$, respectively. SK, used as the positive control, exhibited a significantly higher clot lysis percentage of $65.37 \pm 2.18\%$, which was statistically significant ($p < 0.05$) compared to the clot lysis of $4.82 \pm 0.92\%$ seen with the negative control, NSS.

3.3 | DPPH Radical Scavenging Activity

The methanol extract of Mon-Pu demonstrated the highest DPPH radical scavenging activity across most of the tested concentrations (Table 3). The effective concentration that causes a 50% decrease in the initial DPPH concentration is defined as the IC_{50} . A substance with a lower IC_{50} is considered to exhibit significant antioxidant activity. As presented in Table 4, the IC_{50} value for the methanolic extract of Mon-Pu was $3.14 \pm 0.05 \mu\text{g}/\text{mL}$, indicating substantial antioxidant activity. In comparison, the aqueous extract had an IC_{50} value of $8.33 \pm 0.27 \mu\text{g}/\text{mL}$, which reflects a comparatively lower antioxidant potential. At higher concentrations (200–400 $\mu\text{g}/\text{mL}$), both extracts displayed nearly constant inhibition values, which may be attributed to antioxidant saturation and/or limited solubility of phenolic compounds at elevated concentrations. The IC_{50} of ascorbic acid, used as a reference antioxidant, was $2.50 \pm 0.05 \mu\text{g}/\text{mL}$. These values were calculated based on the linear regression equation.

3.4 | Phytochemical Profile of Mon-Pu Extracts

The analysis of methanolic Mon-Pu extract reveals the presence of two principal phenolic compounds, gallic acid and quercetin, as evidenced by the chromatogram and mass spectrum obtained using selective ion monitoring (SIM), a technique employed for highly sensitive and quantitative analysis of specific known compounds (Figures 5 and 6). The RT mass-to-charge ratios (m/z) were 3.72 (169) and 13.71 (301), respectively, corresponding to the reference standards. On the other hand, LC-ESI-MS failed to detect chlorogenic acid, epicatechin, vanillic acid, and coumaric acid. The concentrations of gallic acid and quercetin in the extract were $15.07 \pm 1.80 \mu\text{g}/\text{g}$ and $6.48 \pm 0.41 \mu\text{g}/\text{g}$, respectively.

In addition, we identified five compounds through a comparison of RTs and fragmentation patterns from the scan mode, which is used for broad analysis to identify unknown compounds (Figures 7 and Table 5), with previously reported data available in databases such as PubMed, Google Scholar, and Web of Science.

4 | Discussion

The present findings advance the understanding of natural antithrombotic agents by demonstrating that bioactive constituents in Mon-Pu and other Southern Thai vegetables may contribute to diverse mechanisms of cardiovascular protection. These results are consistent with previous research highlighting the cardiovascular benefits of polyphenol-rich diets [21–23]. Importantly, this is the first study to report fibrinolytic and thrombolytic activities in Mon-Pu extracts, underscoring their potential as natural sources for thrombolytic agents. In addition,

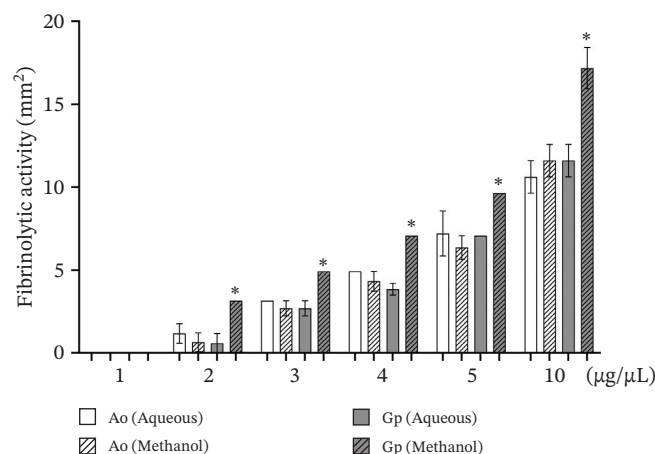


FIGURE 3 | The fibrinolytic activity of Mon-Pu and cashew extracts at various concentration data are presented as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Data were analyzed by the program GraphPad Prism (Version 9.0). * $p < 0.05$ indicates a significant difference when compared with other extracts at the same concentration. Gp: *G. perakensis* (Mon-Pu); Ao: *A. occidentale* (cashew).

the antithrombotic effects observed in cashew extracts are consistent with the earlier report of fibrinolytic and thrombolytic properties in cashew leaves [17], reinforcing the potential value of these indigenous plants for cardiovascular applications.

Thrombosis and related disorders are often linked to the production of reactive oxygen species (ROS), which contribute to oxidative stress, leading to endothelial damage, atherosclerosis progression, myocardial infarction, and ischemia-reperfusion injury [24]. Antioxidants play a crucial role in maintaining vascular health by neutralizing ROS and preventing thrombus formation. To assess the antioxidant potential of Mon-Pu extracts, the DPPH radical scavenging assay was performed. The reduction in absorbance of the DPPH solution is directly related to the concentration of antioxidants [25]. The results showed that higher concentrations of Mon-Pu extracts enhanced DPPH radical scavenging activity, indicating strong antioxidant effects. It indicates that although some heat-sensitive compounds may be lost during extraction, bioactive compounds are retained. These findings are consistent with the study by Panpipat et al. [26], which reported that Mon-Pu extracts exhibited high antioxidant activity.

Numerous studies have highlighted the effectiveness of polyphenols [27], flavonoids [27, 28], alkaloids [29], and cardiac glycosides [30], which are effective against thrombosis and coronary artery diseases. In this study, the phytochemical analysis of the methanolic Mon-Pu extract identified several

phenolic compounds, including gallic acid, quercetin, procyanidin, ferulic acid, and kaempferol. These compounds are classified as polyphenols, natural plant-derived compounds known for their antioxidant and antithrombotic properties [31]. In contrast, quinic acid and malic acid are organic acids, not polyphenols, and may play different biological roles.

Phenolic compounds, which are secondary metabolites in plants, are primary antioxidant constituents of natural plant products, encompassing both phenolic acids and flavonoids [32]. The significant antioxidant capacity of polyphenols is attributed to their multiple phenolic hydroxyl groups, which neutralize free radicals [33]. Our findings align with previous studies identifying gallic acid in *G. hypoleucum* using the same solvent extract [34]. In addition, a study has shown that the total phenolic content of *G. wallichianum* extracts was 160 mg/100 g [35]. Gallic acid, a well-known phenolic compound with remarkable antioxidant effects, has been linked to the inhibition of platelet aggregation [36]. In addition to these properties, the presence of gallic acid and quercetin in Mon-Pu extract likely contributes to its clot lysis activity. Gallic acid has been shown to exert fibrinolytic effects by enhancing t-PA activity through suppressing PA inhibitor-1 (PAI-1), thereby facilitating plasmin generation and promoting the degradation of fibrin clots [37]. While quercetin is also a constituent of the extract, the majority of evidence for its fibrinolytic action is derived from in vivo studies, which

TABLE 2 | Fibrinolytic and proteolytic activities with F/P ratios for cashew and Mon-Pu extracts.

Extract concentration (10 µg/µL)	Cashew (<i>A. occidentale</i>)			Mon-Pu (<i>G. perakensis</i>)		
	Fibrinolysis (mm ²)	Proteolysis (mm ²)	F/P	Fibrinolysis (mm ²)	Proteolysis (mm ²)	F/P
Aqueous	13.83 $\pm$ 2.10	17.31 $\pm$ 2.36	0.76	12.72 $\pm$ 1.82	12.59 $\pm$ 0.00	1.01
Methanol	12.59 $\pm$ 0.00	12.59 $\pm$ 0.00	1.00	20.72 $\pm$ 1.54	16.06 $\pm$ 2.05	1.29

Note: F/P, fibrinolysis/proteolysis ratio. Values are expressed as mean $\pm$ SD ($n = 3$).

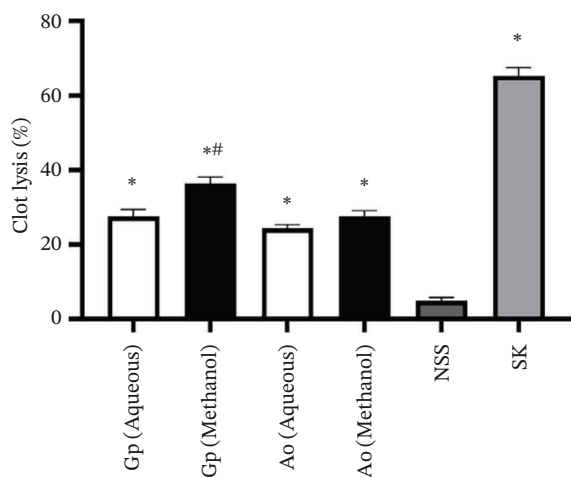


FIGURE 4 | Thrombolytic activity of Mon-Pu and cashew extracts. Data are presented as mean $\pm$ SEM. Data were analyzed using GraphPad Prism (Version 9.0). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ indicates a significant difference compared to the negative control (NSS). ** $p < 0.05$ indicates a significant difference compared to the aqueous extract of the same plant. Gp: *G. perakensis* (Mon-Pu); Ao: *A. occidentale* (cashew); SK: streptokinase. Values are mean $\pm$ SEM from triplicate analysis of ten samples ($n = 10$).

demonstrate its capacity to increase t-PA release and reduce PAI-1 expression, thus supporting thrombolytic activity [38, 39]. Therefore, the observed clot lysis effect of Mon-Pu extract may be primarily attributed to the direct action of gallic acid, with quercetin providing additional support based on its activity in biological systems.

Given these results, Mon-Pu extracts appear to be promising candidates for nutraceutical or pharmaceutical development targeting cardiovascular health. Their demonstrated antioxidant and antithrombotic activities indicate their potential application in functional foods, dietary supplements, or as adjunct therapies to reduce the risk of cardiovascular disease and thrombotic events. The identification of major bioactive compounds, including gallic acid, quercetin, and kaempferol, further strengthens their candidacy for health-promoting products.

While these findings are promising, the study's in vitro design limits direct applicability to humans. To enhance translational relevance, future research should incorporate well-established in vivo models of thrombosis and oxidative stress to comprehensively assess the bioavailability, pharmacokinetics, safety, and therapeutic efficacy of Mon-Pu extracts. Such studies may utilize animal models, including arterial or venous thrombosis models, to evaluate the effects of the extract on thrombus formation, dissolution, and associated biomarkers. In addition,

TABLE 3 | DPPH test results for Mon-Pu extracts.

Concentration ($\mu\text{g/mL}$)	% Inhibition	
	Methanolic extract	Aqueous extract
400	94.93	99.82
200	94.46	92.64
100	92.56	93.36
50	94.14	91.74
25	94.30	92.64
12.5	94.14	71.27
6.25	86.22	40.75
3.125	54.54	21.36
1.5625	36.33	14.36
0.78125	22.54	nd
0.390625	10.03	nd

Note: The data were regressed with variations in concentration as the X value and % antioxidant activity as the Y value according to the variation in the test method. The nd expressed as not detected ($n = 3$).

TABLE 4 | IC_{50} values for Mon-Pu extracts.

Mon-Pu extracts	Line equation	Y value	X value or IC_{50} ($\mu\text{g/mL}$)
Methanolic extract	$y = 12.324x + 12.084$ $R^2 = 0.9728$	50.00 —	3.14 ± 0.05 —
Aqueous extract	$y = 5.2585x + 6.1275$ $R^2 = 0.9975$	50.00 —	8.33 ± 0.27 —

Note: The data were derived using the linear regression equation $y = bx + a$, where x represents concentration and y represents % antioxidant activity. IC_{50} values (x) are expressed as mean $\pm$ SD ($n = 3$). R^2 indicates the coefficient of determination for the linear fit.

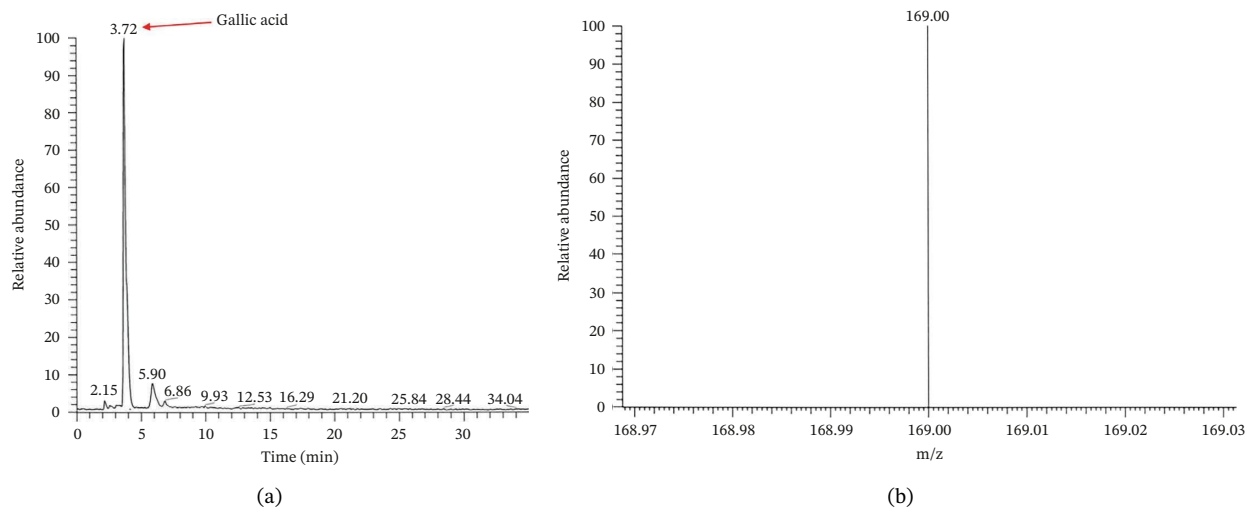


FIGURE 5 | Detection of phenolic compounds of Mon-Pu methanolic extract by LC-MS. (a) Chromatogram of gallic acid. (b) Mass spectrum of gallic acid.

TABLE 5 | Identification of compounds based on LC-ESI-MS scan mode analysis with retention times and fragmentation patterns.

RT (min)	Compound	Mass spectrometry (m/z)
2.87	Quinic acid	109.45, 191.83 , 287.72, 379.67, 405.40, 413.44
5.94	Malic acid	124.35 , 183.89, 205.82, 287.33, 433.51, 498.2
7.24	Procyanidin	141.52, 194.04, 284.21, 327.57 , 412.32, 448.58, 553.41, 513.69, 663.27
10.45	Ferulic acid	179.80, 193.76 , 223.45, 301.76, 323.57, 353.53, 414.80, 425.50
12.16	Kaempferol	51.38, 225.72, 284.20 , 301.35, 338.82, 389.04, 691.26, 730.21

Note: Gallic acid and quercetin were confirmed separately by selective ion monitoring (SIM) mode (Figures 5 and 6). RT, retention time; m/z, mass-to-charge ratio.

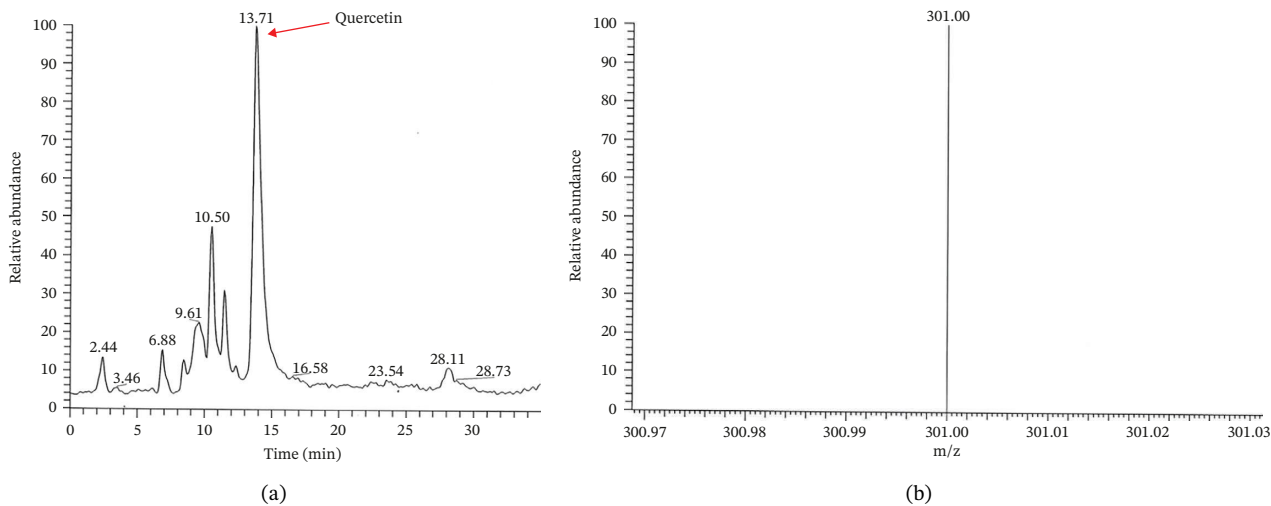


FIGURE 6 | Detection of flavonoid compounds in Mon-Pu methanolic extract by LC-MS. (a) Chromatogram of quercetin. (b) Mass spectrum of quercetin.

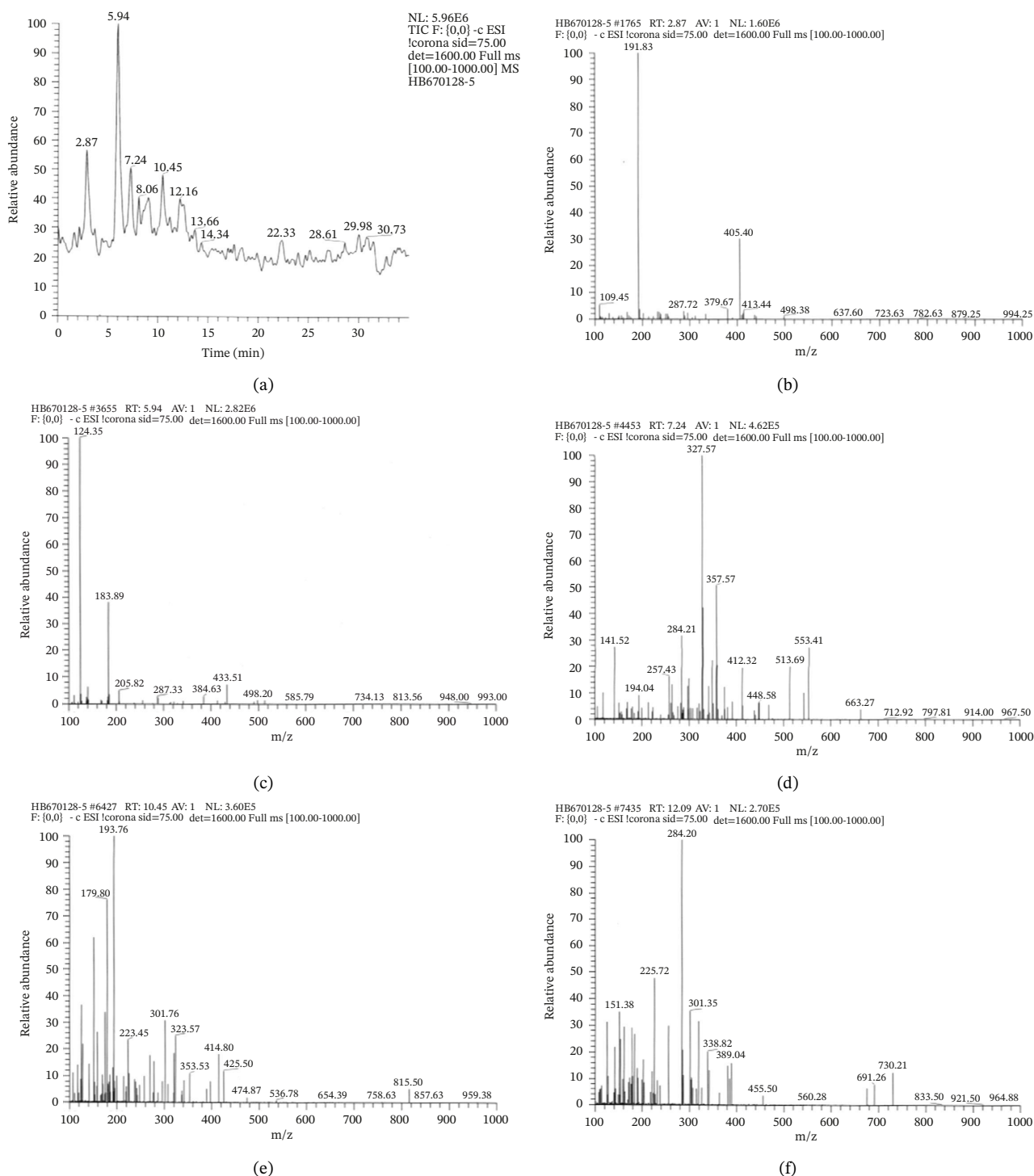


FIGURE 7 | The chromatogram and mass spectrum from LC-ESI-MS-based analysis. (a) Scan mode chromatogram, (b) m/z 191.83 at RT 2.87, (c) m/z 124.35 at RT 5.94, (d) m/z 327.57 at RT 7.24, (e) m/z 193.76 at RT 10.45, (f) m/z 284.20 at RT 12.16.

mechanistic studies in living systems are warranted to elucidate the molecular pathways underlying the observed antithrombotic and antioxidant activities, with emphasis on in vivo biomarkers such as coagulation parameters, platelet reactivity, and oxidative stress indicators. Furthermore, the phytochemical composition of *Mon-Pu* extracts may also be influenced by environmental factors including soil composition, climate, and plant maturity, which could affect both reproducibility and biological potency.

5 | Conclusions

The fibrinolytic, thrombolytic, and antioxidant activities of *Mon-Pu* extracts indicate significant potential to improve cardiovascular health through multiple mechanisms. Key bioactive polyphenols such as gallic acid and quercetin may offer an integrated approach to preventing thrombotic disorders by targeting both clot breakdown and oxidative stress. These findings

highlight Mon-Pu extracts as promising natural agents for developing functional foods or supplements aimed at reducing cardiovascular risk. Further research is needed to clarify mechanisms, confirm in vivo efficacy, and evaluate safety to support their therapeutic or nutraceutical application.

Author Contributions

Suriyan Sukati: conceptualization, project administration, supervision, methodology, investigation, resources, software, data curation, formal analysis, validation, visualization, funding acquisition, and writing—original draft. Surachai Techaoei: conceptualization, data curation, resources, methodology, investigation, formal analysis, validation, visualization, and writing—original draft. Warachate Khobjai: conceptualization, methodology, investigation, validation, visualization, editing and review, and writing—review and editing. Muhammad Junaid: conceptualization, methodology, validation, and writing—review and editing.

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Disclosure

All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author on reasonable request.

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