Review Article
Natural Products for Antithrombosis

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Received 24 October 2014; Revised 22 March 2015; Accepted 24 March 2015

Academic Editor: Angelo A. Izzo

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Thrombosis is considered to be closely related to several diseases such as atherosclerosis, ischemic heart disease and stroke, as well as rheumatoid arthritis, hyperuricemia, and various inflammatory conditions. More and more studies have been focused on understanding the mechanism of molecular and cellular basis of thrombus formation as well as preventing thrombosis for the treatment of thrombotic diseases. In reality, there is considerable interest in the role of natural products and their bioactive components in the prevention and treatment of thrombosis related disorders. This paper briefly describes the mechanisms of thrombus formation on three aspects, including coagulation system, platelet activation, and aggregation, and change of blood flow conditions. Furthermore, the natural products for antithrombosis by anticoagulation, antiplatelet aggregation, and fibrinolysis were summarized, respectively.

1. Introduction
The hemostatic system, which comprises platelet aggregation, coagulation, and fibrinolysis, is a host defense mechanism that preserves the integrity of the high pressure closed circulatory system in mammals after vascular damages [1]. Under normal physiological conditions, the thrombi formation, controlled by the regulatory system, is temporary and spatial [2–5]. However, when pathological processes overwhelm the regulatory system of hemostasis or a shift in the hemostatic balance towards the procoagulant side, thrombosis is initiated [6]. Under this hypercoagulable state, excessive quantities of thrombi will be formed, which will ultimately lead to parts or total blockage of blood vessels [7, 8]. The development of clots in the artery, vein as well as microvascular circulation is the most frequent cause of morbidity and mortality worldwide [9, 10]. The formation of thrombi in the arterial circulation usually occurs in individuals at high risk of cardiovascular diseases [11] and coronary myocardial infarction and ischemic stroke are the main results of atherosclerosis and thrombosis in the coronary arteries [12]. Furthermore, peripheral arterial diseases including mesenteric artery embolism and limb arterial thrombosis are also closely related to the arterial thrombosis. Venous thromboembolism (VTE), consisting of deep vein thrombosis (DVT) and its complication, pulmonary embolism (PE), is a relatively common condition that associated with serious symptoms [13, 14]. In reality, venous thrombosis is the second leading cause of death in patients with cancer. In addition, disseminated intravascular coagulation and microangiopathy hemolytic anemia (thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS)) are associated with microvascular thrombotic disorders [6]. Therefore, more and more studies have been focused on preventing thrombosis for the treatment of those thrombotic diseases.

In recent years, antithrombotic drugs, which can be classified into three major categories including anticoagulation, antiplatelet aggregation, and fibrinolysis, have been intensively studied and developed as potential therapeutic approaches for arterial and venous thrombosis [15, 16]. Among these clinical used drugs, heparin [17], warfarin [18], and their derivates are mainly applied in inhibition of the blood coagulation factors, while plenty of antiplatelet drugs such as aspirin (ASP), clopidogrel, and abciximab have been
used in reducing the risk of cardiovascular diseases [19–22]. Furthermore, fibrinolytic agents, such as streptokinase, tissue plasminogen activator (t-PA), and reteplase, are engaged to remove and dissolve the formed blood clots [23, 24]. Despite intense investigation over the last 40 years into the discovery and development of more effective antithrombotic drugs, the effect of these therapies on mortality rates still remained small [25]. And this situation will probably become more challenging in the future as the incidences of obesity, diabetes, and the metabolic syndromes rapidly increase. The reasons of low cure rates of these drugs mainly lie in drug resistance, limited efficacy in some patients, and side effects such as higher bleeding risk and gastrointestinal dysfunctions [26]. A study in United Kingdom, researchers indicated that the responsible drug for over 60% of the deaths caused by adverse drug reactions is ASP [27]. The side effects of ASP include bleeding, gastrointestinal toxicity, and thrombocytopenia. Cilostazol, a potent inhibitor of cyclic adenosine monophosphate-(cAMP-) phosphodiesterase 3 (PDE3), has serious side effects such as headache and palpitation [28]. Apixaban is an oral selective direct factor Xa (FXa) inhibitor and its most common adverse event is bleeding [29], and other adverse events reported are hypersensitivity reactions, syncope, nausea, dizziness, and so forth. Therefore, there is a rising urgent need for novel therapeutic approach to reduce current adverse effects of antithrombotic drugs without impairing their efficacy.

Nowadays, much effort has been focused on the discovering of natural products as effective supplements or even substitutes to those currently used antithrombotic drugs [30]. These natural products, composing of natural plants [31–33], traditional Chinese medicines (TCMs) [34, 35], and functional foods [36–38] as well as some special animal materials [39], have been found to possess remarkable antithrombotic property both in experimental and clinical stages. It is known to all that TCMs have a long history for treating many kinds of human diseases including thrombotic diseases and blood stasis syndromes. In reality, in Shennong’s Classic of Materia Medica (Shennong Bencao Jing in Chinese) [40], 83 of 365 TCMs were recorded with the function of “HuoXueHuaYu,” which means to promote blood circulation for removing blood stasis. Nowadays, there are some natural products that have been used in clinic for the treatment of thrombotic diseases. For example, Shimotsu-To, which is a combined prescription of four herbal extracts, Paeonia lactiflora, Rehmannia glutinosa, Angelica sinensis, and Ligusticum chuanxiong, has been used in clinic for improving abnormal blood coagulation, fibrinolysis, and atherosclerosis [41]. Kang naoxue shuan (in Chinese) tablet, which consists of Flos Carthami, Radix Angelicae Sinensis, Hirudo, and so forth, can protect cerebral ischemia through antiplatelet aggregation and reduction of blood viscosity [42]. Besides, Ginkgo biloba leaves tablets are widely used in treating ischemic cerebrovascular diseases [43]. The main reasons for applying natural products to the treatment of thrombotic diseases are that they comprise multiple constituents and each constituent may have multiple targets; they may exert pleiotropic and synergistic effects that have positive functions for increasing the therapeutic efficacy. Besides, the constituents of natural products usually have less side effects on the gastrointestinal system [44].

This review will provide an overview on the formation mechanisms of thrombosis and the antithrombotic properties exerted by natural products and describe the pathways by which their activities may contribute to reduce thrombotic risks.

2. The Formation of Thrombosis

Thrombus can be classified into four groups based on different positions and constituents [45]: (1) pale thrombus, mainly occurs in fast-flowing blood with numerous platelets; (2) red thrombus, constituting of fibrin and erythrocyte in slow-flowing blood; (3) mixed thrombus, a continuous process of thrombus formation; (4) hyaline thrombus (also called microthrombus), the formation of cellulose in microcirculation small vessels. On the other hand, venous thrombosis, arterial thrombosis, and microvascular thrombosis are more likely to be distinguished depending on different blood vascular systems [46].

Thrombus formation, including platelet adhesion, activation, secretion, and aggregation as well as tissue factor (TF) initiating thrombin generation and fibrin formation, is highly complex [1]. When the vessel wall is breached or the endothelium is disrupted, collagen, and TF become exposed to the flowing blood, thereby initiating formation of a thrombus. Exposed collagen triggers the accumulation and activation of platelets, whereas exposed TF initiates the generation of thrombin, which not only converts fibrinogen to fibrin but also further activates platelets [8]. In this paper, the formation of thrombi is described in brief on three aspects, including coagulation system, platelet activation, and aggregation, and the change of blood flow conditions.

2.1. Coagulation System. Blood coagulation and platelet adhesion and activation are critical for cessation of blood loss at sites of vascular injury in the high-pressure closed circulatory system [47]. Upon vessel injury, coagulation system can be activated via either the contact activation (or intrinsic) pathway or by the TF (or extrinsic) pathway and converge on a common (intrinsic + extrinsic) pathway, which starts at the level of factor X (FX) to lead to thrombin and fibrin formation [48]. The extrinsic pathway is initiated by excessive exposure of TF which is a 263-residue membrane-bound glycoprotein [49] and as receptor and cofactor for factor VII (FVII) and its active form VIIa (FVIIa) [3, 50, 51]. On binding of FVIIa to TF, complex (TF-FVIIa) acquires catalytic activity and converts factors IX (FIX) and X (FX) to their active derivatives factors IXa (FIXa) and Xa (FXa), respectively [52]. Simultaneously, the intrinsic pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen, prekallikrein, and FXII. FXII firstly becomes FXIIa; and FXIIa converts FXI to FXa. FXa activates FIX, which with its cofactor FVIIIa forms the tenase complex and then activates FX to Fxa [53]. In the common pathway, Fxa derived from both intrinsic and extrinsic processes with FVa on membrane surface in complex with prothrombinase complex activates thrombin
formation which finally converts fibrinogen to fibrin polymers [54, 55] (Figure 1).

2.2. Platelet Activation and Aggregation. The intact vascular endothelium is a semipermeable barrier that controls the diffusion of plasma molecules, regulates vascular tone and inflammatory, and releases gaseous signal molecule including nitric oxide (NO) and prostacyclin (PGI₂) as well as endothelial CD₃₉ to prevent platelet aggregation or dilate blood vessels under physiological conditions. However, dysfunctional or impaired endothelium is characterized by the loss of such antiplatelet properties and tends to mediate and accelerate thrombosis. The exposure binding sites of collagen and von Willebrand factor (vWF), a multimeric plasma glycoprotein, allow the platelet membrane glycoprotein (GPIb-IX-V or GPVI) to adhere on it in the first place. After the initial adhesion of platelets to the extracellular matrix, platelets undergo shape change and the activation process requires a rapid response to autocrine and paracrine mediators, including adenosine diphosphate (ADP), thrombin (THR), epinephrine, and thromboxane A2 (TXA₂) [56]. Furthermore, platelet granule secretions lead to the local release of ADP/adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT), Ca²⁺, adhesion proteins (e.g., fibrinogen, fibronectin, thrombospondin, vitronectin, P-selectin, and GPIIb/IIIa), and coagulation factors (factor V, factor XI, plasminogen activator inhibitor type I, plasminogen, and protein S), all of which contribute to perpetuate and amplify the thrombotic response [57]. These platelet agonists binding to specific membrane receptors (e.g., collagen binds to GPVI or α₂β₁, THR interacts with protease activated receptors, and ADP binds at least two ADP receptors on platelets) [58–60] activate phospholipase Cβ (PLCβ), resulting in the production of diacylglycerol (DAG) and inositol trisphosphate (IP₃). DAG and IP₃ activate protein kinase C (PKC) and mobilize cytoplasmic Ca²⁺, respectively. Then TXA₂ is produced as a consequence of increased cytoplasm Ca²⁺-levels and the high concentration of Ca²⁺ is necessary for the activation of PLA2 through phosphorylation by p-38 mitogen-activated protein kinase (MAPK) [61]. Platelet aggregation is regulated in the final part of the pathway by activation of the platelet heterodimer GPIIb/IIIa receptor, the most abundant proteins on the platelet surfaces. Fibrinogen, the main ligand for the GPIIb/IIIa receptor, binding to GPIIb/IIIa also triggers an “inside out” signaling, causing amplification of the initial signal and further platelet activation. In the final phase of thrombus formation, fibrinogen is converted to fibrin by thrombin, leading to the stabilization of the platelet aggregates with more platelets and blood cells (leukocytes and red blood cells), thus getting trapped and contributing to growth of thrombus [62].

2.3. Change of Blood Flow Conditions. Physiologically, plasma separates blood vessel from the tangible components such as erythrocyte, leukocyte, and platelet in blood. Once the blood flow slows down, platelet will move to the edge of blood.
vessel as well as adhere to the impaired endometrial, coagulator factors will be activated, and thrombin accumulates and amounts to a high concentration to facilitate thrombus formation. Furthermore, the blood viscosity [63], which will result in a lower erythrocytic deformability and a stronger platelet aggregation, will increase under slow blood flow condition. This cycling process between increasing erythrocytic deformability and slowing down blood flow finally promotes the adherence and aggregation of platelet. As a result, it is easy to form thrombus in vein with slow blood flow, where the concentration of coagulation factors and thrombin are very high locally [64, 65]. On the contrary, in artery where coagulation factors and thrombin can be scattered by fleet blood flow and it is less likely to achieve effective concentrations, so the thrombus formation in artery mainly relies on the adherence, activation, and aggregation of platelet rather than the impacts of coagulation factors and thrombin [66].

3. Antithrombotic Effects of Natural Products

Studies have demonstrated that natural products become increasingly crucial in reducing the thrombotic risks and treating various cardiovascular diseases. As previously mentioned, drugs for treating thrombosis can be divided into three categories: (1) anticoagulants, which prevent the coagulation system and interfere with further plaque expansion; (2) antiplatelet agents, which decrease platelet aggregation and inhibit thrombus formation; (3) fibrinolytic drugs, which dissolve the formed thrombus directly [67].

3.1. Anticoagulation. The extrinsic and intrinsic coagulation systems are initiated after vascular disruption via TF and collagen, respectively [8]. In clinical treatment, inhibition of coagulation system is an effective way to prevent the pathological thrombus formation.

3.1.1. Inhibition of Tissue Factors. TF as a membrane protein and the main initiator of the coagulation cascade is essential for thrombus formation [68]. TF expression in endothelial cells is induced by different inflammatory mediators including tumor necrosis factor- (TNF-)-α [69], interleukin- (IL-) 1β [70], or histamine [71]. In reality, reducing TF expression significantly impairs thrombus formation, and agents focused on inhibition of TF activation become increasingly used effective clinical methods to treat coagulation diseases.

It has been reported that Chaenomeles sinensis has antithrombotic and antiplatelet aggregation activities [72]. Thirteen components were isolated and purified from the fruits of C. sinensis and five of them including hoverchertoside C (IC_{50} = 14.0 μg), luteolin-7-O-β-D-glucuronide (IC_{50} = 31.9 μg), hyperin (IC_{50} = 20.8 μg), avicularin (IC_{50} = 54.8 μg) and quercitrin (IC_{50} = 135.7 μg) can inhibit the TF expression of rat plasma after the addition of CaCl_{2} in vitro. Furthermore, the TF inhibitory activity of the C-ring pentacyclic flavonol was evidently stronger than that of C-ring hexacyclic flavonol [73]. Rhizoma Ligustici Chuanxiong (with the main active component ligustrazine) is widely used in treating cardiovascular diseases, pulmonary hypertension, chronic renal failure and liver cirrhosis [74]. Shang et al. reported the inhibitory effects of ligustrazine on the expression of TF and vWF in human blood induced by THR in vitro. The result showed that ligustrazine suppressed TF expression not only in quiescent condition but after being induced by THR, and also decreased vWF formation after being induced by THR. These results provide a scientific basis for Rhizoma Ligustici Chuanxiong to be used as an antithrombotic agent [75]. In addition, a sesquiterpene glycoside (3-O-α-L-rhamnopyranosyl-(→4)-α-L-rhamnopyranosyl-(1→2)-α-L- (4-trans-feruloyl)-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl) isolated from the leaves of Erióbrotus japonica Lindley (Rosaceae) showed a strong TF inhibitory activity (IC_{50} = 2 μM) in vitro and another component ferulic acid illustrated a weak inhibitory activity (IC_{50} = 369 μM). This active sesquiterpene glycoside was composed of three parts including nerolidol, carbohydrate and feruloyl moieties, and the nerolidol moiety was mainly responsible for the inhibitory effect against TF [76].

In addition, estrogen replacement therapy could protect cardiovascular system and decrease the incidence of related diseases [77]. α-Zearanol (ZAL), which is one of the natural phytoestrogens usually found in beans and grain, could decrease the contents of TF and its expression on vascular endothelium in rat plasma ex vivo with similar to or better than that of positive drug 17β-estradiol [78].

3.1.2. Inhibition of the Coagulation Pathways. The pathways of the coagulation system mainly consist of two distinct cascades (intrinsinc and extrinsic coagulation pathways) ultimately contributes to the formation of the key protease thrombin which in turn converts fibrinogen into fibrin to stabilize the formed platelet-rich plug. In experiment models, activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) are tested to indicate the activation of intrinsic, extrinsic and their common (intrinsic + extrinsic) pathway, respectively [79]. The anticoagulation effects by inhibition of the coagulation pathways of natural products are summarized in Table 1.

The green algae Monostroma arcticum (MA), with polysaccharide as its important bioactive substance, is widely distributed in China. A polysaccharide HAF0 (average molecular weight of 9.36 kDa) isolated from MA showed the inhibition effect on the intrinsic and/or common coagulation pathway with prolonging APTT and TT [80]. Polygala fallax Hesm. (PFH) is used as a folk medicine for antiaging, preventing myocardial ischemia and regulation of immune system. The anticoagulation and antithrombotic effects of the total saponins from PFH was mainly contributed to the inhibition of intrinsic coagulation system by prolonging APTT, plasma recalcification time (RT) as well as THR-induced fibrinogen clotting time, but did not impact on PT [81]. In reality, the anticoagulation mechanisms for most of the drugs mainly rely on inhibition of both intrinsic and extrinsic, or common coagulation pathways. Hyperoside, isolated from the leaves of Rhododendron brachycarpum, was observed ex vivo in mice with dose-dependent prolongation of the APTT and PT as well as inhibited platelet aggregation induced by THR and collagen in vitro, ADP in vivo [82]. Polysaccharide from Umbilicaria esculenta inhibited the thrombus formation...
Table 1: Inhibition on the coagulation pathways of natural products.

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IN, EX, and CO represent for intrinsic, extrinsic, and common coagulation pathways, respectively; APTT: activated partial thromboplastin time; TT: thrombin time; PT: prothrombin time; RT: recalcification time; CT: coagulative time; BT: bleeding time.

in a dose-dependent manner using an arteriovenous shunt thrombosis model in rats, and the more prolongation of APTT suggested a more obvious inhibition of the intrinsic than the extrinsic coagulation systems [83]. Withaferin A (WFA), an active compound from Withania somnifera, is widely studied on its effects on inflammatory, cardiovascular and central nervous system [84]. It is reported that WFA significantly prolonged APTT as well as PT, inhibited the activities and production of thrombin and FXa following extending in vivo and ex vivo bleeding time, and inhibited the production of TNF-α induced plasminogen activator inhibitor type 1 (PAI-1), an important component of the coagulation system that down-regulates fibrinolysis in the circulation [85]. Those results indicated that WFA possessed antithrombotic activities and might be developed as a new anticoagulant agent [86]. Wogonin (WGN) as well as its metabolite wogonoside (WGNs) is the flavonoids from Scutellaria baicalensis Georgi [87]. Treatment with WGN and WGNs resulted in prolonging APTT and PT as well as inhibition of the activities and production of THR and FXa in tumor necrosis factor- (TNF-) α activated human umbilical vein endothelial cells [88]. Pawlaczyk et al. studied the anticoagulant and antiplatelet activities of different fractions of Erigeron canadensis L. The mixture parts of polysaccharide-polyphenolic macromolecules inhibited both intrinsic and extrinsic coagulation pathways, as well as platelet aggregation.
induced by collagen in vitro. While in the carbohydrate part, only glucuronic acid and galacturonic acid showed weak anticoagulant activity [89]. In addition, the anticoagulant effect of total glycosides of paenony included prolonging APTT, PT, and TT in vitro confirmed that intrinsic, extrinsic, and common coagulation pathways were all inhibited [90].

3.2. Anti-Platelet Aggregation. The inhibition of platelet function has been widely studied for a long time in an effort to prevent and treat thrombosis, especially in antiplatelet aggregation. Andrographolide, the active component of Andrographis paniculata, could inhibit PAF-induced human blood platelet aggregation in a dose-dependent manner (IC50 = 2 μM) [91]. Bupleurum from the aerial parts of Bupleurum falcatum showed an 8-fold potent inhibitory effect (IC50 = 47.5 μM) compared to that of ASP (IC50 = 420 μM) on collagen-induced platelet aggregation, and comparable inhibitory effects as ASP on AA-induced platelet aggregation [92]. In Maione's study, Tanshinone IIA (TIIA) selectively inhibited rat platelet aggregation induced by reversible ADP stimuli (3 μM) in a concentration-dependent manner (0.5–5 μM). Nevertheless, TIIA was less active against the aggregation induced by irreversible ADP (10 μM) and collagen (10 μg/mL) stimuli [93]. Apart from single bioactive component, studies have also provided evidences for antiplatelet aggregation effects of crude extracts of natural products. The 80% aqueous-ethanol extract of Abies webbiana was found to inhibit both ADP- and epinephrine-induced human platelets aggregation, thereby suggesting therapeutic potential of this plant against thromboembolic conditions [94]. In Gadi's study, crude aqueous extract (CAE) of parsley was evaluated for its antiplatelet aggregation activity in rats in vitro and ex vivo. CAE dose-dependently inhibited platelet aggregation in vitro induced by THR, ADP, collagen and epinephrine. The oral administration of CAE (3 g/kg) significantly (P < 0.001) inhibited platelet aggregation ex vivo and prolonged bleeding time (P < 0.001) without changes of the platelet amount [95]. In terms of the mechanisms for antiplatelet therapies, they are mainly composed of platelet membrane protein inhibitors, impacting nucleotide and arachidonic acid system as well as inhibition of platelet granules secretion.

3.2.1. Inhibition of Platelet Membrane Receptors. Development of definite platelet receptor inhibitors contributed to clinical treatment of antiplatelet aggregation, for example, ADP P2Y12 receptor antagonists include ticlopindole and clopidogrel; GPIIb/IIIa antagonists include abciximab, tirofiban, and eptifibatide [96]. Based on the variety of protein structures, functions and ligand properties, platelet receptors can be classified into three groups including integrin, adhesion and agonist receptors. A large number of natural products and their constituents are reported as platelet receptors antagonists (Table 2).

GPIIb/IIIa, a heterodimeric receptor of the integrin family expressed at high density (50000–80000 copies/cell) on the platelet membrane, determines the final process during platelet aggregation. So many new antiplatelet aggregation drugs mainly focus on inhibition of this dominant receptor [151]. Spatholobus suberectus is a widely used TCM to promote blood circulation for the treatment of diseases related to the blood stasis syndromes [152]. It has been demonstrated that 95% ethanol extract of S. suberectus significantly inhibited ADP- and collagen-induced platelet aggregation in human platelet by inhibiting fibrinogen binding to the GPIIb/IIIa receptor and further suppressing the formation of TXA2 [106]. Garlic is a common used spicy food all over the world, and a garlic preparation aged garlic extract (AGE) is reported to have inhibition effect of platelet aggregation [153]. Allison et al. [113] investigated the antiplatelet aggregation mechanism of AGE by testing their adhesion to fibrinogen using Rose Bengal and 51Cr uptake, fluorescence activated cell sorting (FACS) analysis and measurement of intracellular cAMP contents in human platelet after induced by ADP. The results showed that AGE at concentrations of 3.12% to 12.5% (v/v) can inhibit the binding of platelets to fibrinogen by approximately 40% in the Rose Bengal assay (P < 0.05) as well as 61.5%–72% in the 51Cr experiments (P < 0.05), and significantly decrease the amount of PAC-1 binding to GPIIb/IIIa by approximately 72% in the FACS analysis with increasing platelet cAMP (P < 0.01) level. These findings suggested that AGE inhibits platelet aggregation via inhibition of the GPIIb/IIIa receptor and an increase of cAMP level. In Jeon's study, two bioactive compounds isomaltol and pentagalloyl glucose were separated from bark of Rhus verniciflua Stokes, and their antiplatelet mechanism were evaluated using receptor expression on platelet membranes, including GPIIb/IIIa (CD41), GPIIIb/IIIa-like expression (PAC-1) and P-selectin (CD62), and intracellular calcium mobilization responses. The results indicated that pentagalloyl glucose had a significant inhibitory effect on the expression of P-selectin, but isomaltol had no such effect. Furthermore, isomaltol and pentagalloyl glucose decreased the expression of GPIIb/IIIa, which appeared to have anti-GPIIb/IIIa activity [118].

Adhesion receptors, which mainly refer to collagen receptors, mediate the platelet binding to injury endothelium including α2β1 (GPIIa/IIa) and GPVI. Glaucocalyx A (GLA) is a biologically active ent-kauranoid diterpenoid isolated from Rabdosia japonica var. glaucocalyx, a traditional Chinese medicinal herb. GLA can significantly inhibit platelet aggregation in response to most of the platelet agonists including collagen, THR and ADP [154]. The inhibitory effect of GLA on collagen-stimulated platelet aggregation was notably potent, even occurred at as low as 0.01 μg/mL. GLA inhibited platelet aggregation induced by collagen-related peptide (CRP), a GPVI specific agonist in a dose-dependent manner and reduced collagen-induced phosphorylation of three major molecules, tyrosine kinase Syk, LAT, and phospholipase Cγ2 in GPVI signaling pathway. Therefore, GLA can be developed and used as an collagen receptor antagonist for antiplatelet aggregation [108]. Salvianolic acid B (SB) is an active component isolated from Danshen (Salvia miltiorrhiza), a TCM widely used for the treatment of cardiovascular disorders. Ma et al. demonstrated that α2β1 might be one of the direct target proteins of SB on platelets, and the signal cascade network of SB after binding with integrin α2β1 might include regulation of intracellular Ca2+ level, cytoskeleton-related proteins such as coronin-1B and...
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ADP: adenosine diphosphat; PAF: platelet activating factor; THR: thrombin; AA: arachidonic acid; SFLLRN: thrombin receptor activating peptide; GP IIb/IIIA: Glycoprotein IIb/IIIA; TXA₂: thromboxane A₂; TXB₂: thromboxane B₂; cAMP: cyclic adenosine monophosphate; (3H)SQ29548: TXA₂ receptor antagonist.
cytoskeleton structure of platelets [109]. A traditional Korean formula called modified Je-Ho-Tang (MJHT), which is composed of Mume Fructus, Amomi Tsako Fructus, Santali Albi Lignum and Amomi Fructus, could promote blood flow and eliminate blood stasis. The hot-water extract of MJHT dose-dependently inhibited collagen-induced whole blood aggregation and adhesion by shear stress in flow conditions. Besides, the extract significantly inhibited the conformational change of GPIIb/IIIa (PAC-1), the activation of P-selectin and mobilization of platelet Ca\(^{2+}\) [120].

Once adhere to the sites of vascular injury, platelets are involved in the process of activation and aggregation by releasing of agonists such as ADP, 5-HT, TXB\(_2\) to amplify the thrombus. Therefore, inhibition of the agonist receptor can attenuate the formation of thrombus. Two active components, acetin and II-3,1-5,II-5,II-7,1-4',II-4'-hexahydroxy-(1-3,II-8)-flavonyllavananol from the leaves of Garcinia nervosa var. pubescens King, showed strong inhibitory effects on platelet-activating factor (PAF) receptor [111]. Another agonist receptor of THR could be strongly inhibited by Eryloside F, a novel steroidal disaccharide metabolite of Erylosis formosus, and finally led to inhibit human platelet aggregation in vitro [117]. Piper longum L. has been used as a crude drug to improve intestinal disorder as well as the activity of peripherally poor blood circulation in Asia [155]. Piperlongumine, a constituent of P. longum, could concentration-dependently inhibited platelet aggregation induced by TXA\(_2\) receptor agonist U46619, but slightly inhibited THR-induced aggregation. Piperlongumine also inhibited U46619-induced phosphatidylinositol hydrolysis and the binding of (H)SQ29548 (TXA\(_2\) receptor antagonist) to TXA\(_2\) receptor, so it is assumed that piperlongumine act as a TXA\(_2\) receptor antagonist to inhibit platelet aggregation [119]. Pomolic acid (PA), a triterpenoid isolated from Licania pittieri, has shown a potent ability to inhibit ADP- and epinephrine-induced human platelet aggregation. According to the mechanism study, PA could be a potent competitive antagonist of P2Y\(_{12}\) receptor [121].

3.2.2. Impacting on Nucleotide System. CAMP plays a modulatory role in PLC-mediated secretion and aggregation of human platelets. The levels of cAMP are tightly controlled and dependent on both its synthesis rate by adenylate cyclase (AC) and its hydrolysis rate by PDE [156]. In addition, cAMP levels may be increased by peroxisome proliferator-activated receptors (PPARs) activation [157]. Intracellular cyclic guanosine monophosphate (cGMP) levels are rapidly increased by soluble guanylyl cyclase (sGC), which modulates multiple signaling pathways, including cGMP-dependent receptor proteins, cGMP-regulated PDE and cGMP-dependent protein kinases. The increasing in cGMP levels is accompanied by a decrease in intracellular Ca\(^{2+}\) mobilization while the decrease in Ca\(^{2+}\) levels inhibits the conformation change of GPIIb/IIIa into its active form and thus decreases platelet binding to fibrinogen [158]. In a word, the increasing in cAMP and cGMP levels may exert a strong platelet inhibitory effect by decrease of intracellular Ca\(^{2+}\) levels.

Cordycepin (3’-deoxyadenosine), the major active component in Cordyceps militaris, has significant inhibition effect on human platelet aggregation. Cordycepin may increase cAMP and cGMP levels and subsequently inhibit the intracellular Ca\(^{2+}\) as well as TXA\(_2\) but without affecting on PLC-γ2 or IP\(_3\) [159]. In another study, cordycepin-enriched (CE-) WB801C from Cordyceps militaris dose-dependently inhibited ADP-induced platelet aggregation with IC\(_{50}\) of 18.5 µg/mL. The possible inhibition mechanism was that CE-WB801C elevated cAMP involved in IP\(_3\), R (Ser\(^{157}\)) phosphorylation to inhibit Ca\(^{2+}\) mobilization and VASP (Ser\(^{157}\)) phosphorylation to inhibit α\(_{IIb}/β_3\) activation [160]. The ancient plant Ginkgo biloba possesses many biological activities such as radical scavenging, blood flow improvement and vasoprotection. Ginkgolide C, one of the active components in G. biloba, can significantly increase the formation of cAMP and cGMP as well as suppressing the level of intracellular Ca\(^{2+}\) and TXA\(_2\). In addition, zymographic analysis confirmed that pro-matrix metalloproteinase-9 (pro-MMP-9, 92-kDa) released from human platelets can be activated by Ginkgolide C to form an activated MMP-9 (86-kDa), which can significantly inhibit platelet aggregation stimulated by collagen [161]. Furthermore, another active component of G. biloba, queratin prevented platelet aggregation by inhibition of PDE\(_3\) [162]. It should be mentioned that PDEs can limit the intracellular levels of cyclic nucleotides by catalyzing the hydrolysis of cAMP and cGMP, thus regulating platelet function. The inhibition of PDEs may therefore exert a strong platelet inhibitory effect [163]. Oligoparin A from Oligoporus tephroleucus, an edible mushroom cultivated in Korea, inhibited collagen-induced platelet aggregation in a concentration-dependent manner, but not affecting ADP- and THR-induced platelet aggregation. Further study revealed that oligoparin A can induce the dynamic increase of cAMP and cGMP in platelet. Rat blood in vitro pretreatment with oligoparin A significantly blocked collagen-induced ERK2 phosphorylation as well as diminished the binding of fibrinogen to its cognate receptor, integrin α\(_{IIb}/β_3\) [164].

3.2.3. Inhibition of Platelet Granules Secretion. Platelet granules mainly consist of α-granules, dense granules and lysosomes which serve an essential role in promoting platelet aggregation by releasing numerous activated factors such as Ca\(^{2+}\), 5-HT, ATP, ADP, P-selectin, and so forth [165]. Inhibitions of platelet granules secretion by natural products are summarized in Table 3.

The concentration of cytosolic Ca\(^{2+}\) plays a fundamental role in mediating dense granule release and platelet aggregation. Crocetin, a major ingredient of saffron, against platelet aggregation were mainly contributed to inhibiting Ca\(^{2+}\) mobilization via reducing both intracellular Ca\(^{2+}\) release and extracellular Ca\(^{2+}\) influx, as well as inhibiting secretion of 5-HT, an independent risk factor for platelet aggregation and for thrombus formation [122]. Geiji-Bokryung-Hwan (GBH), Korean traditional formulation, consisting of Cinamomoni Ramulus, Poria Cocos, Mountain Cortex Radicis, Paeniae Radix and Persicae Semen. GBH potently inhibited thrombin, CRP, U46619 (a TXA\(_2\) mimic), ADP, or SFLLRN
Table 3: Inhibition of the platelet granules secretions of natural products.

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ADP: adenosine diphosphate; THR: thrombin; AA: arachidonic acid; CRP: collagen-related peptide; 5-HT: 5-hydroxytryptamine; IP3: inositol-1,4,5-trisphosphate; TXA\(_2\): thromboxane A\(_2\).

(a thrombin receptor agonist peptide) induced platelet aggregation by acting on a certain step of the signal transduction pathway. Park et al. confirmed that GBH inhibited IP3-mediated Ca\(^{2+}\) mobilization without altering tyrosine phosphorylation of PLC-γ2 [124]. Magnolol was isolated from Magnolia bark for the treatment of anxiety, neural and cardiovascular disorders [166], the antiplatelet aggregation mechanism of magnolol contribute to an inhibitory effect on 5-HT releasing [126]. Curdione, one of the major sesquiterpene compounds from Rhizoma Curcumae, had a potent protective effect on acute liver injury in mice and potentially to be an active constituent for strengthening the anti-inflammatory or cancer chemo-preventive capacity [167].

In the antiplatelet aggregation test, curdione preferentially inhibited PAF- and THR-induced platelet aggregation in a concentration-dependent manner (IC\(_{50}\) = 60–80 µM). Curdione can inhibit P-selectin expression, intracellular Ca\(^{2+}\) mobilization as well as causing an increase of cAMP levels in PAF-activated platelets [132].

P-selectin, shows a crucial function in mediating platelet adhesion to the damage vessels, is localized in the α-granules and released when activation of platelet. Black soybean (BB) significantly inhibited collagen-induced platelet aggregation by attenuating 5-HT secretion and P-selectin expression, as well as inhibiting TXA\(_2\) formation in vitro [125]. Ligustrazine ferulate, the main active component of Rhizoma Ligustici Chuanxiong had distinct antithrombotic effect. Ligustrazine ferulate reduced the expression of platelet P-selectin as well as suppression of platelet adhesion to neutrophil [127]. Soshiho-tang (SH), which consists of seven herbal drugs, had antithrombotic and antiplatelet activities. Lee et al. reported that SH significantly inhibited various agonist-induced platelet aggregations and completely inhibited 5-HT secretion and TXA\(_2\) formation. Furthermore, SH presented antithrombotic activity by prolonging the occlusion time of thrombus formation when applied in a FeCl\(_3\)-induced thrombus formation model [123]. Fuentes et al. demonstrated for the first time that guanosine from Solanum lycopersicum
possessed antiplatelet (secretion, spreading, adhesion and aggregation) activity induced by ADP as well as collagen 
in vitro and inhibited platelet inflammatory mediator of atherosclerosis (sCD40L), while depression of CD40L
expression can prevent thromboembolic-related disorders [131].

3.2.4. Impacting on Arachidonic Acid System. TXA₂, intensely induces platelet activation and vasoconstriction, is generated from arachidonic acid (AA) which released when membrane phospholipids are broken down by diverse agonists such as collagen, thrombin and ADP. The enzymes related to TXA₂ production are cyclooxygenase (COX-1) and thromboxane synthase (TXAS), which are located at microsomes. COX-1 produces prostaglandin (PGG₂) from substrate AA, TXAS produces TXA₂ from PGG₂ that oxidized from PGG₂ by
endoperoxidase. Therefore, inhibition of COX-1 or TXAS is a very useful marker to evaluate the antiplatelet effect of
compound. For instance, COX-1 inhibitor aspirin and TXAS inhibitor ozagrel are being used as antiplatelet agents [168].
Another metabolic pathway of AA is the lipoxygenase (LOX) pathway that forms hydroxycisatetraenoic acids (HETE)
and leukotrienes. TXB₂ and 6-keto-PGF₁₀ are the stable metabolites of TXA₂ and PGI₂, respectively. When the
ratio of TXA₂/PGI₂ is above normal conditions, thrombus formation will occur. On the other hand, when the ratio of
TXA₂/PGI₂ is lower than normal conditions, the processes of platelet aggregation or thrombus formation will be self-
limited and a bleeding tendency may occur. A variety of natural products (Table 4) including berberine [138], hesperetin
[139] and ethyl acetate extract of Caesalpinia sappan L. [145] inhibited platelet aggregation by keeping balance of TXA₂
and PGI₂.

As mentioned above, interference of the activation of the associated enzymes such as COX-1, COX-2, TXAS and
LOX during arachidonic acid pathway is regarded as an effective way to inhibit platelet aggregation. Obovatol, a major
biphenolic component of Magnolia obovata leaves, presented antiplatelet activity by inhibiting COX-1 and LOX activities
to suppress production of TXB₂, PGE₂ and 12-HETE [136]. Morroniside, extracted and purified from Cornus officinalis
Sieb.et Zucc, significantly inhibited the activation of COX as well as TXB₂ generation, and had a selective antiplatelet effect
on ADP-induced aggregation [146, 147]. Coy et al. isolated 26 neolignans (14 bicyclooctane-type and 12 benzo[4,5]furan-
type) from three Lauraceae species (Pleuranthodythrum cinereum, Ocotea macrophylla, and Nectandra amazonum) and evaluated
their antiplatelet aggregation property in vitro through inhibition of COX-1, COX-2, 5-LOX and agonist-induced
aggregation of rabbit platelets. The results showed that benzo[4,5]furan neolignans were found to be the COX-2 selective
inhibitors, whereas bicyclooctane neolignans selectively inhibited the PAF-action as well as COX-1 and 5-LOX.
The neolignan 9-nor-7, 8-dehydro-isolicarbin B, and cinerin C were found to be the most potent COX-2 inhibitor and
PAF-antagonist, respectively. In addition, nectamazin C (bicyclooctane-type neolignan) exhibited dual 5-LOX/COX-
2 inhibition [148]. Abe et al. screened for inhibitors of human platelet aggregation and human 5-LOX from the Myoga
(Zingiber mioga Roscoe) extracts. Experimental results indicated that miogatrial, miogadial, sesquiterpene and polygo-
dial were potent inhibitors of human platelet aggregation and human 5-LOX, and their 3-formyl-3-butenal structure was
essential for the activities [149]. In addition, Ginsenoside Rk1
from white ginseng decreased the 12-HETE level involved in AA pathway, which is related to 12-LOX translocation
resulting from the decreased of Ca²⁺ levels [150].

3.3. Fibrinolysis. The conversion of fibrinogen to fibrin
and the consequent formation of a stable fibrin clot are the
ultimate events in the coagulation and thrombotic cascades
[169]. The agents available for clinical treatment on fibrinolysis
can be classified into two groups: plasmin-like proteases
which can directly hydrolyse fibrin, for example, nattokinase
and lumbrokinase; and plasminogen activators, for example,
tissue type plasminogen activator (t-PA) and streptokinase
[170]. In recent years, some effective thrombolytic agents have
been purified and characterized from foods or animal mate-
rials such as Japanese natto, douche (a traditional Chinese
soybean food) [171] and earthworm [172].

In 1983, a high fibrinolytic active enzyme named lum-
brokinase was firstly separated from artificial breeding earth-
worm in Japan [173]. This fibrinolytic enzyme had a dual
functions included dissolving fibrin directly and activate
plasminogen. Furthermore, Mihara et al. [172] isolated a
strong fibrinolytic enzyme from Lumbricus rubellus which
contained abundant asparagine and aspartic acid with little
proline or lysine. In addition, Xiong et al. separated and puri-
fied a fibrinolytic enzyme (33 kDa) with strong fibrinolysis
effects and proteolytical activity from Eisenia foedita [174].

Nattokinase (27.3 kDa to 35 kDa) is a kind of serine
proteases which is produced in the fermentation process of
Bacillus natto or Bacillus subtilis var. natto. Nattokinase pos-
esses a significant fibrinolytic property and the main mech-
anism was to dissolving fibrin directly as well as activating
plasminogen to increase the intrinsic plasmin formation.
In the expectation to be developed as a new generation of fibrinolytic agents and health food, nattokinase has lots of advan-
tages such as high safety, low cost and fast acting [175, 176].
Another serine protease (31 kDa with a single polypeptide
chain) with fibrinolytic activity named CSP was purified from
the culture supernatant of the fungus Cordyceps sinensis. CSP
was found to be a plasmin-like protease, but not a plasmino-
gen activator through preferentially cleaving the Aα chain of
fibrinogen and the α-chain of fibrin [170].

Pinus densiflora, an evergreen needle-leafed tree indige-
nous to Asia Pacific, has been used for the treatment of mul-
tiple ailments such as cardiovascular disease, cancer, diabetes
and antihypertension. It was reported that pine needle extract
would facilitate fibrinolysis, decrease the blood plasma
cholesterol and triglyceride in cholesterol fed rat, and it’s
helpful in removing blood clots [177]. On the other hand,
Huang et al. screened for the fibrinolytic activities of 6
kinds of authentic medicinal materials from Guangxi
(China) by fibrin plate method in vitro. As a result, Puer-
aria lobata, Trichosanthes kirilowii, Lonicerajaponica, and
Desmodium styracifolium showed fibrinolytic activity, and
in particular the fibrinolytic activity of D. styracifolium
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<td>Neolignans of three Lauraceae species (Pleurothrytum cinereum, Ocotea macrophylla, and Nectandra amazonum)</td>
<td>Rabbit blood (<em>in vitro</em>); agonist: PAF, ADP and AA</td>
<td>Inhibition of COX-2 by Benzoafuran neolignans; inhibition of PAF-action, COX-1, 5-LOX by bicyclooctane; inhibition of COX-2, PAF-action by neolignan 9-nor-7,8-dehydro-isolicarin B and cinerin C; inhibition of 5-LOX/COX-2 by Nectamazin C</td>
<td>[148]</td>
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<tr>
<td>Extracts of Myoga (Zingiber mioga Roscoe)</td>
<td>Human blood (<em>in vitro</em>); agonist: ADP and AA</td>
<td>Inhibition of 5-LOX by miogatrial, miogadial, sesquiterpene and polygodial</td>
<td>[149]</td>
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<tr>
<td>Ginsenoside Rk1 of white ginseng</td>
<td>Rabbit blood (<em>in vitro</em>); agonist: AA</td>
<td>Decreasing of 12-HETE, 12-LOX, and Ca&lt;sup&gt;2+&lt;/sup&gt; levels</td>
<td>[150]</td>
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</tbody>
</table>

AA: arachidonic acid; ADP: adenosine diphosphate; THR: thrombin; PAF: platelet activating factor; COX-1: cyclooxygenase-1; COX-2: cyclooxygenase-2; TXAS: thromboxane synthase; LOX: lipoxigenase; TXA<sub>2</sub>: thromboxane A<sub>2</sub>; TXB<sub>2</sub>: thromboxane B<sub>2</sub>; 5-HT: 5-hydroxytryptamine; PLC-γ2: phospholipase C-γ2; PGD<sub>2</sub>: prostaglandin D<sub>2</sub>; ATP: adenosine triphosphate; ROS: reactive oxygen species; PGI<sub>2</sub>: prostacycline 2; 12-HETE: 12-hydroxy-5,8,10,14-eicosatetraenoic acid.
was similar to that of positive drug urokinase \[178\]. In addition, two components (1-palmitoyl-2-oleoyl-3-\(\text{O-\(\alpha\)-D-glucopyranosylglycerol} and 1-myristoyl-2-oleoyl-3-\(\text{O-\(\alpha\)-D-glucopyranosylglycerol) were purified from \text{Sargassum fulvellum} and the fibrinolytic effect was identified \text{in vitro} \[179\].

4. Conclusion

Thrombosis remains a final pathway to disease and death in some of our most common diseases such as myocardial infarction and stroke. Although substantial progress has been made in understanding the biology of thrombus formation and the pathophysiology of thrombosis, all the pharmacological agents available for prevention or treatment have been in use for decades or have been replaced with newer variants that offer a modest incremental improvement. Natural products have been reported with apparent inhibitory activity on thrombotic diseases both in experimental and clinical stages, which provide a useful preventive approach or an adjunct to current pharmacological treatments for thrombotic diseases. Advances in the knowledge of both the mechanisms of thrombus formation and of the biological functions of natural products will provide new insights to promote human health.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21275169, 81202886, and 21175159) and Project no. CQDXWL-2014-Z007 supported by the Fundamental Research Funds for the Central Universities.

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