Review of Literature

2.1 Natural products towards the discovery of potential future antithrombotic drugs

Natural products and the active components of plants have a long history of use in modern medicine and in certain systems of traditional medicine, and are valuable source of therapeutic agents for millennia [1-5]. Medicinal plants serve as a greater resource for new medication and their potential currently became a topic of interest to all the researchers across the globe [6-9]. The World Health Organization (WHO) has recommended medicinal plants to be used more effectively in healthcare system as they generally considered as safe because they are "natural" (WHO, 2005). Furthermore, even stakeholders in pharmaceutical and neutraceutical industries see a high potential in natural products as drug leads [6,10-12] Therefore, during the past decade there has been an increasing trend of scientific research on pharmacological re-assessment of traditional medicinal plants used to treat various ailments as well as development of new drugs and pharmaceuticals from natural products [1,10].

Thrombosis remains a final pathway to disease and death in some of our most common diseases such as myocardial infarction and stroke [13,14]. 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 [13,15,16]. 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.

This chapter is dealt with the literature survey on medicinal plants and their active components in the treatment of thrombosis-associated cardiovascular diseases (CVDs).

2.2 Cardiovascular diseases and natural products

The hemostatic system, which comprises platelet aggregation, fibrinolysis, and coagulations, maintains the integrity of the high pressure closed circulatory system in mammals after vascular damages [17]. The thrombus formation inside the blood vessels is controlled by the regulatory system which is temporary and spatial under normal physiological conditions [18-21]. However, when pathological processes overwhelm the regulatory system of hemostasis or a shift in the hemostatic balance towards the pro-coagulant side, thrombosis event is initiated [22,23]. Under this hypercoagulable state, excessive quantities of thrombi form, that results in partial or complete blockage of blood vessels to reduce the blood flow [24,25]. The development of clots in the artery,

vein, as well as in microvascular circulation-a condition known as thrombosis is the most frequent cause of morbidity and mortality worldwide [26-28]. The formation of thrombi in the arterial circulation usually occurs in individuals at high risk of cardiovascular diseases [29,30]. The coronary myocardial infarction and ischemic stroke leads to atherosclerosis and thrombosis in the coronary arteries [31]. Furthermore, peripheral arterial diseases including mesenteric artery embolism and limb arterial thrombosis are also closely related to the arterial thrombosis. Venous thromboembolism (VTE) consists of deep vein thrombosis (DVT) whereas pulmonary embolism (PE) is a relatively common condition of CVD [32-34]. Noteworthy, venous thrombosis is the second leading cause of death in patients suffering from cancer. Furthermore, disseminated intravascular coagulation and micro angiopathy hemolytic anemia (thrombotic thrombocytopenic purpura and hemolytic uremic syndrome) are also associated with microvascular thrombotic disorders [23]. Therefore, substantial efforts must be given on the prevention of thrombosis for the treatment of thrombotic diseases.

The three major categories of antithrombotic drugs including anticoagulants (blood thinners), inhibitors of platelet aggregation (antiplatelet agents), and fibrinolytic (clot bursting) have found tremendous potential therapeutic application to mitigate the problem of arterial and venous thrombosis [35-38]. Despite of intense investigation over the last two decades into the discovery and development of more effective antithrombotic drugs, the benefit of these therapies on mortality rates still remains small [39-41]. This situation will probably become more aggressive and challenging in near future as the incidences of obesity, diabetes, and the metabolic syndromes contributing to CVDs have been rapidly increasing. Drug resistance, limited efficacy in some patients, interaction with food components (drug-food interaction), low therapeutic index, and side effects such as higher bleeding risk and gastrointestinal dysfunctions are some of the limiting factors contributing to the less success stories of these life saving drugs [42,43]. Therefore, there is a worldwide intense competition for the development of more effective antithrombotic drugs of high therapeutic index to efficiently tackle the problem of CVDs.

Nowadays, much effort has been focused on the discovering of natural products as effective supplements or even substitutes to those currently used antithrombotic drugs [15,44,45]. These natural products are composed of herbal drugs [46-48], traditional Chinese medicines (TCMs) [15,49], traditional Indian medicine also known as

Ayurveda, and functional foods [50-53] have been found to possess remarkable antithrombotic property both in experimental and clinical stages. For example, Shimotsu-To, which is a combined prescription of four herbal extracts, *Paeonia lactiflora*, *Rehman-nia glutinosa*, *Angelica sinensis*, and *Ligusticum chuanxiong*, has been used in clinic for improving abnormal blood coagulation, fibrinolysis, and atherosclerosis [54]. Kang naox-ueshuan tablet, which consists of *Flos Carthami*, *Radix Angelicae Sinensis*, *Hirudo*, and so forth, can protect cerebral ischemia through inhibition of platelet aggregation and reduction of blood viscosity [55] Besides, *Ginkgo biloba* leaves tablets have been widely used in treating ischemic cerebrovascular diseases in China [52,56].

Some of the major advantages of the therapeutic application of natural products against thrombotic diseases are 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 marginal side effect on the gastrointestinal system and therefore they may be considered as safe [57].

This chapter will provide an overview on the mechanisms of thrombus formation and the antithrombotic properties of natural products derived from medicinal plants. Further, the antithrombotic mechanism of medicinal plants and their identified active components will also be discussed.

2.3 Thrombosis and platelet aggregation

Thrombus can be classified into four groups depending on its occurrence in different blood vascular system such as vein and artery, and constituents [24,58] These groups are (i) pale thrombus, mainly occurs in fast-flowing blood with numerous platelets; (ii) red thrombus, constituting of fibrin and erythrocyte in slow-flowing blood; (iii) mixed thrombus, a continuous process of thrombus formation; and (iv) 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 [59].

Thrombus formation events including platelet adhesion, activation, secretion, and aggregation as well as tissue factor (TF)-induced thrombin generation and fibrin formation, is a highly complex process [17,60,61]. 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 of blood vessels / tissues

triggers the accumulation and activation of platelets, whereas exposed TF initiates the generation of thrombin which converts fibrinogen to fibrin in addition to activating circulating platelets [24,62,63].

2.3.1 Coagulation system

Blood coagulation and platelet adhesion as well as activation are critical for cessation of blood loss at sites of vascular injury in the high-pressure closed circulatory system [64-66]. 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 and extrinsic) pathway, which starts at the level of factor X to lead to thrombin and fibrin formation [67,68].

The extrinsic pathway is initiated by excessive exposure of TF which is a 263-residue membrane-bound glycoprotein [69-71] and serves as a receptor and cofactor for factor VII and its active form VIIa [71,72]. The binding of FVIIa to TF leads to TF-FVIIa complex formation which acquires catalytic activity and then converts factors IX and X to their active derivatives factors IXa and Xa, respectively [73-75]. Simultaneously, the intrinsic pathway begins with formation of the primary complex on free collagen by high-molecular-weight kininogen, prekallikrein, and FXII. The latter is cleaved to FXIIa whichin turn converts FXI to FXIa. The activated FXI activates FIX, which then with its cofactor FVIIIa forms a tenase complex to activate FX to FXa [76,77]. In the common pathway, FXa derived from both the intrinsic and extrinsic pathways along activates thrombin formation which finally converts fibrinogen to fibrin polymers [77-79] (Fig. 2.1).

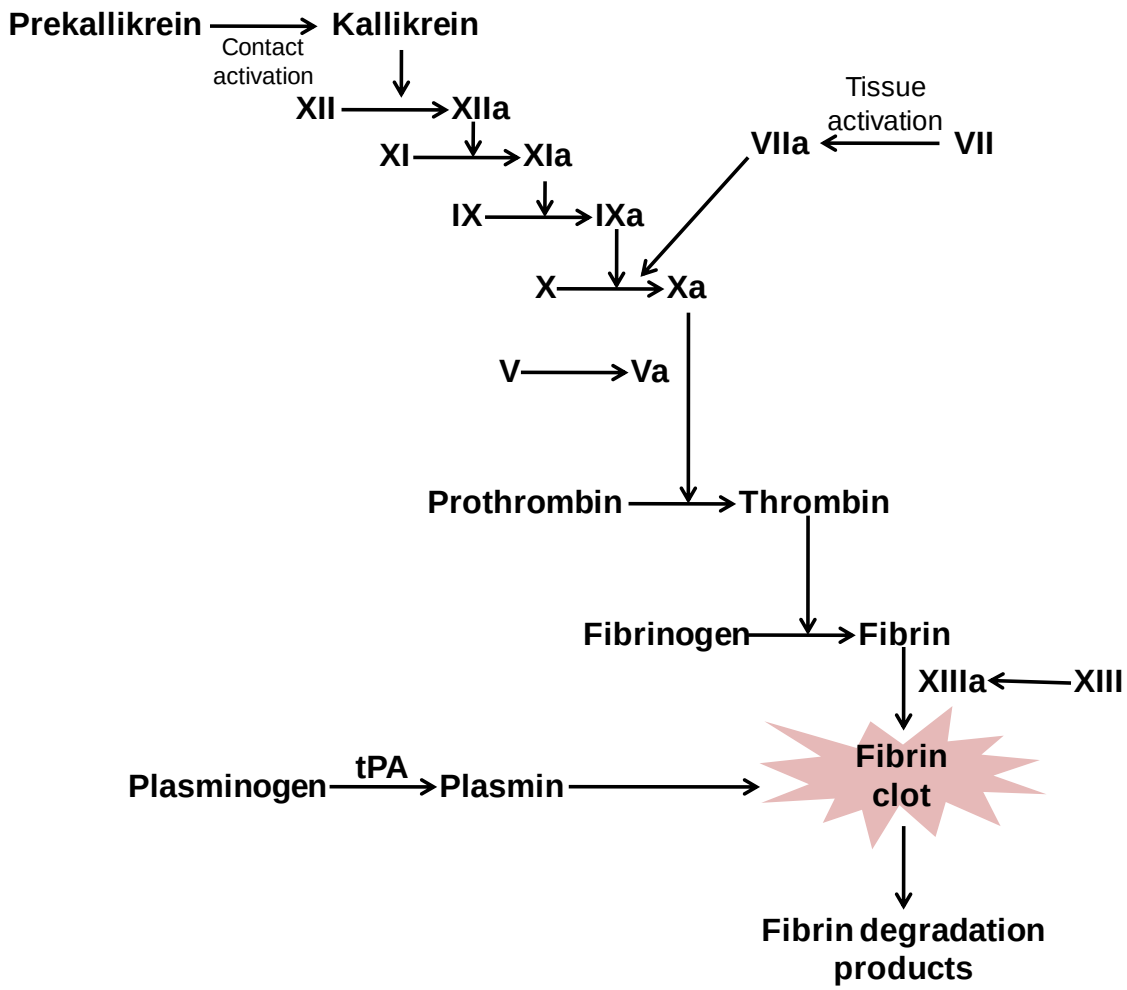

Fig. 2. 1 Blood coagulation pathway

2.3.2 Platelet activation and aggregation.

The intact vascular endothelium is a semipermeable barrier that controls the diffusion of plasma molecules, regulates vascular tone and inflammatory molecules, and releases gaseous signal molecules including nitric oxide (NO) and prostacyclin (PGI_2) as well as endothelial CD_{39} to prevent platelet aggregation or dilate blood vessels under physiological conditions [80-82]. However, dysfunctional or impaired endothelium is characterized by the loss of antiplatelet properties and tends to mediate and accelerate thrombosis [83-85]. The exposure binding sites of collagen and von Willebrand factor (vWF) which is a multimeric plasma glycoprotein allow the platelet membrane glycoprotein (GPIb-IX-V or GPVI) to adhere on it in the first place [83,86,87]. After the initial adhesion of platelets to the extracellular matrix, platelets undergo shape change and the activation process requires a rapid response to autocrine or paracrine mediators, including adenosine diphosphate (ADP), thrombin (THR), epinephrine, collagen, and thromboxane A_2 (TXA_2) [88-90]. These platelet agonists binding to specific membrane

receptors (e.g., collagen binds to GPVI or THR interacts with protease activated receptors, and ADP binds at least two ADP receptors on platelets and activates phospholipase C (PLC) [91-93]. This result in the production of diacylglycerol (DAG) and inositol trisphosphate (IP_3) [94-96]. DAG and IP_3 together activate protein kinase C (PKC) and mobilize cytoplasmic Ca^{2+}, respectively, [97] Then TXA_2 is produced as a consequence of increased cytoplasm Ca^{2+}-levels and the high concentration of Ca^{2+} is necessary for the activation of PLA_2 through phosphorylation by p-38-mitogen-activated protein kinase (MAPK) [98-100]. 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 [101,102]. Fibrinogen binding to GPIIb/IIIa also triggers an "inside out" signaling, causing amplification of the initial signal and further platelet activation [103,104]. 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 [17,24,105,106].

2.3.3 Antithrombotic effects of plant derived natural products

Studies have demonstrated that natural products become increasingly crucial in reducing the thrombotic risks and treatment of various types of CVDs. There are three types of commercial antithrombotic drugs - (1) anticoagulant, (2) antiplatelets, and (3) fibrinolytic or clot bursting [35,36,41,107,108].

2.3.3.1 Anticoagulants derived from medicinal plants

The extrinsic and intrinsic pathways of coagulation are initiated after vascular disruption via TF and collagen, respectively [24]. In clinical treatment, inhibition of coagulation system is an effective way to prevent the pathological thrombus formation [109].

2.3.3.2 Inhibition of tissue factors by natural products.

It has been reported that *Chaenomeles sinensis* has antithrombotic and platelet aggregation inhibition activities [110]. Thirteen components were isolated and purified from the fruits of *C. sinensis* and five of them including hover trichoside C (IC_{50}= 14.0 g), luteolin-7-O—D-glucuronide (IC_{50}= 31.9 g), hyperin (IC_{50}=20.8 g), avicularin (IC_{50}= 54.8 g), and quercetin (IC_{50}=135.7 g) against thrombin can inhibit TF expression in rat plasma after the addition of $CaCl_2$ in *in vitro* conditions. Further, the TF inhibitory activity of the C-ringpentacyclic flavonol was found to be superior as compared to C-ring hexacyclic flavonol [110]. In 2000, Shang et al.[111] reported the

inhibitory effects of ligustrazine on the expression of TF and vWF in human blood induced by THR. In addition, a sesquiterpene glycoside isolated from the leaves of *Eriobotrya japonica* Lindley (Rosaceae) in *in vitro* conditions showed a potent TF inhibitory activity (IC_{50}= 2 µM) whereas another component ferulic acid isolated from the same plant exhibited a weak inhibitory activity (IC_{50}= 369 µM) [112]. In addition, Zearalanol (ZAL), which is one of the natural phytoestrogens usually found in beans and grain, was also shown to decrease the quantity of TF by inhibiting *ex vivo* expression of TF on vascular endothelium in rat plasma with a potency similar to or better than that of commercial drug 17 -estradiol [113].

2.3.3.3 Effect on coagulation pathways by natural products.

The pathways of the coagulation system mainly consist of two distinct cascades (intrinsic and extrinsic coagulation pathways) that ultimately contribute to the formation of the key protease thrombin which in turn converts fibrinogen into fibrin to stabilize the formed platelet-rich plug [24,114]. In experiment models, activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) are tested to determine the activation of intrinsic, extrinsic and common (intrinsic and extrinsic) pathway, respectively, [115-117]. The anticoagulation effects of some plant-derived natural products are summarized in Table 2.1.

2.3.4 Platelet aggregation inhibition property of plant-derived natural products

Platelets play a central role in physiology, during haemostasis, and in pathology in the initiation of thrombosis and development of atherosclerosis [62,96]. Cardiovascular risk factors, such as hypercholesterolemia, hypertension, smoking, and diabetes, result in oxidative stress in the vascular wall, endothelial dysfunction and platelet hyperactivity [118]. Andrographolide, the active component of *Andrographis paniculata*, has been shown to inhibit PAF-induced human blood platelet aggregation in a dose-dependent manner with an IC_{50}value of ≈ 2 µM [119]. Spent hop extract (SHE) inhibited the ADP-induced platelet aggregation and decreased platelet hyperactivity in *in vitro* condition [120].

Table 2.1. Inhibition on the coagulation pathways by some plant-derived natural products.

Natural products	**Model**	**Coagulation pathways**	**Effects**	**Reference**
Borneol	Rat blood (*in vivo*)	EX & CO	Prolonging PT and TT and inhibition venous thrombosis	[121]
Polysaccharide of *Umbilicaria esculenta*	Rat blood (*in vitro*)	IN, EX & CO	Prolonging APTT, PT, and TT	[122]
Wogonin and wogonoside of *Scutellaria baicalensis* Georgi	Human blood	IN & EX	Prolonging APTT and PT and inhibition of thrombin	[123]
Hyperoside of *Rhododendron brachycarpam*	Rat blood (*in vivo*)	IN & EX	Prolonging APTT and PT	[121]
Sulfated (1 →3)- -L-arabinan of *Codium vermilara*	Human blood	IN, EX & CO	Prolonging APTT, PT, and TT	[124]
Sulfated polysaccharides of *Hizika fusiformis*	Rat/Rabbit blood	IN	Prolonging rats BT, CT *in vivo,* and	[125]
Dilinoleic acid, safflower yellow, compatibility preparation	Rat blood (*in vivo*)	IN & EX	Prolonging APTT, TT, CT, and BT	[126]
Saline extract of *Hirudinaria manillensis*	Rat blood (*in vivo*)	IN, EX & CO	Prolonging APTT, PT, and TT	[127]
Gloriosa superb	Rat blood	EX, CO	Inhibit fibrin clot formation by inhibiting thrombin	[128]
Phlorotannins STP-1 and STP-2 of *Sargassum thumbergii* Kuntze	Rabbit blood	IN, EX & CO	Prolonging APTT, TT, PT, CT, and BT	[129]

Withaferin A of *Withania somnifera*	Human blood	IN & CO	Prolonging APTT and PT and inhibition of thrombin	[130]
Total saponin of *Polygala fallax*	Rabbit blood	IN	Prolonging APTT and RT and fibrinogen clotting time but without PT	[131]
Synclisia scabrida	Rat blood	IN, CO	Prolongs PT	[132]

Abbreviations: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: re-calcification time; CT: coagulative time; BT: bleeding time

Bupleurumin, a flavonoids from the aerial parts of *Bupleurum falcatum* showed an 8-fold potent inhibitory effect (IC_{50} = 47.5 µM) as compared to that of aspirin (IC_{50} = 420 µM) on collagen-induced platelet aggregation; however, the inhibitory potency of aspirin and Bupleurumin on arachidonic acid-induced platelet aggregation was found to be almost identical [133]. Tanshinone IIA (TIIA),an active compound from medicinal plant, in a concentration-dependent manner (0.5– 5 µM) selectively inhibited the ADP (3 µM) -induced platelet aggregation in a rat model [134]. Nevertheless, TIIA was less active against the irreversible aggregation induced by high concentration of ADP (10 µM) and collagen (10 g/mL) stimuli [134].

Apart from single bioactive component, evidences have also been presented to show the platelet aggregation inhibitory effects of crude extracts of plants. 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 [135]. In another study, crude aqueous extract (CAE) of *Petroselinum crispum* (parsley) was evaluated for its antiplatelet aggregation activity in *in vitro* as well as in *in vivo* rat model. In *in vitro* CAE dose-dependently inhibited the THR, ADP, collagen and epinephrine-induced platelet aggregation [136]. The cordycepin-enriched (CE) WIB801C from *Cordyceps militaris* also dose-dependently inhibited the ADP-induced platelet aggregation with an IC_{50}value of 18.5 g/mL [137].

Development of definite platelet receptor inhibitors from herbal compounds contributed to the clinical treatment of platelet aggregation. For example, ADP $P2Y_{12}$ receptor antagonists include ticlopidine and clopidogrel; GPIIb/IIIa antagonists include abciximab, tirofiban, and eptifibatide. Based on the structures, functions and ligand properties of platelet receptors, they can be classified into three groups -integrin, adhesion and agonist receptors. A large number of natural products from medicinal plants are reported as platelet receptors antagonists (Table 2.2).

Table 2.2. Inhibition of platelet membrane receptors by natural products from plants.

Natural products	**Experimental models**	**Possible mechanism(s)**	**Reference**
2,3,5,4 -Tetrahydroxystilbene-2-O-D-glucoside of *Polygonum-multiflorum*	Human blood (*in vitro*)	Inhibition of Fc RIIa, Akt (Ser473), and GSK3 (Ser9) phosphorylation	[48]
95% ethanol extract of *Spatholobus suberectus*	Human blood (*in vitro*);	Blockage of fibrinogen binding to the GP IIb/IIIa, suppression of TXA2 formation	[138]
Glaucocalyxin A of *Rabdosia japonica (Burm. f.) var. glaucocalyx (Maxim.) Hara*	Human blood (*in vitro*);	Inhibition of tyrosine phosphorylation of Syk, LAT, phospholipase C 2, and P-selectin secretion	[139]
Salvianolic acid B of *Salvia miltiorrhiza*	Rat blood (*in vitro and in vivo)*	Exerting binding affinity to decreasing of intracellular Ca^{2+}, and impacting on cytoskeleton-related proteins level	[140]
Indole-3-carbinol of cruciferous vegetables	Human blood (*in vitro*)	Inhibition of fibrinogen binding to GP IIb/IIIa and decreasing the levels of TXB2, prostaglandin E2	[141]
Essential oils of five *Goniothalamus species*	Human blood (*in vitro*);agonist: ADP, AA, and collagen	Possessing strong PAF antagonistic activity	[142]
15–20% ethanol extract of aged garlic (*Allium sativum*)	Human blood (*in vitro*); agonist is ADP	Inhibition of fibrinogen binding to GP IIb/IIIa and increasing the level of cAMP	[143]
Tetramethylpyrazine of *Ligusticam wallichii Franch*	Human blood (*in vitro*); agonists are ADP, collagen, and U46619	Inhibition of fibrinogen binding to GP IIb/IIIa and decreasing the levels of intracellular Ca^{2+} as well as TXB_2	[144]

Aqueous extract of *Agrimonia pilosa*	Human blood (*in vitro*); agonist is ADP	Inhibition of fibrinogen binding to GP IIb/IIIa and decreasing the level of P-selectin	[145]
N-butanol extract of *Toona sinensis Seed*	Human blood (*in vitro*); agonist is thrombin	Inhibition of fibrinogen binding to GP IIb/IIIa and decreasing the level of intracellular Ca^{2+}	[146]
Eryloside F of *Erylus formosus*	Human blood (*in vitro*); agonist is THR, SFLLRN, and U-46619	Possessing strong THR antagonistic activity	[147]
Isomaltol and pentagalloyl glucose of *Rhus verniciflua* Stokes	Human blood (*in vitro*); agonists are ADP, AA, and collagen	Decreasing the expression of GPIIb/IIIa	[148]
Piperlongumine of *Piper longum* L.	Rabbit blood (*in vitro*); agonists are U4619 and THR	Inhibition of U46619-induced phosphatidyl inositol hydrolysis as well as the binding of (3H)SQ29548 to TXA2 receptor	[149]
Hot-water extract of modified Je-Ho-Tang (*Mume Fructus, Amomi Tsaoko Fructus, Santali Albi Lignum, and Amomi Fructus*)	Human blood (*in vitro*); agonist is collagen	Inhibiting adhesion and decreasing the expression and P-selectin monoclonal, Ca^{2+} mobilization	[150]
Pomolic acid of *Licania pittieri*	Human blood (*in vitro*); agonist is ADP	Competitive antagonism of ADP-induced platelet aggregation	[151]

Abbreviations:ADP: adenosine diphosphat; PAF: platelet activating factor; THR: thrombin; AA: arachidonic acid; SFLLRN: thrombin receptor activating peptide; GP IIb/IIIa: Glycoprotein IIb/IIIa; TXA_2: thromboxane A_2; TXB_2: thromboxane B_2; cAMP: cyclic adenosine monophosphate; (^{3}H)SQ29548: TXA_2 receptor antagonist.

The cAMP plays a modulatory role in PLC-mediated secretion and aggregation of human platelets. The cAMP level is tightly controlled and dependent on both its rate of synthesis by adenylate cyclase (AC) as wells itsrate of hydrolysis rate phosphodiesterase [152]. In addition, cAMP level may be increased by peroxisome proliferator-activated receptors (PPARs) activation [153]. Intracellular cyclic guanosine monophosphate (cGMP) levelis 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 increase in cGMP levels is accompanied by a decrease in intracellular Ca^{2+} mobilization while the decrease in Ca^{2+} level inhibits the conformation change of GPIIb/IIIa into its active form and thus decreases platelet binding to fibrinogen [154]. The ancient plant *Ginkgo biloba* possesses many biological activities. Ginkgolide C, one of the active components in *G. biloba*, can significantly increase the formation of cAMP and cGMP with a corresponding decrease in the levels of intracellular $Ca^{2+}$and TXA_2.[155]. 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 significantly inhibits the collagen-stimulated platelet aggregation [155]. Furthermore, quercetin, another active component of *G. biloba*, prevents platelet aggregation by inhibition of PDE_3 [156].

Oligoporin A from *Oligoporus tephroleucus*, an edible mushroom cultivated in South Korea, inhibited the collagen-induced platelet aggregation in a concentration-dependent manner, but it did not affect ADP- and THR-induced platelet aggregation.[157]. Further study to reveal the mechanism of antiplatelet activity revealed that oligoporin A can induce the dynamic increase of cAMP and cGMP levels in platelets. Studies have shown that pretreatment of rat blood (*in vitro)* with oligoporin A significantly blocked collagen-induced ERK2 phosphorylation as well as diminished the binding of fibrinogen to its cognate receptor, integrin IIb/IIIa [157].

TXA_2, intensely induces platelet activation and vasoconstriction, is generated from arachidonic acid (AA) [158,159]. The former component is released when membrane phospholipids are broken down by diverse agonists such as collagen, thrombin and ADP. The enzymes related to TXA_2 production are cyclooxygenase (COX-1) and thromboxane synthase (TXAS), which are located at microsomes. The COX-1 produces prostaglandin

(PGG_2) from substrate AA, TXAS produces TXA_2 from PGH_2 that oxidized from PGG_2 by endoperoxidase. Therefore, inhibition of COX-1 or TXAS is a very useful marker to evaluate the antiplatelet effect of natural compounds [160]. For instance, COX-1 inhibitor aspirin and TXAS inhibitor ozagrel are being used as antiplatelet agents [161,162]. Another metabolic pathway of AA is the lipoxygenase (LOX) pathway that forms hydroxyl leicosa tetraenoic acids (HETE) and leukotrienes. TXB_2 and 6-keto-PGF1 are the stable metabolites of TXA_2 and PGI_2, respectively. When the ratio of TXA_2/PGI_2 is above normal conditions, thrombus formation will occur. On the other hand, when the ratio of TXA_2/PGI_2 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, for example, berberine [163](Huang et al., 2002), hesperetin [164] and ethyl acetate extract of *Caesalpinia sappan* L. [165] inhibited platelet aggregation by keeping balance of TXA_2 and PGI_2.

As mentioned above, the interference of the activation of the associated enzymes of arachidonic acid pathway, such as COX-1, COX-2, TXAS and LOX is regarded as an effective way to inhibit platelet aggregation. Obovatol, a major biphenolic component of *Magnolia obovata* leaves, demonstrated antiplatelet activity by inhibiting the COX-1 and LOX activities to suppress production of TXB_2, PGD_2 and 12-HETE [166,167]. Morroniside, extracted and purified from *Cornus officinalis* Sieb, significantly inhibited the activation of COX as well as TXB_2 generation, and had a selective inhibitory effect on ADP-induced platelet aggregation [168,169]. 26 neolignans (14 bicyclooctane-type and 12 benzofuran-type) from three Lauraceae species (*Pleurothyrium cinereum,Ocotea macrophylla*, and *Nectandra amazonum*) showed antiplatelet aggregation property in *in vitro* conditions via inhibition of COX-1, COX-2, 5-LOX and agonist-induced aggregation of rabbit platelets [170]. The results showed that benzofuran neolignans was found to be the selective inhibitors COX-2, whereas bicyclooctane neolignans selectively inhibited the PAF-action as well as COX-1 and 5-LOX. The neolignan 9-nor-7, 8-dehydro-isolicarin 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 inhibition of 5-LOX and COX-2 [170].

Abe et al. [171] screened for presence of inhibitors for human platelet aggregation and

human 5-LOX from the Myogaplant (*Zingiber mioga* Roscoe) extracts. Their study has shown that miogatrial, miogadial, sesquiterpene and polygodial isolated from *Zingiber mioga* were potent inhibitors of human platelet aggregation and human 5-LOX, and was found that 3-formyl-3-butenal structure was essential for exhibiting the above properties [171].

2.3.5 Fibrinolysis by natural products form plants

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 [172]. The agents available for clinical treatment on fibrinolysis (clot bursting) can be classified into two groups- (i) plasmin-like proteases which can directly hydrolyse fibrin, for example, nattokinase and lumbrokinase, and (ii) plasminogen activators, for example, tissue type plasminogen activator (t-PA) and streptokinase [172]. In recent years, some effective thrombolytic agents have been purified and characterized from foods or animal materials such as Japanese natto, douche (a traditional Chinese soybean food) [173] and earthworm (Zhao, 2012); however, due to limited work there are not many reports on thrombolytic agents from plant sources [15,174].

Pinus densiflora, an evergreen needle-leafed tree indigenous to Asia Pacific, has been used for the treatment of multiple ailments such as cardiovascular disease, cancer, diabetes and antihypertension. It was reported that pine needle extract facilitated fibrinolysis, decreased the blood plasma cholesterol and triglyceride in cholesterol fed rat, and was helpful in removing the blood clots [174]. In 2016, Aruna and colleagues screened for the presence of fibrinolytic activity in 5 kinds of authentic medicinal materials from India by clot solubilzation assay [175]. Five medicinal plants *Achyranthes Aspera, Eclipta alba, Hemidesmus indicus, Hibiscus rosa sinensis,* and *Zingiber offcinale* demonstrated indirect fibrinolytic activity by tissue plasminogen activation. Further they indicated that the aqueous and ethanol extract of *Hibiscus rosa sinensis* showed that excellent fibrinolytic activity [175].

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