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An overview on antithrombotic compounds derived from natural products

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Abstract. The use of medicinal plants is becoming more common as a result of their various therapeutic effects, which have been proven by scientific research aimed at discovering new substances that can be used as an alternative to synthetic molecules that have undesirable side effects, limiting their use in some cases. The objective of this review is to explore plants with antithrombotic compounds as well as the methods used to estimate their activity such as Cinnamomum cassia, Zingiber officinale, Syzygium aromaticum, Artemisia herba alba, Curcuma longa, Camellia sinensis, Allium sativum, Melilotus officinalis, Ferula communis, Dactylicapnos torulosa, Ginkgo biloba and Caesalpinia ferrea. A comprehensive literature search was undertaken to provide insight into the potential use of these antithrombotic drugs and a brief assessment on the use of natural substances in the treatment of thrombosis was also conducted.

Keywords: Thrombosis, Physiopathology, Treatment, Methods estimation, Antithrombotic plants

1 Introduction

Hemostasis is an interaction process between coagulation and anticoagulants that keeps blood within the wounded vascular system during periods of injury. This natural phenomenon comprises a complex mechanism that contains three fundamental steps: vasoconstriction, temporary blockage of a break by a platelet plug, and blood coagulation, or formation of a fibrin clot. Even though the process of thrombosis uses hemostatic mechanisms is undesired because it occurs even if there is no discontinuity of the vascular wall [1]. Anticoagulant drugs are required for the short-term treatment of arterial and venous thrombotic disorders such as deep vein thrombosis, pulmonary emboli, ischemic stroke, hypercoagulable states, strokes and heart attacks as well as the long-term prevention of recurrences [2][3]. However, heparins, vitamin K antagonists and their derivatives have been the major mainstays of anticoagulant therapy in clinical trials.

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Although their effectiveness is undeniable, the deleterious life-threatening side effects of these drugs have also been well documented [4]. Despite its low efficacy in patients with coronary artery disease and recurrent thrombotic events, antiplatelet treatment based on acetylsalicylic acid and clopidogrel is also indicated due to its preventive action and few side effects [5]. Most thrombolytic drugs, such as tissue-type plasminogen activator (t-PA), plasmin-like proteins (e.g. nattokinase) and urokinase, have a limited half-life in human blood circulation and can produce adverse effects like hemorrhage [6]. As a result, the quest for novel antithrombotic compounds is relevant, therefore, it is a necessity and demand of time to explore alternative anticoagulants. Plants are a safer source of medicines [3]. Herein, compounds extracted from herbs have complex structures and are pharmacologically active in living organisms, thereby aiding pharmacotherapy [7]. The aim of this study is to provide an overview of recent studies on antithrombotic substances derived from natural products and to elucidate the pathophysiology mechanism of thrombosis as well as the antithrombotic activity estimation methodologies.

Pathophysiology of thrombosis

Blood coagulation represents a complex, yet well coordinated series of events that involve tissue factor bearing cells and platelets [8]. The regulation of platelet vessel wall interactions, coagulation proteases, and fibrinolytic factors takes place on the endothelial surface [9]. Platelet activation is initiated by several agonists, including collagen via glycoprotein (GP) VI receptors and thrombin via protease-activated receptors (PAR) 1 and 4. Therefore, several important processes occur after activation (Figure 01). Arachidonic acid is first converted to thromboxane A2, a powerful proaggregatory and vasoconstrictor agent [10]. Second, platelets then degranulate. Dense granules, containing adenosine diphosphate (ADP), fuse with the cell membrane. ADP then acts on platelet P2Y1 and, most importantly, P2Y12 receptors, further stimulating and amplifying platelet activation. Similarly, alpha granules, containing P-selectin as well as other proinflammatory and procoagulant factors, fuse with the membrane. P-selectin binds to a range of inflammatory cells, including neutrophils and monocytes [11]. Third, through calcium mobilization and dephosphorylation of vasodilator-stimulated phosphoprotein (VASP), platelets undergo shape shift from discoid to stellate forms, implying physical aggregation. Conformational change in the GP IIb/IIIa receptor, which causes cross-links with other GP IIb/IIIa, enhancing platelet-platelet binding, and also stimulating other platelets to activate via outside-in signaling [11,12]. Herein, platelets and vascular and blood cells also release extracellular vesicles by outward blebbing, which are small particles released by almost all cell types when activated or injured, and composed by multiple bioactive molecules such as RNA, miRNA, cytokines, transcription and growth factors. Phosphatidylserine is exposed in the exterior layer of some extracellular vesicles, eliciting a 50- to 100-fold stronger procoagulant activity than activated platelets [13]. In addition to platelets and extracellular vesicles, activation of the coagulation cascade is critical in thrombosis (Figure 2). Broadly divided into two converging pathways, activation of the extrinsic pathway by tissue factor

and/or contact activation of the intrinsic pathway coagulation cascade leads to activation of factor X, a component of the prothrombinase complex, resulting in thrombin generation (Figure 3). Thrombin converts soluble fibrinogen to insoluble fibrin, which produces interweaving strands, further stabilized by factor XIII. There is interplay between the coagulation cascade and platelets. Not only can thrombin activate platelets via PAR1 and PAR4, but also conversely platelets themselves can catalyze thrombin generation through membrane scramblase activity. Although there is considerable overlap between platelet and coagulation-mediated effects, the primary mechanism of initiation differs by setting. Platelet activation is often the most apparent whereas coagulation cascade activation predominates in the venous circulation [14].

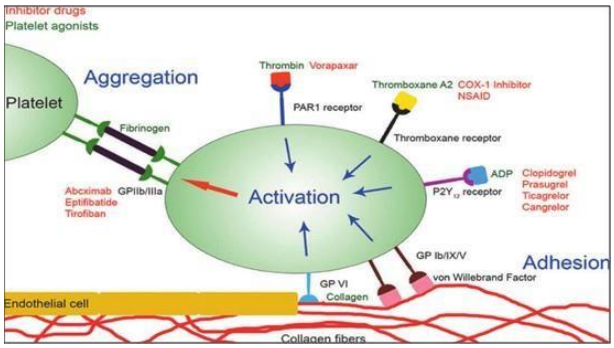

Fig. 1. Platelet agonists involved in platelet activation and site of action of inhibitory drugs. ADP = adenosine diphosphate; COX = cyclo-oxygenase; GP = glycoprotein; NSAID = nonsteroidal anti-inflammatory drug; PAR = protease-activated receptor [15].

Thrombosis treatment

Antithrombotic therapies currently include anticoagulant, antiplatelet and thrombolytic drugs. Among these, anticoagulant and antiplatelet drugs such as heparins, vitamin K antagonists, acetylsalicylic acid and clopidogrel, which directly hinder the formation of thrombosis, are the most regularly used in clinics [4][5]. However, the majority of them suffer from unsatisfactory efficacy and some negative side effects. Thrombosis remains a significant challenge for modern science, necessitating a huge demand for the development of effective and safer drugs [17]. This review explored various phytochemicals from herbal plants that are found to have biological activities such as anticoagulant properties (Table 01). The possibility of interactions between prescription pharmaceuticals and conventional remedies should never be ignored by doctors. Care should be taken when herbal physicians prescribe these herbal remedies to patients already on anticoagulant therapy. Physicians' ongoing efforts to report and identify potential interactions between conventional medicine and prescription drugs could increase awareness of interactions and improve the standard of patient treatment.

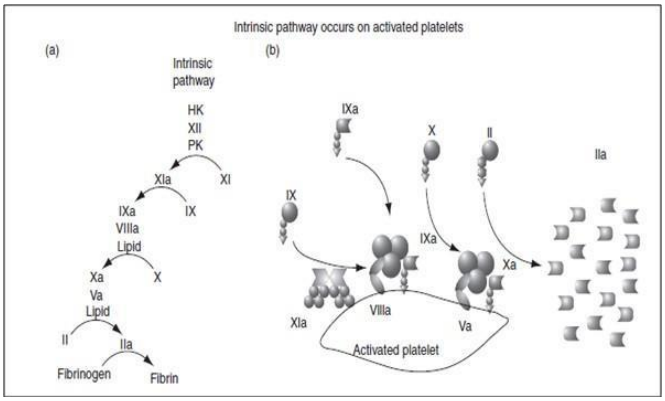

Fig. 2. Traditional (a) and contemporary (b) paradigms of coagulation highlighting both the biochemical and cell-based components required for thrombin generation [16].

Estimation methods of antithrombotic activity

The antithrombotic effect may be related to anticoagulant, antiplatelet or thrombolytic activity [17]. Each of these actions is evaluated by a specific method. Anticoagulant activity is frequently assessed by measuring prothrombin time (PT), which gives an indication of the concentration of prothrombin in the blood; it is the time required for coagulation and it varies between 11 and 15 seconds as well as the activated partial thromboplastin time (aPTT) and the thrombin time, which indicates the rate of conversion of fibrinogen into fibrin [19][32]. The response to platelet aggregation is monitored using either a turbidimetric method with an optical agregometer, the degree of platelet aggregation is determined after addition of an aggregating agent and normalized assuming the platelet poor plasma accounted for 100% light transmission and that the PRP (platelet-rich plasma) represented 0% light transmission [18]; or an in vivo test by monitoring the effect of the substance administered to the animal on induced arterial thrombosis by evaluating the number of embolisms and the duration of the embolization [31].

In addition, the inhibitory effect of certain substances can be evaluated on the enzymes involved in the thrombotic process such as VKORC1 (Vitamin K epoxide reductase complex 1) which is involved in the activation of coagulation factors II, VII, IX, X and the regeneration of vitamin K [28]. In addition, Zebrafish assay is considered as an effective tool to study hemostasis and thrombosis [29]. The zebrafish is an organism that lends itself to a number of unique and powerful approaches not possible in mammals. It has primarily been used to investigate areas distinct from coagulation. Therefore, the zebrafish is developing into a powerful in vivo model to study hemostasis, and its features as a model organism are well suited to develop high-throughput screens to identify novel mediators of hemostasis and thrombosis, validate candidate genes identified in human populations, and characterize the structure/function relationship of gene

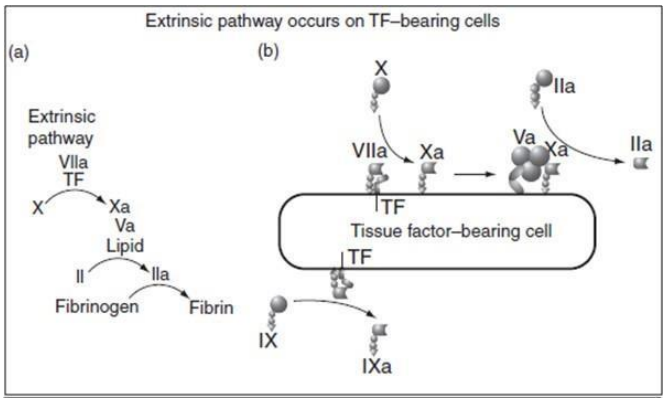

Fig. 3. The assembly of coagulation proteins on activated platelets leads to a burst of thrombin (IIa) generation-a prerequisite event in thrombus growth and propagations, and fibrin formation [16].

products. Loss of function studies have demonstrated conservation of function for a number of zebrafish coagulation factors. These include positive and negative regulators of coagulation, as well as important components of the thrombus itself, such as von Willebrand factor, fibrinogen, and thrombocytes. Zebrafish thrombocytes have a sparse cytoplasm with large nuclei. The ultrastructure analysis of thrombocytes demonstrated that the cytoplasm contains many vesicles that open to the cell surface, similar to the open canalicular system in mammalian platelets (Figure 4) [33]. Such analyses have also been leveraged to aid in the understanding of human variation and disease, as well as to conduct in-vivo structure/function investigations [34].

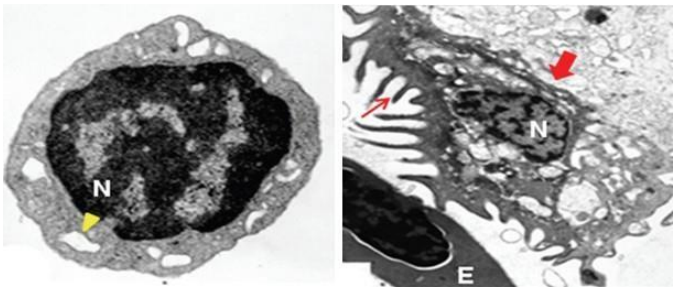

Fig. 4. Zebrafish thrombocyte electron micrographs. (a) Zebrafish thrombocyte. Open canalicular-like system is shown by arrowhead; N: nucleus; (b) An activated thrombocyte. Thrombocyte in an aggregation reaction; activated thrombocyte is shown by a thick arrow, thrombocyte in the aggregate shows filopodia shown by a thin arrow; E: erythrocyte [35].

Table 01: List of plants with antithrombotic activity

Medicinal plant	Origin	Family	Part used	Extract type	Composition	Plant properties	Active ingredient	Action mechanism
Cinnamomum cassia [18]	Korea	Lauraceae	/	Ethanolic	4-hydroxybenzoic acid, eugenol, coumarin, amygdalactone, cinnamic alcohol, cinnamaldehyde, 2-hydroxycinnamaldehyde, 2-methoxycinnamaldehyde coniferaldehyde, cinnamic acid, icariside DC, dihydrocinnacasside, lyoniresinol 3a-O-b-d-glucopyranoside	Bactericidal Sedative Hypotensive Peripheral vasodilation platelet anti-aggregation	Eugenol Coniferaldehyde	Eugenol was reported to suppress TXA2 formation from AA in platelets and thus was presumed to decrease platelet aggregation indirectly by inhibiting the synthesis of one of the strongest agonists and directly by blocking the TXA2 receptor
Zingiber officinale [19]	Sudan	Zingibaraceae	Rhizome	Aqueous	Gingerols, beta-bisabolene, zingiberene zingiberol, zingiberenol, ar-curcumene, beta-sesquiphellandrene, beta-sesquiphellandrol (cis and trans), numerous monoterpene hydrocarbons, alcohols aldehydes	Antipyretic Analgesic, Antitussive Cardiac inotropic Sedative	/	/
Syzygium aromaticum [3][20]	India	Myrtaceae	Flowers	Aqueous	phenolic molecules : hidroxibenzoic acids, flavonoids, hidroxiphenyl propens, hidroxicinamic acids and eugenol. gallic acid derivatives	Analgesic Antioxidant Anticancer Antiseptic Anti-depressant Antispasmodic Anti-inflammatory Antiviral Antifungal Antibacterial	Eugenol Polysaccharides	Eugenol seems to slow blood clotting. Polysaccharides isolated from clove may have antithrombic effects in vitro
Artemisia herba alba [21][22]	Saudi Arabia	Asteraceae	Aerial parts	Methanolic	Cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone	Antidiabetic Antimicrobial Antioxidant Antisickling	/	The methanol extract of *A. herba-alba* prolonged clotting time as recorded via the PT test and this prolongation of PT indicates the inhibition of the extrinsic and/or common pathway of coagulation, demonstrating its anticoagulant activity

Curcuma longa [22][23][24]	Korea	Zingiberaceae	/	/	Curcumin Bisdemethoxycurcumin	Antiinflammatory Antiproliferative Antiangiogenic	Curcumin Bisdemethoxy-curcumin	Curcumin and BDMC prolonged aPTT and PT significantly and inhibited thrombin and FXa activities. They inhibited the generation of thrombin or FXa In vivo, these anticoagulant effects of curcumin were better than those of BDMC indicating that methoxy group in curcumin positively regulated anticoagulant function of curcumin
Camellia sinensis [1][3]	India	Theaceae	Leaves	Aqueous	Polyphenolic compounds: Epigallocatechin-3-gallate (EGCG)	Anti-inflammatory Anti-oxidative Anti-carcinogenic Cardiovascular benefits	Epigallocatechin-3-gallate (EGCG)	Green tea and EGCG significantly prolonged mouse tail bleeding time in conscious mice. They inhibited adenosine diphosphate- and collagen-induced rat platelet aggregation in a dose-dependent manner. The antiplatelet activity may result from the inhibition of thromboxane A2 formation. Because ATP release from a dense granule is inhibited by catechins in washed platelets, thromboxane A2 formation may have been inhibited by preventing arachidonic acid liberation and thromboxane A2 synthase
Allium sativum [3][4][25]	Iraq	Amaryllidaceae	Bulb	Aqueous	Propyl /-disulphide, alliin and allicin	Anticoagulant Antioxidant Antibiotic Hypocholesterolaemic Hypoglycemic Hypotensive	Allicin	The prolongation of PT suggests inhibition of the extrinsic coagulation pathway. The chemical constituents of garlic can actually reduce fibrin formation and help to decrease fibrin that already exists in the blood. Some researchers have even gone as far as to state that Garlic is more effective at preventing blood clots than aspirin therapy
Melilotus officinalis [26][27]	Iraq	Fabaceae	Leaves	Ethanolic	Phenolic and amino coumarin derivatives (7-hydroxy coumarin and 6-amino coumarin)	Digestive, Eye inflammation, Hemorrhoids, Stomach ulcer and disorders	Coumarin	Fibrinolysis causing activate fluidity of blood due to lyse intravascular thrombi, increasing the stimulation of fibrinolysis may cause systemic destroying of fibrinogen and coagulation factors so minimal concentration of coumarin involved in ethanolic extract of *M. officinalis* (sweet clover) block multiple steps of the coagulation cascade by being competitive inhibitors of Vitamin-K in the biosynthesis of prothrombin Dicourmal or bis 4-hydroxy coumarin gave the higher anticoagulant activity with (2800 sec.) due to presence of methoxyl group so the modification of 4-hydroxy coumarin by adding methoxyl group will increase anticoagulation effect; dicumarol, inhibits the clotting mechanism by interaction the production process of an important factors which were produced by liver and necessary for vitamin K activity

Ferulla communis [28]	France	Apiaceae	/	/	Daucane esters or drimane ethers (non poisonous chemotype) prenylated coumarins (poisonous chemotype)	Antivitamin K Rodenticide	Ferulenol Ferprenin	Ferulenol and ferprenin are 4-oxygenated coumarin derivatives synthesized by F. communis L. Coumarin derivatives are known to be potential inhibitor of vitamin K epoxide reductase activity responsible for the recycling of vitamin K, a cofactor essential for the coagulation process. The presence of ferulenol and/or ferprenin was therefore associated with the toxicity of the 'poisonous' chemotype of F. communis L. Indeed, the symptomatology associated with F. communis L. poisoning is coherent with an anti-VKORC1 activity of ferulenol and/or ferprenin. The involvement of ferulenol in the hemorrhagic syndrome was shown by in vivo studies in rats. Nevertheless, the mechanism was not described, except that consumption of ferulenol was associated to decrease in vitamin K-dependent clotting factors.
Dactylicapnos torulosa [29]	China	Papaveraceae	Aerial parts	/	(**1**–**3**), Torulosine A, 1-Methoxypseudoprotopine and 1,14-Dimethoxyprechilenine together with 33 (**4**–**36**) known compounds	/	/	The antithrombotic effect of compounds may be related to their inhibitory effect on platelet aggregation
Ginkgo biloba [30][31]	France	Ginkgoaceae	/	/	Ginkgo flavones glycoside, terpenlactones and other special active components	Antioxidant functions Anti-inflammatory Hepatoprotective	Ginkgo flavone Glycosides Terpene Lactones (ginkgolide, bilobalide)	Ginkgo biloba extract decreases the main parameters of platelet thrombus formation (number of emboli and duration of embolization) The mechanism is in relation with the Endothelium Derived Relaxing Factor (EDRF) and Prostacyclin (PGI 2) linked to platelet disaggregation
Caesalpinia ferrea [32]	Brazil	Caesalpinioideae	Barks	Polysaccharides	Uronic acid and low content of protein and phenolic compounds	/	Polysaccharides, containing arabinose, galactose, rhamnose and uronic acid	PE-Cf and polysaccharide fractions inhibited platelet aggregation induced by ADP and FIII showed greater efficacy, it is possible to speculate that the content of polyphenols and/or higher molecular weight may be contributing to the higher antiplatelet effect of FIII. In addition, FIII effect was similar to clopidogrel, suggesting an antagonism of ADP receptors. In respect to C. ferrea fractions, only FIII slightly prolonged the bleeding time

2 Conclusion and perspectives

The current review emphasizes plant-derived anticoagulant substances. It has been discovered that the phytochemicals found in medicinal plants have biological effects including anticoagulant qualities. Therefore, the use of herbal medicines offers an alternative to the drawbacks of currently available anticoagulants like warfarin and heparin, which have bleeding complications, as well as the unpredictability of the dosing of the newer anticoagulant drugs in some patient populations, such as patients with underlying chronic diseases. Traditional remedies are widely used, but their safety and efficacy have not been rigorously examined. The review looked at a variety of herbal remedies that need to be exploited more in order to reach the desired level. These reports may be a better target for the creation of natural anticoagulant drug substitutes.

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