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REVIEW ARTICLE

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Possible antithrombotic properties of propolis

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ABSTRACT

The formation of thrombi in blood vessels results in thrombosis that is responsible for a considerable morbidity and mortality because it is associated with arterial diseases such as myocardial infarction, stroke, and peripheral occlusive disease in addition to venous thromboembolic disorders. Pharmaceutical agents such as anticoagulant agents and antiplatelet agents are applied for the prevention of the recurrence of thrombotic disorders. However, the use of these pharmaceuticals can result in side effects such as bleeding, as well as renal and hepatic disorders. The prevention and treatment of diseases using functional foods and alternative medicines have recently attracted an attention. Functional foods and alternative medicines with possible antithrombotic properties that have been used for many years are now receiving a significant focus in terms of the treatment and prevention of thrombosis. As they have already been used for so long, they are likely to be safe. Propolis is a hive product comprising resinous materials collected by bees from plants, and it includes various chemical compounds. Various biological activities of propolis have been indicated, and propolis is used as folk medicine and health supplement worldwide. This report reviews the possibility of using propolis as an antithrombotic agent.

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Introduction

Blood normally flows through blood vessels unobstructed. Nevertheless, blood components such as cells and plasma can leak from wounds in damaged vessels, and severe bleeds can be fatal. Blood clots at wound sites of damaged vessels stop bleeding in a process called hemostasis that includes platelets and a cascade of coagulation factors [1]. In a broad sense, the fibrinolytic system and its component factors are a part of the hemostatic system because fibrinolysis removes clots that are no longer needed for hemostasis to complete [2]. Vascular endothelial cells that line the inside of blood vessels exert powerful antithrombotic actions that enable blood to circulate throughout the body [3]. However, blood clots also form at the sites of damaged vascular endothelium, where they can cause thrombus and other thrombotic disorders. Such clots are called thrombi, and the situation involving them is called thrombosis [3]. Thrombi in the coronary arteries, lungs, and brain vessels can be fatal [4–6]. Namely, thrombosis is a pathological clot that results when hemostasis is excessively activated in the absence of bleeding. The clotting process in a healthy person is regulated as needed, and thrombus formation is avoided. Nevertheless, clots can form easily in the blood vessels of persons with risk factors such as dyslipidemia, diabetes mellitus, obesity, psychological stress, a sedentary lifestyle, and cigarette smoking [7–14]. These factors also increase the risk of atherosclerosis, in which blood vessels become clogged with fatty plaque deposits. Atherosclerosis additively increases the risk of thrombosis [15].

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The prevention of thrombosis has been prioritized in the developed countries, where (unhealthy) lifestyles have increased the risk of thrombotic events and the incidence of thrombosis [16,17]. Various drugs that are used to prevent thrombotic diseases in developed nations can prevent thrombosis [18], but these also prevent the hemostatic system from working properly, resulting in increased susceptibility to bleeding. Thus, milder agents with minimal side effects such as bleeding are desirable. The discovery of natural products and alternative medicines that have antithrombotic properties is now a significant target, and the application of such products to prevent thrombosis is anticipated [19–22]. In general, antithrombotic activity generally refers to antiplatelet action and the anticoagulant action of plasma and occasionally fibrinolytic activity [18]. Antithrombotic substances comprise anticoagulants that halt the coagulation system and interfere with further clot expansion, antiplatelet agents that decrease platelet aggregation and inhibit thrombus formation, and fibrinolytic enzymes that directly dissolve thrombus [23]. Whether or not these compounds exert antithrombotic effects through actions on blood coagulation factors, platelets and the fibrinolytic system should be experimentally assessed *in vitro* and *in vivo*. This review outlines the possible antithrombotic properties of propolis and natural substances found in propolis.

Chemical Composition and Representative Biological Activities

Chemical composition

Propolis is a hive product produced by mixing bee saliva with resinous materials that bees collect from various plants or confer trees [24]. Honeybee propolis is famous and popular; not only honeybees but also other types of bees produce propolis [25–30]. Propolis is a folk medicine and health supplement all over the world, and various biological activities have been indicated [24,31]. Propolis includes various chemical substances. The general ratios (%) of these substances are as follows: resins and balsam—50%, beeswax—30%, pollen—5%, essential and aromatic oils—10%, and other substances that include organic compounds [32]. In general, propolis contains polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes, and ketones, among other compounds [32]. However, the composition and biological activities of propolis greatly depend on the location of the bees, the season when resins are collected, and the plant sources of the resins [33]. For example, propolis samples from Europe and North America mainly comprise flavonoids, phenolic acids, and their esters [34]. Brazilian propolis contains various biologically active organic compounds in abundance such as artepillin C [35], whereas Chinese propolis is highly antioxidative and abundant in benzyl caffeate [36]. Therefore, the chemical heterogeneity of propolis is easy to understand, and the effects of propolis are closely associated with the production area. Table 1 summarizes the geographic origins, main plant sources, and chemical compounds. Examples of seasonal differences are as follows: Isla et al. [37] reported the seasonal variations of the antioxidant activity of propolis from Argentina. Samples corrected in November had the most antioxidant activity. They found a correlation between antioxidant capacity and flavonoid content. Regueira et al. [38] showed different effects of the dry and rainy seasons on the antibacterial activity and chemical composition of Brazilian red propolis, and Souza et al. [39] found that seasonal variations influence the Mg, Fe, Na, Ca, and Cu contents of Brazilian propolis. Thus, the quality of propolis within the same region varies depending on the season.

Table 1. Geographic origins, main plant sources and main bioactive compounds.

Geographic origin	Plant source	Main bioactive compounds	Reference Nos.
Europe, North America, and temperate regions of Asia	*Populus* spp., most often *P. nigra* L.	Polyphenols, phenolic acids, and their esters	[34,87]
Russia	*Betula verrucosa* Ehrh.	Polyphenols	[87]
Brazil	*Baccharis* spp., predominantly *B. dracunculifolia* DC.	Prenylated cinnamic acid derivatives, Diterpenic acids	[35,88]
Cuba	Venezuela Clusia spp.	Polyprenylated benzophenones	[89]
Pacific region (Okinawa, Taiwan)	Unknown	C-prenylated flavanones, Furofuran lignans	[90]
Kenya	Unknown	Polyphenols	[91]
Greece and Cyprus	Unknown	Flavonoids, terpenes	[92]

Effects of propolis on the immune system

Propolis exerts biological activities, including effects on immune systems. Brazilian propolis increases tumor necrosis factor-α (TNF-α) production in mice [40]. Ethanol extracts of Brazilian propolis upregulate toll-like receptor 2 (TLR-2) and TLR-4 expression and interleukin-1β (IL-1β) production in Bagg Albino c (BALB/c) mice [41]. Sforcin et al. reported that the ethanol extract of Brazilian propolis enhances NK cell activity. A water extract of propolis induces IL-1 production by peritoneal macrophages *in vitro* [42], and propolis extracts stimulate neutrophil chemotactic activity [43,44].

Antioxidant activity

The wide spectrum of the biological activities of propolis largely results from its antioxidative effects. Many studies have investigated the antioxidant activities of propolis [45], and those of phenolic acids and flavonoids in propolis and relationships among their chemical structures have been reviewed [46]. The antioxidant activity of propolis is due to its abundant content of polyphenols such as phenolic acid and flavonoids [45]. The antioxidant activity of propolis greatly depends on the production area and the plant source from which it is derived. For example, the antioxidant activity of Brazilian red propolis is more potent than that of green and brown propolis [47–49]. Although most of the studies regarding the antioxidant properties of propolis have been in cell culture or experimental animals, a few studies have examined the antioxidant effects of propolis in humans [50–52].

Antimicrobial activity

Antimicrobial activity of propolis is very well documented. Propolis exerts antimicrobial activity against a wide range of bacteria, fungi, and viruses [24]. Propolis is more active against Gram-positive bacteria than Gram-negative bacteria [53,54]. It was shown that propolis from different geographic regions has a considerable antiviral activity by acting at different levels and interfering with the replication of some viruses such as herpes simplex types 1 and 2, adenovirus type 2, influenza virus, or human immunodeficiency virus [53]. Antifungal activity is also influenced by the chemical variation of propolis. Some studies have shown the effect of propolis from different geographic origins against different fungi [54].

Anti-cancer activity

Propolis is effective against cancers of the brain, head and neck, skin, breast, liver, pancreas, kidney, bladder, prostate colon, and blood [55]. The key mechanism underlying the anticancer activity is thought to depend on the inhibition of matrix metalloproteinases, antiangiogenesis, metastasis prevention, cell cycle arrest, induction of apoptosis, and modulation of deleterious side effects induced by chemotherapy. The anticancer activities of propolis vary depending on the botanical source and geographic origin [56]. Brazilian red propolis possesses cytotoxic activities [57] against human hepatocellular carcinoma cell lines [58] and mouse skin tumors *in vivo* [59]. Greek propolis exerts antiproliferative activity against human colon adenocarcinoma cells (HT-29) [60].

Effects of propolis on oxidative stress

Oxidative stress is closely associated with diabetes, hypertension, and obesity [61–64], which might be the consequences and causes of the high rates of mortality due to cardiovascular diseases among humans and animals [65,66]. Propolis exerts inhibitory activities on oxidative stress. Yuan et al. showed the cytoprotective effects of Brazilian green propolis against oxidative stress induced by oxidized low-density lipoprotein in human umbilical vein endothelial cells (HUVECs) [67]. Sun et al. [68] reported the potential protective effects of bioactive constituents from Chinese propolis against acute oxidative stress induced by hydrogen peroxide in cardiac H9c2 cells. Mujica et al. [50] described the role of propolis in oxidative stress and lipid metabolism in a randomized controlled trial. Their study showed the effects of an orally administered propolis solution on the oxidative status and modulation of lipids in a human population [51]. These studies provide an important basis for the application of propolis to the prevention and treatment of cardiovascular diseases. These effects have been attributed to natural compounds in propolis [69].

Propolis polyphenol and its metabolism in the body

Polyphenols are classified according to their chemical structures as flavonoids, simple phenols, hydrolyzed tannins, and condensed tannins [70]. Flavonoids, simple phenols, and condensed tannins have attracted a notice as targets for functional food development, and propolis contains many flavonoids [71]. Many flavonoids exist in propolis as glycosides that comprise an aglycone combined with sugar, and the dynamics *in vivo* differ depending on the type of aglycone and its sugar chain. That is, when orally consumed polyphenols are taken up by intestinal epithelial cells, some glycosides

are hydrolyzed by lactose-phlorizin hydrolase and P-glucosidase in intestinal epithelial cells to produce aglycone [72]. Thereafter, aglycones such as catechin and flavonol passively diffuse into the epithelium, and most of the aglycones taken into the intestinal epithelial cells that enter glucuronidation, sulfate conjugation, and methylation in the circulating blood. Although many points remain unclear about the mechanism(s) through which polyphenols exhibit various physiological functions and low tissue concentrations in vivo, polyphenols enter into the body through oral ingestion circulation in blood and exert various actions [72].

Antiplatelet Activity of Propolis

Platelets adhere to the disrupted surfaces of damaged blood vessels and release biologically active constituents that induce further aggregation to stop bleeding although platelets cannot self-aggregate under normal circumstances. Thus, platelets play important roles in not only hemostasis but also the pathogenesis of cardiovascular and cerebrovascular disorders [73]. Since activated platelets aggregate and form thrombus and platelet activation is relevant to various cardiovascular and cerebrovascular disorders, the inhibition of platelet activation is clinically important for patients with thrombosis, cardiovascular diseases, and cerebrovascular disorders. However, the antiplatelet agents that are currently applied to clinically treat and prevent these disorders are associated with many side effects, among which bleeding is the most critical. Safer antiplatelet drugs are needed for such situations. Medicines derived from natural products and alternative medicine such as propolis might offer solutions.

Caffeic acid phenethyl ester (CAPE) is an active component of propolis produced in Europe, the Far East, and New Zealand [71,74]. The findings of many studies *in vitro* and *in vivo* have shown that CAPE has diverse biological activities [74]. Caffeic acid phenethyl ester is thought to be responsible for the anticancer, antioxidant, immunomodulatory, antibacterial, antiviral, anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects of propolis [74,75]. Therefore, propolis might have a potential for various clinical applications.

Some studies have found that CAPE exerts the effects on platelet activation. Hsiao et al. [76] examined the influence of 15–100 pM CAPE in washed human platelets and platelet plug formation *in vivo* and found that its concentration dependently inhibits collagen-induced platelet activation. Platelet aggregation stimulated by the glycoprotein VI agonist, convulxin, as well as the $\alpha_2\beta_1$ integrin agonist, aggretin, is also inhibited by CAPE (25 μM). The inhibition of collagen-induced platelet activation by CAPE is accompanied by $[Ca^{2+}]_i$ mobilization, phosphoinositide breakdown, the activation of protein kinase C, and mitogen-activated protein kinases such as extracellular signal-regulated kinase 2 (ERK 2), c-Jun amino-terminal kinase, and p38 mitogen-activated protein kinase (MAPK), as well as v-Akt murine thymoma viral oncogene (AKT) phosphorylation, and thromboxane A (TXA_2) formation. In addition, CAPE (25 μM) interferes with FITC-collagen binding to platelet membranes. Platelet inhibition by CAPE is, at least in part, mediated by binding to collagen receptors such as a2pi integrin and GP VI [76]. Zhou et al. [77] showed that a CAPE analog (CAPE-NO_2) inhibits collagen-induced platelet aggregation, and they also suggested that this is associated with the downregulation of thromboxane B_2 (TXB_2), cyclooxygenase 1 (COX-1), and 5-hydroxytryptamine (SHT) and the elevation of NO and cyclic guanosine monophosphate (cGMP) [77]. Zhang et al. [78] found that an aqueous extract of propolis dose dependently inhibited the platelet aggregation induced by the agonists, adenosine diphosphate (ADP), thrombin receptor activator peptide, and collagen. Among CAPE, galangin, apigenin, quercetin, kaempferol, ferulic acid, rutin, chrysin, pinostrobin, and pinocembrin are the components of propolis; only CAPE, galangin, and pinostrobin inhibited platelet aggregation. These findings indicated that propolis components including CAPE might have therapeutic value for fighting thrombotic disease. One study *in vivo* showed that CAPE (5 mg/kg) significantly prolonged the latency of platelet plug induction in mice [76], but clinical findings have not been reported.

Bojic et al. recently showed that the ethanolic extracts of propolis reduce ADP-induced platelet aggregation using whole-blood platelet aggregation assays [79]. Their study showed the antiaggregatory potential of propolis ethanolic extracts in low micromolar concentrations on whole blood samples. Martina et al. [80] found that oral propolis (65 mg/kg/day) prolonged tail bleeding time using mouse tail bleeding assays, which reflects platelet aggregation activity *in vivo*. They showed that the activity of oral aspirin (10.4 mg/kg) was similar. Although further studies are needed to confirm the beneficial effects *in vivo*, these findings suggested that propolis supplementation can influence

platelet aggregation and consequently thrombus formation and might have the potential to prevent cardiovascular diseases if proven in human studies.

Effects of Propolis on Fibrinolytic System

Fibrinolysis is a process in which clot degradation is modulated after damaged vascular tissue is repaired and replaced. The fibrinolytic system removes fibrin from the vascular system and thus prevents the enlargement of pathogenic hemostatic clots and vessel occlusion [2]. Therefore, attenuating fibrinolysis can lead to an increased risk of thrombosis. Fibrinolysis is regulated by plasminogen activator (PA) and plasminogen activator inhibitor 1 (PAI-1) [81]. The liver, adipose tissues, muscle, bone, and hematopoietic cells produce PAI-1, which inhibits tissue plasminogen activator during blood fibrinolysis [82]. Thrombus becomes difficult to dissolve when plasma PAI-1 concentrations are elevated, and persistent blood clots lead to thrombosis. Since plasma PAI-1 is elevated in patients with metabolic syndrome including obesity and diabetes, PAI-1 might be associated with a thrombotic tendency in such patients [82]. That is, a link between PAI-1 and metabolic syndrome has been established, and elevated plasma PAI-1 levels are now considered as a true component of the syndrome.

Chronic low-grade inflammation has been linked to the progression of obesity and related diseases [83]. Elevated plasma PAI-1 is closely associated with chronic inflammation in the adipose tissues of obese patients. Therefore, controlling PAI-1 elevation associated with chronic low-grade inflammation might help to prevent thrombosis caused by lifestyle-related diseases. Low-grade inflammation was used to induce a thrombotic tendency in an animal model and found that an orally administered ethanol extract of Brazilian propolis inhibits plasma PAI-1 elevation during inflammation [84]. The ethanol extract of Brazilian propolis suppressed PAI-1 production in cultured HUVEC stimulated with inflammatory TNF-α that increases PAI-1 release into the medium [85]. Chrysin suppresses the TNFα-induced increase in PAI-1 secretion, and chrysin is the most potent inhibitor of Brazilian propolis in terms of PAI-1 release from HUVEC [86]. However, as the content of chrysin in Brazilian propolis is very low, other contents of orally consumed Brazilian propolis might also be associated with the suppression of PAI-1 production. Further studies are needed to elucidate how propolis affects PAI-1 production and to identify specific molecules in Brazilian propolis that suppresses an increase in PAI-1.

Application of Propolis to the Prevention and Therapy of Thrombosis

Although studies of the effects of propolis on thrombosis are relatively rare, expectations that propolis will be able to treat and prevent thrombosis are enhanced. We considered that the possible antithrombotic properties of propolis should be announced to other investigators to further develop this field.

As described above, antithrombotic actions are progressed on suppressing platelet aggregation and PAI-1 production. Table 2 summarizes these antithrombotic effects of propolis. Studies using whole blood have shown that adding a small amount

Table 2. Antithrombotic effects of propolis and compounds in propolis.

Effects	Experimental target	Geographic origin of propolis	Active compounds	Reference Nos.
Inhibition of platelet aggregation	Isolated platelets	China	Unknown (water extract)	[78]
Inhibition of platelet aggregation	Isolated platelets	-	CAPE	[76]
Inhibition of platelet aggregation	Isolated platelets	-	CAPE analog	[77]
Inhibition of platelet aggregation	Platelet in whole blood	Various regions	Unknown (ethanol extract)	[79]
Prolonged tail bleeding	Mice	Unknown	Unknown	[80]
Inhibition of PAI-1 production from endothelial cells	Cultured endothelial cells	Brazil	Chrysin	[85,86]
Attenuation of PAI-1 production	Mice	Brazil	Unknown	[84]

of propolis (5 μg in terms of flavonoids) to whole blood inhibits platelet aggregation [79]. Oral propolis (65 mg/kg/day) prolongs tail bleeding time that reflects platelet aggregation activity *in vivo* [80]. In addition, food containing 0.5% ethanol extract of propolis inhibits PAI-1 production in mice [84]. To date, the antithrombotic effects of propolis have not been clinically studied, but the propolis concentrations used in these reports will be useful for future clinical studies.

Similar to other biological properties, the antithrombotic properties of propolis should be directly associated with its chemical configuration, which varies according to regional vegetation, pollen collection season, collection techniques, and bee species. Active components in propolis and their effects on blood coagulation factors, platelets, and fibrinolytic system will require further investigation before propolis can be clinically applied. The validation and quality control of propolis that possesses antithrombotic properties are also important for clinical applications.

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