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Antioxidant Activity of Tannin Components from *Vaccinium vitis-idaea* L.

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Abstract

Reactive oxygen molecules have been implicated as important pathological mediators in many clinical disorders and periodontal disease. To provide possible alternative treatment of periodontal disease, six tannins isolated from *Vaccinium vitis-idaea* L. were evaluated for anti-lipid peroxidation, anti-superoxide formation and free radical scavenging activity.

The results showed that cinnamtannin B_1 displayed the strongest anti-lipid peroxidation activity, proanthocyanidin A-1 displayed the strongest superoxide scavenging activity, and epicatechin-(4β⟶6)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin had the strongest anti-superoxide formation effect.

We conclude that tannins isolated from *V. vitis-idaea* L. exhibited multiple antioxidant activity, and could be used for the treatment of periodontal disease.

Reactive oxygen species such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) are constantly formed in the human body. The radicals react with cell membranes, induce lipid peroxidation, and have been implicated as important pathologic mediators in many clinical disorders (Slater & Cheeseman 1987). Ischaemia–reperfusion injury, diabetes mellitus (Sugawara et al 1992), coronary arteriosclerosis (Kok et al 1991), ageing and carcinogenesis (Oberley & Oberley 1986; Yagi 1987), and more recently periodontal disease (Whyte et al 1989; Shapira et al 1991; Kimura et al 1993) have been linked with radical oxidative damage.

Inflammatory periodontal disease is generally recognized as a bacterial plaque-associated disease. Slots (1977) described a predominance of Gram-negative anaerobic rods from advanced periodontitis. The pathogenesis of periodontal disease is a result of complex interactions between pathogenic bacteria and the host's immune response. Shapira et al (1991) demonstrated that superoxide formation of peripheral polymorphonuclear leucocytes was increased in rapidly progressive periodontitis.

Tannins are polyphenols widely present in plants. Since polyphenols are known to demonstrate biological activity, especially antioxidant properties, they are considered to be important active components of medicinal plants. *Vaccinium vitis-idaea* L. is an ericaceous plant used as a remedy for gonorrhoea, dysuria and diarrhoea (Mitsuhashi 1988). The leaf and berries of this plant have been shown to have antiviral and anti-inflammatory effects (Fokina et al 1993; Tunon et al 1995), some of the reported constituents being arbutin, hyperin, hydroquinone, isoquarcetin, and tannins (Perry 1980). To provide a possible alternative treatment of periodontal disease, six tannins isolated from *V. vitis-idaea* L. were evaluated for their anti-lipid peroxidation, anti-superoxide formation, and free radical scavenging activities.

Materials and Methods

Materials

Tannins from *Vaccinium vitis-idaea* L. were isolated according to the method of Morimoto et al (1988). The most abundant compounds were selected for study: procyanidin B-1, procyanidin

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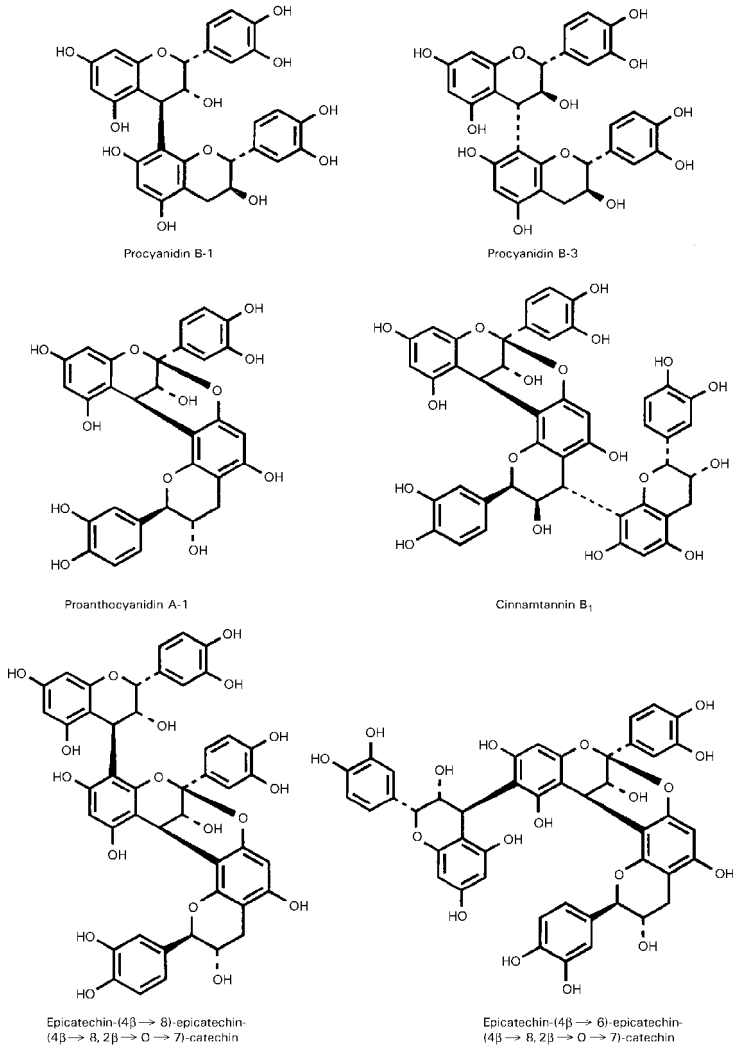

Figure 1. Structures of six tannins isolated from *Vaccinium vitis-idaea* L.

B-3, proanthocyanidin A-1, cinnamtannin B_1, epicatechin-(4β⟶8)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin, and epicatechin-(4β⟶6)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin. The structures of these compounds are shown in Figure 1.

Anti-lipid peroxidation activity: thiobarbituric acid test

The effect of test components on lipid peroxidation was determined by the method described by Yoshiyuki et al (1981). The reaction mixture contained liver homogenate, Tris–HCl buffer (pH 7·2), ascorbic acid, $FeCl_2$ and various concentrations of test components. After incubation, distilled water and thiobarbituric acid were added and then shaken vigorously. *n*-Butanol was added to extract the products of malondialdehyde and samples were measured at 532 nm (Wong et al 1987) using a Hitachi U-2000 spectrophotometer.

Anti-superoxide formation: xanthine oxidase inhibition test

Xanthine oxidase activity was evaluated by the spectrophotometric measurement of the formation of uric acid from xanthine (Chang et al 1994). Samples were dissolved in dimethylsulphoxide to 10^{-2} M, and diluted with 50 mM KH_2PO_4 buffer (pH 7·8) to obtain each concentration. Superoxide formation was calculated by measuring uric acid production by spectrophotometry at 295 nm. The IC50 value of each compound was calculated.

Free radical scavenger activity: cytochrome C test

Superoxide anions were assayed spectrophotometrically by a cytochrome C reduction method described by McCord & Fridovich (1969). Xanthine oxidase converts xanthine to uric acid and yields superoxide anions, and these radicals

Table 1. Summary of the multiple antioxidant effects (IC50) of six tannins isolated from *Vaccinium vitis-idaea* L.

Compound	IC50 (μM) values for various antioxidant assays		
	Thiobarbituric acid test	Xanthine oxidase inhibition test	Cytochrome C test
Proanthocyanidin A-1	15·27	416·5	10·14
Procyanidin B-1	7·76	461·0	29·53
Procyanidin B-3	10·75	630·0	30·39
Cinnamtannin B_1	2·25	450·5	16·62
Epicatechin-(4β⟶8)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin	7·90	428·5	27·78
Epicatechin-(4β⟶6)-epicatechin-(4β⟶8, 2β⟶O7)-catechin	9·29	308·0	26·77

directly reduce ferri-cytochrome C to ferro-cytochrome C, having an absorbance change at 550 nm. When test compounds showed superoxide scavenger activity, there was a decrease in the reduction of ferri-cytochrome C.

Results

The antioxidant activity (IC50 value) of the six tannin components isolated from *V. vitis-idaea* L. is summarized in Table 1.

In the thiobarbituric acid test, the IC50 values ranged from 2·25 to 15·27 μM. All of the compounds showed anti-lipid peroxidation activity, with cinnamtanin B_1 displaying the most potent activity. In the xanthine oxidase inhibitor test, the IC50 values ranged from 308 to 630 μM, with epicatechin-(4β⟶6)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin displaying strong activity, while procyanidin B-3 did not show anti-superoxide formation activity. In the cytochrome C test, the IC50 values ranged from 10·14 to 30·39 μM. Proanthocyanidin A-1 had the strongest superoxide scavenger activity, followed by cinnamtannin B_1.

Discussion

The tissue damage caused by reactive oxygen species may include DNA damage, lipid peroxidation (Halliwell & Gutteridge 1984), protein damage (Bartold et al 1984), oxidation of important enzymes (Varani et al 1985), and stimulation of pro-inflammatory cytokine release by monocytes and macrophages (Shirikawa et al 1989). The major type of defence in live systems against oxidative damage is the use of antioxidant enzymes to convert reactive oxygen species into non-toxic compounds. The use of antioxidants is an effective procedure for preventing oxidative damage.

The biological activities of tannins include marked anti-tumour, antiviral and anti-HIV activities, inhibition of lipid peroxidation and plasmin activity, mediation of DNA nicking, amelioration of renal failure, and several others (Yokozawa et al 1993). The anti-radical and antioxidant activities vary greatly among different classes of compounds, even in those of the same type.

The $FeCl_2$–ascorbic acid stimulated lipid peroxidation method is an indirect measure of lipid peroxidation which is susceptible to interference by endogenous and exogenous substances; it should be regarded as an indication rather than as an absolute measure of total tissue lipid peroxide levels (Draper & Hadley 1990). Xanthine oxidase converts hypoxanthine to xanthine and then xanthine to uric acid in the presence of molecular oxygen to yield superoxide anion and hydrogen peroxide. Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and oedema as well as changes in vascular permeability (McCord & Fridovich 1968; Hearse et al 1986). Limitation of superoxide anion regeneration by the enzymatic pathway would be beneficial in ischaemia and oedema. Our results indicate that tannins have an inhibitory effect on radical generation. The cytochrome C reduction test is an indirect evaluation for the formation of superoxide anions and may be influenced by xanthine oxidase inhibitors to give a pseudo-positive reaction.

According to Morimoto et al (1988) procyanidin B-1 (a flavan-3-ol tannin) and procyanidin B-3 are singly-linked proanthocyanidins. Proanthocyanidin A-1 and cinnamtannin B_1 are doubly-linked proanthocyanidins, epicatechin-(4β⟶8)-epicatechin-(4β⟶8, 2β⟶O⟶7)-catechin and epicatechin - (4β⟶6)-epicatechin - (4β⟶8, 2β⟶O⟶7)-catechin are condensed tannins possessing a proanthocyanidin A-1 unit. Previous studies have shown that procyanidins and proanthocyanidins have anti-complementary activity (Shahat et al 1996) and anti-lipid peroxidation effects on rat

heart mitochondria (Hong et al 1995). Cinnamtannin B_1 showed cytotoxicity against melanoma cells (Kashiwada et al 1992). In our study, the multiple antioxidant activities of these six tannins were evaluated. Cinnamtannin B_1 displayed the strongest activity in anti-lipid peroxidation, proanthocyanidin A-1 displayed the strongest superoxide scavenging activity, and epicatechin-(4$\beta \longrightarrow$6)-epicatechin-(4$\beta \longrightarrow$8, 2$\beta \longrightarrow$O$\longrightarrow$7)-catechin had the strongest anti-superoxide formation effect.

We conclude that the tannins extracted form *V. vitis-idaea* L. exhibit anti-lipid peroxidation, anti-superoxide formation, and superoxide scavenger activities. This may be useful for the treatment of oxidative damage of tissues caused by the generation of reactive oxygen species, including periodontal disease.

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