

Antimicrobial activity of tannin components from *Vaccinium vitis-idaea* L.

K. Y. Ho, C. C. Tsai, J. S. Huang, C. P. Chen, T. C. Lin and C. C. Lin

Abstract

Reactive oxygen species have been implicated as important pathological mediators in many clinical disorders, including periodontal disease. As a possible alternative for the treatment of periodontal disease, the antimicrobial activity of six tannins isolated from *Vaccinium vitis-idaea* L., with confirmed antioxidant activity, were assayed by the agar dilution method against selected periodontal pathogens, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. The results showed that epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin had strong antimicrobial activity against *P. gingivalis* and *P. intermedia*, but not *A. actinomycetemcomitans*. The other tannins tested did not show antimicrobial activity. We conclude that tannins isolated from *V. vitis-idaea* L. with antimicrobial activity could potentially be used for the treatment of periodontal disease.

Introduction

Human inflammatory periodontal disease is generally recognized as a bacterial plaque-associated disease. The pathogenesis of periodontal disease is a result of complex interactions between pathogenic bacteria and the host's immune response. Periodontal therapy directed towards removing local irritants combined with proper oral hygiene has been demonstrated to be effective in the treatment of periodontal disease (Tagge et al 1975; Rosling et al 1976). However, mechanical treatment may not predictably eliminate putative pathogens such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (van Winkelhoff et al 1996). Thus, mechanical therapy has more recently been augmented with local or systemic administration of antimicrobial agents.

Reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻) are continuously formed in the human body. It is likely that the role of ROS is common to both bacterial- and host-mediated pathways of tissue damage. The radicals induce lipid peroxidation (Halliwell & Gutteridge 1984), and have been implicated as important pathological mediators in many clinical disorders (Slater & Cheeseman 1987). Ischaemia-reperfusion injury, diabetes mellitus (Sugawara et al 1992), coronary arteriosclerosis (Kok et al 1991), aging and carcinogenesis (Oberley & Oberley 1986; Yagi 1987), and more recently periodontal disease (Whyte et al 1989; Shapira et al 1991; Kimura et al 1993) are also related with radical oxidative damage.

Vaccinium vitis-idaea L. is an ericaceous plant used as a remedy for gonorrhoea, dysuria and diarrhoea (Mitsushashi 1988). The leaf and berries of this plant have been shown to have anti-viral and anti-inflammatory effects (Fokina et al 1993;

Graduate Institute of Dental
Sciences and Department of
Periodontics, Kaohsiung Medical
University, Kaohsiung, Taiwan
K. Y. Ho, C. C. Tsai, J. S. Huang

Department of Pharmacy, Ta-Jen
Pharmaceutical College, Ping-
Tung, Taiwan
C. P. Chen, T. C. Lin

Graduate Institute of Natural
Products, Kaohsiung Medical
University, Kaohsiung, Taiwan

C. C. Lin
Correspondence: Chun-Ching
Lin, No. 100, Shih-Chuan 1st
Road, Graduate Institute of
Natural Products, Kaohsiung
Medical University, Kaohsiung,
Taiwan 807. E-Mail:
aalin@ms24.hinet.net

Tunon et al 1995). The active constituents include arbutin, hyperin, hydroquinone, isoquarcetin and tannins (Perry 1980). We previously demonstrated that tannins isolated from *V. vitis-idaea* L. exhibited multiple antioxidant activity (Ho et al 1999). However, to our knowledge, there is little information about the use of these antioxidants as antimicrobial agents in the treatment of periodontal disease. In this study, six tannins isolated from *V. vitis-idaea* L. were evaluated for antimicrobial activity against periodontal pathogens, *A. actinomycetemcomitans*, *P. gingivalis* and *Prevotella intermedia*.

Materials and Methods

Microorganisms

A. actinomycetemcomitans (ATCC 29523), *P. gingivalis* (ATCC 33277) and *P. intermedia* (ATCC 25611) were generously provided by the University of Pennsylvania School of Dental Medicine, PA. The stock strains were suspended in brain heart infusion (BHI) medium containing 15% glycerol and kept in the freezer at -70°C .

Plant material

Tannins from *V. vitis-idaea* L. were isolated according to the method of Morimoto et al (1988). The tannins studied were: procyanidin B-1, procyanidin B-3, proanthocyanidin A-1, cinnamtannin B₁, epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin, and epicatechin-(4 β →6)-epicatechin-(4 β →8, 2 β →O→7)-catechin (Figure 1).

Antimicrobial activity

Antimicrobial activity was assayed by the agar dilution method (Baron & Finegold 1990) using BHI agar (Difco Laboratories) supplemented with 0.5% yeast extract, 10 mg mL⁻¹ vitamin K, 5 mg mL⁻¹ hemin and 5% sheep red blood cells. Before testing, bacteria were inoculated in the same medium and incubated for 3–4 days at 35°C. The cultures were then harvested and adjusted with Brucella broth to $2\text{--}5 \times 10^7$ colony-forming units (CFU) mL⁻¹. The highest concentration of tannin or tetracycline in serially-diluted solution was 100 $\mu\text{g mL}^{-1}$. Media containing tannin was prepared by adding 1 vol of twofold serially-diluted tannin solution (in 50% dimethylsulfoxide) to 9 vols BHI agar that had been warmed to 50°C and supplemented with vitamin K, hemin and sheep red blood cells as described above. Agar plates containing tannin, a control plate without

tannin and a positive control plate containing tetracycline were spot inoculated with 10^4 CFU mL⁻¹ with a Steers Replicator (Ericsson & Sherries 1971) immediately after preparation of the inocula. The agar plates were allowed to stand until the inoculum spots were completely absorbed, and were then placed in a GasPak anaerobic jar and incubated at 35°C for 3–4 days. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of tannin at which no growth of organisms was observed. All data represent at least three replicated experiments per microorganisms.

Results and Discussion

The antimicrobial activity (MIC) of the six tannin compounds isolated from *V. vitis-idaea* L. is summarized in Table 1. The MIC of tannins against *A. actinomycetemcomitans* was $\geq 100 \mu\text{g mL}^{-1}$, and was classified as having no antimicrobial activity. For *P. gingivalis* and *P. intermedia*, the MIC of tannins was 25–100 $\mu\text{g mL}^{-1}$ or greater. Epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin displayed significant antimicrobial activity, while other tannins were considered as having no antimicrobial activity. Tetracycline displayed strong antimicrobial activity (MIC 0.4–1.6 $\mu\text{g mL}^{-1}$).

The types of tissue damage caused by ROS include DNA mutation, 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 monocyte and macrophage (Shirikawa et al 1989). The major type of defence in living systems against oxidative damage is the use of antioxidant enzymes to convert ROS to non-toxic compounds. Antioxidants are classed as scavenging antioxidants such as β -carotene, retinol, ascorbic acid, α -tocopherol, urate and those substances containing sulfhydryl groups. Preventive antioxidants function largely by sequestering transition metal ions and preventing Fenton reactions, and enzyme antioxidants such as catalase and superoxide dismutase, function by catalysing the oxidation of other molecules (Chapple 1997).

Shapira et al (1991) reported that superoxide formation by peripheral polymorphonuclear leucocytes is increased in rapidly progressive periodontitis patients. In periodontal diseases, proteases and free radicals are released from inflammatory cells, particularly polymorphonuclear leucocytes, which may damage adjacent periodontal tissues (Asman et al 1994). Furthermore, periodontal disease-related bacteria including *Porphy-*

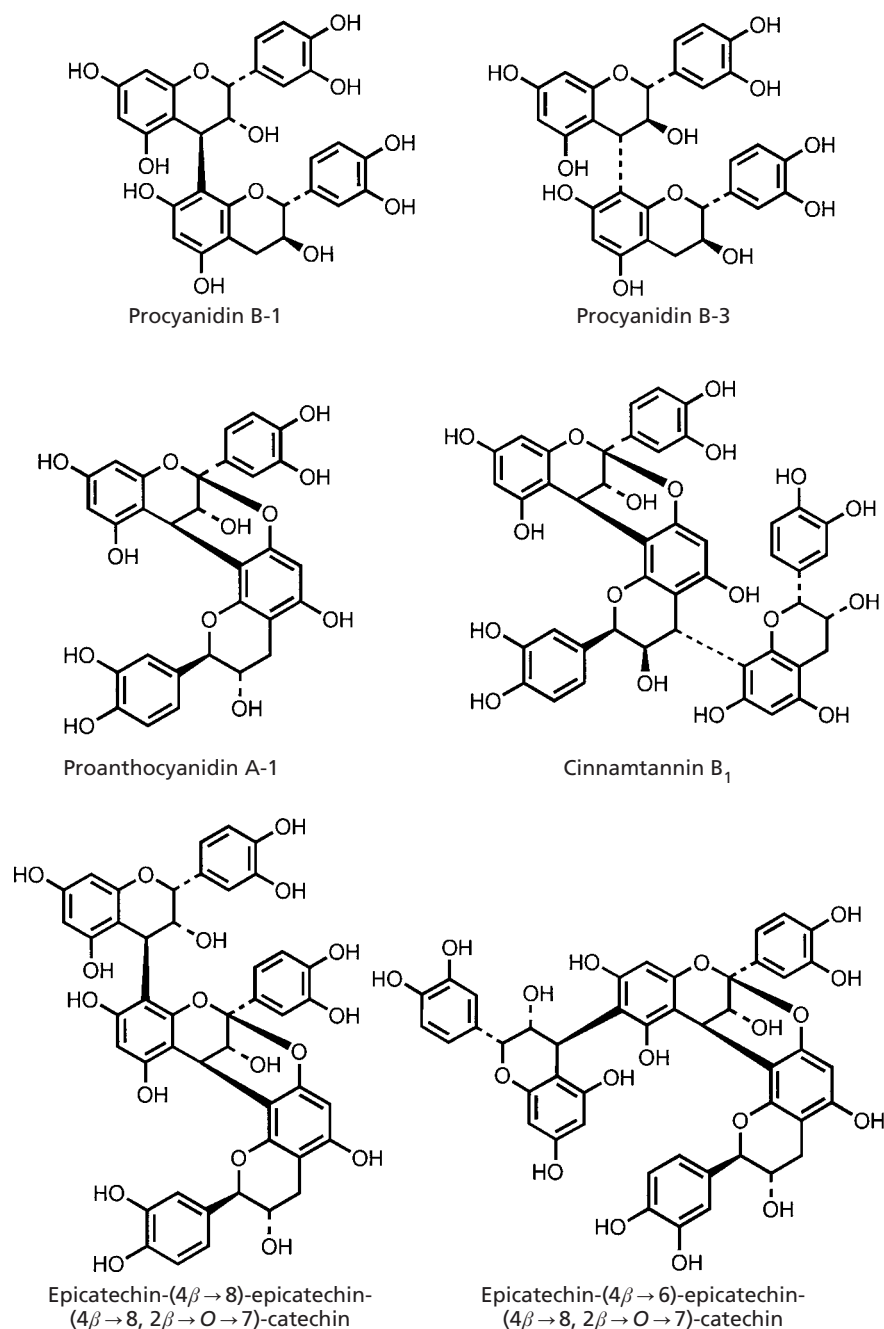


Figure 1 Structures of tannins isolated from *Vaccinium vitis-idaea* L.

romonas and *Prevotella* species, and *A. actinomycetem-comitans* are capable of producing collagenase (Robertson et al 1982). The killing of collagenase-producing bacteria and inhibition of collagenase activity by antibiotics (McCulloch et al 1990) and tea catechin (Makimura et al 1993) may help prevent periodontal disease.

Tannins are polyphenols and are widely present in plants. The biological activity of tannins include marked anti-tumour, anti-viral and anti-HIV activity, inhibition of lipid peroxidation and plasmin activity, mediation of DNA-nicking and amelioration of renal failure (Yokozawa et al 1993). The anti-radical and antioxidant activity varies greatly among different classes of com-

Table 1 Effect of tannins and tetracycline on pathogens of human periodontitis.

Microorganism	Minimum inhibitory concn ($\mu\text{g mL}^{-1}$)						
	1	2	3	4	5	6	7
<i>Actinobacillus actinomycetemcomitans</i>	> 100	100	> 100	> 100	> 100	> 100	1.6
<i>Porphyromonas gingivalis</i>	> 100	100	> 100	100	25	100	1.6
<i>Prevotella intermedia</i>	> 100	> 100	> 100	100	25	100	0.4

1, Procyanidine B-1; 2, procyanidine B-3; 3, proanthocyanidin A-1; 4, cinnamtannin B₁; 5, epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin; 6, epicatechin-(4 β →6)-epicatechin-(4 β →8, 2 β →O→7)-catechin; 7, tetracycline.

pounds. *V. vitis-idaea* L. has been shown to have antiviral and anti-inflammatory effects (Fokina et al 1993; Tunon et al 1995). Since periodontal disease is related to inflammation processes, and ROS have been implicated as important pathological mediators in inflammation, the antimicrobial activity of tannins components of *V. vitis-idaea* L. were evaluated in this study.

According to Morimoto et al (1988), the epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin and epicatechin-(4 β →6)-epicatechin-(4 β →8, 2 β →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 inhibitory effects on lipid peroxidation of rat heart mitochondria (Hong et al 1995). Cinnamtannin B₁ showed cytotoxicity against melanoma cells (Kashiwada et al 1992). The strong antimicrobial effect of epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin might be associated with the condensed structure or the collagenase inhibitory effects (Makimura et al 1993). Further research is necessary to determine why other tannins exhibited multiple antioxidant activity, but not antimicrobial activity.

Tetracycline is probably the most commonly used antibiotic for the treatment of periodontitis, but is not recommended for children under 9 years of age. In the prevention of recurrent periodontitis and refractory periodontitis, some resistance has been noted (McCulloch et al 1990). Although epicatechin-(4 β →8)-epicatechin-(4 β →8, 2 β →O→7)-catechin had less potent antimicrobial activity than tetracycline in this study, it show marked antimicrobial activity against some periodontal pathogens, and may be useful as an oral antiseptic for the prevention of periodontal disease.

We conclude that certain tannins extracted from *V. vitis-idaea* L. exhibit antimicrobial activity and may be useful for the treatment of tissue damage caused by the

generation of ROS, including the treatment of periodontal disease. Further studies on delivery systems for periodontal use, stability in the diseased periodontal environment in-vivo and side-effects on soft and hard tissues are necessary.

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