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The antimicrobial activity of compounds from the leaf and stem of *Vitis amurensis* against two oral pathogens

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ABSTRACT

Nine compounds isolated from the leaf and stem of *Vitis amurensis* Rupr. (Vitaceae) were evaluated for their antimicrobial activity against two oral pathogens, *Streptococcus mutans* and *Streptococcus sanguis*, which are associated with caries and periodontal disease, respectively. The results of several antimicrobial tests, including MIC, MBC, and TBAI, showed that three compounds inhibited the growth of the test bacteria at concentrations ranging from 12.5 to 50 μg/mL. Among these compounds, compound **5**, *trans*-ε-viniferin, displayed the strongest activity against *S. mutans* and *S. sanguis* with MIC values of 25 and 12.5 μg/mL, respectively. This is the first report on the antimicrobial activity of stilbenes and oligostilbenes isolated from the leaf and stem of *V. amurensis*. Thus, this result suggests that natural antimicrobial compounds derived from *V. amurensis* may benefit oral health as plaque-control agents for the prevention of dental caries and periodontal disease.

Dental caries is considered to be a preventable disease. Prevention requires minimizing the frequency of ingesting simple carbohydrate foods and beverages, regular oral hygiene measures to remove plaque and the introduction of topical fluoride in the form of toothpaste.[1,2] It is still considered one of the most prevalent infectious diseases in the world. Although the nature of the disease and the methods of treatment have advanced tremendously during the past century, the disease is still not controlled worldwide.[3]

Streptococcus mutans and *Streptococcus sanguis* are very important oral microflora. *S. mutans* is the main microorganism that causes dental caries, dental plaque, and cariogenicity.[4] This organism is also involved in a causative agent of endocarditis. *S. sanguis* indirectly affects the caries process through its competition with *S. mutans* for the inhabitation of extant oral niches during dental plaque formation.[5,6] Unlike *S. mutans*, which is frequently associated with dental caries, *S. sanguis* is believed to have low cariogenic potential. However, this strain also has been implicated in cases of subacute bacterial endocarditis and reported drug-resistance.[7]

Recently, bacterial resistance to antibiotics has caused great concern worldwide. The application of antibiotics for the prevention and treatment of dental caries would be harmful for the patients and could promote the development of multidrug-resistant (MDR) strains of bacteria.[2] Numerous efforts have been made to find new antibacterial compounds from various sources, such as microorganisms, animals, and plants.[8] A literature survey showed promising data on the feasibility of using available natural products as preventive measures for dental caries.[3] In the course of screening natural products, the 70% EtOH extract of the leaf and stem of *Vitis amurensis* showed potent inhibitory activity against *S. mutans* and *S. sanguis*. *V. amurensis*, a wild-growing grape distributed in Korea, Japan, and China, has been used for centuries as traditional oriental medicine.[9] The root and stem have been used to relieve pain from injury, rheumatalgia, stomachache, neuralgic pain, and abdominal pain.[10] In particular, the root of *V. amurensis* has been reported to possess antioxidant, anti-inflammatory, and antitumor activities.[11] Various phytochemicals have been reported in *V. amurensis*, including the polyphenols, catechin, procyanidin, and amuresin.[12,13] Although various in vitro studies have been performed to investigate the mode of actions of these phytochemicals and their effects, effects on oral health and disease prevention have not been previously reported.

In further screens for growth inhibitory activity against oral pathogens, an EtOAc fraction of *V. amurensis* demonstrated preferential antimicrobial activity against the oral pathogens *S. mutans*

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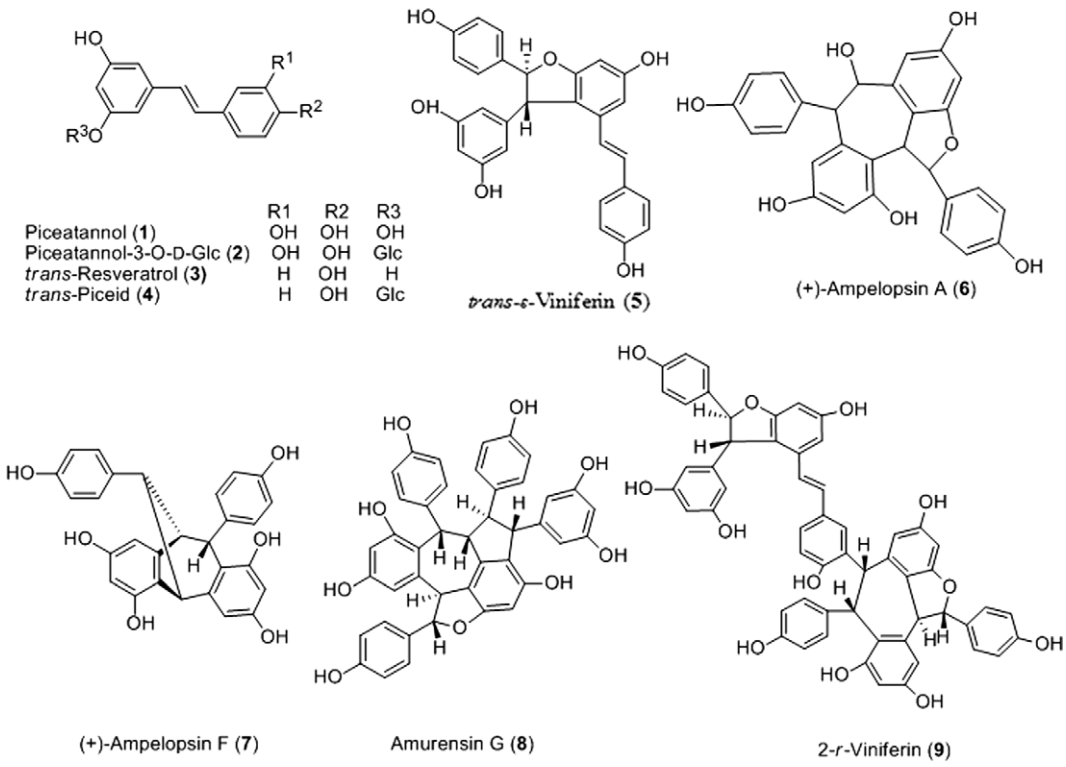

Figure 1. Structures of stilbenes and oligostilbenes (**1**–**9**) from leaf and stem of *Vitis amurensis*.

and *S. sanguis* at 0.5 and 0.25 mg/mL, respectively (data not shown). Subsequently, efforts have been made to discover antimicrobial compounds among the nine compounds (**1**–**9**) previously isolated from the EtOAc fraction of *V. amurensis*, which include: piceatannol (**1**), piceatannol-3-*O*-D-Glc (**2**), *trans*-resveratrol (**3**), *trans*-resveratrol-3-*O*-D-Glc (*trans*-piceid, **4**), *trans*-ε-viniferin (**5**), (+)-ampelopsin A (**6**), (+)-ampelopsin F (**7**), amurensin G (**8**), and 2-*r*-viniferin (**9**)[9] to serve as plaque-control agents for the prevention of dental caries (Fig. 1).

Two strains, *S. mutans* and *S. sanguis*, were incubated in Todd–Hewitt broth (BD, USA) at 37 °C in a 5% CO_2 incubator. Antimicrobial activity was determined using the disk diffusion assay[14,15] and by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in accordance with NCCLS guidelines and Koo et al.[16,17] Adherence inhibition tests were conducted according to the method of Myrella et al.[18] and Farid. et al.[3,19]

As shown in Table 1, compounds **1**, **3**, and **5**–**9** demonstrated inhibitory activity against the Gram-positive cariogenic oral streptococci, *S. mutans* and *S. sanguis* in a dose-dependent manner. It appears that the glycosylations **2** and **4** of piceatannol and resveratrol, respectively, did not inhibit microbial growth. Among the seven compounds, *trans*-ε-viniferin (**5**) displayed the strongest activity against *S. mutans* and *S. sanguis* with MIC values of 25 and 12.5 μg/mL, respectively. Furthermore, MBC and TBAI values of *trans*-ε-viniferin (**5**) also indicated the strongest activity against the two oral pathogens (Table 2). Compounds **1**, **3**, and **8** showed considerable activity against *S. mutans* with MIC values of 50 μg/mL. These compounds also displayed activity against *S. sanguis* with MIC values of 50, 25, and 12.5 μg/mL, respectively, while the MIC of the positive control (erythromycin) was 12.5 μg/mL (Table 2). Compound **3**, **8**, and **9** showed good inhibition of adherence by *S. mutans* at sub-MIC concentrations (MIC value of 50 μg/mL; TBAI value of 25 μg/mL) (Table 2). This result is consistent with the effective inhibition of glucosyltransferase B and C by these compounds; the sucrose-dependent adherence and accumulation of cariogenic streptococci is mediated by water-insoluble glucans synthesized by these enzymes.[20]

The potent growth inhibition of **9** was observed only against *S. mutans* with an MIC value of 50 μg/mL. However, **9** showed the

Table 1
Antimicrobial activity of compounds isolated from the leaf and stem of *V. amurensis* against oral pathogens by disk diffusion assay[a]

Compounds (μg/disk)	*Streptococcus mutans*					*Streptococcus sanguis*				
	50	100	200	400	600	50	100	200	400	600
Piceatannol (**1**)	++	++	+++	++++	++++	++	++	+++	++++	++++
Piceatannol-3-*O*-D-Glc (**2**)	–	–	–	–	–	–	–	–	–	–
trans-Resveratrol (**3**)	+	++	+++	+++	++++	+	+	++	++	+++
trans-Resveratrol-3-*O*-D-Glc (**4**)	–	–	–	–	–	–	–	–	–	–
trans-ε-Viniferin (**5**)	++	++	+++	++++	++++	++	+++	+++	+++	+++
(+)-Ampelopsin A (**6**)	–	–	+	++	+++	–	–	+	++	+++
(+)-Ampelopsin F (**7**)	–	+	++	++	+++	–	–	+	++	++
Amuresin G (**8**)	–	+	+	++	++	+	++	++	++	++
2-*r*-Viniferin (**9**)	–	+	++	++	++	–	+	+	++	++

[a] The antimicrobial activity was represented as followed: –, no inhibitory zone; +, <11 mm; ++, <15 mm; +++20 mm; ++++, over 20 mm.

Table 2
Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and total bacterial adherence inhibition (TBAI) of the compounds from *V. amurensis*[*]

Compounds	*Streptococcus mutans*			*Streptococcus sanguis*		
	MIC (μg/mL)	MBC (μg/mL)	TBAI (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	TBAI (μg/mL)
Piceatannol (**1**)	50	100	50	50	100	50
trans-Resveratrol (**3**)	50	50	25	25	100	100
trans-ε-Viniferin (**5**)	25	50	25	12.5	50	50
(+)-Ampelopsin A (**6**)	200	200	100	>400	>400	>200
(+)-Ampelopsin F (**7**)	100	200	100	>400	>400	>200
Amuresin G (**8**)	50	50	25	12.5	100	50
2-*r*-Viniferin (**9**)	50	50	25	200	400	100
Erythromycin	<1.5	<1.5	0.78	12.5	50	25

[*] The experiment was repeated three times and the concentrations are shown as average values of three independent determinations.

weak activity against *S. sanguis* with an MIC values of 200 μg/mL. Compounds **6** and **7** did not have potent antimicrobial effect (Table 2). Except for compounds **2** and **4**, the seven antimicrobial compounds exhibited higher MIC values than that of the crude EtOAc fraction, suggesting that these compounds contributed significantly to the activity observed in the EtOAc fraction. Compounds **3** and **5** represent the most active compounds in the EtOAc fraction, and they are capable of suppressing the growth of the test oral pathogens. Compounds **3** and **5** were previously reported to be the major compounds of the EtOAc fraction and they have LOX-1 inhibitory activity.[21]

The glycosides (**2** and **4**) of piceatannol and resveratrol did not inhibit microbial growth. This demonstrates that glycosylation system may protect macrolide-producing microorganisms during antibiotic biosynthesis or be a mechanism of macrolide resistance in pathogens.[22] Thus we infer that glycosylation of piceatannol and resveratrol resulted in virtually the complete loss of antibacterial activity.

Erythromycin had a considerably higher antimicrobial activity than the isolated compounds against *S. mutans*, whereas these antibiotics showed low antimicrobial activity against *S. sanguis*. The MIC value of erythromycin against *S. sanguis* was the same as those of compounds **5** and **8** (Table 2). Yoshihiko et al. have reported that *S. sanguis* is resistant to erythromycin.[7]

Recent reports have shown the presence of several stilbenoids and oligostilbenoids in the stem of *V. amurensis*. Considerable attention has been focused on naturally occurring oligostilbenes because they have been found to have multiple bioactivities.[23,24] Herein, one stilbene (**3**) and one oligostilbene (**5**), the main compounds found in the leaf and stem of *V. amurensis*, presented the most potent antimicrobial activity. Therefore, they may have potential for further development as natural antiplaque agents. Interestingly, phenolic compounds have been found to complex with proteins and inactivate microbial adhesions, enzymes, and cell envelope transport proteins.[25] Thus, plant-derived antimicrobial compounds may serve as alternatives to the commonly used chemicals for dental plaque and oral disease control.

In conclusion, the results suggest that antimicrobial compounds from natural leaf and stem may benefit oral health as plaque-control agents for the prevention of dental caries and periodontal disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.020.

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