Korean J. Plant Res. 21(6) : 431~434(2008)

Anticariogenic activity of piceatannol isolated from *Callistemon citrinus* fruit against *Streptococcus mutans*

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Abstract - Dental caries is the destruction of the enamel of teeth by *Streptoccus mutans*. *S. mutans* has been isolated from human dental plaque and is associated with the initial development of enamel lesions. We have studied the antibacterial action of the fruit of *Callistemon citrinus* against a cariogenic bacterium, *S. mutans*. From the fruit of *C. citrinus*, piceatannol (3,3',4',5-tetrahydroxystilbene) was isolated by repeated column chromatography with SiO_2 and Sephadex LH-20. Its structure was elucidated by instrumental analysis using 1D-NMR, 2D-NMR and EI-MS. This compound was isolated from the fruit of *C. citrinus* for the first time. The anticarcinogenic activity of this compound was determined by using agar well-diffusion method and minimal inhibition concentration (MIC).

Key words - *Callistemon citrinus*, *Streptococcus mutans*, piceatannol, anticariogenic activity

Introduction

Callistemon citrinus (Myrtaceae) is a shrub with a distinct citrus aroma (thus the common name is lemon bottlebrush). Ethanolic leaf extracts of *C. citrinus* were shown to have a strong anti-Mycobacterium tuberculosis activity (Frame *et al*., 1998). In addition, a compound isolated as a component of the steam-volatile oils from *C. citrinus*, leptospermone, showed a moderately active herbicide and thus produced unique bleaching symptoms on susceptible weed species (Mitchell *et al*., 2001). The water-distilled essential oil from the leaves and flowers of *C. citrinus* contained the active components. The chemical composition was determined by GC, GC/MS and GC/FTIR, and identified as 1,8-cineole, α-pinene and α-terpineol (Chane-Ming *et al*., 1998).

In the course of study to search anticariogenic agents (Lee *et al*., 2002; Park *et al*., 2005), we found that the extracts from *C. citrinus* had antibacterial activity against *Streptococcus mutans* which is a bacterial species in oral flora and is associated with the initial development of enamel lesions and producing dental caries (Hamada and Slade, 1980; Loesche, 1986). Numerous chemicals and antibiotics are being used as antibacterial agents against *S. mutans* to reduce plaque-mediated diseases including dental caries (Gjermo *et al*., 1973). However, many of these agents are known to have undesirable side effects such as teeth staining, vomiting and diarrhea (Chen *et al*., 1989). For this reason, great concern is focused on natural products including wood extractives as natural antifungal agent (Park *et al*., 2004). Moreover, many researches are reported on screening natural products including medicinal plants for anticariogenic agents (Skanaka *et al*., 1989; Bae *et al*., 1992).

Here, we report the isolation and identification of compound from the fruit of *C. citrinus* and also evaluated its anticariogenic activity.

Materials and Methods

Plant materials and extraction

The fruit of *C. citrinus* were collected from Jeju Province, Korea during June, 2002. A voucher specimen was deposited at the Korea Forest Research Institute, Suwon, Korea. Dried the fruit of *C. citrinus* was finely ground, extracted twice with ethanol (EtOH) and then evaporated to give the crude extract. The crude extracts of the fruit was successively partitioned with various organic solvents in the order of *n*-hexane, methylene chloride (CH_2Cl_2) and ethyl acetate (EtOAc).

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Isolation of compound

The methylene chloride fraction was subjected to column chromatography on silica gel column eluted with CH_2Cl_2-MeOH (30:1, v/v) to yield 3 sets of fraction (CC1 ~ CC3). Fraction CC3 was rechromatographed on Sephadex LH-20 column with MeOH-H_2O (1:1, v/v) to give 3 subfractions (CC3-1 ~ CC3-3). Compound was obtained from second fraction (CC3-2) among three subfractions as brown amorphous powder.

EI-MS *m/z* : 244 (M^+, molecular ion) 173, 81, 63. ^{1}H-NMR (500 MHz, CD_3OD) : δ 6.17 (1H, *t*, *J* = 2.3 Hz, H-4), δ 6.45 (2H, *d*, *J* = 2.5 Hz, H-2,6), δ 6.74 (1H, *d*, *J* = 8.5 Hz, H-5'), δ 6.75 (1H, *d*, *J* = 16 Hz, H-7), δ 6.84 (1H, *dd*, *J* = 2, 9 Hz, H-6'), 6.89 (1H, *d*, *J* = 16 Hz, H-8), 6.98 (1H, *d*, *J* = 2 Hz, H-2').

^{13}C-NMR (125 MHz, CD_3OD) : δ 159.6 (C-3,5), 146.5 (C-4'), 146.4 (C-3'), 141.3 (C-1), 131.1 (C-1'), 129.7 (C-8), 127.0 (C-7), 120.2 (C-6'), 116.4 (C-5'), 113.8 (C-2'), 105.7 (C-2, 6), 102.6 (C-4).

HMBC correlations : H-2,6→C-7, H-2'→C-8/C-8, H-5'→C-1', H-8→C-1/ C-7/C-1'/C-2'.

Instrument analysis

^{1}H and ^{13}C NMR with TMS as internal standard. NMR spectra were obtained using a Varian UI 500 spectrometer at the operating frequency of 500 MHz (^{1}H) and 125 MHz (^{13}C) at Korea Basic Science Institute in Seoul. EI-MS: JEOL JMS-600W, direct inlet at 70 eV.

Anticariogenic acivity test

S. mutans (KCTC 3065) was used to measure antibacterial activity of the extracts by paper disc diffusion method. *S. mutans* was cultured in BHI broth (Difco) at 37℃ for 48hr and *ca*. 1×10^6 cell numbers of bacteria were mixed uniformly with BHI agar medium in petri dish. Paper discs soaked in the crude drug extract were carefully placed on the bacteria agar medium in petri dishes. The cultivation was carried out at 3 7℃ incubator for one day and the inhibition zone against *S. mutans* was measured.

The minimal inhibition concentration (MIC) in the samples were measured according to the methods of Namba *et al*. (1982) and Hwang *et al*. (2004). Samples (10 mg) were dissolved in ethanol (1 mL) and prepared in a series of diluted solution. The solution (0.1 mL) was then added to 0.9 mL of sterile liquid BHI with 0.1 mL of a bacterial suspension (ca. 2×10^5 colony forming units ((CFU)/mL) to each test tube. The tube was mixed and incubated at 37℃ for 48hr. The MIC was defined as the lowest concentrations of a crude extract at which no visible growth of *S. mutans* was observed.

Results and Discussion

Identification of compound isolated from the fruit of *C. citrinus*

The structure was elucidated by spectral data such as MS, ^{1}H- and ^{13}C-NMR spectra and 2D-NMR spectra. Compound obtained from the methylene chloride soluble fraction of the ethanol extract of the fruit of *C. citrinus* was isolated as brown amorphous powder. The ^{1}H-NMR spectrum of compound showed signals assignable to one doublet representing two proton at δ 6.45 (2H, *d*, *J* = 2.5 Hz, H-2,6) and two *trans* olefinic protons at δ 6.75 (1H, *d*, *J* = 16 Hz, H-7) and 6.89 (1H, *d*, *J* = 16 Hz, H-8). A total of 12 carbons appeared in the ^{13}C-NMR spectrum, which induced three oxgenated-olefine tertiary carbon (δ 159.6, 146.5, 146.4), two olefine tertiary carbon (δ 141.3, 131.1) and seven olefine methine carbon (δ 129.7, 127.0, 120.2, 116.4, 113.8, 105.7, 102.6). Carbons with protons and their protons were precisely matched by the HMQC experiment. In the HMBC spectrum (Figure 1), major correlations were observed between H-8 and C-1 (141.3), C-7 (127.0), C-1' (131.1), and C-2' (113.8). From the above evidence, the structure of compound was concluded to be 3,3',4',5-tetrahydroxystilbene, piceatannol (Figure 2) which isolated from the fruit of *C. citrinus* for the first time. All the spectral data of this compound were in good agreement with those of Oh *et al*. (2001) and Li *et al*. (2005).

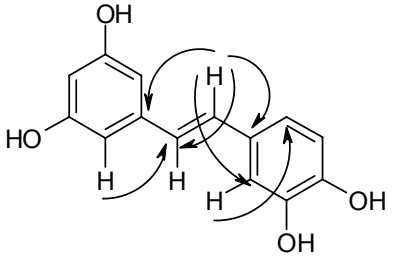

Fig. 1. HMBC correlations for the compound isolated from the fruit of *C. citrinus*.

Table 1. Anticariogenic activities of crude extracts, methylene chloride fraction and piceatannol from *C. citrinus* against *S. mutans*.

Samples	Diameter of inhibition zone (mm)					MIC ($ug \cdot mL^{-1}$)
	5*	3.75	2.5	1.25	0.62	
Crude extracts	19.1	17.5	15.8	12.5	10.0	31.3
CH_2Cl_2 fraction	18.5	16.3	15.2	13.1	9.8	31.3
Piceatannol	21.3	20.3	17.9	15.3	12.1	15.6

*mg/disk

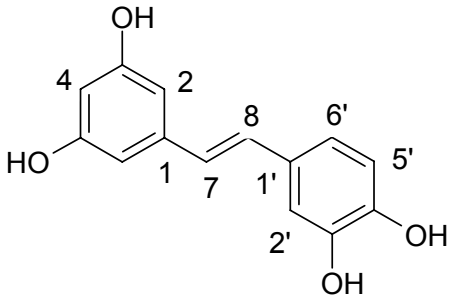

Fig. 2. Chemical structure of the compound isolated from the fruit of *C. citrinus.*

Anticariogenic acivity

The anticariogenic activity of the fruit extracts, its CH_2Cl_2 fraction, and piceatannol from the *C. citrinus* are shown in Table 1. The diameters of inhibition zone (mm) of fruit extracts its CH_2Cl_2 fraction, and piceatannol were 19.1, 18.5 and 21.3 mm (at 5 mg/disk), respectively. The MIC values of the fruit extract and its CH_2Cl_2 fraction and piceatannol are 31.3, 31.3 and 15.6 $ug \cdot mL^{-1}$, respectively, indicating that piceatannol had the highest anticariogenic activity. Piceatannol initially isolated from the heartwood of *Vouacapoua* species was previously identified as an antileukemic agent (Zheng and Ramirez, 1999). However, it is first time to report on the anticariogenic activity.

Compared with other researches, our results show that the *C. citrinus* plant has a strong inhibitory effect on the growth of *S. mutans*. Although the growth inhibition zones of baicalein and ganhuangenin (at 100 ㎍/disc) isolated from *Scutellaris baicalensis* were 11.2 and 10.2 mm, respectively (Moon *et al*., 1997), the MIC value of piceatannol had two times higher than that of sanguinarine isolated from *Sanguinaria canadensis*, being used for the industrial mouthwash products (Southard *et al*., 1984). Methanol extracts from *Coptis chinensis* also showed inhibitory effects on the growth of *S. mutans* with the MIC value of 130 $ug \cdot mL^{-1}$ (Jang *et al*., 2000). From the results we suggest, the fruit extracts and isolated compound, piceatannol, from *C. citrinus* could be used to effectively control dental caries.

Literature Cited

Bae, K., W. Seo, T. Kwon, S. Baek, S. Lee and K. Jin. 1992. Anticariogenic β-Carboline alkaloids from *Commelina communis*. Arch. Pharm. Res. 15: 220-223.

Chane-Ming, J.P.R.Vera and D. J. Fraisse. 1998. Chemical composition of essential oil of *Callistemon citrinus* (Curtis) Skeel from Reunion. J. Essent. Oil Res. 10: 429-431.

Chen, C.P., C.C. Lin and T. Namba. 1989. Screening of Taiwanese crude drugs for antibacterial activity against *Streptoccus mutans*. J. Ethnopharmacol. 27: 285-295.

Frame, A.D., E. Rios-Olivares, L. De Jesus, D. Ortiz, J. Pagan and S. Mendez. 1998. Plants from Puerto Rico with anti-Mycobacterium tuberculosis properties. P R Health Sci. J. 17: 243-252.

Gjermo, P., G. Oloosa and L. Arskang. 1973. Effect on dental plaque formation and some in vitro properties of 12 bisbiguanides. J. Periodont. Res. 12: 81-88.

Hamada, S. and H.D. Slade. 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. 44: 331-384.

Hwang J.K., J.Y. Chung, N.I. Baek and J.H. Park. 2004. Isopanduratin A from *Kaepferia panduata* as an active antibacterial agent against cariogenic *Streptococcus mutans*. Int. J. Antimicrob. Ag. 23: 377-381.

Jang, G.H., B.Y. Ahn, S.H. Oh, D.S. Choi and Y.J. Kwon. 2000. Anticariogenic effects of *Coptis chinensis* Franch extract. Kor. J. Food Sci. Technol. 32: 1396-1402. (in Korea)

Lee, W.Y., J.K. Ahn and Y.J. Kwon. 2002. The screening of antibacterial activity against *Streptococcus mutans* and glucosyltransferase inhibitory activity from woody plants.

KFRI J. For. Sci. 65: 30-46. (in Korea)

Li, Y., D.M. Zhang and S.S. Yu. 2005. A new stilbene from *Cercis chinensis* Bunge. J. Inter. Plant Biol. 47: 1021-1024.

Loesche, W.J. 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev. 50: 353-380.

Mitchell G, D.W. Bartlett, T.E. Fraser, T.R. Hawkes, D.C. Holt, J.K. Townson and R.A. Wichert. 2001. Mesotrione: a new selective herbicide for use in maize. Pest Manag. Sci. 57: 120-128.

Moon, Y.H., Y.H. Lee, B.S. Min and K.H. Bae. 1997. Antibacterial constituents from *Scutellariae radix* against *Streptococcus mutans* OMZ176. Kor. J. Parmacogn. 28: 99-103. (in Korea)

Namba, T., M. Tsunezuka and M. Hattori. 1982. Dental caries prevention by traditional Chinese medicines. Planta medica. 44: 100-106.

Oh, S.J., N.I. Baek and H.Y. Kim. 2001. Piceatannol, antioxidant compound isolated from the root of *Rheum undulatum* L. J. Korean Soc. Agric. Chem. Biotechnol. 44: 208-210. (in Korea)

Park, J.S., H.H. Baek, D.H. Bai, T.K. Oh and C.H. Lee. 2004. Antibacterial activity of Fennel (*Foeniculum vulgare* Mill.) seed essential oil against the growth of *Streptococcus mutans*. Food Sci. Biotechnol. 13: 581-585.

Park, Y.K., W.Y. Lee, S.Y. Park, J.K. Ahn and M.S. Han. 2005. Anticariogenic activity of *Callistemon citrinus* extract against *Streptococcus mutans*. Mokchae Konghak 33: 72-77.

Sakanaka, S., M. Kim, M. Taniguchi and T. Yamamoto. 1989. Antibacterial substances in Japanese extract against *Streptococcus mutans*, a cariogenic bacterium. Agr. Biol. Chem. 53: 2307-2311.

Southard, G.L., R.T. Boulware, D.R. Walbonr and E.E. Thorne. 1984. Sanguinarine, a new antiplaque agent: retention and plaque specificity. J. Am. Dent. Assoc. 108: 338-343.

Zheng, J. and V.D. Ramirez. 1999. Piceatannol, a stilbene phytochemical, inhibits mitochondrial F0F1-AtPase activity by targeting the F1 complex. Biochem. & Biophysi. Re. Commun. 261: 499-503.

(Received 30 May 2008 ; Accepted 8 August 2008)