Antibacterial and Radical Scavenging Activities of 1-C-(*p*-Hydroxyphenyl)-Glycerol from *Trichosanthes kirilowii*

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1-C-(*p*-Hydroxyphenyl)-glycerol (1) was isolated and purified by column chromatography and recrystallization from the rhizome of *Trichosanthes kirilowii*, firstly in this species. Isolated compound showed inhibitory effects on the growth of five bacteria (*Bacillus cereus*, *Escherichia coli*, *Streptococcus faecalis*, *etc)* completely at the concentration higher than 10 ppm as well as delayed the growth of three bacteria (*Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*) at the concentration of 25 ppm for 60 h. Moreover, this compound showed potent antioxidant activity against DPPH radical (IC_{50} = 56.0 ppm)

Key words: *antibacterial, antioxidant, 1-C-(p-hydroxyphenyl)-glycerol, DPPH,* Trichosanthes kirilowii

Incidences of food-borne illnesses are major problem worldwide, estimating 6 to 81 million cases [Mead *et al.*, 1999]. It has been a threat for both consumers and the food industry despite the use of preserving processes. Meanwhile, consumers are concerned about the safety of foods containing preservatives. Therefore, there has been a growing interest in new and effective techniques to reduce cases of food-borne illnesses. Antimicrobial substances from natural sources like plants have been investigated to achieve higher levels of food safety [Otshudi *et al.*, 1999; Essawi and Srour, 2000].

Trichosanthes kirilowii is perennial climber, belonging to the *Cucurbitaceae* family, has a stem of about 5 m in length and bloom white flowers from July to September. An edible starch can be obtained from the rhizome. Its fruit, seed, and rhizome have been used in oriental medicine as an anti-inflammatory agent, a cough medicine, and an expectorant [Namba, 1994; Jiangsu, 1986].

In the present study, we isolated for the first time a compound from the rhizome of *T. kirilowii* and identified its structure through spectroscopic methods. In addition, isolated compound was evaluated for antimicrobial activity against the food-borne microorganisms and radical scavenging activity against DPPH.

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Abbreviations: DPPH, 1,1-Diphenyl-2-picrylhydrazyl; DEPT, Distortionless Enhancement by Polarization Transfer; HMBC, Hetero nuclear Multiple-Bond Connectivity.

Materials and Methods

Plant material. The rhizome of *T. kirilowii* was collected from Jeju (Korea) in August, 2005 and identified by Prof. Jae-Hong Pak. Extra pure grade solvents were purchased from Duksan Pure Chemicals (Ansan, Korea).

Reagents. DPPH was purchased from Sigma-Aldrich. All chemicals used were of analytical grade.

Instruments. Melting points were measured on a Thomas Scientific Capillary Melting point Apparatus and are uncorrected. IR spectra were recorded on a Bruker Vertex 70 infrared Fourier transform spectrophotometer (KBr) and UV spectra were measured on a Varian Cary100 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR at 500 and 125 MHz, respectively and 2D-NMR data were obtained on a Bruker AM 500 spectrometer in acetone-d_6. EIMS was obtained on a JEOLJMS-700 mass spectrometer. TLC was conducted on precoated Kieselgel 60F_{254} plates (Art. 5715; Merck) and the spots were detected either by examining the plates under a UV lamp or treating the plates with a 10% ethanolic solution of phosphomolybdic acid (Wako Pure Chemical Industries) followed by

heating at 110°C.

Extraction and isolation. The air-dried rhizome of *T. kirilowii* (1.0 kg) was cut into pieces and extracted at room temperature with 80% MeOH (4 L × 3) for 7 days. The combined extract was evaporated to dryness under reduced pressure at below 40°C. After filtration and concentration, the resultant extract (45.0 g) was suspended in H_2O (2 × 300 mL). The resulting suspension was diluted with H_2O and partitioned with organic solvents ($CHCl_3$ and *n*-BuOH) of different polarities to afford soluble-$CHCl_3$ (9.0 g), soluble-*n*-BuOH (11.0 g), and soluble-H_2O (18.0 g) extracts, respectively. The $CHCl_3$ extract (9.0 g) was chromatographed over silica gel using *n*-hexane : EtOAc and $CHCl_3$: MeOH gradient to give eight fractions (P1-P8). The fourth fraction (P4) (500 mg) was subjected to the silica gel column chromatography with *n*-hexane : ether (30 : 12 : 1) to afford 24 subfractions. Subfractions 10-15 were subjected to silica gel chromatography (*n*-hexane : ether = 25 : 15 : 1) and purified by recrystallization (hexane-ether mixture) to yield 1-C-(*p*-hydroxyphenyl)-glycerol (**1**) (62 mg).

1-C-(*p*-Hydroxyphenyl)-glycerol (**1**): colorless needle; mp 135-137°C; UV (CH_3OH) λmax (log e) 206 (2.51), 255 (4.15) nm; IR (KBr) ν_{max} 3351, 2935, 1462 cm^{-1}; ^{1}H-NMR (acetone-d_6, 500 MHz) δ 2.99 (1H, m, H-2), 3.73 (1H, dd, J = 3.5, 9.0 Hz, H-3a), 4.10 (1H, dd, J = 6.9, 9.0 Hz, H-3b), 4.62 (1H, d, J = 4.1 Hz, H-1), 6.73 (2H, d, = 8.5 Hz, H-2' and H-6'), and 7.14 (2H, d, J = 8.5 Hz, H-3' and H-5'); ^{13}C NMR (acetone-d_6, 125 MHz) δ 55.1 (C-2), 72.3 (C-3), 86.5 (C-1), 116.5 (C-2' and C-6'), 128.7 (C-3' and C-5'), 133.2 (C-1'), and 158.1 (C-4').

Microorganisms and cultures. All of bacterial strains were obtained from the Korean Culture Center of Microorganism (KCCM). Gram positive bacteria: *Bacillus substilis* (ATCC 9372), *B. cereus* (ATCC 27348), *Staphylococcus aureus* (ATCC 13301). Gram negative bacteria: *Escherichia coli* (ATCC 15489), *Vibrio parahaemolyticus* (ATCC 33844), *Pseudomonas aeroginosa* (ATCC 10490), *Streptococcus faecalis* (ATCC 29200), and *Staphylococcus epidermidis* (ATCC 12228). All bacterial strains except *V. parahaemolyticus* were grown at 32°C in a tryptic soy (TS) broth or a TS agar (Difco, Detroit, MI, USA). *V. parahaemolyticus* strain was grown at 32°C in a TS broth or a TS agar supplemented with 3% (w/v) NaCl.

Disc diffusion method. Inhibition tests were performed using the diffusion technique of Piddok (Piddok, 1990). Weighed aliquots of each dry sample were dissolved in methanol, and a 20 μL portion of each solution was placed on 8 mm Whatman paper disc to give concentrations of 6.25, 12.5, 25, 50, and 100 μg. The disc was then placed on an agar plate previously seeded with the microorganism and, after incubation at 32°C for 12 h, the zone of inhibition was measured.

Assessment of growth inhibition. The eight bacteria were subcultured on the appropriate agar media. The colonies of the bacteria colonies were cultured in TS broth at 32°C for 12 h. The liquid media (10 mL) were prepared by supplementing 1-C-(*p*-hydroxyphenyl)-glycerol (**1**) diluted with methanol at 0, 10, 25, and 50 ppm, followed by inoculation with 200 mL each of the above broth culture, and incubated at 32°C for 72 h. Optical density (OD) was measured at 12 h intervals during incubation using a spectrophotometer (Varian, Cary100, Australia) at 600 nm (Kim *et al.*, 2002; Lee *et al.*, 2001).

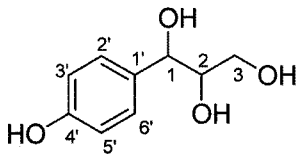

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Fig. 1. Structure of isolated compound 1 from the rhizome of *T. kirilowii*.

Table 1. Antibacterial activities of the isolated compound from the rhizome of *T. kirilowii*

Compound	Microorganisms	Zone of inhibition (mm)				
		100[a]	50	25	12.5	6.25
1-C-(*p*-Hydroxy-phenyl)-glycerol	*Bacillus cereus*	18	15	13	10	9
	Bacillus substilis	12	10	9	-[b]	-
	Staphylococcus epidermidis	19	15	12	10	-
	Streptococcus faecalis	20	17	13	11	10
	Vibrio parahaemolyticus	18	15	13	11	9
	Pseudomonas aeroginosa	16	13	11	-	-
	Escherichia coli	20	17	13	11	9
	Staphylococcus aureus	15	10	9	9	-

[a]Concentrations of isolated compound (μg/disc), [b]diameter of inhibition zone <8.5 mm.

Measurement of scavenging activity of DPPH radical. Antioxidant activity of the isolated compound was measured on the basis of the scavenging activity of the stable DPPH free radical following the method described by Braca *et al* (Braca *et al.*, 2001). Various concentrations of the compound were added to 0.15 mM DPPH in EtOH, and the mixture was shaken vigorously. Absorbance at 517 nm was determined after 30 min, and the radical scavenging effect was calculated as [(Ao – Ae)/Ao] × 100, where Ae and Ao were absorbance of the sample with and without the isolated compound.

Results and Discussion

The 80% MeOH extract obtained from the rhizome of *T. kirilowii* (1.0 kg) was fractionated into $CHCl_3$ and *n*-

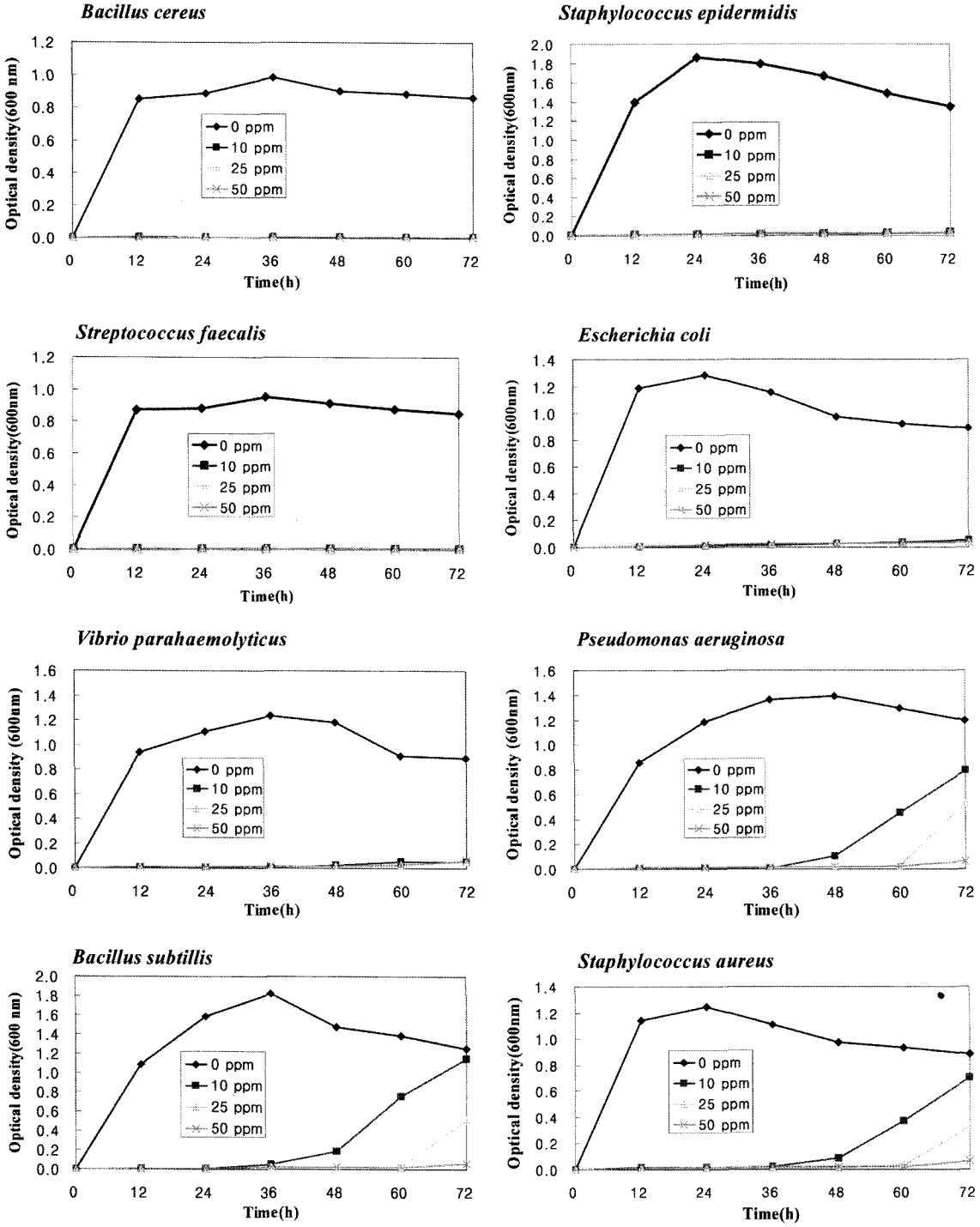

Fig. 2. Growth inhibition by isolated compound 1 from the rhizome of *T. kirilowii* on several food-borne microorganisms for 72 h at 32°C.

BuOH layers through solvent fractionation, and one compound was isolated through repeated chromatographic separation of $CHCl_3$ fractions. Structural identification of the compound was carried out through the interpretation of several spectral data, and compared with the data described in the literature (Katakawa *et al.*, 2000; Lundgren *et al.*, 1982, 1985). Isolated compound was readily identified as 1-C-(*p*-hydroxyphenyl)-glycerol (**1**) and it was the first to be reported from this plant.

Compound **1** was obtained as colorless needle and in the EIMS, the molecular ion peak showed at *m/z* 184. IR spectrum showed strong hydroxyl absorption bands at 3351 cm^{-1}. The ^{1}H-NMR spectrum of compound **1** exhibited characteristic proton signals, two oxymethine proton (δ 2.99 and 4.62) and one methylene proton (δ 3.73 and 4.10) of the glycerol skeleton. In particular, the mutually coupled signals at δ 7.14 (2H, d, J = 8.5 Hz, H-2' and H-6') and 6.73 (2H, d, J = 8.5 Hz, H-3' and H-5') indicated the presence of a *p*-hydroxyphenyl group. In addition, ^{13}C-NMR and DEPT experiments indicated the presence of two oxymethine carbon (δ 55.1 and 86.5), one methylene carbon (δ 72.3), two equivalent aromatic carbons (δ 116.5 and 128.7) of the symmetrical benzene-ring, and two quaternary carbons (δ 133.2 and 158.1). The *p*-hydroxyphenyl group was placed at C-1 due to HMBC correlation of H-2' and 6' with C-1. From the above spectroscopic evidence, the structure of compound **1** was determined to be 1-C-(*p*-hydroxyphenyl)-glycerol.

In vitro antibacterial activity of the isolated compound against the food-borne microorganisms was assessed by the presence of inhibition zones and growth inhibition. Isolated compound showed antibacterial activity against four bacteria (*B. cereus, S. faecalis, E. coli,* and *V. parahaemolyticus*) at 6.25 μg/disc and four bacteria (*B. substilis, S. epidermidis, P. aeroginosa,* and *S. aureus*) at 12.5 μg/disc (Table 1).

The compound showed inhibitory activity on the growth of five bacteria (*B. cereus, S. faecalis, E. coli, S. epidermidis,* and *V. parahaemolyticus*), completely at the concentration higher than 10 ppm for 72 h, and delayed the growth of three bacteria (*B. substilis, P. aeroginosa,* and *S. aureus*) at the concentration of 25 ppm for 60 h (Fig. 2).

DPPH radical was chosen to test the antioxidant activity of the isolated compound from the rhizome of *T. kirilowii*. For measurement of antioxidant activity, UV/Vis spectrophotometry method was used to observe DPPH radicals. DPPH, which creates stable free radicals, loses its color when DPPH radicals capture radicals (RO·, ·OH). The change in absorbance produced by the reduced DPPH was used to evaluate the ability of isolated compound to act as free radical scavengers (Brand-Williams *et al.*, 1995; Blois, 1958). Compound **1** showed antioxidant activity against DPPH radicals (IC_{50} = 56.0 ppm, Fig. 3).

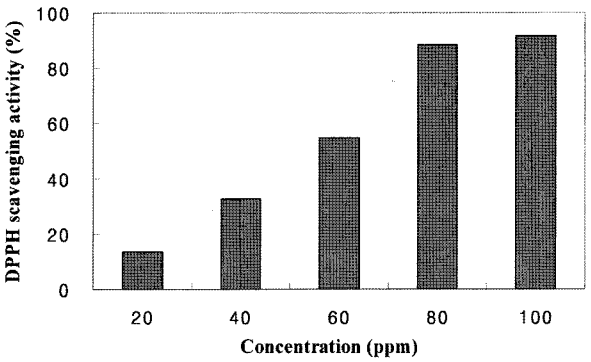

Fig. 3. DPPH radical scavenging activity of isolated compound 1 from the rhizome of *T. kirilowii*.

In conclusion, compound **1** was isolated from the rhizome of *T. kirilowii* and its structure was identified through spectroscopic methods. The compound showed strong antibacterial activity against food-borne microorganisms and antioxidant activity against DPPH radicals.

References

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **181**, 1199-1200.

Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, and Morelli I (2001) Antioxidant principles from Bauhinia terapotensis. *J Nat Prod* **64**, 892-895.

Brand-Williams W, Cuvelier ME, and Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* **28**, 25-30.

Essawi T and Srour M (2000) Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacol* **70**, 343-349.

Jiangsu New Medicinal College Edita (1986) The Dictionary of Chinese Materia Medica. *Shanghai Science and Technology Press* 1782.

Katakawa J, Tetsumi T, Kamei S, Iida T, and Katai M (2000) Phenolic compounds of fruit of *Coix lachryma-jobi* L. *Natural Medicines* **54**, 257-260.

Kim JH, Lee DS, Lim CW, Park HY, and Park JH (2002) Antibacterial activity of sea-mustard, *Laminaria japonica* extracts on the cariogenic bacteria, *Streptococcus mutans*. *Korean J Fish Soc* **35**, 191-195.

Lee HO, Baek SH, and Han DM (2001) Antimicrobial effects of *Chamaecyparis obtusa* essential oil. *Korean J Microbiol Biotechnol* **29**, 253-257.

Lundgren LN, Popoff T, and Theander O. (1982) Arylglycerol glucides from *Pinus sylvestris*. *Acta Chemica Scandi., Series B: Organic Chem and Biochem* **B36**, 695-699.

Lundgren LN, Shen Z, and Theander O (1985) Dilignol glycosides from *Pinus massoniana* lamb. *Acta Chemica*

Scandi., Series B: Organic Chem and Biochem **B39**, 241-248.

Mead PS, Slutsker L, Dietz V, McCaig LF, Breese JS, Shapiro C, Griffin PM, and Tauxe RV (1999) Food related illness and death in the United States. *Emerg Infect Dis* **5**, 607-625.

Namba T (1994) The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Picture, I, Hoikusys, Osaka, Revised Edition, 271.

Otshudi AL, Foriers A, Vercruysse A, Van Zeebroeck A, and Lauwers S (1999) *In vitro* antimicrobial activity of six medicinal plants traditionally used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo (DRC). *Phytomedicine* **7**, 167-172.

Piddok LJV (1990) Techniques used for the determination of antibacterial resistance and sensitivity in bacteria. *J Appl Bacteriol* **68**, 307-318.