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SHORT COMMUNICATION

Isolation of 8-hydroxyquinoline from *Sebastiania corniculata* and Antimicrobial Activity against Food-borne Bacteria

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Abstract Antimicrobial activities of the five fractions obtained from the methanol extract of *Sebasticania corniculata* plant were evaluated against five food poisoning bacteria using the agar diffusion method. The chloroform fraction possessed strong antimicrobial activities against five food poisoning bacteria. 8-Hydroxyquinoline was isolated from the chloroform fraction by the various chromatography analyses. When to the agar diffusion method was used, 8-hydroxyquinoline showed potent antimicrobial activities against five food poisoning bacteria. In the case of minimum bactericidal concentration or minimum inhibitory concentration, 8-hydroxyquinoline showed significantly higher antimicrobial activity against five food poisoning bacteria. Thus, the extract of *S. corniculata* and 8-hydroxyquinoline could be useful for the development of eco-friendly food supplemental agents.

Keywords Antimicrobial activity · Food poisoning bacteria · *Sebasticania corniculata* · 8-Hydroxyquinoline

Food-borne illness is a growing health problem in the developed/developing countries (Lu et al., 2012). Health problems have high-impact on the health and economies of developed/developing countries (Lu et al., 2012). Outbreaks of food-borne illness are a critical issue, resulting in increased costs to the food industry and national health systems (Lu et al., 2012). In this regard, synthetic antimicrobials are widely used to control the food-borne diseases in the developed/developing countries. However, synthetic antimicrobials may develop resistant strains and side effects. Thus, interest on natural antimicrobials has risen (Yang et al., 2013). Scientists have begun to investigate natural antimicrobials to control the food-borne diseases, with naturally occurring antimicrobial agents found in various plants (Larhsini et al., 2001; Kim et al., 2013). Except for the conventional therapy of antimicrobial agents, many studies have reported that medicinal plants have biological and pharmacological activities and are increasingly used as alternatives to traditional drugs (Lee and Ahn, 1998; Kim et al., 2004; Ranilla et al., 2010).

Plant-derived materials containing alkaloids, flavonoids, quinones, and terpenoids are widely distributed in food-borne illness (Lee and Ahn, 1998; Kim et al., 2004; Yang et al., 2013). The genus *Sebasticania* species (family Euphorbiaceae) are perennial herbs cultivated throughout East Asia and tropical America, and are widely used in traditional medicine, due to their therapeutic activities against a wide range of viral (Kott et al., 1999), microbial (Khera et al., 2003), nociceptive (Luzzi et al., 2000), and spasmodic effects (Yunes et al., 1990). Although some scientists have reported on the biological and pharmacological activities in the extract of the whole plant of *Sebasticania corniculata* (Vahl) Muell as well as isolation of bioactive compound, only few have been identified to carry antimicrobial activity. Objective of the present study is to isolate antimicrobial constituent of the extract of *S. corniculata* against food poisoning bacteria.

The whole plant of *Sebasticania corniculata* was purchased from the International Biological Material Research Center (Korea). A voucher specimen was authenticated by Prof. Jeong-Moon Kim and deposited in the herbarium at College of Agricultural and Life Sciences, Chonbuk National University. The whole plant of *S. corniculata* (6 kg) was ground in a blender, extracted twice with methanol (15 L) at room temperature for 1 day, and filtered the methanol extract through Tokyofilter paper No. 2 (Tokyo Roshi, Japan) in vacuum. The combined filtrates were then concentrated at 45°C in vacuum, using a rotary vacuum

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evaporator (EYELA autojack NAJ-100, Japan). The concentrated materials (20 g) were sequentially divided into hexane (1.2 g), chloroform (3.7 g), ethyl acetate (2.9 g), butanol (3.3 g), and water (8.9 g) fractions for subsequent bioassay. All fractions were concentrated via rotary evaporator, except the water fraction was freeze-dried.

The chloroform fraction (12 g) partitioned from the methanol extract was subjected to silica gel column chromatography (Merck 70-230 mesh, 800 g, 6.0 i.d. × 85 cm, USA), and was eluted by a stepwise gradient of chloroform/methanol (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50, v/v) giving five fractions (CH 1-CH 5). The active fraction (CH 3) showed the strongest antimicrobial activity against five food poisoning bacteria. This fraction was further chromatographed on a silica gel column and eluted with chloroform/methanol (5:1, v/v), and the column fractions were analyzed via thin layer chromatography and pooling fractions with similar patterns of thin layer chromatography. Subsequently, the active fraction (CH 33, 3.0 g) was chromatographed on a Sephadex LH-20 column (Pharamacia, USA) by chloroform/acetone/methanol (25:2:2, v/v) giving six fractions (CH 331-CH 333). To purify the active fraction (CH 332, 314 mg), preparative high performance liquid chromatography (prep HPLC, LC-908W-C60, Japan Analytical Industry Co., Japan) was conducted for separating the active constituent used, and the eluates were examined for antimicrobial activity. The first column was a JAI GS Series Column (GS310 50 cm + GS310 50 cm, 21.5 mm i.d. × 50 cm L, Japan Analytical Industry Co., Japan) with a flow rate of 8 mL/min and detection at 254 nm, using chloroform/acetone (20:2, v/v). Due to CH 3323 activity (203 mg), eluates were further chromatographed on a JAI W Series Column (W-253 50 cm + W-252 50 cm, 20.0 mm i.d. × 50 cm L, Japan Analytical Industry Co., Japan) under the same conditions indicated above. The active principle (CH 33232, 104 mg) was finally isolated by assessing the antimicrobial activities of the eluates and its structure was determined by various spectroscopic analyses. UV spectra were obtained in methanol by a Waters 490 spectrometer (USA.) with EI-Mass spectra (JEOL JMS-DX 30 spectrometer, JEOL, Japan). ^{1}H- and ^{13}C-NMR were measured using a JNM-EX 600 (Jeol Ltd., Japan) spectrometer in deuterated chloroform with tetramethylsilane as an internal standard at 600 and 150 MHz. Chemical shifts were expressed in δ (ppm). Using ^{1}H-^{1}H correlation spectrum, as well as ^{13}C-^{1}H correlation spectrum unambiguous ^{1}H and ^{13}C NMR chemical shifts were obtained.

The methanol extract, five fractions, and 8-hydroxyquinoline were tested against *Bacillus cereus* ATCC 14579, *Listeria monocytogenes* ATCC 15313, *Salmonella typhimurium* IFO 14193, *Shigella sonnei* ATCC 25931, and *Staphylococcus aureus* KCCM 11335. Bacterial strains were obtained from Korean Culture Center of Microorganisms and aerobically cultured at 37°C for 24 h in Nutrient broth (NB, Difco, USA). *Staphylococcus aureus* was cultured in Tryptic Soy broth (TSB, Difco, USA).

The agar diffusion method was used for determining antimicrobial activities of the five fractions from the methanol extract of *S. corniculata*. Microorganisms were incubated in NB or TSB at 37°C for 24 h to yield 1.0×10^{7} CFU/mL as compared to 0.5 McFarland standard. A suspension of the incubated microorganisms (0.1 mL of 1.0×10^{7} CFU/mL) was spread on Mueller Hinton Agar (MHA, Difco, USA) plates. Each sample was dissolved in methanol, and sterilized paper discs were impregnated with 40 μL of each sample. The methanol served as negative control, was injected on sterilized paper disc at 40 μL. After drying in fume hood, the injected paper discs were placed on the inoculated MHA plates. These plates were incubated under aerobic conditions at 37°C for 24 h. Antimicrobial activity was expressed as the diameters of the inhibition zones (mm), and treatments were performed in triplicate. Values are means ± SD of three parallel measurements.

Minimum bactericidal concentration (MBC) or Minimum inhibitory concentration (MIC) of 8-hydroxyquinoline was determined by broth microdilution techniques, with 8-hydroxyquinoline (10 mg) dissolved in methanol (10 mL) as a stock solution and was serially diluted ranging from 100 to 1 μg/mL. Each dilution (50 μL) was dispensed into a 96-well microplate, which had been injected with 100 μL of Mueller Hinton broth, and was then inoculated with 50 μL of the bacterial suspensions. The final concentration of each strain was adjusted to 10^{7} CFU/mL (absorbance values of 0.08 to 0.10 at 625 nm, according to McFarland turbidity standards). The MIC values, determined through turbidity reading at 600 nm, are defined as the lowest concentration of a substance at which visible growth of the microorganisms are inhibited. The MBC is the lowest concentration without colony formulation on the agar plates, determined by spreading 100 μL of each microorganism on a MHA plate. These plates were incubated at 37°C for 24 h. All experiments were performed in triplicate. Experimental results are produced as mean values ± standard deviations. Statistical significance was accepted at a level of $p < 0.05$ (SAS Institute, 1990).

The yield of the methanol extract of the *S. corniculata* whole plant was 8.41%, and the methanol extract of *S. corniculata* was divided into five fractions: hexane, chloroform, ethyl acetate, butanol, and water fractions. The highest yield was obtained from the water fraction (44.5%), following chloroform fraction (18.5%), butanol fraction (16.5%), ethyl acetate fraction (14.5%), and hexane fraction (6.0%). The antimicrobial activities of the methanol extract and five fractions derived from *S. corniculata* are given in Table 1. The methanol extract and chloroform fraction exhibited antimicrobial activities against *B. cereus*, *L. monocytogenes*, *S. aureus*, *S. typhimurium*, and *S. sonnei* at 10 mg/disc. However, hexane, ethyl acetate, butanol, and water fractions had no antimicrobial activities against all tested microorganisms. The negative control did not exhibit antimicrobial effects against five food poisoning bacteria. Therefore, the chloroform fraction was selected to isolate the active compound of *S. corniculata*.

To isolate the active compound of chloroform fraction, silica gel column chromatography, thin layer chromatography, and prep HPLC were performed with mixed organic solvents. Structural

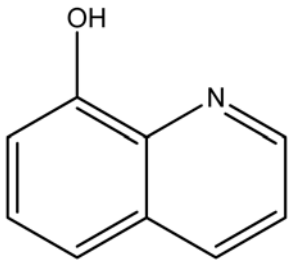

Fig. 1 Structure of 8-Hydroxyquinoline

determination of the isolate was made by spectroscopic analyses, including UV, electron impact mass spectrometry (EI-MS), ^{1}H NMR, ^{13}C NMR, and 2D-NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and DEPT) as well as by direct comparison with the authentic reference compound. The active compound was identified as 8-hydroxyquinoline based on the following evidence: 8-hydroxyquinoline (Fig. 1) (C_9H_7NO, MW 145); EI-MS (70 eV) *m/z* (% relative intensity): M^+ 145 (100), 144 (2), 117 (85), 116 (14), 90 (7), 89 (6); ^{1}H-NMR (CD_3OD, 400 MHz); 8.75-8.76 (1H, *m*, *J* = 5.84 Hz, H-2), 8.20-8.22 (1H, *m*, *J* = 10 Hz, H-4), 7.42-7.46 (1H, *m*, *J* = 17.8 Hz, H-5), 7.38-7.40 (1H, *d*, *J* = 7.6 Hz, H-3), 7.32-7.34 (1H, *m*, *J* = 9.52 Hz, H-6), 7.07-7.10 (1H, *m*, *J* = 8.8 Hz, H-7), 5.42 (OH, *s*, H-8); ^{13}C-NMR (CD_3OD, 100 MHz); 152.7 (C-8), 150.3 (C-2), 138.8 (C-9), 135.3 (C-4), 129.0 (C-10), 126.2 (C-6), 121.3 (C-3), 120.3 (C-5), 112.0 (C-7). The present findings are similar to those of Lee et al. (2010).

To test the antimicrobial activity of active compound isolated from *S. corniculata*, the antimicrobial activity of 8-hydroxyquinoline was evaluated by the agar diffusion method at 0.5 mg/disc and compared with that of tetracycline served as positive control (Table 1). Against all test microorganisms, 8-hydroxyquinoline had antimicrobial activities (25.4±1.3 against *B. cereus*, 27.5±1.5 against *L. monocytogenes*, 27.2±1.3 against *S. aureus*, 26.7±0.9 against *S. sonnei*, and 33.4±1.5 against *S. typhimurium*). In the present study, these results indicated that the growth-inhibitory activity of *S. corniculata* whole plant could be attributed to 8-hydroxyquinoline against five food poisoning bacteria.

The MBC and MIC values of 8-hydroxyquinoline were revealed by determining antimicrobial activities (Table 2). The MBC values of 8-hydroxyquinoline are 12.5 μg/mL against *B. cereus*, 25 μg/mL against *L. monocytogenes* and *S. aureus*, and 75 μg/mL against *S. sonnei* and *S. typhimurium*. The MIC values of 8-hydroxyquinoline are 10 μg/mL against *B. cereus*, *L. monocytogenes*, and *S. aureus*, 25 μg/mL against *S. sonnei*, and 50 μg/mL *S. typhimurium*. The growth-inhibiting activity of 8-hydroxyquinoline against five food-borne bacteria was then compared to that of the commercially available antibiotic, tetracycline (Table 2). The MBC values of tetracycline are 12.5 μg/mL against *B. cereus*, 20 μg/mL against *S. aureus*, 50 μg/mL against *L. monocytogenes*, *S. sonnei*, and *S. typhimurium*. The MIC values of tetracycline are 5 μg/mL against *B. cereus* and *S. aureus*, 20 μg/mL against *L. monocytogenes*, and 25 μg/mL against *S. sonnei* and *S. typhimurium*. These results clearly demonstrated that the antimicrobial activity of 8-hydroxyquinoline was lower than that of tetracycline against five food-borne bacteria. In previous studies, MIC and MBC values of *C. colocynthis* extract were 0.20 and 0.41 mg/mL against *Escherichia coli* and 0.23 and 0.41 mg/mL against *Pseudomonas aeruginosa* (Marzouk et al., 2009). Although the insecticidal properties of 8-hydroxyquinoline derived from *S. corniculata* were previously reported (Lee et al., 2010), the present study is the first to evaluate the antimicrobial activities of *S. corniculata* extracts and 8-hydroxyquinoline against food-borne bacteria.

Based on the Material Safety Data Sheet provided by Sigma-Aldrich (2013), the oral LD_{50} values of 8-hydroxyquinoline (1,200 mg/kg) indicated a low acute toxicity to mammals. In conclusion, the *S. corniculata* whole plant and 8-hydroxyquinoline could be used as a source of natural antimicrobial agents potentially suitable for the replacement of synthetic preservatives. Future

Table 1 Antimicrobial activities of materials derived from *Sebastiania corniculata* and isolated compound

Materials	Food Poisoning Bacteria				
	B. cereus	*L. monocytogenes*	*S. aureus*	*S. typhimurium*	*S. sonnei*
Methanol extract[1)]	13.3±1.5[4)]	14.2±1.6	13.6±1.3	13.5±1.2	13.1±1.6
Hexane fraction[2)]	-[5)]	-	-	-	-
$CHCl_3$ fraction[2)]	15.1±1.3	16.2±1.4	14.4±1.8	14.1±1.6	13.3±1.4
EtOAc fraction[2)]	-	-	-	-	-
BuOH fraction[2)]	-	-	-	-	-
Water fraction[2)]	-	-	-	-	-
8-Hydroxyquinoline[2)]	25.4±1.3	27.5±1.5	27.2±1.3	26.7±0.9	33.4±1.5
Tetracycline[3)]	40.5±1.2	29.8±2.1	30.4±0.9	27.5±1.5	24.8±1.1

[1)]Dose: 10 mg/disc.
[2)]Dose: 0.5 mg/disc.
[3)]Dose: 0.1 mg/disc.
[4)]Values (mm) were expressed as means ± SD of three parallel measurements, $p < 0.05$.
[5)]-, no activity.

studies should be conducted to evaluate the antimicrobial action of 8-hydroxyquinoline and to develop formulations to improve its antimicrobial potency and stability.

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