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Research Report

Isolation of Antibacterial Compounds from *Parasenecio pseudotaimingasa*

Eun Ju Cho[1], Ji Yeon Choi[1], Ki Ho Lee[2], and Sanghyun Lee[2*]

[1]*Department of Food Science and Nutrition, Pusan National University, Busan 609-735, Korea*
[2]*Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea*

*Corresponding author: slee@cau.ac.kr

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Abstract. The aim of this research was to investigate the antibacterial activity of *Parasenecio pseudotaimingasa*, a perennial plant belonging to the Asteraceae, tribe Senecioneae. The methanol extract, *n*-hexane fraction, and *n*-butanol fraction of *P. pseudotaimingasa* leaves all exhibited antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*, forming inhibition zones greater than 11 mm in discs. Among them, the *n*-hexane fraction showed the highest antibacterial activity against *E. coli* and *S. aureus*, with an inhibition zone greater than 14 mm. Open-column chromatography was used to isolate the phytochemical constituents from the *n*-hexane fraction; spectroscopic analysis elucidated their structures as β-sitosterol and daucosterol. Further testing of β-sitosterol and daucosterol also revealed the antibacterial effects against both *E. coli* and *S. aureus*, suggesting their potential as antibacterial agents.

***Additional key words*:** bacteria, extraction, fractionation, isolation, reflux, sterol

Introduction

Various pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus* are related to the occurrence of infectious diseases. *E. coli* is a common cause of urinary tract infections and bacteremia in humans, and is frequently resistant to aminopenicillins, such as amoxicillin and ampicillin (Allen et al., 1999; Karlowsky et al., 2002; Landgren et al., 2005). In addition, *S. aureus* is a common cause of infection in hospitalized patients (Westh et al., 2004). The outer cell membrane of Gram-negative bacteria such as *E. coli* is known to be covered with a lipopolysaccharide layer of 1-3 μm thickness, while the surface of Gram-positive bacteria such as *S. aureus* has a peptidoglycan layer on which teichoic acid, teichuronic acid, and proteins are covalently bound (Sonohara et al., 1995).

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). Plant products, either as pure compounds or as standardized extracts, provide promising opportunities for new anti-infective drugs. There is an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action that can be used to treat new and re-emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to natural products, looking to develop better antimicrobial drugs (Benkeblia, 2004; Kang et al., 2005).

Parasenecio pseudotaimingasa is a perennial plant belonging to the Asteraceae, tribe Senecioneae. About 80 species in this tribe are distributed across Korea, Eastern Asia, Russia, and America (Koyama, 1969). *P. pseudotaimingasa*, whose young sprouts are edible, grows to 60-100 cm tall with leaves 27-32 cm in length. The leaf edges are divided into three palmate lobes, and a short petiole surrounds the stem like a sheath. In July and August, *P. pseudotaimingasa* produces inflorescences with yellow flowers 7 mm in diameter (Lee, 2003).

Previous studies on plants within the same genus plants have reported on the ecological characteristics of *P. firmus* (Jin and Ahn, 2010), HPLC analysis of *Cacalia firma* constituents (Park et al., 2009), chemical compositions of mountainous vegetables (Agung et al., 2011; Lee et al., 2011), and aldose reductase activity of *P. pseudotaimingasa* (Kim et al., 2011). To the best of our knowledge, there are no reports in the literature on the antibacterial activity of extracts from *P. pseudotaimingasa*. This paper deals with the isolation and identification of phytochemical compounds from *P. pseudotaimingasa* and their antibacterial activities against *E. coli* and *S. aureus*.

Materials and Methods

Plant Materials

The leaves of *P. pseudotaimingasa* (Nakai) K. J. Kim were collected on Mt. Baekun, Korea in 2009 and were botanically authenticated by Prof. Y. H. Ahn of Chung-Ang University, Korea. A voucher specimen (No. LEE 2009-08) was deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

Instruments and Reagents

Electron ionization mass spectrometry (EI-MS) was performed with a JEOL JMS-600W (Tokyo, Japan) mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 500 NMR (Rheinstetten, Germany) spectrometer in $CDCl_3$ or pyridine using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in Hertz (Hz). TLC analysis was conducted with Kiesel gel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 followed by charring at 60℃. Silica gel (200-400 mesh, Merck, Germany) and Sephadex LH-20 (25-100 μm, Amersham Biosciences, Sweden) were used for the isolation of constituents. All other chemicals and reagents were of analytical grade.

Extraction, Fractionation, and Isolation

The air-dried leaves of *P. pseudotaimingasa* (692 g) were extracted with MeOH (10 L × 3) under reflux. The resulting extracts were combined and concentrated under reduced pressure to yield 141 g of residue. Combined MeOH extract was then suspended in H_2O and partitioned successively with equal volumes of *n*-hexane (27.8 g), MC (2.9 g), and *n*-BuOH (21.0 g). A portion of the *n*-hexane fraction (10.0 g) was chromatographed on a silica gel column and eluted with a gradient of *n*-hexane and EtOAc to afford compounds **1** (*n*-hexane and EtOAc, 97:3) and **2** (*n*-hexane and EtOAc, 30:70).

Compound **1** - white powder; EI-MS *m/z*: 414 $[M]^+$ (100.0), 396 (42.5), 381 (21.8), 329 (25.0), 303 (28.9), 289 (4.0), 273 (25.3), 255 (48.0), 231 (15.9), 213 (25.2), 159 (25.6), 145 (25.8); ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 2.

Compound **2** - white powder; FAB-MS *m/z*: 577 $[M+H]^+$; ^{1}H- and ^{13}C-NMR (300 MHz, pyridine): see Table2.

Microorganisms and Media Preparation

E. coli and *S. aureus* used in this study were provided by the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). Strains were maintained at 37℃ intrypticase soy agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ). Original cultures were maintained at -70℃. The TSA culture medium contained 15 g of a pancreatic casein digest, 5 g of a papaic soybean digest, 5 g NaCl, and 15 g agar in 1 L distilled water. The pH of the medium was adjusted to 7.3.

Antibacterial Activity

The antibacterial activity of *P. pseudotaimingasa* was tested by the disc agar diffusion method (Davidson et al., 1989). TSA plates were inoculated with 0.1 mL of culture, and sterile filter paper discs (8 mm) containing 50 μg·30 μL^{-1} of *P. pseudotaimingasa* extracts, fractions, and compounds were distributed on the surface. Inhibition zones were determined after an incubation period of 24 h at 37℃.

Results and Discussion

This study evaluated the antibacterial activities of *P. pseudotaimingasa* against *E. coli* and *S. aureus*. Inhibitory abilities of the methanol extract and fractions from *P. pseudotaimingasa* on microbial growth are summarized in Table 1. The extract and fractions inhibited the growth of *E. coli* and *S. aureus*, forming inhibition zones larger than 11 mm. The *n*-hexane fraction exhibited the greatest antibacterial activity against *E. coli* and *S. aureus*, forming an inhibition zone of 14 mm.

A chromatographic separation of the active *n*-hexane fraction led to the isolation of compounds **1** and **2**. ^{1}H-NMR spectra of **1** and **2** revealed a sterol skeleton. Spectra exhibited two angular methyl singlets of H-18 and -19 at δ 0.67-0.68 and 0.95-1.01; three doublets of H-21, -26 and -27 at δ 0.92-1.00, 0.81-0.94, and 0.86-0.90, respectively; and an olefinic proton broad doublet signal of H-6 at δ 5.34-5.35. ^{13}C-NMR spectra of compounds **1** and **2** had resonance spectra of 27 and 33, respectively. C-5 and -6 signals of compounds **1** and **2** were observed at δ 141.0-141.3 and 121.9-122.3, respectively. Compounds **1** and **2** had similar structural signals. A typical glucose moiety pattern was observed in the ^{1}H- and ^{13}C-NMR spectra of compound **2**; HMBC analysis revealed a glucose at C-3 (β-linkage) of aglycone with an anomeric proton at δ 4.11 (d, J = 7.8 Hz) (Table 2). The structures of compounds **1** and **2** were elucidated as β-sitosterol (stigmast-5-en-3-ol) and daucosterol

Table 1. Antibacterial activities of the MeOH extract and fractions from *P. pseudotaimingasa*.

Sample (50 μg·30 μL^{-1})	Clear Zone (mm)	
	E. coli	*S. aureus*
MeOH extract	11	11
n-Hexane fraction	14	14
n-BuOH fraction	14	12

Table 2. ^{1}H- and ^{13}C-NMR spectral data for compounds **1** and **2** from *P. pseudotaimingasa*.

NO.	1		2	
	δ_H	δ_C	δ_H	δ_C
1	-	37.4	-	37.3
2	-	31.8	-	29.4
3	3.52 m	71.9	-	78.5
4	2.27 m	42.4	-	38.4
5	-	141.0	-	141.3
6	5.35 m	121.9	5.63 m	122.3
7	-	32.0	-	31.8
8	-	32.0	-	30.6
9	-	50.3	-	50.7
10	-	36.7	-	36.8
11	1.99 m	21.2	-	20.4
12	-	39.8	-	40.3
13	-	42.4	-	42.7
14	-	57.0	-	56.9
15	-	24.5	-	23.8
16	-	28.4	-	26.7
17	-	56.1	-	56.6
18	0.68 s	12.0	0.67 s	12.4
19	1.01 s	19.1	0.95 s	19.6
20	-	36.3	-	34.5
21	0.92 d (6.3)	18.9	1.00 d (6.3)	19.4
22	-	34.1	-	32.6
23	-	26.2	-	24.9
24	-	46.0	-	46.4
25	-	29.1	-	28.9
26	0.81 d (6.3)	19.1	0.94 d (5.4)	19.8
27	0.86 d (4.2)	19.6	0.90 d (6.3)	20.1
28	-	23.2	-	21.7
29	0.80 t (5.8)	12.1	0.89 m	12.5
Glc-1			5.00 brs	103.0
Glc-2			-	75.8
Glc-3			-	79.0
Glc-4			-	72.1
Glc-5			-	78.9
Glc-6			-	63.2

(β-sitosterol-3-*O*-β-D-glucoside), respectively, by comparison of the spectral data as described in the literature (Lee et al., 2011; Park et al., 2009; Umlauf et al., 2004). This is the first report on the isolation of compounds **1** and **2** from *P. pseudotaimingasa* (Fig. 1).

The antibacterial activities of β-sitosterol (**1**) and daucosterol (**2**) against *E. coli* and *S. aureus* are summarized in Table 3.

Table 3. Antibacterial activities of compounds **1** and **2** from *P. pseudotaimingasa*.

Compound (50 μg·30 μL^{-1})	Clear Zone (mm)	
	E. coli	*S. aureus*
1	12	12
2	11	12

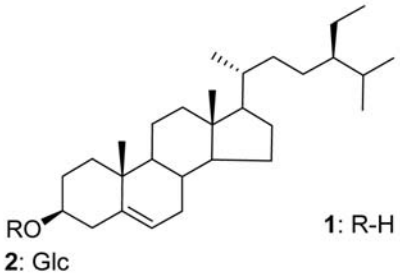

Fig. 1. Structures of compounds **1** and **2** from *P. pseudotaimingasa*.

β-Sitosterol, a common plant sterol, exhibits estrogenic, antiulcer, antitumor, and anti-inflammatory activities (Lee et al., 2007; Park et al., 2001; Yuk et al., 2007). In addition, previous studies have shown that β-sitosterol from *Heliotropiumm arifolium* and *Sansevieria hyacinthoides* has exhibits antibacterial activity (Jain et al., 2001; Kim, 2003; Sultana et al., 2011). Furthermore, daucosterol, a β-sitosterol glycoside, is reported to improve immune function by stimulating human peripheral blood leucocyte proliferation. Daucosterol increases the activity of helper T-cells, cytokines, interleukin 2, γ-interferon, and natural killer cells, and possesses anti-cancer and anti-seizure activity (Baek et al., 2000). It has also been found to inhibit succinic semialdehyde dehydrogenase and succinic semialdehyde reductase, enzymes that degrade gamma amino butyric acid (GABA), suggesting a possible role as an anticonvulsant by elevating GABA levels in the central nervous system (Baek et al., 2000; Kim et al., 2004; Lee et al., 2007). Thus, although β-sitosterol and its glucoside, daucosterol, have demonstrated potential utility in a variety of disorders, the antibacterial activity of phytosterols from *P. pseudotaimingasa* has remained untested until now. Medicinal plants have been cultivated for increased yields of bioactive components. The phytochemical composition of many plants has changed over time, with domestication of agricultural crops resulting in the enhanced content of some bioactive compounds (Schmidt et al., 2008).

There is enormous potential for developing antimicrobials from plants compounds, which may not produce the toxicity associated with synthetic antimicrobials. In conclusion, β-sitosterol (**1**) and daucosterol (**2**) were isolated from the leaves of *P. pseudotaimingasa*, and their antibacterial activities

were confirmed. These biologically active constituents have potential as inhibitory substances against *E. coli* and *S. aureus*.

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