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Antibacterial Activity of *Zanthoxylum schinifolium*

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Abstract – The aim of this research was to investigate the industrial application of *Zanthoxylum schinifolium*. Antibacterial activities of the *n*-hexane, methylene chloride (MC), ethyl acetate, and butanol fractions of *Z. schinifolium* were tested against *Escherichia coli*, *Staphylococcus aureus* and *Helicobacter pylori*. Among the fractions, the *n*-hexane and MC fractions showed the stronger antibacterial activity against *H. pylori*, with an inhibition zone greater than 10 mm in disc assays. Further testing of bergapten and lupeol from the MC fraction of *Z. schinifolium* revealed the antibacterial effects against *E. coli*, *S. aureus* and *H. pylori*, suggesting their potential use as antibacterial agents.
Keywords – *Zanthoxylum schinifolium*, antibacterial, bergapten, lupeol

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 to 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).

Zanthoxylum schinifolium is a deciduous shrub growing to 5-7 m, native to China, Japan, and Korea. It is in flower in August, and the seeds ripen in November. The flowers of *Z. schinifolium* are dioecious (Paik *et al*., 2005). In China, the herb of *Z. schinifolium* was used to invigorate the circulation of blood and was regarded as a drug for various pains, such as fracture and injuries from falls (Cui *et al*., 2009). In previous papers, two alkaloids were isolated from *Z. schinifolium* fruits and exhibited strong feeding deterrent activity against two stored product insects, *Tribolium castaneum* and *Sitophilus zeamais* (Liu *et al*., 2009). *Z. schinifolium* essential oils were found to possess strong insecticidal activity against the maize weevils of *Sitophilus zeamais* and estragole was the major compound of the essential oil of fresh fruits (Wang *et al*., 2011). Moreover, collinin and oxynitidine from the bark showed significant activity of anti-HBV DNA replication (Chang *et al*., 1997). Phytochemicals including coumarins, triterpenoids, and flavonoids from the extract of *Z. schinifolium* were found (Cheng *et al*., 2002). Biological activities such as anti-diabetes, monoamine oxidase inhibition, anti-platelet, lipid peroxidation inhibition, and nitrite-scavenging have been reported from this plant (Fang *et al*., 2010; Lee *et al.*, 2011).

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This paper describes the *in vitro* antibacterial activity of constituents from *Z. schinifolium* leaves.

Experimental

Plant materials – The leaves of *Z. schinifolium* were collected at Gwangreung (June, 2011) by Korea National Arboretum (KNA), Republic of Korea and a voucher specimen was deposited at the Herbarium of the Department of Integrative Plant Science, Chung-Ang University, Korea.

Instruments and reagents – Mass spectrometry (MS) was measured with a Jeol JMS-600W (Tokyo, Japan) mass spectrometer. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 500 NMR spectrometer (Rheinstetten, Germany) in $CDCl_3$ using TMS as an internal standard. Chemical shifts were reported in parts per million (d), and coupling constants (*J*) were expressed in Hertz (Hz). TLC analysis was conducted with Kiesel gel 60 F254 (Art. 5715, Merck & Co., Inc., Germany) plates (silica gel, 0.25-mm layer), with compounds visualized by spraying with 10% H_2SO_4 followed by charring at 60 ºC. All other chemicals and reagents were of analytical grade.

Extraction, fractionation, and identification – The leaves of *Z. schinifolium* were extracted with methanol (MeOH) under reflux. The MeOH extracts were combined, suspended in H_2O, and then partitioned successively with equal volumes of *n*-hexane, methylene chloride (MC), ethyl acetate (EtOAc), and butanol (*n*-BuOH). Compounds **1** and **2** were detected in the MC fraction (fr.) of *Z. schinifolium* by TLC analysis. Compounds **1** and **2** were identified from the MC fr. by prep-TLC. Their structures were identified by ^{1}H-NMR spectrum as below.

Compound 1 – White powder; EI-MS: *m/z* 216 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.25 (3H, s, OMe), 6.25 (1H, d, *J* = 9.5 Hz, H-3), 7.02 (1H, br s, H-3'), 7.11 (1H, s, H-8), 7.59 (1H, br s, H-2'), 8.14 (1H, d, *J* = 9.5 Hz, H-4).

Compound 2 – White powder; EI-MS: *m/z* 426 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.76 (3H, s, H-28), 0.79 (3H, s, H-23), 0.83 (3H, s, H-25), 0.94 (3H, s, H-24), 0.97 (3H, s, H-27), 1.03 (3H, s, H-26), 1.68 (3H, s, H-30), 2.38 (1H, dd, *J* = 5.2, 11.0 Hz, H-19), 3.19 (1H, br d, *J* = 11.0 Hz, H-3), 4.55 (1H, s, H-29b), 4.67 (1H, s, H-29a).

Microorganisms and media preparation – *E. coli*, *S. aureus* and *Helicobacter pylori* strains 26695 used in this study were provided by the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea), and the *H. pylori* was provided by the Korean Type Culture Collection (KTCC, Jinju, Korea). The *E. coli* and *S. aureus* were maintained at 4 ºC in trypticase soy agar (TSA; Becton, Dickinson and Company, USA). 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. *H. pylori* were cultured in brucella broth (Difco, USA) containing 10% horse serum (Welgene, Daegu, Korea) and for testing, were grown on a medium prepared with (per liter) BD Bacto Tryptone (10 g), BD Bacto Peptamin (10 g), BD Bacto Dextrose (1 g) and BD Bacto Yeast Extract (2 g) (Becton, Dickinson and Company [BD], Franklin Lakes, NJ, USA), sodium chloride (5 g), and sodium bisulfite (0.1 g). Microaerophilic conditions were maintained in a 10% CO_2 incubator at 37 ºC with humidity greater than 95%.

Antibacterial activity – The antibacterial activity of *Z. schinifolium* was tested by the disc agar diffusion method (Davidson and Parish, 1989). Plates of medium were spread with 0.1 mL of culture broth, and sterile filter paper discs (8 mm) containing 50 μg/30 μL of the *Z. schinifolium* fractions and compounds were placed on the agar surface. Inhibition zones were determined after 24 h at 37 ºC.

Results and Discussion

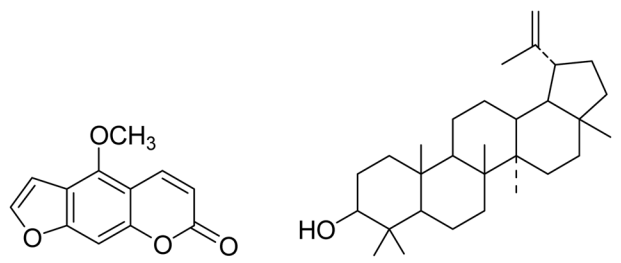
The microbial growth-inhibitory activities of *Z. schinifolium* against *E. coli*, *S. aureus*, and *H. pylori* were evaluated (Table 1). The *n*-hexane and MC fractions inhibited the growth of *H. pylori*, forming inhibition zones larger than 10 mm clear zones. Especially, the *n*-hexane and MC fractions of *Z. schinifolium* showed the greatest antibacterial activity against *H. pylori,* showing 10 and 11 mm of inhibition zone, respectively.

Compounds **1** and **2** were identified as bergapten and lupeol (Fig. 1), respectively, from the MC fraction of *Z. schinifolium* by comparison of the spectral data with published values (Fernández *et al*., 2001; Liu *et al*., 2004;

Table 1. Antibacterial activities of the solvent fr. from *Z. schinifolium*

Sample (50 μg/30 μL)	Clear zone (mm)		
	E. coli	*S. aureus*	*H. pylori*
n-Hexane fr.	–	–	11
MC fr.	–	–	10
EtOAc fr.	–	–	–
n-BuOH fr.	–	–	–

–: Not determined.



1 2

Fig. 1. Structures of compounds **1** and **2** from *Z. schinifolium*.

Choi *et al.*, 2005; Lee *et al.*, 2006). The antibacterial activities of bergapten (**1**) and lupeol (**2**) against *E. coli*, *S. aureus* and *H. pylori* are summarized in Table 2. At 50 μg/30 μL, bergapten (**1**) produced zones of growth inhibition greater than 11 mm in diameter around *S. aureus* and *H. pylori* colonies. Especially, bergapten (**1**) exhibited the highest antibacterial activity against *S. aureus* with inhibition zone exceeding 13 mm. And lupeol (**2**) produced zones of growth inhibition greater than 11 mm in diameter around *E. coli* and *H. pylori* colonies.

Bergapten (**1**), a furanocoumarin contained in grapefruit, has a strong inhibitory effect on CYP3A4 with a very low IC_{50} (Ho *et al.*, 2001). Previous paper investigated the pro-apoptotic effects induced by high dose of bergapten in the absence of UV rays, in human breast cancer cells (Panno *et al*., 2009). In Panno *et al*. (2010) paper, this compound induced a lowering of PI3K/AKT survival signal in MCF-7 cells even in presence of IGF-I stimulation. Meanwhile, lupeol (**2**) from the medicinal plant *Crataeva nurvala* Buch-Ham (Capparidaceae) has been shown to exhibit antihepatotoxic, antioxidant, and antitumor activities in rats (Nagaraj *et al*., 2000; Saleem *et al*., 2001; Sudharsan *et al.*, 2005). It is also noteworthy that lupeol at its effective therapeutic doses exhibit no toxicity to normal cells and tissues (Goyal and Rani, 1989; Saleem, 2009).

Based on these findings, bergapten (**1**) and lupeol (**2**) hold potential therapeutic value for a variety of disorders. The development of antimicrobials from plant compounds attracts interest based on the premise that natural products may be less toxic than synthetic antimicrobials. In conclusion, the antibacterial activities of bergapten (**1**) and lupeol (**2**) in *Z. schinifolium* were confirmed. These biologically active plant constituents may have industrial and health-related applications in the control of *E. coli*, *S. aureus* and *H. pylori*. It suggests that bergapten (**1**) and lupeol (**2**) could be useful antibacterial agents to inhibit pathogenic bacteria.

Table 2. Antibacterial activities of compounds **1** and **2** from *Z. schinifolium*

Sample (50 μg/30 μL)	Clear zone (mm)		
	E. coli	*S. aureus*	*H. pylori*
1	8	13	11
2	11	9	12

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