Natural Product Sciences
17(1) : 23-25 (2011)

Antimicrobial Effects of *Lonicera japonica* against Gram Positive and Gram Negative Anaerobic Bacteria

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Abstract – It has been shown that the butanol extract of *Lonicera japonica* has antimicrobial and other potentially useful biological activities. The purpose of this study was to determine the *in vitro* activity of *Lonicera japonica* compared to other antimicrobial agents against anaerobic bacteria. Specifically, the *in vitro* activity of the butanol extract was investigated against 104 clinical isolates of anaerobic bacteria using an agar dilution method and the results were compared to erythromycin, cefoxitin, imipenem, clindamycin, and metronidazole. It was found that *Lonicera japonica* and imipenem were the most active antimicrobial agents tested.
Keywords – *Lonicera japonica,* anaerobic bacteria, antimicrobial effects

Introduction

Lonicera japonica Thunb. (Caprifoliaceae) is a species of honeysuckle native to north eastern Asia, including Japan, Korea, northern and eastern China, and Taiwan. *L. japonica* is traditionally used as a medicinal plant (Peng *et al*. 2000), and many pharmacological studies and clinical practices have demonstrated that *L. japonica* possesses many biological effects, including hepatoprotective, cytoprotective, antimicrobial, antioxidative, antiviral, and anti-inflammatory activities (Chang *et al*. 1995). The constituents of this plant have been investigated and they were found to contain iridoid glucosides and polyphenolic compounds (Kakuda *et al*. 2000). The primary polyphenolic components in *L. japonica* are hyperoside, chlorogenic acid, luteolin, and caffeic acid (Chang *et al*. 1995). Research has shown that hyperoside, chlorogenic acid, and other flavones can be used to scavenge free radicals in addition to having anti-inflammatory activities.

The primary components of *L. japonica* have medicinal properties: flower buds have anticancer, anti-microbial, and anti-inflammatory properties (Zhang *et al*. 2008); the leaf has antioxidant properties and inhibitors of tyrosinase (Byun *et al*. 2004); the stem also has inhibitory activities against tyrosinase and xanthine oxidase in addition to nitrite scavenging potential (Wang and Helliwell, 2001). However, research on the antibacterial properties of *L. japonica* is still scarce; few scientific articles have reported on these activities (Cai *et al*. 2004). Despite its various medicinal properties, no reports to date have published on the chemical composition and antibacterial properties derived from the flower of *L. japonica*.

In this study, the chemical composition of the n-Butanol extract from the flower of *L. japonica* was examined by HPLC, and the antimicrobial activities of buthanolic Thunb. extracts was tested. Butanol was chosen as the solvent for the extraction because it is generally accepted that butanol is superior to methanol and acetone for extracting biologically active components (e.g. flavonoids) from tea (Cai *et al*. 2004).

Materials and Methods

Preparation of the BuOH fraction of *L*. japonica and microorganisms – *L. japonica* was obtained from an oriental drug store (Chungju, Korea). The chopped material was refluxed with distilled water; the water extract was then partitioned with n-butanol (BuOH) and filtered. The filtrate was evaporated *in vacuo* to dryness. The yield based on the dry weight of *L. japonica* was 5.7% w/w. In the BuOH fraction, loniceroside A, loniceroside B, lonicerin, and loganin were found to be the primary constituents. The content percentages in this fraction were determined by HPLC and are as follows: 2.5% for loniceroside A, 2.2% for loniceroside B, 2.1% for lonicerin, and 4.7% for loganin. Antibiotics were

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Table 1. Antibacterial activities of the butanol extract from *L. japonica* and other antimicrobial agents against anaerobic bacteria

Microorganism (no. of isolates)	Antimicrobial agent	MIC (mg/L)		
		MIC_{50}	MIC_{90}	Range
Bacteroides fragilis (n = 15)	Butanol extract	0.125	1.0	0.125 – 1.0
	Erythromycin	0.125	0.5	0.125 – 1.0
	Cefoxitin	4.0	16	1.0 – > 16
	Imipenem	0.032	0.5	0.032 – 1.0
	Clindamycin	0.032	0.125	0.032 – 1.0
	Metronidazole	1.0	2.0	1.0 – 2.0
Bacteroides ovatus (n = 21)	Butanol extract	1.0	2.0	2.0 – 1.0
	Erythromycin	0.5	1.0	0.5 – 2.0
	Cefoxitin	0.5	4.0	1.0 – 8.0
	Imipenem	0.15	0.5	0.15 – 1.0
	Clindamycin	2.0	4.0	0.5 – 8.0
	Metronidazole	4.0	32	2.0 – > 64
Clostridium difficile (n = 16)	Butanol extract	0.064	0.5	0.032 – 0.5
	Erythromycin	1.0	2.0	0.25 –8.0
	Cefoxitin	0.125	1.0	0.125 – 2.0
	Imipenem	0.125	0.5	0.064 – 0.5
	Clindamycin	1.0	2.0	1.0 – 4.0
	Metronidazole	0.032	0.064	0.032 – 0.5
Clostridium perfringenes (n = 14)	Butanol extract	0.5	1.0	0.125 – 2.0
	Erythromycin	0.25	0.5	0.25 – 1.0
	Cefoxitin	1.0	4.0	1.0 – 32
	Imipenem	0.125	1.0	0.064 – 1.0
	Clindamycin	0.5	2.0	0.25 – 4.0
	Metronidazole	2.0	8.0	1.0 – 16
Propionibacterium acnes (n = 22)	Butanol extract	0.064	0.25	0.032 – 0.25
	Erythromycin	0.5	1.0	0.5 – 2.0
	Cefoxitin	4.0	32	2.0 – > 64
	Imipenem	0.25	2.0	0.25 – 4.0
	Clindamycin	2.0	8.0	1.0 – 16
	Metronidazole	1.0	4.0	0.5 – 4.0
Peptostreptococci (n = 16)	Butanol extract	1.0	2.0	1.0 – 2.0
	Erythromycin	0.25	1.0	0.25 –2.0
	Cefoxitin	0.5	2.0	0.125 – 4.0
	Imipenem	0.25	1.0	0.25 – 4.0
	Clindamycin	4.0	16	0.125 – 4.0
	Metronidazole	4.0	32	2.0 – > 32

obtained from suppliers: erythromycin (Serva, Feinbiochemia GmbH & Co., Heidelberg, Germany), cefoxitin (The Upjohn Co., Chicago, USA), imipenem (Merck & Co., Inc., West Point, USA), clindamycin and metronidazole (Sigma Chemical Co., St. Louis, MO, USA). The 140 strains of anaerobic bacteria that were used in this study were clinical isolates obtained between April 2007 and February 2009 from the Korea University Hospital (KUH) (Seoul, South Korea). All isolates were identified using standard criteria (Holdeman *et al*. 1997; Shutter *et al*. 1980). The species and number of strains tested are detailed in Table 1.

Antimicrobial activity – The MICs (Minimal Inhibitory Concentration) were determined as described earlier by Rhee using an agar dilution method (Rhee, 2004). In brief, both cyclic dipeptides were dissolved in water with

8% methanol, and the pH was adjusted to 4.0 with 2M NaOH. A pH-adjusted 8% methanol-water solution without the dissolved substances was used as a negative control. Antibiotic concentrations that were tested ranged from 0.125 mg/l to 128 mg/l. Colony suspensions equivalent to a 0.5 McFarland standard were prepared and inoculated onto mediums containing antibiotics using a Cathra Systems replicating device (MCT Medical, Inc., St. Paul, MN, USA) to yield a final inoculum of 10^4 CFU/spot. The plates were incubated in ambient air at 35 °C for 24 h. The MICs were defined as the lowest antibiotic concentration that completely inhibited growth.

Results and Discussion

The antimicrobial activities of the butanol extract of *L. japonica* Thunb. and five antimicrobial agents were compared against selected anaerobic bacterial strains and are shown in Table 1. *Lonicera japonica* and imipenem were highly active against *Bacteroides fragilis, Bacteroides ovatus, C. difficile, C. perfringenes*, and *Propionebacterium acnes,* with MIC_{90}s (where 90% of isolates were inhibited) ranging from 0.032 mg/l to 0.5 mg/l. However, only the MICs of three strains among the 15 *B. fragilis* strains were high (8 mg/l). Furthermore, the butanol extract was active against all anaerobic groups, with all MIC_{50}s ≤ 0.5 mg/l except for *B. ovatus* and *Peptostreptococci*. The butanol extract was least effective against *Peptostreptococci* with a MIC_{50} of 1.0 and a MIC_{90} of 2.0 mg/l; the MICs of clindamycin and metronidazole were 16.0 and 4.0 mg/l (MIC_{50}), 4.0 and 64 mg/l (MIC_{90}), respectively.

Although imipenem was active against all gram negative and positive anaerobic bacteria, 70% of *Peptostreptococci* and 10% of *Clostridium perfringens* were resistant to this agent. It is important to note that clindamycin was active against 70% of *B. fragilis*, while all other strains were resistant to this agent. Erythromycin was active against 80% of strains overall, and only 30% of *C. difficile* strains were resistant to this agent. In contrast, metronidazole was the most active against *C. difficile* only, with a MIC_{50} of 0.032 mg/l and a MIC_{90} of 0.064 mg/l. However, the other strains were resistant to this agent.

In present study, it was found that *L. japonica* was effective against anaerobic gram negative and gram positive bacteria. As expected, *L. japonica* was more active against various anaerobic bacteria than various antimicrobial agents such as erythromycin, cefoxitin, imipenem, clindamycin, and metronidazole. In addition, the MIC of the butanol extract against the *C. difficile* was 0.064 mg/l which is less than that of ALP20 (a new penem antibiotic, Astra Clinical Research Center, Sodertalje, Sweden) which was against *C. difficile* using the same MIC (4.0 mg/l) test as reported by Carl *et al* (1989). The present results indicate that of the approved antimicrobials tested, imipenem had the greatest activity against gram negative bacteria (*B. fragilis* and *B. ovatus*) with MICs ranging from 0.032 to 1.0 mg/l. It is also important to note that the MICs of the butanol extract of *L. japonica* ranged from 0.032 to 2.0 mg/l in all strains.

In summary, this study is the first to demonstrate that *Lonicera japonica* exhibits activity against anaerobic bacteria. In conclusion, *Lonicera japonica* is a potent inhibitor of anaerobic bacteria *in vitro*. Additional investigations like these with other honeysuckle parts of *Lonicera japonica* is warranted, particularly with regard to bioavailability, toxicity, and stability, it has potential to be beneficial in treatments for bacterial infections.

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Received November 12, 2010
Revised February 15, 2011
Accepted February 19, 2011