Original Research Article

Korean J. Plant Res. 24(6) : 719～723 (2011)

Study on Antimicrobial Activity of Extracts from *Fritillaria unibracteata* Hsiao et K.C. Hsia and *F. ussuriensis* Maxim.

Jung-Wook Moon, Chen-zhi, Won-Seob Song[1]* and Seung Hwa Baek[2]

College of Life and Geographical Sciences, Qinghai Normal University, Xining 810008, China
[1]College of Life Science and Natural Resources, Sunchon National University, Suncheon 540-742, Korea
[2]Department of Biofood Science and Biotechnology, Chungbuk Provincal University, Okcheon 373-806, Korea

Abstract - Antimicrobial activities of methanol, ethanol, water, and CH_2Cl_2 extracts from *Fritillaria unibracteata* Hsiao et K.C. Hsia and *F. ussuriensis* Maxim. were investigated by disk-agar diffusion method. The result showed comparatively strong antimicrobial activity against several microorganisms. The extracts from *F. unibracteata* and *F. ussuriensis* dose-dependently increased the activity. However, water and CH_2Cl_2 extracts showed no antimicrobial activity against 7 microorganisms. Especially, against the most sensitive microorganism *Staphylococcus epidermidis*, methanol extracts at highest concentration of 20 mg/mL exhibited the largest clear zone on plate by 6-12 mm and ethanol extracts on plate by 6-10 mm.

Key words - Antimicrobial activity, Disk-agar diffusion method, *Fritillaria unibracteata*, *F. ussuriensis*

Introduction

In recent years, with the rapid development of natural products research and the general improvement in life quality, people are increasing food demand. Consumers pay initially more attention to the biological function of the food itself and its freshness when buying food, the food materials on the market go through a procedure of minimum heating and processing only in order to meet consumer demand (Branen, 1975), however, while the biological function of the food is guaranteed by this processing method, the microorganisms existing in materials can survive at same time (Jang *et al*., 1999; Jang and Han, 2002). To reduce the damage of these microorganisms on human body and prevent microbial contamination (Kim *et al*., 2004) on food, the food preservatives are added to inhibit the bacteria proliferation. Due to the low prices of artificial preservatives and the synthetic preservatives used mostly on the market (Nakamura *et al*., 1991; Ramos *et al*., 2003) many scholars reinforce the plants study considering the food safety, hoping to find a natural preservative (Seeram *et al*., 2006). Because natural preservatives can avoid toxicity issues caused by synthetic preservatives, purification researches on antibacterial compounds from plant resources are getting more and more concerns (Song *et al*., 2003). Currently, it is reported that natural preservatives have been extracted from the chives, chrysanthemum, aromatic plants, *Ulmus pumila*, *Houttuynia cordata* (Wang *et al*., 1993).

Fritillaria unibracteata Hsiao et K.C. Hsia and *F. ussuriensis* Maxim. have the effect of clearing heat and moistening lungs (Chen *et al*., 2007), and eliminating carbuncle, so that it is commonly used in the Hyperactivity dry cough (Gao *et al*., 2000), dry cough with little phlegm, laboring cough, bloody sputum (Li *et al*., 2002), breast abscess, and lung abscess (Zhang *et al*., 2004; Li and Li, 1998; Bauer *et al*., 1996). The prior in vitro studies on the genus of *Fritillaria* suggested that verticinone, imperialine and imperialine 3-β-glucoside, 3β-acetylimperialine and sinpeinine A may have effective anti-asthmatic activity (Lin *et al*., 2006), antitussive, expectorant and anti-inflammatory activities (Wang *et al*., 2012). Alkaloids were found to be the major biologically active ingredients in genus of *Fritillaria*.

This study was conducted to determine mainly the antimicrobial activity of various solvent extracts including methanol extract, ethanol extract, dichloromethane extract and water extract from *F. unibracteata* and *F. ussuriensis*, providing the basic information for further development and utilization of the medicinal plants.

*Corresponding author. E-mail : chinakor@empas.com

Materials and Methods

Materials

Fritillaria unibracteata samples were supplied by Prof. Chen Zhi of China-Korea Qinghai-Tibetan Plateau Institute, and *F. ussuriensis* samples were purchased from the materials storehouse of Korean traditional medicine.

Apparatus

Apparatus used in this study are listed in the following Table 1.

Bacteria

The bacteria used in the experiment were as follows: 6 kinds of Gram positive, and 1 kind of Fungi. They are *Staphylococcus epidermidis*, *Staphylococcus aureus, Pityrosporum ovale, Listeria monocytogenes*, *Bacillus subtilis*, *Corynebacterium ammoniagenes*, *Candida albicans*, supplied by Korea Culture Center of Microorganisms, KCCM).

Table 1. Apparatus used in experiment.

Apparatus	Maker
Shaking incubator	VS-8480SR, VISION, Korea
Centrifuge	VS-24SMTi, VISION, Korea
Micro high speed refrigeration	Micro17R, HANIL SME, Korea
Rotary evaporator	R-215, BUCHI, Germany
Heating bath	B-491, BUCHI, Germany
Auto clave	SS325, TOMY, U.S.A
Ultrasonic cleaner	SUC-200M, Tae-Sheng, Korea
Incubator	VS-1203P3, VISION, Korea
Clean bench	KMC-1400LSN, VISION, Korea

Table 2. Information on bacteria, gram positive and fungi.

Strains	Strains No.	Gram Strain
Staphylococcus epidermidis	KCCM 35494	Gram (+)
Staphylococcus aureus	KCCM 11335	Gram (+)
Pityrosporum ovale	KCCM 11894	Gram (+)
Listeria monocytogenes	KCCM 19111	Gram (+)
Bacillus subtilis	KCCM 11316	Gram (+)
Corynebacterium ammoniagenes	KCCM 1174	Gram (+)
Candida albicans	KCCM 11282	Fungi

Medium

Bacteria used in this experiment are Gram-positive and Fungi. The following Table 3 presents the different media compositions in each bacterium.

Extraction and enrichment

Dry matters of *Fritillaria unibracteata* and *F. ussuriensis* are prepared and placed for 7 days at room temperature. Each 200 g from *F. unibracteata* and *F. ussuriensis* dry sample materials were extracted with 4 different solvents at each 500 ml, including 85% methanol, ethanol, methylene (CH_2Cl_2) chloride and distilled water for 8 hours at 60℃. Each extract of 50 g was obtained from two herbs with 4 different solvents. The crude extract concentrate was evaporated to obtain from the supernatant at 45℃~50℃. Methanol extracts, ethanol extracts, CH_2Cl_2 extracts, and water extracts of the two herbs are obtained.

Plate preparation of medium

In order to prepare aseptic conditions for the experiment, firstly experimental apparatus were sterilized with high-pressure autoclave (121℃, 15 min), all the experimental operations were carried out on the clean table. Bacteria provided from Korea Culture Center of Microorganisms (KCCM), which was incubated in the incubator (31℃, 24 hour) after transplantation on medium, and then placed in freezer at 0℃~2℃ alternatively. Media preparation was conducted in accordance with Table 3-3, and then sterilized by high-pressure autoclave (121℃, 15 min). Under aseptic

Table 3. Compositions of media and incubation protocols used for the experiment.

Strain	Media
Gram (+)	Peptone 1.5 g/L Beef extract 5 g/L Agar 20 g/L Distilled water 1 L
Fungi	Potato extract 20 g/L Glucose 20 g/L Agar 20 g/L Distilled water 1 L

operating condition, bacteria were inoculated by platinum (pt) on aseptic media in container, and then incubated in the oscillating incubator (37℃, 200 rpm) for 24 hours.

Determination of antimicrobial activity

The antimicrobial activity of *Fritillaria unibracteata* and *F. ussuriensis* is determined by disk-agar diffusion method (Bauer, 1996). The various extracts treated from the corresponding reagents were methanol extracts, ethanol extracts, CH_2Cl_2 extracts, and water extracts given with concentrations of 5, 10, 15, 20 mg/mL. The extracts were filtered by 0.45 ㎛ membrane filter, Millipore Co., USA. Extracts of different concentrations from every solvent were added on a sterile-treated filter paper (filter paper disc 8 mm, Whatman, USA), and the paper were attached on the medium. And then, the containers treated with the extracts were incubated for 24~28 hours in the adapted temperatures (37℃), measured the diameter of clear inhibition (mm) of filter paper around after 24 hours, compared the antibacterial activity by extract.

Results

Antimicrobial activity of *F. unibracteata* and *F. ussuriensis* show in Table 4 and 5, respectively. The inhibition zone of methanol and ethanol extracts in *F. unibracteata* at different concentrations was ranged as follows: *Staphylococcus epidermidis* 3~12 mm, *S. aureus* 3~10 mm, *Pityrosporum ovale* 3~11 mm, *Listeria monocytogenes* 3~9 mm, *Bacillus subtilis* 3~6 mm, *Corynebacterium ammoniagenes* 3~10 mm, *Candida albicans* 3~10 mm (Table 4). The result indicated that *F. unibracteata* extracts show the highest antimicrobial activity against *Staphylococcus epidermidis,* and followed by against *Pityrosporum ovale*, *S. aureus*, and *Candida albicans*.

The inhibition zone of methanol and ethanol extracts in *F. ussuriensis* Maxim. at different concentrations was *Staphylococcus epidermidis* 4~11 mm, *S. aureus* 2~8 mm, *Pityrosporum ovale* 2~8 mm, *Listeria monocytogenes* 3~7 mm, *Bacillus subtilis* 3~10 mm, *Corynebacterium ammoniagenes* 3~7 mm, *Candida albicans* 2~9 mm (Table 5). The data showed the highest antimicrobial activity against *Staphylococcus*

Table 4. Antimicrobial activity of bulb extracts of *F. unibracteata* by methanol extract and ethanol extract.

Strains	Clear zone on plate (mm)							
	Methanol extracts				Ethanol extracts			
	5 ㎕	10 ㎕	15 ㎕	20 ㎕	5 ㎕	10 ㎕	15 ㎕	20 ㎕
Staphylococcus epidermidis[1)]	-	3	8	12	-	-	5	10
Staphylococcus aureus[1)]	-	3	6	8	-	-	5	10
Pityrosporum ovale[1)]	-	3	7	11	-	-	4	9
Listeria monocytogenes[1)]	-	-	4	9	-	-	3	6
Bacillus subtilis[1)]	-	-	4	6	-	-	3	6
Corynebacterium ammoniagenes[1)]	-	-	3	10	-	-	4	7
Candida albicans[2)]	-	5	8	10	-	3	5	10

[1)]Gram positive, [2)]Fungi; The clear zone expressed was included a size of disc-paper (8 mm of diameter).

Table 5. Antimicrobial activity of bulb extracts of *F. ussuriensis* by methanol extract and ethanol extract.

Strains	Clear zone on plate (mm)							
	Methanol extracts				Ethanol extracts			
	5 ㎕	10 ㎕	15 ㎕	20 ㎕	5 ㎕	10 ㎕	15 ㎕	20 ㎕
Staphylococcus epidermidis[1)]	-	4	7	11	-	-	3	9
Staphylococcus aureus[1)]	-	2	5	8	-	-	2	7
Pityrosporum ovale[1)]	-		4	7	-	-	2	8
Listeria monocytogenes[1)]	-	-	3	7	-	-	3	6
Bacillus subtilis[1)]	-	-	3	10	-	-	4	8
Corynebacterium ammoniagenes[1)]	-	-	3	6	-	-	-	7
Candida albicans[2)]	-	-	2	8	-	-	5	9

[1)]Gram positive, [2)]Fungi; The clear zone expressed was included a size of disc-paper (8 mm of diameter).

epidermidis suggesting similar activity to extracts in *F. unibracteata.* Dichloromethane.

Discussion

The extracts from *F. unibracteata* and *F. ussuriensis* dose-dependently increased antimicrobial activity. It is shown from Table 4 and Table 5 that methanol and ethanol extracts of *Fritillaria unibracteata* have inhibitory effect, the strongest inhibition showed at the highest concentration of 20 mg/㎕. Besides, methanol and ethanol extracts showed same activity significant *Bacillus subtilis* and *Candida albicans*. Methanol extracts from *F. unibracteata* showed stronger activity than ethanol extracts, against *Staphylococcus epidermidis*, *Listeria monocytogenes*, and *Corynebacterium ammoniagenes.* However, activity of methanol extract against *Staphylococcus aureus* was lower than that of ethanol extract inhibition to *Staphylococcus aureus*. However, dichloromethane extract and water extract have no inhibitory effect.

No inhibitory effect was observed in dichloromethane and water extracts from *F. unibracteata* and *F. ussuriensis*.

This experiment can be summarized as follows; *Staphylococcus aureus* was the sensitive microorganism to the dichloromethane and water extracts from *F. unibracteata* and *F. ussuriensis*. The bacterium is known to be a common respiratory pathogen (Nakamura *et al.*, 1991; Jang *et al.*, 1999), and has some inhibitory role to *Listeria monocytogenes* which is a common pathogen causing food poisoning (Wang *et al.*, 1993). *Candida albicans* as a common pathogen causing gastroenteritis was more susceptible to the extracts (Branen *et al.*, 1975; Gao *et al.*, 2000). Therefore, it was concluded that methanol and ethanol extract of *F. unibracteata* have potent anti-microbial activity for the supportive treatment on respiratory diseases, food poisoning, and gastroenteritis diseases.

Literature Cited

Bauer, A.W., M.M. Kibby, J.C. Sherris and M. Turck. 1996. Antibiotic susceptibility testing by standardized single disc method. American J. Clin. Pathol. 45:493-496.

Branen, A.L. 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J. American Oil Chem. Soc. 52:59-63.

Chen, M.H., H.C. Wang, W.M. Zhu and Z. Chen. 2007. Phamacological research on Fritilaria. J. Anhui Agri. Sci. Bull. 13(1):103-105.

Gao, S.M., Y. Xia, and F.P. Tan. 2000. The pharmacological action of cultured bulb of *Fritillaria unibracteata* Hsiao et K.C. Hsia. J. Plant Res. Env. 9(1):4-8.

Jang, D.S., K.H. Park, J.R. Lee, T.J. Ha, Y.B. Park, S.H. Nam and M.S. Yang. 1999. Antimicrobial activities of *Sesquiterpene lactones* isolated from *Hemisteptia lyrata*, *Chrysanthemum* zawadskii and *Chrysanthemum* boreale. J. Korean Soc. Appl. Biol. Chem. 42:176-179 (in Korean).

Jang, J.K. and J.Y. Han. 2002. The antioxidant ability of grape seed extracts. Korean J. Food Sci. Technol. 34:524-528 (in Korean).

Kim, J., S.A. Kim, W.K. Yun, E.J. Kim, M.K. Woo and M.S. Lee. 2004. Antioxidative effect of ethanol extract for 5 kinds of spice. J. Korean Soc. Food Sci. Nutr. 33:1426-1431 (in Korean).

Li, P., L.J. Zeng, S.L. Li and et al. 2002. The extraction of imperialine and imperialine-3 beta-glucoside from *Fritillaria pallidiflora* Schrenk and quantitative determination by HPLC-evaporative light scattering detection. Phytochem Analy. 3(3):158-161.

Li, X. and Y.R. Li. 1998. Mensurate the alkaloid content of *Fritillaria thunbergii* Miq by acid dye colorimetry. China Pharm. 8(10):45-47.

Lin, B.Q., H. Ji, P. Li, Y. Jiang and W. Fang. 2006. Selective antagonism activity of alkaloids from bulbs *Fritillariae* at muscarinic receptors: functional studies. J. Enthnopharmacol. 551:125-130.

Nakamura, S., A. Kato and K. Kobayashi. 1991. New antimicrobial characteristics of lysozyme-dextran conjugate. J. Agric. Food Chem. 39:647-650.

Ramos, A., A. Visozo, J. Piloto, A. Garcia, C.A. Rodriguez and R. Rivero. 2003. Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. J. Ethnopharmacol. 87:241-246.

Seeram, N. P., S.M. Henning, Y. Niu, R. Lee, H.S. Scheuller and D. Heber. 2006. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. J. Agric. Food Chem. 54:1599-1603.

Song, J.H., M.J. Kim, H.D. Kwon and I.H. Park. 2003. Antimicrobial activity of fractional extracts from *Houttuynia cordata* root. J. Korean Soc. Food Sci. Nutr. 32:1053-1058 (in Korean).

Wang, D.D., S. Wang, X. Chen, X. Xu, J. Zhu, L. Nie and X. Long. 2012. Antitussive, expectorant and anti-inflammatory activities of four alkaloids isolated from Bulbus of *Fritillaria wabuensis*. J. Ethnopharmacol. 139:189-193.

Wang, L.L., B.K. Yang, K.L. Parkin and E.A. Johson. 1993. Inhibition of *Listeria monocytogenes* by monoacylglycerols synthesized from coconut oil and milk fat by lipase-catalyzed glycerolysis. J. Agric. Food Chem. 41:1000-1005.

Zhang, Y.H., H.L. Ruan, H.F Pi, H.F. J. Z. Wu, H.D. Sun and T. Fujita. 2004. Structural elucidation of fritillahupehin from bulbs of *Fritillaria hupehensis* Hsiao et K.C. Hsia. J. Asian Nat. Products Res. 6(1):29-34.

(Received 24 June 2011 ; Revised 20 December 2011 ; Accepted 28 December 2011)