Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014-15; 6(4); 845-849

ISSN: 0975-4873

Research Article

In vitro Antibacterial Activity of Three *Bupleurum* Plants

Byongsoon Lee[1], Seongkyu Choi[2], *Kyeong Won Yun[2]

[1]*Department of Health Care & Science, Jeonju University, Jeonju 560-759, Korea*
Department of Oriental Medicine Resources, Sunchon National University, Suncheon 540-950, Korea

Available Online: 22nd November, 2014

ABSTRACT
The antibacterial activity of three *Bupleurum* plants (*Bupleurum falcatum, Bupleurum falcatum* 'Mishima' and *Bupleurum latissimum*) was tested against three gram-positive and three gram-negative bacteria using the disk-diffusion method. The ether and ethyl acetate fraction of crude methanol extract from the three *Bupleurum* plants showed potent antibacterial activity against the tested microorganisms. The ethyl acetate fraction of *B. falcatum* exhibited a higher antibacterial activity than the other two *Bupleurum* plants. In general, the ethyl acetate fraction of crude methanol extract of *B. falcatum* and *B. falcatum* 'Mishima' showed a higher growth inhibitory activity than the ether fraction against tested gram-positive and gram-negative bacteria. In contrast, the ether fraction of *B. latissimum* showed a higher antibacterial activity than the ethyl acetate fraction. Antibacterial assay showed that *Salmonella typhimurium* was the most sensitive microorganism with the lowest MIC (minimum inhibitory concentration). In addition, the extract of the three *Bupleurum* plants displayed significant antibacterial activity against *Bacillus cereus* and *Bacillus subtilis*.

Keywords: *Bupleurum falcatum*, *Bupleurum falcatum* 'Mishima', *Bupleurum latissimum*, antibacterial activity, medicinal plant

INTRODUCTION

Recently, the natural substances extracted from plant have received particular attention and the food industry is continuously developing to satisfy a consumer's desires along with an improvement in living standards. So, the studies to find the new functional ingredients from natural substances and to suppress the propagation of a toxic microorganism to food have been conducted.[1,2] The bacterial strain discovered from egg, beef and dairy products in United States, Europe as well as developing country was *Samonella enterica, Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum*.[3,4] Synthetic preservatives have been added to food for long time, but the use of synthetic preservatives is rapidly declined because of the side effect. For such a reason, the development of the food preservative from the natural substance is positively necessary.[5]

The genus of *Bupleurum* is one of the large genuses of Apiaceae and is widely distributed at Europe and Asia.[6] *Bupleurum* spp. is simple leaf and its venation is parallel venation, and its flower color is yellow and it have been used in traditional Chinese herbal medicine as the major component for curing liver diseases.[7] It was reported that about 150 species of the genus are distributed at the only northern hemisphere.[8] *Bupleurum falcatum, B. longiradiatum, B. euphorbioides, B. latissimum* and *B. scorzonerifolium* are spread in Korea as 5 syntaxon.[9]

The roots of *B. falactum* contain several saikosaponins,[10] and their various pharmacological activities have been reported.[11,12,13,14] The anticomplementary,[15] macrophages Fc receptor up-regulating[16] and antiulceractivities[17,18] have been observed as their pharmacological activities. *Bupleurum falcatum* 'Mishima' is a cultivar of the *Bupleurum falcatum* was introduced from Mishima island of Japan and cultivated at Goheunggun, Jeollanam-do, Korea. *Bupleurum latissimum* is an endemic species of Korea. It was observed at shoreline of Ulleungdo island by 1970 and was vanished because of the environmental variation. It has been observed since 2000.[19]

The aim of this study is to assess the antibacterial activity of ether fraction and ethyl acetate fraction from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum* against 6 strains by using an agar disk diffusion procedure.

MATERIALS AND METHODS

Plant materials: The three plants were collected as following: *Bupleurum falcatumm* was collected from Jeongseongun, Gangwon-do (37° 22' 47" N, 128° 39' 42" E), *B. falcatum* 'Mishima' was collected from Goheunggun, Jeollanam-do (34° 36' 40" N, 127° 17' 6" E) and *B. latissimum* was collected from Ulleungdo island, Gyeongsangbuk-do (37° 30' 23" N, 130° 51' 26" E), Korea. The collected plant roots were air-dried in shadow for two weeks.

Bacterial strains and conditions: The ether and ethyl acetate fractions of the three *Bupleurum* plants against the following bacteria: *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium*. The bacteria were cultured on nutrient broth agar.

**Author for correspondence*

Table 1: Antibacterial activities against *Bacillus cereus* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC (mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	8.3±0.0^{b}	9.2±0.4^{b}	10.5±0.7^{b}	11.0±0.4^{ab}	12.2±1.1^{a}	0.1
Ethyl acetate fraction	-	8.3±0.0^{d}	10.8±0.1^{c}	12.2±0.1^{b}	13.6±0.6^{a}	13.9±0.7^{a}	0.1
B. falcatum 'Mishima'							
Ether fraction	-	-	9.2±0.4^{b}	10.3±0.0^{b}	10.9±0.4^{ab}	11.3±0.3^{a}	0.25
Ethyl acetate fraction	-	-	9.9±0.1^{c}	11.6±0.8^{b}	11.4±1.3^{b}	12.7±0.6^{a}	0.25
B. latissimum							
Ether fraction	-	8.6±0.4^{b}	9.7±0.3^{b}	10.9±0.2^{ab}	12.3±0.1^{ab}	13.3±0.2^{a}	0.1
Ethyl acetate fraction	-	-	-	9.3±0.2^{c}	10.8±0.6^{b}	11.5±0.4^{a}	0.5

Different letter indicates significant differences (p<0.05) within a row.
- : Not detected.

Table 2: Antibacterial activities against *Bacillus subtilis* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC (mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	8.4±0.2^{c}	9.0±0.3^{bc}	9.9±0.4^{b}	11.1±0.2^{a}	11.6±0.6^{a}	0.1
Ethyl acetate fraction	-	8.4±0.2^{c}	9.0±0.3^{bc}	9.9±0.4^{b}	11.1±0.2^{a}	11.6±0.6^{a}	0.1
B. falcatum 'Mishima'							
Ether fraction	-	8.7±0.6^{d}	9.3±0.6^{cd}	9.8±0.5^{bc}	10.7±0.1^{ab}	11.5±0.5^{a}	0.1
Ethyl acetate fraction	-	-	9.3±0.6^{c}	9.8±0.5^{bc}	10.7±0.1^{ab}	11.5±0.5^{a}	0.25
B. latissimum							
Ether fraction	-	8.7±0.1^{c}	9.0±0.2^{c}	10.4±0.1^{b}	11.4±0.4^{a}	11.8±0.2^{a}	0.1
Ethyl acetate fraction	-	-	9.0±0.2^{c}	10.4±0.1^{b}	11.4±0.4^{a}	11.8±0.2^{a}	0.25

Different letter indicates significant differences (p<0.05) within a row.
- : Not detected.

Table 3: Antibacterial activities against *Staphylococcus aureus* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC(mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	-	8.8±0.2^{c}	9.5±0.4^{bc}	9.9±0.7^{b}	10.9±0.6^{a}	0.25
Ethyl acetate fraction	-	-	9.2±0.9^{c}	10.9±0.1^{b}	11.4±0.2^{ab}	12.0±0.5^{a}	0.25
B. falcatum 'Mishima'							
Ether fraction	-	-	8.5±0.1^{c}	9.0±0.4^{bc}	9.6±0.1^{b}	10.8±0.2^{a}	0.25
Ethyl acetate fraction	-	-	8.3±0.0^{b}	8.7±0.0^{b}	10.2±0.4^{a}	10.9±0.4^{a}	0.25
B. latissimum							
Ether fraction	-	-	8.2±0.1^{c}	9.0±0.5^{bc}	9.9±0.1^{ab}	10.6±0.0^{a}	0.25
Ethyl acetate fraction	-	-	8.2±0.1^{b}	8.4±0.1^{b}	9.6±1.2^{ab}	8.9±0.7^{a}	0.25

Different letter indicates significant differences (p<0.05) within a row.
- : Not detected.

Plant extracts for antibacterial activity: 100 gram of each plant sample was extracted for 20min in 500ml methanol at room temperature. After being kept at room temperature for 30 min, the solution was filtered through Whatman No.2 filter paper. The crude methanol extract was partitioned with 500ml of hexane and then the layer was concentrated (hexane fraction). The remaining layer was

Table 4: Antibacterial activities against *Escherichia coli* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC(mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	-	8.5±0.1^{c}	9.8±0.2^{b}	10.3±0.1^{ab}	10.8±0.4^{a}	0.25
Ethyl acetate fraction	-	8.2±0.1^{d}	9.2±0.4^{c}	10.6±0.1^{b}	11.5±0.0^{ab}	12.0±0.3^{a}	0.1
B. falcatum 'Mishima'							
Ether fraction	-	-	8.7±0.6^{b}	9.3±0.9^{b}	10.0±0.4^{a}	10.4±0.1^{a}	0.25
Ethyl acetate fraction	-	-	8.8±0.6^{c}	10.6±0.2^{b}	11.4±0.3^{ab}	11.7±0.2^{a}	0.25
B .latissimum							
Ether fraction	-	-	8.7±0.0^{c}	9.8±0.2^{b}	10.6±0.3^{ab}	10.9±0.0^{a}	0.25
Ethyl acetate fraction	-	-	-	8.5±0.2^{c}	9.7±0.1^{b}	10.4±0.4^{a}	0.5

Different letter indicates significant differences (p<0.05) within a row.
- : Not detected.

Table 5: Antibacterial activities against *Pseudomonas aeruginosa* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC (mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	8.2±0.1^{b}	8.5±0.4^{b}	8.9±0.4^{b}	10.3±0.2^{a}	10.6±0.3^{a}	0.1
Ethyl acetate fraction	-	-	8.6±0.6^{c}	10.0±0.1^{b}	10.6±0.4^{ab}	11.2±0.5^{a}	0.25
B. falcatum 'Mishima'							
Ether fraction	-	-	8.4±0.1^{c}	9.0±0.3^{bc}	9.6±0.2^{ab}	10.3±0.0^{a}	0.25
Ethyl acetate fraction	-	-	8.5±0.2^{c}	9.6±0.2^{b}	10.6±0.1^{a}	11.0±0.4^{a}	0.25
B. latissimum							
Ether fraction	-	-	8.4±0.2^{c}	9.0±0.8^{bc}	9.4±0.3^{b}	10.5±0.4^{a}	0.25
Ethyl acetate fraction	-	-	8.1±0.0^{b}	8.7±0.4^{ab}	9.4±0.1^{ab}	9.0±1.1^{a}	0.25

Different letter indicates significant differences (p<0.05) within a row.
- : Not detected.

successively fractionated with 500ml ethyl ether and then ethyl acetate (ether and ethyl acetate fraction). The remaining residue was the water fraction. Each fraction was concentrated *in vacuo* to 30ml at 30℃ and tested for antibacterial activity. Antibacterial activity was measured only with the ether and ethyl acetate fractions.

Determination of antibacterial activity: Each bacterial strain was grown in a nutrient broth at 30℃ for 24h prior to testing. For the Disc plate method, 0.1ml of the bacterial cell suspensions were poured uniformly on the nutrient broth agar plate. The paper disks containing the extracts were carefully placed on the seeded Petri dishes. The diameters of inhibition zones were measured in millimeters after the strains were incubated at 30℃ for 24h or 48h.[20] And the minimum inhibitory concentration (MIC) was determined as the lowest concentration that caused an inhibition zone. Concentrations of the extract were 0.05, 0.10, 0.25, 0.50, 1.00 and 1.50 mg/ml.

Statistical analysis: The experiments were conducted in triplicate and the results are expressed as mean ± standard deviation (SD). Differences between means were tested through Duncan's multiple range test. Statistical analysis was performed with the software program SPSS (Version 18.0).

RESULTS AND DISCUSSION

This study describes the antibacterial activity of three *Bupleurum* plants against three gram-positive and three gram-negative bacteria. Tables present diameter of inhibition zone and MIC value exerted by the ether and ethyl acetate fraction of methanol extract from the three *Bupleurum* plants toward the tested microorganisms. The ether and ethyl acetate fraction of crude methanol extract from the three *Bupleurum* species showed that the inhibitory effect against the microorganisms with the diameters of clear zone ranging between 8.1 ㎜ and 13.9 ㎜. Our findings showed that the increase of concentration of the three *Bulpeurum* plant extracts caused increasing inhibition of the bacterial growth. The ethyl acetate fraction of crude methanol extract from *B. falcatum* showed a significantly higher growth inhibitory activity than that of the other two plants. The ethyl acetate fraction of crude methanol extract from *B. falcatum* and *B. falcatum* 'Mishima' showed a significantly higher growth

Table 6: Antibacterial activities against *Salmonella typhimurium* of the ether and ethyl acetate fraction of methanol extract from *Bupleurum falcatum, B. falcatum* 'Mishima' and *B. latissimum*

	Clear zone (±S.D., mm) at various concentrations (mg/ml)						MIC (mg/ml)
	0.05	0.10	0.25	0.50	1.00	1.50	
B. falcatum							
Ether fraction	-	-	8.4±0.4^{c}	9.5±0.3^{b}	10.9±0.4^{ab}	11.4±0.1^{a}	0.25
Ethyl acetate fraction	-	8.3±0.1^{d}	9.3±0.2^{c}	10.3±0.3^{b}	11.2±0.4^{ab}	11.6±0.4^{a}	0.1
B. falcatum 'Mishima'							
Ether fraction	-	8.4±0.3^{c}	9.0±0.1^{bc}	9.3±0.3^{bc}	9.7±0.1^{b}	10.7±0.2^{a}	0.1
Ethyl acetate fraction	-	8.2±0.1^{c}	8.5±0.6^{bc}	9.5±1.4^{ab}	10.4±1.1^{a}	10.4±0.6^{a}	0.1
B. latissimum							
Ether fraction	-	8.3±0.0^{c}	8.7±0.4^{bc}	9.6±0.7^{b}	10.6±0.1^{a}	11.5±0.1^{a}	0.1
Ethyl acetate fraction	-	8.2±0.1^{c}	8.4±0.1^{bc}	9.3±0.3^{b}	10.8±0.6^{a}	10.6±0.1^{a}	0.1

Different letter indicates significant differences ($p<0.05$) within a row.
- : Not detected.

inhibitory activity against gram-positive and gram-negative bacteria than the ether fraction. In contrast, the ether fraction of crude methanol extract of *B. latissimum* showed a higher growth inhibitory activity than the ethyl acetate fraction. The results from the disk-diffusion method followed by measurements of MIC indicated that *Salmonella typhimurium* is the most sensitive microorganism with the lowest MIC value. Another sensitive microorganism is *Bacillus cereus* and *B. subtilis.* In general, our results are consistent with previous observations that gram-positive bacteria are typically more sensitive than gram-negative bacteria to extracts of some medicinal plants [21] and *Eucalyptus globulus*,[22] but gram-negative bacteria are often more sensitive than gram-positive bacteria.[23] In previous study, the plants belonging to the family Apiaceae were shown antibacterial activity.[24,25] As comparison the previous study, the three *Bupleurum* plants is less antibacterial activity than other Apiaceae plants, but the three *Bupleurum* plants is shown a valuable antibacterial activity.

The plants of *Bupleurum* have been used as oriental medicine resources for treatment of various diseases. In this study, we report for the first time that the comparative study on the antibacterial activity of the three *Bupleurum* plants. It is shown that antibacterial extracts from the three *Bupleurum* plants can be assumed to be useful as an antibacterial agent against bacterial disease.

CONCLUSION

The present study elucidates a potent antibacterial agent from extract of *Bupleurum falcatum, Bupleurum falcatum* 'Mishima' and *Bupleurum latissimum*. The ether and ethyl acetate fraction from the three *Bupleurum* plants showed significant antibacterial activity on three gram-positive and three gram-negative bacteria. This is the first study to compare the antibacterial activity of the three *Bupleurum* plants used as medicine resources in Korea. According to the results of this study, roots of the three *Bupleurum* species can be used as a potential source of natural antimicrobial resources. Nevertheless further studies are needed for enlightening the chemicals responsible for antimicrobial activity.

REFERENCES

1. Bae JH. Antimicrobial effect of *Pulsatilla koreana* extracts on food-born apathogen. The Korean Journal of Nutrition 2004; 37:655-661.
2. Chung KS, Kim JY, Kim YM. Comparison of antibacterial activities of garlic juice and heat-treated garlic juice. Korean Journal of Food Science and Technology 2003; 35:540-543
3. Greig JD, Ravel A. Analysis of foodborne outbreak data reported internationally for source attribution. International Journal of Food Microbiology 2009; 130:77-87.
4. Tauxe RV. Emerging food born pathogens. International Journal of Food Microbiology 2002; 78:31-41.
5. Dillon VM, Board RG. Future prospects for natural antimicrobial food preservation systems. *In* Dillon VM. (eds.), Natural antimicrobial systems and food preservation. CAB Press, Wallingford, UK. 1994; pp. 297-305.
6. Kim. M, So S, Park H, Seo E, Kwon H, Song H. Ecology of *Bupleurum latissimum* Population. Journal of the Korea Society of Environmental Restoration Technology 2006; 9:78-85.
7. Yen MH, Lin CC, Chang CH, Lin SC. Antiinflammatory and hepatoprotective activity of saikosaponin-f and the root extract of *Bulpeurum kaoi*. Fitoterapia 1994; 65:409-417.
8. Moon KS. Content and utilization of medicinal plants. The Sicence-Encyclopedia Publishing Company. 1991; pp. 435-439.
9. Park SH, Seo E, Han K, Kim M, Park KR. Numerical taxonomic analyses of *Bupleurum latissimum* (Apiaceae). Korean Journal of Plant Taxonomy 2008; 38;31-42.
10. Aimi N, Fujimoto H, Shibata S. The chemical studies on oriental plant drugs. XVIII. The constituents of *Bupleurum* spp. (3). Saikogenins E and G. Chemical and Pharmaceutical Bulletin 1986; 16:641-646.

11. Takagi K, Shibata M. Pharmacological studies on *Bupleurum falcatum* : Ⅱ. Antiinflammatory and other pharmacological actions of crude saikosides. Yakugaku Zasshi 1969; 89:1367-1378.
12. Abe H, Sakaguchi M, Odashima S, Arichi S. Protective effect of saikosaponin-d isolated from *Bupleurum falcatum* L. on CCl_4-induced liver in the rat. Naunyn-Schmiedeberg's Arch. Pharmacology 1982; 320:266-271.
13. Abe H, Orita M, Konishi H, Arichi S, Odashima S. Effects of saikosaponin-d on aminonucleoside nephrosis in rats. European Journal of Pharmacology 1986; 120:171-178.
14. Mizoguchi Y, Tsutsui H, Yamamoto S, Morisawa S. Effects of saiko (Chai-Hu) on antibody response and mitogeninduced lymphocyte transformation *in vitro*. Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 1985; 2:330-336.
15. Yamada H, Ra KS, Kiyohara H, Cyong JC, Otsuka Y. Structural characterisation of anti-complementary pectic polysaccharide from the roots of *Bupleurum falcatum* L. Carbohydrate Research 1989; 15:209-226.
16. Matsumoto T, Cyong JC, Kiyohara H, Abe A, Hirano M, Danbara H, Yamada H. The pectic polysaccharide from *Bupleurum falcatum* L. enhance immune-complexes binding to peritomeal macrophages through Fc receptor expression. International Journal of Immunopharmacology 1993; 15:683-693.
17. Sun XB, Matsumoto T, Yamada. Effect of a polysaccharide fraction from the roots of *Bupleurum falcatum* L. on experimental gastric ulcer models in rats and mice. Journal of Pharmacy and Pharmacology 1991; 43:699-704.
18. Yamada H, Sun XB, Matsumoto T, Ra KS, Hirano M, Kiyohara H. Purification of anti-ulcer polysaccharides from the roots of *Bupleurum falcatum* L. Planta Medica 1991; 57:555-559.
19. Kim M, So S, Park H, Seo E, Kwon H, Song H. Ecology of *Bupleurum latissimum* Population. Journal of the Korea Society of Environmental Restoration Technology 2006; 9:78-85.
20. Bauer AW, Kibby MM, Sherries JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. Journal of Clinical Pathology 1966; 45:493-496.
21. Mothana RAA, Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology 2005; 96:177-181.
22. Boulekbache-Makhlouf L, Slimani S, Madani K. 2Total phenolic content, antioxidant and antibacterial activities of fruit of *Eucalyptus globulus* cultivated in Algeria. Industrial Crops and Products 2013; 41:85-89
23. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-born pathogens. Letters in Applied Microbiology 1998; 26:118-122.
24. Kwon YS, Kobayashi A, Kajiyama SI, Kawazu K, Kanzaki H, Kim CM. Antimicrobial constituents of *Angelica dahurica* roots. Phytochemistry 1997; 44:887-889.
25. Kaur GJ, Arora DS. In vitro antibacterial activity of three plants belonging to the family Umbelliferae. International Journal of Antimicrobial Agents 2008; 31:380-399.