Antimicrobial and Antioxidant Activity of *Saposhnikovia divaricata, Peucedanum japonicum,* and *Glehnia littoralis*

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Kim *et al.*: Antimicrobial and Antioxidant Activity of Umbelliferae Plants

***Saposhnikovia divaricata*, *Peucedanum japonicum*, and *Glehnia littoralis* of the family Umbelliferae, have long been used as traditional herbal medicine in Asian countries. This study is the first to compare and**

analyse the relationship between antimicrobial activity, antioxidant activity, and total polyphenol content of *Saposhnikovia divaricata*, *Peucedanum japonicum,* and *Glehnia littoralis*. The four fractions of hydro-methanol extract of these 3 plants were evaluated using the disc diffusion method to determine the minimum inhibitory concentration against 5 different bacterial strains. The ethyl acetate fraction was the most effective against the bacterial strains investigated. The 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging activity was evaluated to measure antioxidant activity. The antioxidant activity of the ether and ethyl acetate fractions was in the order of *Saposhnikovia divaricata*>*Peucedanum japonicum*>*Glehnia littoralis*. The total polyphenol content of *Saposhnikovia divaricata* was greater than that of *Peucedanum japonicum* and *Glehnia littoralis*. Even though the 3 plants belong to the same family and are used for similar medical purposes, their antimicrobial activity, antioxidant activity, and total polyphenol content was different. This study would help researchers to uncover the critical aspects of the activities possessed by plants.

Key words: *Glehnia littoralis*, *Peucedanum japonicum*, *Saposhnikovia divaricata*, antimicrobial activity, antioxidant activity, minimum inhibitory concentration, DPPH free radical scavenging activity

Saposhnikovia divaricata (also known as *Ledebouriella seseloides*, SD), *Peucedanum japonicum* (PJ), and *Glehnia littoralis* (GL) have long been used in traditional medicine under the name of *Bangpung* in Korea, which is also known as *Fang Feng* in traditional Chinese herbal medicine and *Bofu* in Japan. These plants belong to the Umbelliferae family and are assumed to have similar effects. These are commonly used as ingredients in many polyherbal preparations designed to dispel "wind" and to induce sweat, alleviate rheumatic conditions, and relieve spasms. They have also been used to treat cough and neurological diseases. The active constituents reported from these plants were essential oils, mannitol, bitter glycoside, chromones, coumarins, and polyacetylenes[1].

Investigations of the antimicrobial activity of medicinal plants has been an ongoing activity. Compounds isolated from plants that possessed antimicrobial activity include terpenoids, saponins, phenolics and phenylpropanoids. Many plants used as aromatic herbs and spices have been reported to have antimicrobial activity and antioxidant activity, while phenolic compounds are often used as aromatizants or antioxidants in the food industry[2-6]. It is well-known that free radicals induce oxidative stress, which might cause damage to lipids, proteins, and nucleic acids in the body and result in various diseases. The harmful effects of free radicals can be attenuated by antioxidants, and the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay is a common method to determine the antioxidant potential of plant extracts[7-9]. Antioxidant activity of phenolic compounds widely distributed in plants could be of value to human health. Total phenolic content and antioxidant activities are strongly correlated, and a relationship between phenolic compounds and antimicrobial activity has also been reported[10-13]. However, no studies have compared the antimicrobial activity, antioxidant activity and phenolic contents of SD, PJ and GL.

The main components of SD, PJ and GL reported were phenolics including coumarine derivatives (psoralen, bergapten, imperatorin), coumarin isomer, chromone and its derivatives (divaricatol, ledebouriellol, hamaudol) and flavonoids (rutin, ferulate). These phenolic compounds are known to have various biological effects including analgesic and antiinflammatory activity[14,15].

The present study was conducted to assess the differences in the antimicrobial activity, DPPH free radical scavenging activity, and total polyphenol content of the three aforementioned species of the Umbelliferae family. To accomplish this, the disc diffusion method was applied to measure the minimum inhibitory concentration (MIC) of partitioned fractions of the methanol extract against five different bacterial strains, including three Gram-positive and two Gram-negative bacteria. To measure the radical scavenging activity, we used a DPPH assay, while the total polyphenol content was determined using the modified Folin-Denis method[16]. L-Ascorbic acid, DPPH, Folin-Denis' reagent, tannic acid and sodium carbonate were

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purchased from Sigma-Aldrich. All solvents and other chemicals were purchased from Dae-Jung and Junsei Chemical.

SD root was purchased from a commercial market, while PJ and GL were collected from Jindo Island in the southern portion of Korea. The materials were authenticated by Prof. K. W. Yun and Voucher specimens were deposited in the Herbarium of Sunchon National University, Korea. The root part of the plants was used for all experiments. The collected samples were air-dried for 14 d, after which they were extracted with hydro-methanol.

The tested microorganisms included three Gram-positive bacteria (*Bacillus subtilis* ATCC 9327, *Lysteria monocytogene* ATCC 15313 and *Staphylococcus aureus* ATCC 13301) and two Gram-negative bacteria (*Escherichia coli* ATCC 15489, and *Salmonella typimurium* KCCM 11862). The Gram-positive and the Gram-negative bacteria were cultured on nutrient broth agar at 30° for 18-24 h.

The air-dried roots were pulverized using an electric mill, after which 200 g of the powdered root was macerated with 1000 ml of methanol/water (80: 20 v/v) for 24 h. The percolates were then filtered through Whatman No. 2 filter paper. Next, the crude hydro-methanol extract was subsequently fractionated with 500 ml of hexane, after which the top hexane layer (comprising the hexane fraction) was concentrated. The remaining layer was successively partitioned with 500 ml of diethyl ether, ethyl acetate and water in a separating funnel (forming the ether, ethyl acetate and water fractions). Each fraction was subsequently concentrated *in vacuo* to 30 ml at 30° and tested for antimicrobial, antioxidant activity and the total polyphenol content.

Each bacterial strain was grown in a nutrient broth at 30° for 18-24 h prior to testing, then subcultured three times for another 18-24 h. The turbidity of bacterial cell suspensions was brought to 0.3 optimal density at 660 nm by adding sterile broth and was then used for the tests. Next, 10 ml of the bacterial cell suspensions was poured uniformly onto nutrient agar plates and then paper disks (8.0 mm in diameter) containing the hexane, ether, ethyl acetate, or water fractions of the extracts were carefully placed on the bacteria-seeded Petri dishes. The diameters of the resulting zones of inhibition were measured in mm after the cultures were incubated for 24 or 48 h at 30°. The diameter of inhibition zone was measured[17,18]. The MIC was determined as the lowest concentration that caused an inhibition zone.

The DPPH free radical scavenging activity was evaluated using the Blois method (1958)[19], with slight modification. Briefly, 160 µl of each fraction sample was mixed with 40 µl of 1.5×10^{-4} M DPPH solution (in methanol) solution. The mixtures were gently mixed and allowed to stand at room temperature for 30 min, and the absorbance at 520 nm were measured using a microplate spectrophotometer reader (Molecular Devices). The antioxidant activity of each fraction was expressed in terms of IC_{50} values (the concentration required to inhibit DPPH radical formation by 50 %). L-Ascorbic acid was used as reference.

The total polyphenol content was determined using the Folin-Denis method, with slight modification[16]. The fractions were centrifuged at 1200 rpm for 10 min, and the supernatant collected. The clear supernatant of the samples (0.5 ml) were mixed with 8 ml of distilled water, after which 0.5 ml of Folin-Denis' reagent was added. After 3 min, 1 ml of sodium carbonate (10 % in distilled water) was added and the solution was allowed to stand for 2 h at 22° in darkness. The absorbance was then measured at 700 nm using an UV-Vis spectrophotometer (HP-8453, USA). A standard curve prepared from tannic acid (50-300 mg/l) was used for quantification and the total polyphenol content was expressed as mg tannin/g dry weight.

All tests and analyses were carried out in triplicate and each experiment was repeated three or four times. The data shown here represent the mean±standard deviation. The statistical significance was determined by analysis of variance (ANOVA) followed by Duncan's multiple range test.

The antimicrobial activity and MIC of fractions of methanol extracts from the three test plants root are shown in Table 1. The ether and ethyl acetate fractions of SD and PJ showed activity, while GL primarily showed activity in the ethyl acetate fraction. Overall, the ethyl acetate fraction showed a strong inhibitory effect against bacterial growth and Gram-negative bacteria were more sensitive than Gram-positive bacteria to the three test plants. The MIC values indicated that *E. coli* was the most sensitive investigated microorganism (MIC=0.1 mg/ml) to all three test plants. Additionally, SD and PJ were more effective against both Gram-positive and Gram-negative bacteria, while GL was only effective against Gram-negative bacteria. In the case of PJ, the ethyl acetate

fraction was effective against all five bacterial strains at a MIC of 0.1 mg/ml (Table 1).

Antioxidant activity was determined by DPPH assay and expressed as IC_{50} (μg/ml), which was the amount of sample needed to scavenge 50 % of initial concentration of the free radical. Results of the IC_{50} values revealed that ether and ethyl acetate fraction of methanol extract from the three plants had higher scavenging activity when compared with the hexane and water fraction (Table 2). Specially, the order of DPPH antioxidant activity of the ethyl acetate fraction was PJ (5.42 μg/ml)>GL (16.66 μg/ml)>SD (30.54 μg/ml; Table 2).

Phenolic compounds have been investigated in many medicinal plants. The total phenolic content of the three plants was shown in Table 3. It was observed that the amount of total phenolic content in ether and ethyl acetate fraction was high, and low in hexane and water fraction. It showed that in ethyl acetate fraction of PJ (57.42 mg/g) presented higher total phenolic followed by GL (31.66 mg/g) and SD (26.32 mg/g).

Plant derived bioactive substances especially with antioxidant and antimicrobial activities could be of potential use to pharmaceutical and food industry and to the consumers[4,20,21]. SD, PJ and GL have been used in a variety of herbal medicines under the assumption that they all have similar effects. However, the present study demonstrated that there are notable differences among the antimicrobial activities, DPPH free radical scavenging activities and total polyphenol contents of these plants. SD is known to have tumor cell growth inhibitory effects and antioxidant activities. Additionally, chromone extract of SD has antirheumatoid effects that occur via inhibition of nuclear factor-kappaB (NF-kB) and mitogen-activated protein kinases (MAPK)[9,22,23]. PJ has antiobesity effects that occur via phenolic compounds such as neochlorogenic acid, chlorogenic acid and rutin, as well as antiplatelet activity induced by khellactone and monoamine oxidase inhibitory effects exerted by coumarine[24-27]. GL has antioxidant effects, antiinflammatory effects that occur via coumarin derivative imperatorin, antibacterial

TABLE 1: MIC OF EACH FRACTION OF HYDRO-METHANOL EXTRACTS OF *S. DIVARICATA*, *P. JAPONICUM* AND *G. LITTORALIS* AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Species	Minimum Inhibitory Concentration (MIC, mg/ml)				
	Gram-positive			Gram-negative	
	B. subtilis	*S. aureus*	*L. monocytogene*	*S. typhimurium*	*E. coli*
S. divaricata					
Hexane	1.5	-	-	-	0.2
Ether	0.5	0.3	2.0	0.2	0.2
Ethyl acetate	0.2	0.2	0.2	0.1	0.1
Water	1.0	-	-	-	-
P. japonicum					
Hexane	-	-	-	-	0.3
Ether	2.0	0.5	1.5	0.5	0.3
Ethyl acetate	0.1	0.1	0.1	0.1	0.1
Water	-	-	-	-	-
G. littoralis					
Hexane	-	-	-	-	-
Ether	-	-	-	-	0.5
Ethyl acetate	2.0	1.5	-	0.2	0.1
Water	-	-	-	-	-

'-' not detected

TABLE 2: DPPH FREE RADICAL SCAVENGING ACTIVITY OF EACH FRACTION OF HYDRO-METHANOL EXTRACT OF *S. DIVARICATA*, *P. JAPONICUM* AND *G. LITTORALIS*

Species	IC_{50} (μg/ml)[a]				
	Hexane	Ether	Ethyl acetate	Water	Ascorbic acid
S. divaricata	145.53±12.17	5.30±0.05	30.54±1.33[a]	186.91±4.28[a]	3.04±0.05
P. japonicum	246.71±43.65[a]	46.03±5.85	5.42±0.05	104.97±5.90	
G. littoralis	44.64±3.22	71.43±16.00[a]	16.66±0.29	-	

[a]Means±standard deviation with the same letters within a column are not significantly different at p=0.05 according to Duncan's multiple range test. '-' not detected

TABLE 3: TOTAL POLYPHENOL CONTENT OF EACH FRACTION OF HYDRO-METHANOL EXTRACT OF *S. DIVARICATA*, *P. JAPONICUM* AND *G. LITTORALIS*

Species	Total polyphenol content (mg/g dw)[a,b]			
	Hexane	Ether	Ethyl acetate	Water
S. divaricata	12.53±2.17[a]	53.30±1.24[a]	26.32±0.91[b]	7.81±2.78[b]
P. japonicum	6.71±2.65[b]	21.71±2.16[b]	57.42±2.05[a]	12.97±1.41[a]
G. littoralis	17.64±1.22[a]	11.43±1.57	31.66±1.31[b]	2.42±0.81

[a]Means±standard deviation with the same letters within a column are not significantly different at p=0.05 according to Duncan's multiple range test. [b]dw : dried weight

and antifungal effects, and antiinflammatory activity owing to NF-kB and MAPK activity. GL has polyine compounds, which are not phenolic, but have been reported to have antibacterial and antifungal activities[28-32]. Other active constituents of GL include lignin and neolignan (polyphenolic substance) and biphenyl ferulate (flavonoid)[14,15]. The antimicrobial effects of the three plants could be of use against infectious disease. In the present study, the disc diffusion method indicated that ethyl acetate fraction is the most effective fraction against both Gram-positive and Gram-negative bacteria. Overall, Gram-negative bacteria were more sensitive to the three tested plants than Gram-positive bacteria, since the fractions of the three plants exhibited lower MIC values against Gram-negative bacteria. However, for Gram-positive bacteria, SD and PJ showed better effect than GL. Due to the differences in the activity and chemical properties, these plants should be used for specific purposes when employed as food additives and/or for medicinal purposes. DPPH free radical scavenging activity measures the degree of inhibition of DPPH free radicals. Regarding the antioxidant activity, SD in ether fraction and PJ in ethyl acetate fraction exhibited the highest antioxidant activity. The relation between antioxidant and total phenolic content for the three plant examined was shown (Table 2 and 3). All the three plants have antioxidant activity and their active components might be related to phenolic compounds, which have antioxidative and antimicrobial activity[13]. Lipopolysacchride (LPS) is a component of cell wall of Gram-negative bacteria and uses commonly for inflammation-associated researches. Anomalin, isolated from SD and a pyranocoumarin derivative, has antiinflammatory effect against LPS-induced macrophage activation. Also there is a report of antiinflammatory effect by GL on LPS-treated RAW 264.7 cells. Interestingly, there are no reports on the LPS or inflammation related research from SD[29,32].

The results of the present study suggest that there are differences in the antimicrobial activity, antioxidant activity, and total polyphenol content among the three species of plants in the Umbelliferae family, which should be considered prior to their use for specific biotechnological, nutraceutical or pharmaceutical applications. It should be noted that this is a preliminary study and further investigations are needed to determine the mechanism of action of the investigated plants.

Conflicts of interest:

The authors declare that there are no conflicts of interest.

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