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Antimicrobial Effect of *Portulaca oleracea* Extracts on Food-Borne Pathogens

Ji-Hyun Bae

Department of Food Science and Nutrition, Keimyung University, Daegu 704-701, Korea

Abstract

This study was performed to investigate the antimicrobial effects of *Portulaca oleracea* extracts against food-borne pathogens. First, the *Portulaca oleracea* was extracted with methanol at room temperature, and then further fractionated by using petroleum ether, chloroform, ethyl acetate and methanol, respectively. The antimicrobial activity of the *Portulaca oleracea* extracts was determined using a paper disc method against food-borne pathogens and food spoilage bacteria. The ethyl acetate extracts of *Portulaca oleracea* showed the highest antimicrobial activity against *Staphylococcus aureus* and *Shigella dysenteriae*. There was also a synergistic effect of the combined extracts of *Portulaca oleracea* and *Indigofera kirilowii* as compared to each extract alone. Finally, the growth inhibition curve of ethyl acetate extracts of *Portulaca oleracea* against *Staphylococcus aureus* and *Shigella dysenteriae* was determined. The ethyl acetate extract of *Portulaca oleracea* showed strong antimicrobial activity against *Staphylococcus aureus* at the concentration of 4,000 ppm. The 4,000 ppm of ethyl acetate extract from *Portulaca oleracea*, retarded the growth of *S. aureus* by more than 24 h and *Shigella dysenteriae* up to 12 h at 37°C.

Key words: *Portulaca oleracea*, antimicrobial activity, food-borne pathogens

INTRODUCTION

Recent improvements in the availability of different and varied foods have increased the demand for safe foods, and the developments of processed foodstuffs have resulted in food additives being widely used. In particular, synthetic products are commercially used as preservatives for inhibiting microbe growth. However, with the increased concern of consumers on the safety of food additives, natural additives are replacing chemical compounds as food additives, including preservatives. This tendency requires the development of substances that are harmless to humans and have wide-ranged antimicrobial activity, necessitating studies on the antimicrobial activities of natural substances for use as food preservatives. It is widely believed that natural substances have a large amount of antimicrobial ingredients, and both foreign and domestic researchers have studied them (1-3). In Korea, many papers have reported antimicrobial activity of weed extracts (4), of medicinal & esculent plants (5-8), of herbs (9), and of the protective effects of herbs against food poisoning bacteria (10).

Purslanes (*Portulaca oleracea*) is an annual plant and is also called machihyun, that grows wild by the roadside and in vegetable gardens. In Korea, the plant has been eaten with spices and been used as an herb. In folk medicine, purslane was as an antidote for pest-poisoning and poisonous snake bites (11). It is known that L-noradrenaline, dopamine, L-dopa, potassium, and several kinds of organic acids, glutamic acid, aspartic acid, and alanine are contained in purslanes (12), and a large quantity of ω-3 fatty acids, similar to those found abundantly in fish oil, are also reported to be found in its leaves, stems, and roots (13). Purslane is eaten after being collected in summer and fall, when the leaves and stems are thick, after being dipped into boiling water for an instant, and is reported to be effective in treating various kinds of inflammations because of its bacteriocidal action against the *Shigella* species (14). In this study, the authors investigated the effects of purslane extract, which have been used in Korea for a long time and are known to be nontoxic to humans, on the growth of various food-borne pathogens and spoilage microorganisms.

MATERIALS AND METHODS

Materials

The purslanes used in this study were collected in Korea. The authors purchased dried purslanes in an herbal medicine pharmacy (Yakjeon-golmok, Daegu, South Korea) in September 2003. The dried purslanes were mildly washed with water twice in order to remove impurities,

E-mail: jhb@kmu.ac.kr
Phone: +82-53-580-5875, Fax: +82-53-580-5885

and then were finely ground as samples.

Strains and culture medium

The strains used for measuring the antimicrobial activity of purslane extract included two Gram (+) bacteria and seven of Gram (-) bacteria provided by the Korea Research Institute of Bioscience and Biotechnology of the Korea Institute of Science and Technology (Table 1). Tryptic soy broth and tryptic soy agar (Difco, USA) were used for all the strains as a viable culture media, and the strains were cultured at 37°C for 18~24 h.

Extraction of antimicrobial substances

Five hundred grams of the dried purslane were extracted with 1 L of methanol at room temperature for 6 h, and the methanol extracts filtered through Whatman No. 2 filter paper (Whatman International Ltd., England) in order to remove impurities. The filtered solution was vacuum evaporated with an EYELA (N-N. Series, Japan) at 45°C. The concentrated methanol extract was then further fractionized with petroleum ether, chloroform, ethyl acetate, and methanol, respectively. The methanol extract and various organic solvents were poured into separatory funnels, shaken by hand for 5 minutes, and left at room temperature for 15 minutes for separation. The hot-water extract of purslane was extracted by organic solvent, and the leftover was added to distilled water and boiled at 100°C for 30 minutes, and then filtered by the above-mentioned method. The filtered solution was vacuum evaporated by using an EYELA (N-N. Series, Japan) at 45°C, and was diluted at proper concentrations for the experiment.

Measurement of antimicrobial activity of purslane extract

In order to detect antimicrobial activity, a paper disc (3 mm diameter) was used in this experiment (15). The strains cultured in tryptic soy broth (TSB), adjusted to an absorbance at 620 nm using a spectrophotometer (Nontron instruments, Italy) with a 0.4 OD value, were mixed uniformly in the cultured plates in which Tryptic Soy Agar (TSA) was poured, and were hardened at room temperature. On the media, sterilized paper discs were placed in proportion to the number of samples and were fastened to the media. Then, the purslane extracts from petroleum ether, chloroform, ethyl acetate, methanol, and hot-water were diluted to be 100 ppm, 250 ppm, 500 ppm, and 1,000 ppm, respectively and 20 μL of each were gradually absorbed into the plates. As the control sample, 70% ethanol without purslane extract was spotted by the above-mentioned method. All the prepared plates were cultured at 37°C for 24 h, and the sizes of the clear zone (mm) formed around the discs were measured in order to determine the degree of the antimicrobial activity of each fraction.

Table 1. List of microorganisms used for antimicrobial activity test

	Strains
Gram positive bacteria	*Staphylococcus aureus* ATCC 25923 *Bacillus cereus* ATCC 27348
Gram negative bacteria	*Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853 *Salmonella typhimurium* ATCC 14028 *Salmonella enteritidis* ATCC 13076 *Shigella sonnei* ATCC 25931 *Shigella dysenteriae* ATCC 9199 *Shigella flexneri* ATCC 12022

Measurement of the synergistic effect in antimicrobial activity

In order to identify whether the antimicrobial activity of purslane extract increased when mixed with other antimicrobial plant extracts, the authors mixed the purslane extract with the extract of *Indigofera kirilowii.* The ethyl acetate extract of purslane (250 ppm), which exhibited the highest antimicrobial activity in the preliminary experiment, and the ethyl acetate extract of *Indigofera kirilowii* (250 ppm) were mixed together and were compared with the antimicrobial activities of the ethyl acetate extract of purslane (500 ppm) and the ethyl acetate extract of *Indigofera kirilowii* (500 ppm) alone. *Staphylococcus aureus* and *Shigella dysenteriae* were used as the experimental bacteria. As a control, 20 μL of 70% ethanol was also used as the equal amount to that of each sample.

Growth inhibition by ethyl acetate extract

The ethyl acetate extract of purslane was sterilized by membrane filtration (0.2 μm, pore size. Toyoroshi kaisha, Ltd., Japan) and each extract was added to broth at the concentrations of 1,000, 2,000 and 4,000 ppm. Then, the culture solution with a 0.4 O.D. value was inoculated into the broths under sterile conditions to make an ultimate concentration of 10^9 after dilution at 37°C for 72 h. The optical density of the test organism was measured at 620 nm at 12 h intervals (16).

RESULTS AND DISCUSSION

Yields of the various organic solvents and hot water extract of purslane

The yields of the purslane fractions of petroleum ether, chloroform, ethyl acetate, methanol, and hot-water extracts of purslane were 0.4%, 0.9%, 0.7 %, 3.4%, and 4.2%, respectively (Table 2). Therefore, the yield of petroleum

Table 2. Yield of organic solvents and water extracts of *Portulaca oleracea*

Fraction	Dried weight (g)	Yield (%)
Petroleum ether	2.04	0.4
Chloroform	4.52	0.9
Ethyl acetate	3.52	0.7
Methanol	17.1	3.4
Hot water	21.1	4.2

ether was lowest while that of the hot-water extract was highest.

Antimicrobial activity of the organic solvents and hot-water extract of purslane

In this study, the antimicrobial activities of the organic solvents and hot-water extracts of purslane were investigated by applying them to food-borne pathogens and food putrefaction bacteria by using paper discs. The antimicrobial activities of petroleum ether, chloroform, ethyl acetate, methanol, and hot-water extracts of purslane for Gram (+) strains are shown in Table 3, and the activity increased when the concentration of various extracts of purslane spotted into the discs increased. In other words, the size of inhibition zone showing antimicrobial activity increased when concentration increased, and the ethyl acetate extract showed the highest activity because the activity of the extract at 1,000 ppm in concentration was 30 mm for *S. aureus*. The petroleum ether extract of purslane showed no antimicrobial activity to the strains used in this study, while the chloroform extract of purslane showed antimicrobial activity only to *Shigella dysenteriae* at 1,000 ppm in concentration. The ethyl acetate extract of purslane showed antimicrobial activity to all the strains used in this study and exhibited activity even at a 250 ppm concentration. The ethyl acetate fraction of the ethanol extracts from mustard leaf was reported to show the highest antimicrobial activity to *Staphylococcus aureus* (17); in this study, the ethyl acetate extract of purslane also showed the strongest antimicrobial activity to *S. aureus*. The antimicrobial activities against Gram (-) strains by various purslane extracts in this study are shown in Table 4. The methanol and hot-water extracts of purslane showed the most effective antimicrobial activities to *Shigella dysenteriae*, while the ethyl acetate extract of purslane showed strong antimicrobial activities to all the strains. In particular, *Shigella dysenteriae* was most susceptible to the ethyl acetate extract, and the clear zone was 30 mm at 1,000 ppm (Fig. 1). As mentioned above, the ethyl acetate extract of purslane had wide-ranged antimicrobial activity to Gram (+) and (-) strains. Kim (18) reported that the methanol extract of Chinese pepper was more susceptible to *E. coli*, a Gram (-) bacteria, than to Gram (+) strains. In this study, the antimicrobial activity of purslane extract could not be tested when its concentration was less than 100 ppm. The petroleum ether and chloroform extracts failed to show clear antimicrobial activities against any of the strains, and the chloroform extract only exhibited antimicrobial activity when the concentration was over 1,000 ppm. It is reported that saponins, organic acids, tannin, sugar, glucosides, and several alkaloids are eluted from the ethyl acetate extracts of plants, and it is considered that the ethyl acetate extract of purslane, showing the highest antimicrobial activity in this study, contained such substances. Hong et al. (19) reported that the butanol extract of the barks of elm tree inhibited the growth of *S. aureus*, *S. faecalis* and *Bacillus* sp. which are all Gram (+) strains while showing no inhibitory effects on the growth of *E. coli*, a Gram (-) strain, or against *Candida albicans*, Eumycetes. However, purslane in this study showed no difference in activity between Gram (+) and Gram (-) bacteria.

Synergistic effects of *Indigofera kirilowii* and purslane extracts

The antimicrobial activities of the combined ethyl acetate extracts of *Indigofera kirilowii* and purslane are shown in Table 5. The antimicrobial activity of the two

Table 3. Antimicrobial activities of each solvent fraction of *Portulaca oleracea* against Gram positive bacteria

Strains	Clear zone on plate (mM)[1]					
	Fraction conc. (ppm)	PE	C	EA	M	W
Staphylococcus aureus	100	-[2]	-	-	-	-
	250	-	5	23	9	4
	500	5	7	27	10	5
	1,000	6	9	30	11	6
Bacillus cereus	100	-	-	-	-	-
	250	-	8	18	6	2
	500	7	9	19	9	3
	1,000	8	10	20	10	4

[1]Diameter. [2]No inhibitory zone was formed.
PE: Petroleum ether extract, C: Chloroform extract, EA: Ethyl acetate extract, M: Methanol extract, W: Water extract.

Table 4. Antimicrobial activities of each solvent fraction of *Portulaca oleracea* against Gram negative bacteria

Strains	Clear zone on plate (mM)[1]					
	Fraction conc. (ppm)	PE	C	EA	M	W
Escherichia coli	100	-[2]	-	-	-	-
	250	-	-	12	-	-
	500	-	6	13	5	-
	1,000	-	7	15	7	4
Pseudomonas aeruginosa	100	-	-	-	-	-
	250	-	-	12	-	-
	500	-	9	15	3	4
	1000	-	10	17	5	5
Salmonella typhimurium	100	-	-	-	-	-
	250	-	-	9	-	-
	500	-	7	11	3	2
	1,000	-	8	16	4	3
Salmonella enteritidis	100	-	-	-	-	-
	250	-	-	9	-	-
	500	7	6	14	3	3
	1,000	9	8	19	5	4
Shigella sonnei	100	-	-	-	-	-
	250	-	-	11	6	6
	500	5	7	15	8	7
	1,000	6	8	25	9	9
Shigella dysenteriae	100	-	-	-	-	-
	250	-	5	15	9	7
	500	9	7	19	12	9
	1,000	13	9	30	14	13
Shigella flexneri	100	-	-	-	-	-
	250	-	5	14	5	3
	500	4	8	17	7	4
	1,000	7	9	22	8	6

[1]Diameter. [2]No inhibitory zone was formed.
PE: Petroleum ether extract, C: Chloroform extract, EA: Ethyl acetate extract, M: Methanol extract, W: Water extract.

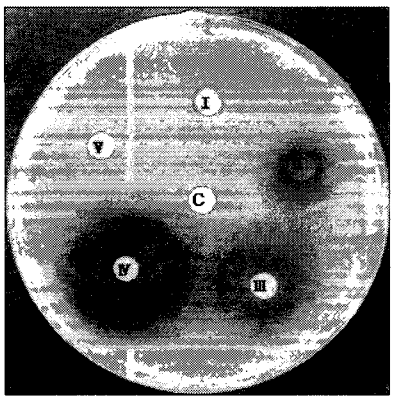

Fig. 1. Antimicrobial activities of various extracts of *Portulaca oleracea* against *Shigella dysenteriae* at the concentration of 1,000 ppm.
C: Control (70% ethanol), I: Petroleum ether, II: Aqueous extract, III: Methanol, IV: Ethyl acetate, V: Chloroform.

combined plants was more effective against *Shigella dysenteriae* than either of the extracts applied separately: the antimicrobial activity was strongest when the ethyl acetate extracts of the two plants were mixed (250 ppm and 250 ppm, respectively) (21 mm) than when the ethyl acetate extract of purslane (500 ppm) was solely applied (17 mm) (Fig. 2).

Effect of the ethyl acetate extract of purslane on the growth of Gram (+) and Gram (-) bacteria

The ethyl acetate extract of purslane was added to TSB medium at the concentrations of 0, 1,000, 2,000 and 4,000 ppm, and was inoculated into *Staphylococcus aureus* as the Gram (+) and *Shigella dysenteriae* as the Gram (-), and were cultured for 72 h. The results of measuring the growth of the strains at different time intervals are shown in Fig. 3 & 4. The *Staphylococcus aureus* grew rapidly from the 12th hour of the culture in control medium without the ethyl acetate extract of purslane, while the growth of the strain was inhibited for 24 h in the medium where at least 1,000 ppm of the extract was applied (Fig. 3). Chung (20) reported that the ethanol extract of *Opuntia ficus indica* inhibited bacterial growth at concentrations of at least 3.0 mg/mL, while Park et al. (21) reported that the water-soluble ex-

Table 5. Antimicrobial activity of combinded extracts of *Indigofera kirilowii* and *Portulaca oleracea*

Strains	Clear zone on plate (mM)[1]			
	Control	*Indigofera kirilowii* (500 ppm)	*Portulaca oleracea* (500 ppm)	Both[2] (each 250 ppm)
Staphylococcus aureus	-[3]	13	26	29
Shigella dysenteriae	-	15	17	21

[1]Diameter.
[2]*Indigofera kirilowii* and *Portulaca oleracea*.
[3]No inhibitory zone was formed.

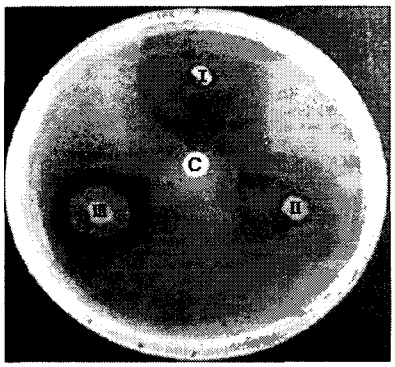

Fig. 2. Antimicrobial activities of various extracts of Agrimonia *Portulaca oleracea* and methanol extract of *Indigofera kirilowii* against *Shigella dysenteriae* at the concentration of 250 ppm.
I: *Portulaca oleracea* (250 ppm) and *Indigofera kirilowii* (250 ppm), II: *Portulaca oleracea* (500 ppm), III: *Indigofera kirilowii* (500 ppm).

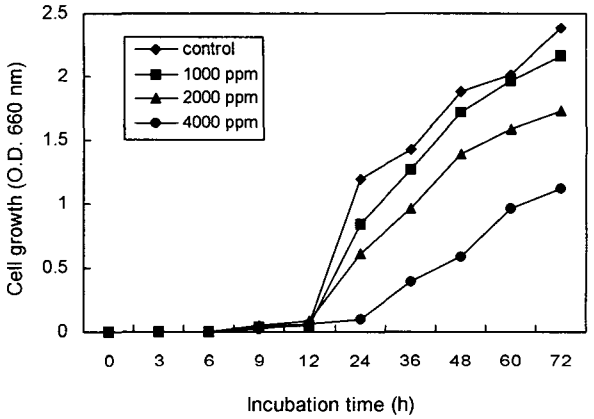

Fig. 3. Effect of ethyl acetate extracts of *Portulaca oleracea* on the growth of *Staphylococcus aureus*.

tract of mustard leaf began to inhibit microbial growth at concentrations between 1,000 and 1,200 ppm. Jeon et al. (22) reported that the methanol extract of Whiteman's Foot inhibited the growth of *Staphylococcus aureus*, and in this study also, the ethyl acetate extract of purslane inhibited the growth of the strain. Shin et al. (23) reported that the ethanol extract of *Perillae folium* inhibited the growth of *S. typhimurium* for 36 h, while Chung and Jung (24) reported that the extract of *Ganoderma lucidum* showed specific antimicrobial activity to *S.*

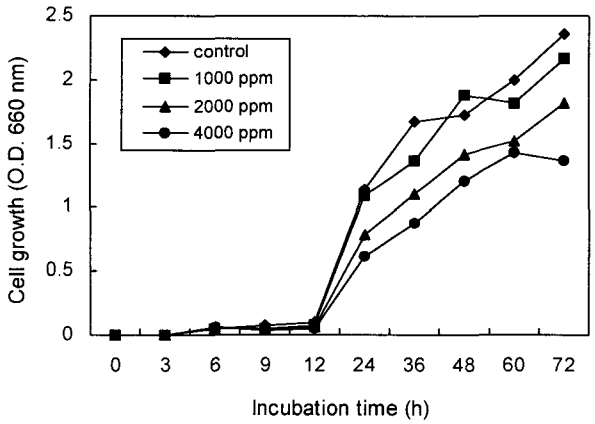

Fig. 4. Effect of ethyl acetate extracts of *Portulaca oleracea* on the growth of *Shigella dysenteriae*.

typhimurium. Therefore, it can be concluded that the growth of food-borne pathogens can be inhibited effectively when various antimicrobial substances extracted from natural sources are combined. Fig. 4 shows the results of the growth inhibition of *Shigella dysenteriae* induced by the ethyl acetate extract of purslane for 72 h. The strain grew rapidly from the 12th hour of culture in the control sample without the extract, while growing slowly in the medium when the extract at 4,000 ppm in concentration. Therefore, the ethyl acetate extract of purslane effectively inhibits growth of *Staphylococcus aureus* and *Shigella dysenteriae*.

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