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A Study of Antioxidant and Antibacterial Activates of the Extraction of *Perscaria hydropiper* L.

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ABSTRACT: This purpose of this study is to examine the antioxidant and antimicrobial activities of *Persicaria hydropiper* L. extract in 70% ethanol and in water, a medicinal herb, as an effort to examine the potential of medicinal herbs for development of antioxidants and natural preservative substitutes. The total polyphenol content in the 70% ethanol extract and in the water extract were 19.88 mg/g and 7.46 mg/g, respectively. The DPPH radical scavenging activity was 90.99% and 64.98% in the 70% ethanol extract and water extract, in which 70% ethanol extract showed a higher activity. The antioxidant effect of *Persicaria hydropiper* L. extract appears to be very good and due to its excellent growth inhibitory effect on food-poisoning-causing microorganisms in the food, it is thought to be utilized as a potential natural preservative substitute in many areas.

Keywords: *Persicaria hydropiper* L., antimicrobial activity, antioxidant, DPPH radical scavenging activity, natural preservative, spoilage microorganisms

INTRODUCTION

Persicaria hydropiper L. is a dicotyledon belonging to the joint weed family with a hairless erect stem growing to a height to 30～80 cm and branching. Its leaves are similar to those of willow with the length of 5～10 cm or so. Red and white flowers come out at the tip of the branch in a nodding spike-shape during July～October. *Persicaria hydropiper* L. is listed as 'a raw material that can be used in food' in the Korean Food Code, of which leaves are used as a spice in Japan and as a yellow dye in Europe, and of which fruits are used as a pepper substitute in Europe[1,2].

The main components of the *Persicaria hydropiper* L. leaf extract are flavonoids, which have been shown to have the antioxidant activity, hemostatic effect, inhibitory effect for lipid peroxidation and antimicrobial effect[3,4]. Flavonoids are widely distributed in higher plants and flavone from which the name is based on was derived from a greek word, flavus (yellow) and they belong to polyphenol compounds with the skeletal configuration of C6-C3-C6. The structure of C3-unit determines the difference in the degree of oxidation, whereby they are classified into flavon, flavonal, flavane, flavanone, chalcone, auron and anthocyanine. These flavonoids loaded with yellow pigments are known to have superior anti-viral, anti-inflammatory, anti-cancer and antioxidant effects[5,6] and it was also reported that the phenolic substances greatly affect the antimicrobial activity[7].

Polyphenol compounds are secondary metabolic compounds widely distributed in the plant kingdom,

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which possess bioactive functions such as anti-cancer, anti-hypertensive, anti-diabetic, anti-inflammatory, antioxidant and antimicrobial effects. They mainly exist in the cell membranes and vacuoles as an ester-type, free-type or coupled-type with a number of phenolic hydroxyl groups of various molecular weights in diverse structures, which enables them to easily bind to various compounds[8].

In particular, food poisoning caused by food-poisoning-causing microorganisms and food spoilage bacteria which are most sensitive to temperature occurs to millions of people each year around the world, which leads to death in many people[9,10]. Therefore, given that food is the source of essential nutrients for survival of human and provides bioactive compounds necessary for health promotion and disease prevention, synthetic preservatives have been developed as food additives to utilize them as a means for inhibiting the growth of food-poisoning-causing microorganisms and food spoilage bacteria to enhance the shelf life and safety of the processed food, and the amount of their usage is continuously on the rise[11]. However, synthetic preservatives are chemical synthetics, which are, in a strict sense, foreign substances, not the original food ingredients[12] and the consumers' perception of synthetic preservatives is acting very negatively on the synthetic preservatives in that they are known to be accumulated in the body over a long period of time even in a small amount[13,14].

The Codex Alimentarius Commission[15] provides the methods and guidelines for assessing the amount of dietary intake of food additives, which were recommended to each country, and also in Korea, "Guidelines for assessment of daily intake of food additives in Koreans," were presented by the Korea Food and Drug Administration in 2004[16]. Thus, as an effort to overcome the safety issues of synthetic preservatives, studies on the utilization of natural substances with antimicrobial properties have been attempted for a long time in both domestic and international levels[17,18]. Domestic research on the bioactive extracts isolated from medicinal herbs and algae[19] or on screening of their antimicrobial activity by applying them on food is actively ongoing [20,21], however, currently there are few studies on the direct application of *Persicaria hydropiper* L. extract on food microorganisms.

Therefore, research on *Persicaria hydropiper* L. which was reported to have a potential to play a role as a natural preservative and antimicrobial agent even in a low concentration[22] is urgently required to demonstrate the effectiveness of its bioactive substances scientifically that can be applied to various food in the future. In this study, the total phenolic content, DPPH scavenging activity and ABTS radical scavenging activity of *Persicaria hydropiper* L. were measured representing its antioxidant activity and to examine its antimicrobial activity, *B. subtilis*, *S. aureus*, *E. coli*, *S. enterica*, etc., which are food spoilage bacteria, were applied to explore its potentials as a natural antioxidant and natural food preservative.

METHODS

Measurement

1) Extraction of the Materials and Samples

The dried *Persicaria hydropiper* L. used in this experiment was purchased from the market in June 2015, finely ground, filtered through a 50 mesh sieve and stored refrigerated in a polyethylene bag for future use. For extraction of bioactive substances from water pepper, 70% ethanol and water were used as solvents, and for the primary extraction, 10 times the mass of the solvent per 50 g ground sample were added and mixed, which was subject for stirring-mediated leaching for 24 hours at room temperature, and using the same solvents, the secondary and tertiary extractions were made in the same way, and then, the entire extracted solution was filtered with a filter paper (Whatman No.2). The filtrate was concentrated under reduced pressure in rotation in the 60℃ water bath using a Rotary Vacumm Evaporator (HS-2005-N, Hahn Shin Scientific co., Kyunggi, Korea), and the obtained viscous extract was freeze-drier (Ilshin Lab co., Korea) and stored in the –70℃ deep freezer (WUF-500, Daihan Scientific, Gangwon, Korea) to be used later in the experiment. All experiments were repeated three times to calculate the average value.

2) Strains

Strains used in the experiment were *Bacillus cereus* (KCTC 3624), a strain of disseminating bacteria, which are widely distributed in nature and spoil food, *Staphylococcus aureus* (KCTC 3881), a Gram-positive bacterial strain, which produce an entrotoxin that causes food poisoning, *Escherichia coli* (KACC 13821), a Gram-negative bacterial strain, which is an indicator strain for contamination and a food spoilage bacterial strain, and *Salmonella enterica* (KCTC 12401), the most common and typical Gram-negative bacteria among the infective strains, which inhabit in the intestines of mammals and birds and they were divided into two Gram-positive strains and two Gram-negative strains, as shown in Table 1. All strains used in the experiment were distributed from the Korea Research Institute of Bioscience and Biotechnology Biological Resource Center (KCTC) and the Korea Agriculture Microbial Resource Center (KACC).

3) Measurement of anti-oxidant activity

(1) The total content of polyphenol compounds

The total polyphenol content depending on the amount of the *Persicaria hydropiper* L. concentrate added was measured by AOAC method. 1 mL *Persicaria hydropiper* L. extract was taken, to which 1 mL 2% (w/v) Na_2CO_3 solution was added, which was allowed to sit for 3 minutes, and then, 0.2 mL 50% Folin-Ciocalteu reagent was added for the reaction to take place and the absorbance was measured at 760 nm. The total polyphenol content was presented after converting it to tannic acid according to the standard curve created using tannic acid.

(2) Radical scavenging activity determined by DPPH method

Table 1. List of microbial strains used for antimicrobial test

Strain		ATCC NO.
Gram positive bacteria	*Bacullus cereus*	KCTC 3634
	Staphylococus aureus	KCTC 3881
Gram negative bacteria	*Escherichia coli*	KACC 13821
	Salmonella enterica	KCTC 12401

The electron-donating capacity depending on the amount of the *Persicaria hydropiper* L. concentrate added was examined by a following method modified from Blois method, in which DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich, St Louis, MO, USA) radical scavenging activity was measured. 15×10^{-4} M DPPH solution was added to 0.1 mL *Persicaria hydropiper* L. concentrate, which was allowed to sit for 30 minutes in the dark room at room temperature, and then the absorbance was measured at 517 nm. BHA (0.1 mg/mL) was used as the control sample.

(3) Radical scavenging activity determined by ABTS method

ABTS (2,2'-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid, Sigma-Aldrich, St. Louis, MO, USA) radical scavenging activity of the *Persicaria hydropiper* L. sample was measured by the method used by Pellegrin. Cations of ABTS were produced by incubating 7.4 mM ABTS and 2.6 mM potassium persulphate in the dark room for a day, and the resulting solution was diluted with 1 × PBS to make the absorbance value at 732 nm to 0.7±0.03 (Mean±SD). 50 μL sample (500 μg/mL) was added to 950 μL diluted ABTS solution, and then the absorbance was measured.

4) Measurement of antimicrobial activity

(1) Bacterial culture

Strains were used after three subcultures. The subcultured bacteria were inoculated into the nutrient broth for culturing for 24 hours in a 30 ℃ incubator, which was inoculated again into the aforesaid medium and the absorbance at 535 mm was adjusted to about 0.5 before use.

(2) Measurement of antimicrobial activity by paper disc method

Search for the antimicrobial activity from the *Persicaria hydropiper* L. extract (Water and 70% ethanol) was performed by a method adapted from the paper disc method. Each strain cultured in the slant culture medium 20 hours ahead was taken by a scoop of platinum loop and inoculated into a 10 mL test tube containing TSB (Tryptic soy broth, Difco), which was cultured for 24 hours at 30℃. 15 mL of

each nutrient agar and TSB agar were split into 90 mm petri dishes, hardened after sterilization and dried overnight inside a clean bench (Na-Nu Hi-Tech Co., Korea), on which 0.1 mL of each activated strain indicated was plated with a bent glass rod. The *Persicaria hydropiper* L. extract dissolved in ethanol was absorbed in the sterile paper discs (8 mm Advantec, Toyo Roshi Co., Japan) in concentrations of 1,000, 2,000 and 4,000 μg/disc and after evaporation of the solvent in the clean bench, the paper discs were placed on the surface of the plates where the strains were spread, which were incubated at a 4℃ cold room for 1 hour and then, the plates were incubated for 24 hours in a 37℃ incubator. The antimicrobial activity was measured by the diameter (mm) of the growth inhibitor zone and the experiment was triplicated.

(3) Measurement of the growth inhibitory effect in liquid culture

Using the *Persicaria hydropiper* L. extract in 70% ethanol, the growth inhibitory effect was measured in liquid culture. 0.1 mL of each activated culture solution was inoculated into the liquid culture media which were adjusted to contain the extract samples (w/v) in the concentrations of 0 ppm, 100 ppm, 200 ppm, 400 ppm and 1,000 ppm, respectively, followed by culturing at 30℃ for a day, during which the degree of bacterial growth was measured every 2 hours.

For the degree of bacterial growth, the absorbance at 600 nm was measured with Digital VIS/ Ultraviolet Spectrophotometer (CE 292, series 2, Cecil instruments, Cambridge England) and the degree of bacterial growth was represented by the changes in the absorbance over the time course.

Data Analysis

The All data in this study were shown as Mean± SD using the values measured three times repeatedly and validation of significance was analyzed by Duncan's multiple range test after performing student's *t*-test and ANOVA. SPSS (Statistical Package for Social Sciences, SPSS, Inc., Chicago, IL, USA, ver 20.0) was used and validation for the difference in the significance between samples was carried out in the level of $p<0.05$.

RESULTS

The Effect of Antioxidant Activity

1) The total polyphenol content

The total polyphenol content in *Persicaria hydropiper* L. was analyzed and the results are shown in Table 2.

In this study, the total polyphenol content in *Persicaria hydropiper* L. was measured and it was found that 7.46±0.01 mg/g was present in the water extract and 19.88±0.08 mg/g was present in the 70% ethanol extract, suggesting that the total polyphenol content varies depending on the extraction conditions, and higher content was measured from the 70% ethanol extract. The total polyphenol content in *Persicaria hydropiper* L. was measured higher than 17.93 mg/g in Phellinus, 10.98 mg/g in green tea, 10.31 mg/g in clove, 8.86 mg/g in cinnamon and 8.55 mg/g in Japanese alder (*jeokyang*) according to the study of Moon JS et al[7]. According to the findings of Kim DI et al[23] that the difference in the antioxidant activity is determined by polyphenol content, it is thought that *Persicaria hydropiper* L. can play an important role in the absorption and neutralization of free radicals and degradation of hydrogen peroxide as an antioxidant.

2) DPPH radical scavenging activity and ABTS radical scavenging activity

The results of the DPPH radical scavenging activity and ABTS radical scavenging activity of *Persicaria hydropiper* L. by solvent are as shown in Table 3. The DPPH radical scavenging activity of *Persicaria hydropiper* L. was 90.99±0.03% in 70% ethanol and 64.98± 1.81% in water, which was proportional to the total

Table 2. Total polyphenol contents according to the different solvent extracts from *Persicaria hydropiper* L.

(mg/g)

	Water extract	70% Ethanol extract
Persicaria hydropiper L.	7.46±0.01[2)***]	19.88±0.08

[1)] Mean±SD (n=3).
[2)] Student`s *T*-test, *** $p<0.001$.

Table 3. DPPH radical scavenging activities and ABTS radical scavenging activities according to the different solvent extracts from *Persicaria hydropiper* L.

(%)

Variables	DPPH Radical scavenging activities	ABTS Radical scavenging activities
Ascorbic acid[1)]	94.38±0.48[2)a3)]	99.14±0.50[a]
Water extract	64.98±1.81[b]	8.06±0.55[c]
70% Ethanol extract	90.99±0.03[ab]	27.42±0.60[b]

1) Positive control.
2) Mean±SD (n=3).
3) Means with different letters in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

polyphenol content.

In addition, DPPH radical scavenging activity is an antioxidant effect derived from the phenolic compounds and it was reported that the greater is the reducing power of such material, the greater is the DPPH radical scavenging activity[24]. In a study of Kim DI et al[23], the antioxidant effect was examined through the DPPH radical scavenging activity, and the results demonstrated that high radical removing effects of 89.6% and 94.6% were measured for ginkgo stems and Cornus fruits, respectively, which can be compared with the antioxidant power of water pepper. Ha GJ et al[25] reported that the DPPH radical scavenging activity and ABTS radical scavenging activity of *Artemisia argyi* H. were similar depending on the concentration of the extract.

However, the result of this study showed that the ABTS scavenging activity of *Persicaria hydropiper* L. by solvent was as low as 27.42±0.60% in 70% ethanol and 8.06±0.55% in water, suggesting a big difference from the DPPH radical scavenging activity. This result was consistent with the result from the study of Jeon JM et al[26] reporting that the part showing the highest DPPH radical scavenging activity was different from the part showing the highest ABTS cation scavenging activity, and such differences in the scavenging activities are derived from the fact that they are different kinds of radicals in spite of being radicals in common. That is, the scavenging activities of DPPH and ABTS are different in that the former removes free radicals and the latter removes cations, and their differences are resulted from the difference in the strength of bonding between those two substrates and their corresponding reactants[27].

Antimicrobial Activity

1) The antimicrobial effect measured by paper disc diffusion

Dried *Persicaria hydropiper* L. was finely ground and the concentrate extracted in 70% ethanol and water at room temperature was diluted and added to make the concentrations of the extract to 1,000, 2,000 and 4,000 μg/disc and its antimicrobial effect on pathogenic microorganisms was examined and the results are as shown in Table 4 and Table 5, respectively.

In *B. cereus*, *S. aureus* and *E. coli*, the higher was the concentration of the 70% ethanol *Persicaria hy-*

Table 4. Antimicrobial activity of different concentration from 70% ethanol extract of *Persicaria hydropiper* L.

(mm)

Conc. (μg/disc)	Clear zone on plate			
	B. cereus	*S. aureus*	*E. coli*	*S. enterica*
1,000	13.0	11.0	10.5	-[1)]
2,000	20.0	14.0	15.0	11.0
4,000	23.0	22.0	22.0	16.0

1) - : No activity.

Table 5. Antimicrobial activity of different concentration from water extract of *Persicaria hydropiper* L

(mm)

Conc. (μg/disc)	Clear zone on plate			
	B. cereus	*S. aureus*	*E. coli*	*S. enterica*
1,000	-[1)]	10.0	-	-
2,000	-	13.0	-	10.0
4,000	-	16.0	21.0	16.0

1) - : No activity·

dropiper L. extract absorbed in the disc, the bigger was the size of the inhibition zone, which represents antimicrobial activity, and the respective antimicrobial activities were 13.0, 11.0 and 10.5 mm at the concentration of 1,000 μg/disc, but in *S. enterica*, it was 11.0 mm at the concentration of 2,000 μg/disc.

In *B. cereus*, *S. aureus*, *E. coli* and *S. enterica*, the size of the inhibition zone was 23.0, 22.0, 22.0 and 16.0 mm, respectively at the concentration of 4,000 μg/disc in particular, suggesting a high antimicrobial activity. However, the water extract didn't show any antimicrobial activity against *B. cereus*, and in *S. aureus*, the antimicrobial activity corresponding to the size of the inhibition zone of 10.0, 13.0 and 16.0 mm was appeared at the concentration of 1,000, 2,000 and 4,000 μg/disc, respectively. However, in *S. enterica*, 70% ethanol extract showed 11.0 and 16.0 mm and the water extract showed 10.0 and 16.0 mm at the concentration of 2,000 and 4,000 μg/disc, respectively, in which no significant difference was observed. Taken together, the antimicrobial power of 70% ethanol extract was superior to the water extract, but it was found that the antimicrobial power against *S. enterica* was relatively low compared with other strains.

2) The growth inhibitory effect in liquid culture

The bacterial strains were inoculated into liquid cultures with different concentrations of *Persicaria hydropiper* L. extract and cultured at 30℃ for 24 hours and the growth inhibitory effect was examined by measuring the degree of the bacteria growth every 2 hours using Disital VIS/Ultraviolet spectrophotometer.

The results of measuring the growth inhibitory effect of the 70% ethanol extract alone which showed an excellent antimicrobial activity in the paper disc method are shown in Figure 1～4.

As shown in Figure 1, while the growth of *B. cereus*, a Gram-positive bacterial strain, was not inhibited at the concentrations of 100～200 ppm or lower, the bacterial growth was significantly inhibited at 400 ppm, which was mostly suppressed at 1,000 ppm.

As shown in Figure 2, the growth of *S. aureus*, a Gram-positive bacterial strain, was significantly inhibited for 10 hours at 200 ppm, for 16 hours at 400 ppm and 24 hours for 1,000 ppm, but as shown in Figure 3, the growth of *E. coli*, a Gram-negative bacterial strain, was inhibited for 24 hours only at 1,000 ppm, which was similar to the result as *B. cereus*.

As shown in Figure 4, *S. enterica*, a Gram-negative bacterial strain, showed slightly higher absorbance at 1,000 ppm compared with other strains, which was similar to the result obtained by the paper disc

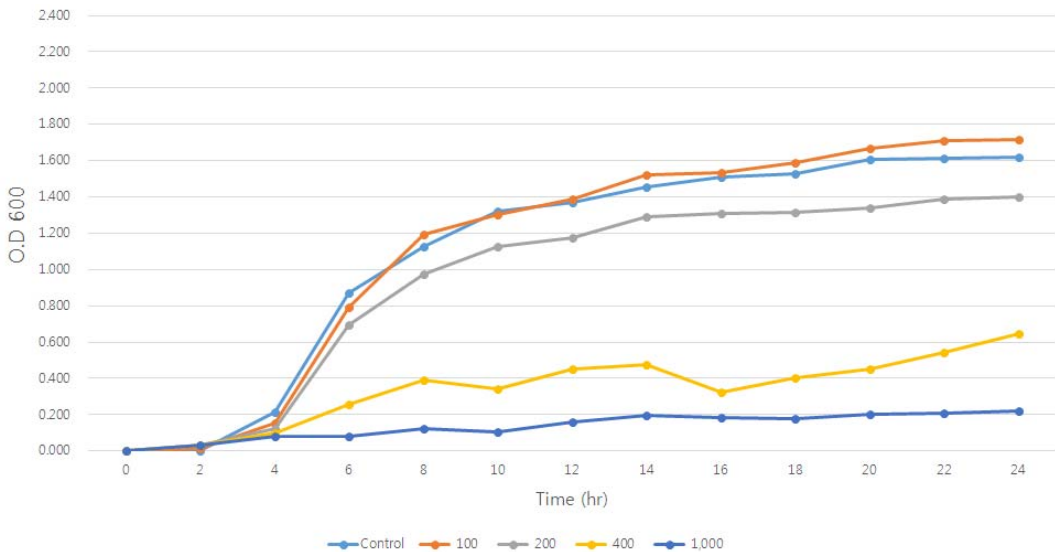

Figure 1. Inhibitory effect of 70% ethanol extract from *Persicaria hydropiper* L. on the growth of *Bacillus cereus* at 30℃.

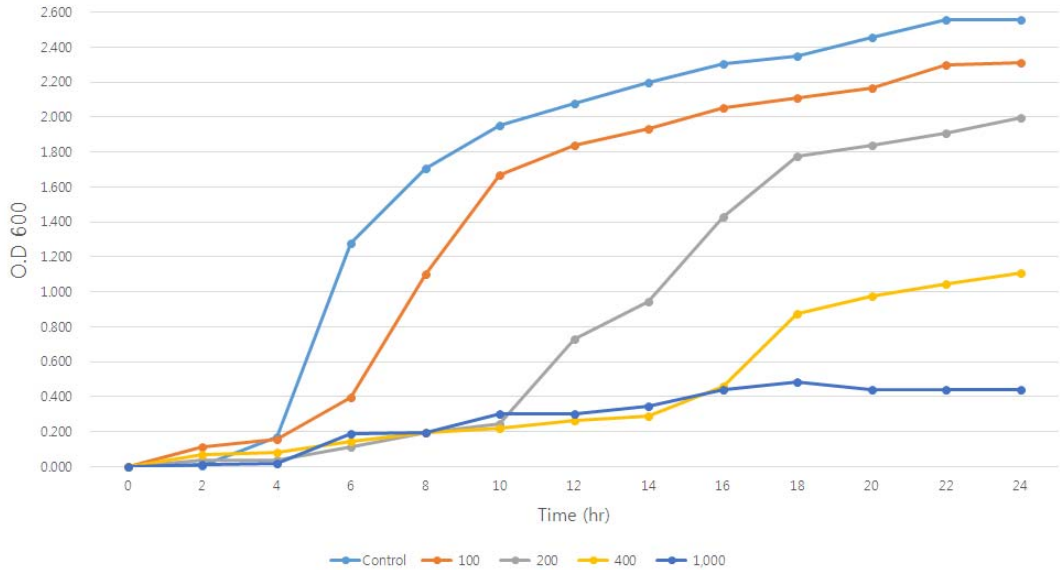

Figure 2. Inhibitory effect of 70% ethanol extract from *Persicaria hydropiper* L. on the growth of *Staphylococus aureus* at 30℃.

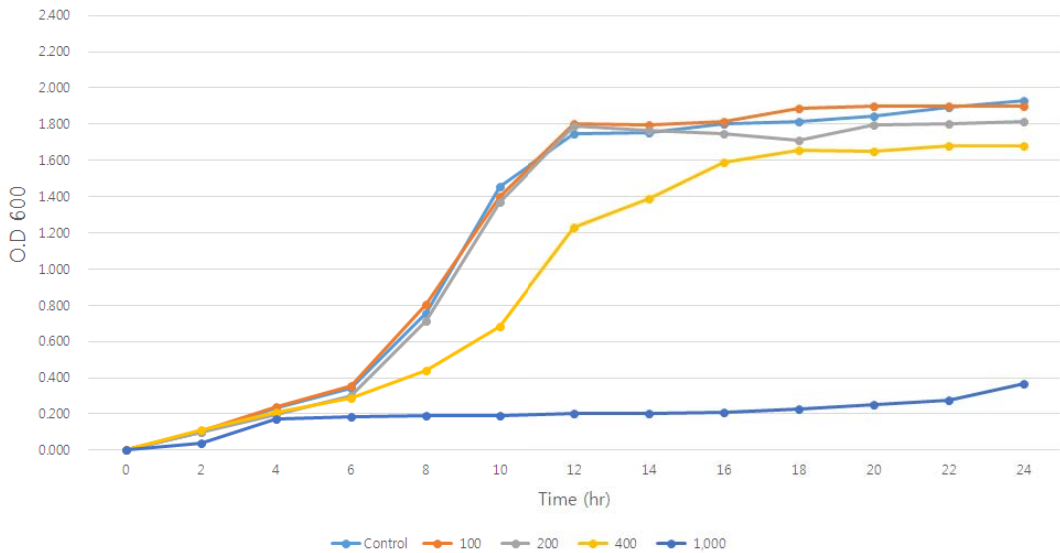

Figure 3. Inhibitory effect of 70% ethanol extract from *Persicaria hydropiper* L. on the growth of *Escherichia coli* at 30℃.

method.

The study of Ahn ES et al[28] showed that 75% ethanol extracts of *Hwangbaek*, clove, nut gall, rigida and paeony had high antimicrobial activities depending on the concentrations added, which is similar to the report that the highest growth inhibitory activity was observed at 1,000~2,000 ppm or better. When compared with these results from previous studies, the antimicrobial effect of *Persicaria hydropiper* L. extract in 70% ethanol is deemed to be very good and the applicability of *Persicaria hydropiper* L. as a natural antimicrobial material is considered to

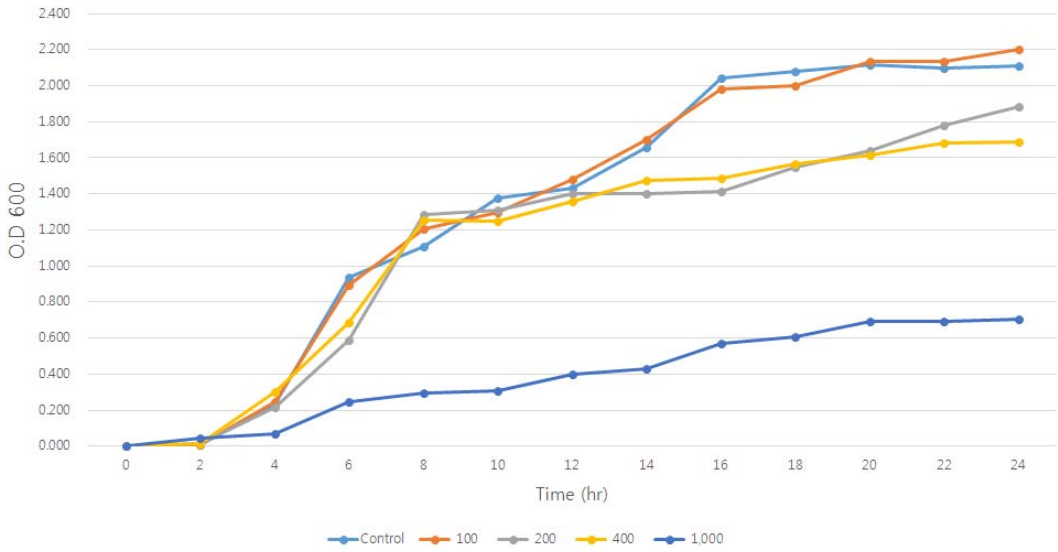

Figure 4. Inhibitory effect of 70% ethanol extract from *Persicaria hydropiper* L. on the growth of *Salmonella entrica* at 30℃.

be very high.

DISCUSSION AND CONCLUSIONS

Discussion of Findings

This purpose of this study is to examine the antioxidant and antimicrobial activities of *Persicaria hydropiper* L. extract in 70% ethanol and in water, a medicinal herb, as an effort to examine the potential of medicinal herbs for development of antioxidants and natural preservative substitutes and the results are as follows:

The first conclusion, the result of the antioxidant activity demonstrated that the total polyphenol content in the 70% ethanol extract and in the water extract were 19.88 mg/g and 7.46 mg/g, respectively. The DPPH radical scavenging activity was 90.99% and 64.98% in the 70% ethanol extract and water extract, respectively, in which 70% ethanol extract showed a higher activity. However, the ABTS scavenging activity was as low as 8.06% and 27.42% in the water extract and 70% ethanol extract, respectively. Such differences in their scavenging activities are derived from the fact that DPPH is a free radical, whereas ABTS is a cation to be removed and they are also affected by concentration-dependent differences.

The second conclusion, in order to examine the antimicrobial activity of *Persicaria hydropiper* L. against a total of four food spoilage microorganisms, antimicrobial activity was measured by paper disc method, in which 70% ethanol extract and water extract of *Persicaria hydropiper* L. were diluted to appropriate concentrations and added to make the concentrations to 1,000, 2,000 and 4,000 μg/disc, and the results showed that in *B. cereus*, *S. aureus* and *E. coli*, the higher was the concentration of the *Persicaria hydropiper* L. extract in 70% ethanol absorbed in the disc, the bigger was the size of the inhibition zone, which represents antimicrobial activity, and the respective antimicrobial activities were 13.0, 11.0 and 10.5 mm at the concentration of 1,000 μg/disc, and at the concentration of 4,000 μg/disc, the size of the inhibition zone was 23.0, 22.0, 22.0 and 16.0 mm in *B. cereus*, *S. aureus*, *E. coli* and *S. enterica*, respectively, but the size of the inhibition zone using the water extract absorbed in the discs at the concentration of 4,000 μg/disc, was 16.0, 21.0 and 16.0 mm in *S. enterica*, *E. coli* and *S. aureus*, respectively, indicating its reduced antimicrobial power compared with the 70% ethanol extract.

The third conclusion, the results of the growth inhibitory effect of the 70% ethanol extract in liquid culture showed that the growth of *B. cereus*, a Gram-positive bacterial strain, was rapidly inhibited at 400

~1,000 ppm, and the growth of *S. aureus* was significantly inhibited for 10 hours at 200 ppm, 16 hours at 400 ppm and 24 hours for 1,000 ppm. *E. coli*, a Gram-negative bacterial strain, showed a higher antimicrobial activity at 1,000 ppm and the growth of *S. enterica* was inhibited only at 1,000 ppm, which showed the highest absorbance among the microorganisms examined.

Implications

In order to examine the antimicrobial activity against food spoilage microorganisms, antimicrobial activity was measured by paper disc method and the result showed that in *B. cereus*, *S. aureus* and *E. coli*, the higher was the concentration of the *Persicaria hydropiper* L. extract in 70% ethanol absorbed in the disc, the bigger was the size of the inhibition zone, which represents antimicrobial activity, but the size of the inhibition zone using the water extract absorbed in the discs at the concentration of 4.000 μg/disc, was 16.0, 21.0 and 16.0 mm in *S. enterica*, *E. coli* and *S. aureus*, respectively, indicating its reduced antimicrobial power compared with 70% ethanol extract. The results of the growth inhibitory effect in liquid culture showed that the growth of *B. cereus* and *S. aureus*, Gram-positive bacterial strains, was inhibited, the antimicrobial effect against *E. coli*, a Gram-negative bacterial strain was high at 1,000 ppm and the growth of *S. enterica* was inhibited only at 1,000 ppm, which showed the highest absorbance among the microorganisms examined.

The antioxidant effect of *Persicaria hydropiper* L. extract appears to be very good and due to its excellent growth inhibitory effect on food-poisoning-causing microorganisms in the food, it is thought to be utilized as a potential natural preservative substitute in many areas.

Limitations and Future Research

Despite the implications that the current study can provide, there are several limitations that future research should address. The current study was conducted only in extract of *Persicaria hydropiper* L; therefore, it is limited to apply the findings to other extracts, According to above results, 70% ethanol extract of *Persicaria hydropiper* L. was found to have very effective antioxidant effect which is thought to be due to its high total polyphenol content and DPPH radical scavenging activity and it is likely to be utilized as a natural preservative substitute in many areas due to its high growth inhibitory effect on food-poisoning-causing microorganisms in the food. The functional studies that can be applied to food are needed in the future, and it is expected to be used and developed as a natural food additive in the food industry.

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