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Antioxidant and Antimicrobial Activities of the Ethanol Extract of *Allium victoriallis* L. var. *platyphyllum*

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Abstract This study was conducted to evaluate *in vitro* antioxidative and antimicrobial properties of ethanolic extracts from *Allium victoriallis* L. var. *platyphyllum* (AVP) with 6 different parts. The antioxidative activities of these samples were determined using the 4 separate methods that involved reducing power, DPPH, hydrogen radical scavenging, and lipid peroxidation with use of a β-carotene/linoleic acid system. The leaf part ethanolic extracts (1,000 ppm) showed the strongest inhibitory potential for reducing power, DPPH, and hydroxyl radicals to 99.8, 49.4, and 52.8%, respectively. Inhibition values of linoleic oxidation were calculated as 58.0, 39.5, and 38.0% for seed, flower, and leaf ethanolic extracts (1,000 ppm), respectively, from AVP. In addition, the ethanolic extracts of the root part showed the most effective antimicrobial activity. The inhibition zones of the root ethanolic extracts (200 μg/disc) of AVP against *Bacillus cereus* and *Staphylococcus aureus* were 17 and 14 mm, respectively. In a micro-dilution assay, *B. cereus*, *S. aureus*, and *Vibrio parahaemolyticus* exhibited sensitivity to root part ethanolic extracts with an minimum inhibition concentration (MIC) value of 20, 28, and 18 mg/mL, respectively. Therefore, the AVP extracts may be suitable as antimicrobial and antioxidative agents in the food industry.

Keywords: *Allium victoriallis* L. var. *platyphyllum*, antioxidant activity, antimicrobial activity, foodborne pathogen

Introduction

Given that antimicrobial substances are exist in sufficient quantities in many different plants (1,2), a variety of research has been conducted to investigate the antimicrobial activities in plants or herbal medicines along with their application as food preservative additives (3,4). For many centuries, several species from the Liliaceae family, which includes 700 species, have been used as vegetables and spices, and as folk medicines for curing various diseases. For example, *Allium* species, which is the largest and the most important representative genus of the Liliaceae family, including *A. sativum* (garlic) and *A. cepa* (onion) has been shown in previous studies to act as an antithrombotic (5,6), an antidiabetic (7), a hypocholestero-lemic (8), a hypolipidemic (9,10), and an anticancer agent (11).

Allium victoriallis L. var. *platyphyllum* (AVP), which belongs to Liliaceae, is an herbaceous perennial plant grown on Jirisan(Mt.), Odaesan(Mt.), and on Ulleungdo (Island) in Korea. AVP has been traditionally used as an edible and medicinal wild herb for the treatment of gastritis and heart disease in Korea (12,13). It has been established that AVP exerts pharmacological activities such as anti-hepatotoxic and anti-hyperlipemic activity in the rat (14). This herbal plant is reported to contain 2-3% carbohydrates and ascorbic acid in its leaf and sulfur-containing substances in bulk (15). In addition, it has been reported that 2 flavonoids (astragalin and kaempferol 3, 4'-di-*O*-β-D-glucoside) were isolated in bulbs of AVP (12). However, research for antimicrobial and antioxidative potentials of AVP was not well known.

In the present study, the antioxidative activities of the ethanolic extracts from AVP with different parts are determined using separate systems. In addition, the antimicrobial activity of these extracts against 6 foodborne pathogens was assessed in an agar diffusion and turbidity assay.

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Materials and Methods

Sample preparation The AVP were obtained from a 'Seorrim Form' in June 2005 and divided into 6 parts (flower, flower stalk, seed, leaf, stem, and root). All parts were stored at −70°C, prior to lyophilize for 72 hr and pulverized with blender. Dried AVP sample (10 g) was extracted by stirring with 100 mL of 70% ethanol at 25°C at 100×g for 24 hr and filtering through filter paper. The residue was then extracted with 2 additional 100 mL portions of 70% ethanol as described above. The combined ethanolic extract was then evaporated at 40°C to dryness, dissolved in 10% ethanol to a concentration of 100 ppm and stored at 4°C for further use.

Antioxidant activity

***Reducing power*:** The reducing power was determined according to the method of Yildirm *et al.* (16). Extracts in distilled water (2.5 mL) were mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (10 mg/mL). The mixture was then incubated at 50°C for 20 min, added with 2.5 mL trichloroacetic acid (100 mg/mL), and centrifuged at 1,000×g for 10 min. The upper layer (5 mL) was mixed with 5 mL distilled water and 1 mL ferric chloride (1 mg/mL), and the absorbance was measured at 700 nm: higher absorbance indicates

higher reducing power.

DPPH radical scavenging capacity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity was evaluated by a slightly modified method of Blois (17). Trolox was used as positive controls. The percent inhibition of the DPPH radical by the sample extracts was calculated by the following formula: DPPH radical scavenging activity (%)=$[(A_0-A_1)/A_0]\times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the test sample.

Hydroxyl radical scavenging activity: Competition between deoxyribose and the sample against hydroxyl radical generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system was measured to determine the hydroxyl radical scavenging activity (18). The reaction mixture consisted of 0.1 mL of 0.05 M $FeSO_4H_2O$, 0.1 mL of 0.05M EDTA, 0.2 mL of 0.05 M 2-deoxyribose, 1.2 mL of 0.1 M phosphate buffer (pH 7.4), 0.2 mL of 0.05 M H_2O_2, and 0.2 mL of sample solution at different concentration (0.1, 0.5, and 1 mg/mL). The reaction was started by the addition of hydrogen peroxide. After incubation at 37°C for 2 hr, the reaction was stopped by adding 1 mL of 2.8% trichloroacetic acid and 1 mL of 1% thiobarbituric acid. After boiling the mixture for 15 min followed by ice-cooling, the absorbance was measured at 530 nm. Hydroxyl radical scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radical. Hydroxyl radical scavenging activity (%)=$[(A_0-A_1)/A_0]\times 100$, where A_0 is the absorbance of the control reaction (all reagents except the test compound), and A_1 is the absorbance of the test sample.

Lipid peroxidation inhibitory activity in β-carotene/linoleic acid system: Lipid peroxidation inhibitory activity was determined by the β-carotene/linoleic acid system method (19). The antioxidant activity of the sample extracts and pure compounds was evaluated by the following formula: β-carotene/linoleic acid antioxidant activity (%)=$[1-(A_c-A_s)/A_c]\times 100$, where A_c is the absorbance of the control, and A_s is the absorbance in the presence of the test sample.

Antimicrobial activity

Microorganisms: The AVP extracts submitted to evaluation of antimicrobial activity with the bacteria strains: *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* KCTC 3710, *Salmonella choleraesuis* ATCC 13312, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* KTCT 3726, and *Vibrio parahaemolyticus* ATCC 17802. The cultures of *V. parahaemolyticus* were incubated at 37°C for 18 hr in Nutrient broth (NB, Difco, Detroit, MI, USA) with NaCl added at a final concentration of 3% (Na-NB broth). The others strains were incubated at 37°C in Nutrient broth for 18 hr. All bacterial cultures were incubated in aerobic conditions.

Agar diffusion method: The agar diffusion method was performed using cotton swabs for each bacterial suspension (0.1, OD_{600}) and inoculated in plates where the bacteria were spread uniformly on the agar surface. The dried plant extracts were dissolved in 10% ethanol solvent to a final concentration of 100 mg/mL and sterilized through filtration by syringe filter (0.45 μm). There is no inhibition effect for the tested microorganism growth at the final concentration of 10% ethanol. The 8 mm diameter discs were impregnated with 200 μg/disc of AVP extracts and placed on the inoculated agar. The plates were incubated at 37°C for 12 hr and next, examined to verify the inhibition. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated in triplicate.

Turbidity assay: The antimicrobial activity of the extracts was evaluated by using a turbidity assay in culture broth. One-hundred μL of all tested pathogens (0.1, OD_{600}) was inoculated into 5 mL of NB media along with each part ethanolic extracts from AVP and then incubated at 37°C for 24 hr. The control was inoculated with only each pathogen without sample extracts and ampicilline and tetracycline were used as positive substances. After incubation, the OD was measured at 600 nm using spectro-photometer. All experiments were performed in triplicate.

Statistical analysis Results are the mean values of 3 replicates of the same sample. Statistical analysis was performed using analysis of variance.

Results and Discussion

The part ethanolic extracts from AVP were individually assessed for their possible antioxidative capacities by employing 4 complementary tests: DPPH radical, hydroxyl radical scavenging, reducing power, and lipid peroxidation.

DPPH radical scavenging activity Figure 1A shows the DPPH radical scavenging abilities of each ethanolic extract of AVP with different parts. The ability to scavenge DPPH radicals of AVP ethanolic extracts was in the order of leaf (49.2%)>seed (38.0%)>root (34.0%)>flower (33.6%)> flower stalk (32.4%)> and stem (28.0%) at the concentration of 1,000 ppm.

Reducing power activity Reducing activity is generally associated with the presence of reductones, which have been shown to exert an antioxidant effect by donating a hydrogen atom and breaking the free radical chain. The reducing power for each ethanolic extracts of AVP was assessed with different parts. In the results (Fig. 1B), the leaf extracts (1,000 ppm) showed the strongest activity, at 99.8%, a level of activity identical to that of Trolox as a positive control. The seed and root parts extracts also showed activity, at 77.1 and 51.4%, respectively.

Hydroxyl radical scavenging activity The hydroxyl radical scavenging activities of AVP extracts were measured by investigating the competition between deoxyribose and AVP extracts against hydroxyl radicals generated from the Fe^{2+}-H_2O_2 system (20). Hydroxyl radicals attack deoxyribose and set off a series of reactions, and thiobarbituric acid reactive substance (TBARS) eventually formed. 2-Deoxyriboe is oxidized by OH formed through a Fenton reaction and is then degraded into malondialdehyde (18). According to the results (Fig. 2), the extents to which hydroxyl radicals generated by Fe^{2+}-H_2O_2 decompose deoxyribose were 42.5, 40.9, 49.6, 52.8, 47.2, and 47.0% in flower, flower stalk, seed, leaf, stem, and root at a concentration of 1,000 ppm, respectively, which were higher than Trolox (100 ppm, 37.8%) as a positive control. In this study,

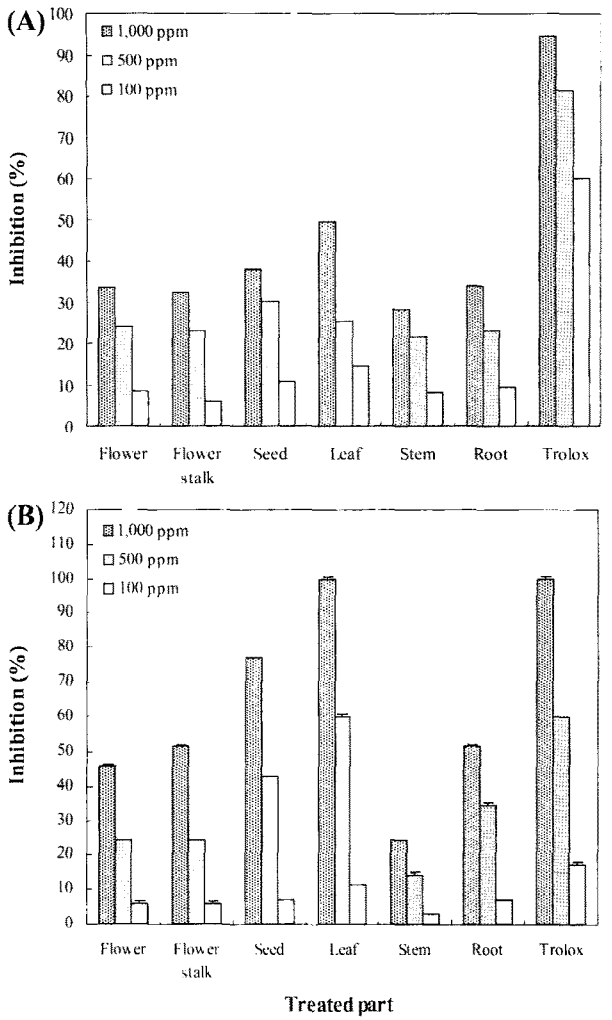

Fig 1. Antioxidative activities of AVP ethanolic extracts with different parts on DPPH radical (A) and reducing power (B). Each value is expressed as mean±SD, n=3. Trolox was used as positive control.

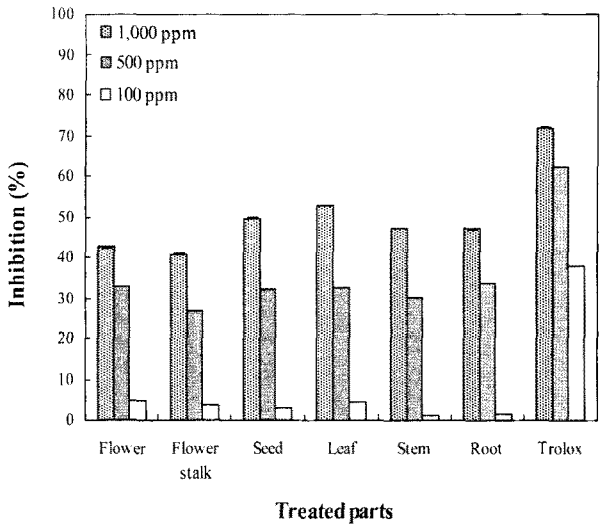

Fig. 2. Hydroxyl radical scavenging activity of AVP ethanolic extracts with different parts. Each value is expressed as mean ±SD, n=3. Trolox was used as positive control.

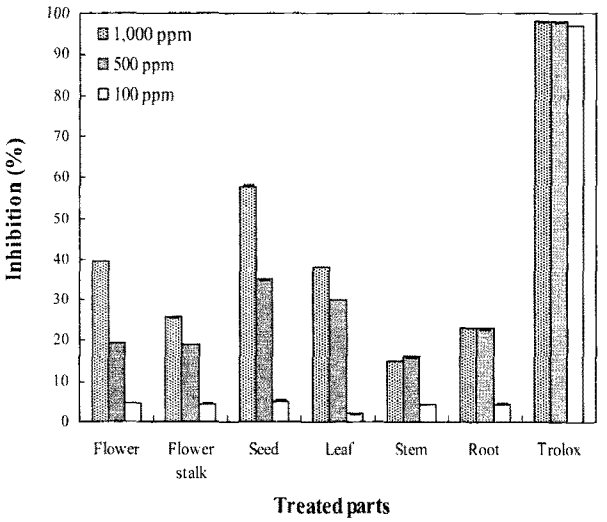

Fig. 3. Lipid peroxidation inhibitory activities of AVP ethanolic extracts with different parts. Each value is expressed as mean ±SD, n=3. Trolox was used as positive control.

among the tested part, leaf extract exhibited the strongest inhibition effects on deoxyribose oxidation. Although the hydroxyl radical scavenging abilities of the extracts were significantly less than those of Trolox, it was evident that the extracts can serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

In a previous study, the total phenolic contents from different parts of AVP ranged from 3.07 to 24.29 mg of GAE/g extract, and the total phenolic contents from different parts ranged from 2.53 to 20.00 GAE/g (21). The highest total phenolic and flavonoid content was observed in the leaf (24.29 mg GAE/g extract and 20.00 mg RE/g extract, respectively) and seed (19.79 mg GAE/g extract and 16.29 mg RE/g extract, respectively) parts. The most effective content appeared to be flavonoids and other phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Some studies have reported that their metal chelating capabilities and radical scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation and low-density lipoprotein (LDL) oxidation (22,23). In fact, many *in vitro* data suggested that polyphenolic compounds including flavonoids possess anti-inflammatory, anti-allergic, antiatherosclerosis, anti-diabetes, and anticarcinogenic properties (24). These biological properties are thought to be related to the antioxidant activity of these compounds (25).

Inhibitory effect of lipid peroxidation Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. It has been suggested that it is an important event in the deterioration of foods during storage (26). To determine the inhibitory activity of APV extracts on lipid peroxidation, the β-carotene/linoleic acid system was used. As shown in Fig. 3, in particular seed part extracts (1,000 ppm) showed remarkable inhibition for the oxidation of linoleic acid, at 58.0%. The inhibitory effect of the different part extracts of AVP followed the order seed > flower > leaf > flower stalk > root > stem.

Table 1. Screening of antimicrobial activities of different part of AVP extracts against some foodborne pathogens using an agar diffusion method[1)]

Tested pathogen	Used parts							
	Flower	Flower stalk	Seed	Leaf	Stem	Root	Ampicilline	Tetracycline
S. aureus	-[2)]	-	11.0±0.1	-	-	14.1±0.3	13±0.5	17±0.3
L. monocytogenes	10.0±0.4	10.3±0.2	11.0±0.1	12.0±0.1	-	13.0±0.1	15±0.3	23±0.5
S. choleraesuis	-	-	-	-	-	10.0±0.4	23±0.5	14±0.5
B. cereus	8.5±0.1	8.5±0.1	9.0±0.2	8.5±0.1	-	17.0±0.1	-	16±0.3
B. subtilis	8.5±0.1	9.0±0.2	11.0±0.1	12.0±0.1	-	12.0±0.1	12±0.3	19±0.3
V. parahaemolyticus	-	10.0±0.4	10.0±0.4	12.0±0.1	-	12.0±0.1	-	17±0.3

[1)]The results were presented as diameter (mm) of clear zone produced by treatment of samples at the concentration of 200 μg/disc, respectively. Each value is expressed as mean±SD, n=3. Ampicilline and tetracycline (12.5 μg/disc) were used as positive control.
[2)]No inhibition.

Antioxidative activity of some *Allium* species has been reported elsewhere (27-29). Tepe *et al.* (30) have assessed the antioxidative activity of 5 *Allium* species from Turkey and showed the inhibition activity of the oxidation of linoleic acid by *A. atroviolaceum* and *A. dictyoprosum*, in β-carotene/linoleic acid system. In the present study, the leaf and seed extracts from AVP presented particularly high phenolic contents and antioxidative activity from used assay systems. It is also well known as a free radical playing an important role in the antioxidation of unsaturated lipids in foodstuffs (31,32). Lipid peroxidation can be catalyzed by enzymes (lipoxygenases, cyclooxygenases) or

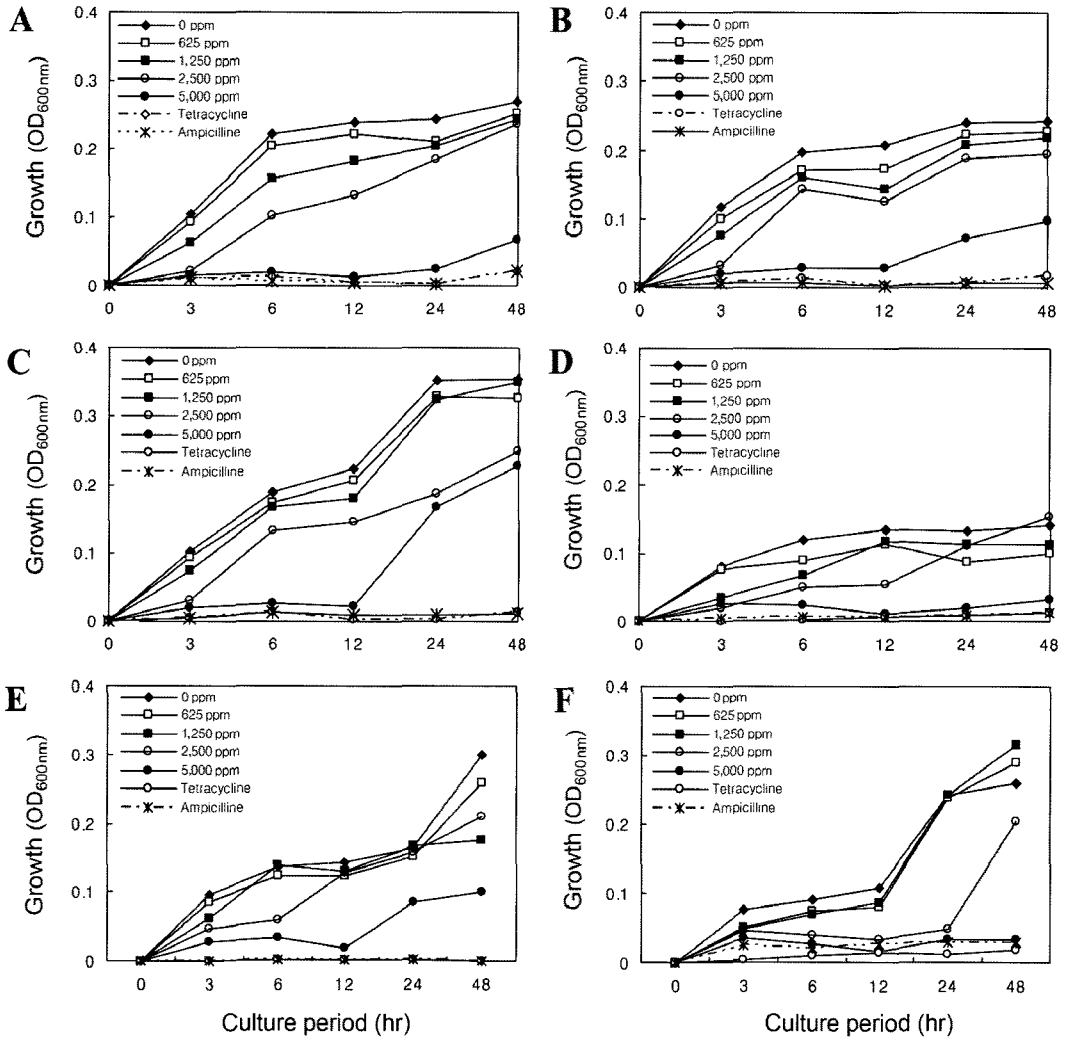

Fig. 4. Growth kinetics of foodborne pathogens on culture broth in the absence and in the presence of the roots ethanolic extracts from AJN. (A) *S. aureus*, (B) *S. choleraesuis*, (C) *B. subtilis*, (D) *B. cereus*, (E) *L. monocytogenes*, and (F) *V. parahaemolyticus*. Data represented the average values of 3 replications. Ampicilline and tetracycline (12.5 μg/mL) were used as positive control.

initiated by sources of reactive oxygen species (e.g., NADPH oxidase, transition metal ions, radiation). When exposed to oxidative stress, unsaturated lipids can be attacked by free radicals and oxidized into lipid peroxides. Consequently, a pernicious radical chain mechanism allows producing a much larger number of lipid hydroperoxide molecules in the propagation steps. From this point, AVP extracts showed the antioxidative effects against lipid peroxidation to scavenge the hydroxyl radicals at the stage of initiation of peroxyl radicals.

Antimicrobial activity The antibacterial activities of the different part extracts of AVP were determined against 6 bacterial strains (Gram-positive; *L. monocytogenes*, *B. cereus*, *B. subtilis*, and *S. aureus*, Gram-negative bacteria; *S. choleraesuis* and *V. parahaemolyticus*), which are all considered as common foodborne pathogens. The antimicrobial activity was determined using an agar diffusion method.

In agar diffusion method, all part extracts (200 μg/disc) showed antimicrobial activity against tested foodborne pathogens except for the stem part (Table 1). In particular, the root part had the highest inhibition activity against *B. cereus* (17.0±0.1 mm) and *S. aureus* (14±0.1 mm), respectively. It showed activity identical to that of ampicilline and tetracycline (12.5 μg/disc) as a positive control. However, the stem part did not show any inhibitory activity. The leaf part showed slight activity against *L. monocytogenes*, *B. subtilis*, and *V. para-haemolyticus* strains.

From the above results, the root part ethanolic extracts of AVP that presented the strongest inhibition zone in the agar diffusion method were added to NB media to determine the growth inhibitory activity against these pathogens using a turbidity assay. The results (Fig. 4) showed that AVP strongly inhibited the growth of all of the tested pathogens in a dose-dependent manner. In particular, *B. cereus*, *S. aureus*, and *V. parahaemolyticus* exhibited sensitivity to the root part ethanolic extracts with an minimum inhibitory concentration (MIC) value of 20, 28, and 18 mg/mL, respectively. Although the growth rates of *S. choleraesuis*, *B. subtilis*, and *L. monocytogenes* were inhibited until 12 hr and dramatically increased at 24 hr. However, the growth rates of *S. aureus*, *B. cereus*, and *V. parahaemolyticus* were inhibited for the 24 hr culture period. This implies that AVP is effective as a natural preserving source against *S. aureus*, *B. cereus*, and *V. parahaemolyticus*. Chung *et al.* (33) reported that *A. sativum* (allicin) inhibits the growth of foodborne and clinical pathogens as a food preservative; its major active component was thiosulfinate compound (34). Moreover, garlic has been reported to reduce blood lipids and to have anticancer effects.

In conclusion, although further investigation addressing safety issues and activity relating to AVP extracts in necessary, this result suggests that the leaf, seed and root from AVP can be used as antioxidative agents and as an alternative phytochemial preserves in the food industry. Further studies will be conducted to identify the active components of AVP.

Acknowledgments

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