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RESEARCH NOTE

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Antimicrobial Activities of White, Red, and Extruded Ginsengs with Different Extraction Conditions

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Abstract White, red, and extruded ginsengs were studied against 8 strains of food-borne pathogens and/or food spoilage microorganisms. The ginseng powders were extracted with different extractants and screened for antimicrobial activity using the disc diffusion and broth dilution techniques. The results showed that the yield of extraction was higher with increase of aqueous solution content and temperature. Preliminary screening revealed that the red ginseng extracts were most active, that has been found to be highly effective against all tested microbe except *Listeria monocytogenes*. Moreover, *Bacillus subtilis* has shown highly susceptible, which the diameters of inhibition zone values of 28 extracts were between 7 and 14 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) recorded for the different crude ginseng extracts against microorganism using ranged from 6.25 to 100 mg/mL, indicated that the methanol extract of ginseng were more effective than ethanol and water extracts. The 60% methanol extract of red ginseng had the greatest effects against *B. subtilis* with MIC and MBC values at 6.25 mg/mL.

Keywords: antimicrobial activity, antibiotic, minimum inhibitory concentration, minimum bactericidal concentration, microorganism

Introduction

Food-borne diseases are still a major problem in the world that resulting from consumption of food contaminated with pathogen has been of vital concern to public health (1). *Salmonella* sp., *Listeria monocytogenes,* and *Escherichia coli* accounted for the largest number of outbreaks, cases, and deaths. Particularly, *L. monocytogenes* is a bacterium responsible for the severe food-borne illness, Listeriosis. Most reports associate listeriosis with the consumption of contaminated ready-to-eat foods such as dairy products, processed or cured meat and poultry, salads, seafood, and uncooked eggs (2). Moreover, microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality. There is considerable interest in the possible use of such natural alternatives as food additives either to prevent the growth of foodborne pathogens or to delay the onset of food spoilage by synthetic chemical preservatives (3).

Panax ginseng C.A. Meyer (Araliaceae) has long been recognized a traditional tonic herb to treat physical and mental disorders (4). Commercial ginseng is classified into fresh, white, and red ginseng. White ginseng is made by peeling the fresh ginseng roots and drying them without steaming. Red ginseng is made by steaming (98-100°C for 2-3 hr) without peeling to enhance its preservation and efficacy (5). Pharmacological studies show that the bioactivities of the different ginsenosides vary, depending on the extraction conditions. Most of extraction procedure use methanol or ethanol as solvent (either pure or an aqueous solution) and often couple with heat, refluxing, or sonication (6-8). Approximately 200 substances in *P. ginseng* include ginsenosides, polyacetylenes, alkaloids, polysaccharides, oligosaccharides, oligopeptides, flavonoids, lipids, vitamins, and minerals (9). The multiple biological actions of ginseng are generally attributed to ginsenosides (ginseng saponins), the most active ingredient of ginseng. Korean ginseng contains 30 different ginsenosides, of which more than 10 individual ginsenosides have been proven for their medical actions, such as antiaging, anticarcinogenic, and antidiabetic (10,11).

Furthermore, the previous researches reported that ingredients of ginseng extract could inhibit the microorganisms. For example, a polyacetylene compound from the ginseng of Canary Islands, could inhibitory to *Staphylococcus aureus* and *Bacillus subtilis* but not other bacteria or yeast (12). Acidic polysaccharides of ginseng have been shown to inhibit *Helicobacter pylori*. Other proteins isolated from the Panax family (panaxagin and quinqueginsin) also exhibit antifungal activity (13).

Therefore the purpose of this study was to compare the antimicrobial activity effect of solvent extraction condition on white, red, and extruded white ginseng extracts against common food-borne pathogen and/or spoilage microorganism, evaluating by minimum inhibitory concentration determination and agar diffusion disc method.

Materials and Methods

Materials Powdered *Panax ginseng* (white ginseng and red ginseng) used in this work were purchased from Dong-Jin Drug and Food Co., Gumsan, Korea. All antibiotics were obtained from Merck (Calbiochem, Darmstadt, Germany). Media (nutrient broth, nutrient agar, Mueller Hinton broth, and Mueller Hinton agar) for culture were obtained from Becton, Dickinson (Sparks, MD, USA), all other reagents were commercial grade and were obtained from commercial suppliers.

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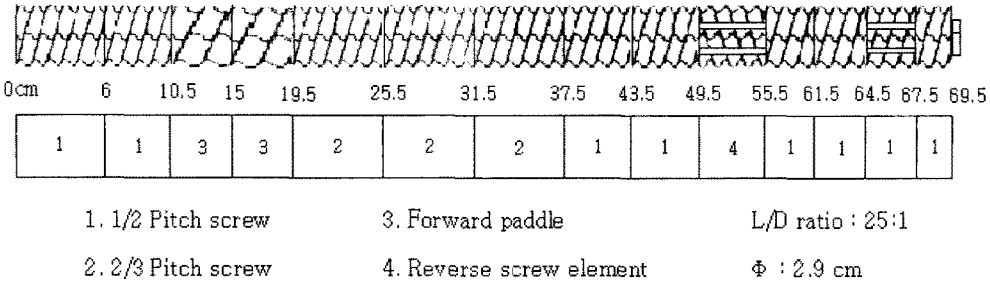

Fig. 1. Screw configuration for extrusion of ginseng powder.

Extrusion Extruded ginseng was made from white ginseng powder using co-rotating intermeshing twin screw extruder (Incheon Machinery Co., Incheon, Korea). The ginseng powder was extruded in extruder of which screw configuration with an L/D ratio is shown in Fig. 1. The process conditions applied were as follows: screw speed 250 rpm, feed rate 140 g/min, barrel temperature 120°C, moisture content 20%, screw diameter 29 mm, and die diameter 3 mm. The extrudate was directly dried in an oven at 50°C for 8 hr. The dried extrudate was ground to powder using a mechanical grinder and then sieved through 35 mesh and stored in a glass jar at 4°C until analyzed.

Extraction The powdered ginsengs (20 g) were macerated with water and 100, 80, and 60% of ethanol and methanol (1:8) at 25 and 35°C. The mixture was shaken for 24 hr at 110 rpm in water bath at 25 and 35°C. The extracts were filtered through Whatman No.1 filter paper using a Buchner funnel and the solvent removed under reduced pressure with a rotary evaporator (Buchi R-200; Labortechnik, Flawil, Switzerland); this process was repeated in 3 times. The dried crude extracts were stored at 4°C until testing. The extracts were dissolved in water or 100% dimethyl sulfoxide (DMSO) before use. The yield of extract was calculated by weight of ginseng extract (g)/weight of ginseng powder (g) ×100%.

Antimicrobial activity ***Microbial strains*:** The microorganisms tested were obtained from Korean Collection for Type Cultures, Korea. They were *Staphylococcus epidermidis* (KCTC 3958), *B. subtilis* (KCTC 1023), *L. monocytogenes* (KCTC 3710), *E. coli* (KCTC 1041), *Pseudomonas aeruginosa* (KCTC 1636), *Salmonella typhimurium* (KCTC 1925), and *Candida albicans* (KCTC 7270).

***Agar diffusion disc method*:** The antimicrobial activities of isolates to the different extracts were tested using the disc-agar method standardized by the National Committee for Clinical Laboratory Standards (14). The microorganisms were grown overnight at 37°C in 30 mL of nutrient broth. The cultures were adjusted with sterile saline solution (0.85% NaCl) to obtain turbidity comparable to that of McFarland No. 0.5 standard. Petri plates were prepared by pouring 20 mL of Mueller Hinton agar (MHA) and allowed to solidify. Plates were dried and 0.1 mL of inoculums suspension was poured and uniformly spread. Thirty μL containing 500 mg/mL of each ginseng extracts were impregnated onto paper disc (6 mm in diameter) placed on the top of agar plate. The plates were incubated at 37°C for 24 hr. Gentamycin, streptomycin, and tetracycline, at 5 mg/mL, were included as positive controls for bacteria. Ketoconazole was used as positive control for *C. albicans* and water or 100% DMSO served as negative controls. The antimicrobial activity was assessed based on measurement of the diameter (mm) of the clear zone around the paper disc. All measurements were done in triplication.

***Minimum inhibitory concentration (MIC)*:** The estimate of the MIC was carried out by the broth dilution method (15). The test extract was dissolved and was incorporated into Mueller Hinton broth to get concentration of 100 mg/mL and serially diluted to achieve 50, 25, 12.5, and 6.25 mg/mL, respectively. The tubes were inoculated with 0.5 mL microorganism suspension of 10^6 CFU/mL. MIC value were defined as the lowest extract concentration that prevents visible bacterial growth after 24 hr of incubation at 37°C. Gentamycin, streptomycin, tetracycline, ketoconazole (5 mg/mL), and control with no extracts were used as reference. MIC assays were carried out in duplicate.

***Minimum bactericidal concentration (MBC)*:** To confirm the activity, the MBC was set up with subcultures made from each MIC broth that appears visually clear. The tubes were diluted (1:4) in fresh Mueller Hinton broth and then subculture on to surface of the MHA. Plates were incubated overnight at 37°C. The MBC was defined as the lowest concentration of plant extract that completely prevented microbial growth. Each MBC experiment was repeated 2 times.

Result and Discussion

Extraction yield Ginseng extraction yield have been compared to ginseng type (white, red, or extruded) and extraction condition (solvent or temperature). Seven different extraction solvents were used: 100% water, 100% ethanol, 100% methanol, 80% ethanol, 80% methanol, 60% ethanol, and 60% methanol. Extraction yield was calculated based on the original weight of ginseng powder. Extraction yield vary due to different solubility. The results are summarized in Fig. 2 and 3 that illustrate the yield of different ginseng extracts at 25 and 35°C, respectively. All of the 3 ginseng powder was extracted, the higher water content in organic solvent resulted in higher extraction yield. Methanol extraction showed higher yield than ethanol extraction, while the yields for water gave the highest for both 25 and 35°C. It can be observed that extraction yield increased with increasing extraction temperature. The yields of ethanolic extruded, white, and red ginseng extracts at 25°C were 2.90, 7.91, and 12.61%,

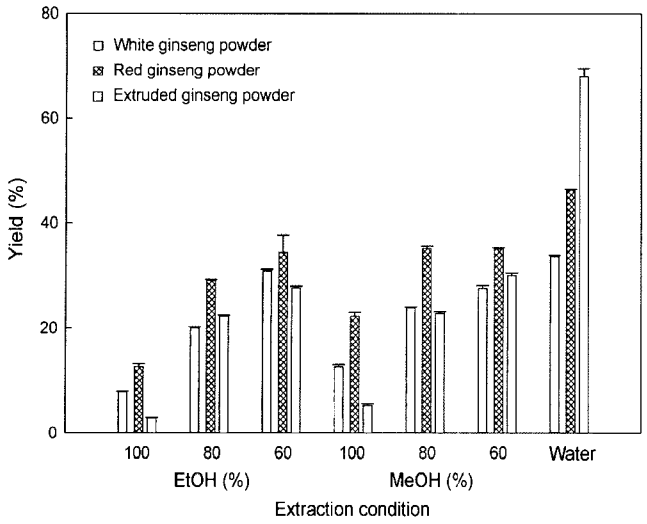

Fig. 2. Yield (%) of crude ginseng extracts at 25°C.

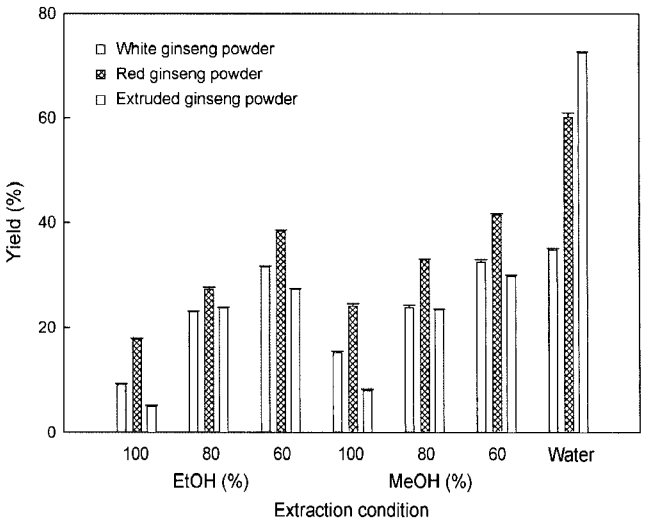

Fig. 3. Yield (%) of crude ginseng extracts at 35°C.

respectively, and the corresponding yields at 35°C were 4.98, 9.12, and 17.50%, respectively. This can be attributed to different affinities of the extraction solvents for total ginseng constituents in terms of their different extraction conditions, such as polarity of extracting solvents, and temperature (16).

In the case of white ginseng powder, the highest extraction yield of 34.72% was obtained at 100% water and 35°C, while red and extruded ginseng extracts were 59.94 and 72.54%, respectively. When ethanol and methanol were used, the highest yields were extracts from red ginseng powder for all condition. In case of 80% ethanol/methanol, the yields of white ginseng extracts were higher than that extracted from extruded ginseng. It was the opposite in case of 100 and 60% ethanol/methanol, that the extraction yield of extruded ginseng was lower than white ginseng, i.e., 12.55 and 5.24%, respectively (obtained from 100% methanol at 25°C).

Antimicrobial activity The antimicrobial activity of screening of crude ginseng extracts was evaluated by agar disc diffusion method, using 2 strains of Gram-positive bacteria (*S. epidermidis, B. subtilis, L. monocytogenes*), 3 strains of Gram-negative bacteria (*E. coli, P. aeruginosa, S. typhimurium*) and 1 strain of yeast (*C. albicans*). The results of the present study are shown in Table 1. Among Gram-positive, *B. subtilis* was the most sensitive organism to ginseng extracts, that 28 extracts were presented. Red ginseng extracted by pure methanol at 25°C was most effective to inhibitory, that average zone of inhibition of 14 mm (Fig. 4). Whereas only 4 and 3 extracts were inhibitory to *S. epidermidis* and *L. monocytogenes*, respectively, that minimal zone of inhibition ranged from 7 to 10 mm. Against *E. coli*, white ginseng methanol extract at 25°C created a zone of inhibition of about 14 mm (Fig. 5), red ginseng ethanol extract at 25°C 10 mm, and extruded ginseng ethanol extract at 35°C 9 mm. The extraction of red ginseng by 100% ethanol at 35°C of 500 mg/mL was more effective to against *P. aeruginosa* when compared with other extracts. All of ginseng extracts has low inhibitory effect on *S. typhimurium*. Mostly, only ginseng extract obtained from pure solvent were presented clear zone with *C. albicans*. The result found that red ginseng ethanol at 35°C had the most growth inhibition.

Furthermore, the result showed that out of 3 solvents used for extraction, the ethanol extracts displayed a broader spectrum of antimicrobial activity whereas the water extract of ginseng showed no antimicrobial activity against all of strain microorganism at the concentration used. Red ginseng has shown better results in comparison with white and extruded ginseng. The negative control (water and 100% DMSO) used in this study did not affect the growth of any of the tested microbial strain.

The MIC of ginseng extracts war tested against micro-organism that determine its lowest but still efficacious concentration, using 6.25, 12.5, 25, 50, and 100 mg/mL of extracts. Result showed (Table 2 and 3) that red ginseng extract by 60% methanol at 35°C was highest to inhibit *S. epidermidis* with MIC value of 6.25 mg/mL, whereas *L. monocytogenes* obtained from red ginseng extract by 60% ethanol at 25°C with same MIC value. The extraction of red ginseng by 80 and 60% methanol (at both 25 and 35°C) had shown most active against to *B. subtilis*. MIC value of this study for *P. aeruginosa* and *S. typhimurium* were 50 and 100 mg/mL, respectively. It was interesting to note that complete inhibition against *C. albicans* was obtained from 4 ginseng extracts (as red and extruded ginseng), MIC value at 6.25 mg/mL. In addition, *B. subtilis* was the most sensitive strain as it was inhibited by broth dilution method of the extracts tested, that found to be MBC value as 6.25 mg/mL (from red ginseng extract by 60% methanol at 35°C), while *E. coli* was the most resistant strain that none presented activity against *E. coli*.

Extracts from plants are widely used in the food industry. They usually contain more than a single compound with antimicrobial activity. Hence, using extracts has as a consequence to take advantage of all active compounds present in extracts as reported Hao *et al.* (17). Several of ginseng constitute have been deemed responsible for the antimicrobial property (18). Saponin isolated from red ginseng was to inhibit the growth of *S. aureus* (19). Extraction is the first essential step for the isolation of

Table 1. Zone of inhibition (mm) of crude ginseng extracts (500 mg/mL) against microorganism[1)]

Solvent	Concentration (%)	Temp. (°C)	Type of ginseng	*S. epidermidis*	*L. monocytogenes*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhimurium*	*C. albicans*
Ethanol	100	25	W	-	-	-	-	-	8	-
			R	9	-	13	-	-	-	8
			E	7	-	8	-	-	8	9
		35	W	-	-	-	-	8	-	7
			R	-	-	12	-	10	7	10
			E	-	9	8	9	-	7	-
	80	25	W	-	-	-	-	-	-	-
			R	-	-	10	10	-	-	-
			E	-	-	-	-	8	-	-
		35	W	-	-	-	-	-	-	-
			R	-	-	11	-	-	-	-
			E	-	-	7	-	-	-	-
	60	25	W	-	-	9	-	-	-	-
			R	7	-	12	-	-	-	-
			E	-	-	-	-	-	-	-
		35	W	-	10	9	-	-	-	-
			R	-	-	12	-	8	-	-
			E	-	-	8	-	-	-	-
Methanol	100	25	W	-	-	8	-	-	-	-
			R	-	-	14	-	-	-	9
			E	7	-	8	-	-	8	8
		35	W	-	-	10	-	-	-	-
			R	-	-	12	-	-	-	9
			E	-	-	7	-	-	-	-
	80	25	W		-	-	14	-	-	-
			R	-	-	11	-	-	-	-
			E	-	-	-	-	-	-	-
		35	W	-	-	10	-	-	-	-
			R	-	-	11	-	-	-	-
			E	-	-	8	-	-	-	-
	60	25	W	-	-	9	-	-	-	-
			R	-	-	12	-	-	-	-
			E	-	-	9	-	-	-	-
		35	W	-	9	13	-	-	-	-
			R	-	-	12	-	-	-	8
			E	-	-	10	-	-	-	-
Gentamycin				27	28	29	28	26	27	NT
Streptomycin				25	24	25	23	20	23	NT
Tetracycline				10	29	26	26	14	30	NT
Ketoconazole				NT	NT	NT	NT	NT	NT	23

[1)]Ginseng extracts- W, white ginseng extract; R, red ginseng extract; E, extruded ginseng extract; -, no distinct zone of inhibition; NT, no test.

many bioactive components from the natural product. Other researchers reported that the extraction yield of ginsinoside in ethanol-water solution are the higher than extraction yields in water (20). Furthermore, the extraction yield of ginsenoside decreased slowly with increasing of solvent concentration. Our results show that ginseng extracts were slightly more active against Gram-positive than Gram-negative bacteria, as Gram-negative organisms are less susceptible to the action antibacterials. The basis of varying degree of sensitivity of test organisms of bacteria may to be due to the impermeability of membrane of Gram-negative bacteria, since the outer membrane acts as effective barrier against many hydrophobic substances including long-chain fatty acid (21,22)

In conclusion, the present study provided the potential antimicrobial properties of extracts from 3 kind ginseng powder (white, red, and extruded). The red ginseng extracts have shown better effect on antimicrobial, while extruded ginseng was more effective than white ginseng. The methanol extracts of different ginsengs possessed

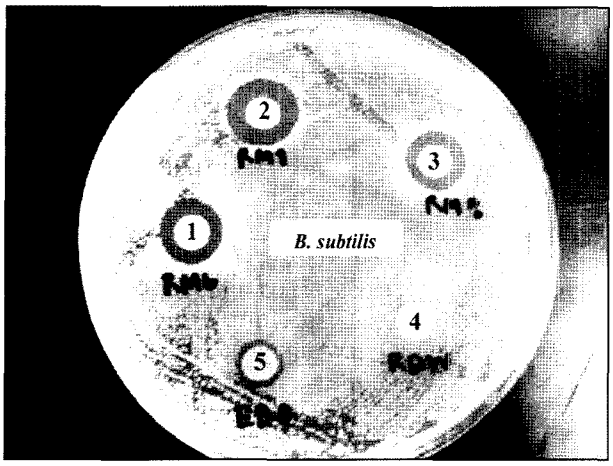

Fig. 4. The inhibition zone on MHA showing potential of some ginseng extracts for growth inhibition of *B. subtilis*. 1-4, Red ginseng extracted by 60% methanol, 100% methanol, 80% methanol, water, respectively; 5, 100% ethanol extruded ginseng extract.

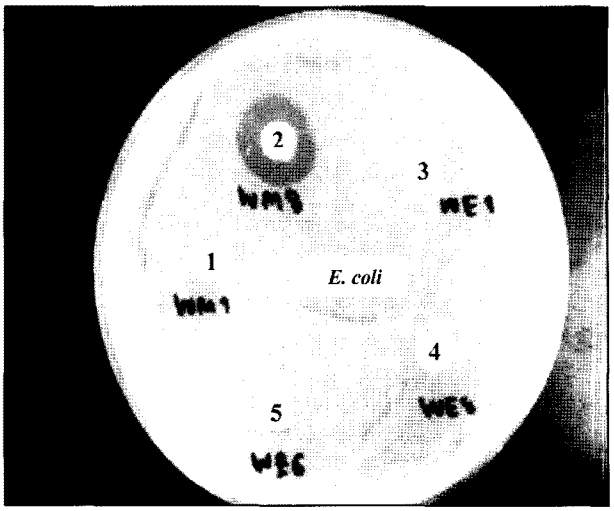

Fig. 5. The inhibition zone on MHA showing potential of some ginseng extracts for growth inhibition of *E. coli*. 1-5, White ginseng extracted by 100% methanol, 80% methanol, 100% ethanol, 80% ethanol, 60% ethanol, respectively.

broader spectrum of activity than extracts of ethanol with tested by dilution assay. This result considered that ginseng extract may be useful in controlling the number of food-borne and food spoilage microorganisms in the food system.

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Table 2. Susceptibility of ginseng extracts (white, red, and extruded ginseng) at 25°C to antimicrobials by dilution test[1)]

Ginseng	Solvent	Conc. (%)	*S. epidermidis*		*L. monocytogenes*		*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. typhimurium*		*C. albicans*	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
White	EtOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	>100	-	50	>100	100	>100	>100	-	>100	-	100	>100	>100	-
	MeOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	25	>100	50	100	50	>100	>100	-	>100	-	>100	-	>100	-
	DW		>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
Red	EtOH	100	100	>100	>100	-	25	100	>100	-	100	100	100	>100	50	50
		80	>100	-	>100	-	100	>100	>100	-	>100	-	100	>100	>100	-
		60	100	>100	6.25	25	>100	-	>100	-	>100	-	100	>100	6.25	>100
	MeOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	100	>100	50	100
		80	100	>100	50	>100	6.25	12.5	>100	-	>100	-	>100	-	6.25	100
		60	50	100	100	100	6.25	6.25	>100	-	>100	-	>100	-	50	>100
	DW		>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
Extruded	EtOH	100	>100	-	>100	-	>100	-	>100	-	50	100	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	50	>100	50	100	50	>100	>100	-	>100	-	100	>100	6.25	>100
	MeOH	100	>100	-	>100	-	50	100	>100	-	>100	-	>100	-	>100	-
		80	50	100	50	>100	>100	-	>100	-	100	>100	100	>100	>100	-
		60	50	>100	50	>100	12.5	50	>100	-	>100	-	100	>100	6.25	>100
	DW		>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-

[1)]MIC, minimum inhibition concentration; MBC, minimum bactericidal concentration; -, no test.

Table 3. Susceptibility of ginseng extracts (white, red, and extruded ginseng) at 35°C to antimicrobials by dilution test[1)]

Ginseng	Solvent	Conc. (%)	*S. epidermidis*		*L. monocytogenes*		*B. subtilis*		*E. coli*		*P. aeruginosa*		*S. typhimurium*		*C. albicans*	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
White	EtOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	>100	-	50	>100	>100	-	>100	-	>100	-	100	>100		
	MeOH	100	>100	-	>100	-	>100	-	>100	-	100	>100	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	25	>100	50	100	50	100	>100	-	>100	-	>100	-		
	DW		>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
Red	EtOH	100	25	100	>100	-	25	100	>100	-	100	100	100	>100	50	100
		80	>100	-	>100	-	100	>100	>100	-	>100	-	100	>100	>100	-
		60	100	>100	>100	-	50	>100	>100	-	>100	-	100	>100	12.5	>100
	MeOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	100	>100	50	100
		80	100	>100	25	100	6.25	12.5	>100	-	100	100	>100	-	6.25	100
		60	6.25	50	100	100	6.25	6.25	>100	-	>100	-	>100	-	>100	-
	DW		>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
Extruded	EtOH	100	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		80	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-	>100	-
		60	50	>100	50	100	25	100	>100	-	>100	-	100	>100	6.25	>100
	MeOH	100	50	50	50	>100	>100	-	>100	-	>100	-	>100	-	>100	-
		80	50	100	50	>100	>100	-	>100	-	100	>100	>100	-	>100	-
		60	50	>100	50	>100	50	100	>100	-	>100	-	100	100	6.25	>100
	DW		>100	-	>100	-			>100	-	>100	-	>100	-	>100	-

[1)]MIC, minimum inhibition concentration; MBC, minimum bactericidal concentration; -, no test.

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