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Original Research Article

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Gas chromatographic-mass spectrometric analysis, antimicrobial and antioxidant effects of ethanolic garlic extract

Na-Hye Park[1,2†], Hye Ri Jang[1,2†], Seung-Jin Lee[1], Naila Boby[1], Seung-Chun Park[1*]

***Corresponding author:**

Seung-Chun Park

[1]College of Veterinary Medicine, Kyungpook National University, 41566, Bukgu, Daegu, Republic of Korea.

† contributed equally

Abstract

To investigate the possibility of garlic extract as a feed additive for the prevention and treatment of *Salmonella* infection.

Garlic (*Allium sativum*) extracts were prepared by extracting fresh crushed garlic with different concentrations of ethanol. The extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). To examine their biological activity, antimicrobial analysis was carried out by measuring minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Salmonellaenterica* Typhimurium. Antioxidant activity was examined by ABTS radical scavenging assay.

The yield of garlic extract and the contents of antimicrobial components were confirmed to be acceptable when the ethanol concentration used for the extraction was below 75%. GC-MS analysis confirmed the predominant components of garlic extract to be allyl trisulfide, 2-hydroxy-gamma-butyrolactone, 1,3-dihydroxyacetone dimer, propanoic acid, and 2-propanone. The MIC was 10 mg/mL for all extracts and the IC50 (50% inhibitory concentration) was 1.6 mg/mL. The antioxidant activity was the highest for 20 mg/mL 100% ethanol garlic extract (82.1%).

Among the various ethanolic extracts, 75% ethanol extract was the most efficient in terms of the recovery rate and antimicrobial and antioxidant activities. Our data suggested that garlic extract can be used as a feed additive against salmonella infections.

Keywords: Garlic extract; Antimicrobial effect; Antioxidant Effect; feed additive

Introduction

Mortality caused by bacterial infection is the major cause of production damage in Korean farms [1]. Infection by *Salmonella enterica* Typhimurium, a gram-negative bacilli, usually occurs via the oral route and causes digestive system disorders in humans and animals [2]. *S. enterica* Typhimurium is found in pigs, chicken, and eggs; human infection occurs via the food chain. Although antibiotic treatment is used to prevent bacterial infection in livestock and used as growth promoters, this can induce side effects such as antibiotic resistance [3-5].

Recently, natural antibiotics from plant extracts have been reported as a substitute for conventional antibiotics with the purpose of suppressing the emergence of antibiotic-resistant bacteria. Among these plants, garlic has been reported to exhibit various pharmacological effects: improvement of arteriosclerosis and brain function; antimicrobial, anti-cancer, antiviral, and antioxidant activities; enhancing immunity and blood coagulation; and liver function recovery [6]. In particular, garlic has been reported to be effective against gram-positive, gram-negative, and acid bacteria [3-5]. Garba et al. [7] reported that the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of water extracts of garlic for *Escherichia coli* were 100 and 200 mg/mL, respectively. Saha et al. [8] reported that the MIC of garlic water extract (produced by extracting garlic with water)for *E. coli* ATCC 52922 was 700 μg/mL. Yoon et al. [9] compared the antimicrobial activities of garlic ethanol extracts at concentrations of 6.25, 12.5, 25, 50, and 100%. The antimicrobial activity of garlic is known to result from allicin (allyl 2-propenyl thiosulfinate), which is produced by the decomposition of the nonspecific sulfur amino acid allin (S-propenyl-L-cysteine sulfoxide) by allinase enzyme [10].

Previous studies have indicated that the major component of garlic is allicin, an organosulfur compound. Organosulfur compounds are sulfur-containing organic compounds, which are expected to have antibacterial effects similar to penicillin and sulfazoles [11]. Stoll et al. [12] reported that garlic was the major source of allicin (allyl 2-propenyl thiosulfinate), which was degraded by allinase into diallyl thiosulfinate and diallyl disulfide when garlic tissue was degraded

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[12]. Therefore, this study aimed to confirm the antimicrobial activity of the organosulfur compounds produced in the extraction process of garlic.

Although the antimicrobial activity of garlic extract has been tested against various bacteria, there have been no studies of their pharmacodynamic anti-*Salmonella* activity. In this study, the rate of production of organosulfur compounds based on the yield and ethanol concentration of the extracts was determined by using ethanol as an extraction solvent. Based on these results, the antimicrobial and antioxidant activities of the garlic extract were investigated according to ethanol concentration and their suitability as a feed additive to *S. enterica* typhimurium was determined.

Materials and methods

Preparation of garlic extract

Fresh garlic (*Allium sativum*) was homogenized and dried. Mixtures of ethanol and water (ethanol concentrations: 0, 25, 50, 75, and 100%) were added to dried garlic powder (3 g). The powder was extracted at room temperature for 3 h by an EYELA cute mixer CM-1000. The extract was centrifuged at 1500 rpm for 1 h and the supernatant was lyophilized. Finally, the samples were adjusted to a final concentration of 100 mg/mL with mixtures of 0, 25, 50, 75, and 100% ethanol and water, as appropriate, and filtered through an 11-μm filter (Whatman, UK).

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GS-MC) was used to analyze and compare the volatile components in the extracts. The GC-MS analysis of garlic extract was performed using an Agilent gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a 5975 GC-MS selective detector (Agilent Technologies).

Agilent J & B DB-5MS was used as the column (30 m length 0.25 mm i.d. and 0.25-m film thickness fused silica capillary column). The column temperature was maintained at 70 C for 1 min, 300 C for 20 min, and analyzed for a total of 6 h. The inlet and detector temperatures were 250 C and helium was used as carrier gas to maintain a flow rate of 1 mL/min. The injection method was analyzed in splitless mode. The ionization energy of the mass selective detector was 70 eV and the scanning mass range was 10–800 m/z [13].

Measurement of MIC and MBC

MIC values of the garlic extracts and colistin, used as a control antibiotic, were determined by the broth microdilution method of Clinical Laboratory Standard Institutes (CLSI) against the *S. enterica* Typhimurium ATCC14028 strain. The highest concentrations of garlic extract and colistin were 2048 and 1024 μg/mL, respectively.

S. enteric Typhimurium (105 CFU/mL) was diluted and incubated overnight at 37 C. *E. coli* ATCC 52922 was used as a quality control strain. The MIC was measured as the lowest concentration at which no bacterial growth occurred. The MBC was defined as the minimum concentration of antibiotics that reduced the number of pathogens by at least 99.9% [14].

ABTS radical scavenging activity test for antioxidant effect

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay is used to confirm the antioxidant activity. Because it has free radicals in its oxidized form, the compound is an electron donor. Specifically, 7.0 mmol ABTS ammonium salt and 2.45 mmol potassium persulfate were added to distilled water and left at RT for 12–16 h to obtain a dark blue solution, which loses its color when reacted with antioxidant substances [15]. In each well of a 96-well plate, 10 μL/well of the serially diluted sample was added, followed by 200 μL/well of 7 mM ABTS solution. The reaction was allowed to proceed at RT for 6 min in the absence of light, and the absorbance was measured at 734 nm by using an ELISA reader (VersaMax ELISA Microplate Reader CC, USA). Trolox was used as a standard. The antioxidant capacity was calculated using the following formula [16].

ABTS radical scavenging activity (%) = {1-(Control OD-Sample OD)/Control OD} 100

Control OD: Optical density of ABTS solution without sample at 734 nm

Sample OD: Optical density of ABTS solution with sample at 734 nm

The IC50 (half-maximal inhibitory concentration) was used to measure the potency of the biological function of garlic extract. It is the concentration of the substance that can result in 50% inhibition: the higher the IC50 value, the lower the antioxidant capacity.

Results and discussion

This study aimed to confirm the antimicrobial and antioxidant activity of garlic extracts as feed additive after the determination of the ethanol concentration suitable for extraction, according to the yield.

The recovery rate of garlic extract in several elution solvents

Previous studies [14] compared the extracts of different polarity solvents, including water, alcohol, chloroform, and petroleum ether. The results indicated that the ethanol and petroleum ether extracts contained high levels of allicin [17]. However, their experiment did not confirm the yield in relation to various ethanol concentrations and their respective antimicrobial and antioxidant activities. Therefore, this experiment was conducted to confirm the optimization ofgarlic extraction based on ethanol content.

The yields of the 25%, 50%, 75%, and 100% ethanol garlic extracts were compared with those of the water extracts (Table 1). Ethanol is known to be an effective solvent for the extraction of both hydrophobic and hydrophilic materials of plants [18]. The recovery rates of the water extract and 25, 50, and 75% ethanol extracts were not significantly different (all 19–22%). However, the 100% ethanol extract produced a recovery of 4.0%.

Table 1. Yields of different garlic extracts (*Allium sativum*) from solutions with different ethanol concentrations

Ethanol concentration (%)	0	25	50	75	100
Yield[a] (%)	21.7±1.86	21.2±3.07	19.1±2.54	20.9±1.19	4.0±0.46

[a]The yield is the ratio of the amount actually produced when a target substance is obtained from a raw material through a certain chemical process and is generally expressed as a percentage. The data represent the mean values ± SD (standard deviation) of three replicates. 100 mg/mL garlic extracted powder.

Gas chromatography-mass spectrometry analysis

The physiologically active components of ethanol garlic extracts were compared through GC-MS analysis (Table 2). The results showed that allyl trisulfide, 2-hydroxy-gamma-butyrolactone, 1 3-dihydroxyacetone dimer, propanoic acid, and 2-propone were detected in all extracts (Table 3 and Fig. 1). These substances are produced by the degradation of alline [10, 12] and have been reported to have beneficial effects, such as antioxidant and anticancer activities [19-26].

The highest content (11.56%) of allyl trisulfide was found in the water extract and the lowest content (2.73%) was found in the 100% ethanol garlic extract. The 1,3-dihydroxyacetone dimer was found to increase in proportion to the ethanol concentration in all extracts except for the 100% ethanol garlic extract. The highest value (19.8%) was observed in 75% ethanol garlic extract. In contrast, the 2-hydroxy-gamma-butyrolactone content was 4.94 ± 1.08%, irrespective of ethanol concentration.

Therefore, 75% ethanol garlic extract was the most suitable for the extraction of the physiologically active ingredients. With the exception of 100% ethanol extract, a higher concentration of ethanol tended to produce a general increase in physiologically active ingredients.

Both the yield and GC-MS analysis indicated that 75% ethanol concentration was the most suitable for extraction.

Table 2. Phyto-components identified by GC-MS analysis of garlic ethanol extracts of varying concentrations

Parameter	RT (min)	Name of compound	Peak area (%)
0% ethanol garlic extract	24.57	Trisulfide, allyl trisulfide	11.56
	30.46	1,3-Dihydroxyacetone dimer	7.58
	32.12	2-Hydroxy-gamma-butyrolactone	4.64
	16.53	Acetic acid	3.59
	16.76	Propanoic acid	5.84
	19.96	Trisulfide, 1-allyl-3-methyltrisulfane	3.13
	24.15	2-Methoxy imino imidazolidine	5.91
	12.07	2-Propanone	3.04
	28.97	2-Propenoic acid	18.84
	36.06	3,6,9,12-Tetraoxatetradecan-1-ol	4.83
25% ethanol garlic extract	24.58	Trisulfide, allyl trisulfide	10.22
	30.46	1,3-Dihydroxyacetone dimer	10.54
	32.12	2-Hydroxy-gamma-butyrolactone	4.93
	12.56	Acetaldehyde	10.6
	28.97	Cyclododecane	9.38
	17.23	Diallyl disulfide	3.18
	16.77	Propanoic acid	8.38
	44.2	1,2-Propanediol	3.09
	24.15	2-Methoxyiminoimidazolidine	5.67
	12.08	2-Propanone	5.16

50% ethanol garlic extract	24.57	Trisulfide, allyl trisulfide	6.04
	30.46	1,3-Dihydroxyacetone dimer	12.86
	32.12	2-Hydroxy-gamma-butyrolactone	6.4
	12.55	Acetaldehyde	14.66
	30.57	Acetamide	2.34
	16.54	Acetic acid	4.25
	28.98	Dodecyl acrylate	4.63
	16.77	Propanoic acid	9.45
	24.15	2-Methoxyiminoimidazolidine	5.57
	12.09	2-Propanone	9.34
75% ethanol garlic extract	24.58	Trisulfide, allyl trisulfide	5.09
	30.46	1,3-Dihydroxyacetone dimer	19.77
	32.12	2-Hydroxy-gamma-butyrolactone	5.33
	12.54	Acetaldehyde	10.78
	16.51	Acetic acid	8.67
	16.76	Propanoic acid	8.69
	47.54	Heptadecane	7.31
	44.2	Hexadecanoic acid	3.98
	12.08	2-Propanone	8.18
	33.86	4H-Pyran-4-one	5.16
100% ethanol garlic extract	32.12	2-Hydroxy-gamma-butyrolactone	3.41
	12.55	Acetaldehyde	6.06
	16.47	Acetic acid	8.96
	30.47	Dihydroxyacetone	14.43
	16.76	Propanoic acid	4.98
	44.19	n-Hexadecanoic acid	3.59
	12.08	2-Propanone	6.71
	37.84	3-Furancarboxaldehyde	4.2
	33.86	4H-Pyran-4-one	6.09
	48.33	9,12-Octadecadienoic acid	3.14

Table 3. Common phytocomponents identified in extracts of garlic (*Allium sativum*)

Name of compound	Activities	Reference
Allyl trisulfide	Anti-cancer effects, platelet aggregation, blood pressure reduction, antioxidant,	[19-22]
	cholesterol lowering, papain inactivation, detoxification, glutathione transferase activity	
1,3-Dihydroxyacetone dimer	Catalyzes the formin reaction	[23]
2-Hydroxy-gamma-butyrolactone	Antioxidant, analgesic, anti-diabetic, antibacterial, and antifungal activity	[24]

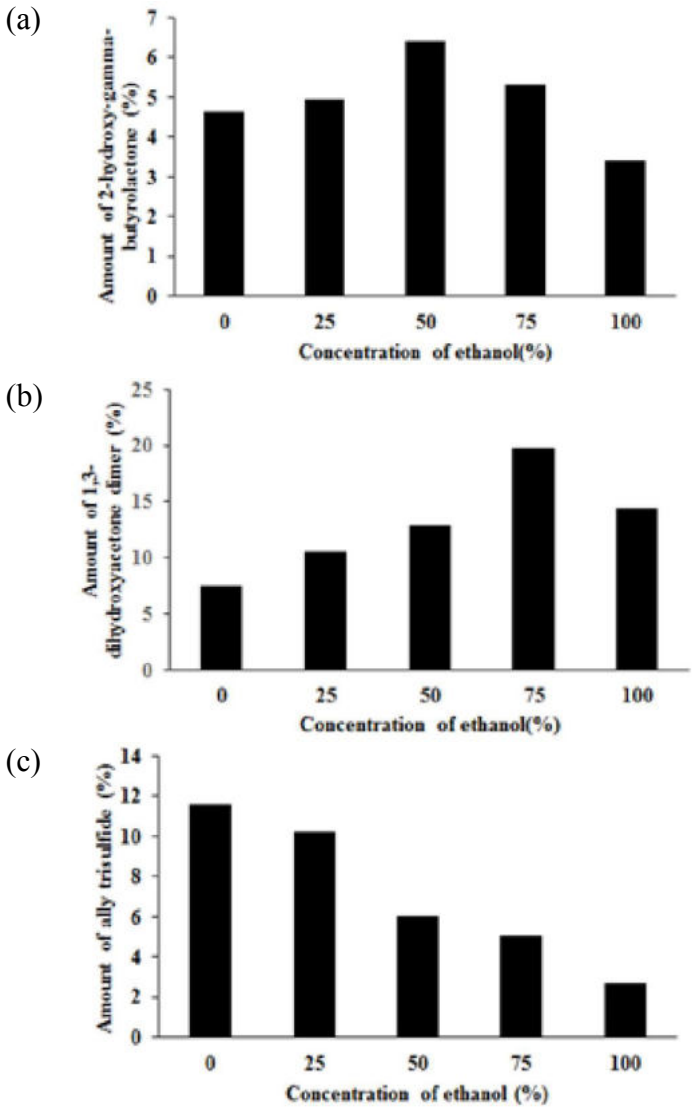

Figure 1. Relative contents (%) of common compounds in different concentrations of garlic extract: ally trisulfide (A); 2-hydroxy-gamma-butyrolactone (B); 1,3-dihydroxyacetone dimer.

Antibacterial effect of ethanol extract

In order to classify the antimicrobial activity of ethanol extracts according to their concentration, the antimicrobial activity of water, 25%, 50%, 75%, 100% ethanol garlic extract, and the antibiotic colistin was compared. The antimicrobial activity of garlic extract on Salmonella was 0.025 MIC and 0.05 MBC of colistin. Although the antimicrobial effect was lower than that of the antibiotic, it was similar to that reported previously for plant extracts [5]. In this study, the MIC of all ethanol extracts was 10 mg/mL. Additionally, the MBC of all ethanol extracts was confirmed to be greater than 20 mg/mL, except for the 100% ethanol garlic extract (Table 4). The MBC value was the highest for 100% ethanol extract, but was not significantly different from that of the other concentrations. As mentioned above, the substances responsible for the antimicrobial activity of the garlic extract were identified as trisulfide and 2-hydroxy-gamma-butyrolactone (Table 3). Except for the 100% ethanol extract and 2-hydroxy-gamma butyrolactone, a higher ethanol concentration resulted in a higher composition of the antimicrobial contents (Table 2). Therefore, there were no significant differences in antimicrobial activity at all concentrations owing to the relative difference according to the ethanol concentration of these two components.

Thus, although the antimicrobial activity of garlic was confirmed, the effect of ethanol concentration on the antimicrobial activity was not identified. Additionally, antioxidant analysis was conducted to

investigate the optimum extraction conditions for the use of garlic extracts as a feed additive.

Table 4. Antimicrobial activities of colistin and different concentrations of garlic extracts against *S. enterica* typhimurium

	Colistin	Ethanol concentration (%)*				
		0	25	50	75	100
MIC (mg/mL)	0.25	10	10	10	10	10
MBC(mg/mL)	1 ± 0.0	> 20	> 20	> 20	> 20	20

*Ethanol concentration in solvent, which includes 100 mg/mL ethanol garlic extract (*A. sativum*)

Antioxidant activity of ethanol extracts

The antioxidant activities of the extracts at various ethanol concentrations are summarized in Table 5. The antioxidant activity of 100% ethanol garlic extract was 82.07%, which was approximately equivalent to 3.5 mM trolox. For the 100% ethanol garlic extract, 10 mg/mL showed antioxidant activity similar to that of 1.8 mM trolox. The results of this test indicated that the ethanol extract exhibit a concentration-dependent antioxidant activity.

The treatment with Trolox (0.117, 0.234, 0.469, 0.938, 1.875, 3.75, 7.5, and 15 mM) revealed an IC50 value of 1.611 mM (Figure 2). The IC50 values of ethanol garlic extract are presented in Table 5; a higher concentration of ethanol garlic extract resulted in a higher antioxidant effect. According to the study by Son et al. [25], the antioxidant activity of the black garlic was higher than that of the control. However, the IC50 value in the DPPH radical scavenging assay was 1.07 mg/mL. It was presumed that the antioxidant activity was reduced by the reaction [25, 26]. Therefore, the consumption of large amounts of garlic, which can promote antioxidant effects from unheated garlic and prevent cancer and aging, is recommended.

Table 5. The IC50 (half-maximal inhibitory concentration) of different concentrations of ethanol garlic extracts against ABTS free radicals

	IC_{50} of ABTS free radical (mg/mL)				
Ethanol percentage	0	25	50	75	100
IC_{50} (mg/mL)	71	41	23	20	18

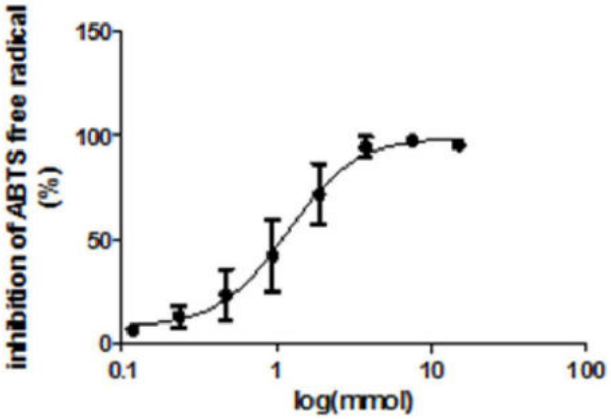

Figure 2. IC50 values for inhibitors in TECA test of trolox

Conclusion

This study was performed to investigate the possibility of using garlic extracts as feed additives with antimicrobial activity to prevent salmonella food poisoning. Through analysis of the yields and GC-MS, the ethanol concentration range with the optimum extraction efficiency was 0–75% (yield 17–22%). However, the antimicrobial activity of garlic extracts did not significantly differ according to the ethanol concentration. This is because the content of trisulfide and 2-hydroxy-gamma-butyrolactone, which are antibacterial substances, was unchanged, as shown through GC-MS analysis (Table 2). The results of the antioxidant experiments conducted to confirm the effects as feed additives showed that the antioxidant activity of garlic extracts increased as the ethanol concentration increased (Table 5). In conclusion, 75% ethanol garlic extract was considered to be the most suitable feed additive in terms of the overall yield, antimicrobial effect, and antioxidant activity.

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Conflict of interest

Authors declare that they have no conflict of interest.

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