

Full Length Research Paper

Shallot (*Allium ascalonicum* L.) oil: Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria

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Shallot (*Allium ascalonicum* L.) oil was studied for its major diallyl sulfide content and its antimicrobial activity against food-borne pathogenic bacteria including *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, and *Vibrio cholerae*. The oil had a very low concentration of diallyl monosulfides (1.59%) in comparison with the other diallyl sulfides (24.66% for diallyl disulfide, 16.08% for diallyl trisulfide, and 10.88% for diallyl tetrasulfide). Shallot oil and all four major diallyl sulfides inhibited all of the test bacteria. Among them, *E. coli* O157:H7 and *B. cereus* was the most and the least sensitive strains, respectively. The oil had a bacteriocidal effect on *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *V. cholerae* but had a bacteriostatic effect on *B. cereus* and *S. enterica*.

Key words: Antimicrobial activity, diallyl sulfide, shallot oil.

INTRODUCTION

Food-borne pathogenic bacteria are bacteria associated with foods and causing illness. Many species of bacteria falls in this group including *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Vibrio cholerae* and *Yersinia enterocolytica*. Patients suffering from the illness can develop one or more of the following: nausea, abdominal pain, vomiting, diarrhea, gastroenteritis, fever or even death. Severity of the symptoms depends on types of infecting pathogens and health status of infected persons (Buzby, 2001).

Fresh, raw and minimally cooked foods are main resources of food-borne pathogenic bacteria. They have been consumed widely both in developed and developing countries. Thus, food-borne disease outbreaks can be occurred in every parts of the world. The disease not only affects people's health and well-being, but it also has an economic impact on individuals and countries. A large amount of money has been spent to prevent and cure the disease. For example, in the United States, such bacteria

are responsible for over 76 million cases of food-borne illness and 323,000 hospitalizations at a cost of approximately 7 - 10 billion US dollars annually (Mead et al., 1999).

Currently, prevention of food contamination with food-borne pathogenic bacteria relies mainly on a broad spectrum of antimicrobial chemicals such as chlorine, chloride oxide, acidified, sodium chlorite, trisodium phosphate and cetylpyridinium chloride (Oyarzabal, 2005; Ricke et al., 2005). However, these synthetic chemicals do not meet increasing consumers' demand for natural food products. Furthermore, safety of chemical additives has become a major concern of consumers because of their toxicity reported recently (Veschetti et al., 2003). For these reasons, the exploration of natural and safe antimicrobial substances to replace synthetic chemicals receives increasing attention. Plant essential oils are potential alternatives because many of them have been a part of the human diet for hundreds of years and thus have been generally recognized as safe (GRAS) by the Food and Drug Administration (FDA).

Shallot (*Allium ascalonicum* L.) belongs together with garlic (*Allium sativum*), onion (*Allium cepa*), chive (*Allium schoenoprasum*) and shallot (*Allium oschaninii*) to the *Alliaceae* family. It has been used in many diets and in folk medicine since ancient time. Shallot is recognized for

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Table 1. Contents of diallyl sulfides in shallot oil.

Sulfide compound	Concentration	
	($\mu\text{g/g}$) ^a	(%) ^b
Diallyl monosulfide	79.3 \pm 3.02	1.59
Diallyl disulfide	1,228.6 \pm 10.41	24.66
Diallyl trisulfide	800.9 \pm 7.23	16.08
Diallyl tetrasulfide	542.1 \pm 6.65	10.88
Total sulfides	4982.0 \pm 11.15	
Percent of diallyl sulfides ^c	53.2	

^a Results are mean \pm S.D. values of five replicates.

^b Calculated from (concentration of each diallyl sulfides \times 100)/total sulfides.

^c Calculated from (sum of four diallyl sulfides \times 100)/total sulfides.

its hypoglycemic (Jalal et al., 2007), hypocholesterolemic (Tappayuthpijarn et al., 1989) and antioxidant (Leelarungrayub et al., 2006) effects. It was also reported to have anti bacteria and antifungal activity (Amin and Kapadnis, 2005). The health related and antimicrobial effects of plants in *Allium* species are believed to depend substantially on four diallyl sulfides including diallyl monosulfide (DMS), diallyl disulfide (DDS), diallyl trisulfide (DTS) and diallyl tetrasulfide (DTTS) which are the sulfur-containing compounds found in the plants (Block et al., 1992; Kim et al., 2004). In this study, shallot oil was examined for its diallyl sulfides content, antimicrobial activity and mode of action against several strains of food-borne pathogenic bacteria.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacteria used in this study were *B. cereus* ATCC 4342, *C. jejuni* ATCC 49349, *E. coli* O157:H7 ATCC 35159, *Listeria monocytogenes* ATCC 19111, *S. enterica* ATCC 8326, *S. aureus* ATCC 15923, and *V. cholerae* ATCC 14101. All of them were grown at 37°C in BHI (Brain Heart Infusion) broth. Bacterial stock cultures were stored as frozen cultures at -80°C in BHI broth containing 20% glycerol (v/v).

Plant materials and sample preparation

Shallot (*Allium ascalonicum* L.) was purchased from herb shops in Ubon Ratchathani Province, Thailand. Its essential oil was prepared according to the method described by Ravid and Putievsky (1985). Fresh plant materials were steam distilled for 3 h in a 100 L direct steam pilot plant apparatus. The recovered oil (about 4.2 - 2.5 g/kg of elephant garlic) was stored at -80°C until use.

Preparation of diallyl sulfides

Four diallyl sulfides (DMS, DDS, DTS, and DTTS) were obtained as follows. DMS (purity 97%) were purchased from Aldrich Chemical (Milwaukee, WI). DDS, DTS, and DTTS were prepared by fractional distillation from crude DDS (purity 80%) obtained from Aldrich Chemical. Identification of DTS and DTTS was confirmed by the

method of Sparrins et al. (1988). All of the diallyl sulfides were stored at -80°C until use.

Analysis of diallyl sulfides in shallot oil

One mg of shallot oil was redissolved in 10 ml of acetonitrile immediately before compositional analysis by the method of Lawson et al. (1991). Total sulfides and the four major diallyl sulfides of shallot oil were quantified by reverse-phase high performance liquid chromatography set at 240 nm with a Supelcosil LC-18, 250 \times 4.6 mm \times 5 μm column. The mobile phase used was acetonitrile: water: tetrahydrofuran (70:27:3) at a flow rate of 1 ml/min.

Antimicrobial activity determination

Shallot oil, DMS, DDS, DTS, and DTTS were examined for their antimicrobial activities against the food-borne pathogens named above using the microtiter broth microdilution method described by Amsterdam (1996) with some modifications. Briefly, the tested compounds were initially adjusted to 200 $\mu\text{g/ml}$ and then subjected to a doubling dilution series in a microtiter plate containing BHI broth. The bacteria to be tested (5 μl aliquots) were added to the wells containing the diluted compounds to obtain a final concentration of 10^4 CFU/ml. Controls (without tested compounds and without tested bacteria) were included for each plate. After incubation at 37°C, bacterial growth was inspected at 24 and 48 h. Results were reported as the minimal inhibitory concentration (MIC) required causing no growth of the bacteria. Each MIC value was obtained from five experiments.

Examination of mode of action

Shallot oil (at a final concentration equal to the MIC value) was added to 4.9 ml of bacterial cultures (10^3 CFU/ml). After incubation at 37°C for 24 h, 100 μl of the mixtures were inoculated into 4.9 ml of fresh BHI broth. As a control, 100 μl of untreated cultures of bacteria at a concentration of 10^3 CFU/ml were transferred to 4.9 ml of fresh BHI broth. The optical density at a wavelength of 600 nm ($\text{OD}_{600\text{nm}}$) of the tested and control cultures was determined at the time of inoculation and after incubation at 37°C for 24 and 48 h.

RESULTS AND DISCUSSION

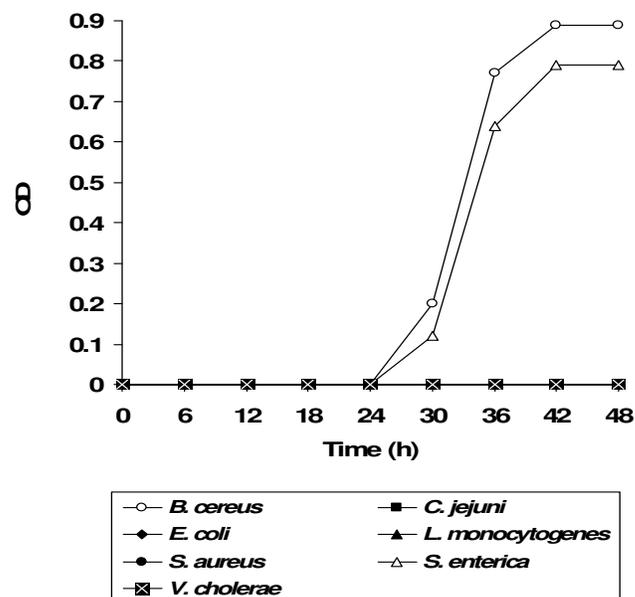
The concentrations of the four major diallyl sulfides (DMS, DDS, DTS, and DTTS) and total sulfides in shallot oil were determined (Table 1). Approximately half of the total sulfides found in the shallot oil (53.2%) were the four major sulfides, among which DDS and DMS were the most and the least abundant respectively. The percentage of diallyl sulfides in shallot oil from this study was very similar to that in garlic oil reported by Lawson et al. (1991), Tsao and Yin (2001), and Rattanachaikunsopon and Phumkhachorn (2008) which were 54.4, 52.7 and 53.6% respectively, but higher than that in Chinese leek oil (41.7%) (Tsao and Yin, 2001) and chives oil (42.3%) (Rattanachaikunsopon and Phumkhachorn, 2008). Shallot oil was able to inhibit all tested bacterial strains (Table 2). The MIC values varied depending on strains of bacteria. Of all of the tested bacteria, *E. coli* O157:H7 was the most sensitive strains to shallot oil whereas *B.*

Table 2. MICs of shallot oil and standard diallyl sulfides against various strains of food-borne pathogenic bacteria.

<i>V. cholerae</i>	MIC ($\mu\text{g/ml}$)				
	Shallot oil	DMS	DDS	DTS	DTTS
<i>Bacillus cereus</i> ATCC 4342	25	100	12.5	1.57	0.4
<i>Campylobacter jejuni</i> ATCC 49349	12.5	50	6.25	0.78	0.4
<i>Escherichia coli</i> O157:H7 ATCC 35159	3.13	25	1.57	0.2	0.05
<i>Listeria monocytogenes</i> ATCC 19111	6.25	50	3.13	0.78	0.2
<i>Salmonella enterica</i> ATCC 8326	12.5	50	6.25	0.78	0.4
<i>Staphylococcus aureus</i> ATCC 15923	6.25	25	3.13	0.4	0.2
<i>Vibrio cholerae</i> ATCC 14101	6.25	50	3.13	0.78	0.2

cerus was the least sensitive one. When the standard DMS, DDS, DTS and DTTS were tested for their antimicrobial effects against all of the bacterial strains, a wide range of MICs was observed (Table 2). The values reduced with each additional sulfur atom. Of all diallyl sulfides examined, DMS was the only diallyl sulfide showing a higher MIC against all of the bacteria than shallot oil. Since concentration of DMS in shallot oil was very low (Table 1) and its antimicrobial activity against was quite weak (Table 2), its contribution to antimicrobial activity of the oil could be negligible. Therefore, the other three diallyl sulfides (DDS, DTS and DTTS) were major agents in shallot oil responsible for its antimicrobial activity.

This study showed that the MICs of DMS, DDS, DTS and DTTS varied depending on bacterial strain. For example, the MICs of DMS, DDS, DTS and DTTS for the inhibition of *E. coli* O157:H7 were 25, 1.57, 0.2 and 0.05 $\mu\text{g/ml}$, respectively whereas those of *B. cereus* were, 100, 12.5, 1.57 and 0.4 $\mu\text{g/ml}$, respectively. Furthermore, the MICs of DMS, DDS, DTS and DTTS were also dependent on the determination method. This statement was supported by the comparison of the MICs of DMS, DDS, DTS and DTTS for the inhibition of *E. coli* O157:H7 obtained from this study with those obtained from our previous study which was 72, 20, 12, and 4 $\mu\text{g/ml}$ respectively (Rattanachaikunsopon and Phumkhachorn, 2008). In this study, we used BHI broth instead of Mueller-Hinton broth, and we used 10^4 CFU/ml instead of 5×10^5 CFU/ml as the initial concentrations of the test bacteria.

**Figure 1.** Recovery in fresh BHI broth of food-borne pathogenic bacteria inhibited by shallot oil.

When the test bacteria inhibited by shallot oil were transferred to fresh BHI broth, different results were observed. *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *V. cholerae* did not resume their growth in the fresh BHI broth within 24 and 48 h (Figure 1). These results suggest that shallot oil has bacteriocidal mode of action on these bacteria. On the other hand, *B. cereus* and *S. enterica* were able to grow in the fresh BHI broth within 24 and 48 h (Figure 1), indicating the bacteriostatic mode of action of the oil on both strains. Explanation on the difference in mode of action of shallot oil on different bacterial strains is still mysterious. To clarify this matter, further investigations are required.

The findings presented in this report showed that the shallot oil has potential to be used as natural antimicrobial substance to improve the safety of foods. Its use for inhibiting food-borne pathogenic bacteria in food models are now ongoing in our laboratory.

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