Diallyl Sulfide Content and Antimicrobial Activity against Food-Borne Pathogenic Bacteria of Chives (Allium schoenoprasum)

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Chives, a member of the Alliaceae family, have been used in food and medicine in Thailand for a long time. Diallyl sulfides (diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide) are believed to be responsible for the antimicrobial activity of plants in this family. In this study, chive oil was examined for its diallyl sulfide content and its antimicrobial activity against some strains of food-borne pathogenic bacteria. Chive oil had a very low concentration of diallyl monosulfide in comparison with the other diallyl sulfides. They inhibited all pathogenic bacteria used in this study with a different degree of inhibition. Chive oil was also shown to be able to inhibit Escherichia coli O157:H7 in a food model. This study is the first report describing not only the diallyl disulfide content of chive oil, but also its antimicrobial activity against food-borne pathogens in both a test tube and food model.

Key words: chives; food-borne pathogenic bacteria; diallyl sulfide

The bacteria associated with food and causing illness are called food-borne pathogenic bacteria. They comprise both gram negative and gram positive bacteria. Common foodborne bacterial pathogens are Bacillus cereus, Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Salmonella spp., Shigella spp., Staphylococcus aureus, Vibrio cholerae and Yersinia enterocolytica. Contamination of food with these bacteria usually arises from improper handling, preparation or storage of food. Symptoms of bacterial infection typically occur one day or more after the ingestion of contaminated food. Patients can develop one or more of the following: nausea, abdominal pain, vomiting, diarrhea, gastroenteritis, fever or even death.1

In Thailand, food-borne illness is very common because of bad hygiene practices and the habit of eating raw or partially cooked food. It not only affects people’s health and well-being, but it also has an economic impact on individuals and the country. Food-borne disease outbreaks from such bacteria as Escherichia coli impose a substantial burden on the health-care system and markedly reduce the economic productivity of the country. Treatment for such illness still relies on antibiotics which are expensive and unsafe. Prolonged and improper use of antibiotics can lead to bacteria developing drug resistance which can be transferred to environmental and other human pathogenic bacteria.2–5 Finding an alternative treatment for food-borne disease has recently become an urgent issue. Plants and herbs are among the potential options because of their natural origin and antimicrobial activity.6–12

Chives (Allium schoenoprasum) are bulb-forming herbaceous perennial plants belonging, together with garlic, onion, shallot and leek, to the Alliaceae family. They can be easily grown with little care and found fresh throughout Thailand all year-round. They are grown for their leaves which are used for both culinary and medicinal purposes. They are used for culinary purpose as a condiment providing a somewhat milder flavor than that of neighboring Allium species. They are used in traditional dishes in many countries such as Thailand, France and Sweden. They have a beneficial effect on the circulatory system with medicinal use, acting upon it by lowering the blood pressure. They are used to relieve the pain from sunburn and a sore throat. Reports concerning the antimicrobial activity of chives, especially against fungi, can be found, although they are scarce. Diallyl sulfides (diallyl monosulfide, diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide), which are the sulfur-containing compounds found in chives, are believed to be responsible for the health-promoting effects and antimicrobial activity.13,14

Unlike other members in the Alliaceae family such as garlic and onion, little attention has been paid to the antimicrobial property of chives. It is therefore interest-
ing to study that property of chives which are considered to be herbaceous plants like garlic. In this study, chive oil was examined for its diallyl sulfide content and antimicrobial activity against food-borne pathogens and its characteristics compared with those of garlic oil. The antimicrobial activity in a food model of chive oil against *E. coli* O157:H7, a common pathogenic bacteria found in Thai food, is also reported in this study.

**Material and Methods**

**Bacterial strains and culture conditions.** The bacteria used in this study were *Bacillus cereus* ATCC 4342, *Campylobacter jejuni* ATCC 49349, *Clostridium botulinum* ATCC 19397, *Escherichia coli* O157:H7 ATCC 35150, *Listeria monocytogenes* ATCC 19111, *Salmonella enterica* ATCC 8326, *Staphylococcus aureus* ATCC 25923, and *Vibrio cholerae* ATCC 14101. All of them were obtained from American Type Culture Collection (ATCC). Unless otherwise stated, all the bacteria used in this study, except for the *Clostridium* strain, were grown at 37 °C in BHI (brain heart infusion) broth. The *Clostridium* strain was propagated anaerobically in a cooked meat medium at 37 °C. Bacterial stock cultures were stored as frozen cultures at −80 °C in appropriate culture media containing 20% glycerol (v/v). Throughout the experiments, the strains were subcultured every 2 weeks on an agar medium and kept at 4 °C. Before use, a liquid culture prepared from a single colony was transferred twice into the appropriate broth and incubated according to the conditions was stated.

**Plant materials and sample preparation.** Fresh chives (*Allium schoenoprasum*) and garlic (*Allium sativum*) were purchased from herb shops in Ubon Ratchathani province, Thailand. Leaves of the chives and bulbs of garlic were used for preparing essential oils according to the method described by Ravid and Putievsky.\(^{15}\) Each fresh plant material was steam-distilled for 3 h in a 100 liters direct steam pilot plant apparatus. The oil recovered from chives (about 1.5–3.8 g/kg of chive leaves) and from garlic (about 2.4–4.5 g/kg of garlic bulbs) was stored at −80 °C until needed for use.

**Preparation of diallyl sulfides.** Four diallyl sulfides (diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide) were used. Diallyl monosulfide (97% purity) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Diallyl disulfide, diallyl trisulfide and diallyl tetrasulfide were each prepared by fractional distillation from crude diallyl disulfide (80% purity) obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Diallyl trisulfide and diallyl tetrasulfide were identified by the method previously described.\(^{16}\) Each diallyl sulfide was stored at −80 °C until needed for use.

**Analysis of diallyl sulfides in chive and garlic oils.** One mg of essential oil prepared from each plant was redissolved in 10 ml of acetonitrile immediately before a compositional analysis by the method of Lawson, Wang and Hughes.\(^{17}\) Total sulfides and all four diallyl sulfide components of chive and garlic oils were quantified by using reverse-phase high-performance liquid chromatography set at 240 nm with a Supelcosil LC-18, 250 mm × 4.6 mm × 5 μm column. The mobile phase used was acetonitrile:water:tetrahydrofuran (70:27:3) at a flow rate of 1 ml/min. The total sulfides measured were diallyl monosulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, diallyl pentasulfide, diallyl hexasulfide, methyl allyl disulfide, methyl allyl trisulfide, methyl allyl tetrasulfide, methyl allyl pentasulfide, methyl allyl hexasulfide, dimethyl trisulfide, dimethyl tetrasulfide, and dimethyl pentasulfide.

**Examination of the antimicrobial activity.** The antimicrobial activities of chive oil, garlic oil, diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide were examined against the food-borne pathogens already mentioned. Tetracycline was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and included in this experiment. The minimal inhibitory concentration (MIC) was determined by the broth microdilution method. Aerobic isolates were prepared for inoculation with cation-adjusted Mueller-Hinton broth. Anaerobic isolates were prepared for inoculation with Wilkins-Chalgren anaerobe broth. Microdilution wells were inoculated with approximately 5 × 10^5 CFU/ml for aerobes and approximately 1 × 10^6 CFU/ml for anaerobes. The plates were incubated for at least 16 h at approximately 37 °C for aerobes and for at least 46 to 48 h at approximately 37 °C in an anaerobic atmosphere for the anaerobes. Each MIC value was obtained from five experiments.

**Antimicrobial activity of chive oil in a food model.** Boiled chicken breasts were sterilized by autoclaving at 121 °C for 10 min and then cut aseptically into portions of 10 g. Each piece of meat was rubbed throughout with chive oil, except that used as the control. To each food sample was applied a suspension of *E. coli* O157:H7 (about 10^4 CFU/g of food), before massaging briefly to distribute the bacterial inoculum. The inoculated food sample was packaged in a vacuum bag before being incubated at 37 °C.

To prepare a suspension of the *E. coli* O157:H7 inoculum, a culture of *E. coli* O157:H7 in the exponential phase in BHI broth was harvested, washed once in 0.9% NaCl, and resuspended in the same solution. The bacterial suspension was stored at 4 °C. At various time points, each inoculated food sample was aseptically transferred to a sterile Stomacher bag, and 10 ml of 0.9% NaCl was added to the bag. After homogenizing in Stomacher apparatus for 30 s, dilutions of each sample in 0.9% NaCl aq. were subjected to a plate counting assay.
Results

Analysis of diallyl sulfides in chive and garlic oils

The concentrations of diallyl sulfides in the chive oil and garlic oil are shown in Table 1. Chive oil contained each of the diallyl sulfides at a lower level than that in garlic oil. In both essential oils, the diallyl sulfides having the highest and lowest concentrations were diallyl disulfide and diallyl monosulfide, respectively. Although the concentrations of total diallyl sulfides in chive oil (4,892 ± 18.4 μg/g) and garlic oil (4,966 ± 20.7 μg/g) were similar, the percentage of all diallyl sulfides in chive oil (42.3%) was lower than that in garlic oil (53.6%).

Examination for antimicrobial activity

Chive oil and garlic oil were found to inhibit all of the food-borne pathogenic bacteria tested in this study (Table 2). MIC values against the pathogens varied depending on the strain of bacteria. For all of the bacteria, the values for garlic oil were lower than those for chive oil, indicating that garlic oil had higher antimicrobial activity than chive oil. In respect of chive oil and garlic oil, L. monocytogenes and E. coli O157:H7 were respectively the most sensitive and least sensitive strains. A wide range of MICs was observed for diallyl sulfides, the value being reduced with each additional sulfur atom (Table 3). Diallyl monosulfide was the only diallyl sulfide showing a higher MIC against all the pathogenic bacteria than chive oil and garlic oil. Table 3 also shows the MICs of tetracycline against the food-borne pathogens. They ranged from 0.5 to 2 μg/ml, being closer to the MICs of diallyl tetrasulfide than to those of the other diallyl sulfides.

Antimicrobial activity of chive oil in the food model

The antimicrobial activity of chive oil against E. coli O157:H7 in the food model with studied and the results shown in Fig. 1. When E. coli O157:H7 was inoculated on chicken breast to which chive oil had been applied, the number of bacterial strains was decreased from 10^8 CFU/ml to 0 CFU/ml within 24 h after the bacterial inoculation. However, E. coli O157:H7 inoculated on food without chive oil increased in cell number from 10^6 CFU/ml at the time of bacterial inoculation to 2.5 × 10^8 CFU/ml 24 h after inoculation.

Discussion

Diallyl sulfides are considered to be responsible for the antimicrobial activity of plants in the Alliaceae family. It was therefore interesting to compare the concentration of diallyl sulfides in chives with that in garlic. The percentage of diallyl sulfides in garlic determined in this study (53.6%) was very similar to that reported previously: 54.4% \(^{17}\) and 52.7% \(^{18}\). The
percentage of diallyl sulfides found in chives in this study (42.3%) was close to that found in Chinese leek reported by Tsoa and Yin (41.7%). Since concentration of diallyl monosulfide in chive oil and garlic oil was very low (Table 1) and its antimicrobial activity was quite weak (Table 2), its contribution to the antimicrobial activity of both oils could be negligible. Therefore, the other three diallyl sulfides (diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide) were major agents in chive and garlic oils responsible for their antimicrobial activity. In this study, the sum of the concentrations of diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide in garlic oil was significantly higher than that in chive oil. This may explain why garlic oil showed greater antimicrobial activity than chive oil.

Besides the four diallyl sulfides (diallyl monosulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide), other sulfides were found in garlic oil and chive oil, including diallyl pentasulfide, diallyl hexasulfide, methyl allyl disulfide, methyl allyl trisulfide, methyl allyl tetrasulfide, methyl allyl pentasulfide, methyl allyl hexasulfide, dimethyl trisulfide, dimethyl tetrasulfide, and dimethyl pentasulfide. Due to their volatility and very low content in garlic oil and chive oil, they tended to be lost during the preparation process. Therefore, their contribution to the antimicrobial activity of garlic oil and chive oil could be ignored.

Chive oil exhibited an inhibitory effect on all food-borne pathogenic bacteria tested in this study with a varying degree of inhibition, suggesting that it had antimicrobial activity against both gram positive and gram negative bacteria. Although many plants and herbs have been reported to have antimicrobial activity against food-borne pathogens, our results provide another herb of choice for fighting these pathogens. The possibility of developing bacterial resistance to plants and plant products has sent the message to scientists to add more to the list of plants and plant products inhibiting food-borne pathogens. The broad inhibitory spectrum of chive oil and several other plants make them more interesting to use for preventing and treating food-related illness than substances with very specific activity against bacteria such as nisin which only has an inhibitory effect on a few strains of gram positive bacteria.

Chive oil was also found to reduce the number of food-borne pathogenic bacteria in a food model. We chose E. coli O157:H7 in this study to inoculate into food because it is one of the major pathogenic bacteria contaminating Thai food and causing food-related illness and death among Thai people. We also chose 37 °C as the temperature to store food in this experiment because this is the optimal growth temperature for E. coli O157:H7 and it is very close to the room temperature of food markets in Thailand (ranging from 35 °C to 38 °C), where the food normally kept at room temperature, and not refrigerated. Since E. coli O157:H7 was the least sensitive strain to chive oil among the bacteria used in this study and chive oil was found to inhibit the bacterial strain, it could be assumed that chive oil has the possibility to inhibit other bacterial pathogens in food.

In summary, chive oil was shown in this study to inhibit several strains of food-borne pathogens in the laboratory level and E. coli O157:H7 in food. These results may encourage us to use chives or chive oil as an alternative to treat illness associated with food-borne pathogens.

References

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