Antimicrobial Activity of Elephant Garlic Oil against *Vibrio cholerae* in Vitro and in a Food Model

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***Vibrio cholerae*** is a major foodborne pathogen in Thailand. It is present in raw and lightly cooked foods, and it causes cholera. Natural products inhibiting it can be used to improve the safety of foods. In this study, elephant garlic oil was studied for its major diallyl sulfide content and its antimicrobial activity against *V. cholerae*. The oil had a very low concentration of diallyl monosulfides (1.62%) in comparison with the other diallyl sulfides (25.09% for diallyl disulfide, 16.04% for diallyl trisulfide, and 10.58% for diallyl tetrasulfide). In an *in vitro* study, the oil was found to have a bactericidal effect on all tested strains of *V. cholerae*, with varied minimal inhibitory concentrations (MICs) ranging from 3.13 to 25 μg/ml. It was also found that elephant garlic oil retarded the growth of the bacteria or reduced the bacterial cell load in the food model, depending on its concentration.

**Key words:** elephant garlic oil; antimicrobial activity; *Vibrio cholerae*; sausage

*Vibrio cholerae* is a curved-shaped gram negative bacillus with a polar flagellum. It can be subdivided into serogroups based on somatic O antigens. Although most pathogens belong to the O1 serogroup, members in the O139 serogroup are also known to be disease causing bacteria. *V. cholerae* O1 is further divided into two biotypes (El Tor and classical), as well as three serotypes (Ogawa, Inaba, and Hikajima). Cholera is an infectious gastroenteritis caused by *V. cholerae*. The symptoms range from mild diarrhea to severe, potentially life-threatening diarrhea and vomiting. The disease is usually seen in communities with poor sanitation, and is spread via *V. cholerae* contaminated water and food. Although the major reservoir for cholera was long assumed to be humans themselves, considerable evidence indicates that aquatic environments can serve as reservoirs. 

Outbreaks of cholera have decimated communities since ancient times. The disease continues to be endemic in South East Asia and parts of Africa and South America. The latest outbreak of cholera, considered to be one of the worst outbreaks of the disease, occurred in 2008 in Zimbabwe. The Ministry of Health of Zimbabwe has reported a total of about 12,000 cholera cases with 484 deaths, affecting all provinces in the country. 

Because of bad hygiene practices and the habit of eating raw or lightly cooked food, cholera is very common in Thailand. It occurs every year in various regions throughout the country. The Department of Disease Control, Ministry of Public Health, Thailand reported that in the year 2007 cholera affecting 1,428 persons with seven deaths was found in 13 provinces of Thailand, including Tak, Ranong, Nakorn Sawan, Khon Kaen, Samut Sakorn, Bangkok, Nakorn Prathom, Surat Thani, Chinhuri, Rayong, Nonthaburi, Udon Thani, and Loei. The identification of causative bacteria isolated from patients’ feces showed that *V. cholerae* O139 was responsible for the disease in six patients while the remainder of the infections were caused by *V. cholerae* O1 El Tor Ogawa (1,153 cases), *V. cholerae* O1 El Tor Inaba (268 cases), and *V. cholerae* O1 El Tor Hikajima (1 case). Outbreaks of the disease not only affect people’s health and well-being, but also have an economic impact on individuals and the country.

Raw and minimally processed foods sold to consumers in ready-to-eat form generally do not contain preservatives or antimicrobial substances and rarely undergo any heat processing before consumption. Thus they can be sources of foodborne pathogens. The addition of antimicrobial substances in the foods can be used to improve the safety of foods.

In recent years, there has been considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Plants and plant products represent a source of natural antimicrobial substances to be used in foods. The antimicrobial activities of plant essential oils have been known for a long time. Many studies have reported their activities against foodborne pathogenic bacteria, but evidence on the antimicrobial effects of elephant garlic oil on *V. cholerae* has not been presented.

Elephant garlic (*Allium ampeloprasum*) or “kratium tone” in Thai belongs with garlic (*Allium sativum*), onion (*Allium cepa*), chive (*Allium schoenoprasum*), and shallot (*Allium oschaninii*) to the Alliaceae family. It is a variety of garlic with very large cloves and a tender, mild, slightly sweet flavor. It is popularly used in cooking throughout Thailand because it is very easy to grow at home and can be used by consumers who want just a hint of garlic flavor in food. For medicinal use, elephant garlic is believed to have anti-hepatotoxic activities and can

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Abbreviations: MIC, minimal inhibitory concentration; EG, elephant garlic; DMS, diallyl monosulfide; DDS, diallyl disulfide; DTS, diallyl trisulfide; DTTS, diallyl tetrasulfide
protect against trichothecene (T-2) toxin induced epidermal damage in mice.\textsuperscript{13,15} Reports on the antimicrobial activity of the elephant garlic, especially against fungi, can be found, but they are scarce.\textsuperscript{14} The antimicrobial activities and health related effects of plants in the genus \textit{Allium} are believed to depend substantially on four major diallyl sulfides, diallyl monosulfide (DMS), diallyl disulfide (DDS), diallyl trisulfide (DTS), and diallyl tetrasulfide (DTTS), which are the sulfur-containing compounds found in the plants.\textsuperscript{9,11,15}

This study aimed to investigate the potential of using elephant garlic oil to inhibit or kill \textit{V. cholerae} both in the test tube and in food. The experiments were designed to examine the diallyl sulfide content, antimicrobial activity, and mode of action of elephant garlic oil against several strains of \textit{V. cholerae}. The effects of the addition of elephant garlic oil on the growth of \textit{V. cholerae} in a food model were also studied.

**Material and Methods**

\textit{Bacterial strains and culture conditions.} A total of 15 strains of \textit{V. cholerae} were included in the study. Four of them were reference strains obtained from the American Type Culture Collection (ATCC), \textit{V. cholerae} ATCC 14033 (serogroup O1), \textit{V. cholerae} ATCC 14734 (serogroup O1), \textit{V. cholerae} ATCC 51394 (serogroup O139), and \textit{V. cholerae} ATCC 35971 (non-O1, non-O139) strains. The other bacteria were clinical strains with serogroup O1 (VC 1 to VC 6) and serogroup O139 (V7 to V11). The strains, serological groups, and sources of the bacteria are presented in Table 1. The identity of these strains was confirmed by a variety of biochemical, physiological, and serological tests, as described previously.\textsuperscript{16,17} In addition, strains identified as \textit{V. cholerae} O1 were biotyped according to the standard procedure.\textsuperscript{17}

All the bacteria used in this study 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). Throughout the experiments, the strains were subcultured every 2 weeks on BHI agar and kept at 4°C. Before use, liquid cultures prepared from a single colony were transferred twice into fresh BHI broth and incubated at 37°C for 24 h.

\textit{Plant materials and sample preparation.} Elephant garlic (\textit{Allium ampeleoprasum}) was purchased from herb shops in Ubon Ratchathani Province, Thailand. Its essential oil was prepared according to the method described by Ravid and Putievsky.\textsuperscript{18} Fresh plant materials were steam distilled for 3h in a 100-liter 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.

\textit{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 Sparnins et al.\textsuperscript{19} All of the diallyl sulfides were stored at −80°C until use.

\textit{Analysis of diallyl sulfides in elephant garlic oil.} One mg of elephant garlic oil was redissolved in 10 ml of acetone mixture immediately before compositional analysis by the method of Lawson et al.\textsuperscript{20} Total sulfides and the four major diallyl sulfides of elephant garlic oil were quantified by 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.

\textit{Antimicrobial activity determination.} Elephant garlic oil, DMS, DDS, DTS, and DTTS were examined for their antimicrobial activities against the \textit{V. cholerae} strains named above using the microtiter broth microdilution method described by Amsterdam,\textsuperscript{21} with some modifications. Briefly, the tested compounds were initially adjusted to 200μg/ml and then subjected to a doubling dilution series in a microtiter plate containing BHI broth. The bacteria to be tested (5μl aliquots) were added to the wells containing the diluted compounds to obtain a final concentration of 10^3 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 to cause no growth of the bacteria. Each MIC value was obtained from five experiments.

\textit{Examination of mode of action.} Elephant garlic oil (at a final concentration equal to the MIC value) was added to 4.9 ml of \textit{V. cholerae} cultures (10^5 CFU/ml). After incubation at 37°C for 24 h, 100μl of the mixtures were inoculated into 4.9 ml of fresh BHI broth. As a control, 100μl of untreated culture of \textit{V. cholerae} at a concentration of 10^5 CFU/ml was transferred to 4.9 ml of fresh BHI broth. The optical density at a wavelength of 600 nm (OD_{600nm}) of the tested and control cultures was determined at the time of inoculation and after incubation at 37°C for 24 and 48 h.

\textit{Antimicrobial activity of elephant garlic oil in a food model.} Nham, a traditional Thai pork sausage, was used as the food model. Four hundred grams of food sample were prepared by mixing the ingredients including ground pork (62.5%), boiled, sliced pork rinds (27.5%), salt (2.5%), sugar (2.5%), and ground roasted rice (10%). The resulting mixture was divided into two groups of four samples (50-g samples). Four different concentrations of elephant garlic oil (0, 500, 1,000, and 1,500 ppm) were added separately to 50-g samples of both groups of food. \textit{V. cholerae} ATCC 14734 was then inoculated in every sample of only one group at an initial level of 10^3 CFU/ml. All 50-g food

\begin{table}
\centering
\caption{Strains, Serological Groups, and Sources of \textit{V. cholerae} Used in This Study}
\begin{tabular}{lll}
\hline
\textbf{V. cholerae} & \textbf{Serological classification} & \textbf{Source} \\
\hline
ATCC 14033 & Serogroup O1, biotype El Tor, serotype Inaba & American Type Culture Collection \\
ATCC 14734 & Serogroup O1, biotype El Tor, serotype Ogawa & American Type Culture Collection \\
ATCC 51394 & Serogroup O139 & American Type Culture Collection \\
ATCC 35971 & Non-O1, non-O139 & Clinical strain from Tak Province \\
VC1 & Serogroup O1, biotype El Tor & Clinical strain from Samut Sakorn Province \\
VC2 & Serogroup O1, biotype El Tor & Clinical strain from Chonburi Province \\
VC3 & Serogroup O1, biotype El Tor & Clinical strain from Udorn Thani Province \\
VC4 & Serogroup O1, biotype El Tor & Clinical strain from Surat Thani Province \\
VC5 & Serogroup O1, biotype El Tor & Clinical strain from Bangkok Province \\
VC6 & Serogroup O1, biotype El Tor & Clinical strain from Chonburi Province \\
VC7 & Serogroup O139 & Clinical strain from Nonthaburi Province \\
VC8 & Serogroup O139 & Clinical strain from Bangkok Province \\
VC9 & Serogroup O139 & Clinical strain from Khon Kaen Province \\
VC10 & Serogroup O139 & Clinical strain from Ranong Province \\
VC11 & Serogroup O139 & Clinical strain from Ranong Province \\
\hline
\end{tabular}
\end{table}
were used to calculate the concentration of the bacteria in food. This

described above. The numbers of bacterial cells grown on TCBS agar

confirmed to be V. cholerae O1 biotype El Tor by the methods
described above. The numbers of bacterial cells grown on TCBS agar
were used to calculate the concentration of the bacteria in food. This
experiment was performed in triplicate.

**Results**

*Diallyl sulfides in elephant garlic oil*

The concentrations of the four major diallyl sulfides (DMS, DDS, DTS, and DTTS) and total sulfides in elephant garlic oil were determined (Table 2). The total sulfides measured were diallyl monosulfide, diallyl disulfide, diallyl trisulfide, diallyl tetradsulfide, diallyl pentasulfide, diallyl hexasulfide, methyl allyl disulfide, methyl allyl trisulfide, methyl allyl tetrasulfide, methyl allyl pentasulfide, methyl allyl hexasulfide, dimethyl trisulfide, dimethyl tetradsulfide, and dimethyl pentasulfide. Approximately half of the total sulfides found in the elephant garlic oil (53.3%) were the four major sulfides, among which DDS and DMS were the most and the least abundant respectively.

**Antimicrobial activity**

An examination of the antimicrobial activity of elephant garlic oil against reference strains and clinical strains of V. cholerae revealed that it inhibited all tested bacterial strains (Table 3). The MIC values varied depending on the strain of bacteria. The clinical strains of V. cholerae O1 which gave the lowest (3.13 μg/ml) and highest (12.5 μg/ml) MIC values were VC2 and VC5 respectively, while VC10 and VC 9 were the clinical strains of V. cholerae O139 giving the lowest (3.13 μg/ml) and highest (25 μg/ml) MIC values respectively. Among all the tested bacteria, VC2 (O1 strain) and VC10 (O139 strain) were the strains most sensitive to elephant garlic oil, whereas VC9 (O139 strain) was the least sensitive. When standard DMS, DDS, DTS, and DTTS were tested for their antimicrobial effects against the various strains of V. cholerae, a wide range of MICs was observed (Table 3). The values reduced with each additional sulfur atom. Of all the diallyl sulfides examined, DMS was the only one showing a higher MIC against all of the bacteria than elephant garlic oil.

**Mode of antimicrobial action**

To study mode of action of elephant garlic oil against the various V. cholerae strains used in this study, recovery of V. cholerae inhibited by the oil for 24 h was examined in fresh BHI broth. It was found that the bacteria inhibited by the oil did not resume growth in the fresh BHI broth within 24 h or 48 h. As for the control, the untreated V. cholerae cells grew in the fresh BHI broth. After 24 h of incubation at 37°C, the OD values of the control cultures ranged from 0.30 to 0.72. These results suggest that elephant garlic oil has a bacteriocidal mode of effect on V. cholerae.

**Antimicrobial activity of elephant garlic oil in the food model**

The growth and survival of V. cholerae ATCC 14734 in nham was monitored over a 5-d period in the presence of various concentrations of elephant garlic oil and the results were compared with nham to which no elephant garlic oil was added. The results are reported in Fig. 1. In the presence of elephant garlic oil, V. cholerae ATCC 14734 had an initial concentration of 3 log CFU/g and grew quickly in the nham, reaching a concentration of about 8 log CFU/ml at day 3. After that, the amount of bacteria increased very slowly. The addition of elephant garlic oil to the nham caused a decrease of about 4 log units of bacterial cell load (Fig. 1). The addition of elephant garlic oil to nham at concentrations of 1,000 and 1,500 ppm produced reductions in V. cholerae cells in the food. The bacterial cell reduction was found to be dose-dependent. The presence of 1,000 ppm of elephant garlic oil in nham resulted in a reduction of the bacteria from 3 log CFU/g to about 2 CFU/g at day 5 (Fig. 1). When 1,500 ppm of elephant garlic oil was added to the nham, V. cholerae in the food began to be undetectable at day 4 (Fig. 1).

**Table 2. Contents of Diallyl Sulfides in Elephant Garlic Oil**

<table>
<thead>
<tr>
<th>Sulfide compound</th>
<th>Concentration (µg/g)</th>
<th>(%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diallyl monosulfide</td>
<td>81.2 ± 3.31</td>
<td>1.62</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>1,256.4 ± 10.63</td>
<td>25.09</td>
</tr>
<tr>
<td>Diallyl trisulfide</td>
<td>803.4 ± 6.28</td>
<td>16.04</td>
</tr>
<tr>
<td>Diallyl tetrasulfide</td>
<td>530.0 ± 3.85</td>
<td>10.58</td>
</tr>
<tr>
<td>Total sulfides</td>
<td>5,007.8 ± 9.66</td>
<td></td>
</tr>
<tr>
<td>Percent of diallyl sulfidesb</td>
<td>53.3</td>
<td></td>
</tr>
</tbody>
</table>

a Results are mean ± S.D. values of five replicates.

b Calculated by (concentration of each diallyl sulfides × 100)/total sulfides.

c Calculated from (sum of four diallyl sulfides × 100)/total sulfides.

**Table 3. MICs of Elephant Garlic Oil and Diallyl Sulfide Standards against Various Strains of V. cholerae**

<table>
<thead>
<tr>
<th>V. cholera</th>
<th>MIC (µg/ml)</th>
<th>Elephant garlic oil</th>
<th>DMS</th>
<th>DDS</th>
<th>DTS</th>
<th>DTTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 14013</td>
<td>6.25</td>
<td>50</td>
<td>3.13</td>
<td>0.78</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>ATCC 14734</td>
<td>6.25</td>
<td>50</td>
<td>3.13</td>
<td>0.78</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>ATCC 51394</td>
<td>12.5</td>
<td>50</td>
<td>6.25</td>
<td>0.78</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>ATCC 35971</td>
<td>12.5</td>
<td>100</td>
<td>6.25</td>
<td>1.57</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>VC1</td>
<td>6.25</td>
<td>100</td>
<td>3.13</td>
<td>0.78</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>VC2</td>
<td>3.13</td>
<td>25</td>
<td>1.57</td>
<td>0.2</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>VC3</td>
<td>6.25</td>
<td>50</td>
<td>3.13</td>
<td>0.4</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>VC4</td>
<td>6.25</td>
<td>50</td>
<td>3.13</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>VC5</td>
<td>12.5</td>
<td>50</td>
<td>6.25</td>
<td>0.78</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>VC6</td>
<td>6.25</td>
<td>25</td>
<td>3.13</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>VC7</td>
<td>6.25</td>
<td>50</td>
<td>3.13</td>
<td>0.78</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>VC8</td>
<td>12.5</td>
<td>50</td>
<td>3.13</td>
<td>0.78</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>VC9</td>
<td>25</td>
<td>100</td>
<td>12.5</td>
<td>1.57</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>VC10</td>
<td>3.13</td>
<td>25</td>
<td>1.57</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>VC11</td>
<td>12.5</td>
<td>100</td>
<td>3.13</td>
<td>0.4</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

To study mode of action of elephant garlic oil against the various V. cholerae strains used in this study, recovery of V. cholerae inhibited by the oil for 24 h was examined in fresh BHI broth. It was found that the bacteria inhibited by the oil did not resume growth in the fresh BHI broth within 24 h or 48 h. As for the control, the untreated V. cholerae cells grew in the fresh BHI broth. After 24 h of incubation at 37°C, the OD values of the control cultures ranged from 0.30 to 0.72. These results suggest that elephant garlic oil has a bacteriocidal mode of effect on V. cholerae.
Fig. 1. Effects of Elephant Garlic Oil at Different Concentrations on the Growth and Survival of V. cholerae ATCC 14734 in Nham.

Discussion

The concentration of the diallyl sulfides in elephant garlic oil examined in this study (53.3%) was found to be very close to those in garlic oil reported previously by Lawson et al.,20) Rattanachaikunsopon and Phumkhachorn,11) and Tsao and Yin,26) 54.4%, 53.6% and 52.7% but respectively, but it was higher than those in Chinese leek oil (41.7%)22) and chives oil (42.3%).11) Since the concentration of DMS in elephant garlic oil was very low (Table 2) and its antimicrobial activity against was rather weak (Table 3), its contribution to the antimicrobial activity of the oil might have been negligible. Therefore, the other three diallyl sulfides (DDS, DTS, and DTTS) were perhaps major agents in elephant garlic oil responsible for its antimicrobial activity.

In this study, the antimicrobial activities of DMS, DDS, DTS and DTTS against various strains of V. cholerae were studied. The MIC values varied depending on the strain of bacteria, ranging from 25 to 100 µg/ml for DMS, from 1.57 to 12.5 µg/ml for DDS, from 0.2 to 1.57 µg/ml for DTS, and from 0.005 to 0.4 µg/ml for DTTS. Compared to our previous study with V. cholerae ATCC 14101,11) the MICs for DMS (72 µg/ml), DDS (24 µg/ml), DTS (12 µg/ml), and DTTS (4 µg/ml) against the bacterium were higher than the values obtained from the present study, especially with DTS and DTTS. The difference in the MICs was perhaps due to the strain of bacteria and the method used to determine the values. In this study, we used BHI broth instead of Mueller-Hinton broth, and we used $10^6$ CFU/ml instead of $5 \times 10^5$ CFU/ml as the initial concentrations of the tested bacteria, but further investigations are needed to confirm the actual causes of the differences in the MICs obtained from our previous study and from this study.

V. cholerae are known to have various serotypes. In Thailand, V. cholerae O1 El Tor Ogawa is the main causative agent of the infection. However, V. cholerae O1 El Tor Inaba, V. cholerae O1 El Tor Hikajima, and V. cholerae O139 has also been found to be associated with epidemics of cholera in Thailand. An in vitro study of the antimicrobial activity of elephant garlic oil against V. cholerae showed that the oil inhibited all tested pathogenic and nonpathogenic strains of V. cholerae. The degree of its inhibitory effect on the bacteria varied from strain to strain. All V. cholerae inhibited by elephant garlic oil did not resume their growth in fresh BHI broth within 48 h, indicating a bacteriocidal mode of action. Several plants and plant essential oils having bacteriocidal effects on V. cholerae have been reported, including black tea,23) Malus sativa,24) Cyndenia oblonga,24) Persea gratissima,24) Punica granatum,24) and essential oils extracted from Lepechinia caulescens25) and lemon.26) Although plants and plant products have been reported to have antimicrobial activity against V. cholerae, our results provide another plant of choice for fighting the pathogen. The possibility of the development of bacterial resistance to plants or plant products27,28) has sent a message to scientists to add more to the list of plants and plant products inhibiting bacteria. The ability of elephant garlic oil to inhibit V. cholerae, which are gram-negative bacteria, makes it more interesting for use to prevent food-related illness caused by V. cholerae and other gram-negative bacteria that cannot be inhibited by nisin, the only bacteriocin accepted by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) in 1969 as a food preservative.

The results obtained from the in vitro study of the antimicrobial activity of elephant garlic oil against V. cholerae encouraged us to study its inhibitory effects on bacteria in the food environment. Our previous success in using chive oil to inhibit Escherichia coli O157:H7, a major gram-negative pathogenic bacterium, in food models11) was another main reason driving us to test the potential of elephant garlic oil to inhibit the V. cholerae present in foods. In this study, nham, a traditional Thai pork sausage, was used as the food model. Because it is often consumed raw, it is a good source of foodborne pathogens, including V. cholerae. The addition of elephant garlic oil to nham resulted in a retardation in bacterial growth or a reduction in the bacterial cell load, depending on the concentration of elephant garlic oil added the food. The concentrations of elephant garlic oil used to inhibit the growth of V. cholerae in the in vitro (62.5 ppm) and in vivo (1,500 ppm) experiments were substantially different. The ratio of the inhibitory concentrations was about 24-fold. These results do not surprise us, because similar results have been obtained in studies of the antimicrobial activity of many essential oils against bacteria in vitro and in foods. For essential oils exhibiting antimicrobial activity in vitro, it has generally been found that greater concentrations of the oils are needed to achieve the same effects in foods. The ratio has been recorded to range from 100-fold (in soft cheese) to 10-fold (in pork liver sausage).29) Although the causes of this phenomenon are still unknown, several explanations have been reported. The food matrices might serve as barriers to protect bacterial cells from inhibitory substances. In addition, the greater availability of nutrients in foods compared to laboratory media enable bacteria to repair damaged cells faster. Both the intrinsic properties of food (water content, protein content, pH, salt, and other additives) and extrinsic factors (temperature, characteristics of the microorganisms) have been found to be relevant in this respect.29)
To our knowledge, this study is the first to show the success in using plants or plant products to inhibit and kill pathogenic *V. cholerae* in a food model. Although it provides only preliminary data on the use of elephant garlic oil in food to prevent the foodborne illness associated with *V. cholerae*, it may be an important step towards development of an effective application of the oil in the food to prevent cholera and other foodborne diseases.

References