Twelve essential oils were tested in vitro for antimicrobial activities against several strains of Campylobacter jejuni, a pathogen causing food-borne diseases worldwide. Using disk diffusion and minimal inhibitory concentration determination assays, we noted that coriander oil exhibited the strongest antimicrobial activity against all tested strains. The oil had a bactericidal effect on the target bacteria. In evaluating the antimicrobial potency of coriander oil against C. jejuni on beef and chicken meat at 4°C and 32°C, it was found that the oil reduced the bacterial cell load in a dose-dependent manner. The type of meat and temperature did not influence the antimicrobial activity of the oil. This study indicates the potential of coriander oil to serve as a natural antimicrobial compound against C. jejuni in food.

Key words: coriander oil; antimicrobial activity; Campylobacter jejuni

Bacteria of the genus Campylobacter are curved-shaped, gram-negative bacilli that are oxidase-positive and catalase-positive and motile by means of either unipolar or bi-polar flagella. They require a microaerophilic atmosphere for growth. They are now recognized as one of the main causes of human gastroenteritis in both developing and industrialized countries. Annually, approximately 400 million cases of Campylobacter-associated gastroenteritis occur worldwide. Due to under-reporting, the actual case numbers are estimated to be many times more than the documented ones. The disease not only affects people’s health and well-being, but also has an economic impact on individuals and countries. Substantial economic losses are documented annually due to clinical treatment costs and lost working hours.

Of the 17 species of the genus Campylobacter, C. jejuni is the most common cause of bacterial gastroenteritis. C. jejuni infections are seen most commonly as acute enteritis with diarrhea, malaise, abdominal pain, stomach cramp, nausea, vomiting, and fever. In addition, the infections can lead to severe human health complications, including Guillain-Barre syndrome, an acute neurological disease marked by increasing paralysis. Furthermore, life-threatening systemic Campylobacter diseases are diagnosed more and more frequently. Human infections mainly result from consumption of contaminated food, milk, or water. Raw meat, especially poultry, sold in retail markets has been reported to be commonly contaminated by Campylobacter. Contact with contaminated meat is considered the major cause of food-borne Campylobacter enteritis.

At present, the reduction or elimination of contaminated Campylobacter on raw poultry meat is achieved using synthetic chemicals such as chlorine, chlorine oxide, acidified sodium chlorite, trisodium phosphate, and cetylpyridinium chloride, but these synthetic chemicals do not meet increasing consumers’ demand for natural food products. Furthermore, the safety of chemical additives has become a major concern of consumers because of the toxicity reported recently. For these reasons, the exploration of natural and safe antimicrobial substances to replace synthetic chemicals is receiving increasing attention. Plants and plant products represent a source of natural antimicrobial substances to be used in foods because many of them have been a part of the human diet for hundreds of years and have been reported to possess antimicrobial activity.

Essential oils are aromatic oily liquids obtained from many parts of plants, including the flowers, buds, seeds, leaves, twigs, bark, wood, fruit, and roots. Although many methods have been used to obtain essential oils from plant materials, the most commonly used method is steam distillation. Some essential oils have long been recognized to possess antimicrobial properties. Research on using essential oils to control populations of food-borne pathogenic bacteria is increasing due to the demand for natural and safe food additives. Many of them have promising ability to reduce the bacteria both in vitro and in foods. These data encouraged us to search for a potential essential oil for use to control Campylobacter jejuni in food.

In this study, plant essential oils were evaluated for their ability to inhibit the growth of C. jejuni. The most active one was further studied for its potential to serve as an antimicrobial agent when applied to chicken meat and beef experimentally contaminated with C. jejuni.
Material and Methods

Bacterial strains and culture conditions. The C. jejuni strains used in this study were a reference strain, C. jejuni ATCC 29428, and five clinical strains, CC1 to CC5, isolated from diarrheic stools of humans. The bacteria were cultured on Preston agar (Oxoid, Wesel, Germany) and incubated at 37 °C for 24 h in anaerobic jars with gas generating kits for Campylobacter for a microaerobic atmosphere (approximately 6% O2 and 10% CO2). Liquid cultures of the bacteria were prepared in BHI (brain heart infusion) broth (Oxoid) and incubated under the conditions mentioned above. All isolates were stored at −80 °C in a freezer by the Microbank system (PRO-LAB Diaganostics, Cheshire, UK).

Preparation of essential oils. The plants used in this study are listed in Table 1. They were herbs and spices readily found throughout Thailand. All of them were purchased from herb shops in Ubon Ratchathani Province, Thailand. Parts of the plants described in Table 1 were used to prepare essential oils by steam distillation. Two hundred g of each sample was cut into small pieces and homogenized in 200 ml of distilled water using a blender. The homogenate was subjected to essential oil extraction using a vertical steam distillation unit. The extracted essential oil was kept in a dark bottle and stored at 4 °C until use.

Antimicrobial activity test. The antimicrobial activity of each essential oil against the C. jejuni strains was carried out by the disk diffusion method. Diluted bacterial culture (100 μl, 10⁸ CFU/ml) of the bacterial strains was spread on the surface of Preston agar before placement of sterile filter paper disks 6 mm in diameter (Schleicher and Schuell, Florham Park, NJ) on the agar. Fifteen μl of each essential oil was spotted on the paper disk. Oxytetracycline (5 μg/disk) was used as a positive control and distilled water as a negative control. After incubation at 37 °C in a microaerophilic atmosphere for 24 h, the diameters of the growth-free zones around the disks were measured and subtracted from the diameter of the paper disk, giving the sizes of the inhibition zones beyond the paper disk. Each essential oil was tested in three replicates.

Determination of minimal inhibitory concentration (MIC). Essential oils were examined for their antimicrobial activities against the C. jejuni strains using the microtiter broth microdilution method described by Amstel and 20 with some modifications. Briefly, a series of twofold dilutions of each essential oil, ranging from 2% v/v to 0.03% v/v, was prepared in a microtiter plate containing BHI broth supplemented with 0.5% v/v of Tween 80. The bacteria to be tested were added to the wells containing the diluted oil to obtain a final concentration of 10⁵ CFU/ml. Controls (without tested compounds or tested bacteria) were included for each plate. After incubation at 37 °C in a microaerophilic atmosphere for 24 h, the optical density at a wavelength of 600 nm (OD600nm) of the tested and control cultures was determined at the time of inoculation and after incubation at 37 °C in a microaerophilic atmosphere for 24 h.

Examination of mode of action. Each essential oil (at a final concentration equal to the MIC value) was added to 4.9 ml of C. jejuni cultures (10⁸ CFU/ml). After incubation at 37 °C in a microaerophilic atmosphere for 24 h, 100 μl of the mixtures inoculated into 4.9 ml of fresh BHI broth. As a control, 100 μl of untreated culture of C. jejuni at a concentration of 10⁸ CFU/ml was transferred to 4.9 ml of fresh BHI broth. The optical density at a wavelength of 600 nm (OD600nm) of the tested and control cultures was determined at the time of inoculation and after incubation at 37 °C in a microaerophilic atmosphere for 24 h.

Sample preparation. The meat samples used in this study were chicken breast and lean beef. All were obtained from a local market in Ubon Ratchathani Province, Thailand. They were trimmed into square pieces approximately 36 cm² (6 cm × 6 cm × 1 cm). The samples were vacuum-packaged and frozen in sterile Whirl-Pak retain bags (The Lab Depot, Dawsonville, GA). The packaged samples were shipped frozen by overnight courier to Thai irradiation Center, Thailand Institute of Nuclear Technology, Pratumthani Province, Thailand, and sterilized with 12 Kgy of gamma radiation using Carrier type gamma irradiator model JS 8900 IR-155. Upon return, the samples were frozen at −20 °C and maintained at that temperature until use.

Bacterial and oil treatment preparation. Pieces of meat prepared as above were minced in a sterile blender, and portions of 100 ± 0.1 g were placed in polyethylene bags. The meat samples were inoculated with C. jejuni ATCC 29428 to a final concentration of 10⁵ CFU/g of meat. Prior to bacterial inoculation of the meat, different concentrations of coriander oil (0.1%, 0.25%, and 0.5% v/w) were added to the samples. The samples were homogenized using a Stomacher 400 (Seward, London, UK) at normal speed for 5 min. All of the bags containing samples of meat were kept at 4 °C (refrigeration temperature) and 32 °C (average room temperature in Thailand) and examined every 30 min for 3 h after bacterial inoculation for the presence of C. jejuni. Sterile water (instead of coriander oil) was added to the untreated controls, and this was inoculated with the test bacteria and placed under the same conditions as the other samples. Three individual replicates of each experiment were performed in all cases.

Bacteria enumeration. Portions of 10 g of meat were weighed and homogenized in a plastic bag with 10 ml of phosphate buffer (pH 7) in the stomacher for 30 s. Only the liquid part of the homogenate was collected, and it was serially diluted with the phosphate buffer. Appropriate dilutions of each sample were spread on Preston agar, a selective medium for C. jejuni. The plates were then incubated at 37 °C for 24 h in a microaerobic atmosphere. Standard microbiological and biochemical tests for C. jejuni were performed to confirm the identity of the isolates.

Results

Antimicrobial activity

The antimicrobial activities of essential oils against the reference and clinical strains of C. jejuni are presented in Table 2. All essential oils inhibited the C. jejuni strains, with different degrees of inhibition. For all of the essential oils, C. jejuni ATCC 29428 was most sensitive strain, while clinical strain CC2 was the least sensitive. Of all the tested oils, coriander oil was the most inhibitory of all C. jejuni strains, exhibiting maximum inhibition zones up to 26.8 mm, while holy basil oil had the weakest antimicrobial activity against all the tested bacterial strains, yielding the smallest inhibition zones, varying from 8.3 to 11.0 mm.

Table 1. List of Plants and Parts Used in Essential Oil Extraction

<table>
<thead>
<tr>
<th>Common names</th>
<th>Thai names</th>
<th>Scientific names</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil</td>
<td>Ho Ra Pa</td>
<td>Ocimum basilicum</td>
<td>Leaf</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Ob Cheay</td>
<td>Cinnamonum verum</td>
<td>Bark</td>
</tr>
<tr>
<td>Clove</td>
<td>Kan Plu</td>
<td>Eugenia Caryophyllus</td>
<td>Flower bud</td>
</tr>
<tr>
<td>Coriander</td>
<td>Puk Chee</td>
<td>Coriandrum sativum</td>
<td>Seed</td>
</tr>
<tr>
<td>Elephant garlic</td>
<td>Kra Team</td>
<td>Allium ampelegrosam</td>
<td>Bulb</td>
</tr>
<tr>
<td>Ginger root</td>
<td>Kra Chai</td>
<td>Bosenbergia pandurata</td>
<td>Root</td>
</tr>
<tr>
<td>Garlic</td>
<td>Kra Team</td>
<td>Allium sativum</td>
<td>Bulb</td>
</tr>
<tr>
<td>Greater galangal</td>
<td>Ka</td>
<td>Alpinia galangal</td>
<td>Root</td>
</tr>
<tr>
<td>Holy basil</td>
<td>Kra Prao</td>
<td>Ocimum sanctum</td>
<td>Leaf</td>
</tr>
<tr>
<td>Kaffir lime</td>
<td>Ma Grood</td>
<td>Citrus hystis</td>
<td>Fruit peel</td>
</tr>
<tr>
<td>Lemon grass</td>
<td>Tra Krai</td>
<td>Cymbopogon citratus</td>
<td>Stem</td>
</tr>
<tr>
<td>Tumeric</td>
<td>Ka min</td>
<td>Curcuma longa</td>
<td>Root</td>
</tr>
</tbody>
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Determination of MIC

The MICs of all the essential oils obtained by the microtiter broth microdilution method are shown in Table 3. In general, there was a correlation between the

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Determination of MIC

The MICs of all the essential oils obtained by the microtiter broth microdilution method are shown in Table 3. In general, there was a correlation between the
results from the disk diffusion assay (Table 2) and those from the MIC determination assay (Table 3). Of all the tested oils, coriander oil had the lowest MIC values, while holy basil oil had the highest MIC values for all strains of *C. jejuni*, indicating the strongest and the weakest antimicrobial activities for coriander oil and holy basil oil respectively. The lowest MIC value (0.03% v/v) was obtained when *C. jejuni* ATCC 29428 was inhibited by coriander oil. An MIC value of more than 2% v/v was obtained only when clinical strains CC2 and CC4 were inhibited by holy basil oil.

**Examination of mode of action**

The modes of action of the essential oils against various *C. jejuni* strains were examined. For each essential oil, it had the same mode of action against all strains of *C. jejuni* used in this study. After *C. jejuni* cells inhibited with the essential oils were transferred to fresh BHI broth, the cultures were examined for optical density at a wavelength of 600 nm at the time of transfer and at 24 h after transfer. *C. jejuni* cells inhibited by basil, faggertroot, and holy basil oils were found to be able to re-grow in the fresh culturing broth after transfer for 24 h, indicating the bacteriostatic mode of action of the oils. However, the remaining oils exhibited a bactericidal mode of action against *C. jejuni*, because the bacterial cells inhibited by the oils did not resume growth in fresh BHI broth after transfer for 24 h. The growth of the bacteria was still undetectable after transfer for 48 and 72 h.

**Effects of coriander oil on *C. jejuni* in food**

Based on the most active essential oil determined by the disk diffusion and MIC determination assays, coriander oil was evaluated as an antimicrobial compound against *C. jejuni* ATCC 29428, the most sensitive strain to all of the oils, in food systems. The survival of *C. jejuni* ATCC 29428 on ground chicken meat and beef was monitored over a 3-h period in the presence of various concentrations of coriander oil, and the results were compared with both types of meat to which no coriander oil was added, at 4°C and 32°C.

Over the 3-h observation period, the bacterial cell numbers on the chicken meat without coriander oil were quite stable at 5 log CFU/ml at 4°C (Fig. 1). The addition of coriander oil to the meat resulted in a reduction in bacterial populations. The effects of the coriander oil on the *C. jejuni* present on chicken meat were dose-dependent. The oil at concentration of 0.5% v/w killed all of the bacteria on the meat, while...
0.1% and 0.25% v/w oils reduced the bacterial cell loads on the meat from 5 log CFU/ml to 3 and 1 log CFU/ml respectively. In all cases, most of the bacterial cells on the meat were killed by the oil in the first 30 min.

The two types of meat (chicken meat and beef) did not influence the antimicrobial strength of the coriander oil (Fig. 1). No _C. jejuni_ cells were detected on chicken meat or beef when 0.5% v/w coriander oil was used. Furthermore, the numbers of bacterial cells left on the two types of meat were very similar at the end of the experiments, when 0.1% and 0.25% v/w of coriander oil were used.

The antimicrobial activity of coriander oil against _C. jejuni_ on chicken meat and beef was not affected by temperature. At 4°C and 32°C, the response patterns of the bacterium to all concentrations of coriander oil were very similar (Fig. 1). With 0.5% v/w coriander oil, the bacterium was not detected on the meats after 30 min at either temperature (Fig. 1). With 0.1% and 0.25% v/w coriander oil, the numbers of viable cells left on the chicken meat and the beef were not different when the experiments were performed at 4°C and 32°C.

**Discussion**

According to an in vitro study of the antimicrobial activities of the essential oils against several strains of _C. jejuni_, coriander oil possessed the most powerful inhibitory effect and had a bactericidal effect on the bacteria tested. Several plants and plant essential oils having bacteriocidal effects on _C. jejuni_ have been reported, including marigold, ginger root, jasmine, patchouli, gardenia, cedar wood, carrot seed, celeray seed, mugwort, spikenard, and orange bitter oils.15,21) Although plants and plant products have been reported to have antimicrobial activity against _C. jejuni_, our results provide another plant of choice to inhibit the pathogen. The possibility of resistance development of bacteria to plants or plant products22,23) has sent the message to scientists to add more to the list of plants and plant products inhibiting bacteria. The ability of coriander oil to inhibit _C. jejuni_, which are gram-negative bacteria, makes it more interesting for use to prevent food-related illness caused by _C. jejuni_ and other gram-negative bacteria, which cannot be inhibited by nisin, the only bacteriocin that accepted by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) in 1969 as a food preservative.

Coriander is considered both an herb and a spice since both its leaves and seeds are used as seasoning condiments. It has traditionally used as an analgesic, aphrodisiac, antiarthritic, anti-inflammatory, diuretic, antispasmodic, circulatory stimulant, and anti-diabetic.24) It has also been noted for its cholesterol-lowering (hypolipidemic) effects.25) The essential oil extracted from coriander seed has been reported to have antimicrobial activity against both gram-negative and gram-positive food-borne pathogenic bacteria, including *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*.26) Hence it is of interest to examine the antimicrobial activity of the oil against _C. jejuni_ both in vitro and in food.

The disk diffusion assay and the MIC determination assay are rapid and practical approaches to screen large numbers of potential antimicrobials, but they do not account for the potential effects of a food matrix. Rajkovic _et al._ reported that carvacrol, which inhibited

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**Fig. 1.** Survival of _C. jejuni_ in Ground Chicken Meat and Beef Exposed to Coriander Oil at Concentrations of 0.10, 0.25, and 0.50% v/w, and Stored at 4°C and 32°C. Counts are means ± S.D. (n = 3). Bars indicate error of standard deviation (p < 0.05).
the growth of Bacillus cereus and Bacillus circulans in nutrient broth, failed to exhibit any antimicrobial properties when combined with potato puree.\textsuperscript{27} Interference between food matrices (juice and dip) and the antimicrobial potency of chitosan hydrolysates was also observed.\textsuperscript{28} For these reasons, it is necessary to evaluate the antimicrobial potency of coriander oil in food. In this study, raw chicken meat was used as the food model to evaluate the antimicrobial activity of coriander oil against \textit{C. jejuni} in food. Coriander oil was found to inhibit \textit{C. jejuni} on chicken meat in a dose-dependent pattern. The concentrations of the oil used to inhibit the growth of \textit{C. jejuni} in the \textit{in vitro} (0.03\% v/v) and food (0.5\% v/w) experiments were substantially different. The ratio of the inhibitory concentrations was about 17-fold. These results are similar to those obtained in studies of the antimicrobial activity of many essential oils against bacteria \textit{in vitro} and in foods. For essential oils exhibiting antimicrobial activity \textit{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).\textsuperscript{15} Although the causes of this phenomenon are still unknown, several explanations have been offered. The food matrices might serve as barriers to protect bacterial cells from inhibitory substances. In addition, the greater availability of nutrients in foods as 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, the characteristics of the microorganisms) have been found to be relevant in this respect.\textsuperscript{15}

Types of meat were also studied for their effects on the antimicrobial activity of coriander oil. The concentration of the oil inhibiting \textit{C. jejuni} on chicken meat and beef was the same, indicating that the two types of meat did not influence the antimicrobial strength of the oil. Similar results have also been found when oregano oil was used to inhibit \textit{Photobacterium phosphoreum} in cod and salmon fillets.\textsuperscript{29} The same concentration of thyme oil was also found to inhibit \textit{E. coli} O157:H7 in lettuce, romaine, and carrot.\textsuperscript{30}

Temperature is a factor affecting the antimicrobial potency of essential oils. Essential oils that inhibit target bacteria over a wide range of temperature are required for more applications. Beuchat \textit{et al.} reported that low temperature enhanced the inhibitory ability of plant extracts, but this is not the case for some essential oils.\textsuperscript{31} For example, Mytle \textit{et al.} demonstrated the effectiveness of clove oil in inhibiting \textit{L. monocytogenes} on chicken frankfurters at 5°C and 15°C.\textsuperscript{32} Our study also found similar activity of coriander oil in inhibiting \textit{C. jejuni} on chicken meat and beef at 4°C and 32°C.

In conclusion, this study indicates the potential of coriander oil to serve as a natural antimicrobial against \textit{C. jejuni}. Its availability, low cost, and effectiveness over a wide range of temperature contribute to its advantages as a food preservative and prospective alternative to currently used chemical-based inhibitors. However, in order to use it effectively at the industrial level, more research is needed to better characterize its potential as an antimicrobial compound.

Acknowledgments

The clinical strains of \textit{C. jejuni} (CC1 to CC5) were kindly donated by Sunprasithpisarong Hospital, Ubon Ratchathani Province, Thailand.

References