Antibacterial activity of Terminalia chebula fruit extract against Campylobacter spp. isolation from chickens

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Abstract

The effect of ether, ethanolic and aqueous extracts of Terminalia chebula on Campylobacter spp. were examined using an agar-well diffusion method on semi-solid Brucella agar. Ethanolic extracts of Terminalia chebula showed significant antibacterial activity and had a minimal inhibitory concentration (MIC) of 25 mg/ml. The ethanolic crude extract was evaluated for its efficacy against Campylobacter spp. in broiler chicks. Each chick was orally inoculated with $1 \times 10^6$ colony-forming units of Campylobacter spp. at 9-day-old. Crude extract was given 50 mg/chick for 3 days by crop gavage. Chickens received the crude extract 24 h before receiving the Campylobacter spp. All chickens were killed at 11 days of age. The result demonstrated that the number of Campylobacter colonies from caeca were as follow: the second group : $8.0728 \pm 0.6159$, the third group : $5.7186 \pm 2.0540$, the fourth group : $7.2026 \pm 0.9631$ colonies per g of caecal content, respectively. Nevertheless, no Campylobacter colony was detected in the first group. The result revealed that chickens which received the crude extract prior inoculated with the Campylobacter had lowest isolate rate with scientific significant ($p<0.05$).

Key words: Terminalia chebula fruit, extract, Campylobacter spp., chickens

INTRODUCTION

Campylobacter spp. is an important causes of campylobacteriosis, a gastro-intestinal tract infection in humans. (Reilly & Gilliland, 2003) Campylobacter spp. is part of the intestinal flora of several animals (Reilly & Gilliland, 2003), especially chickens. Original source of human infections is caused by contaminated poultry products. The use of antibiotics in modern intensive animal production for therapy and prevention of diseases could not be a rational solution to reduce Campylobacter incidence. Several studies have actually pointed out the partial association between the veterinary use of antibiotics and the emergence of resistant strains of Campylobacter related to human enteritis (Desmonts et al., 2003). Currently, the use of antibiotic in feed to prevent colonization of Campylobacter in chickens has been prohibited throughout Western European countries. One challenging alternative to relieve this problem is to use the traditional medicinal plants to prevent the bacteria infection in chicken.

Terminalia chebula is routinely used as traditional medicine to cure several ailments such as fever, cough, diarrhea, agastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections (Dash & Bhagwan, 1991). Plant fruits appear to have evoled complex antibiotic compounds to cure various diseases like cancer, cardiovascular, digestive and pathogenic bacteria. Antibacterial activity of T. chebula extracts against several bacterial strains have been reported (Malkzadeh et al., 2001; Kim et al., 2006; Chattopadhyay et al., 2007; Bag et al., 2009). Due to this medicinal plant possesses a high amount of tannin (40%). The antimicrobial properties of this substance are well established (Scalbert, 1991). The high phenolics content in Terminalia chebula powder can be associated with this activity (Chattopadhyay et al., 2008). The objective of this work was to characterise the effect of T. chebula extract on Campylobacter colonization in broiler chickens.
MATERIALS AND METHODS

Plant material: T. chebula fruits were collected at the end of January 2009 from local markets in Khon Kaen, Thailand. The specimens were deposited at department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University.

Preparation of plant extract: The T. chebula fruits were cut into small pieces and dried in an oven at 50 °C for 24 h and afterwards ground with an electric mill.

Extraction: The method of Dupont et al. (2006) was adopted for extraction with little modification. Ethanolic extracts: This dried puld (50 g) was soaked separately in 250 ml of ethanol for 48 h at room temperature. At the end of extraction, each extract was filtrate by Whatman filter paper no.1. The ethanol extract was dried at low temperature under reduced pressure in a rotary evaporator to obtain a residue of crude extract. The ethanolic extracts were resuspended in water at the ratio of 2:10 (g/ml). The extracts were then sterilized by membrane filtration. Aqueous extracts: Dried plant material (50 g) was extracted in boiling distilled water (250 ml) for 10 min, then the extract was filtered after cooling. The aqueous extracts were resuspended in water at the ratio of 2:10 (g/ml). The extracts were then sterilized by membrane filtration.

Bacterial isolate: A local isolate, ten strains of Campylobacter isolated from chickens. Identification was by colony morphology, Gram staining, microaerophilic growth (at 37 °C), oxidase, catalase, urease, nitrate, H2S and hippurate hydrolysis test and nalidixic sensitivity. The inoculum size was adjusted to approximately 10^8 colony-forming units (cfu) per ml.

Antibacterial effectiveness in vitro

Agar-well diffusion method: The antibacterial tests were performed using agar-well diffusion assay (Perez, Paul & Bazerque, 1990). Bacterial suspensions from above were adjusted to a McFarland turbidity of 0.5 (approximately 1.0 x 10^8 cfu ml^-1), 150 µl of an individual active culture of Campylobacter isolation was transferred into 14 ml of semi-solid brucella agar 0.75%(w/w) (Oxoid, Hampshire, England) at 50 °C. The inoculated medium was swirled to distribute the Campylobacter and held at room temperature for 30 min. Subsequently 6 mm- diameter wells and 4 mm- in depth were bored in the agar and 35 µl volumes of 200 mg ml^-1 of each reconstituted extract was pipetted into wells. Sterilized water (35 µl) was used as negative control and 5-% glacial acetic acid (15 µl) was used as a positive control. The plates were incubated at 37 °C under microaerophilic atmosphere for 48 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells.

Minimal inhibitory concentration (MIC): The same procedures were use to test MIC. Concentrate extracts of plants were added at two-fold serial dilution (7.8 to 200 mg ml^-1) to 0.85 % normal saline. A 35 µl different of concentrate extracts of plants dilution were transferred into each well. The plates were incubated at 37 °C under microaerophilic atmosphere for 48 h Observations were performed at least concentration in duplicate and the result expressed as the lowest concentration of the extract that inhibited the bacterial growth after incubation was taken as the MIC of a crude extract.

Inhibitory effect of the ethanolic extract on Campylobacter colonization in broiler chickens (in vivo): The ethanolic extract of T. chebula against isolated Campylobacter strains in vivo was also determined (Banhart et al., 1999). The 1-day-old 40 broiler chickens were divided into four groups by completely randomized design. Each group consisted of 10 chickens. The experimental animals were treated as follows: each chicken was orally inoculated with 1 x 10^6 cfu of isolated Campylobacter (A2) at 9-day-old except the first group. Crude extract was given at 2x/bird (x = MIC values of crude extract) for 3 days by crop gavage. The first group was a healthy control group which Campylobacter was not challenged. The second group was a control
group, the extract was not given. The third group was given with the ethanolic extract at the concentration of 2x/bird (x = MIC values of crude extract) (dissolved in 0.5 ml distilled sterile water) for 3 days by crop gavage. Chickens received the crude extract 24 h before received the *Campylobacter*. The fourth group was given with commercial herbal medicine product at the concentration of 2x/bird (x = MIC values of crude extract) (dissolved in 0.50 ml distilled sterile water) for 3 days by crop gavage. Chickens received the crude extract 24 h before received the *Campylobacter*. After 11 days of treatment, all chickens were killed and the caecum were removed. Rate of *Campylobacter* isolated from caecal content of each treatment was compared.

**Statistical analysis :** For data on effect of the ethanolic extract on *Campylobacter* colonization in broiler chickens. ANOVA was conducted using independent-sample t-test procedure of SPSS. Number of bacterial count was transformed to log$_{10}$ before analyses. Number of *Campylobacter* spp. count (log$_{10}$) in caecal content of the broiler chickens at 11 days after treatment were regarded as dependent variables. The statistical model for the caecal bacterial contents were included the effect of treatments (group 1, 2, 3 and 4) and the interaction between treatments. The difference between the value with $P<0.05$ were considered as statistical significance.

**Results and Discussion :** As shown in Table 1, ethanolic extracts of *T. chebula* were significantly more active than aqueous extracts. There was no significant difference between isolates in sensitivity to the extracts. The antibacterial activity of the ethanolic fruit extract on the test organism using agar-well diffusion. The MIC values of 25 mg/ml as can be seen in Fig.1. The activity of the ethanolic extract against *Campylobacter* spp. in broiler chicks. The result demonstrated that the number of *Campylobacter* colonies from caecum were as follow : the second group : 8.0728 ± 0.6159, the third group : 5.7186 ± 2.0540, the fourth group : 7.2026 ± 0.9631 colonies per g of caecal content, respectively. Nevertheless, no *Campylobacter* colony was detected in the first group (Table 2). The results also indicated that the number of *Campylobacter* colonies of the third and the fourth group were decreased significantly when compared with the control group ($P<0.05$). The third group also contained significantly less *Campylobacter* colonies than the fourth group ($P<0.05$). The result revealed that birds which receiving the crude extract before receiving the *Campylobacter* had lowest isolate rate with scientific significant ($p<0.05$). The present finding is hence highly encouraging in recognizing a plant of interesting antibacterial activity. Sato et al. (1997) have reported that 50% ethanol extract of fruiting bodies of *T. chebula* Retz. exhibits antibacterial activity against MRSA and the compounds responsible for this activity are gallic acid and its ethylestr. Kim et al. (2006) have studied the effect of *T. chebula* fruits on six intestinal bacteria and found that ethanedioic acid isolated from fruit of *T. chebula* had moderate inhibitory activity whereas elagic exerted a potent inhibitory activity against intestinal *E. coli*. Due to this medicinal plant composed of a high amount of tannin (40%). The antimicrobial properties of this substance were well established (Scalbert, 1991). The high phenolics content in *T. chebula* powder can be associated with this activity. But flavonoids and carotenoids may also have an important role (Chattopadhyay et al., 2008).

**Conclusion :** Thus *T. chebula* has high potential for further development as a feed additive for *Campylobacter* decontamination in poultry industry and further studies both on the extract and/or its chemical constituents are needed to pinpoint of the findings.

**Acknowledgements :** The author would like to thank Khon Kaen University for financial support.
Table 1. Inhibition effect of *T. chebula* extracts on the growth of *Campylobacter* spp. was demonstrated by using agar-well diffusion method. The diameter of inhibition zone was measured in mm. Distilled sterile water was used as negative control. Acetic acid was used as positive control. Isolated *Campylobacter* from native chickens.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Isolate <em>Campylobacter</em>&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
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<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>21</td>
</tr>
<tr>
<td>Aqueous</td>
<td>16</td>
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Table 2. Number of *Campylobacter* count in caecal content of the broiler chickens that were treated with *T. chebula* and commercial extract for 11 days.<sup>*</sup>

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean colony number (n=10) (Log cfu/g)&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (No treated both <em>Campylobacter</em> and extract)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control (treated <em>Campylobacter</em> only)</td>
<td>8.0728 ± 0.6159&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Given of <em>Terminalia chebula</em></td>
<td>5.7186 ± 2.0540&lt;sup&gt;•&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Given of commercial extract</td>
<td>7.2026 ± 0.9631&lt;sup&gt;•&lt;/sup&gt;</td>
</tr>
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<sup>*</sup>Mean in the same column bearing different superscripts differ (*P*<0.05)

Figure 1. Minimal inhibitory concentration (MIC) of crude extract from *T. chebula* in 95% ethanol against *Campylobacter* spp. growth on semi-solid brucella agar.

![Image of minimal inhibitory concentration (MIC) of crude extract from *T. chebula* in 95% ethanol against *Campylobacter* spp. growth on semi-solid brucella agar.]

*Terminalia chebula*

1 = 200 mg/ml. 2 = 100 mg/ml. 3 = 50 mg/ml. 4 = 25 mg/ml. 5 = 12.5 mg/ml. 6 = 6.25 mg/ml.
7 = 3.12 mg/ml. 8 = 1.56 mg/ml. 9 = 0.78 mg/ml. 10, 11 = Distilled water
Reference


