Antibacterial and therapeutic effects of a combination of *Coptidis rhizoma* and *Galla rhois* extracts in piglets challenged with *Campylobacter coli*

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Abstract: The antibacterial effects of a combination of *Coptidis rhizoma* and *Galla rhois* extracts (CGE) were evaluated in piglets. The minimum bactericidal concentration of CGE was 2.0 mg/mL. Thirty 5-week-old piglets were challenged with *Campylobacter* (*C.*) *coli* after allocation to three different groups, a control and two treatment groups fed with CGE at 2.0 or 4.0 g/kg feed for 7 days. On day 7, *C. coli* in the feces of the CGE-treated groups were significantly lower than in the control (p < 0.01). These results suggest that CGE can be used to control *C. coli* in piglets.

Keywords: *Campylobacter coli*, *Coptidis rhizoma*, *Galla rhois*, piglet

*Campylobacter* spp. are one of the most dominant zoonotic bacteria which cause human gastroenteritis in many developing and industrialized countries [11]. In the European Union, the number of confirmed-campylobacteriosis cases in human amounted to 220,209 in 2011. In addition, human campylobacteriosis in the United States was estimated to affect over 1.3 million persons every year [15].

Both *Campylobacter* (*C.*) *jejuni* and *C. coli* asymptotically colonize in the intestinal tract of birds and mammals, including food production animals which pose an important risk for human *C. coli* from contaminated carcasses at slaughter and of milk [12]. Pigs are a natural reservoir of *Campylobacter* spp. with *C. coli* as the dominant species. The prevalence of *C. coli* infection in pigs is known to be between 50 and 100%, and excretion levels of this pathogen ranged from $10^2$ to $10^7$ colony forming units (CFU)/g feces [6].

Recent studies have shown a rapid increase in the prevalence of antibiotic resistance to *Campylobacter* spp. due to the use of antimicrobial agent for the prevention and treatment of bacterial infection in farming livestock [10]. Ultimately, the rise of antibiotic-resistant *C. coli* on livestock could impact on human health.

Recently, medicinal herbs have received more attention to resolve the problem of antibiotic resistance after the ban on antibiotics as growth promoters in animal feed. Bioactive components in medical herbs have been applied in clinical and therapeutic areas [3, 7]. *Coptidis rhizoma* has been used in oriental medicine as an antibacterial and anti-inflammatory agent from long time ago [14]. The extract of *Coptidis rhizoma* contains a high level of berberine, an alkaloid possessing various antimicrobial activities in a variety of pathogenic microorganisms [8]. In addition, *Galla (G.) rhois* has long been used in traditional Asian medicine and is a harmless natural material that contains a number of tannin-derived components, including methyl gallate and gallic acid which have an antibacterial activity [1].

Although many previous studies [9, 12, 13] investigated the antimicrobial effects of medicinal herbs against *Campylobacter* spp., few studies exist to investigate the antibacterial and therapeutic effects for the combination of herbs on livestock infected by the bacteria. The present study evaluated the antibacterial and remedial potentials of an herbal combination containing *Coptidis rhizome* and *G. rhois* on piglets challenged with *C. coli*.

* C. *coli* (ATCC 33559) kept at ~80°C was recovered on Mueller-Hinton (MH) agar with 5% sheep blood (Oxoid, UK) for 48 h at 37°C under microaerobic conditions (6% O2, 7% CO2, 80% N2, 7% H2). Liquid cultures were obtained by inoculation of colonies in Brucella broth (Becton, Dickinson and Company, USA) and cultivation under the same conditions for 24 h. For pig inoculation, colonies of *C. coli* were cultivated in Brucella broth (Becton, Dickinson and Com-

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pany) and incubated for 16 h under microaerobic conditions. After incubation, 0.5 mL of the culture with an optical density of approximate 0.3 at 600 nm were inoculated in 20 mL Brucella broth (Becton, Dickinson and Company) and cultivated for 4 h in order to obtain a solution of about $5.0 \times 10^7$ CFU/mL. Cell numbers were determined by performing standard plate counts according to ISO 10272-2 [5].

*Coptidis rhizoma* and *G. rhois* were purchased from the Korea National Animal Bio Resource Bank (Jinju, Korea) and ground to a powder after air-dry in a dark room. Each 100 g of *Coptidis rhizoma* and *G. rhois* powder was soaked in 400 mL of 70% aqueous methanol (v/v) for 24 h under mantle-reflux. The solvent was removed under reduced pressure using a rotary vacuum evaporator (EYELA N-1000 S; Tokyo Rikakikai, Japan). The extracts were filtered using a Whatman No.1 filter paper, and the filtrates were freeze-dried with a vacuum freeze dryer (MCFD 8508; Ilshin Lab, Korea) and blended into powder using a mill (Kinematica, Switzerland) with 90 standard mesh. The extract powders were mixed in the inverse ratio of the minimum bactericidal concentration (MBC) of each herbal extract against *C. coli*, and the combination was designated as CGE.

To determine the minimum inhibitory concentration (MIC) and MBC of the herbal extracts, a modified microdilution method was used to determine the MIC of the methanol extracts from *Coptidis rhizoma* and *G. rhois* against *C. coli*. The extract (20 µL) was diluted to final concentrations ranging from 0.1 to 2.0 mg/mL in a 96-well microtiter plate and with and without the addition of 30 µg/mL erythromycin and 100 µg/mL neomycin (Sigma-Aldrich, Germany). After plates were incubated for 48 h under conditions mentioned above, colonies of *C. coli* were counted and expressed as log CFU/g fecal samples. Statistical analyses were carried out using a one-way analysis of variance (ANOVA) and Student’s t-test. All data were expressed as the mean ± SD. The values at $p < 0.05$ were considered to be statistically significant.

As shown in Table 1, the MIC and MBC against *C. coli* were low in order of CGE, *Coptidis rhizoma* and *G. rhois* extract. As CGE was mixed with the inverse ratio of MBC of *Coptidis rhizoma* and *G. rhois* extract, the herbal mixture consisted of *Coptidis rhizoma* and *G. rhois* extract at a rate of 8 : 5 (w/w). In a previous study [9], the MIC of aqueous Chinese soft leek extracts against *C. coli* was 2.0 mg/mL. In another previous study [12], the MBC of *Eleutherine americana* extract against *Campylobacter* spp. ranged from 31.25 to 1,000 µg/mL. In preceding study on the anti-*Campylobacter* effects of herbal extracts [13], the MIC and MBC of the extract from *Drypetes gosseweileri* were 0.78 and 3.125 µg/mL, correspondingly, and those of the extract from *Parkia biglobosa* was both 1.56 mg/mL. Considering the herbal extract method and bacteria species, the MIC and/or MBC of CGE in this study were lower than that of the above herbal extracts, except for the *Parkia biglobosa* extract.

Figure 1 shows the change in *C. coli* numbers in feces of piglets administered with different concentrations of CGE during the experimental period. At the 1st day after treat-

<table>
<thead>
<tr>
<th>Herbal extract</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coptidis rhizoma</em></td>
<td>0.15</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Galla rhois</em></td>
<td>0.25</td>
<td>4.0</td>
</tr>
<tr>
<td>CGE</td>
<td>0.10</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Table 1.** MIC and MBC of *Coptidis rhizoma* extract, *Galla rhois* extract and CGE on *Campylobacter coli*
Efficacy of CGE on piglets infected with *C. coli* 261

**Fig. 1.** *Campylobacter coli* counts in fecal contents of piglets.

\[\begin{array}{c}
\text{Control treated with normal feed (n = 10); A Group A treated with 2.0 g/kg feed CGE [a mixture of *Coptidis rhizoma* extract and *Galla rhois* extract (8 : 5, w/w)] (n = 10); B Group B treated with 4.0 g/kg feed CGE (n = 10).} \\
\end{array}\]

- In conclusion, the results in this study demonstrate that CGE at a concentration of 2.0 g/kg in feed may be used to reduce *C. coli* counts in feces of piglets. Therefore, CGE could be an effective candidate for the treatment of *C. coli* infection in piglets.

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