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Short Communication: Bioactive Materials

Antimicrobial Effect of Emodin Isolated from *Cassia tora* Linn. Seeds against Food-Borne Bacteria

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Abstract The antimicrobial activities of emodin and its derivatives (anthraquinone, alizarin, and alizarin-3-methyliminodiacetic acid) were evaluated using a paper disc diffusion method against food-borne bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus intermedius*, *Salmonella typhimurium* and *Shigella sonnei*). Emodin isolated from *C. tora* seeds has an antimicrobial activity against *Bacillus cereus*, followed by alizarin-3-methyliminodiacetic acid (13.0±2.5 mm) and alizarin (11.5±1.2 mm). Furthermore, emodin showed the antimicrobial activity against *S. sonnei* and *S. typhimurium*. In conclusion, *C. tora* seed and its active component derivatives are useful for the development of natural products on food supplemental agents and pharmaceuticals.

Keywords antimicrobial activity · *Cassia tora* seeds · emodin · food-borne bacteria

For the last decades, food industry has emphasized effort to develop their products modified from the current market. Some techniques have been devised to prevent the microorganism proliferation from the food degradation and the risk of pathogens (Appendini and Hotchkiss, 2002). The concerns about food safety have being continued because of the outbreaks of new food-borne disease caused by pathogenic microorganism. Although chemical or artificial preservatives are already used to inhibit or inactivate growth of various kinds of pathogenic microorganisms, some of them have been caused to unwanted effects such as allergic diseases (Fleming-Jones and Smith, 2003; Powella et al., 2011; Liang et al., 2012). The natural substances as alternatives may be employed (Kim et al., 2013). Therefore, these play the important roles in future food preservation markets.

Cassia tora Linn. is an annual small shrub which grows in Asian countries. It is commonly known as 'Sicklepod' (Maity et al., 1998). The seed extracts of *C. tora* have been used in Chinese medicine as an aperient, anti-asthenic and diuretic agent and also to improve visual activity (Asolkar et al., 1992; Maity et al., 1998). The seeds of *C. tora* contain several anthraquinone glycosides and naphthopyran glycosides. The seed extract is also reported for its hypotensive activity. Many medicinal properties such as antihepatotoxic, antimicrobial, and antimutagenic activities have been attributed this plant (Wong et al., 1989; Choi et al., 1997; Yen and Chung, 1999; Patil et al., 2004). To substantiate the claim, this study was initiated to evaluate the antimicrobial effects of *C. tora* seeds and active components against food-borne bacteria.

Alizarin, alizarin-3-methyliminodiacetic acid, and emodin were purchased from Fluka Chemical (Germany) and anthraquinone and tetracycline were provided from Sigma Chemical (USA). The other chemicals were of reagent grade. The seeds of *C. tora* were purchased from a local market in Jeonju and the extraction and partition of *C. tora* seeds were performed as modified from Kim et al. (2004). *C. tora* seeds (5 kg) were extracted twice with methanol (10 L) in the shaking incubator at 30°C for 48 h, and filtered using a rotary vacuum evaporator (EYELA, NAJ-100, Japan). The yield of *C. tora* extract was 10.56% (528 g). Next, the combined filtrate (10 g) was partitioned in hexane (1.5 g), chloroform (2.1 g), ethyl acetate (1.2 g), butanol (1.3 g), and water soluble (3.1 g). The methanol extracts and five fractions were stored to

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refrigeration at 4°C.

To isolate active component from *C. tora* seeds, the chloroform fraction (10 g) was loaded on silica gel column chromatography (6.5×100 cm, Merck 70-230 mesh, 900 g) and successively eluted with chloroform:methanol (gradient, v/v). The separated fractions were analyzed via thin layer chromatography (TLC, silica gel 60 F_{254}, chloroform:methanol, 30:1, v/v) and some fractions showing similar patterns were combined. As a result, seven fractions (CT-1 to CT-7) were produced and the CT-3 fraction (3.3 g) showed strong antimicrobial activities against food-borne bacteria. The CT-3 fraction was further chromatographed on a silica gel column and eluted with chloroform:methanol (30:1, v/v). The CT-31 fraction (1.35 g) showed potent antimicrobial activities against food-borne bacteria. This bioactive fraction (CT-31) was then chromatographed over a Sephadex LH-20 column (Pharmacia, 5×80 cm) using chloroform:acetone:methanol (50:1:2, v/v/v). This operation was repeated three times. Again, an active fraction (CT-311) (1.12 g) exhibited strong antimicrobial activities against food-borne bacteria. This fraction was chromatographed over a Polyclar AT column (Touzart and Matignon, 100 g) packed with chloform:acetone (50:1, v/v) and eluted with an increasing ration of methanol (1, 2, 5, 10, and 20%). The active fraction (CT-3114), containing antimicrobial activities against food-borne bacteria, was finally purified successively on a Sephadex LH-20 column (Pharmacia) eluted with chloroform:methanol (6:4, v/v). Finally, CT-31144 fraction (116 mg) was isolated. The chemical structure of the isolated active component was determined using spectroscopic analysis methods (Fig. 1). The ^{1}H- and ^{13}C- Nucleic Magnetic Resonance (NMR) spectra were recorded in deuterochloroform ($CDCl_3$) using a JNM-ECA 600 spectrometer at 600 and 150 MHz (with trimethylsilane as an internal standard), respectively, with chemical shifts expressed in δ (ppm). In addition, EI-MS spectra were obtained with a JEOL JMS-DX 30 spectrometer (Japan) and IR spectra were obtained on a Bio-Rad (CA) FT-80 spectrophotometer.

The antimicrobial effects of materials were evaluated against food-borne bacteria (the gram positive bacteria: *Bacillus cereus* ATCC 14579, *Listeria monocytogenes* ATCC 15313, *Staphylococcus intermedius* ATCC 29663; the gram negative bacteria: *Salmonella typhimurium* IFO 14193 and *Shigella sonnei* ATCC 25931). Bacterial strains were obtained from the Korean Culture Center of Microorganisms (Korea). Bacterial strains were aerobically cultured at 37°C for 24 h in Nutrient broth (NB). The agar diffusion method was employed for the determination of antimicrobial activities of the materials (NCCLS, 2008). Briefly, microorganisms were incubated in NB at 37°C for 24 h, to yield approximately 1.0×10^{7} CFU/mL as compared to the turbidity of the McFarland turbidity standard. A suspension of the incubated microorganisms (0.1 mL of 1.0×10^{7} CFU/mL) was spread on Mueller Hinton agar (MHA) plates, and sterilized paper discs (8 mm in diameter) were impregnated with 40 μL of the sample and placed on the inoculated MHA plates. Tetracycline was served as a positive control at 0.1 mg/disc. These plates were then incubated under aerobic conditions at 37°C for 24 h. Treatments were performed in triplicate, and antimicrobial effect was expressed as the diameters of the inhibition zones (mm). Values are presented as means ± SD of three parallel measurements. All treatments were repeated three times. These results are expressed as mean values ± standard deviations (SD). According to SAS (version 6), statistical significance was accepted at a level of $p<0.05$.

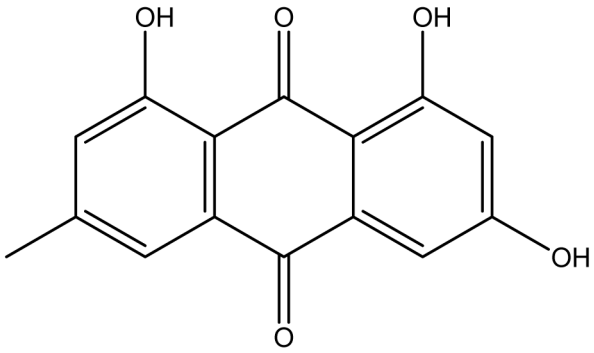

Fig. 1 Structure of Emodin.

In this study, the yield of the methanol extract of *C. tora* seeds was 10.56%. The five fractions derived from the *C. tora* extract varied. The highest yield was obtained from the water soluble fraction (31%), followed by chloroform fraction (21%), hexane fraction (15%), butanol fraction (13%), and ethyl acetate (12%), respectively. Furthermore, to isolate active component from the chloroform fraction derived from *C. tora* extract, the step of isolation and identification were processed. As a result, the isolated component was characterized as emodin (6-methyl-1,3,8-trihydroxyanthraquinone) (Fig. 1). Emodin ($C_{15}H_{10}O_5$, MW: 270.24, organe needles from ethanol, mp 260-263 ºC): IR (KBr) μ_{max} 3425 (O-H), 1667, 1627 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.46 (3H, s, Ar-CH_3), 6.65 (1H, d, J=2.5 Hz, H-4), 7.12 (1H, s, H-7), 7.24 (1H, d, J=2.5 Hz, H-4), 7.55 (1H, s, H-5), 12.05 (1H, s, OH), 12.18 (1H, s, OH); 13C-NMR ($CDCl_3$) δ 191.9, 182.2, 167.0, 165.9, 161.9, 149.3, 136.6, 134.0, 124.8, 121.2, 114.4, 110.0, 109.7, 108.6, 21.8. The isolation and spectral analyses of emodin from *C. tora* have already reported from the study of anthraquinones isolated from *Cassia obtusifolia* seeds and the current findings are similar to those of a study conducted by Yang et al. (2003).

To establish structure-activity relationship on emodin derivatives and antimicrobial activity against food-borne bacterial, the antimicrobial activities of emodin isolated from *C. tora* seeds and its derivatives (anthraquinone, alizarin, and alizarin-3-methyl-iminodiacetic acid) were evaluated using a paper disc diffusion method. These results were showed in Table 1. Based on the clean zone (mm) of each sample against food-borne bacteria, at 1 mg/disc, emodin isolated from *C. tora* seeds has a moderate antimicrobial activity (20.0±1.4 mm) against *Bacillus cereus*, and has a weak antimicrobial activities (14.6±1.4 and 14.4±1.8 mm) against *Shigella sonnei* and *Salmonella typhimurium* whereas the antimicrobial activities against *Listeria monocytogenes* and *Staphylococcus intermedius* had not showed. Compared with the

Table 1 Antimicrobial effects of compound derived from *Cassia tora* seed extract

Materials[1)]	Clean zone (mm)				
	Microorganisms[2)]				
	Bc	Lm	Si	Ss	St
Anthraquinone (backbone)	-[4)]	-	-	-	-
Alizarin	11.5±1.2[3)]	-	11.0±2.1	-	-
Alizarin-3-methyliminodiacetic acid	13.0±2.5	-	23.5±1.6	-	-
Emodin (isolated component)	20.0±1.4	-	-	14.6±1.4	14.4±1.8
Tetracycline[5)]	38.5±1.2	27.7±2.3	20.0±2.6	21.7±1.4	25.0±1.8

[1)]Dose: 1 mg/disc.
[2)]Bc, *Bacillus cereus* ATCC 14579; Lm, *Listeria monocytogenes* ATCC 15313; Si, *Staphylococcus intermedius* ATCC 29663; Ss, *Shigella sonnei* ATCC 25931; St, *Salmonella typhimurium* IFO 14193.
[3)]Values (mm) were expressed as means ± SD of three parallel measurements, $p < 0.05$.
[4)]-, no activity.
[5)]Tetracycline was served as a positive control at 0.1 mg/disc.

antimicrobial activities of emodin and its derivatives (anthraquinone, alizarin, and alizarin-3-methyliminodiacetic acid) against food-borne bacteria, against *B. cereus*, the emodin (20.0±1.4 mm) has the highest antimicrobial effect, followed by alizarin-3-methyl-iminodiacetic acid (13.0±2.5 mm) and alizarin (11.5±1.2 mm). Only, against *S. intermedius*, alizarin (11.0±2.1 mm) and alizarin-3-methyliminodiacetic acid (23.5±1.6 mm) had moderate or weak antimicrobial effect. In case of *S. sonnei* and *S. typhimurium*, the antimicrobial activity of emodin was only showed. In addition, all samples had inactivation against *L. monocytogenes* and the antimicrobial activity of anthraquinone was not showed against all food-borne bacteria. However, in comparison with antimicrobial activity of tetracycline as a positive control, against all food-borne bacteria, tetracycline had a potent antimicrobial activity than emodin and its derivatives.

According to previous studies, the emodin (LC_{50} values 1.4, 1.9, and 2.2 mg/L) obtained from *C. obtusifolia* had the potent larvicidal activity against three mosquito species (*Culex pipiens pallens*, *Aedes aegypti*, and *A. togoi*), respectively (Yang et al., 2003). In addition, Chukwujekwu et al. (2005) reported that the bioactive minimum inhibitory concentration (MIC) values of emodin were 7.8 and 3.9 ppm against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. The findings of toxicity of *C. tora* seeds and emodin derivatives toward human and mammal have not been reported. Taken together, the emodin has possessed a wide range of biological activities. Furthermore, *C. tora* seed and its active component derivatives are useful for the development of natural products on food supplemental agents and pharmaceuticals.

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