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Antimicrobial Properties of Turmeric (*Curcuma longa* L.) Rhizome-Derived *ar*-Turmerone and Curcumin

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Abstract The growth responses of six bacterial strains exposed to materials extracted from turmeric (*Curcuma longa*) rhizomes were examined using impregnated paper disk agar diffusion. Methanol extracts of turmeric rhizomes exhibited strong inhibitory activity against *Clostridium perfringens* and weak inhibitory activity toward *Escherichia coli* at 5 mg/disk. However, in tests conducted with *Bifidobacterium adolescentis*, *B. bifidum*, *B. longum*, and *Lactobacillus casei*, the methanol extract showed no inhibitory response. The biologically active constituent isolated from the turmeric rhizomes extracts was characterized as *ar*-turmerone using various spectroscopic analyses including EI-MS and NMR. The responses varied according to the dosage, chemicals, and bacterial strain tested. At 2 and 1 mg/disk, *ar*-turmerone strongly inhibited the growth of *C. perfringens* and moderately inhibited the growth of *E. coli* without any adverse effects on the growth of four lactic acid-bacteria. Of the commercially available compounds originating from turmeric rhizomes, curcumin exhibited strong and moderate growth inhibition against *C. perfringens* at 2 and 1 mg/disk, respectively, and weak growth inhibition against *E. coli* at 1 mg/disk. However, little or no activity was observed for borneol, 1,8-cineole, and sabinene against all six bacteria strains tested. The observed inhibitory activity of the turmeric rhizome-derived curcumin and *ar*-turmerone against *C. perfringens* and *E. coli* demonstrate one of the important pharmacological activities of turmeric rhizomes.

Key words: *Clostridium perfringens*, *Escherichia coli*, growth-inhibiting activity, *ar*-turmerone, turmeric (*Curcuma longa*) rhizome

Introduction

Intestinal microfloras are classified as beneficial or harmful bacteria based on their effects on human health. In particular, harmful bacteria such as *Clostridium*, *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, and *Veillonella* not only produce carcinogenic substances *de novo* but also change metabolites from dietary sources into tumor initiators or promoters (1-3). The infectious diseases caused by clostridia have a broad spectrum of clinical severity that ranges from mild outpatient illness to sudden death. Among clostridia, *Clostridium perfringens* has been associated with sudden death, toxicity, and gastrointestinal disease in humans (1, 3). In contrast, bifidobacteria are often taken as useful indicators of human health under most environmental conditions, based on the fact that they play important metabolic roles such as amino acid and vitamin production, pathogen inhibition and immuno-potentiation, and their association with longevity (4-7). Accordingly, it is desirable to both inhibit the growth of potential pathogens such as clostridia and increase the numbers of bifidobacteria in the human digestive system. Selective promoters of bifidobacteria or inhibitors of harmful bacteria are especially important for human health, since intake of these materials can normalize physiological functions that have been disturbed, thereby preventing and treating various diseases caused by pathogens in the gastrointestinal tract (8, 9).

In recent years, much attention has been focused on selective, plant-derived growth modulators in the intestine, based on the fact that most plant-extracted materials are relatively nontoxic to humans (2, 10). Since many of these materials are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of potentially safer anti-microbial agents (2, 10). Therefore, much effort has been focused on identifying plant materials with potential use products as commercial antimicrobial agents. In East Asia, the rhizome of turmeric (*Curcuma longa* L.) has long been considered to have natural medicinal properties as an analgesic in the treatment of menstrual disorders, rheumatism, and traumatic diseases due to the number of mono-terpenoids, sesquiterpenoids, and curcuminoids it contains (11). Furthermore, it has been noted that the materials of turmeric rhizome have antiplatelet (12), fungicidal (13), and repellent (14) properties. The antiplatelet constituents and insect repellent in turmeric rhizome are curcuminoids (12) and turmerones (15), respectively. However, relatively little work has been done on the inhibitory activities of the rhizome of turmeric toward harmful intestinal bacteria, despite its known antibacterial and antioxidant properties. Active compounds isolated from turmeric rhizomes may be a good source of lead compounds for antimicrobial agents.

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Materials and Methods

Chemicals Borneol, 1,8-cineole, and sabinene were purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). All chemicals were of reagent grade.

Bacteria strains and culture conditions The bacterial

strains used in this study were *Bifidobacterium adolescentis* ATCC 15073, *B. bifidum* ATCC 29521, *B. longum* ATCC 15707, *C. perfringens* ATCC 13124, *E. coli* ATCC 11775, and *Lactobacillus casei* ATCC 27216 isolated from human feces. Stock cultures of these strains were routinely stored as bacteria culture (Brain Heart Infusion broth, pH 7.6; deMan Rogosa Sharpe broth, pH 5.7) in 25% glycerol at -80°C and subcultured on Eggerth Gagnon (EG) agar (Eiken Chemical, Tokyo, Japan) when required. The plates were incubated anaerobically at 37°C for 2 days in an anaerobic chamber with an atmosphere of 80% N_2, 15% CO_2, and 5% H_2. The bacteria were then grown in EG broth (pH 6.8).

Extraction and isolation Dried rhizome (4.5 kg) from turmeric was purchased from material collected in March 2005 in Jeonju (Jeonbuk, Korea). It was finely powdered, extracted twice with methanol (10 L) at room temperature for two days and filtered. The combined filtrate was concentrated under vacuum at 34°C and yielded about 9.76% (based on the weight of the dried rhizome). The extract (439 g) was sequentially partitioned into hexane (160.7 g), chloroform (116.4 g), ethyl acetate (72.6 g), butanol (24.2 g), and water (65.1 g) for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation at 35°C, while the water portion was freeze-dried. The active component of the hexane fraction was isolated as described by Kim *et al.* (13).

Because of its strong antimicrobial activity against *C. perfringens*, the hexane fraction (10 g) was chromatographed on a silica gel column (70-230 mesh, 600 g, 6.5 i.d. × 65 cm; Merck, Rathway, NJ, USA), and successively eluted with chloroform/methanol (50:1 and 20:1). Column fractions were collected and analyzed by thin layer chromatography (TLC, SILC/UV 254, 025 mm; Macherey-Nagel, Germany). Fractions with a similar TLC pattern were pooled. The antimicrobial fraction (1.9 g) was successively rechromatographed on a silica gel column, using hexane/ethyl acetate (30:1). For further separation of the biologically active constituent, a preparatory HPLC (JAI Recycling Preparative HPLC, LC 908W-C 60; Analytical Industry Co., Ltd., Japan) was used. The column was μPorasil (19 i.d. × 300 mm, Waters, Millipore, MA, USA) using hexane/ethyl acetate (100:1) at a flow rate of 5 mL/min and measured at 243 nm. Finally, a potent bioactive compound (248 mg) was isolated. Structural determination of the active isolate was made by spectroscopic analysis. ^{1}H- and ^{13}C-NMR spectra were recorded in deuterochloroform with a Bruker AM-500 spectrometer at 400 and 100 MHz, respectively. Chemical shifts were reported as δ values downfield from an internal standard of Me_4Si. Mass spectra were obtained on a Jeol GSX 400 spectrometer (Jeol JMS-AX 302 WA; Tokyo, Japan).

Growth-Inhibition Assay To assay of the effect of the test materials on the growth of test microorganisms, one loopful of bacteria was suspended in 1 mL of sterilized physiological saline. An aliquot (0.1 mL) of the bacterial suspension was seeded on EG agar. A sample of the test material in 100 L of methanol was applied using a Drummond glass microcapillary to a paper disk (Advantec 8 mm-diameter and 1 mm-thickness). After evaporation of the solvents, the disks were placed on the agar surface inoculated with the test bacteria. All plates were incubated anaerobically at 37°C for 2 days. The control disks received 100 L of methanol, which exhibited no adverse effects on the organisms used. All tests were performed in triplicate. The inhibitory responses were classified as previously described (2): potent response, ++++, zone diameter >30 mm; strong response, +++, zone diameter 21-30 mm; moderate response, ++, zone diameter 16-20 mm; weak response, +, zone diameter 10-15 mm; and little or no response, -, zone diameter <10 mm.

Results and Discussion

The growth-inhibiting activities of the five fractions obtained from methanol extracts of turmeric rhizomes were tested against six different bacterial strains. The methanol extract exhibited strong inhibitory activity against *C. perfringens* and weak activity against *E. coli* at 5 mg/disk. However, in tests conducted with *B. adolescentis*, *B. bifidum*, *B. longum*, and *L. casei*, the methanol extracts showed no inhibitory effect (Table 1). We next tested various fractions of the methanol extract at a dose of 5 mg/disk. The hexane and ethyl acetate fraction exhibited a strong inhibitory activity against *C. perfringens*, but little to no inhibitory activity against *B. adolescentis*, *B. bifidum*, *B. longum*, *E. coli*, and *L. casei* (Table 1). Little to no inhibitory activity was observed with the chloroform, butanol, and water fractions against

Table 1. Growth-inhibiting effects of turmeric rhizome-derived materials against human intestinal bacteria

Material[1)]	Bacterial strain[2)]					
	B. bifidum	*B. longum*	*B. adolescentis*	*C. perfringens*	*E. coli*	*L. casei*
Methanol Extract	-[3)]	-	-	+++	+	-
Hexane Fraction	-	-	-	+++	+	-
Chloroform Fraction	-	-	-	-	-	-
Ethyl acetate Fraction	-	-	-	+++	-	-
Butanol Fraction	-	-	-	-	-	-
Water Fraction	-	-	-	-	-	-

[1)]Exposed to 5 mg/disk. [2)]Cultured on Eggerth-Gagnon agar at 37°C for 2 days in an atmosphere of 80% N_2, 15% CO_2 and 5% H_2.
[3)]Inhibitory zone diameter 21-30 mm, +++; 16-20 mm, ++; 10-15 mm, +; and <10 mm, -.

the six bacterial strains tested.

Purification of the biologically active compound from the hexane fraction was performed by silica gel column chromatography and HPLC. Bioassay-guided fractionation of the turmeric rhizome extracts provided an active constituent identified by spectroscopic analyses, including EI-MS, ^{1}H-NMR, and ^{13}C-NMR (Fig. 1-3), and by direct comparison with an authentic reference compound. The active constituent was characterized as the sesquiterpene ketone *ar*-turmerone. The compound was identified on the basis of the following evidence; EI-MS (70 eV), *m/z* (% rel int) M^+ 216 (100), 201 (30), 132 (21), 119 (65), 117 (14), 83 (81), 55 (17): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (3H, d, J = 7 Hz), 1.84 (3H, d, J = 2 Hz), 2.03 (3H, d, J = 2 Hz), 2.25 (3H, s), 2.63 (2H, d, J = 2 Hz), 3.22 (1H, m), 6.10 (1H, m), 7.06 (4H, s): ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.8, 21.0, 22.6, 27.6, 37.0, 53.5, 125.1, 127.7, 130.0, 136.6, 144.6, 157.0, 202.5. The spectroscopic analyses of *ar*-turmerone isolated from the hexane fraction are identical to the data of *ar*-turmerone isolated from turmeric rhizome (13, 15).

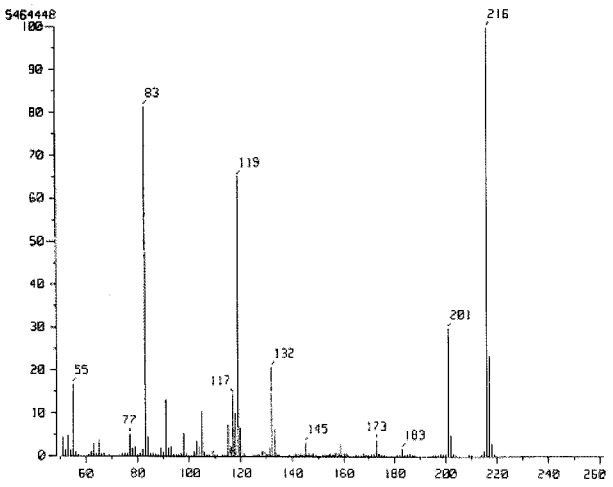

Fig. 1. Mass spectra of *ar*-turmerone isolated from turmeric rhizomes.

The growth-inhibiting activity of *ar*-turmerone against the six bacterial strains was examined using the impregnated paper disk method (Table 2). The responses varied according to the dosage and the bacterial strain tested. With regard to *C. perfringens*, *ar*-turmerone exhibited strong growth inhibition (+++) at 2 and 1 mg/disk and moderate growth inhibition (++) at 0.5 mg/disk. Furthermore, this isolate revealed moderate activity against *E. coli* at 2 and 1 mg/disk and weak activity against *E. coli* at 0.5 mg/disk. However, *ar*-turmerone exhibited little or no inhibition toward *B. adolescentis*, *B. bifidum*, *B. longum*, and *L. casei* at 2 mg/disk (Table 2). The results of the current study indicate that the growth-inhibiting activity of *ar*-turmerone is more pronounced toward *C. perfringens* and *E. coli*, as compared to bifidobacteria and lactobacilli. The isolate did not adversely affect the growth of the bifidobacteria and lactobacilli tested. These results for the growth inhibitory activity of *ar*-turmerone confirm its superiority and usefulness as an antimicrobial agent.

To determine the antimicrobial activities of other components identified in turmeric rhizomes (13), borneol, 1,8-cineole, curcumin, and sabinene were tested against the six intestinal bacteria (Table 2). Of the four compounds, curcumin exhibited strong and weak growth inhibition against *C. perfringens* and *E. coli*, respectively, at 2 and 1 mg/disk. Furthermore, curcumin exhibited moderate growth inhibition against *C. perfringens* at 0.5 mg/disk. However, little or no activity was observed for borneol, 1,8-cineole, and sabinene against *B. adolescentis*, *B. bifidum*, *B. longum*, *C. perfringens*, and *L. casei* at 2 and 1 mg/disk. The results of the current study indicate that the growth-inhibiting activities of the hexane and ethyl acetate fractions derived from turmeric rhizome extracts against *C. perfringens* and *E. coli* can be attributed primarily to *ar*-turmerone and curcumin, respectively.

This study is the first to report the antimicrobial

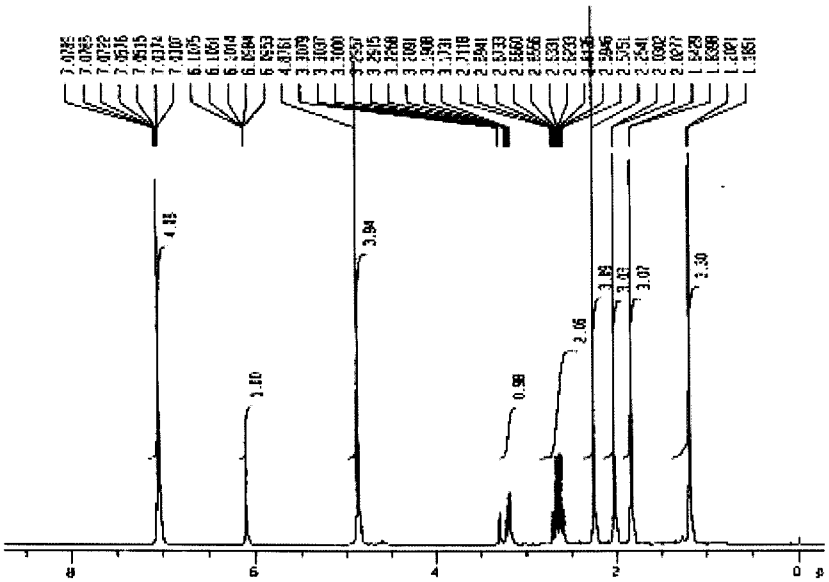

Fig. 2. ^{1}H NMR spectra of *ar*-turmerone isolated from turmeric rhizomes.

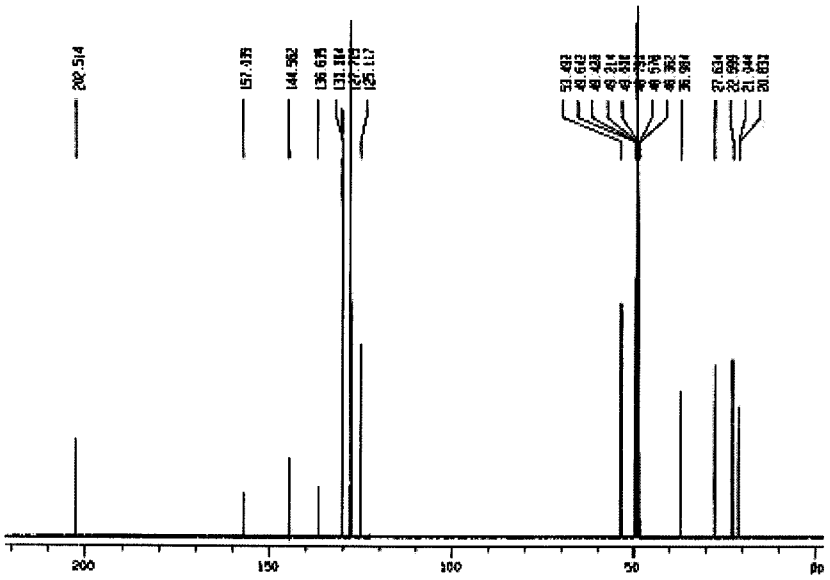

Fig. 3. ^{13}C NMR spectra of *ar*-turmerone isolated from turmeric rhizomes.

Table 2. Growth-inhibiting effects of isolated turmeric rhizome-derived components and cinnamaldehyde against human intestinal bacteria

Compound	Dose (mg/disk)	Bacterial strain[1)]				
		B. bifidum	*B. longum*	*C. perfringens*	*E. coli*	*L. casei*
ar-Turmerone	2.0	-[2)]	-	++++	++	-
	1.0	-	-	+++	++	-
	0.5	-	-	++	+	-
	0.1	-	-	+	-	-
Curcumin	2.0	-	-	+++	+	-
	1.0		-	++	+	
	0.5		-	++		
	0.1	-	-	-	-	-
Borneol	2.0	-	-	-	-	-
	1.0	-	-	-	-	-
1,8-Cineole	2.0	-	-	-	-	-
	1.0	-	-	-	-	-
	0.5	-	-	-	-	-
Sabinene	2.0	-	-	-	-	-
	1.0	-	-	-	-	-

[1)]Cultured on Eggerth-Gagnon agar at 37°C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2. [2)]Inhibitory zone diameter >30 mm, ++++; 21-30 mm, +++; 16-20 mm, ++; 10-15 mm, +; and <10 mm, -.

function of components isolated from turmeric rhizomes. Extract of turmeric rhizomes is used in foods as a condiment. It is also used as an essential ingredient in medicine as a carminative, antihelminthic, laxative and as a cure for liver ailments (16). The use of turmeric rhizomes as an antiplatelet agent, insect repellent, insecticide, and antibacterial agent has long been known (13, 15-19). Among these components, turmeric oils and curcuminoids constitute a major group of secondary metabolites. In previous studies, turmerone and *ar*-turmerone isolated from turmeric rhizomes have been reported to be repellent toward *Tribolium castaneum* (Hbst.) (17, 20), and showed inhibitory effects on arachidonic acid-induced platelet aggregation. Thromboxane A_2 is transformed *via* the COX pathway from arachidonic acid, suggesting that turmerone and *ar*-turmerone may have, at least partly, an effect on the COX pathway in platelets (12). Curcuminoids such as curcumin, demethoxy-

curcumin, and bismethoxycurcumin have potent anti-oxidant, antibacterial, antitumorigenic, and anti-inflammatory properties (16, 18, 21, 22).

In conclusion, the current results indicate that the materials extracted from the turmeric rhizomes have growth-inhibiting effects *in vitro* against specific bacteria from the human intestine. The observed inhibitory action of the turmeric rhizomes components toward *C. perfringens* and *E. coli* may be indicative of at least one of the pharmacological actions of the turmeric rhizomes. In this regard, further research is necessary to establish whether this activity remains *in vivo* after human consumption of turmeric rhizomes by humans.

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