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Selective Responses of Three *Ginkgo biloba* Leaf-Derived Constituents on Human Intestinal Bacteria

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The selective responses of *Ginkgo biloba* leaf-derived materials against six intestinal bacteria was examined using an impregnated paper disk method and compared with that of bilobalide, ginkgolides A and B, kaempferol, and quercetin. The components of *G. biloba* leaves were characterized as kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside), kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside, and quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) by spectroscopic analysis. The growth responses varied with each bacterial strain tested. At 2 mg/disk, kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) and quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) revealed potent inhibition against *Clostridium perfringens*, and kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside showed a clear inhibitory effect on *Escherichia coli*. At 0.5 mg/disk, quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) showed a strong activity against *C. perfringens,* but weak activity was exhibited by kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) against *C. perfringens* and kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside against *E. coli*. No inhibition was observed from treatments conducted with bilobalide, ginkgolides A and B, kaempferol, or quercetin. Furthermore, these isolated compounds did not inhibit *Bifidobacterium bifidum*, *B. longum*, *B. adolescentis*, or *Lactobacillus acidophilus*.

KEYWORDS: ***Ginkgo biloba*; intestinal bacteria; kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside); kaempferol 3-*O*-(2″-*O*-β-*D*-glucopyranosyl)-α-*L*-rhamnopyranoside; quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside)**

INTRODUCTION

Various microorganisms are resident in the human intestinal tract in a highly complex ecosystem with considerable species diversity (*1, 2*). They not only participate in the normal physiological functions, but also contribute significantly to the genesis of various disease states, by biotransforming a variety of ingested or endogenously formed compounds to useful or harmful derivatives (*1, 2*). Accordingly, these biotransformations may influence drug efficacy, toxicity, carcinogenesis, and aging (*3, 4*). Differences in the intestinal bacteria between patients and healthy subjects, and between younger and elderly subjects, have been observed. A normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seem to play a large role not only in metabolism, but also host defense against infection, aging, and immunopotentiation (*3, 4*). In contrast, the microbiota of cancer patients is composed of a high concentration of clostridia and eubacteria with few lactic acid bacteria. It has also been reported that elderly subjects harbor fewer bifidobacteria and more clostridia than younger subjects. Thus, any disturbance of the microbiota may cause a variety of diseases of abnormal physiological states (*5*−*7*).

Much current concern about human health has been focused on plant-derived bifidus factors which promote the growth of bifidobacteria or growth inhibitors against harmful bacteria such as clostridia, eubacteria, and *Escherichia coli* because plants constitute a rich source of bioactive chemicals and many of them are largely free from harmful adverse effects (*8, 9*). Earlier findings have already reported and confirmed that among 78 oriental plant species, the methanol extract of *Ginkgo biloba* leaves revealed a potent growth-inhibiting activity toward *Clostridium perfringens* (*10*). This plant species is not only important as an insecticide, but is considered to possess some medicinal properties, such as an antioxidative agent and an inhibitor of arachidonic acid (*9, 11, 12*). However, relatively little work has been carried out on the effects of *G. biloba* leaf-derived materials on growth of intestinal microorganisms compared to other areas of intestinal microbiology despite its excellent pharmacological action.

We assessed the selective responses of three *G. biloba* leaf-derived constituents on human intestinal bacteria in order to develop new and safer types of modulators in human intestine. In addition, the antibacterial activities of six components derived

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from *G. biloba* are also discussed in relation to the results obtained.

MATERIALS AND METHODS

Chemicals. Kaempferol and quercetin were obtained from Sigma Chemical (St. Louis, MO). Bilobalide and ginkgolides A and B isolated from *G. biloba* were kindly provided by Prof. Moo-Key Kim from the Faculty of Biotechnology, College of Agriculture, Chonbuk National University, Chonju, South Korea. All other chemicals were of reagent grade.

Bacterial Strains and Culture Conditions. The bacterial strains used in this study were *Bifidobacterium bifidum* ATCC 29521, *B. longum* ATCC 15707, *B. adolescentis* ATCC 15073, *Clostridium perfringens* ATCC 13124, *Escherichia coli* ATCC 11775, and *Lactobacillus acidophilus* KCTC 3145 isolated from human feces. Stock cultures of these strains were routinely stored on Eggerth-Gagnon liver extract-Fieldes slant at −80 °C, and, when required, were subcultured on Eggerth-Gagnon (EG) agar (Eiken Chemical Co., Ltd, Tokyo, Japan) (*13*). The plates were incubated anaerobically at 37 °C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2 in an anaerobic chamber (Coy Lab., Grass Lake, MI). The bacteria were then grown in EG broth (pH 6.8).

Isolation and Identification. Leaves of *G. biloba* (10 kg) were dried in an oven at 60 °C for 2 days, finely powdered, extracted twice with 60% aq Me_2CO at room temperature, and filtered (Toyo filter paper No. 2, Japan). The extract was concentrated via rotary evaporation at 35 °C to yield about 13.4% dry weight of the leaves (*14*). The extract (20 g) was sequentially partitioned into hexane, EtOAc, BuOH, and H_2O portions for subsequent bioassay with *C. perfringens* and *B. fragilis*. The organic solvent portions were concentrated to dryness by rotary evaporation at 35 °C, and the H_2O portion was freeze-dried. For isolation, 5 mg of each *G. biloba* leaf-derived fraction in methanol was applied to paper disks (Advantec 8 mm diameter and 1 mm thick, Toyo Roshi, Japan).

Kaempferol 3-O-α-(6‴-p-Coumaroylglucosyl-β-1,4-rhamnoside). The EtOAc extract (51 g) was chromatographed over Amberlite IRN-78 column (Prolabo, 100 g, USA) using a stepwise gradient of H_2O/MeOH (0, 10, 20, 40, and 60%) and then MeOH/0.05 N HCl. The active 40% fractions (22.5 g) were monitored by TLC. The active 40% fraction was chromatographed over a silica gel 60 column (Merck, 1.0 kg, USA) packed with EtOAc and eluted with a stepwise gradient of EtOAc/MeOH (0, 10, 20, 30, and 40%). The active fraction (3.2 g) was chromatographed over a Polyclar AT column (Touzart and Matignon, 100 g, USA) packed with $CHCl_3$/MeOH (5:1) and eluted with the same solvent. The compound was finally purified successively on a Sephadex LH-20 column (Pharamacia, USA) and cellulose (Merck) eluted with MeOH. Pure compound (475 mg) was obtained as an amorphous solid.

Kaempferol 3-O-(2″-O-β-D-Glucopyranosyl)-α-L-rhamnopyranoside. The concentrated BuOH extract (48 g) was chromatographed over a Sephadex LH-20 column (Pharmacia, 800 × 49 mm, USA) and a Sepralyte RP-18 column (Analytichem, prep. grade, 40 μm, 713 × 18.5 mm, USA) with different MeOH/H_2O gradients as mobile phases. HPLC column, Hypersil ODS, 3 μm (Knauer, USA): mobile phase, MeOH (A) and 0.5% *ortho*-H_3PO_4 in H_2O (B). A linear gradient was run from 38 to 48.2% MeOH in 12 min. Pure compound (113 mg) was obtained. Acetylation of the compound was carried out by stirring 500 mg of the substance with 500 μL of Ac_2O in 500 μL of pyridine overnight at room temperature to afford a nonacetate of the compound. Complete hydrolysis of the compound was achieved in 2 M trifluoroacetic acid by refluxing for 60 min. Identification of the aglycons and the sugars was completed via TLC on silica gel 60 F_{254} (Merck, USA). Diphenylboric acid 2-amino-ethyl ester (1% in MeOH) was used for the detection of the aglycons.

Quercetin 3-O-α-(6‴-p-Coumaroylglucosyl-β-1,4-rhamnoside). The H_2O fraction (189 g) was defatted with C_6H_6 and chromatographed over an Amberlite IRN-78 column (100 g) using a stepwise gradient of H_2O/MeOH (0, 10, 20, 30, 40, 50, and 60%) and then MeOH/0.05 M HCl. Active fractions (42 g) were monitored by TLC. The active 30% fraction was chromatographed over a silica gel 60 column (1.0 kg) packed with $CHCl_3$ and eluted with a stepwise gradient of $CHCl_3$/MeOH (0, 10, 20, 30, 40, and 50%). The active fraction (4.5 g) was chromatographed over a Sephadex LH-20 column (Pharmacia, 800 × 49 mm, USA) using a stepwise gradient of H_2O/MeOH (20, 25, 30, 35, and 40%). This operation was repeated five times. The active fraction (250 mg) was chromatographed over a Polyclar AT column (100 g) packed with $CHCl_3$/MeOH (5:1) and eluted with an increasing ratio of MeOH (25, 30, 35, 40, 45, and 50%). The compound was finally purified on a Sephadex column (915 g) eluted with MeOH. Pure compound (200 mg) was obtained.

Structural determination of the active isolate was based on the spectroscopic analysis. ^1H- and ^{13}C NMR spectra were recorded with a Bruker AM spectrometer, and chemical shifts are given in ppm. UV spectra were obtained on a Waters 490 spectrometer. IR spectra were obtained on a Biorad FT-80 spectrophotometer, and mass spectra were obtained on a JEOL JMS-DX 30 spectrometer. The method of Park et al. was used to visualize the isolated compounds (*12*).

Microbiological Assay. For assaying the inhibitory effect of each test sample on the microorganisms, one loopful of bacteria was suspended in 1 mL of sterile physiological saline. An aliquot (0.1 mL) of the bacterial suspensions was seeded on EG agar. Samples of the extract dissolved in methanol were applied to paper disks by using a Drummond glass microcapillary (Advantec, 8 mm, Toyo Roshi, Japan). After evaporation, the disks were placed on EG agar surface and incubated at 37 °C for 2 days in an anaerobic chamber. Control disks were treated with methanol only. All inhibition tests were conducted in triplicate. The growth responses of test samples were determined by comparison with those of the control. The inhibitory responses were classified as previously described: inhibitory zone diameter >30 mm, ++++; 21−30 mm, +++; 16−20 mm, ++; 10−15 mm, +; and <10 mm, − (*15, 16*)

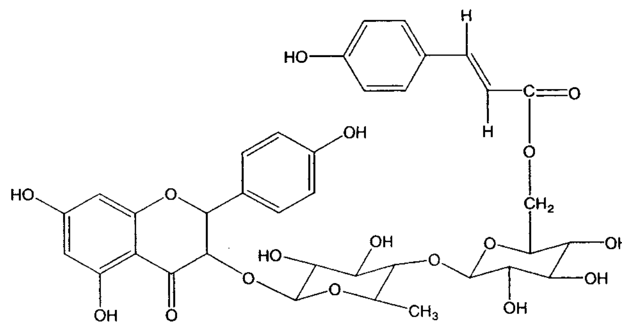
Table 1. Growth-Inhibiting Effects on Human Intestinal Bacteria by Various Fractions Obtained from Me_2CO Extracts of *Ginkgo biloba* Leaves

material[a]	bacterial strain[b]				
	B. longum	*B. bifidum*	*B. adolescentis*	*C. perfringens*	*E. coli*
Me_2CO extract	++[c]	-	++	++++	++++
hexane fraction	-	-	-	-	-
EtOAc fraction	+	-	-	++++	+
BuOH fraction	-	-	-	-	++++
H_2O fraction	+	-	-	++++	-

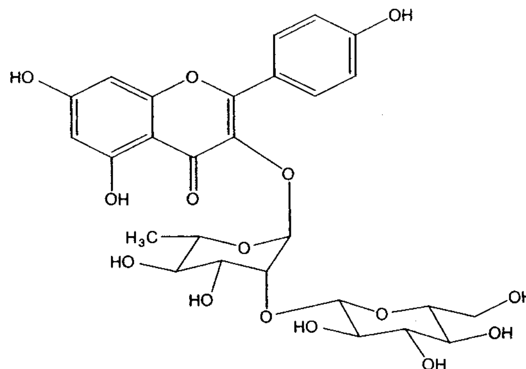
[a] Exposed to 5 mg/disk. [b] Cultured on Eggerth-Gagnon agar at 37 °C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2. [c] Inhibitory zone diameter >30 mm, ++++; 21–30 mm, +++; 16–20 mm, ++; 10–15 mm, +; and <10 mm, -.

RESULTS

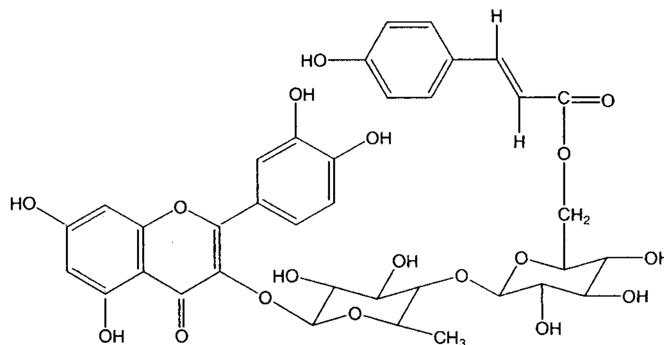
Growth-inhibiting activities of human intestinal bacteria to the fractions obtained from Me_2CO extracts of *G. biloba* leaves were assayed by using the impregnated paper disk method (**Table 1**). The Me_2CO extracts showed a very strong activity (++++) against *C. perfringens* and *E. coli*, and moderate activity (++) against *B. longum* and *B. adolescentis*. However, in tests conducted with *B. bifidum,* which is predominantly in the intestines of infants, the Me_2CO extracts showed no inhibitory response. EtOAc and H_2O fractions obtained from Me_2CO extracts showed a strong activity against *C. perfringens*, and the BuOH fraction exhibited a strong activity against *E. coli*. However, the hexane fraction had no inhibitory response against any intestinal bacteria tested. Purification of the biologically active compounds from the fractions were chromatographed via repeated silica gel, Amberlite IRN-78, Polyclar AT, Sephadex LH-20, and Sepralyte RP-18 column chromatography, and the isolates were bioassayed. Three active isolates showed a strong and moderate activity. Structural determinatios of the isolates were made by various spectroscopic analyes, and the compounds were characterized as kaempferol 3-*O*-α-(6‴-*p*-



Kaempferol-3-*O*-α–(6‴-p-coumaroylglucosyl-β-1,4-rhamnoside), **K1**



Kaempferol-3-*O*-(2″-*O*-β -D-glucopyranosyl)-α-L-rhamnopyranoside, **K2**



Quercetin-3-*O*-α–(6‴-p-coumaroylglucosyl-β-1,4-rhamnoside), **Q3**

Figure 1. Structures of three compounds isolated from *Ginkgo biloba* leaves.

coumaroylglucosyl-β-1,4- rhamnoside), **K1**, kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside, **K2**, and quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside), **Q3** (**Figure 1**). The compounds were identified on the basis of the following evidence.

K1. Mp 335−339 °C; $[\alpha]_D^{20}$ −60° (EtOH; *c* 1). UV λ_{max}^{EtOH} nm: 355sh, 310, 263; +NaOAc: 370sh, 304, 267; +NaOAc−H_3BO_3: 355sh, 310, 263; +$AlCl_3$: 396sh, 307sh, 302, 275, 225; +$AlCl_3$−HCl: 397sh, 307sh, 303, 275, 225. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.92 (3H, *d*, *J* = 6.0 Hz, Me rhamnose), 3.06−4.24 (*m*, sugars protons), 4.35 (1H, *d*, *J* = 8 Hz, H-1 GLC), 5.61 (1H, *d*, *J* = 2.0 Hz, H-1 rha), 6.11 (1H, *d*, *J* = 2.5 Hz, H-6), 6.10 (1H, *d*, *J* = 16.0 Hz, H-8 coum), 6.25 (1H, *d*, *J* = 2.5 Hz, H-8), 6.72 (2H, *d*, *J* = 9.0 Hz, H-3 coum and H-5 coum), 6.91 (2H, *d*, *J* = 9.0 Hz, H-3′ and H-6′), 7.37 (2H, *d*, *J* = 9.0 Hz, H-2 coum and H-6 coum), 7.45 (1H, *d*, *J* = 16.0 Hz, H-7 coum), 7.72 (2H, *d*, *J* = 9.0 Hz, H-2′ and H-6′), 8.49 (s, OH phenolic). CIMS 70 eV, *m/z* (rel int.): 741 [M + H]$^+$ (2), 595 (10), 472 (14), 433 (100), 287 (1). K1 gave ^{13}C NMR (50 MHz, DMSO-d_6) spectra similar to those published for for kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-β-1,4-rhamnoside) isolated from *G. biloba* (*12*).

K2. Mp 308−311 °C. FABMS: *m/z* 633 [M + K]$^+$, 617 [M + Na]$^+$, 595 [M + H]$^+$, 287 [aglycon + H]$^+$. UV λ_{max}^{MeOH} nm: 267, 297sh, 346; NaOMe 273, 324, 388; $AlCl_3$ 275, 300, 344, 396; $AlCl_3$−HCl 272, 299, 340, 392; NaOAc 272, 302, 375; NaOAc−H_3BO_3 264, 316sh, 344. ^{1}H NMR (300 MHz, CD_3-OD) δ: 0.94 (3H, *d*, *J* = 5.9 Hz, H-6″), 3.19−3.43 (6H, *m*, sugar protons), 3.70 (2H, *m*, H-6‴), 3.81 (1H, *dd*, $J_{3'',2''}$ = 3.5 Hz, $J_{3'',4''}$ = 9.3 Hz, H-3″), 4.29 (1H, *dd*, $J_{2'',1''}$ = 1.1 Hz, $J_{2'',3''}$ = 3.5 Hz, H-2″), 4.42 (1H, *d*, *J* = 7.7 Hz, H-1‴), 5.73 (1H, *d*, *J* = 1.1 Hz, H-1″), 6.20 (1H, *d*, *J* = 1.9 Hz, H-6), 6.37 (1H, *d*, *J* = 1.9 Hz, H-8), 6.94 (2H, *d*, *J* = 8.9 Hz, H-3′ and H-5′), 7.76 (2H, *d*, *J* = 8.9 Hz, H-2′ and H-6′). ^{1}H NMR (300 MHz, $CDCl_3$) of kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside-nonaacetate (kaempferol 3-*O*-(2″-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside after acetylation) δ: 0.87 (3H, *d*, *J* = 6.2, H-6″), 1.96−2.10 (18H, aliphatic OAc units),

Table 2. Growth-Inhibiting Effects of *Ginkgo biloba* Leaf-Derived Compounds against Intestinal Bacteria

compound	dose (mg/disk)	bacterial strain[a]			
		B. bifidum	*B. longum*	*C. perfringens*	*E. coli*
K1	5.0	-[b]	-	++++	-
	2.0	-	-	++++	-
	1.0	-	-	++	-
	0.5	-	-	+	-
K2	5.0	-	-	-	++++
	2.0	-	-	-	+++
	1.0	-	-	-	++
	0.5	-	-	-	+
Q3	5.0	-	-	++++	-
	2.0	-	-	+++	-
	1.0	-	-	+++	-
	0.5	-	-	+++	-
bilobalide	5.0	-	-	-	-
ginkgolide A	5.0	-	-	-	-
ginkgolide B	5.0	-	-	-	-
kaempferol	5.0	-	-	-	-
quercetin	5.0	-	-	-	-

[a] 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. [b] Inhibitory zone diameter >30 mm, ++++; 21–30 mm, +++; 16–20 mm, ++; 10–15 mm, +; and <10 mm, -.

2.32−2.40 (9H, aromatic OAc units) 3.29 (1H, *m*, H-5″), 3.70 (1H, *m*, H-5‴), 4.05 (1H, A*B*X, $J_{6'''B,\,5'''}$ = 2.4 Hz, $J_{6'''A,\,6'''B}$ = 12.3 Hz, H-6‴ *B*), 4.33 (1H, *A*BX, $J_{6'''A,\,5'''}$ = 3.8 Hz, $J_{6'''A,\,6'''B}$ = 12.3 Hz, H-6‴ *A*), 4.41 (1H, *m*, H-2″), 4.58 (1H, *d*, *J* = 8.0 Hz, H-1‴), 4.85 (1H, *t*, *J* = 10.0 Hz, H-4″), 5.06−5.25 (4H, *m*, H-3″, H-2‴, H-3‴, H-4‴), 5.60 (1H, *d*, *J* = 1.9 Hz, H-1″), 6.20 (1H, *d*, *J* = 2.2 Hz, H-6), 6.26 (1H, *d*, *J* = 2.2 Hz, H-8), 7.27 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 7.89 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′). The ^{13}C NMR (75 MHz, CD_3OD) spectra of K2 were found to be the same as those for kaempferol 3-*O*-(2″-*O*-*β*-D-glucopyranosyl)-α-L-rhamnopyranoside isolated from *G. biloba* (*12*).

Q3. Mp 229−233 °C. UV λ_{max}^{EtOH} nm: 360sh, 316, 300sh, 268, 258; + NaOAc: 370sh, 315, 300sh, 269; + NaOAc−H_3BO_3: 373sh, 315, 300sh, 263; + $AlCl_3$: 410sh, 360sh, 315, 300sh, 272; + AlC_3−HCl: 400sh, 360sh, 315, 300sh; 277. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.91 (3H, *d*, *J* = 6.0 Hz, Me rhamnose), 3.03−4.15 (*m*, sugars protons), 4.28 (1H, *d*, *J* = 8.0 Hz, H-1 GLC), 5.52 (1H, *d*, *J* = 2.0 Hz, H-1 rha), 6.16 (1H, *d*, *J* = 2.0 Hz, H-6), 6.24 (1H, *d*, *J* = 16.0 Hz, H-8 coum), 6.31 (1H, *d*, *J* = 2.0 Hz, H-8), 6.70 (2H, *d*, *J* = 8.6 Hz, H-3 coum and H-5 coum), 6.88 (1H, *d*, *J* = 8.4 Hz, H-5′), 7.25 (1H, *dd*, *J* = 2.0 Hz and 8.4 Hz, H-6′), 7.36 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.41 (2H, *d*, *J* = 8.6 Hz, H-2 coum and H-6 coum), 7.45 (1H, *d*, *J* = 16.0 Hz, H-7 coum). CIMS 70 eV *m*/*z* (rel int.) 757 $[M + H]^+$ (0.3), 611 (1), 595 (0.5), 472 (6.6), 449-(66.7), 303(100). The ^{13}C NMR spectra of Q3 were found to be the same as those for quercetin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-*β*-1,4-rhamnoside) isolated from *G. biloba* (*14*).

The growth-inhibiting activities of the isolated compounds and other components of this plant species toward intestinal bacteria when treated with 5 mg/disk were determined (**Table 2**). Responses varied with the chemical and bacterial strain tested. **K1** and **Q3** showed a very strong growth-inhibiting activity (++++) against *C. perfringens*, but no inhibitory response against other bacteria tested. **K2** exhibited a strong growth-inhibiting activity against *E. coli*, but no inhibitory response against other bacteria. No activity was observed for bilobalide, ginkgolides A and B, kaempferol, or quercetin derived from *G. biloba*.

Because of their potent growth-inhibiting activity toward test bacteria, the isolated compounds were evaluated at low concentrations (**Table 2**). At 2 mg/disk, **K1** and **Q3** produced a very clear inhibitory effect on *C. perfringens* (++++), and **K2** showed a clear inhibitory effect on *E. coli* (+++). Furthermore, any inhibition toward *B. longum* and *L. acidophilus* was not exhibited by these three compounds. At 1 mg/disk, growth of *C. perfringens* was significantly inhibited by **Q3**, whereas moderate inhibitory activity was obtained by **K1**. Furthermore, growth of *E. coli* was moderately inhibited by **K2**. At 0.5 mg/disk, **Q3** showed a strong growth-inhibiting activity (+++) against *C. perfringens,* but weak activity was exhibited by **K1** against *C. perfringens* and **K2** against *E. coli*.

DISCUSSION

The intestinal microbiota in healthy people remains relatively constant but is known to be significantly influenced by physical, biological, chemical, environmental, or host factors (*3*, *17*). Accordingly, alterations to the microbiota may cause abnormal physical conditions or diseases. In our study, the growth-inhibitory activity of *G. biloba* (Ginkgoaceae) leaf-derived materials against five intestinal bacteria in vitro was investigated, and kaempferol 3-*O*-α-(6‴-*p*-coumaroylglucosyl-*β*-1,4-rhamnoside), kaempferol 3-*O*-(2″-*O*-*β*-D-glucopyranosyl)-α-L-rhamnopyranoside, and quercertin 3-*O*-α-(6‴-*p*-coumaroylglucosyl-*β*-1,4-rhamnoside) were identified by comparison of their physical and spectroscopic data with those reported in the literature (*12*, *14*). The ^{1}H and ^{13}C NMR spectra of **K2** showed signals consistent with the presence of glucose, rhamnose, and kaempferol. Triplets at δ 62.4 in the ^{13}C NMR spectra pointed to a free C(6)H_2OH of glucose, and the doublets of the anomeric protons in the ^{1}H NMR at δ 4.42 (*J* = 7.7 Hz) indicated the terminal position of glucose. Both H-1 signals of the rhamnose units were doublets at δ 5.73 (*J* = 1.1 Hz) indicating, by its downfield shifted location, the linkage between rhamnose and C-3 of the aglycon. The *β*-configuration of glucose (diaxial coupling) and the α-configuration of rhamnose (diequatorial coupling) were evidenced by their respective coupling constants $^3J_{1,2}$. Acetylation of **K2** afforded the nonaacetate showing the signal of H-2″ at nearly unchanged position, thus confirming the proposed 2″→1″ linkage. The growth-inhibitory effects of these compounds varied with bacterial strain tested. In this family, a great number of plant extracts have been investigated for the biological properties (*12*, *18*, *19*). Furthermore, materials derived from *G. biloba* have been extensively studied for pharmacological and pesticidal effects (*12*, *14*, *18*).

Infectious diseases caused by clostridia have a broad spectrum of clinical severity that ranges from mild outpatient illness to sudden death. Among the clostridia, *C. perfringens* has been associated with sudden death, toxicity, and gastrointestinal disease in man (*20*, *21*). In contrast, bifidobacteria are often taken as useful indicators of human health under most environmental conditions, because they play important roles in metabolism such as amino acid (*22*) and vitamin production (*17*), aid in the defense against infections (*3*), association with longevity (*23*), antitumor activities (*24*), pathogen inhibition (*25*, *26*), and immunopotentiation (*27*, *28*). Accordingly, it would be desirable to both inhibit the growth of potential pathogens such as clostridia and/or increase the numbers of bifidobacteria in the human gut. Selective growth promoters for bifidobacteria or inhibitors for harmful bacteria are especially important for human health, because intake of these materials may normalize disturbed physiological functions, resulting in the prevention and treatment of various diseases caused by pathogens in the gastrointestinal tract. In recent years, much concern has been focused on selective plant-derived growth modulators in the

intestine, based on the fact that many medicinal plant-derived materials are relatively nontoxic to humans. For example, extracts from ginseng (*Panax ginseng*) and green tea (*Thea chinensis* L.) have been shown to not only enhance the growth of bifidobacteria, but selectively inhibit various clostridia (*29*, *30*). In our study, the growth responses of *G. biloba* leaf-derived compounds varied according to bacterial strain tested. Growth-inhibiting activity was more pronounced in *C. perfringens* and *E. coli*, as compared to that of the bifidobacteria. In the test using 1 mg/disk, **Q3** and **K1** showed very strong and moderate activity, respectively, against *C. perfringens*, and **K2** exhibited moderate activity against *E. coli.* These three compounds did not adversely affect the growth of bifidobacteria and lactobacilli used. These results suggest that the strong and moderate inhibitory activity of the three isolates confirms their superiority and usefulness as bacteriocidal agents.

In conclusion, our results indicate that three components isolated from *G. biloba* leaves have growth-inhibiting effects in vitro against specific bacteria from the human intestine. On the basis of our limited data and some earlier findings, the inhibitory action of these compounds against *C. perfringens* and *E. coli* may be an indication of at least one of the pharmacological actions of *G. biloba* leaves. Further work is necessary to establish whether this activity is exerted in the human large intestine after consumption of *G. biloba* leaves by humans.

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