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Note

Growth-Inhibiting Effects of Various Traditional Drinks of Plant Origin on Human Intestinal Bacteria

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A total of 28 traditional drinks derived from 23 plant species in 19 families were tested for their *in vitro* growth-inhibiting effects against *Bifidobacterium adolescents*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Clostridium perfringens*, and *Escherichia coil* using a paper disc agar diffusion method under anaerobic condition. The responses varied with bacterial strain, plant species and tissue sampled. In a test with *C. perfringens* at 5 and 10 mg/disc, potent growth inhibition was produced from the extracts of *Eucommia ulmoides* stems, *Pinus densiflora* leaves and shoots, *Thea sinensis* leaves (green and oolong teas) and *Zingiber officinale* roots. All materials tested did not adversely affect the growth of *bifidobacteria*, *lactobacilli*, and *E. coli*. These results may be an indication of at least one of the pharmacological activities of these plant-derived drinks.

Key words: *growth inhibition, plant, drink, intestinal bacteria.*

Gastrointestinal ecological investigations have indicated that there are age- and disease-associated differences in intestinal bacteria.[1,2] The normal gastrointestinal microbiota is found to be predominantly composed of lactic acid-producing bacteria which seem to play a large role in metabolism, host defense against infection, aging and immunopotentiation.[1,2] On the other hand, the microbiota of cancer patients, patients with Alzheimer's disease, or elderly subjects are composed of a high concentration of clostridia and eubacteria with few lactic acid-producing bacteria.[1-3] Accordingly, any disturbance of the microbiota may cause a variety of diseases or abnormal physiological states.

In recent years, much concern has been focused on plant-drived bifidus factors which promote the growth of beneficial bacteria and plant-derived growth inhibitors against harmful bacteria such as *Clostridium perfringens* and *Escherichia coli* because plants are the richest source of bioactive chemicals and many of them are largely free from harmful adverse effects.[4,5] In East Asia, various plants have been very closely associated with people's life as food or medicine at first and then to make herbal drinks. However, the effects of traditional drinks on the growth of intestinal bacteria have not been fully investigated in spite of their excellent nutritional and pharmacological activities.[5] Therefore, we assessed the growth-inhibiting activity of various herbal drinks derived from plants toward four lactic acid-producing and two harmful intestinal bacteria.

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Materials and Methods

Bacterial strains and culture conditions. The bacterial strains used in this study were as follows: *Bifidobacterium adolescentis* ATCC 15703, *B. bifidum* ATCC 15696, *Lactobacillus acidophilus* ATCC 4356, *L. casei* ATCC 27216, *Clostridium perfringens* ATCC 13124, and *Escherichia coli* ATCC 11775. Stock cultures of these bacterial strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slant[8] at -80°C and, when required, were subcultured on EG (Eiken Chemical, Japan) agar. The plates were incubated anaerobically at 37°C for 2 days in an anaerobic chamber (Coy Lab., USA) in an atmosphere of 5% H_2+15% CO_2+80% N_2. The bacteria were then grown in PYFG broth.

Plant materials and sample preparation. Twenty eight samples of 23 plant species in 19 families were purchased as commercially available products and are listed in Table 1. These samples were finely powdered using a blender, extracted twice with methanol at room temperature for 2 days and filtered (Toyo filter No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 45°C

Table 1. Traditional drinks of plant origin tested.

Test ma terial	Family name	Tissue sampled[a]	Yield (%)[b]
Panax ginseng	Araliaceae	R	8
Platycodon grandiflorum	Campanulaceae	R	16
Artemisia princeps	Compositae	L	3
Chrysanthemum zawadskii var. *latilobum*	Compositae	L	4
Cichorium intybus	Compositae	R	55
Cornus officinalis	Cornaceae	Fr	54
Dioscorea batas	Dioscoreaceae	R	2
Diospyros kaki	Ebenaceae	L	13
Eucomimia ulmoides	Eucommiaceae	St	1
		L	13
Cassia tora	Fabaceae	Se	10
Pueraria thunbergiana	Fabaceae	R	2
Hordeum vulgare	Gramineae	Se	2
Zea mays	Gramineae	Se	4
Polygonatum odoratum	Liliaceae	R	56
Schizandra chinensis	Schizandraceae	Fr	54
Morus alba	Moraceae	L	7
Pinus densiflora	Pinaceae	L	10
		P	12
		Sh	11
Pleuropterus multiflorus	Polygonaceae	R	5
Zizyphus jujuba	Rhamnaceae	Fr	65
		Se	13
Coffea arabica	Rubiaceae	Se	10
Lycium chinense	Solanaceae	Fr	35
Thea sinensis	Theaceae	L[c]	38
		L[d]	28
Zingiber officinale	Zingiberaceae	R	9

[a]Fr, fruit; L, leaf; P, pollen; R, root; Se, seed; Sh, shoot; and St, stem.
[b](Dried weight of methanol extract/dried weight of sample)× 100.
[c]Green tea.
[d]Oolong tea.

with rotary evaporator. The yield of each plant extract is shown in Table 1.

Microbiological assay. For assay of effects of the tea plants on the growth responses of the test organism, one loopful of bacteria was suspened in 1 m*l* of sterile physiological saline. An aliquot (0.2 m*l*) of the bacterial suspensions was seeded on EG agar. A sample (5, 10 and 20 mg) dissolved in 100 μ*l* methanol was applied by syringe to a paper disc (Advatec 8 mm-diameter and 1-mm thickness, Toyo Roshi, Japan). After evaporation of solvents, the discs were placed on EG agar surface inoculated with test bacteria. All plates were incubated anaerobically at 37°C for 2 days. Control discs received 100 μ*l* methanol. All tests were performed in triplicate. The growth responses of the six bacterial strains to the test materials were determined by comparison with those of controls.

Results and Discussion

The effects of test materials on growth inhibition of harmful intestinal bacteria such as *C. perfringens* and *E. coli* are given in Table 2. The responses varied with plant species, bacterial strain and tissue sampled. In a test with *C. perfringens* at 20 mg/ disc, extracts of *Thea sinensis* leaves (green tea), *Thea sinensis* leaves (oolong tea), and *Zingiber officinale* roots revealed strong growth-inhibiting activity (+++) whereas moderate growth inhibition (++) was produced from extracts of *Panax ginseng* roots, *Artemisia princeps* leaves, *Chrysanthemum zawadskii* whole plants, *Eucommia ulmoides* leaves and stems, *Schizandra chinense* fruits, *Morus alba* leaves, and *Pinus densiflora* leaves and shoots. The other plant extracts exhibited weak or no inhibitory effect against *C. perfringens*. When exposed to 5 and 10 mg/disc, potent inhibitory effect was observed in extracts from *E. ulmoides* stems, *P. densiflora* leaves and shoots, *T. sinensis* leaves (green tea), *T. sinensis* leaves (oolong tea), and *Z. officinale* roots. For *E. coli*, all test materials showed no growth-inhibiting activity toward the organism even at 20 mg/disc.

Infectious diseases caused by clostridia have a broad spectrum of clinical severity that ranges from mild out-patient illness to sudden death. Among the clostridia, *C. perfringens* has been associated with sudden death, toxicity, and gastrointestinal disease in humans by biotransforming a variety of ingested or endogenously formed compounds to harmful agents like *N*-nitroso compounds or aromatic steroids within the gastrointestine.[10,11] Recently, Fujusawa

Table 2. Growth-inhibiting activity of various plant-derived drinks against *Clostridium perfringens* ATCC 13124[a].

Test material	Tissue sampled[b]	Dose, mg/disc		
		5	10	20
P. ginseng	R	-[e]	+	++
C. officinale	F	+	+	+
A. princeps	L	+	++	++
C. zawadskii	L	+	++	++
D. kaki	L	-	+	+
E. ulmoides	L	+	+	++
	S	++	++	++
S. chinensis	F	+	+	++
M. alba	L	+	+	++
P. densiflora	L	++	++	++
	P	-	+	+
	S	++	++	++
T. sinensis	L[c]	++	++	+++
	L[d]	++	++	+++
Z. officinale	R	++	++	+++

[a]Plant species showing activity against *Clostridium perfringens* ATCC 13124 are presented. All test samples showed no growth-inhibiting activity toward *Bifidobacterium adolescentis* ATCC 15703, *B. bifidum* ATCC 15696, *Lactobacillus acidophilus* ATCC 4356, *L. casei* ATCC 27216, and *Escherichia coli* ATCC 11775.
[b]For explanation, see Table 1.
[c]Green tea.
[d]Oolong tea.
[e]The responses were classified as previously described[9]: the strong response, +++, zone diameter >20 mm; moderate response, ++, zone diameter 16~20 mm; weak response, +, zone diameter 10~15 mm; and no response, -, zone diameter <10 mm.

et al.[3] reported that the microbiota of patients with Alzheimer's disease are composed of a high concentration of *C. perfringens* with few bifidobacteria.

Bifidobacteria are often taken as useful indicators of human health under most environmental conditions. They play important roles in metabolism such as amino acid and vitamin production, aid defense against infection, are associated with longevity, antitumor activity, pathogen inhibition, improvement of lactose tolerance of milk products, and immunopotentiation.[12,13] Therefore, effects of test materials on the growth of lactic acid-producing bacteria used were determined (data not shown). Even at as high as 20 mg/disc, all materials tested did not cause adverse growth responses to *B. adolescentis, B. bifidum, L. acidophilus,* and *L. casei.*

It would be desirable to both inhibit the growth of potential pathogens 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 which result in the prevention of diseases caused by pathogens in the gastrointestinal tract. Recent *in vivo* investigations using human volunteers have shown that intake of ginseng extract or green tea extract favorably affected the fecal microbiota and biochemical aspects of feces,[6,14] suggesting an indication of at least one of their pharmacological actions. In our study, the growth-inhibiting activity of some drinks described was significantly pronounced in *C. perfringens.* However, they did not affect the growth of *B. adolescentis* and *B. bifidum,* dominant in intestine of the adult and the infant, respectively. These results suggest that intake of drinks of plant origin might be very helpful, regardless of age.

Based upon our limited data and some earlier findings, daily intake of some drinks described might be expected to alter the growth and composition of the microbial community and modulate the genesis of potentially harmful products due to their mild growth-inhibiting activity *in vitro* against specific intestinal bacteria. Further work is necessary to establish whether this activity is exerted *in vivo* after consumption of these drinks by humans.

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