Growth-inhibiting Effects of Brazilian and Oriental Medicinal Plants on Human Intestinal Bacteria

Moo-Key Kim, Sung-Eun Lee[1] and Hoi-Seon Lee*

Institute of Agricultural Science & Technology, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea
[1]Plant Protection Research Unit, Western Regional Research Center, Albany, CA 94706, U.S.A.

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Methanol extracts of 27 Brazilian plant samples and 10 oriental medicinal plant samples (27 families), using spectrophotometric and paper disc agar diffusion methods under anaerobic conditions, were tested *in vitro* for their growth-inhibiting activities against *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Clostridium perfringens*, and *Bacteroides fragilis*. The responses varied with bacterial strains, plant species, and tissues sampled. In a test with *B. longum* and *B. bifidum* (20 mg/disc), extracts of *Acanthopanax sessilifolinus* stem bark and *Ampelozizyphus amazonicus* leaves strongly inhibited the growth of *B. longum*, whereas other plant samples did not inhibit any intestinal bacteria tested. At 5 mg/disc, adding extracts of *Aralia eleta*, *Euterpe oleracea*, and *Syzygium guineense* to the media strongly inhibited the growth of *C. perfringens* and *B. fragilis* without growth inhibition of *B. adolescentis*, *B. longum*, and *B. bifidum*. Extracts of *Jacaranda mimosifolia* and *Ulmus paraifolia* significantly inhibited the growth of *C. perfringens* and *B. fragilis* as well as *B. adolescentis*. These results may be indications of at least one of the pharmacological actions of the five Brazilian plants but not oriental medicinal plants tested.

Key words: *intestinal bacteria, Brazilian plants, oriental medicinal plants, growth inhibition.*

Various microorganisms in the human intestinal tract have been investigated with regards to their highly complex ecosystem and considerable species diversity. It has been well acknowledged that the microbiota not only participate in normal physiological functions, but may also be the cause of various diseases by biotransforming a variety of ingested or endogenously formed compounds to potentially harmful agents such as *N*-nitroso compounds.[1,2] This biotransformation may influence drug efficacy, toxicity, carcinogenesis, and aging. Gastrointestinal ecological investigations have indicated that there are age- and disease-associated differences in intestinal bacteria.[3,4] Normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seem to play important roles in metabolism, host defense against infection, aging, and immunopotentiation.[3,4] 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.[3-7] It has also been reported that elderly subjects harbor fewer bifidobacteria but more clostridia than younger subjects. Accordingly, any disturbance of the microbiota may cause a variety of diseases.

In recent years, interests have been focused on plant-derived bifidus factors and plant-derived growth inhibitors against harmful bacteria such as *Clostridium perfringens* and *Bacteroides fragilis* because plants are the richest source of bioactive chemicals and many of them are largely free from harmful adverse effects.[8,9] However, relatively little work has been carried out on the effects of Brazilian and oriental medicinal extracts on the growth of intestinal microorganisms compared to other areas of intestinal microbiology in spite of their excellent nutritional and industrial significances.[10-12] Therefore, we assessed the inhibitory responses of human intestinal bacteria to extracts of 37 plant species (27 families) for the development of safer agents.

*Corresponding author
Phone: 82-652-270-2544; Fax: 82-652-270-2550
E-mail: hoiseon@moak.chonbuk.ac.kr

Materials and Methods

Bacterial strains and culture conditions. The bacterial strains used in this study were as follows: *Bifidobacterium longum* ATCC 15707, *B. bifidum* ATCC 29521, *B. adolescentis* ATCC 15073, *Clostridium perfringens* ATCC 13124, and *Bacteroides fragilis*. Stock cultures of these strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slant at -80°C[13] and, when required, were subcultured on Eggerth-Gagnon (EG) agar.[13] The plates were incubated 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., Michigan, USA). The bacteria were then grown in the EG broth (pH 6.8).

Plants and sample preparation. Ten different types of commercially processed oriental medicinal plants and 27 Brazilian plants were dried in an oven at 60°C for 3 days, finely powdered, extracted twice with methanol at room

temperature and filtered (Toyo filter paper No. 2). The combined filtrate was concentrated *in vacuo* at 35°C. Twenty-seven plant extracts were kindly provided by Dr Brandao of Universidade Federal de minas Gerais, Brazil. The yield of plant extractions are shown in Table 1.

Microbiological assay. To assay the inhibitory effect on the organisms, one loopful of bacteria was suspended in 1 ml sterile physiological saline. An aliquot (0.1 ml) of the bacterial suspensions was seeded on EG agar. Samples (5, 10, and 20 mg) dissolved in methanol were applied using Drummond microcapillary to paper discs (Advantec, 8 mm, Toyo Roshi, Japan). After evaporation, the paper discs were placed on EG agar surface and were incubated at 37°C for 2 days in an atmosphere of 5% H_2, 15% CO_2, and 80% N_2. Control discs received methanol. All inhibition tests were triplicated. The growth responses of the test samples as compared to those of the controls.

Results and Discussion

The most important factor in primary screening for bioactive substances may be the starting concentration. In our previous papers,[14,15] we reported that concentrations of 5 to 20 mg/disc of plant extracts did not cause problems such as solubility and detection of their minor active components. In this paper, growth-inhibiting responses of methanol extracts from 37 Brazilian and oriental medicinal plants to *B. adolescentis*, *B. longum*, *B. bifidum*, *C. perfringens*, and *B. fragilis* were investigated *in vitro* Table 2. The growth-inhibiting responses were plant species- and bacterial strain-dependent. At a concentration of 20 mg/disc, extracts of *Jacaranda mimosifolia* root and *Ulmus paraifolia* stem bark showed significant growth inhibition on *B. adolescentis* (+++), whereas moderate activity (++) was obtained in extracts of *Arringeria actisma* root, Fraxinus densata stem bark and *Syzygium guineense* root (Table 2). Of these, *J. mimosifolia* and *U. paraifolia* exhibited strong growth-inhibition (+++) at 5 mg/disc (Table 3). In a test with *C. perfringens*, extracts of *A. actisma*, *Euterpe oleracea*, *J. mimosifolia*, *S. guineense* and *U. paraifolia* strongly inhibited the growth of *C. perfringens* at 10 or 20 mg/disc (Table 3). The weak inhibition (+) was obtained in extracts of *Acanthopanax sessilifolinus*, *E. oleracea*, *Olea uropaea*, *Picrasma quassioides* and *Psychotria vellosiana* at 20 mg/disc. Even at 5 mg/disc, extracts of *J. mimosifolia* and *U. paraifolia* strongly inhibited the growth of *C. perfringens* (+++) but extracts of *E. oleracea* and *S. guineense* revealed moderate activity (++) at the same dose. However, the weak inhibition (+) was achieved in extract of *A. actisma* (Table 3). At 20 mg/disc, extracts of *A. eleta*, *E. oleracea*, *J. mimosifolia*, *S. guineense*, and *U. paraifolia* showed significant growth inhibition (+++) on *B. fragilis*, whereas weak activity (+) was obtained in extracts of *A. sessilifolinus*, *O. uropaea*, *P. quassioides*, and *J. mimosifolia*. Even at 5 mg/disc, extracts of *J. mimosifolia*, *S. guineense*, and *U. paraifolia* exhibited moderate activity (++).

Table 1. List of Brazilian and oriental medicinal plants tested.

Plant species	Family	Tissue sampled	Yield[a] (%)
Acanthopanax sessilifolinus	Araliaceae	stem bark	7.4
Ammobium alatum	Asteraceae	stem bark	6.5
Ampelozizyphus amazonicus	Vitaceae	leaf	8.2
Angelica polymorpha	Umbelliferae	root	9.1
Aralia eleta	Araliaceae	stem bark	4.3
Arringeria actisma	Gramineae	root	5.6
Baccahris trimera	Asteraceae	root	4.3
Castanea crenata	Fagaceae	stem bark	11.4
Callistemon linearis	Myrtaceae	stem bark	2.7
Dalbergia myriantha	Leguminosae	stem bark	8.6
Dioscorea rotununda	Disoscoreaceae	fruit	5.9
Dolichus kilimandscharicus	Leguminosae	root	9.5
Euonymus sieboliana	Celastraceae	stem bark	2.7
Euterpe oleracea	Oleraceae	seed	7.7
Fraxinus densata	Oleraceae	stem bark	7.3
Gnidia subcordata	Thymelaeaceae	stem bark	2.9
Ipomoea stans	Convolvulaceae	stem bark	4.7
Iudigofera anil	Fabaceae	root	7.5
Jacaranda mimosifolia	Bignoniaceae	root	8.6
Kalopanax pictus	Araliaceae	stem bark	13.1
Lagerstroemia speciosa	Lythraceae	root	6.4
Leptadenia madagascariensis	Santalaceae	stem bark	11.5
Morus alba	Moraceae	stem bark	6.3
Myristica fragrans	Myristicaceae	stem bark	9.4
Olea uropaea	Oleaceae	root	8.0
Passiflora suberosa	Passifloraceae	leaf	5.9
Picrasma quassioides	Simaroubaceae	stem bark	6.9
Pseudospondias microcarpa	Gramineae	stem bark	8.2
Psychotria vellosiana	Rubiaceae	leaf	4.3
Rosmarinus officinalis	Rosaceae	leaf	6.0
Rumex obtusifolius	Polygonaceae	leaf	12.2
Salvia officinalis	Labiatae	leaf	7.3
Sclerocarya bierra	Cyperaceae	stem bark	4.8
Solanum aculeatissimum	Solanaceae	root	5.7
Sorbus commixta	Rosaceae	stem bark	9.5
Syzygium guineense	Myrtaceae	root	4.8
Ulmus paraifolia	Ulmaceae	stem bark	7.1

[a](Weight/dried weight of sample)×100.

Addition of extracts of *A. eleta*, *E. oleracea*, and *S. guineense* to the media inhibited the growth of *C. perfringens* or *B. fragilis* without the growth inhibition of *B. adolescentis* (Table 3). Extracts of *J. mimosifolia* and *U. paraifolia* inhibited significantly the growth of *C. perfringens* and *B. fragilis* as well as *B. adolescentis*.

It would be most desirable to both inhibit the growth of potential pathogens and/or increase the number 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 the disturbed physiological functions which result in the prevention or reduction of diseases caused by pathogens in the gastrointestinal tract. Similar *in vitro* results were also reported in extracts of ginseng and green tea.[16,17] Previous *in vivo* investigations[18,19] with human volunteers have shown that intake of

Table 2. Growth-inhibitory activity of intestinal bacteria from extracts of Brazilian and Oriental medicinal plants.

Plant species[a]	Bacterial Strain[b]				
	B. adolescentis	*B. longum*	*B. bifidum*	*C. perfringens*	*B. fragilis*
A. sessilifolinus	-c	+++	-	+	+
A. amazonicus	-	+++	-	-	-
A. actisma	++	-	-	+++	+++
E. oleracea	-	-	-	+++	+++
F. densata	++	-	-	+	-
J. mimosifolia	+++	+	+	+++	+++
O. uropaea	-	+	+	+	+
P. quassioides	-	-	-	+	+
P. vellosiana	-	-	-	+	+
S. guineense	++	-	-	+++	+++
U. paraifolia	+++	-	-	+++	+++

[a]Plant species showing activity are presented: *A. alatum, A. polymorpha, A. eleta, B. trimera, C. crenata, C. linearis, D. myriantha, D. rotununda, D. kilimandscharicus, E. sieboliana, G. subcordata, I. stans, I. anil, K. pictus, L. speciosa, L. madagascariensis, M. alba, M. fragrans, P. suberosa, P. microcarpa, R. officinalis, R. obtusifolius, S. officinalis, S. bierra, S. aculeatissimum* and *S. commixta* did not show any inhibitory effect on the test organism.

[b]Exposed at 20 mg/disc.

[c]Strong response +++, zone diameter >20 mm; moderate ++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; no response -, and zone diameter <10 mm.

Table 3. Growth-inhibiting activity of extracts of Brazilian and Oriental medicinal plants against intestinal bacteria.

Test Material	Bacterial Strain	Dose, mg/disc		
		5	10	20
A. actisma				
	B. adolescentis	-	++	+++
	B. fragilis	+	+++	+++
	C. perfringens	+	+++	+++
E. oleracea				
	B. adolescentis	-	-	-
	B. fragilis	+	+++	+++
	C. perfringens	++	+++	+++
J. mimosifolia				
	B. adolescentis	+++	+++	+++
	B. fragilis	+++	+++	+++
	C. perfringens	+++	+++	+++
S. guineense				
	B. adolescentis	-	++	++
	B. fragilis	+++	+++	+++
	C. perfringens	++	+++	+++
U. paraifolia				
	B. adolescentis	+++	+++	+++
	B. fragilis	+++	+++	+++
	C. perfringens	+++	+++	+++

Table 4. Growth-inhibiting activity of extracts of five species with various solvents against intestinal bacteria.

Plant Species Extracting Solvent	Bacterial Strain[a]		
	B. adolescentis	*C. perfringens*	*B. fragilis*
A. actisma			
Methanol	++	+++	+++
Acetone	+	+	-
Ethyl acetate	-	++	-
Hexane	-	-	+
E. oleracea			
Methanol	-	+++	+++
Acetone	-	++	+
Ethyl acetate	-	-	-
Hexane	-	-	-
J. mimosifolia			
Methanol	+++	+++	+++
Acetone	-	+++	+++
Ethyl acetate	-	++	-
Hexane	-	-	-
S. guineense			
Methanol	++	+++	+++
Acetone	++	+++	++
Ethyl acetate	-	++	
Hexane	-	-	+
U. paraifolia			
Methanol	+++	+++	+++
Acetone	++	+++	++
Ethyl acetate	-	+	-
Hexane	-	-	+

[a]Exposed at 10 mg/disc.

ginseng or green tea favourably affected the faecal microbiota and biochemical aspects of faeces, an indication of at least one phamacological action of ginseng and green tea.[20,21] Accordingly, daily intake of Brazilian or oriental plants could alter the growth and composition of the microbial community and modulate the generation of potentially harmful products such as carcinogenic *N*-nitroso compounds or aromatic steroids within the intestinal tract, thus protecting human from a variety of diseases and helping to maintain optimal health.

Among the various human intestinal microorganisms, bifidobacteria are often taken as useful indicators of human health under most environmental conditions. They play important roles in such human physiology as nutritional production of vitamin and essential amino acid, aid defense against infection, and are associated with longevity, pathogen inhibition, immunity activation, improvement of lactose tolerance of milk products, and antitumorigenic activity.[3,4,22-25] Bifidobacteria growth-promoting factors, better known as bifidus factors, have therefore been extensively studied ever since György *et al.*[26] suggested their

existence in human milk. They are classified into lacteal secretions, fructooligosaccharides, derivatives of lactose, and xylooligosaccharides.[27]

In test with *B. longum* or *B. bifidum*, predominant in the intestines of infants, extracts of *A. sessilifolinus* stem bark and *A. amazonicus* leaves strongly inhibited the growth of *B. longum* at a concentration of 20 mg/disc, but other rest of the plant samples (35 plants) showed weak or no inhibitory responses (Table 2), they did not adversely affect the growth of *B. longum* and *B. bifidum*.

Due to their potent growth-inhibitory activities against intestinal bacteria, the activity of each solvent extract of *A. actisma*, *E. oleracea*, *J. mimosifolia*, *S. guineense*, and *U. paraifolia* was evaluated (Table 4). The results indicate that the growth-inhibiting activity of intestinal bacteria increases with increasing polarity of the solvent. Apparently, methanol is the most effective extractor, followed by acetone, of the growth modulator against intestinal bacteria found in the five plant species. This result is similar to the data reported by Economou *et al.*[28] that show the methanol is a widely used and effective solvent for extraction. Therefore, this result demonstrates that the growth-inhibiting responses is greatly dependent on the type of solvents used for the extraction.

In conclusion, the strong activity of Brazilian plants described herein confirms the usefulness of the plants as growth modulators against intestine bacteria. Additionally, natural product-derived materials were found to be more effective than the synthetic growth modulator against intestinal bacteria. Further research to identify the biologically active substances from *A. actisma*, *E. oleracea*, *J. mimosifolia*, *S. guineense* and *U. paraifolia* which show the most potent growth-inhibiting activity is in progress.

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