Agric. Chem. Biotechnol. **44**(4), 185-189 (2001)

Growth-Inhibiting Effects of Herb Plants on Human Intestinal Bacteria

Moo-Key Kim, Byeoung-Soo Park, Byung-Su Kim and Hoi-Seon Lee*

Research Center for Industrial Development of Biofood Materials and Institute of Agricultural & Technology, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea

Received October 8, 2001

Essential oils of 21 herb plant samples, using spectrophotometric and paper disc agar diffusion methods under anaerobic conditions, were tested *in vitro* for their growth-inhibiting activities against *Bifidobacterium bifidum*, *B. longum*, *Lactobacillus casei*, *Clostridium perfringens*, and *Escherichia coli*. The responses varied with bacterial strains and plant oils. At 10 mg/disk, all essential oils did not inhibit beneficial intestinal bacteria, except for the oil of *Alpinia officinarum* and *Melaleuca alternifolia* against *L. casei*. Due to their strong growth-inhibitory activities against *C. perfringens*, *E. coli*, and *L. casei*, the activites of nine oils were evaluated at low concentrations. In test with *C. perfringens* at 1 mg/disk, the oils of *Amyris balsamifera*, *Curcuma longa*, *M. alternifolia*, and *Trachyspermum ammi* showed moderate activities. Moderate activities against *E. coli* were observed with the oils of *M. alternifolia* and *T. ammi*. These results may be indications of at least one of the pharmacological actions of the four herb plants.

Key words: *essential oils, intestinal bacteria, growth inhibition.*

Different types of microflora between 400-500 flourish in the intestinal tract of human, while approximately 10^{11} microflora per gram are found in the guts.[1] These intestinal microflora constitute a very complex ecosystem of aerobic and anaerobic microbes, and are classified, based on the effects on human health, into two groups, beneficial and harmful bacteria. The valences of beneficial and harmful bacteria are influenced by age, climate, diet, habits, host factors, immune mechanisms, microorganisms, sex, and stress.[2] Intestinal bacteria contribute to aging, host defense against infection, immunopotentiation, proinflammation, and various disease directly or indirectly.[3-4] In particular, beneficial bacteria, which produce lactic acids such as *Bifidobacterum* and *Lactobacillus*, suppress the growth of harmful bacteria by decreasing pH in guts. In addition, They improve human health by preventing the colonization of pathogens and producing vitamins, essential amino acids, folic acid, and antitumorigenic materials.[5-6] On the other hand, harmful intestinal microflora such as *E. coil*, *Proteus*, *Pseudomonas*, *Veillonella*, *Staphylococcus*, and *Clostridium* not only produce carcinogenic substances *de novo* but also changes metabolites from dietary sources into tumor initiators or promoters. Furthermore, they biotransform various ingested food into harmful products including N-nitroso compounds, aromatic steroids, phenols and indoles, which cause, among others, sudden death, proinflammation,[7] toxicity, and aging. We therefore focused on plant-derived growth inhibitors for the prevention of dangerous effects caused by *C. perfringens* and *E. coli* because plants are the richest source of bioactive chemicals with many of them largely free from side effects.[8,9] Growth-inhibitory effects on human intestinal bacteria were investigated using essential oils of 21 herb plants for the development of new safer types of agents.

*Corresponding author
Phone: 82-63-270-2544; Fax: 82-63-270-2550
E-mail: hoiseon@moak.chonbuk.ac.kr

Materials and Methods

Bacterial strains and culture conditions. Bacterial strains used were as follows: *B. bifidum* ATCC 29521, *B. longum* ATCC 15707, *L. casei* ATTC 27216, *C. perfringens* ATCC 13124, and *E. coli* ATTC 11755. Stock cultures of these strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slants at 80°C and, when required, were subcultured on Eggerth-Gagnon (EG) agar. 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 essential oil preparation. Total 21 plant species of 15 families, used not only for flavoring foods but also for their antiseptic or medicinal properties, were purchased from a local market in Seoul (Table 1). The plant species were dried in an oven at 60°C for 2 days and finely powdered using a blender. Essential oil of individual dried plant (300 g) was obtained through steam distillation at 100°C for 5 h, and the extracts were dehydrated with sodium sulphate.

Microbiological assay. To measure the inhibitory effect on the organisms, one loopful of bacteria was suspended in 1 ml sterile physiological saline. An aliquot (0.1 m*l*) of the bacterial suspensions was seeded on EG agar. Samples (1, 2, 5, and 10 mg) dissolved in methanol were applied using Drummond microcapillary on to paper discs (Advantec, 8 mm,

Table 1. Essential oils of 21 herb plants extracted through steam distillation.

Plant species	Common Name	Family	Tissue Sampled
Alpinia officinarum	Galangal	Zingaberaceae	root
Amyris balsamifera	Amyris	Rutaceae	whole plant
Armeniaca vulgaris	Apricot	Rosaceae	whole plant
Azadirachta indica	Neem	Meliaceae	leaf
Cananga odorata var. genuina	Ylang Ylang	Annaceae	leaf
Carya illinoensis	Pecan	Juglandaceae	nut
Citrus aurantium var. amara	Petitgrain	Rutaceae	leaf
Citrus reticulata	Orange	Rutaceae	fruit
Curcuma longa	Oleoresin	Zingiberaceae	root
Illicium verum	Aniseed	Illiciaceae	whole plant
Melaleuca alternifolia	Tea Tree	Myrtaceae	leaf
Myristica fragrans	Nutmeg	Myristicaceae	fruit
Pelargonium graveolens	Rose Geranium	Geraniaceae	leaf
Persea americana	Avocado	Lauraceae	fruit
Pimenta officinalis	Allspice Berry	Myrtaceae	fruits
Piper nignum	Black Pepper	Piperaceae	fruit
Ravensara aromatica	Ravensara	Lauraceae	leaf
Santalum album	Sandalwood	Santalaceae	seed
Sassafras albidum	Sassafras	Lauraceae	root bark
Thuja occidentalis	Thuja	Cupressaceae	leaf
Trachyspermum ammi	Ajowan	Apiaceae	seed

Table 2. Growth-inhibitory activities of 21 essential oils against intestinal bacteria at 10 mg/disk.

Sample	Bacterial Strain				
	B. longum	*B. bifidum*	*L. casei*	*C. perfringens*	*E. coli*
A. officinarum	-[a]	-	++	++	-
A. balsamifera	-	-	-	+++	+++
A. vulgaris	-	-	-	+++	++
A. indica	-	-	-	-	-
C. odorata var. genuina	-	-	-	-	-
C. illinoensis	-	-	-	++	-
C. aurantium var. amara	-	-	-	+++	+
C. reticulata	-	-	-	++	-
C. longa	-	-	-	+++	++
I. verum	-	-	-	-	-
M. alternifolia	-	-	+++	+++	+++
M. fragrans	-	-	-	+	-
P. graveolens	+	-	+	+	-
P. americana	-	-	-	-	-
P. officinalis	-	-	-	-	-
P. nignum	-	-	-	+++	-
R. aromatica	-	-	-	+++	-
S. album	+	-	-	+	-
S. albidum	-	-	-	+	-
T. occidentalis	-	-	-	++	-
T. ammi	-	-	-	+++	+++

[a]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.

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.

Results and Discussion

Growth-inhibitory activities of the essential oils from 21 herb plant samples (15 families) on the intestinal bacteria are shown in Table 2. The growth responses differed according to bacterial strains and plant oils. At 10 mg/disk, all essential oils did not inhibit the growth of beneficial intestinal bacteria, except for the essential oils of *Alpinia officinarum* and *Melaleuca alternifolia*. The essential oils of *Amyris balsamifera*, *Armeniaca vulgaris*, *Citrus aurantium* var. *amara*, *Curcuma longa*, *M. alternifolia*, *Piper nignum*, *Ravensara aromatica*, and *Trachyspermum ammi* showed significant growth inhibition (+++) against *C. perfringens*, and those of *A. officinarum*, *Carya illinoensis*, *Citrus reticulata* and *Thuja occidentalis* showed a moderate activity (++). Furthermore, the oils of *Myristica fragrans*, *Pelargonium graveolens*, *Santalum album*, and *Sassafras albidum* showed weak inhibition (+). In a test with *E. coli*, the oils of *A. balsamifera*, *M. alternifolia*, and *T. ammi* showed significant growth inhibitory activities, and those of *A. vulgaris* and *C. longa* showed moderate activities.

Nine essential oils were selected according to their strong growth-inhibitory activities against *C. perfringens*, *E. coli*, and *L. casei*, and their growth-inhibitory effects were evaluated at low concentrations of 1.0, 2.0, and 5.0 mg/disk (Table 3). At these low concentrations, the essential oils of *A. balsamifera*, *A. vulgaris*, and *C. longa* did not inhibit the growth of *L. casei*, but significantly inhibited the growth of *C. perfringens* and *E. coli*. In particular, the essential oil of *T. ammi* had strong inhibitory effects against *C. perfringens* and *E. coli* at 5.0 mg/disk. In test with *C. perfringens* at 1 mg/disk, all essential oils did not inhibit the growth of beneficial intestinal bacteria such as *L. casei*. However, the essential oils of *A. balsamifera*, *C. longa*, *M. alternifolia*, and *T. ammi* showed moderate activities.

The indigenous microflora has been recognized as a major factor in human's health and diseases.[10-12] Among the various human intestinal microorganisms, *C. perfingens* is believed to

Table 3. Growth-inhibitory activities of 21 essential oils against intestinal bacteria at low concentrations.

Plant Species	Dose, mg/disk			
	Bacterial Strain	1.0	2.0	5.0
A. officinarum	*L. casei*	-[a]	-	+
	C. perfringens	-	+	++
	E. coli	-	-	-
A. balsamifera	*L. casei*	-	-	-
	C. perfringens	++	++	+++
	E. coli	-	+	++
A. vulgaris	*L. casei*	-	-	-
	C. perfringens	+	++	+++
	E. coli	-	+	++
C. aurantium var. amara	*L. casei*	-	-	-
	C. perfringens	-	+	++
	E. coli	-	-	-
C. longa	*L. casei*	-	-	-
	C. perfringens	++	+++	+++
	E. coli	+	++	++
M. alternifolia	*L. casei*	-	+	++
	C. perfringens	++	+++	+++
	E. coli	++	++	+++
P. nignum	*L. casei*	-	-	-
	C. perfringens	-	+	++
	E. coli	-	-	-
R. aromatica	*L. casei*	-	-	-
	C. perfringens	-	+	++
	E. coli	-	-	-
T. ammi	*L. casei*	-	-	-
	C. perfringens	++	+++	+++
	E. coli	++	++	+++

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

be related to aging.[13] *C. perfringens* has alpha toxin and phospholipase C, which plays a significant role in the development of gas gangrene,[14] a dramatic and life-threatening syndrome caused by *C. perfingens* type *A. Clinical* features include localized pain, swelling, myonecrosis, and tachycardia. When medically untreated, the patient goes into shock and dies.[15] Furthermore, *C. perfringens* produces harmful enzymes such as β-glucuronidase as well as produces putrefactive products in the intestine. *E. coli* produces cytotoxic necrotising factor (CNF) that induces cell multinucleation in Vero and HeLa tissue and causes necrosis in the rabbit skin.[16,17] In addition, these harmful bacteria cause proinflammation, toxicity, mutagenesis, carcinogenesis, and aging. *Bifidobacterium* and *Lactobacillus* inhibit the growth of harmful bacteria such as *C. perfringens* and *E. coli* by producing lactic acid, acetic acid, and antibiotics.[18] 2-Pyrrolidone-5-carboxylic acid (PC), an antibiotic, was isolated from the cultures of *L. casei* spp. *casei* LC-10 (LCC) and *L. casei* spp. *pseudoplantaarum* LB1931 (LCP).[19] An antimicrobial substance against various harmful bacterial species such as *Salmonella*, *Listeria*, *Campylobacter*, *Shigella*, and *Vibrio cholerae* was extracted from eight species of bifidobacteria.[20] In addition, bifidobacteria indirectly eliminate the source of procarcinogens[21] or harmful enzymes such as nitroreductase, azoreductase, and β-glucuronidase. However, beneficial bacteria are not always more dominant in the gut than harmful bacteria, because the valences of beneficial and harmful bacteria are influenced by physical, biological, chemical, environmental or host factors. Therefore, populations of beneficial bacteria in the intestine should be kept high, while those of harmful bacteria kept low for good health and longevity. In our study, effects on the growth-inhibition of beneficial bacteria and harmful bacteria were investigated using essential oils of 21 herb plant samples. At 10 mg/disk, all essential oils did not inhibit the growth of beneficial intestinal bacteria, except for the essential oils of *A. officinarum* and *M. alternifolia*. Additionally, the essential oils of *A. balsamifera*, *A. vulgaris*, and *C. longa* did not inhibit growth of *L. casei*, but significantly inhibited the growth of *C. perfringens* and *E. coli* at 1.0, 2.0, and 5.0 mg/disk. These results indicate that the chosen nine essential oils have selective strong inhibiting activities on the growth of harmful bacteria at low concentrations. These results may be indications of one of the pharmacological actions of herb plants. Further investigation will be performed to establish whether these activities are applicable *in vivo*.

Acknowledgments. This research was supported by Research Center for Industrial Development of Biofood Materials in Chonbuk National University, Chonju, Korea. Research Center for Industrial Development of Biofood Materials is designated as a Regional Research Center appointed by the Korea Science and Engineering Foundation (KOSEF), Chollabukdo Provincial Government and Chonbuk National University.

References

1. Moore, W. W. C. and Holdeman, L. V. (1974) Human fecal flora: The normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* **27**, 961-979.
2. Mitsuoka, T. (1984) In *A Color Atlas of Anaerobic Bacteria*, Shobunsha, Tokyo, Japan.
3. Hentges, D. J. (1983) Role of the intestinal microflora in host defense against infection. In *Human Intestinal Microflora in Health and Disease*, Hentges, D. J. (ed.) pp. 311-331, Academic Press, New York, USA.
4. Mitsuoka, T. (1992) Intestinal flora and aging. *Nutr. Rev.* **50**, 438-446.
5. Finegold, S. M., Flora, D. J., Attebery, H. R. and Sutter, V. L. (1975) Fecal bacteriology of colonic poly patients and control patients. *Cancer Res.* **35**, 3407-3417.
6. Ishibashi, N. and Shimamura, S. (1993) Bifidobacteria: research and development in Japan. *Food Technol.* 126-135.
7. Bunting, M., Lorant, D. E., Bryant, A. E., Zimmerman, G. A., McIntyre, T. M., Stevens, D. L. and Prescott, S. M. (1997) Alpha toxin from Clostridium perfringens induces proinflammatory changes in endothelial cells. *J. Clin. Invest.* **3**, 565-574.
8. Allen, C. B. (1998) In *PDR for Herbal Medicines*, Medical Economics Company, New Jersey, USA.
9. Sissons, J. W. (1989) Potential of probiotic organisms to prevent diarrhoea and promote digestion infarm animals. *J. Sci. Food Agric.* **49**, 1-13.
10. Anderson, F. D., Gillespie, W. A. and Richmond, M. H. (1973) Chemotherapy and antibiotic resistance transfer between enterobacteria in the human gastro-intestinal tract. *J. Med. Microbiol.* **6**, 461-473.
11. Hentges, D. F. and Cooperstock, M. (1980) Sixth international symposium on intestinal microecology. *Am. J. Clin. Nutr.* **33**, 2415-2544.
12. Sasaki, S., Ozawa, A. and Hasimoto, K., (1982) In *Recent Advances in Germfree Research*, Tokai Univ. Press, Tokyo, Japan.
13. Mitsuoka, T. (1990) Bifidobacteria and their role in human health. *J. Indus. Microbiol.* **6**, 263.
14. Myhara, R. M., Nilsson, K., Bowmer, E. J. and Cruickshank, P. K. (1988) Gas production from melibiose, raffinose and white bean extracts by bacteria of human fecal origin. *Can. Inst. Food Sci. Technol. J.* **21**, 254.
15. MacLennan, J. D. (1962) The histotoxic clostridial infections of man. *Bacteriol. Rev.* **26**, 177-276.
16. Caprioli, A., Falbo, V., Roda, L., Ruggeri, F. and Zona, C. (1983) Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. *Infect Immunol.* **39**, 1300-1306.
17. De Rycke, J., Gonzales, E., Blanco, J., Oswald, E., Blanco, M. and Boivin, R. (1990) Evidence for two types of cytotoxic necrotising factor in human and animal clinical isolates of *Escherichia coli*. *J. Clin. Microbiol.* **28**, 694-699.
18. Fernandes, C. F. and Shahani, K. M. (1990) Anticarcinogenic and immunological properties of dietary lactobacilli.

J. Food Protect. **53**, 704-710.
19. Huttunen, E., Noro, K. and Yang, Z. (1995) Purification and identification of antimicrobial substances produced by two *Lactobacillus casei* strains. *Int. Dairy J.* **5**, 503-513.
20. Ibrahim, S. A. and Bezkoroviny, A. (1993) Inhibition of *Escherichia coli* by Bifidobacteria. *J. Food Protect.* **56**, 713-715.
21. Gibson, G. R. and Wang, X. (1994) Regulatory effect of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* **77**, 412-420.