

Growth-inhibitory Responses of Human Intestinal Bacteria to Extracts of Oriental Medicinal Plants

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Methanol extracts from 50 species of oriental medicinal plants were prepared and subjected to an *in vitro* screening test for their growth-inhibitory activity towards *Bifidobacterium adolescentis*, *Clostridium perfringens* and *Escherichia coli*, using a paper disc agar diffusion method under O₂-free conditions. The inhibitory activity was both bacteria and plant-species dependent. Extracts from *Pueraria thunbergiana*, *Astragalus membranaceus*, *Eucommia ulmoides*, *Coptis japonica*, *Akebia quinata* and *Rhus chinensis* strongly inhibited growth of *C. perfringens*. A growth-inhibitory effect against *E. coli* was observed from extracts of *C. japonica*. These plant extracts did not affect the growth of *B. adolescentis*. It is concluded that intake of these oriental medicinal plants may be important in the prevention of human diseases caused by intestinal microorganisms by altering the growth and composition of intestinal bacteria and modulating the genesis of potentially harmful metabolites.

KEY WORDS—Oriental medicinal plants; Intestinal bacteria; Growth inhibition; *Bifidobacterium adolescentis*; *Clostridium perfringens*; *Escherichia coli*.

INTRODUCTION

Various kinds of microorganisms are resident in the human intestinal tract as a highly complex ecosystem with considerable species diversity. It is well known that they not only participate in normal physiological functions, but may also contribute to the genesis of various disease states by biotransforming a variety of ingested or endogenously formed compounds to harmful derivatives or by protecting against disease by the generation of beneficial products. Accordingly, this biotransformation may influence drug efficacy, toxicity, carcinogenesis and ageing.

Differences in the diversity of the intestinal bacteria between patients and healthy subjects,^{11,18} and between younger and elderly subjects¹⁵ have been observed. The normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seems to play a large role in metabolism, host defense against infection,

ageing and immunopotentiality.^{16,20} On the other hand, 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 harbour fewer bifidobacteria but larger numbers of clostridia than younger subjects. Accordingly, any disturbance of the microbiota may cause a variety of diseases of abnormal physiological states.

Recently, much interest has focused on oriental medicinal plants in human health because they are largely free from harmful adverse effects and have excellent pharmacological action.^{6,10,22} However, relatively little recent work has been carried out on the effect of oriental medicinal plants on the growth responses of intestinal microorganisms compared to other areas of intestinal microbiology.

In the laboratory study described herein, we assessed the growth-inhibitory responses of human intestinal bacteria to extracts of oriental medicinal plants.

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MATERIALS AND METHODS

Bacteria and culture conditions

The bacterial strains used in this study are *Bifidobacterium adolescentis* ATCC 15073, *Clostridium perfringens* ATCC 13124, and *Escherichia coli* ATCC 11775. Stock cultures of these strains were routinely stored on Eggerth-Gagnon Liver extract-Fieldes slant²¹ at -80°C , and when required were subcultured on Eggerth-Gagnon agar²¹ (Eiken Chemical Co., Ltd, Tokyo, Japan) for the bacteria. The plates were incubated anaerobically for 2 d at 37°C in an atmosphere of 80 per cent N_2 , 15 per cent CO_2 and 5 per cent H_2 in a glove-box (COY Lab., Michigan, USA). On the following day, the bacteria were grown in reinforced clostridial medium¹² (Difco) broth (pH 6.8).

Plant materials and sample preparation

The oriental medicinal plant species belonging to 32 families are anecdotally selected, and listed in Table 1. These samples were obtained from a market in Seoul, Republic of Korea, finely powdered using a blender, extracted twice with methanol at 25°C and filtered (Toyo filter No. 2). The combined filtrate was concentrated *in vacuo* at 35°C . The yield of each plant extraction is shown in Table 1.

Microbiological assay

For assay of effects of the plant extracts on the growth responses of the test organisms, 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 Brucella agar (Difco). A sample in methanol solution (100 μl) was applied by Drummond glass microcapillary to a paper disc (ADVANTEC diam. 8 mm, Toyo Roshi, Japan). After evaporation of solvents, the paper discs were placed on the agar surface. All plates were incubated anaerobically for 2 d at 37°C . Control discs received methanol only. All tests of growth inhibition were replicated three times.

The growth responses to the test samples were determined by comparison with that of controls. The inhibitory responses were classified as follows: strongest response + + +, zone diameter >20 mm; moderate + +, zone diameter 16–20 mm; weak +, zone diameter 10–15 mm; no response –, zone diameter <10 mm.

RESULTS

The growth-inhibitory responses of *B. adolescentis* to test samples, representative of the organism dominant in the intestines of adults, were plant-species dependent. Extracts of *P. amurensis* and *S. acutum* (20 mg/discs) showed potent growth inhibition of *B. adolescentis* (+ +), whereas weak activity (+) was obtained in extracts from *C. tinctorius*, *J. effusus* and *G. elata*. Both *P. amurensis* and *S. acutum* also exhibited growth inhibition (+) at lower levels (10 mg/disc).

In tests with *C. perfringens*, extracts from *C. japonica*, *S. acutum* and *R. chinensis* strongly inhibited the growth of *C. perfringens* (+ + + for 20 mg/discs). Moderate inhibition (+ +) was obtained in extracts of *P. thunbergiana*, *A. membranaceus*, *E. ulmoides*, *P. amurensis* and *A. quinata*. Even at lower levels (10 mg/disc), extracts from *A. membranaceus*, *C. japonica*, *P. amurensis*, *S. acutum* and *R. chinensis* inhibited the growth of *C. perfringens* (+ +).

The growth-inhibitory responses of *E. coli* were also plant-species dependent. Extracts of *C. japonica* (20 mg/disc) showed significant growth inhibition on *E. coli* (+ + +), whereas weak activity (+) was obtained in extracts from *E. ulmoides*, *S. acutum*, *L. chinense*, *R. chinensis* and *S. tenuifolia* var. *japonica*. Even 10 mg/discs of extracts from *C. japonica* and *P. amurensis* exhibited inhibitory activity (+ +).

DISCUSSION

The intestinal microbiota in healthy man remains relatively constant but is known to be greatly influenced by physical, biological, chemical, environmental or host factors. Alterations to the microbiota may cause abnormal physical conditions or diseases. The most important factor in primary screening for bioactive substances may be the starting concentration. In our previous papers,^{1–3} we reported that 10–20 mg/disc of a plant extract did not cause any problems such as solubility and detection of its minor active components. In the present paper, growth-inhibitory responses of methanol extracts from 50 species of oriental medicinal plants to *B. adolescentis*, *C. perfringens* and *E. coli* were investigated *in vitro*.

Among the various human intestinal microorganisms, bifidobacteria are often taken as useful indicators of human health under most environmental conditions. This is based upon the facts

Table 1. Plants tested

Plant species	Family name	Part collected	Yield (%)
<i>Arctium lappa</i>	Compositae	root	15
<i>Atractylodes japonica</i>		root	15
<i>Artemisia messerschmidtiana</i>		cortex	10
<i>Carthamus tinctorius</i>		flower	19
<i>Crataegus maximowiczii</i>	Rosaceae	fruit	39
<i>Chaenomeles sinensis</i>		fruit	21
<i>Ledebouriella seseloides</i>	Umbelliferae.	root	17
<i>Angelica dahurica</i>		root	12
<i>Angelica gigas</i>		root	12
<i>Torilis japonica</i>		seed	3
<i>Bupleurum falcatum</i>		root	15
<i>Anthriscus sylvestris</i>		root	21
<i>Aralia continentalis</i>	Araliaceae	root	12
<i>Acanthopanax sessiliflorus</i>		fruit	7
<i>Kalopanax pictus</i>		stem	10
<i>Pueraria thunbergiana</i>	Leguminosae	root	21
<i>Astragalus membranaceus</i>		root	10
<i>Rheum undulatum</i>	Poligonales	root	50
<i>Pleuropterus multiflorus</i>		root	14
<i>Scirpus fluviatilis</i>	Cyperaceae	root	9
<i>Eucommia ulmoides</i>	Eucommiaceae	leaf	17
<i>Achyranthes japonica</i>		root	8
<i>Clematis florida</i>	Ranunculaceae	root	19
<i>Coptis japonica</i>		root	21
<i>Poncirus trifoliata</i>	Rutaceae	fruit	32
<i>Phellodendron amurense</i>		stem	15
<i>Belamcanda chinensis</i>	Iridaceae	root	20
<i>Imperata cylindrica</i>	Gramineae	root	22
<i>Sinomenium acutum</i>	Menispermaceae	root	3
<i>Juncus effusus</i>	Juncaceae	cortex	5
<i>Codonopsis pilosula</i>	Campanulaceae	root	10
<i>Platycodon grandiflorum</i>		root	29
<i>Akebia quinata</i>	Ladizabalaceae	stem	15
<i>Equisetum hyemale</i>	Equisetaceae	stem	5
<i>Asarum sieboldii</i>	Aristolochiaceae	leaf	13
<i>Lycium chinense</i>	Solanaceae	fruit	20
<i>Zizyphus jujuba</i>	Rhamnaceae	fruit	51
<i>Fraxinus rhynchophylla</i>	Oleaceae	root	35
<i>Typha orientalis</i>	Typaceae	pollen	10
<i>Rhus chinensis</i>	Anacardiaceae	gall	52
<i>Schizonepeta tenuifolia</i> var. <i>japonica</i>	Labiatae	leaf	6
<i>Lithospermum erythrorhizon</i>	Borraginaceae	stem	28
<i>Kochia scoparia</i>	Chenopodiaceae	leaf	7
<i>Gentiana scabra</i>	Gentianaceae	root	29
<i>Gastrodia elata</i>	Orchidaceae	root	10
<i>Bletilla striata</i>		root	6
<i>Cuscuta japonica</i>	Convolvulaceae	fruit	6
<i>Ganoderma lucidum</i>	Poliporaceae	body	5
<i>Morus alba</i>	Moraceae	root	3
<i>Sinapis allba</i>	Brassicaceae	root	10

that they play important roles in metabolism, e.g. amino-acid production,^{19,20} aid defense against infection,¹⁶ are associated with longevity,¹⁵ pathogen inhibition⁹ and immunopotentiality.²⁰ Bifidobacteria growth-promoting factors, usually called bifidus factor, have therefore been extensively studied since György *et al.*¹⁴ suggested their existence in human milk, and *N*-acetylglucosamine derivatives (bifidus factors) are growth factors for the organism. Lactulose, oligosaccharides, peptide or peptide-like and vitamin-like substances have been identified as other bifidus factors.^{5,7,17,25} In contrast, clostridia act by biotransforming a variety of ingested or endogenously formed compounds to harmful products like *N*-nitroso compounds or aromatic steroids within the gut.^{8,13} The microbiota of patients with cancer contains few lactic acid bacteria.²⁴ Accordingly, growth inhibitors for clostridia or growth promoters for bifidobacteria may prevent these diseases.

In our microbial assay, growth-inhibitory responses were plant-species dependent. The addition of extracts from *P. thunbergiana*, *A. membranaceus*, *E. ulmoides*, *C. japonica* and *A. quinata* to the media inhibited the growth of *C. perfringens* or *E. coli* without growth inhibition of *B. adolescentis*. Extracts of *S. acutum* and *P. amurense* were potent inhibitors of the growth of *C. perfringens* and *E. coli* as well as *B. adolescentis*. It would be most 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 normalise disturbed physiological functions resulting in the prevention or reduction of diseases caused by pathogens in the gastrointestinal tract. Similar *in vitro* results were also reported in extracts from green tea and ginseng.^{1,2} Previous *in vivo* investigations^{3,4} using human volunteers have shown that intake of ginseng extract or green tea extract favourably affected the faecal microbiota and biochemical aspects of faeces, suggesting an indication of at least one of the pharmacological actions of ginseng⁶ and green tea.²³ Accordingly, the daily intake of oriental medicinal plants might be expected to alter the growth and composition of the microbial community and may modulate the genesis of potentially harmful products such as carcinogenic *N*-nitroso compounds or aromatic

steroids within the intestinal tract, thus protecting from a variety of diseases and helping to maintain optimal human health.

Further work to identify the biologically active substances of *C. japonica* which show the most potent growth-inhibitory activity is in progress.

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