

## Growth-inhibitory Responses of Human Intestinal Bacteria to Extracts from Indian and African Plants

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**Abstract** : Methanol extracts from 84 Indian plant samples (50 species in 31 families) and 27 African plant samples (20 species in 12 families) *in vitro* were tested for their growth-inhibitory activities against *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Clostridium perfringens*, and *Escherichia coli*, using a paper disc agar diffusion method under O<sub>2</sub>-free conditions. The responses varied with bacterial strain, plant species and plant part. Extracts from *Cymbopogon citratus* whole plants, *Ocimum basilicum* whole plant, *Madhuca indica* flowers, and *Aegle marmelos* leaves among Indian plant samples moderately or strongly inhibited the growth of *Cl. perfringens* whereas moderate growth-inhibitory activity against *E. coli* was obtained from extract of Indian *O. basilicum* whole plants. These plant extracts did not affect the growth of the lactic acid forming bacteria tested. These results may be an indication of at least one of the pharmacological actions of these tropical plants. (Received November 3, 1997; accepted November 21, 1997)

### Introduction

Approximately 400 kinds of microorganisms are resident in the human intestinal tract as a highly complex ecosystem with considerable species diversity. They not only participate in normal physiological functions, but significantly also contribute to the genesis of various disease states by biotransforming a variety of ingested or endogenously formed compounds to useful or harmful derivatives. Accordingly, these biotransformations may influence drug efficacy, toxicity, carcinogenesis and aging.<sup>1,2)</sup>

Differences in the intestinal bacteria between patients and healthy subjects, and between younger and elderly subjects have been observed. The normal gastrointestinal microbiota is found to be predominantly composed of lactic acid bacteria which seem to play a large role in metabolism, host defense against infection, aging and immunopotentialization.<sup>1,2)</sup> On the other hand, the microbiota of cancer patients<sup>3,4)</sup> and patients with Alzheimer's disease<sup>5)</sup> are 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.<sup>6)</sup> Accordingly, any disturbance of the

microbiota may cause a variety of diseases of abnormal physiological states.

Recently, much interest has been focused on plant-driven 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 virtually are the richest source of bioactive organic chemicals.<sup>7,8)</sup> We already reported growth responses of human intestinal bacteria to various oriental medicinal plant extracts.<sup>9-13)</sup> However, the effects of tropical plant extracts on growth of intestinal bacteria remain unknown in spite of their excellent pharmacological action.<sup>14-16)</sup>

In the laboratory study described herein, we assessed the growth-inhibitory responses of human intestinal bacteria to methanol extracts of tropical plant species collected from India and Niger (West Africa).

### Materials and Methods

#### Bacterial strains and culture conditions

The intestinal bacterial strains used in this study were as follows; *Bifidobacterium bifidum* ATCC 29521, *Bifidobacterium longum* ATCC 15707, *Lactobacillus acidophilus*

Key words : intestinal bacteria, indian plants, african plants, tropical plants, growth inhibition

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JCM 315, *Clostridium perfringens* ATCC 13124, and *Escherichia coli* ATCC 11775. Stock cultures of 5 strains were routinely stored on Eggerth-Gagnon Liver Extract-Field slant<sup>17</sup> at -80°C and were subcultured on Eggerth-Gagnon (EG) agar (Eiken Chemical, Japan) when required, and were incubated anaerobically at 37°C for 2 d in an anaerobic chamber (Coy Lab., USA) in an atmosphere of 5% H<sub>2</sub> + 15% CO<sub>2</sub> + 80% N<sub>2</sub>.

#### Plant materials and sample preparation

Eighty-four Indian plant samples (50 plant species in 31 families) consisting of leaf (29), stem (24), root (1), flower (1), fruit (3), seed (5), seed oil (1), oilcake (1) and whole plant (19) were collected during March-April and August-September in India (Table 1). Twenty-seven African plant

**Table 1. Indian plants tested**

Plant species	Family name	Part collected	Yield (%)
<i>Adhatoda vasica</i>	Acanthaceae	leaf	28
		stem	27
<i>Agave americana</i>	Agavaceae	whole	5
<i>Achyranthes aspera</i>	Amaranthaceae	whole	15
		stem	13
<i>Annona squamosa</i>	Annonaceae	seed	13
<i>Nerium indicum</i>	Apocynaceae	root	15
		stem	21
		leaf	14
<i>Thevetia peruviana</i>		leaf	20
<i>Acorus calamus</i>	Araceae	whole	20
<i>Calotropis gigantea</i>	Asclepiadaceae	leaf	23
<i>Eupatorium odoratum</i>	Asteraceae	whole	11
<i>Eupatorium triplinerve</i>		whole	18
<i>Parthenium hysterophorus</i>		whole	19
<i>Bignonia anguiscati</i>	Bignoniaceae	leaf	24
		stem	20
<i>Opuntia elatior</i>	Cactaceae	leaf	9
<i>Cassia auriculata</i>	Caesalpinaceae	leaf	13
		stem	17
		whole	28
<i>Cannabis sativa</i>	Cannabinaceae	whole	22
<i>Artemisia maritima</i>	Compositae	whole	11
<i>Acanthospermum hispidum</i>		whole	9
<i>Cuscuta reflexa</i>	Convolvulaceae	whole	6
<i>Thuja occidentalis</i>	Cupressaceae	leaf	22
		stem	13
<i>Acalypha indica</i>	Euphorbiaceae	leaf	23
<i>Ricinus communis</i>	Euphorbiaceae	leaf	22
<i>Jatropha integerrima</i>		whole	15
<i>Cymbopogon citratus</i>	Gramineae	whole	19
<i>Ocimum americanum</i>	Labiatae	leaf	21
		whole	15
		seed	14
		stem	13
		whole	19
<i>Ocimum basilicum</i>		whole	19
<i>Ocimum sanctum</i>		whole	15
<i>Azadirachta indica</i>	Meliaceae	leaf	26
		seed	17
		stem	23

**Table 1. (Continued)**

Plant species	Family name	Part collected	Yield (%)
<i>Melia azedarach</i>		leaf	21
		seed	17
		stem	24
<i>Swietenia mahagoni</i>		leaf	19
		fruit	20
<i>Acacia ferruginea</i>	Mimosaceae	leaf	18
		stem	17
<i>Prosopis chinensis</i>		leaf	21
<i>Ficus elastica</i>	Moraceae	stem	22
		leaf	13
<i>Bougainvillea spectabilis</i>	Nyctaginaceae	stem	16
		leaf	24
<i>Arachis hypogaea</i>	Papilionaceae	stem	24
		whole	23
<i>Pongamia pinnata</i>		leaf	15
		seed	20
		stem	11
<i>Sesbania grandiflora</i>		leaf	22
		stem	22
<i>Ziziphus mauritiana</i>	Rhamnaceae	leaf	23
<i>Aegle marmelos</i>	Rutaceae	fruit	19
		leaf	25
		stem	24
		leaf	25
<i>Murraya koenigii</i>		stem	28
		leaf	25
<i>Bassia latifolia</i>	Sapotaceae	leaf	15
<i>Madhuca indica</i>		flower	25
		oilcake	12
		leaf	15
		oil	26
		seed	23
		stem	15
<i>Veronica anagallis</i>	Scrophulariaceae	stem	20
<i>Datura metel</i> var. <i>alba</i>	Solanaceae	fruit	20
		leaf	26
		seed	23
		stem	25
<i>Strychnos nux-vomica</i>	Strychnaceae	leaf	19
<i>Clerodendrum inerme</i>	Verbenaceae	whole	26
<i>Lantana camara</i>		leaf	19
<i>Lantana camara aculeata</i>		stem	16
<i>Stachytarpheta indica</i>		whole	22
<i>Vitex negundo</i>		leaf	23
		stem	25

samples (20 plant species in 12 families) composed of leaf (9), stem (6), fruit (1) and whole plant (11) were collected during October in Niger, West Africa (Table 2). The plant materials were dried in shade, finely powdered using a blender and then were extracted twice with methanol at room temperature and filtered (Toyo filter No. 2). The combined filtrate was concentrated *in vacuo* at 35°C. The yield of each sample extraction is shown in Tables 1 and 2.

#### Microbiological assay

For bioassay of growth-inhibitory responses against test

**Table 2. African plants tested**

Plant species	Family name	Part collected	Yield (%)
<i>Amaranthus viridis</i>	Amaranthaceae	whole	27
<i>Blepharis linarifolia</i>		whole	20
<i>Celosia trigyna</i>		whole	32
<i>Calotropis gigantea</i>	Asclepiadaceae	leaf	25
<i>Cassia mimosoides</i>	Caesalpinaceae	leaf	22
<i>Cassia occidentalis</i>		whole	25
<i>Cassia tora</i>		whole	29
<i>Boscia senegalensis</i>	Capparidaceae	leaf	29
		stem	19
<i>Clome viscosa</i>		whole	22
<i>Combretum glutinotum</i>	Combretaceae	leaf	20
		stem	19
<i>Combretum micronthum</i>		whole	26
<i>Guiera senegalensis</i>		leaf	26
		seed	19
<i>Piloitigma vetilicolin</i>		whole	27
<i>Ipomoea asarifolia</i>	Convolvulaceae	whole	26
<i>Azadirachta indica</i>	Meliaceae	leaf	26
<i>Prosopis chinensis</i>	Mimosaceae	fruit	20
		leaf	21
		stem	23
<i>Bougainvillea spectabilis</i>	Nyctaginaceae	leaf	24
		seed	22
<i>Boirerio radiata</i>	Rubiaceae	whole	19
<i>Waltheria indica</i>	Sterculiaceae	whole	29
<i>Balanites aegyptiaca</i>	Zygophyllaceae	leaf	21
		stem	19

intestinal bacteria, the paper disc agar diffusion method was used.<sup>10</sup> The most important factor in primary screening for bioactive substances may be the starting concentration. We already reported that 10-20 mg/disc of plant extract did not cause any problem such as solubility and detection of its minor active components.<sup>10,13</sup> A sample (10 mg) dissolved in methanol was applied by Drummond glass microcapillary to a paper disc (Advantec 8-mm diameter and 1-mm thick, Toyo Roshi). After evaporation, the paper discs were placed on EG agar surface inoculated with each strain. They were incubated at 37°C for 2 d in an atmosphere of 5% H<sub>2</sub> + 15% CO<sub>2</sub> + 80% N<sub>2</sub>. Control discs received methanol. All tests of inhibition were performed in triplicate.

The growth responses of test samples were determined by comparison with those of controls. The inhibitory responses were classified as previously described; strong response +++, zone diameter >20 mm; moderate ++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; and no response ·, zone diameter <10 mm.<sup>10,13</sup>

## Results

Growth-inhibitory activity of intestinal bacteria to extracts from Indian plant samples is shown in Table 3. In a test

with *Bif. bifidum* which is predominant in the intestines of infants, extracts from *Prosopis chinensis* leaves strongly inhibited the growth of this bacteria (+++). With *Bif. longum* which is dominant bacteria in the intestines of adults, moderate growth inhibitory activity (++) was obtained in extracts from *Ocimum sanctum* whole plants, *Aegle marmelos* fruits, and leaves and stems of *P. chinensis*. For *Lact. acidophilus*, extracts from *Arachis hypogaea* whole plants, *Azadirachta indica* stems, and *P. chinensis* leaves strongly inhibited the growth of this bacteria (+++) whereas moderate inhibitory-responses (++) were produced from extracts of *Clerodendrum inerme* whole plants, *O. sanctum* whole plants, and *Sesbania grandiflora* stems.

The growth inhibition to harmful bacteria such as *Cl. perfringens* and *E. coli* also varied with plant species and part sampled (Table 3). Extracts from *A. marmelos* leaves and *Madhuca indica* flowers showed strong inhibitory activity

**Table 3. Growth-inhibitory activity of intestinal bacteria to extracts of Indian plants**

Plant name <sup>a</sup>		Bacterial strain <sup>b</sup>				
		<i>Bif. bifidum</i>	<i>Bif. longum</i>	<i>Lact. acidophilus</i>	<i>Cl. perfringens</i>	<i>E. coli</i>
<i>A. indica</i>	L	· <sup>c</sup>	·	·	+	·
<i>A. hypogaea</i>	Wp	·	·	+++	·	·
<i>A. indica</i>	St	·	·	+++	·	·
<i>C. inerme</i>	Wp	·	·	++	·	·
<i>C. citratus</i>	Wp	·	·	·	++	·
<i>D. metel</i>	L	+	·	·	·	·
<i>E. odoratum</i>	Wp	·	·	·	+	·
<i>E. triplinerve</i>	Wp	·	·	·	+	·
<i>N. indicum</i>	L	·	+	·	·	·
<i>N. indicum</i>	R	·	+	·	·	·
<i>O. americanum</i>	St	·	·	·	+	·
<i>O. americanum</i>	Se	·	·	·	+	·
<i>O. basilicum</i>	Wp	·	·	·	++	++
<i>O. sanctum</i>	Wp	·	++	++	++	·
<i>P. pinnata</i>	L	·	+	·	·	·
<i>P. chinensis</i>	L	+++	·	+++	++	·
<i>R. communis</i>	L	·	+	·	+	·
<i>T. occidentalis</i>	L	·	+	·	·	·
<i>V. anagallis</i>	St	·	+	·	·	·
<i>A. ferruginea</i>	L	·	+	·	·	·
<i>A. marmelos</i>	L	·	·	·	+++	·
<i>A. marmelos</i>	St	·	+	·	+	·
<i>S. grandiflora</i>	L	·	·	+	·	·
<i>S. grandiflora</i>	St	·	+	++	·	·
<i>S. indica</i>	Wp	·	+	·	·	·
<i>A. aspera</i>	Wp	·	·	+	·	·
<i>A. hispidum</i>	Wp	·	·	+	+	·
<i>M. indica</i>	Fl	·	·	·	+++	·
<i>A. marmelos</i>	Fr	·	++	·	+	·

<sup>a</sup>Plant species showing activity are presented.

<sup>b</sup>Exposed to 10 mg/disc.

<sup>c</sup>Strong response +++, zone diameter >20 mm; moderate ++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; no response ·, zone diameter <10 mm.

**Table 4. Growth-inhibitory activity of intestinal bacteria to extracts of African plants**

Plant name <sup>a</sup>		Bacterial strain <sup>b</sup>				
		<i>Bif. bifidum</i>	<i>Bif. longum</i>	<i>Lact. acidophilus</i>	<i>Cl. perfringens</i>	<i>E. coli</i>
<i>C. mimosoides</i>	L	.	+	.	.	.
<i>G. senegalensis</i>	L	.	+	.	.	.
<i>G. senegalensis</i>	S	.	+	.	.	.
<i>B. radiata</i>	Wp	.	+	.	.	.
<i>A. viridis</i>	Wp	.	+	.	.	.
<i>C. tora</i>	Wp	.	++	.	.	.
<i>P. chinensis</i>	L	+++	++	+++	++	+
<i>P. chinensis</i>	St	.	+	.	+	.

<sup>a</sup>Plant species showing activity are presented.

<sup>b</sup>Exposed to 10 mg/disc.

<sup>c</sup>For explanation, see Table 3.

against *Cl. perfringens* whereas moderate activity (++) was obtained in extracts from whole plants of *Cymbopogon citratus*, *Ocimum basilicum* and *O. sanctum*, and *P. chinensis* leaves. In a test with *E. coli*, extracts from *O. basilicum* whole plants only revealed moderate growth-inhibitory activity (++) .

Table 4 revealed the growth-inhibitory activity of African plant samples against intestinal bacteria tested. Similar growth responses with the Indian samples were observed from the African samples. Like Indian *P. chinensis* leaves, extract of the African species revealed also strong and moderate growth-inhibition against the lactic acid bacteria and *Cl. perfringens*, respectively. Extract of *Cassia tora* whole plants exhibited moderate growth inhibition to *Bif. longum*. The other plant samples showed little or no activity against the bacteria used.

## Discussion

In the laboratory study with the human intestinal bacteria, growth-inhibitory responses of extracts from 84 Indian and 27 African plant samples varied with plant species, plant part and bacterial strain. However, there were no differences in growth responses to extracts of same species between India- and Africa-collected plants. Because plants virtually constitute a rich source of bioactive organic chemicals<sup>7,8)</sup> and native herbal practices are replaced by modern medical practices,<sup>18)</sup> in recent years much concern has been focused on plant materials for potentially useful products or lead compounds for synthetic compounds at various medicinal fields. It has been acknowledged that tropical plants have various pharmacological actions. The details along with economic importance of majority of Indian plants are provided by Umrao *et al.*<sup>15)</sup> and Schmelzer.<sup>16)</sup>

Among beneficial bacteria, bifidobacteria have a close re-

lation with the human health. Their proposed physiological effects pertain to improvement of intestinal microbiota by preventing colonization of pathogens, amelioration of diarrhea or constipation,<sup>19)</sup> nutrition production such as vitamins and essential amino acids, improvement of lactose tolerance of milk products,<sup>1)</sup> decrease in serum cholesterol levels, immunity activation, and antitumorigenic activity.<sup>20,21)</sup> Bifidobacteria growth-promoting factors, usually called bifidus factor, have therefore been extensively studied since György *et al.*<sup>22)</sup> suggested their existence in human milk. Extracts of some Oriental medicinal plants such as *Panax ginseng*<sup>10,12)</sup> and *Thea chinensis*<sup>9,23)</sup> were found to have selective growth-promoting activity against only lactic acid bacteria.

Clostridia have harmful effects on human health such as sudden death, toxicity, mutagenesis, carcinogenesis, or aging. They act by biotransforming a variety of ingested or endogenously formed compounds to harmful products like *N*-nitroso compounds or aromatic steroids within the gastrointestinal tract. Because *Cl. perfringens* produces a variety of toxic enzymes such as phospholipase and collagenase disintegrating plasma membrane of host, and toxins which increase permeability of blood vessel of intestine and cause enteritis and colitis, and stimulate aging and tumor genesis of human,<sup>24)</sup> much interest has been focused on selective plant-derived growth inhibitors against *Cl. perfringens* in intestine, based upon the fact that plant-derived materials seem to be less toxic to human.

In our *in vitro* study, extracts from *C. citratus* whole plants, *O. basilicum* whole plants, *M. indica* flowers, and *A. marmelos* leaves strongly inhibited the growth of *Cl. perfringens*. These plant extracts did not affect the growth of the lactic acid bacteria tested. Similar results were reported for oriental medicinal plants<sup>9-13)</sup> and leguminous seeds.<sup>25)</sup> For example, extracts from *Panax ginseng*, *Thea chinensis*, *Pueraria thumbergiana*, *Astragalus membranaceus*, *Eucommia ulmoides*, *Coptis japonica*, and *Akebia quinata* strongly inhibited growth of *Cl. perfringens* without affecting the growth of *Bif. adolescentis*. However, 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 which result in the prevention of diseases caused by pathogens in the gastrointestinal tract. Previous *in vivo* investigations have proved that intake of ginseng extract or green tea extract affected favorably the faecal microbiota and biochemical aspects of feces,<sup>12,23)</sup> suggesting an indication of at least one of their pharmacological actions.

Based upon our data and these earlier findings, daily intake of bioactive tropical plant-derived materials with selectivity might be expected to alter the growth and composition of the microbial community and 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.

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**인도산 및 아프리카산 식물체 추출물의 장내세균에 대한 생육억제 반응**

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**초 록 :** 31과 50종 인도산 식물체 84 시료와 12과 20종 아프리카산 식물체 27 시료의 메탄올 조추출물의 5종 장내세균(*Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Clostridium perfringens*, *Escherichia coli*)에 대한 생육억제활성을 *in vitro* 혐기조건하에서 여지확산법으로 검정한 결과 억제활성은 공시세균과 식물체의 종류 및 부위에 따라 달리 나타났다. 인도산 식물체 시료중, *Cymbopogon citratus* 전부위, *Ocimum basilicum* 전부위, *Madhuca indica* 꽃 및 *Aegle marmelos* 잎 추출물이 *Cl. perfringens*에 대하여 강한 생육저해활성을 나타낸 반면, *O. basilicum* 전부위 추출물은 *E. coli*에 대하여 중간정도의 생육저해활성을 보였다. 그러나, 이들 식물체들은 공시 유산균의 생육에는 영향을 미치지 않았다. 이러한 결과로부터 이들 열대식물들의 약리학적 작용을 일부 설명할 수 있을 것으로 사료된다.

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찾는말 : 장내세균, 인도 식물, 아프리카 식물, 열대식물, 생육저해

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