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Chemical Properties and Physiological Activities of Synnemata of *Beauveria bassiana*

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Abstract Chemical properties and physiological activities of the freeze-dried synnemata of *Beauveria bassiana* were examined. A proximate analysis showed that the synnemata consisted mainly of carbohydrate (49.86%), protein (11.36%), and a moisture content of 30.64%. It contained a low amount of crude ash (4.76%) and fat (3.38%). The carbohydrate was composed mainly of mannose (52.3%), galactose (31.5%), glucose (13.2%), and rhamnose (3%). Trace amounts of arabinose, xylose, and fructose were present. Major amino acids in the synnemata were glutamic acid, glycine, aspartic acid, arginine, threonine, alanine, valine, leucine, lysine, and aspartic acid with the amounts of 30.42, 25.22, 17.17, 15.12, 12.65, 15.23, 12.47, 11.47, 14.24, and 17.17 mg/g, respectively. Among extracts from the synnemata, the hot-water extract showed 67% of anticomplementary activity compared to that of the positive control, followed by ethyl acetate extract (17%) and methanol extract (15%). The hot-water extract also had anticoagulant activity with 55 sec of coagulating time and this fraction exhibited the most potent intestinal immune system modulating activity. The methanol extract showed the highest inhibitory activity (25%) on the 12-O-tetradecanoylphorbol-13-acetate-induced superoxide (O_2^-) generation, followed by hot-water extract (18%) and ethyl acetate extract (10%). The data in the present study indicate that the extract of *Beauveria bassiana* synnemata contains some healthful chemical ingredients and it could provide beneficial physiological activities. These features of the synnemata should be of interest to the food industry as well as other industrial fields.

Key words: *Beauveria bassiana*, synnemata, physiological activity

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Entomopathogenic fungi including the *Beauveria* and *Cordyceps* species have been studied for biocontrol against insect pests and also for the development of bioactive substances. The genus *Cordyceps* (Ascomycotina; Pyrenomycetes; Clavicipitales; Clavicipitaceae), known as a group of entomopathogenic fungi, forms stromata which can be used as Chinese herbal medicines. Hyphomycetous members of *Cordyceps* have the potential as biocontrol agents against insect pests. In particular, *C. militaris* commonly occurs around the world and, therefore, attracts much attention from researchers. This fungus affects the human physiological system: it inhibits glomerular mesangial cell proliferation [22, 43] and has anticarcinogenic and hormonal effects [23, 26]. Cordycepin (3'-deoxyadenosine), isolated from *C. militaris*, exhibited various biological activities such as inhibition of the synthesis of DNA and RNA [6] and an anticancer activity against fibrosarcoma [15].

The genus *Beauveria*, which is known as an imperfect state of *Cordyceps*, has also a potential in herbal medicines as well as biocontrol agents against insect pests. *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycetes) is one of the most commonly occurring entomopathogenic fungal species, and shows strong pathogenicity to Lepidoptera [36], Hymenopterta [11], Coleoptera [27], and Leptinotarsa [9]. The ease of culture and high virulence of this fungus make it a major candidate for use in microbial control of insect pests [7, 17, 24, 27]. This fungus is characterized by the toothed zigzag appearance of the conidiogenous rachis. However, its ascigerous (teleomorphic) state has not yet been discovered.

Traditionally, *B. bassiana*-infected larva of silkworm, so called "Baeggang Jam," has been used as a medicine to treat strokes in Korea [35]. Despite the beneficial features of *B. bassiana*, little study has been done on production of

synnemata of *B. bassiana*, which possibly include healthful chemical ingredients. As a result, the physiological activity of synnemata is still unknown.

Recently, we developed an artificial medium for the mass production of synnemata of *B. bassiana*, thus providing an opportunity for further pharmacological examination. As it is necessary to examine kinds and yields of physiologically active substances present in artificially-cultured synnemata prior to its commercial use, the present study was undertaken to characterize these chemical compositions, their amounts, and their physiological activities.

MATERIALS AND METHODS

Microorganism and Materials

B. bassiana strain, collected from Seorak Mountain, was precultured in Potato Dextrose Broth (200 g of potato infusion form and 20 g of dextrose in 1 l of bidistilled water) for 7 days, followed by inoculation on the silkworm pupae media (80 g of brown rice, 50 g of pupae powder of silkworm, and 130 ml of bidistilled water) [7, 9]. Synnemata of *B. bassiana* produced in the medium were freeze-dried and used for further experiments. RPMI-1640 medium and Hank's balanced salt solution (HBSS) were obtained from Gibco (Grand Island, NY, U.S.A.). Heat inactivated horse serum and fetal bovine serum were obtained from Cell Culture Laboratories (Cleveland, Ohio, U.S.A.), and penicillin, streptomycin, and amphotericin B were purchased from Flow Laboratories (Irvine, Scotland). Alamar Blue™ was a product of Alamar Bio-Sciences Inc. (Sacramento, U.S.A.). Acetyl thiocholine iodide (ASCh) and 5,5'-dithiobis-(2-nitro)-benzoic acid (DTNB) were purchased from Sigma Co. (St. Louis, U.S.A.). The activated partial thromboplastin time (aPTT) reagent was obtained from Boehring Inc. (New York, U.S.A.). 12-O-Tetradecanoylphorbol-13-acetate (TPA) was a product of Research Biochemicals International (Natick, MA, U.S.A.). All other reagents were analytical grade.

Cells and Culture Conditions

PC12 cells, originated from rat pheochromocytoma and human promyelocytic leukemia HL-60 cells, were obtained from the Health Science Research Resources Bank [4]. They were cultured in an RPMI-1640 medium supplemented with 10% heat inactivated horse serum, 5% fetal bovine serum, and 1% antibiotic-antimycotic (Gibco-BRL, New York, U.S.A.). DLD-1 (human colon carcinoma cell line) and LXFL-529 (human lung carcinoma cell line) were originally provided by the National Cancer Institute (NCI, Bethesda, U.S.A.). These cells were cultured in the same medium as for PC12 cells, except for those supplemented with horse serum. The cells were maintained at 37°C in an atmosphere of 95% air/5% CO_2 incubator, and a subculture was created by mechanically removing them from the substratum with squirts of fresh medium.

Preparation of Various Fractions by Systematic Extraction

Freeze-dried synnemata were blanched at 100°C for 5 min and homogenized by Ultra-turrax T-50 (Janke Kunkel IKA-Labortechniker, Germany, 7,000 rpm, 20 min). After centrifugation (8,000 ×g, 30 min), the supernatant was removed and the precipitate was initially fractionated with ethyl acetate, which is a solvent with a low polarity, followed by fractionation with methanol and water in increasing order of their polarity (Fig. 1).

Chemical Analysis

Moisture, crude protein, crude carbohydrate, crude fat, and crude ash in the synnemata were determined according to the methods of AOAC [1]. To determine the crude protein, a 6.25 conversion factor was used. All values expressed are the means of triplicate determinations.

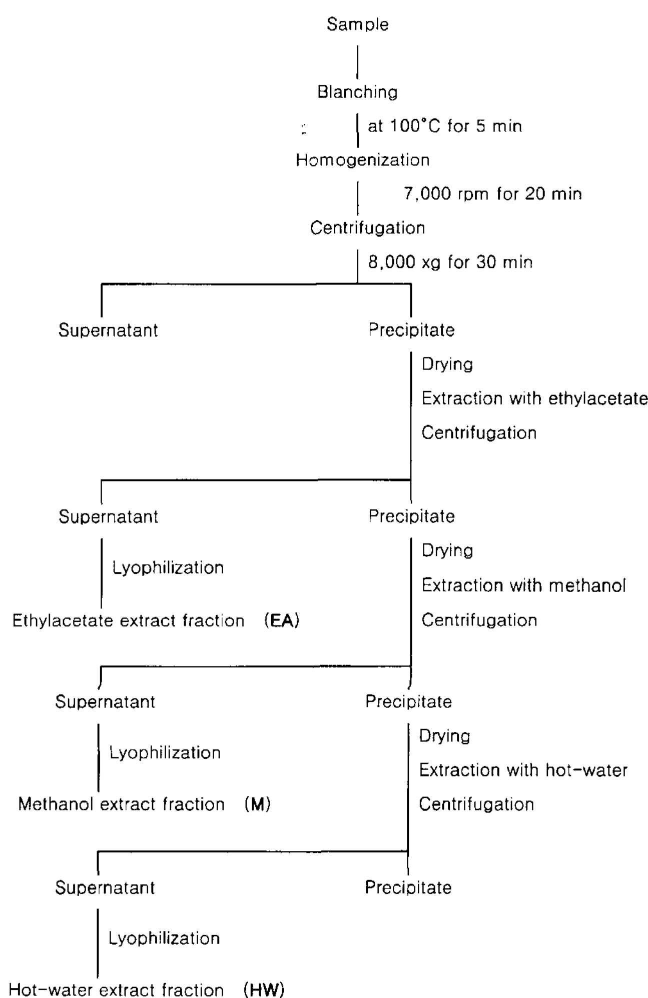

Fig. 1. Preparation of various fractions by systematic extraction.

Analysis of Component Sugar

The component sugar of the polysaccharides was analyzed as alditol acetate after hydrolysis of the polysaccharides with 2 M TFA for 1.5 h at 121°C [17]. It was analyzed by GLC using an SP-2380 capillary column (0.20 mm film, 0.25 mm i.d., 30 m, Supelco, U.S.A.) [42]. The temperature program was as follows: 1 min at 60°C, temperature gradient from 60 to 215°C for 30 min, 18.8 min at 215°C, increase of temperature from 215 to 250°C for 8 min, 5.7 min at 250°C. The molar ratios were calculated from the peak areas and response factors using a flame-ionization detector.

Amino Acid Analysis

For determination of the amino acid composition, 500 mg of sulfosalicylic acid was added to 5 g of sample in 5 ml distilled water. The mixture was centrifuged at 1,300 ×g for 15 min after incubation for 1 h at 4°C. The supernatant was filtered through 0.45 µm filter paper, and pretreated by the modified method described by Lindroth and Mopper [25]. Composition of the amino acid was analyzed by using ODS-µ-Bondapak C_{18} column (3.9 mm i.d., 30 cm) on high performance liquid chromatography (HPLC, Shimadzu, Japan) equipped with a fluorescence detector (Shimadzu, FLD-6A, Japan) according to a modified method of Hodgin *et al.* [13]. All quantitative analyses were performed by comparing peak areas of individual amino acids to those of external standard amino acids (Wako, Japan). All samples were analyzed in triplicate.

Physiological Activity Assay

Anticoagulant and Anticomplementary Activity

Activated partial thromboplastin time (aPTT) was measured at 37°C with an automatic blood coagulator (Clot-1A; Hospitex Diagnostics, Milan, Italy) according to the method described by Fox *et al.* [10]. Briefly, a sample of 100 µl of human citrated platelet-poor plasma (1:10 v/v, 3.8% sodium citrate) was warmed at 37°C, and then 100 µl of a prewarmed activator reagent was added. The mixture was incubated at 37°C for 18 s. Prewarmed 20 mM calcium chloride (100 µl) was then added, and aPTT was recorded as the time for clot formation.

Anticomplementary activity was measured according to the procedure described by Yamada *et al* [39]. Normal human serum (NHS) was obtained from a healthy adult. Various concentrations of the sample in water (50 µl) were mixed with 50 µl of NHS and 50 µl of GVB^{++} (gelatin veronal-buffered saline, pH 7.4) containing 500 µg of Mg^{++} and 150 µg of Ca^{++}. The mixtures were preincubated at 37°C for 30 min, and 350 µl of GVB^{++} was added. IgM-hemolysin-sensitized sheep erythrocytes (250 µl) at 1×10^{8} cells/ml were added to the mixtures diluted serially (10–160 folds), and then incubated at 37°C for 1 h. After addition of phosphate-buffered saline (PBS, pH 7.2) and centrifugation, absorbance of the supernatants was read at 412 nm. The NHS was incubated with water and GVB^{++} as a control. Anticomplementary activity was expressed as the percentage inhibition of the total complementary hemolysis (TCH_{50}) of the control [20]. The degree of inhibition of TCH_{50} was calculated as follows: Inhibition (%)=100× [(TCH_{50} of control−TCH_{50} of sample)/TCH_{50} of control].

Intestinal Immune System Modulating Activity

The activity was measured according to the procedure of Hong *et al.* [14]. A 180 µl of Peyer's patch cell suspension (2×10^{6} cells/ml in RPMI 1640-FBS) prepared from the small intestine of C3H/HeJ mice (5–7 weeks old, Daehan Biolink Co. Korea) was cultured with 20 µl of the test sample in a 96-well flat bottom microtiter plate for 5 days at 37°C in the humidified atmosphere of 5% CO_2-95% air. The resulting culture supernatant (50 µl) was incubated with bone marrow cell suspension (2.5×10^{5} cells/ml) from C3H/HeJ mice for 6 days. After 20 µl of Alamar Blue™ solution was added to each well, the cells were then continuously cultured for 5–24 h [30]. The fluorescence intensity was measured to determine cell numbers with Spectrafluor Plus (Tecan, Austria) at an excitation wavelength of 544 nm and an emission wavelength of 590 nm during cultivation. The intestinal immune system modulating activity was expressed as the stimulation of cell growth of the bone marrow cells compared with that of the control, in which Peyer's patch cells were incubated with distilled water instead of the test sample.

Acetylcholin Esterase (AChE) Inhibition Activity

The AChE Inhibition assay was performed according to the method of Ellman *et al.* [8] using acetylthiocholine iodide as a substrate. To obtain an enzyme source, PC12 cell cultures were homogenized with a Glass-Col homogenizer in 5 volumes of buffer (10 mM Tris-HCl, pH 7.2, containing 1 M NaCl, 50 mM $MgCl_2$, and 1% Triton X-100), and centrifuged at 10,000 ×g for 30 min. The resulting supernatant was used as an enzyme source. The rates of hydrolysis by AChE were monitored spectrophotomatically using a 96-well microtiter plate format. Each freeze-dried extract (10 µl) dissolved in 5% DMSO was mixed with enzyme solution (10 µl) and incubated at 37°C for 15 min. Absorbance was read at 405 nm immediately after addition of 70 µl of Ellman reaction mixture [0.5 mM acetylthiocholine and 1 mM 5,5'-dithiobis-(2-nitro)-benzoic acid in 50 mM sodium phosphate buffer (pH 8.0)] to the mixture of enzyme and extract. Readings were repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. A blank reaction was measured by substituting saline for the enzyme. The percentage of enzyme activity in the presence of inhibitors was calculated by comparison with those of the control's activity (100%). The degree of enzyme inhibition was calculated as follows: Inhibition (%)=100× (1−inhibited reaction/uninhibited reaction).

Inhibition Activity of TPA-Induced O_2^- Generation

An inhibitory test of TPA-induced O_2^- generation in DMSO-differentiated HL-60 cells was performed as previously reported [28, 30]. In order to determine the inhibitory effect of O_2^- generation, a test compound dissolved in 5 µl of DMSO was added to a DMSO-induced differentiated HL-60 cell suspension in PBS (1 ml). After incubation at 37°C for 15 min, the cells were washed twice with PBS to remove unabsorbed test compound. TPA (10 nM) and cytochrome c solution (1 µg/ml) were added to the reaction mixture, which was then incubated for another 15 min. The reaction mixture was placed on ice to terminate the reaction. After centrifugation at 250 ×g, absorbance at 550 nm was measured. Inhibitory effects were expressed by a decreasing ratio of absorbance of test compounds relative to a control experiment.

Cytototoxic Activity Against Tumor Cell Lines

Tumor cells were inoculated over a series of standard 96-well flat bottom microtiter plates and then preincubated to allow them to attach to the microplate. The attached cells were incubated with serially diluted fractions. After continuous exposure to the fractions for 48 h, the culture medium was removed from each well and the cells were fixed with 10% cold TCA at 4°C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 nm and 690 nm with a microtiter plate reader (Biorad, Model3550). All experiments were performed in triplicate.

Table 1. Proximate and chemical properties of synnemata of *Beauveria bassiana.*

Component	Content (%)[c]
Moisture	30.64
Crude protein[a]	11.36
Crude fat	3.83
Crude carbohydrate	49.86
Crude ash	4.76
Component sugar[b]	**Content (mol. %)**
Arabinose	trace
Xylose	trace
Rhamnose	3.0
Mannose	52.3
Galactose	31.5
Glucose	13.2
Fructose	trace

[a]To determine crude protein, a 6.25 conversion factor was used.
[b]Component sugar of polysaccharide was determined by GC as alditol acetate derivatives and analyzed by GLC using an SP-2380 capillary column (0.20 mm film, 0.25 mm i.d., 30 m, Supelco, U.S.A.) equipped with an FID.
[c]All values expressed are means of triplicate determinations.

Results and Discussion

Proximate and Chemical Analysis

Table 1 shows the composition of the synnemata used in the present study. The synnemata consisted mainly of carbohydrate (49.86%), protein (11.36%), and moisture content of 30.64%. Crude ash (4.76%) and fat (3.38%) were found as minor components. The carbohydrate was composed mainly of mannose (52.3%), galactose (31.5%), glucose (13.2%), and rhamnose (3%). In addition, trace amounts of arabinose, xylose, and fructose were observed. Compositional differences were evident between the synnemata of *B. bassiana* and the stromata of an entomopathogenic fungus, *C. militaris*. Glucose was the major component (78.6%) of anticomplementary active exopolymer in liquid-cultured mycelium of *C. militaris*, and carbohydrate in the stromata of *C. militaris* was composed mainly of glucose (88.6%) [37, 41].

Compositional differences were also found in the proximate and chemical analyses between the synnemata of *B. bassiana* and stromata of *C. militaris*. Methionine and lysine were present in the stromata of *C. militaris* in large amounts [41]. Glutamic acid, however, was determined to be the most abundant amino acid in the synnemata of *B. bassiana* (Table 2). The contents of glutamic acid, glycine, aspartic acid, arginine, threonine, alanine, valine, leucine, lysine, and aspartic acid were 30.42, 25.22, 17.17, 15.12, 12.65, 15.23, 12.47, 11.47, 14.24, and 17.17 mg/g, respectively. Other amino acids were detected in a low amount below 10 mg/g.

Table 2. Amino acid composition of synnemata of *Beauveria bassiana.*

Component amino acid[a]	Content (mg/g)[b]
Alanine	15.23
Arginine	15.12
Aspartic acid	17.17
Cysteine	11.25
Glutamine	30.42
Glycine	25.22
Histidine	4.85
Isoleucine	8.27
Leucine	11.47
Lysine	14.24
Methionine	9.86
Phenylalanine	8.64
Serine	10.30
Threonine	12.65
Tryptophan	1.28
Tyrosine	3.62
Valine	12.47

[a]The amino acid was analyzed on an ODS-µ-Bondapak C_{18} column (3.9 mm i.d., 30 cm), and an HPLC (Shimadzu, Japan) equipped with a fluorescence detector (Shimadzu, FLD-6A, Japan) was used.
[b]All analyses were made in triplicate.

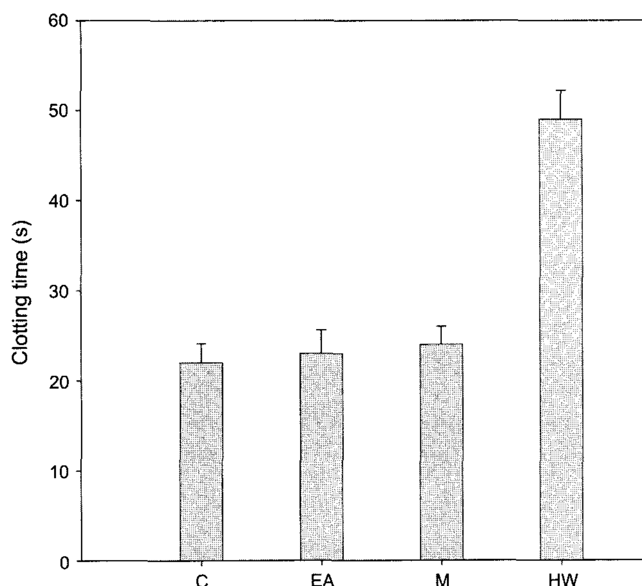

Fig. 2. Anticoagulant activities of synnemata of *Beauveria bassiana* fractionated by solvent extraction.
The clotting time was measured by the aPTT method. The final concentration of the sample was 100 μg/ml. Values represent the means (n=4)±S.D. $p<0.05$, Significance between the control and samples. C, Control (saline); EA, Ethyl acetate extract fraction; M, Methanol extract fraction; HW, Hot-water extract fraction.

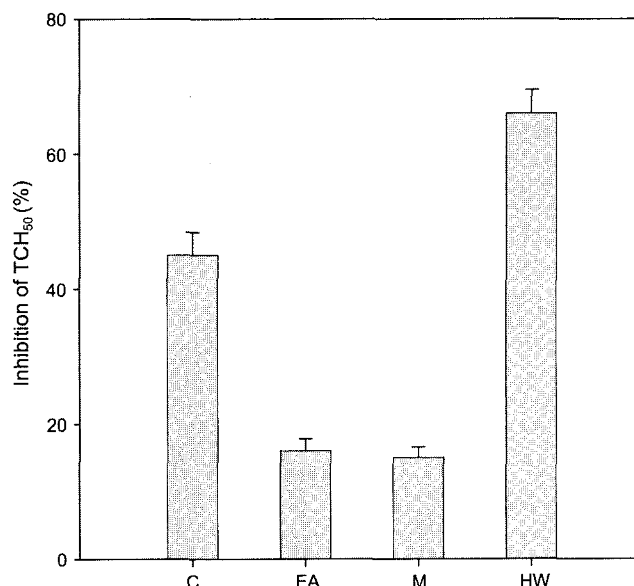

Fig. 3. Anticomplementary activities of synnemata of *Beauveria bassiana* fractionated by solvent extraction.
The percentage of anticomplementary effect for each fraction was expressed as the percentage inhibition of the total complement hemolysis (TCH_{50}) of the control. The final concentration of the sample was 100 μg/ml. Values represent the means (n=4)±S.D. $p<0.05$, Significance between the positive control and samples. C, Positive control (LPS, 100 μg/ml); EA, Ethyl acetate extract fraction; M, Methanol extract fraction; HW, Hot-water extract fraction.

Physiological Activity

Blood coagulation is a major cause of mortality and disability, leading to cerebral hemorrhage, myocardial infarction, peripheral ischemia, arteriosclerosis, and pulmonary embolism [12]. In the last several years, extensive investigations of therapeutic agents have been made in the development of effective anticoagulants. In the present study, anticoagulant activity in various solvent extracts of the synnemata was also examined (Fig. 2), by measuring clotting time by aPTT method, using saline as a control. It was observed that a hot-water extract of the synnemata contained anticoagulant activity (coagulating time of 55 sec). Recently, a new platelet aggregation inhibitor, bassiatin, was found in the cultured broth of *B. bassiana,* which was isolated from a soil sample collected in Yunnan Province, China [19]. The above anticoagulant activity of the synnemata observed in the present study might have been due to the bassiatin. Therefore, drinking of synnemata-boiled water as a food supplement could be helpful in strengthening anticoagulant activity.

Anticomplementary activity in solvent extracts from synnemata was examined by the complement fixation test, and the activity is presented as $ITCH_{50}$ (Fig. 3). Among the extracts, the hot-water extract showed higher activity (67%) than the positive control (45%), and the ethyl acetate extract (17%) and the methanol extract (15%) showed low activities. This anticomplementary activity of the hot-water extract was important, because anticomplementary activity plays an important role in host resistance in the primary humoral mediation of Ag-Ab reactions. Although complement function has been viewed in the context of a predominantly nonspecific resistance mechanism, there is accumulating evidence that the complement is involved in the induction and regulation of specific immune response. Furthermore, complement activation appears to be intrinsically associated with the activation of macrophages and lymphocytes [31], the localization and retention of antigens in germinal centers, the generation of B cell memory [21], cellular cooperation, and the regulation of cyclical antibody production [38].

Among the extracts, the hot-water extract showed the most potent intestinal immune system modulating activity, followed by the methanol extract and the ethyl acetate extract (Fig. 4). Active substances could be extracted by these solvent systems, because all three fractions exhibited intestinal immune system modulating activity. The gut-associated lymphoreticular tissues (GALT) play an important role in host defense, including the IgA response of the intestinal immune system [16]. Peyer's patches are important lymphoid organs of the intestine, and known as inductive sites for IgA production. Therefore, the intestinal immune system, including Peyer's patches, not only contributes to the defense system of the mucosa, but also regulates systemic inflammation, resulting in suppression of allergic reactions and autoimmune diseases [16]. In several studies,

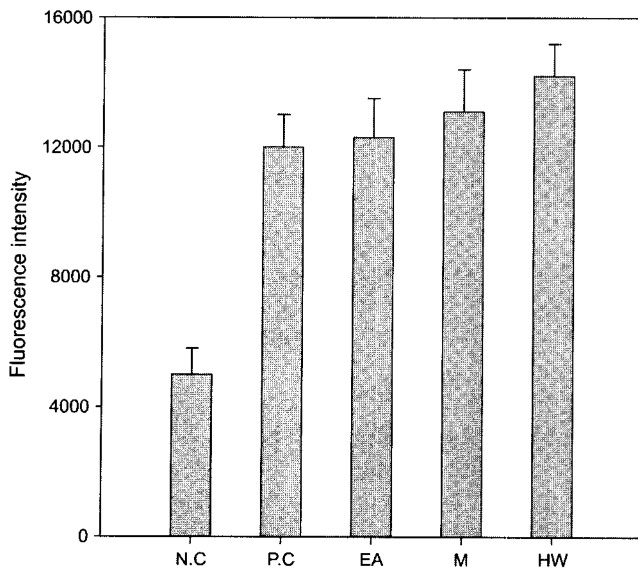

Fig. 4. Intestinal immune system modulating activities of synnemata of *Beauveria bassiana* fractionated by the solvent extraction.

The intestinal immune system modulating effect values for each fraction was expressed as the stimulation of the cell growth of the bone marrow cells, compared with that of the control. The final concentration of the sample was 100 μg/ml. Values represent the means (n=4)±S.D. $p<0.05$, Significance between the positive control and samples. N.C, Negative control (saline); P.C, Positive control (LPS, 100 μg/ml); EA, Ethyl acetate extract fraction; M, Methanol extract fraction; HW, Hot-water extract fraction.

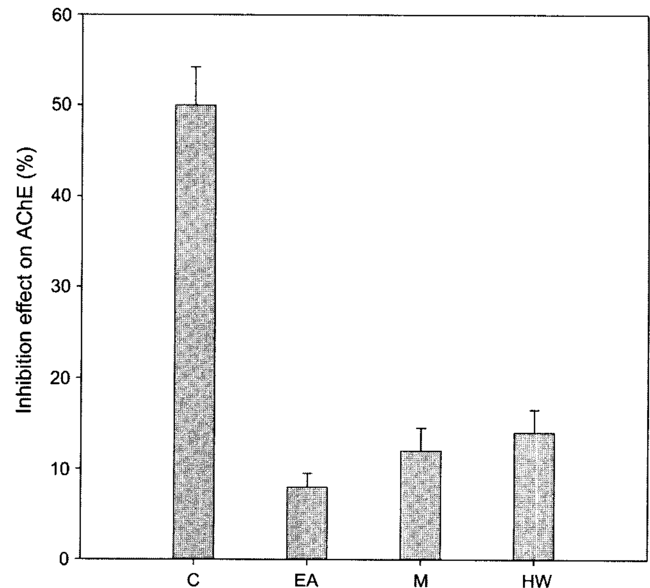

Fig. 5. Inhibitory effect on AChE activities of synnemata of *Beauveria bassiana* fractionated by solvent extraction.

The percentage of enzyme activity for each fraction was calculated in comparison compared to the control activity (100%). The final concentration of sample was 130 μg/ml. Values represent the means (n=4)±S.D. $p<0.05$, Significance between the positive control and samples. C, Positive control (tacrine, 130 μg/ml); EA, Ethyl acetate extract fraction; M, Methanol extract fraction; HW, Hot-water extract fraction.

solvent extract from rhizomes of *Atractylodes lancea* [40] and stromata of *C. militaris* [41] have been shown to have a modulating effect on the intestinal immune system *in vitro*. This action was through activation of T cells in Peyer's patches to stimulate secretion of a hematopoietic growth factor. In the present study, the synnemata of *B. bassiana* also exhibited an intestinal immune system modulating activity. The synnemata, therefore, could provide a beneficial nutritional complement, and the possibility of clinical application as a biomedicine. Further studies on the active substance are necessary before it can be commercially applied.

Acetylcholine esterase (AChE) inhibition activity plays an important role in Alzheimer's disease (AD), which is primarily caused by a cholinergic dysfunction. The degree of cognitive impairments in AD has been reported as correlating well with central cholinergic deficits [5]. This provides a rationale for the most updated approaches to AD drug therapy. Thus, it is suggested that elevation of the acetylcholine (ACh) level might be helpful to improve symptoms of cognitive deficits in AD [3]. Several groups have tried to supplement the acetylcholine (ACh) level in synaptic sites by administration of ACh precursors, cholinergic agonists, or AChE inhibitors [29] such as tacrine (tetrahydroaminoacridine) and galantamine [2], which prevent ACh hydrolysis. Therefore, finding an AChE inhibitor is a prerequisite to treatment of AD. Therefore, we also examined the solvent extracts of the synnemata for an inhibitory effect on AChE (Fig. 5). Among the extracts tested, the hot-water extract exhibited the strongest inhibitory effect (15%). However, the value was lower than that of the positive control (50%). This result indicates that the synnemata of *B. bassiana* is not so beneficial in inhibiting AChE (Fig. 5).

Inhibitory activity of the TPA-induced O_2^- generation was tested. TPA-type tumor promoters are reported to trigger superoxide (O_2^-) generation in epithelial cells and leukocytes [34]. Superoxide (O_2^-) is one of the precursors of several types of reactive oxygen species (ROS) [32], resulting in oxidative stress-related diseases including cancer. The present study also attempted to search for an O_2^- generation inhibitor as a promising candidate for the prevention of these diseases. Among the extracts, the methanol extract revealed the highest inhibitory activity (25%) on the TPA-induced superoxide (O_2^-) generation (Fig. 6), followed by the hot-water extract (18%) and ethyl acetate extract (10%). The activity of the methanol extract was almost the same as that of the positive control, which exhibited about 28% activity. Therefore, the methanol extract deserves to be applied in the food industry field.

The cytotoxic activity of the hot-water extract from the synnemata on DLD-1 (human colon carcinoma cell line) and LXFL-529 (human lung carcinoma cell line) cells was

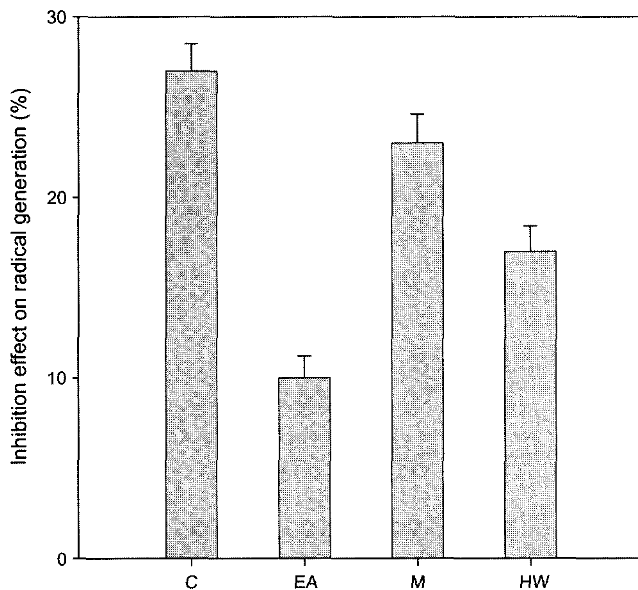

Fig. 6. Inhibitory effect on TPA-induced O_2^- generation of synnemata of *Beauveria bassiana* fractionated by solvent extraction.
The percentage of inhibitory effect for each fraction was calculated in comparison to the control O_2^- generation (100%). The final concentration of the sample was 100 µg/ml. Values represent the means (n=4)±S.D. C, Positive control (genistine, 100 µg/ml); EA, Ethyl acetate extract fraction; M, Methanol extract fraction; HW, Hot-water extract fraction.

proportional to the concentration of the extract (Fig. 7). The concentration of the hot-water extract required to obtain

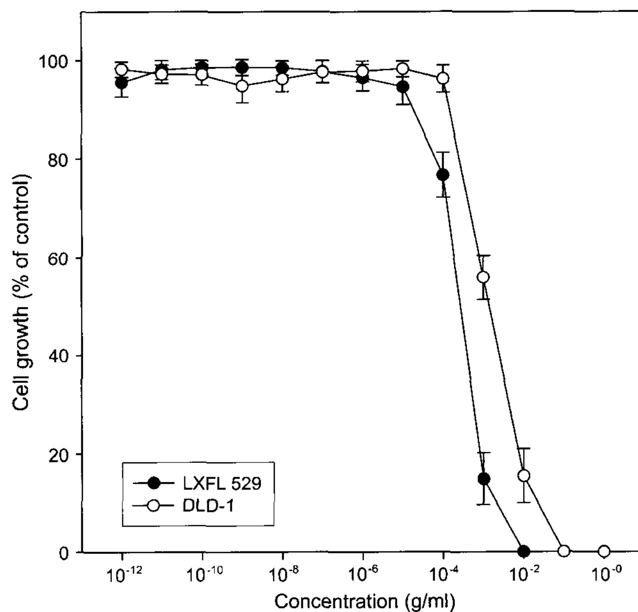

Fig. 7. Effects of hot-water extract on the growth of human carcinoma cells *in vitro*.
The cells were cultured with 10-fold serial dilutions of hot-water extract of synnemata. Cell survival fractions were assessed after continuous exposure for 3 days by SRB assay. Each data represents the mean of at least three experiments. ○, DLD-1; ●, LXFL 529.

a 50% inhibitory growth (EC_{50}) in DLD-1 and LXFL-529 was close to 1 mg/ml, as shown in Fig. 7. Therefore, excess amounts of synnemata would be required to obtain a satisfactory cytotoxic effect against the growth of a cancer cell line. This indicates that the synnemata have little cytotoxic activity.

In summery, physiological activities of the freeze-dried synnemata of *B. bassiana* were evaluated. Hot-water extracts from the synnemata showed various biological activities including anticomplementary activity, anticoagulant activity and the most potent intestinal immune system modulating activity. The methanol extract revealed the highest inhibition activity on the TPA-induced superoxide (O_2^-) generation. Various biological activities shown in the present study indicated that the synnemata of the *B. bassiana* contained some healthful chemical ingredients, and that the intrinsic nature of these active compounds requires further investigation.

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