

SPECIFICATION

TITLE OF THE INVENTION

HEALTHFUL COMPOSITION

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates to a composition having excellent pharmaceutical and health-preserving effects and, more precisely, to a Cordyceps sinensis-derived medicine-type and/or food-and-drink-type composition.

10 PRIOR ART

 Vegetative wasps (plant worms) are microorganisms of Ascomycota, Pyrenomycetes, Clavicipitales, Clavicipitaceae (or Hypocreaceae), Cordyceps, undergoing both a complete metamorphic phase and an incomplete metamorphic phase.

15 Their fruit bodies have been valued from ancient times as drugs marvelously effective for perennial youth and longevity or for nourishment and tonicity. The fruit bodies of vegetative wasps that have heretofore been considered extremely valuable as Chinese medicines are
20 generally powdered and administered.

 According to the present invention, the mycelia of particular vegetable wasps are cultivated and the thus-cultivated mycelia are extracted with hot water. The fact that the resulting extract from such mycelia has a

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cardiotonic effect and an anti-fatigue effect has heretofore been unknown at all.

PROBLEMS TO BE SOLVED BY THE INVENTION

5 The present invention has been made for the purpose of newly developing drugs having excellent cardiotonic, hypotensive, antitussive and anti-fatigue effects or foods and drinks having such effects and for the purpose of developing natural substances that can surely be administered for a long period of time in
10 serious consideration of their safety.

MEANS FOR SOLVING THE PROBLEMS

Having investigated variously in order to attain the above-mentioned objects, we, the present inventors have specifically noted Chinese medicines and vegetative
15 wasps, especially those of Cordyceps sinensis and further studied them and, as a result, have found that the mycelia to be obtained by cultivating them, especially the products to be obtained by extracting the cultivated mycelia with hot water have mild cardiotonic and anti-
20 fatigue effects. On the basis of this finding, we have further studied and at last have achieved the present invention. The present invention is described in detail hereinunder.

DESCRIPTION OF THE DRAWINGS

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Fig. 1 shows the effect of a hot-water extract of the mycelia of Cordyceps sinensis MF-20008 on the contractile force of the right atrial muscle of a rat.

Fig. 2 shows the effect of a hot-water extract of
5 the mycelia of Cordyceps sinensis MF-20008 on the inter-contraction interval of the right atrium of a rat.

Fig. 3 shows the ulcer index (cm) of the ulcer formed in the gastric glands of a rat, to which a hot-water extract of the mycelia of Cordyceps sinensis MF-
10 20008 was administered.

Fig. 4-1 shows the intake of ^3H -thymidine in the primary culture hepatocytes of a rat; and Fig. 4-2 shows the intake of ^3C -valine in the same.

Fig. 5 shows the effect of a hot-water extract of
15 the mycelia of Cordyceps sinensis MF-20008 for the inhibition of the gastric acid secretion in a rat.

To carry out the present invention, it is necessary to cultivate the mycelia of vegetative wasps of Cordyceps sinensis and to collect the thus-cultivated
20 mycelia. All microorganisms of Cordyceps sinensis can be used in the present invention, and these will be referred to as the microorganisms of the invention. For example, Cordyceps sinensis MF-20008 is useful, and this strain has been deposited in the National Institute of Bioscience and
25 Human-Technology of the Agency of Industrial Science and Technology under FERM BP-5149.

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According to the present invention, the mycelia of Cordyceps sinensis are cultivated.

These are cultivated in a medium comprising malt extract, yeast extract, peptone, potato broth, glucose, 5 vitamins, amino acids, nucleic acids, proteins and optionally host components such as insects, etc., by liquid or solid culture, by which the mycelia are fully propagated.

Liquid culture, especially stirring culture by 10 aeration is preferred for cultivating a large amount of the mycelia, which is conducted at pH of from 4 to 7 and at from 15 to 32°C, preferably from 20 to 30°C, while gently stirring the medium at approximately from 50 to 500 rpm, preferably from 100 to 300 rpm, for 3 to 10 days.

15 After the culture, the thus-cultivated mycelia are extracted with hot water. Water is added to the mycelia, and these are, optionally after having been ground, heated at from 60 to 100°C, preferably from 85 to 100°C, more preferably from 90 to 95°C, and thus extracted with the 20 hot water, optionally with stirring. The amount of water to be added is not specifically defined but is preferably from 1 ml to 1 liter, per gram of the dry mycelia.

The residual substances are removed from the thus-obtained hot-water extract, and the resulting liquid 25 extract is used directly or after having been further

processed, as the active ingredient of the present invention. The products to be obtained by processing the liquid extract broadly include the concentrated product, the paste product, the dried product and/or the diluted
5 product of the liquid extract. As the case may be, the hot-water extract or its processed products may be directly used, without removing the residual substances therefrom, for example, as foods and drinks.

The hot-water extract (including its processed
10 products) of the mycelia has mild cardiogenic, hypotensive, antitussive and anti-fatigue effects, and the composition of the present invention containing it can be used as foods and drinks having these effects, foods and drinks for particular health, healthful drinks, healthful
15 foods, nutritious foods, and other various types of foods and drinks, and also as medicines such as cardiacs, hypotensors, antitussives, tonics, etc.

For food-and-drink-type compositions, the active ingredient of the hot-water extract (or its processed
20 product) may be directly used or may be combined with other foods or food components by ordinary methods. The composition of the present invention containing the active ingredient may be in any form of a solid (powder, granules, etc.), a paste, a liquid and a suspension, and
25 it is suitably formulated into healthful drinks along with

sweeteners, sours, vitamins and other various additives for ordinary drinks.

For medicine-type compositions, the active ingredient may be administered as various forms. As the
5 forms, mentioned are various oral compositions, including, for example, tablets, capsules, granules, powders, syrups, etc. These various preparations can be formulated by ordinary methods with known auxiliary additives which are generally used in the technical field of formulating
10 medicines, such as vehicles, binders, disintegrators, lubricants, flavorings, dissolution aids, suspending agents, coating agents, etc. The dose of the active ingredient to be administered varies, depending on the condition, the age and the weight of the person to which
15 it is administered, as well as on the administration route and the form of the preparation, etc., but is generally from 0.1 mg/adult to 1,000 mg/adult for one administration.

The active ingredient of the present invention is
20 derived from natural resources which have been used for many years as Chinese medicines, and this is completely non-toxic or is only slightly toxic. Therefore, this is safe. Its oral administration to rats in a dose of 500 mg/day resulted in no acute toxicity. Therefore, the
25 amount of the active ingredient to be in food-and-drink-

type compositions is not specifically limited for any of disease-preventing use, health-preserving and ordinary use. For medicines, the amount of the active ingredient to be therein may be defined within the above-mentioned
5 range in accordance with the patients. Since the active ingredient of the present invention exhibits no toxicity even when a large amount of it is taken, its dose to be administered may be over the above-mentioned range, if desired, with no problem.

10 The present invention is described in detail by means of the following Production Examples and Examples.

Production Example 1

One liter of M20Y2 medium (comprising 20 g of malt extract (Oxoid) and 2 g of yeast extract (Difco) in one
15 liter and having pH of 5.5) was put into each of 46 3-liter Erlenmeyer flasks, which were then sterilized in an autoclave at 121°C for 15 minutes.

One platinum loop of the mycelia of Cordyceps sinensis MF-20008 (obtained from The Sanming Mycological
20 Institute, Fujian, China), FERM BP-5149, that had been pre-cultivated by M20Y2 agar slant culture was inoculated in each sterilized medium and cultivated therein at 25°C by shaking culture at 180 rpm for 5 days. Thus, 46 liters of the culture was obtained in these 46 flasks.

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46 liters of the culture were centrifuged, and the mycelia thus separated were freeze-dried to obtain 323.6 g of dry mycelia. The dry mycelia were put into a stainless container, 7 liters of water were added thereto, and these were ground with a polytoron, then heated at from 90 to 95°C for 2 hours while stirring and extracted. After the extraction, the residual substances were removed by filtration, and the resulting filtrate was freeze-dried to obtain 130 g of a lightly yellow powder.

10 Production Example 2

In the same manner as in Production Example 1, 150 ml of M20Y2 medium was put into a 500-ml Erlenmeyer flask and sterilized by autoclaving, and 1 ml of freeze-dried Cordyceps sinensis MF-2008 was inoculated therein and cultivated by shaking culture at 25°C and 180 rpm for 5 days. This was used as a seed culture and cultivated in 150 liters of the following medium in the manner mentioned below.

150 liters of D medium (comprising 4.0 g of sucrose, 4.0 g of K_2HPO_4 , 0.5 g of asparagine, 2.0 g of $(NH_4)_2HPO_4$, 2.0 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of $CaCO_3$, 0.1 g of $CaCl_2$ and 4.0 g of yeast extract B-2 in one liter and having pH of 5.6) was, after having been sterilized and filtered, put into a 150-liter fermenter, and 150 ml of the above-mentioned seed culture was inoculated therein

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and cultivated under a controlled condition at 25°C, pH of 5.5 and DO of 50 %, while stirring at 157 rpm, for 3 days. The foams formed during the cultivation were removed by adding a silicone de-foaming agent to the fermenter. 150
5 liters of the culture thus obtained was treated with a clarifier to thereby isolate the mycelia. Thus, an aqueous suspension of the mycelia was obtained. This was heated at from 90°C to 95°C for 2 hours and extracted. Then, this was clarified with a clarifier to isolate a
10 supernatant. This was filtered through 0.45- μ m and 0.22- μ m sterile filters to obtain 80 liters of a lightly yellow hot-water extract (the dry weight of the solid content: 800 g).

Example 1

15 The cultivated mycelia (dry mycelia) of Cordyceps sinensis MF-2008 that had been prepared in Production Example 1 and the hot-water extract thereof (lightly yellow powder) were subjected to a swimming fatigue test with mice, to which these were orally administered.

20 The test animals were male ICR mice (5 to 7 week-age), and these were grouped into three groups and subjected to a fatigue test under the conditions mentioned below.

Administration:

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The hot-water extract of the mycelia of Cordyceps sinensis MF-2008 or the mycelia themselves were mixed with powdery CRF-1 feed at a proportion of 2.5 % or 5 %, respectively, and the resulting powdery feed mix was administered to the mice all the time for 16 days.

Groups of mice: Each group was comprised of 20 mice.

(1) Control group: Fed with only powdery CRF-1 feed.

(2) Group to which the hot-water extract was administered: Fed with the feed mix containing 2.5 % of the hot-water extract.

(3) Group to which the mycelia were administered: Fed with the feed mix containing 5 % of the dry mycelia.

Test schedule:

On the 15th day after the start of the administration, the mice were subjected to a forcedly swimming test. On the 16th day, all the blood was collected from each mouse and subjected to whole blood analysis and serum analysis.

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(1) The swimming fatigue test is as follows: A 36-liter swimming tank (49 cm x 33.5 cm x 22 cm (depth)) was filled with water at 17°C, and 10 ml of a neutral detergent were added thereto. The mice were forcedly swum in the tank under no load, and the swimming test for each mouse was stopped at the time at which the mouse sank in water up to the tip of its nose for 5 seconds. The results obtained are shown in Table 1 below.

Table 1

Group (1) (Control)				
	Gauge No.			
No.	1	2	3	4
1	9' 55"	7' 06"	7' 41"	10' 34"
2	8' 57"	6' 14"	9' 08"	12' 10"
3	7' 54"	8' 45"	7' 34"	8' 24"
4	8' 28"	7' 37"	8' 54"	8' 08"
5	9' 28"	9' 09"	9' 42"	10' 40"

Group (2) (Fed with the feed mix containing the hot-water extract of the cultivated mycelia)				
	Gauge No.			
No.	1	2	3	4
1	9' 16"	12' 33"	11' 22"	9' 56"
2	6' 36"	11' 04"	11' 43"	>15' 00"
3	11' 51"	7' 35"	10' 37"	11' 33"
4	10' 45"	>15' 00"	10' 02"	9' 25"
5	12' 04"	9' 33"	>15' 00"	7' 40"

5

Group (3) (Fed with the feed mix containing the cultivated mycelia)				
	Gauge No.			
No.	1	2	3	4
1	12' 32"	7' 51"	9' 53"	10' 54"
2	9' 00"	6' 33"	>15' 00"	12' 18"
3	Died	9' 07"	7' 28"	10' 49"
4	11' 34"	8' 59"	13' 08"	7' 19"
5	9' 10"	7' 40"	7' 26"	8' 02"

10 The above-mentioned results were analyzed by the
Aspin-Welch test of significance. There was admitted a
significant difference between the first group and the
second group, as the level of significance of the test was
1 %. However, there was admitted no significant
15 difference between the first group and the third group, as
the level of significance of the test was 5 %.

 The swimming fatigue test is one of tests which
are generally employed for the purpose of evaluating the
anti-fatigue effect of test substances on the basis of the
20 variation in the total reaction of test animals.
Therefore, the above-mentioned results are nothing but
ones that have scientifically verified the excellent
effect of the active ingredient of the present invention
for increasing the sustaining capacity for power of
25 locomotion and the excellent anti-fatigue effect of the
same.

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(2) On the 16th day after the start of the administration, the whole blood was collected from each mouse that had been subjected to the above-mentioned swimming fatigue test and analyzed in terms of the number
5 of the platelets ($\times 10,000/\text{mm}^2$), the amount of hemoglobin (g/dl), the number of erythrocytes ($\times 10,000/\text{mm}^2$), the number of leucocytes ($/\text{mm}^2$), and the hematocrit (%).

The results of the analysis of the whole blood are shown in Table 2 below. As is obvious from these results,
10 there was admitted no significant difference between the test groups and the control group in all the test items, as the level of significance of the test was 5 %. (To analyze the whole blood, used was a Student test. To count the number of the platelets in the third group,
15 however, used was an Aspin-Welch test.)

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Table 2

No.	First Group (Control Group)			
	Number of Platelets x 1000/mm ³	Amount of Hemoglobin g/dl	Number of Erythrocytes x 10000/mm ³	Number of Leucocytes /mm ³
1	106.5	14.5	879	4300
2	130.5	15.5	984	4200
3	107.5	16.3	1075	3600
4	120.1	16.4	1104	5700
5	109.4	15	963	6300
6	95.8	15.5	984	5100
7	108.4	14.5	898	2700
8	89.4	15	943	4400
9	114.4	15	1065	4600
Average	109.11	15.41	988.33	4544.44
				57.73

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No.	Second Group (Fed with Hot-water Extract of Cultivated Mycelia of MF-20008)			
	Number of Platelets x 10000/mm ³	Amount of Hemoglobin g/dl	Number of Erythrocytes x 10000/mm ³	Number of Leucocytes /mm ³
1	102.6	15	939	4500
2	121.8	14.7	923	5900
3	136.1	14.5	869	5200
4	97.7	14.5	866	4800
5	77.1	16.4	1011	4200
6	119	15.3	932	6400
7	107.8	13.7	863	4000
8	100.8	16.2	1016	4400
9	121	15.7	1004	5800
10	128	16.1	1015	5000
Average	111.19	15.21	943.8	5020
				55.76

Third Group (Fed with Dry Mycelia of MF-20008)					
No.	Number of Platelets × 10000/mm ³	Amount of Hemoglobin g/dl	Number of Erythrocytes × 10000/mm ³	Number of Leucocytes /mm ³	Hematocrit %
1	88.3	13.7	835	7000	51
2	97.6	15.9	994	3200	56.3
3	103.6	15.3	916	3300	55.3
4	116	14.7	951	7000	53.7
5	155.7	15.5	936	7000	57.4
6	131.6	15.2	943	4100	56.6
7	119.3	16.6	1010	4700	59.3
8	130.9	16.2	991	5200	57.9
9	113.1	15.7	1026	7700	58.8
10	58.6	15.8	994	6400	57.4
Average	111.47	15.46	959.6	5560	56.67

(3) The body weight of each test mouse was weighed during the period of the test, and the variation in the mean weight (g) of the test mice is shown in Table 3 below (Variation in Weight of Mice Tested by Forcedly Swimming Test).

Table 3

Days after the Start of Administration	Mean Body Weight (g)		
	1st Group	2nd Group	3rd Group
0	32.19	32.24	32.14
2	33.74	33.94	33.75
4	34.18	34.37	34.30
7	35.38	35.25	35.02
10	35.81	35.66	35.99
14	36.68	37.52	36.67
16	38.30	38.41	37.64

Example 2

The the hot-water extract of the mycelia of Cordyceps sinensis MF-20008 that had been prepared in Production Example 1 was tested in terms of its effects on the right atrium, the papillary muscle of the right ventricle, the aorta and the bronchi taken out of an SD rat, according to a Tsunoo et al's method (see Kurokawa M. and Tsunoo A., J. Physiol., 407, 135-153, 1988; Tsunoo A. et al., J. Physiol., 433, 163-181, 1991), and the effectiveness of the extract was evaluated.

(1) Specimen of Cardiac Muscle:

By applying the hot-water extract to the specimen of the cardiac muscle of a male SD rat, the contractile force of the cardiac muscle of the right atrium and the inter-contraction interval thereof were measured.

5 The right atrium was taken out of a male SD rat (380 to 450 g) while applying the acting pacemaker thereto. The specimen of the right atrium was fixed in a horizontal perfusion container having a volume of 0.8 ml, and its contractile force was isometrically recorded. As
10 the perfusate, used was a Krebs solution that had been equilibrated with 95 % oxygen and 5 % carbon dioxide, or an aerated salt solution comprising 140 mM of sodium chloride, 5 mM of potassium chloride, 2.6 mM of calcium chloride, 1.3 mM of magnesium chloride, 10 mM of glucose
15 and 5 mM of HEPES. The perfusate was kept at from 36 to 37°C and at a flow rate of from 3 to 4 ml/min.

As a result, the hot-water extract of the mycelia (from 50 to 100 μ g/ml) increased the contractile force of the cardiac muscle of the right atrium by 9 ± 3 % of the
20 control (mean value $\pm$ standard deviation, $n = 5$). The interval of the spontaneous inter-contraction due to the pacemaker was prolonged by 14 ± 10 % of the control ($n = 4$). Fig. 1 and Fig. 2 show the time-dependent increase in the contractile force of the cardiac muscle of the

right atrium and the time-dependent prolongation of the inter-contraction interval of the same, respectively.

In Figs. 1 and 2, the extract solution (60 $\mu\text{g/ml}$) was applied to the test system during the period of time expressed by the thick solid line. The vertical axis indicates the relative value of the contractile force relative to the standard value of the control (before application of the extract solution) of being 1 (one). The horizontal axis indicates the time, on which 0 (zero) means the time at which the extract solution was applied to the test system and the minus number means the time before the application.

As is obvious from the above, the effectiveness of the hot-water extract on the spontaneous contraction of the right atrium was verified by the illustrated data. In addition, the papillary muscle of the right ventricle was taken out of the rat and fixed in a perfusion container in the same manner as above, to which was applied electric stimulation (2 Hz) via a platinum wire, and the effectiveness of the extract on the contraction of the papillary muscle of the right ventricle, which does not spontaneously contract, to be caused by the electric stimulation was examined. As a result, the extract (from 30 to 100 $\mu\text{g/ml}$) increased the tension of the papillary

muscle of the right ventricle to be caused by the electric stimulation by 13 ± 1 % of the control ($n = 3$).

(2) Specimen of Aorta:

By applying the hot-water extract to the specimen
5 of the aorta of a male SD rat, the effect of the extract on the aorta and also the effect thereof of inhibiting the sustaining contraction of the aorta to be caused by 50 mM potassium were examined.

The aorta was taken out of a male SD rat and
10 formed into a spiral section specimen. The specimen was fixed in a perfusion container under the same conditions as above. The 50 mM potassium solution to be used for stimulating the specimen was prepared by substituting sodium chloride in the container by potassium chloride.

15 As a result, the hot-water extract of the mycelia (from 50 to 100 $\mu\text{g/ml}$) exhibited no significant effect on the aorta which was in a static condition. However, this relaxed the sustaining contraction of the aorta caused by 50 mM potassium. Where the contraction tension of the
20 aorta caused by 50 mM potassium was referred to as 100 %, the extract reduced the tension by from 2 to 12 % ($n = 5$).

(3) Specimen of Bronchi:

By applying the hot-water extract to the specimen
of the bronchi of a male SD rat, the effect of the extract
25 of inhibiting the contraction of the bronchi to be caused

by electric stimulation or by 50 mM potassium was examined.

The bronchi were taken out of a male SD rat and formed into a spiral specimen. The specimen was fixed in
5 a perfusion container under the same conditions as above. The 50 mM potassium solution to be used for stimulating the specimen was prepared in the same manner as above. The electric stimulation was applied to the specimen at intervals of 30 seconds via a platinum wire set in the
10 perfusion container.

As a result, the hot-water extract of the mycelia (from 50 to 100 $\mu\text{g/ml}$) inhibited the transient contraction of the bronchi caused by the electric stimulation by from 23 to 65 % of the control ($n = 3$). This also relaxed the
15 sustaining contraction of the bronchi caused by 50 mM potassium. Where the contraction tension of the bronchi caused by 50 mM potassium was referred to as 100 %, the extract reduced the tension by from 9 to 13 % ($n = 3$). This was comparable to or more than the relaxing effect of
20 theophiline (50 μM).

As is obvious from the results in the above-mentioned (1) to (3), it is believed that the hot-water extract of the mycelia has ① a mild effect of enhancing the sustaining contractile force of the cardiac muscle,
25 while prolonging the inter-contraction interval of the cardiac

muscle, ② the extract has a vasodilating effect, while increasing the systemic blood flow, and ③ since the extract inhibits the transient and sustaining contraction of the bronchi, it lowers the respiratory resistance and
5 increases the pulmonary ventilation. Thus, it has been confirmed from these scientific data that the composition of the present invention has a cardiogenic effect, a hypotensive effect and an antitussive effect.

Example 3

10 Using the hot-water extract (freeze-dried powder) of the mycelia of Cordyceps sinensis MF-20008 that had been prepared in Production Example 1, a water-dipped, restrained stress ulcer test with rats was conducted to examine the anti-stress effect of the extract.

15 Test Animals:

 Five-week age, male Sprague-Dawley rats were bought from Nippon Charles River and pre-raised for about one week. The thus-raised six-week age rats were used as test animals.

20 Administration:

 The hot-water extract of the mycelia of Cordyceps sinensis MF-20008 was dissolved in distilled water for injection at a concentration of 50 mg/ml, and this was divided into plural parts each for one-day dose. These
25 were frozen and stored at -20°C. Before use, these were

thawed at room temperature. Distilled water for injection only was applied to the rats of the control group. The solution of the extract or the distilled water was orally administered to rats via a metal gastroscope for rats.

5 The dose of the active ingredient, the dose of the solution (or distilled water) and the administration schedule are shown in Table 4 below. The dose of the solution (or distilled water) was calculated on the basis of the newest body weight of each rat, and the

10 administration was effected, as a rule, at from 9 to 12 in the morning.

Table 4

15

Group	Dose of Active Ingredient (mg/kg/day)	Dose of Solution (or Distilled Water) (ml/kg/day)	Administration Schedule (2)	Number of Animals (heads)
Control	-(1)	4	day 0-4, 7-11	5
	100	2	day 0-4, 7-11	5
	200	4	day 0-4, 7-11	5

(1) Four ml/kg/day of distilled water for injection was

20 administered to each rat of the control group.

(2) The day at which the administration was started was referred to as 0 (zero).

Water-dipped, Restrained Stress Ulcer Test (the stress was applied on days 10-11 overnight):

25 For about 12 hours before the application of the water-dipped, restrained stress thereto, the rats were made to abstain from feed. After this, the rats were put

in a stress gauge and dipped in water up to their xiphoid process. The time for the water-dipping restraint was 15 hours. After the application of the stress thereto, the rats were anesthetized and killed, and the stomach was
5 taken out while ligating the upper portion of the cardiac part of the stomach and the duodenum. A physiological saline solution was injected into the stomach, and the stomach was dipped in a formalin solution. After 15 minutes, the stomach was opened, and the length of the
10 ulcer formed in the gastric glands was measured. After the measurement, the stomach was fixed with formalin and subjected to pathoanatomical inspection and the ulcer formed was scored according to the following criteria.

- Score 0 : No ulcer formed.
- 15 1 : Ulcer formed in the upper portion of the mucosa.
- 2: Ulcer formed in the lamina propria mucosae.
- 3 : Ulcer formed in the lamina muscularis
20 mucosae.
- 4 : Ulcer formed in the tunica serosa ventriculi.

The results are shown in Fig. 3 and Table 5.

Table 5 - Ulcer Index

		Microscore of Gastric Ulcer
5	Control	1
		2 to 3
		2
		2 to 3
		3
	Average	2.4
10	100 mg/ml of extract of mycelia of <u>Cordyceps sinensis</u> MF-20008 applied.	2 to 3
		1
		0
		1 to 2
		1
		1.4
15	200 mg/ml of extract of mycelia of <u>Cordyceps sinensis</u> MF-20008 applied.	1
		1
		2
		2
		3
		1.8
20		

Example 4

As one index of the anti-fatigue effect of the hot-water extract of the cultivated mycelia of Cordyceps sinensis, the activity of the extract on the promotion of the DNA synthesis and the protein synthesis in the primary culture hepatocytes of a rat was examined.

Preparation of an extract containing a hepatocyte growth factor derived from a bovine small intestinal epitheliomucosa and characteristics of the extract:

A methanol-soluble extract of a bovine small intestinal
5 epitheliomucosa consists essentially of low-molecular
substances as estimated by gel permeation chromatography, and it
is referred to as a low-molecular weight fraction (LMW). On
the other hand, an extract to be obtained by extracting a
bovine small intestinal epitheliomucosa with methanol
10 followed by further extracting the resulting residue with
PBS consists essentially of proteinaceous high-molecular
weight substances, and it is referred to as a high-
molecular weight fraction (HMW).

When LMW is added to the primary culture
15 hepatocytes of a rat, it promotes the DNA and protein
synthesis in the presence of insulin in the cells. When a
combination of LMW and HMW is added thereto, the DNA and
protein synthesis is additively accelerated compared to
the basic activity of HMW. In other words, LMW exhibits the
20 essential activity in this assay system, while HMW has an
additive activity. The hot-water extract of Cordyceps
sinensis comprises a low-molecular weight fractions
based on the extraction process. The extract was added to
the primary culture hepatocytes of a rat in the presence
25 of 1×10^{-7} M insulin, and the effect of the

extract of promoting the DNA and protein synthesis in these cells was examined on the basis of the intake of ^3H -thymidine and ^{14}C -valine in the cells.

Results:

5 When the hot-water extract of the mycelia of Cordyceps sinensis was added to the primary culture hepatocytes of a rat in the presence of insulin and HMW, it obviously promoted the synthesis of DNA and protein in the cells. The results are shown in Fig. 4-1 and Fig. 4-
10 2. However, in the absence of insulin and HMW, any significant activity of the extract was not detected. In the presence of only insulin, the activity of the extract was not also detected. From these, it is believed that the extract promotes the synthesis of DNA and protein in
15 the cells in the presence of insulin and HMW.

Example 5

 The hot-water extract of the mycelia of Cordyceps sinensis MF-20008 that had been prepared in Production Example 1 was administered to rats, and the effect of the
20 extract of retarding the secretion of the gastric acid in the rats was examined.

 In this test, used were healthy, male SD rats (Nippon Charles River Co.); the 5-week age rats had been purchased and further pre-raised for 4 days and then used
25 for the test.

The rats were grouped into two groups, a test group and a control group, each being comprised of 4 rats. To the rats of the test group, administered was the hot-water extract of the mycelia of Cordyceps sinensis MF-
5 20008.

The hot-water extract of the mycelia of Cordyceps sinensis MF-20008 was dissolved in pure water (Lot No. YLE9566, produced by Wako Pure Chemicals Co.) at a concentration of 100 mg/ml to prepare a test material to
10 be administered to the rats, and this was frozen and stored.

Pure water or the test material having a predetermined concentration was orally administered to the rats via a metal gastroscope, at 9 in the morning every
15 day. On the 12th day, it was injected into the duodenum after the pylorus had been ligated.

Group	Number of Animals (heads)	Substance Administered	Dose (mg/kg/day)	Administration Route	Administration Schedule
Control Group	4	Pure Water	-	Oral Administration	1 to 11 days
Test Group (extract administered)	4	Test Material	200	Oral Administration	1 to 11 days

The secretion of the gastric juice was measured in the manner mentioned below.

The rats were made to abstain from feed for 17 hours. After this, while they were anesthetized, the
5 abdomen was opened and the pylorus portion was ligated. After 4 hours, the rats were killed by dislocation of cervical vertebrae, their stomachs were taken out, and the gastric juice remained therein was collected. The thus-collected gastric juice was subjected to centrifugation at 3,000 rpm
10 for 5 minutes. The volume of the gastric juice (ml/rat), the acidic degree of the gastric juice (mEq/liter) and the pH thereof were measured. The acidity was measured up to pH 7.0 with a pH meter using 0.01 N NaOH. From the volume of the gastric juice and the acidic degree thereof
15 thus measured, the amount of the acid released per hour (μ Eq/hr) was calculated. The test material was injected into the duodenum immediately after the ligation of the pylorus.

The results are shown in Fig. 5.

20 Example 6

Water was added to 100 g of the powder of the hot-water extract obtained in Production Example 1, 150 g of saccharides, 15 g of honey, 1 g of ascorbic acid, 0.5 g of citric acid and a suitable amount of fragrance to make one
25 kg as a whole. This was sterilized at 95°C for 20 minutes

and filled in bottles in an amount of 100 ml each in a germ-free condition. Thus, a food-and-drink-type healthful drink was prepared.

Example 7

5 De-ionized water was added to 200 g of an aqueous
20 % solution of the powder of the hot-water extract
obtained in Production Example 1, 5 g of tocopherol
acetate, 10 g of thiamine nitrate, 20 g of nicotinic acid
amide, 50 g of anhydrous caffeine and suitable amounts of
10 benzoates and fragrance to make 30 liters as a whole.
After sterilized, this was filled in bottles in an amount
of 30 ml each in a germ-free condition. Thus, a medicine-
type healthful drink was prepared.

Example 8

15 (1) Substance prepared in Production Example 1

50 g

(2) Lactose 90 g

(3) Corn starch 29 g

(4) Magnesium stearate 1 g

20 (1), (2) and (3) (17 g) were mixed and granulated
along with a paste formed from (3) (7 g). (3) (5 g) and
(4) were added to the thus-obtained granules and well
mixed, and the resulting mixture was tabletted under
pressure, using a compression tableting machine, to

obtain 1000 tablets each containing 50 mg of the active ingredient (1).

Advantages of the Invention

5 According to the present invention, there is provided a natural substance-derived composition which is extremely stable and has mild cardiotonic, hypotensive, antitussive and anti-fatigue effects. Though being derived from natural substances, it is possible to stably and industrially provide the composition by culture.

Reference to Deposited Microorganisms under Rule 13-2

1. MF-20008

**a. Name and Address of the Authorized Organ for
Deposition of the Present Microorganisms:**

5 Name: The National Institute of Bioscience and
 Human-Technology of the Agency of Industrial Science
 and Technology of the Ministry of International Trade
 and Industry, Japan
 Address: 1-3, Higashi 1 chome, Tsukuba-shi, Ibaraki-ken
10 305, Japan

b. Date of Deposition in the Organ of a:

June 29, 1994

c. Deposition Number as Rendered by the Organ a:

FERM BP-5149



Claims

1. A composition comprising a hot-water extract of the cultivated mycelia of Cordyceps sinensis.

2. The composition as claimed in claim 1, wherein Cordyceps sinensis MF-20008 is used to obtain the extract.

3. The composition as claimed in claim 1 or 2, wherein the hot-water extract is obtained by extracting the cultivated mycelia with hot water at from 85 to 100°C, preferably from 90 to 95°C.

4. the composition as claimed in any one of claims 1 to 3, wherein the hot-water extract comprises the hot-water extract itself, a liquid moiety thereof to be obtained by removing the extraction residue from the extract, its(their) concentrate(s), paste(s), dilution(s) and/or dried product(s).

5. The composition as claimed in any one of claims 1 to 4, which is a medicine-type one and/or a food-and-drink-type one.

6. The medicine-type composition as claimed in claim 5, which is a cardiac, a hypotensor, an antitussive or an anti-fatigue agent.

7. The food-and-drink-type composition as claimed in claim 5, which is a healthful drink.

ABSTRACT

This invention is a healthful composition comprising a hot-water extract of the cultivated mycelia of Cordyceps sinensis.

The healthful composition of the invention is an oral healthful composition which is highly safe and has excellent cardiotonic, hypotensive, antitussive and anti-fatigue effects.

FIG. 1

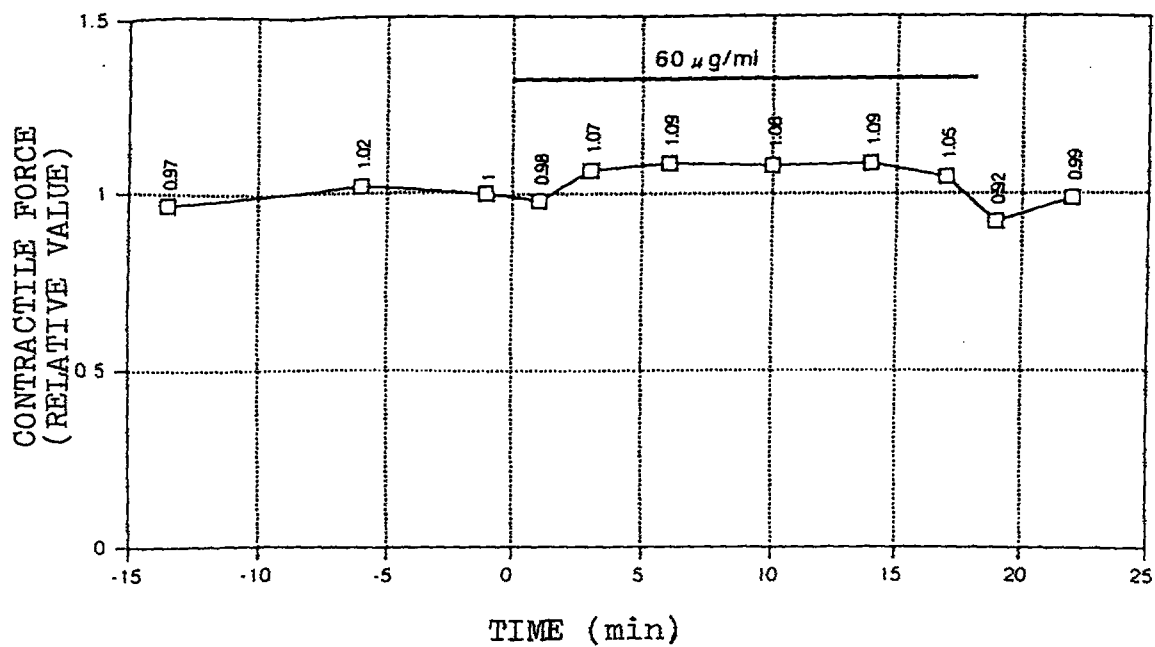


FIG. 2

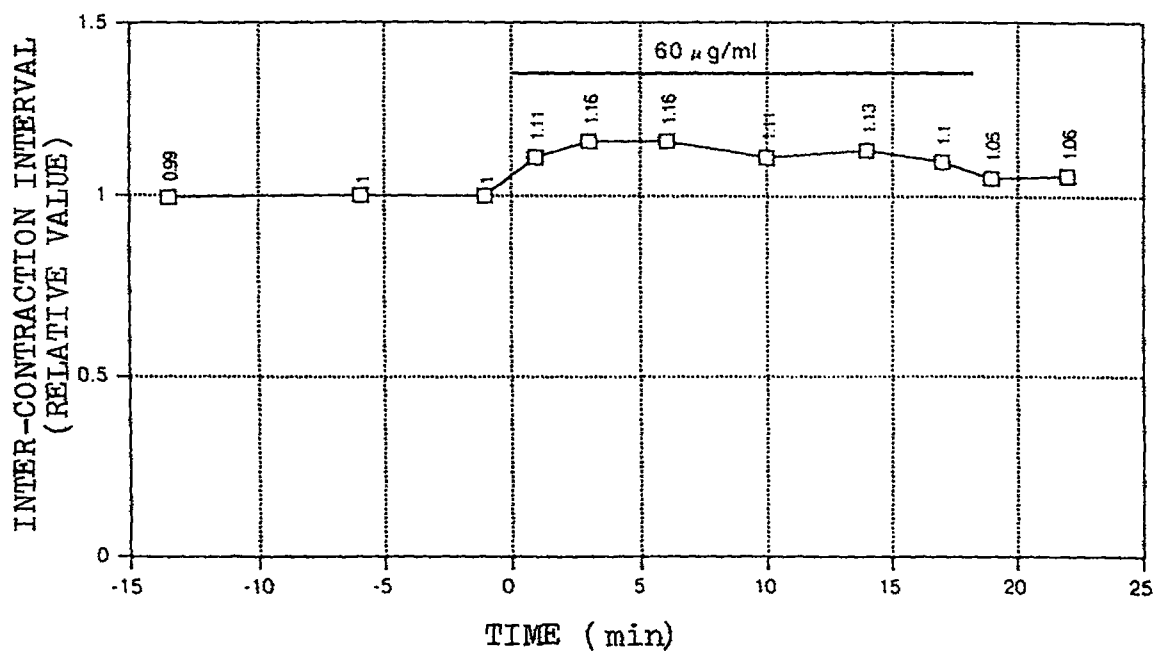


FIG. 3

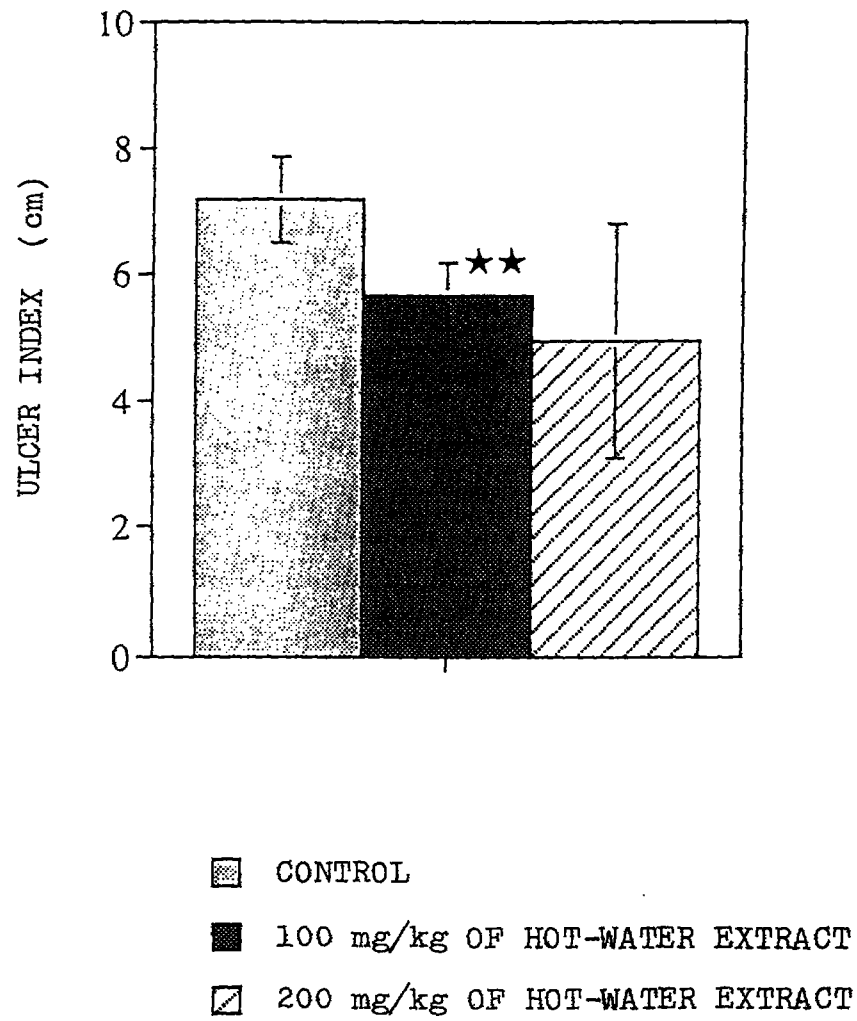


FIG. 4

FIG. 4-1

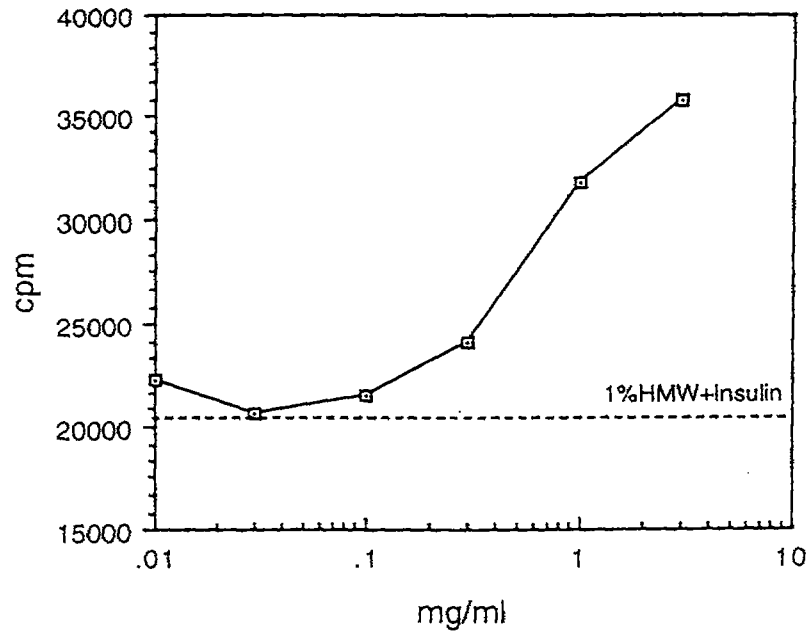


FIG. 4-2

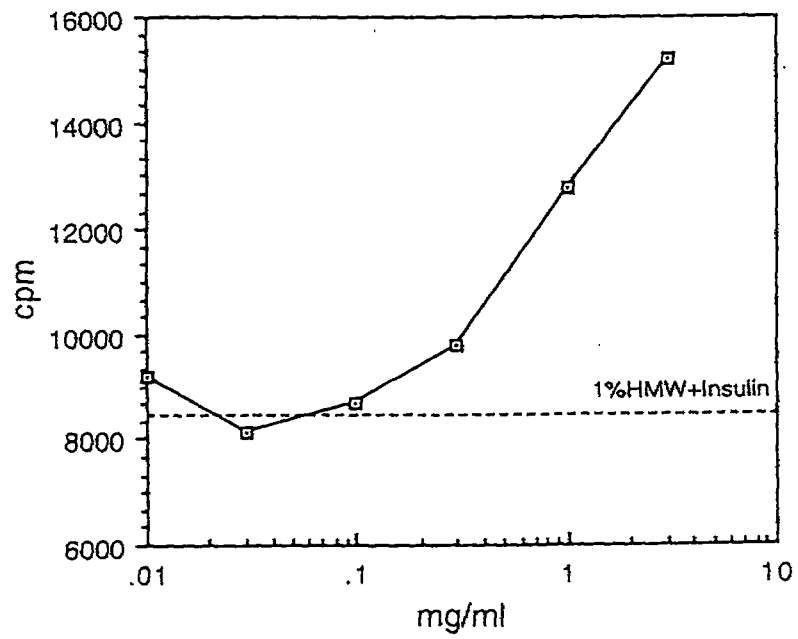
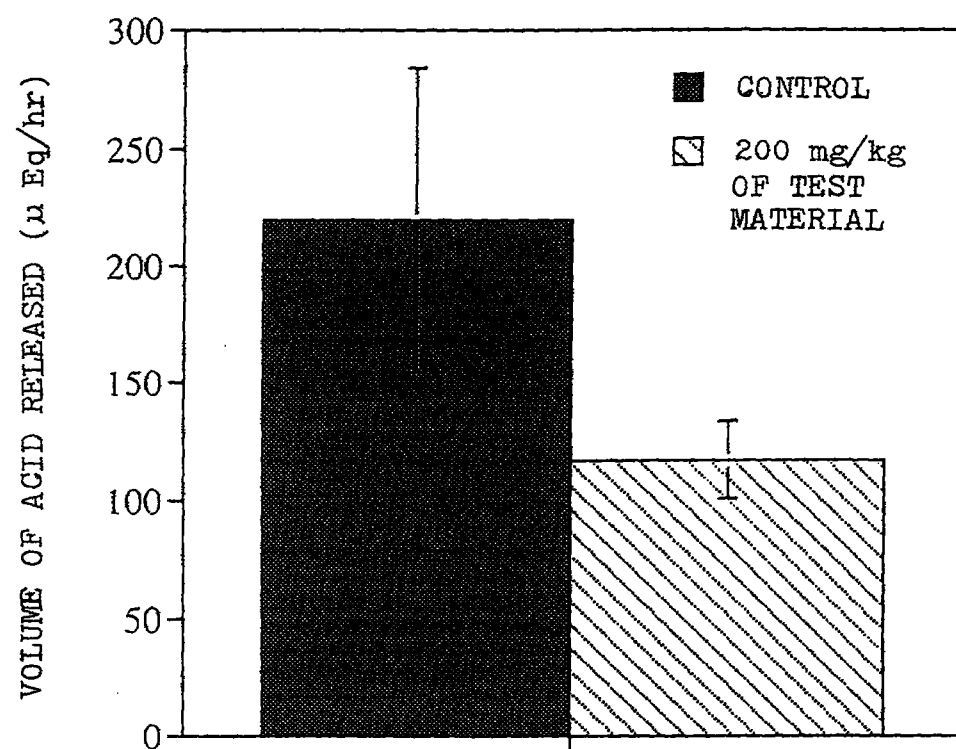


FIG. 5



SPECIFICATION

REPLACEMENT SHEET

24 NOV 1997

Date :

TITLE OF THE INVENTION

HEALTHFUL COMPOSITION

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a composition having excellent pharmaceutical and health-preserving effects and, more precisely, to a Cordyceps sinensis-derived medicine-type and/or food-and-drink-type composition.

PRIOR ART

Vegetative wasps (plant worms) are microorganisms of Ascomycota Pyrenomycetes Clavicipitales Clavicipitaceae Cordyceps, undergoing both a complete metamorphic phase and an incomplete metamorphic phase.

Their fruit bodies have been valued from ancient times as drugs marvelously effective for perennial youth and longevity or for nourishment and tonicity. The fruit bodies of vegetative wasps that have heretofore been considered extremely valuable as Chinese medicines are generally powdered and administered.

According to the present invention, the mycelia of particular vegetable wasps are cultivated and the thus-cultivated mycelia are extracted with hot water. The fact that the resulting extract from such mycelia has a

Fig. 1 shows the effect of a hot-water extract of the mycelia of Cordyceps sinensis MF-20008 on the contractile force of the right atrial muscle of a rat.

Fig. 2 shows the effect of a hot-water extract of the mycelia of Cordyceps sinensis MF-20008 on the inter-contraction interval of the right atrium of a rat.

Fig. 3 shows the ulcer index (cm) of the ulcer formed in the gastric glands of a rat, to which a hot-water extract of the mycelia of Cordyceps sinensis MF 20008 was administered.

Fig. 4-1 shows the intake of ^3H -thymidine in the primary culture hepatocytes of a rat; and Fig. 4-2 shows the intake of ^{14}C -valine in the same.

Fig. 5 shows the effect of a hot-water extract of the mycelia of Cordyceps sinensis MF-20008 for the inhibition of the gastric acid secretion in a rat.

To carry out the present invention, it is necessary to cultivate the mycelia of vegetative wasps of Cordyceps sinensis and to collect the thus-cultivated mycelia. All microorganisms of Cordyceps sinensis can be used in the present invention, and these will be referred to as the microorganisms of the invention. For example, Cordyceps sinensis MF-20008 is useful, and this strain has been deposited in the National Institute of Bioscience and Human-Technology of the Agency of Industrial Science and Technology under FERM BP-5149.

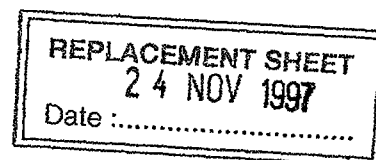
REPLACEMENT SHEET
24 NOV 1997
Date

46 liters of the culture were centrifuged, and the mycelia thus separated were freeze-dried to obtain 323.6 g of dry mycelia. The dry mycelia were put into a stainless container, 7 liters of water were added thereto, and these were ground with a polytoron, then heated at from 90 to 95°C for 2 hours while stirring and extracted. After the extraction, the residual substances were removed by filtration, and the resulting filtrate was freeze-dried to obtain 130 g of a lightly yellow powder.

Production Example 2

In the same manner as in Production Example 1, 150 ml of M20Y2 medium was put into a 500-ml Erlenmeyer flask and sterilized by autoclaving, and 1 ml of freeze-dried Cordyceps sinensis MF-20008 was inoculated therein and cultivated by shaking culture at 25°C and 180 rpm for 5 days. This was used as a seed culture and cultivated in 150 liters of the following medium in the manner mentioned below.

150 liters of D medium (comprising 40.0 g of sucrose, 4.0 g of K_2HPO_4 , 0.5 g of asparagine, 2.0 g of $(NH_4)_2HPO_4$, 2.0 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of $CaCO_3$, 0.1 g of $CaCl_2$ and 4.0 g of yeast extract B-2 in one liter and having pH of 5.6) was, after having been sterilized and filtered, put into a 150-liter fermenter, and 150 ml of the above-mentioned seed culture was inoculated therein



and cultivated under a controlled condition at 25°C, pH of 5.5 and DO of 50%, while stirring at 157 rpm, for 3 days. The foams formed during the cultivation were removed by adding a silicone de-foaming agent to the fermenter. 150 liter of the culture thus obtained was treated with a clarifier to thereby isolate the mycelia. Thus, an aqueous suspension of the mycelia was obtained. This was heated at from 90°C to 95°C for 2 hours and extracted. Then, this was clarified with a clarifier to isolate a supernatant. This was filtered through 0.45- μ m and 0.22- μ m sterile filters to obtain 80 liters of a lightly yellow hot-water extract (the dry weight of the solid content: 800 g).

Example 1

The cultivated mycelia (dry mycelia) of Cordyceps sinensis MF-20008 that had been prepared in Production Example 1 and the hot-water extract thereof (lightly yellow powder) were subjected to a swimming fatigue test with mice, to which these were orally administered.

The test animals were male ICR mice (5 to 7 week-age), and these were grouped into three groups and subjected to a fatigue test under the conditions mentioned below.

Administration:

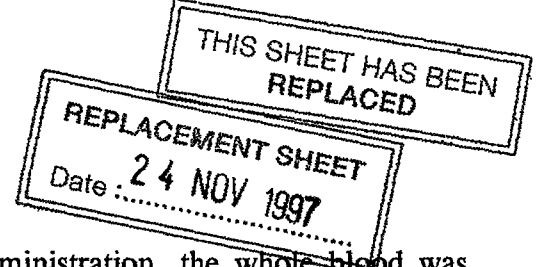
The hot-water extract of the mycelia of Cordyceps sinensis MF-20008 or the mycelia themselves were mixed with powdery CRF-1 feed at a proportion of 2.5% or 5%, respectively, and the resulting powdery feed mix was administered to the mice all the time for 16 days.

Groups of mice: Each group was comprised of 20 mice.

- (1) Control group: Fed with only powdery CRF-1 feed.
- (2) Group to which the hot-water extract was administered: Fed with the feed mix containing 2.5 % of the hot-water extract.
- (3) Group to which the mycelia were administered: Fed with the feed mix containing 5 % of the dry mycelia.

Test schedule:

On the 15th day after the start of the administration, the mice were subjected to a forcedly swimming test. On the 16th day, all the blood was collected from each mouse and subjected to whole blood analysis and serum analysis.



(2) On the 16th day after the start of the administration, the whole blood was collected from each mouse that had been subjected to the above-mentioned swimming fatigue test and analyzed in terms of the number of the platelets ($\times 10,000/\text{mm}^3$), the amount of hemoglobin (g/dl), the number of erythrocytes ($\times 10,000/\text{mm}^3$), the number of leucocytes (mm^3), and the hematocrit (%).

The results of the analysis of the whole blood are shown in Table 2 below. As is obvious from these results, there was admitted no significant difference between the test groups and the control group in all the test items, as the level of significance of the test was 5%. (To analyze the whole blood, used was a Student test. To count the number of the platelets in the third group, however, used was an Aspin-Welch test.)

This Sheet Has Been Replaced



No.	Third Group (Fed with Dry Mycelia of MF-20008)				
	Number of Platelets x 10000/mm ³	Amount of Hemoglobin g/dl	Number of Erythrocytes x 10000/mm ³	Number of Leucocytes /mm ³	Hematocrit %
1	88.3	13.7	835	7000	51
2	97.6	15.9	994	3200	56.3
3	103.6	15.3	916	3300	55.3
4	116	14.7	951	7000	56.7
5	155.7	15.5	936	7000	57.4
6	131.6	15.2	943	4100	56.6
7	119.3	16.6	1010	4700	59.3
8	130.9	16.2	991	5200	57.9
9	113.1	15.7	1026	7700	58.8
10	58.6	15.8	994	6400	57.4
Average	111.47	15.46	959.6	5560	56.67

(2) On the 16th day after the start of the administration, the whole blood was collected from each mouse that had been subjected to the above-mentioned swimming fatigue test and analyzed in terms of the number of the platelets ($\times 10,000/\text{mm}^3$), the amount of hemoglobin (g/dl), the number of erythrocytes ($\times 10,000/\text{mm}^3$), the number of leucocytes ($/\text{mm}^3$), and the hematocrit (%).

The results of the analysis of the whole blood are shown in Table 2 below. As is obvious from these results, there was admitted no significant difference between the test groups and the control group in all the test items, as the level of significance of the test was 5%. (To analyze the whole blood, used was a Student test. To count the number of the platelets in the third group, however, used was an Aspin-Welch test.)