ANTITUMOR ACTIVITY STRENGTHENING METHOD OF CRUDE DRUGS, COMPOSITION CONTAINING CRUDE DRUG STRENGTHENING ANTITUMOR ACTIVITY, EVALUATING METHOD OF ANTITUMOR EFFECTIVENESS BY CRUDE DRUG TREATMENT, AND EVALUATING METHOD OF ANTITUMOR EFFECTIVENESS OF CRUDE DRUG

BACKGROUND OF THE INVENTION

This invention relates to techniques such as strengthening method, evaluating method and the like of antitumor activity of crude drugs such as mushrooms containing polysaccharides having antitumor activity, particularly to techniques for enlarging manifestation of antitumor effects in internal administration or expecting effectiveness of antitumor activity in internal administration and the like.

Various kinds of mushrooms have heretofore been used in the field of Chinese medicines, health foods or the like. For example, it has been known that mushrooms such as GANODERMA (REISHI) (Fomes (SARUNOKOSHIKAKE)), PORIA (BUKURYO), maitake mushroom (MAITAKE, Grifola frondosu Dickson), and the like have antitumor activity such as carcinostatic activity, etc. Many researches have been done about effective ingredients of mushrooms showing antitumor activity, and it has generally been said that polysaccharides contained in mushrooms show effective antitumor activities.

As a matter of fact, in the field of Chinese

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medicines, dried mushrooms are decocted and internally used by drinking the decoction.

Various kinds of Chinese drugs have heretofore been used as natural medicines since they have less side effects as compared with synthesized medicines. In Japanese pharmacopoeia, a number of Chinese drugs have been described as crude drugs.

On the other hand, with regard to antitumor activities of such mushrooms, their decoctions or extracts are ingested to experimental animals such as mouse, etc. by providing with food, and mice of a control group to which no Chinese drug is ingested and mice of a group to which it is ingested are compared and observed to examine, for example, an extinct state, a transferred state of cancer, or vitality percentage of mice or the like whereby their effectivenesses are inspected.

Moreover, in such experimental animals, toxicity and effectiveness are also examined, and after confirmation of their safeties to apply to human beings sufficiently, these medicines are actually administered internally and effectiveness of antitumor activities in human beings are finally confirmed by clinical tests.

SUMMARY OF THE INVENTION

Antitumor activities such as carcinostatic property, cancer transfer-inhibiting property, etc. of mushrooms have heretofore been known as mentioned above, and their

effectivenesses are widely known. They are used by decocting a dried product of mushrooms and drinking the decoction, or mixing an effective ingredient extracted with an alcohol, etc. with other components such as a vitamin preperations to make a form such as health drinks, health foods or medical agents.

According to the administration by an internal means such as drinking a decoction conventionally carried out, the case where its medical effects could be obtained so that the therapeutic effects are apparently observed is unexpectedly little. In agaricus mushroom (AGARIKUSUTAKE, which is also called to as "Agaricus") which has recently attracted attention as having a large medical effect, the same tendency can be observed. Moreover, in maitake mushroom (MAITAKE, Grifola frondosu Dickson) which has recently attracted attention, there is also little example to prove its medical effects.

However, they do never show medical effects, but there is a case where a clearly effective antitumor activity is shown whereas it is in an extremely limited number of examples.

Thus, the present inventor considered whether there is any effective way to obtain the antitumor activities of mushrooms for any person by attaining the mechanism why differences in manifestation of antitumor effects provided by mushrooms occur.

Even when effectiveness of mushrooms having antitumor

activity are confirmed in experimental animals, as mentioned above, the expected effects cannot be shown in almost all the cases when it is actually administered internally to human beings. For confirming the effects thereof by actual and internal administration in clinical experiments, it is necessary to continue internal administration for a certain period of term by controlling the administration system under the constant conditions and to continue observation thereof. Thus, a significant term would be required for obtaining a result whether or not the effects of the mushrooms are shown by internal administration to a patient.

Such a method is certainly effective, but, for example, for searching out a substance having an effective antitumor activity by screening a number of natural products, an enormous amount of days is required so that it is necessary to develop a technique which can evaluate an antitumor activity within a shorter period of time.

Also, when a patient who is under a clinical test suddenly caused complication and a treatment out of control is applied to the patient in haste, confirmation of effectiveness cannot be carried out with regard to the patient. Thus, in a clinical test over a long period of time, a large number of cases where confirmation of effectiveness cannot be carried out occur so that it is not necessarily easy to carry out the clinical test for a long period of time while maintaining effectiveness of the test

itself.

Thus, the present inventor considered that it is necessary to develop a method which can expect manifestation of an antitumor effect with a certain degree when a specimen is administered to human beings without carrying out such a clinical test.

Also, as a substance having an antitumor activity, a large number of materials have been known as crude drugs in Chinese medicine other than mushrooms, and it is more preferred if the above-mentioned problems regarding mushrooms could be solved in view of spreading all of the crude drugs.

An object of the present invention is to effectively develop antitumor activities of crude drugs such as mushrooms and the like containing polysaccharides having antitumor activities.

Another object of the present invention is to expect antitumor activities of crude drugs such as mushrooms and the like when they are administered to human beings without carrying out clinical tests.

Another object of the present invention is to provide a composition containing crude drugs such as mushrooms and the like containing polysaccharides having antitumor activity so as to effectively manifest their antitumor activities.

The above-mentioned and other objects and novel characteristic feature of the present invention are

described in the following present specification in detail.

The present invention relates to an antitumor activity strengthening method which is applied to a crude drug containing polysaccharides having antitumor activity and comprises a roasting step of roasting the above-mentioned crude drug by far infrared rays and a fermentation step of fermenting by adding microorganisms to strengthen the antitumor activity of the above-mentioned crude drug than the case of not effecting the roasting and fermentation.

Subsequent to the above-mentioned fermentation step, the present invention comprises a step of making an oily agent in which the above-mentioned fermentated crude drugs are enclosed with an oily component obtained from plants such as roasted sesame or the like by far infrared rays.

The present invention further comprises using a mushroom which contains ß-glucan.

The present invention further comprises using at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE, Agaricus blazei), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes).

When the above-mentioned agaricus mushroom (AGARIKUSUTAKE) is used, the present invention comprises

applying roasting to raw agaricus mushroom by far infrared rays.

The antitumor activity strengthened crude drug-containing composition of the present invention comprises a crude drug to which the antitumor activity strengthening method of the crude drug with any of the above-mentioned constitutions is applied.

The present invention is an evaluating method of effectiveness on an antitumor activity of the treatment to be applied to the crude drug containing polysaccharides having an antitumor activity, which comprises adding the crude drug to which the above-mentioned treatment is applied to a system which forms lipid peroxide by irradiating ultraviolet rays to an unsaturated fatty acid such as docosahexaenoic acid and evaluating an effect of the treatment for strengthening the antitumor activity of the above-mentioned crude drug to be large as an increased ratio of a formed amount of the above-mentioned lipid peroxide based on an increased ratio of the concentration of the above-mentioned crude drug by the treatment being large.

The present invention comprises mushrooms which contain ß-glucan being used for the above-mentioned crude drug.

The present invention is an evaluating method of effectiveness of the crude drug for evaluating effectiveness of an antitumor effect shown by the crude

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drug containing polysaccharides having an antitumor activity without carrying out clinical tests, which comprises adding the crude drug to a system which forms lipid peroxide by irradiating ultraviolet rays to an unsaturated fatty acid such as docosahexaenoic acid and evaluating and expecting an effect of the treatment for strengthening the antitumor activity by internal administration of the above-mentioned crude drug to be large as an increased ratio of a formed amount of the above-mentioned lipid peroxide based on a ratio of the concentration of the above-mentioned crude drug being large.

The present invention comprises either of the above-mentioned antitumor activity strengthening methods being applied to the above-mentioned crude drug.

The present inventor has carried out various studies on an anti-active oxygen inhibiting substance in Chinese drugs, and through such studies, he has confirmed that active oxygen or lipid peroxides provide remarkable effects on human body and these substances pertains to breakage of tissues in human body whereby they becomes the cause of occurring various infectious diseases.

In the tissues of human body, an enzyme called SOD (superoxide dismutase) exist to protect the tissues of human body from such an active oxygen and to remove the active oxygen. An amount of the SOD decreases with ages and removal of the active oxygen in the body cannot be carried out. Decrease thereof is particularly remarkable

with the age of 40 or more, and removal of the active oxygen taken in the body cannot sufficiently be carried out and it has been clarified in recent years that it becomes the cause of various kinds of adult diseases.

Also, when an excessive active oxygen is formed in the body by chronic or acute stimulation or by a chemical substance, etc., removal of the active oxygen cannot be sufficiently carried out with the amount of SOD existed in the body from the beginning so that the person is suffered from various diseases.

Thus, to various diseases caused by not sufficiently removing the active oxygen due to lack of SOD, it has been considered to effect treatment by administering SOD to solve lack of SOD. However, according to such a treatment method, effects can be admitted in the case of an injection medicine, but not remarkable effect can be admitted in the case of an internal medicine so that it is the present status that the effects have not yet been proved.

With regard to the point that the effects as an internal medicine are not manifested, the present inventor has found that SOD is unstable to gastric juice and the molecular weight of SOD is 30000 or more and it is caused by not being absorbed from digestive organs as such. Also, he has found that there is an action limit that SOD only acts on superoxide (O_2^-) among the existing four kinds of active oxygens.

The present inventor has earnestly studied about a

substance which can remove an excessive active oxygen in the human body and is capable of internally administering, and as a result, he has found a composition which comprises plants oil obtained from a roasted plants which is added to a substance obtained by roasting plant seeds or their germs under suitable conditions and then fermented by adding microorganisms can be used as an active oxygen removing agent which is effective as an internal use (see Japanese Patent No. 2,125,887).

In the above-mentioned plant seeds or their germs, low molecular weight anti-active oxygen substances such as flavonoids, polyphenols, tannin, tocopherol, vitamin B_2, etc. inherently exist. The present inventor has found that such low molecular weight anti-active oxygen substances chemically bind mutually or with the other component, generate a molecular compound or form a complicated complex or a macromolecular compound by adsorption or inclusion, and when they are ingested in such a state, an anti-active oxygen suppressing action expected from a low molecular state anti-active oxygen substance cannot be obtained.

That is, even when it is ingested in such a state, much persons nowadays cannot digest an anti-active oxygen substance to a low molecular weight substance by gastric juice so that the anti-active oxygen suppressing action cannot be obtained. The present inventor stated such a point in "Food Industry", vol. 35, No. 14, "Development and Improvement of DDS, SOD-like Function Foods from Natural

Plants and Seeds and Their Pharmacological and Biochemical Consideration".

The present inventor studied to effectively utilize the above-mentioned anti-active oxygen substance contained in plant seeds or their germs for treatment or prevention of diseases and how to activate the above state anti-active oxygen substances.

As a result, he has found that by heating and roasting plant seeds or germs under mild conditions, the anti-active oxygen substance contained in the plant seeds or their germs is partially liberated from the above-mentioned complex to form an original low molecular weight substance or is partially activated by generating an active functional group due to chemical change. By effecting such treatments, the anti-active oxygen action is significantly strengthened as compared with that before roasting.

On the other hand, many of the antitumor active functions such as carcinostatic property or cancer-transfer inhibiting property of mushrooms are considered to be generally based on polysaccharides such as ß-glucan, etc., but such ß-glucan is different from the above-mentioned low molecular weight anti-active oxygen substance contained in the plant seeds or germs, and it is out of the objects of the above-mentioned series of studies on active oxygen by the present inventor.

However, the present inventor considered whether the fact that, for manifestation of the antitumor effects of

mushrooms, when it is internally administered by drinking a decoction, as mentioned above, it shows remarkable effects for some person but the effects could never be obtained for the other many peoples could be explained by the similar thought as the fact of low molecular weight of the above-mentioned anti-active oxygen substance.

That is, whereas the action and mechanism showing antitumor activity of ß-glucan have not yet sufficiently been elucidated, as mentioned above, the fact that there are differences in appearance of antitumor effects when mushrooms are internally ingested means there are patients to whom an effective ingredient which manifests an antitumor activity of mushrooms easily acted and patients to whom hardly acted.

According to the internal administration form, mushrooms internally ingested are treated by gastric juice sooner or later. Accordingly, he considered that whether the effective ingredient which manifests antitumor activity of the above-mentioned mushrooms effectively act or not markedly related to such a gastric juice treatment. That is, the present inventor considered that there are individual differences in gastric juice treatment and this individual differences would cause differences in manifestation of the antitumor effects.

Thus, the present inventor set forth the following hypothesis between easiness in manifestation of antitumor action in internal use and a gastric juice treatment in

view of the clinical fact that there are individual differences in strength of the gastric juices.

That is, the effective ingredient showing an antitumor activity contained in mushrooms which becomes an object of the gastric juice treatment exists in the state at which the effects are difficultly manifested, and as mentioned above, it is changed to the state in which the effects are easily manifested due to the gastric juice treatment with difference in individuals. Thus, if a person has a strong gastric juice by nature, an ingredient having effects on an antitumor activity contained in ingested mushrooms becomes the state in which the action can be easily shown whereby the antitumor effects are manifested.

However, in a person who has a weak gastric juice by nature, an ingredient having effects on an antitumor activity does not become the state in which the action can be easily shown whereby the antitumor effects are not so manifested.

Much persons nowadays do not have strong gastric juice which can change the effective ingredient showing the antitumor activity of mushrooms to an extent that an action thereof is easily manifested, and as a result, it can be considered that a number of examples which can confirm the effectiveness of the antitumor activity of mushrooms with a certain extent can be scarcely observed by internal administration.

That is, it can be explained that, in the case of a person who has a strong gastric juice by nature, whereas components which are effective for antitumor activity contained in mushrooms are hand in hand like a chain (whereas it is a simile expression) by, for example, polymerization and exist in the unmovable state, i.e., in the non-active state at which they cannot activate, the components are cut by the gastric juice to change to the effective components in the free activated state whereby the antitumor activity can be shown in the body.

It can be considered that much persons nowadays cannot cut the chain in the inactivated state in which the effective ingredients which are effective for cancer are connected with each other as a chain with gastric juice as mentioned above. Thus, it can be so explained that the ingredient effective to cancer are absorbed by the intestines in such a non-activated state that they are hand in hand so that they cannot show their effects in a body.

Thus, the present inventor considered that if a treatment which can previously activate the effective ingredient which is in the non-activated state easily can be applied to mushrooms, the above-mentioned problems would be solved.

The present inventor has tried various means to activate the effective ingredients of such mushrooms in a non-active state, but he could not readily reach an effective means. This is because a sufficient knowledge

cannot be obtained about the state of the effective ingredients how they are in the non-activated state since the actual system is too complex to evaluate.

The present inventor has carried out various experiments as mentioned above, and as the final experiment, he tried to apply the above-mentioned method invented by himself as an activating means of active oxygen suppressing substances such as flavonoids, polyphenols, etc. whereas they are quite different composition from ß-glucan.

When such a method is to be applied, the means is for activating flavonoids and the object to be applied is quite different from ß-glucan so that the non-activated state is considered to be quite different therefrom as a matter of course, and thus, diversion of the method for activating ß-glucan has been considered to be difficult at the beginning.

However, the experimental results were quite different from the expectations by the present inventor and showed that the activation means of the active oxygen suppressing substance is to be effective for changing ß-glucan having the different structure from that of the active oxygen suppressing substances such as flavonoids, etc. to be easily activated.

That is, the present inventor has found that mushrooms which have previously been roasted by far infrared rays are used in the experiment, antitumor effects are improved when it is internally administered as compared with the case where mushrooms which are not roasted by far

infrared rays are used. Moreover, it is found that when the mushrooms roasted by far infrared rays are fermented by adding microorganisms, particularly "Koji" (Aspergillus oryzae) or yeast, the antitumor effects are more improved.

As the mushrooms, mushrooms containing polysaccharides having an antitumor activity, for example, mushrooms such as agaricus mushroom (AGARIKUSUTAKE) which has recently been particularly attracted attention as for its medical effects may be used. In addition, there may be used mushrooms which have heretofore been said to have potent antitumor activity such as Fomes (SARUNOKOSHIKAKE), PORIA (BUKURYO), etc. without problem.

Incidentally, with regard to agaricus mushroom (AGARIKUSUTAKE), it can be found that even when it is carried out far infrared rays roasting and fermentation treatment under various conditions after harvest and drying, sufficient effect cannot be obtained. Thus, it is preferably subjected to treatment under non-dried conditions, i.e., under a raw state, for example, without leaving long after harvest.

Moreover, agaricus mushroom (AGARIKUSUTAKE) is easily spoiled, and if it takes a long time until the far infrared rays roasting treatment, it shall not be allowed to stand for a long term in a raw state. Thus, in such a case, it is preferred to once store in a refrigerator at a low temperature and the treatment such as the far infrared rays roasting, etc. is subjected to within three days from the

harvest.

Moreover, after the above-mentioned far infrared rays roasting and fermentation treatment, it is found that the antitumor effects are more improved when the resulting material is subjected to oily treatment. For example, an oily component obtained from plants such as sesame, etc. subjected to roasting by far infrared rays is added thereto to cover the resulting material after fermentation treatment with the oil component.

As described above, it was initially never expected that the means for making the anti-active oxygen substance contained in plant seeds or germs a low molecular weight compound could be also effectively applied to manifestation of antitumor activity of mushrooms containing polysaccharides having antitumor activities which have quite different structure, etc. from the anti-active oxygen substance. However, it can be clarified that significant effects can be obtained by actually applying to the material through the experiments at this time whereby the present invention has been accomplished.

The present inventor has also found that an antitumor activity which is worth attention can be manifested by carrying out the above-mentioned treatment even when the mushrooms have not attracted specific attention in the point of its antitumor activity. For example, it was found that when maitake mushroom (MAITAKE, Grifola frondosu Dickson), etc. are subjected to roasting by far infrared

rays and fermentation treatment by microorganisms, an antitumor effect comparable to or more than that of agaricus mushroom (AGARIKUSUTAKE) can be obtained.

The antitumor effect of mushrooms such as maitake mushroom (MAITAKE, Grifola frondosu Dickson) has heretofore been known, but it has generally been not considered that it has an effect comparable to that of agaricus mushroom (AGARIKUSUTAKE) which has now been attracted attention. By applying the treatments with the above-mentioned constitution of the present invention to mushrooms, the antitumor effects of mushrooms such as maitake mushroom (MAITAKE, Grifola frondosu Dickson) inherently possessed are sufficiently manifested and the antitumor activity comparable to that of agaricus mushroom (AGARIKUSUTAKE) is shown.

That is, potential antitumor activity possessed by mushrooms could not sufficiently be brought out and its effectiveness could not be clearly confirmed as of today. However, by applying the treatment of the present invention to mushrooms, their antitumor activities are clearly shown and the antitumor activity can be manifested with a degree which could never be expected.

In any of mushrooms, ß-glucan is more or less contained and the above treatment is considered to be a method which is capable of strongly promoting its action and manifestation. Thus, in addition to maitake mushroom (MAITAKE, Grifola frondosu Dickson), the treatment of the

present invention is applied to mushrooms which has usually been used as raw materials for meals such as shiitake mushroom (SHIITAKE, Lentinus edodes), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes), enokitake mushroom (ENOKITAKE, Flammulina velutipes), etc. As a result, it was found that the method can strongly develop potential antitumor activity contained therein.

When the above-mentioned antitumor activity strengthening method of mushrooms of the present invention is applied, ß-glucan of mushrooms which can be usually and simply available with inexpensive can be activated without using expensive and rare mushrooms, and even when an amount of ß-glucan is less than that of the rare mushrooms, significantly effective antitumor activity can be developed as compared with the case where the rare mushrooms are used with the conventional method. This lead to reduction of economical burden for a patient.

Also, the composition containing mushrooms to which such antitumor activity strengthening method is applied is significantly heightened its antitumor activity as compared with the conventional composition in which mushrooms which are said to have an antitumor activity are dried and powdered, and simply mixed.

Moreover, in the course of effecting the above-mentioned series of researches of mushrooms, mushrooms are added by stepwisely increasing the concentration thereof to

the system in which lipid peroxide is formed by irradiating ultraviolet rays to an unsaturated fatty acid such as docosahexaenoic acid, etc. and amounts of the formed lipid peroxides are examined with the respective mushrooms at various kinds of concentrations. As a result, it could be found that the larger the degree of increasing the formed amount of lipid peroxide by the mushrooms is, the stronger the antitumor action by the internal administration shows.

That is, various kinds of mushrooms are added to the system which forms the above-mentioned lipid peroxide with the same concentration, and amounts of the respective lipid peroxides formed are measured. When the amount of the formed lipid peroxide is larger, then the antitumor effects can be shown more potently when it is actually provided by internal administration.

If such a method is employed, whether the antitumor effects are manifested or not when the mushrooms are internally used can be expected without internal administration actually. Even when a clinical test is not carried out to a patient for subjecting to experimental internal administration, the method can be used as a method for evaluating the antitumor effectivity (or effectiveness) by internal administration.

Moreover, for example, various kinds of treatments are applied to mushrooms, and formed amounts of lipid peroxide are compared by adding the mushrooms to which the respective treatments are each carried out, the antitumor

effectivities of the respective treatments can be evaluated.

Also, the above-mentioned explanation is made with respect to mushrooms, the method may be applied to a crude drug containing polysaccharides having antitumor activity other than mushrooms. Application to at least a crude drug containing ß-glucan is effective.

The method can be applied to crude drugs other than mushrooms such as natural crude drugs including crude drugs derived from plants, crude drugs derived from animals and crude drugs derived from minerals. Also, many of Chinese medicines use crude drugs naturally grown, but it may be crude drugs harvested in a farm under suitable conditions or may be those grown by an artificial culture such as water culture, etc.

Moreover, as crude drugs, other than the crude drugs described in, for example, Japanese pharmacopoeia, etc., if it shows effective antitumor activity when the present invention is applied, such natural products or culture crops can be used as crude drugs mentioned in the present specification.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the following, embodiments of the present invention is described in detail by referring to Examples.

With regard to an antitumor activity strengthening method of crude drugs, a composition containing antitumor activity strengthened crude drugs, an evaluating method of

antitumor effectivity of crude drug treatment, and an evaluating method of an antitumor effectivity of crude drugs, explanation will be made by using mushrooms which has been well known as crude drugs containing ß-glucan which is a polysaccharide having an antitumor activity.

(EMBODIMENT 1)

In the embodiment 1 of the present invention, an antitumor activity strengthening method of crude drugs and a composition containing an antitumor activity strengthening crude drugs are described.

In the antitumor activity strengthening method of crude drugs, mushrooms containing ß-glucan are firstly roasted by far infrared rays, and then, microorganisms such as "Koji", etc. are added thereto and fermentation is carried out under the predetermined humidity and predetermined temperature. After completion of the fermentation step, the resulting material is suitably formed to be fine powder, and the fine powder is coated with an oily component obtained by squeezing and pressing sesame which had been roasted by far infrared rays to prepare an oily agent.

Also, a material obtained by successively subjecting mushrooms containing ß-glucan to the above-mentioned far infrared rays roasting step, the fermentation step by microorganisms, and the step of making oily agent becomes a composition which shows an effective antitumor activity by

an internal administration containing mushrooms to which the antitumor activity strengthening method is applied.

In the following, the above-mentioned present invention is to be explained in more detail by referring to Examples.

(EXAMPLES)

As mushrooms to be used in the present Example, mushrooms containing ß-glucan were used. Particularly when mushrooms which contain much amounts of ß-glucan and generally have antitumor effects are used, the antitumor effects are strongly manifested whereby it is preferred.

In the present Example, as such mushrooms, PORIA (BUKURYO), agaricus mushroom (AGARIKUSUTAKE), POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES), maitake mushroom (black, MAITAKE, Grifola frondosu Dickson), matsutake mushroom (MATSUTAKE, Tricholoma matsutake) and shiitake mushroom (SHIITAKE, Cortinellus Shiitake) were used. With respect to agaricus mushroom (AGARIKUSUTAKE), it was found by the present inventor's experiments that it shows potent antitumor activity when it is used in a raw state than the case where it is used after drying, so that it was stored in a refrigerator at a low temperature (4°C) after the harvest and used within 3 days from the harvest.

With regard to the other mushrooms than the agaricus mushroom, not so significant differences in effectiveness were observed when they are used in the raw state and in

the dried state which has heretofore been provided as a Chinese medicine to which the antitumor activity strengthening method of the present invention is applied so that in the present Example, dried materials were used with regard to PORIA (BUKURYO), POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES), maitake mushroom (black, MAITAKE, Grifola frondosu Dickson), matsutake mushroom (MATSUTAKE, Tricholoma matsutake) and shiitake mushroom (SHIITAKE, Cortinellus Shiitake).

Incidentally, in the present embodiment, two kinds of maitake mushrooms were used. Those produced in Japan are those mainly cultured in Niigata prefecture which is a snow country and which are formally called as snow country maitake mushroom (Yukiguni maitake) or black maitake mushroom (Kuromaitake), and in the present specification, it is mentioned as maitake mushroom (black).

On the other hand, maitake mushroom (MAITAKE, Grifola frondosu Dickson) obtained in China is mentioned as POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES) in the present specification and distinguished from the above-mentioned maitake mushroom (black) depending on necessity. In particular, in POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES), a root is mainly used.

The first step of the antitumor activity strengthening method of the present Example is a far infrared rays-roasting step in which the above-mentioned various kinds of mushrooms are roasted by far infrared rays.

The far infrared rays to be used in such a far infrared rays-roasting step are preferably far infrared rays having a wavelength of about 4 to 14 μm. Mushrooms are roasted by using an apparatus such as a pan, a kiln, etc. in which a ceramics which irradiates far infrared rays having a wavelength with such a range is coated at the inner surface thereof, an apparatus such as a pan, a kiln, etc. prepared by mixing ceramic powder which irradiates far infrared rays with stone, gravel, sand, etc. of metal oxides.

For effecting such a roasting, other than agaricus mushroom (AGARIKUSUTAKE), those which are once dried as used in the usual Chinese medicines are used. With regard to the agaricus mushroom (AGARIKUSUTAKE), it is used in a raw state as mentioned above.

The above-mentioned mushrooms are roasted in the above-mentioned apparatus, for example, in an earthen pot prepared from far infrared rays irradiating substances such as granite, ceramics, Tenshouseki stone, etc. and irradiating far infrared rays with a wavelength of 4 to 14 μm with the extent that they are not burned for 30 to 90 minutes while gradually and well stirring.

As the roasting method, it is not limited by the above-mentioned method, and it may be any method so long as it promotes liberation of ß-glucan of mushrooms. For example, as a far infrared rays source to be used for roasting, in addition to the above-mentioned apparatuses, optional ones may be used so long as it comprises a

material which irradiates far infrared rays with a wavelength of 4 to 14 μm such as platinum electromagnetic wave fiber, etc.

Subsequent to such a far infrared rays roasting step, a fermentation step is provided as a second step. After applying the far infrared rays roasting treatment as mentioned above, by using fermentation bacteria such as "Koji" or yeast, fermentation is carried out in a room containing moisture at 30 to 36°C for 48 to 72 hours. It may be carried out by using a fermentation apparatus to shorten the fermentation time.

As microorganisms to be used for fermentation, microorganisms other than "Koji" may be used.

Also, for effecting fermentation, in addition to "Koji", a substance having fermentating power such as matured papaya, juice of pineapple, the rind of a fig, the rind of a grape, a bark of a young bamboo, etc. may be used. Moreover, digestive enzymes such as diastase, pancreatine, etc., proteolytic enzymes derived from microorganisms such as protease, pepsin, trypsin, etc., lytic enzymes of polysasccharide such as hemicellulase, or a precursor substance which forms the above-mentioned digestive enzymes or proteolytic enzymes, etc. may be used for fermentation.

However, according to the experiments by the present inventor, more preferred results can be obtained when fermentation is carried out by using microorganisms, and when fermentation is carried out "Koji" among the

microorganisms in the point of strengthening antitumor activity. After completion of such a fermentation step, the starting materials were pulverized.

For pulverization, commercially available pulverizer may be used, but a machine which generates high temperature at the time of pulverization shall be preferably avoided. It may be carried out by using a pulverization method which does not generate high temperature such as stone mill.

The materials of starting mushrooms to which the far infrared rays roasting step and fermentation step are applied are further subjected to an oily agent-making step. This procedure for making oily agent is to enclose the above-mentioned fine powder of the starting material fine powder after fermentation with an oily component from sesame to which far infrared rays roasting is applied.

As the oily component to be used for making oily agent, there may be used an oil (hereinafter referred to as sesame paste oil) collected from roasted sesame. The sesame paste oil is an oil obtained by subjecting raw sesame to far infrared rays roasting at a temperature not exceeding 100 C slowly for a long time, and grinding down and compressing the sesame after roasting. In such a sesame paste oil, fine solid materials formed by grinding down the sesame are remained as such so that it takes a paste-like appearance.

To such a sesame paste oil is added the above-mentioned pulverized mushrooms after fermentation to

enclose the finely pulverized mushrooms with an oily component of the sesame. By enclosing the mushrooms, permeation force thereof to the cells at the diseased portion can be strengthened as compared with the case where no such an enclosure with the paste oil is carried out. Such an oily agent-making step is an important step to provide an effective target directivity in the viewpoint of drug delivery system (DDS) which is attracted attention in recent years.

Incidentally, the oily agent-making step is an important step as mentioned above, and it is inherently preferred to provide such a step, but if there is some difficulty that to make an oily agent cannot suitably be carried out, such an oily agent-making step may be omitted. The reason why the above can be said is that, as mentioned below, it is inspected by the test that if the far infrared rays roasting step and the fermentation step are certainly carried out, effectiveness of strengthening antitumor activity becomes significantly large even when the oily agent-making step is not carried out as mentioned below.

Also, for making an oily agent, more preferred results can be obtained when a mixed oil in which the above-mentioned sesame paste oil and an oil collected from raw sesame are mixed with a suitable ratio is used.

By mixing the oil collected from raw sesame, a size of an oil droplet ca be made small, whereby a penetrating power to the target cells can be markedly heightened as

compared with the case that the mushrooms are enclosed with the sesame paste oil having a high viscosity and a large oil droplet size.

The oil collected from the raw sesame means an oil obtained by grinding down raw sesame as such and compressing the resulting material, then removing solid material, which corresponds to a commercially available usual sesame oil.

A ratio of mixing the sesame paste oil and the above-mentioned sesame oil obtained from raw sesame may vary depending on the amount of the starting fine powder to be added so that it cannot regulate sweepingly but suitably 1 to 3 parts by weight of the sesame oil based on 1 part by weight of the sesame paste oil. Also, a mixing ratio of the mixing oil and the starting fine powder may be, for example, about 4 to 5 parts by weight of the starting fine powder based on about 1 part by weight of the mixing oil.

The mushrooms in which their antitumor activities had been strengthened by the above-mentioned manner were added to a system which forms lipid peroxide by irradiating ultraviolet rays to docosahexaenoic acid and an amount of the formed lipid peroxide was examined. In addition, their antitumor effects by internal administration were also measured by clinical tests.

Also, to compare the antitumor activity strengthening method of the above-mentioned explanation, formed amount of the above-mentioned lipid peroxide, and effectiveness in

the clinical tests were examined with respect to the case where the far infrared rays roasting alone was applied to the above-mentioned mushrooms, the case where the polysaccharide lytic enzyme fermentation alone was applied to the same, the case where the "Koji" fermentation alone was applied to the same, the case where both of the far infrared rays roasting and the "Koji" fermentation were applied to the same, and the untreated case where no treatment was applied, respectively.

For effecting the above-mentioned polysaccharide lytic enzyme fermentation, various polysaccharide lytic enzymes can be used, but in the present Example, hemicellulase which is a representative one among them was used.

Incidentally, in the case where the far infrared rays roasting alone is applied to mushrooms, the case where the polysaccharide lytic enzyme fermentation alone was applied to the same, the case where the "Koji" fermentation alone was applied to the same, and the case where both of the far infrared rays roasting and the "Koji" fermentation were applied to the same, at the time at which the respective treatments were carried out, the material was pulverized to fine powder with the procedure as mentioned above, and used in the following experiments.

The degree of pulverization of the respective samples is so adjusted that an average pulverized particle size becomes the same degree as others to exclude the effect on

the pulverized particle size. In the case of untreated, the agaricus mushroom (AGARIKUSUTAKE) was used with a raw state and finely chopped, and the other mushrooms than the above, they were used by making fine powders after drying.

Measurement of the formed amount of the above-mentioned lipid peroxide was carried out as mentioned below. A predetermined amount of docosahexaenoic acid was placed in a plural number of test tubes, and the above-mentioned mushrooms weighed previously with different amounts were added to the respective docosahexaenoic acid and they were mixed. In addition, ultraviolet rays were irradiated to the contents in the respective test tubes and amounts of the formed lipid peroxides were measured.

Docosahexaenoic acid was used by previously preparing a diluted solution in which the raw liquid was diluted with ethanol 10-folds and the diluted solution was further diluted to become 200-folds as the final diluted concentration.

The samples of the mushrooms to which the above-mentioned treatments (including untreated) were applied was added to predetermined ethanol so as to become the concentration of the mushroom of 60 mg/ml, and the ethanol was allowed to stand at normal temperature for about 2 to 6 weeks whereby extraction with ethanol was carried out. For using the sample in the test, the extract was used by diluting the final concentration of 0.6 mg/ml.

By mixing 0.05 ml of the diluted docosahexaenoic acid

solution prepared by the manner as mentioned above, 0.1 ml of a mushroom sample and 0.85 ml of ethanol in a test tube, the total amount was made 1 ml. This test tube was irradiated by ultraviolet rays for 3 hours to form a lipid peroxide. The formed lipid peroxide was measured by the TBA reaction (thiobarbituric acid reaction).

By heating the formed lipid peroxide under acidic conditions, liberated malondialdehyde (MDA) was reacted with thiobarbituric acid (TBA) and the formed absorption maximum substance was determined to obtain the formed amount of the lipid peroxide indirectly.

For effecting the TBA reaction, 0.2 ml of a 7% sodium dodecylsulfate, 2 ml of 0.1N hydrochloric acid, 0.3 ml of phosphotungstic acid and 1 ml of the reagent prepared by mixing 0.67% of TBA and acetic acid with a ratio of 1:1 were added to the respective test tubes to which ultraviolet rays had been irradiated, and the TBA reaction was carried out by maintaining at 95° C for 12 minutes after the addition.

Thereafter, the mixture was cooled to room temperature, the TBA reaction layer was extracted with n-butyl alcohol, and the fluorospectrophotometric method was applied to the n-butyl alcohol layer to measure the above-mentioned MDA amount. For effecting the measurement, light with a wavelength of 515 nm was used as an exciting light and fluorometry was carried out at a wavelength of 535 nm. The fluorospectrophotometer used was F2000 manufactured by

Hitachi Ltd. using a 400 V photomultiplier, and the measurement was carried out by making the band pass for an excited light irradiation of 20 nm and the band pass at the fluorescence measurement of 20 nm.

With regard to the effects of docosahexaenoic acid on the mushrooms to which the above-mentioned respective treatments were applied by ultraviolet rays irradiation in the lipid peroxide forming system, they were examined in relation with the MDA value (which is the same as in MDA amount, shown by absorbance) measured by the above-mentioned manner. The results are shown in Table 1.

On the other hand, among the above-mentioned mushrooms, with regard to PORIA (BUKURYO), agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, respective of black maitake mushroom (Kuromaitake) (snow country maitake mushroom (Yukiguni maitake)) and POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES) were used), clinical tests were carried out about breast cancer, stomach cancer and lung cancer in the respective cases of untreated, the polysaccharide lytic enzyme fermentation treatment, "Koji" fermentation treatment, far infrared rays roasting treatment + "Koji" fermentation treatment, far infrared rays roasting treatment + "Koji" fermentation treatment + oily agent-making treatment. The results are shown in Tables 2, 3 and 4.

In Table 2, treatment effects of the above-mentioned mushrooms to which various kinds of methods are applied on

breast cancer patients are shown. Tables 3 and 4 show treatment effects on stomach cancer patients and lung cancer patients of the various kinds of mushrooms to which the same treatments were carried out as in Table 2. Incidentally, in Tables 2 to 4, effective ratios calculated from the following formula are also shown.

Effective ratio = (Effective number + Slightly effective number) x 100/(Effective number + Slightly effective number + non-effective number)

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Table 1 Effects of various mushrooms on MDA production

Kind of treatment	Item to be tested and specimen	MDA value (Average)	Order
	Control (Mushroom not added)	115	–
No treatment	Hoelen	269	4
	Agaricus mushroom	283	3
	Polyporus umbellatus Fries	439	1
	Maitake mushroom (Black)	398	2
	Matsutake mushroom	218	5
	Siitake mushroom	189	6
Far infrared rays roasting	Hoelen	288	4
	Agaricus mushroom	341	3
	Polyporus umbellatus Fries	536	1
	Maitake mushroom (Black)	490	2
	Matsutake mushroom	271	5
	Siitake mushroom	233	6
Polysaccharide lytic enzyme fermentation	Hoelen	284	5
	Agaricus mushroom	375	3
	Polyporus umbellatus Fries	551	1
	Maitake mushroom (Black)	525	2
	Matsutake mushroom	304	4
	Siitake mushroom	265	6
"Koji" fermentation	Hoelen	296	5
	Agaricus mushroom	399	3
	Polyporus umbellatus Fries	594	1
	Maitake mushroom (Black)	528	2
	Matsutake mushroom	302	4
	Siitake mushroom	280	6
Far infrared rays roasting + "Koji" fermentation	Hoelen	334	6
	Agaricus mushroom	489	3
	Polyporus umbellatus Fries	799	1
	Maitake mushroom (Black)	768	2
	Matsutake mushroom	412	4
	Siitake mushroom	381	5
Far infrared rays roasting + "Koji" fermentation + oily agent making	Hoelen	362	6
	Agaricus mushroom	509	3
	Polyporus umbellatus Fries	827	1
	Maitake mushroom (Black)	792	2
	Matsutake mushroom	430	4
	Siitake mushroom	397	5

Respective mushrooms were added 6 mg/ml

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Table 2 Treatment effects of various mushrooms on breast cancer patients

Kind of treatment	Specimen	Breast cancer (200 persons)			Effective Ratio (%)
		Effective	Slightly effective	Not effective	
No treatment	Hoelen	0	3	37	7.5
	Agaricus mushroom	1	2	37	7.5
	Maitake mushroom	1	3	36	10.0
Polysaccharide lytic enzyme fermentation	Hoelen	1	4	35	12.5
	Agaricus mushroom	2	6	32	20.0
	Maitake mushroom	4	5	31	22.5
"Koji" fermentation	Hoelen	1	5	34	15.0
	Agaricus mushroom	3	7	30	25.0
	Maitake mushroom	4	6	30	25.0
Far infrared rays roasting + "Koji" fermentation	Hoelen	2	4	34	15.0
	Agaricus mushroom	4	6	30	25.0
	Maitake mushroom	12	7	31	47.5
Far infrared rays roasting + "Koji" fermentation + oily agent-making	Hoelen	3	5	32	20.0
	Agaricus mushroom	6	8	26	35.0
	Maitake mushroom	14	9	17	57.5

The number in the parenthesis shows the number of total person tested.

$$\text{Effective ratio} = \frac{\text{(Effective + Slightly effective) sample number}}{\text{Total sample number}} \times 100\ (\%)$$

Hereinafter the same as in Tables 3 and 4.

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Table 3 Treatment effects of various mushrooms on stomach cancer patients

Kind of treatment	Specimen	Stomach cancer (150 persons)			Effective ratio (%)
		Effective	Slightly effective	Not effective	
No treatment	Hoelen	0	2	28	6.7
	Agaricus mushroom	0	2	28	6.7
	Maitake mushroom	1	1	28	6.7
Polysaccharide lytic enzyme fermentation	Hoelen	2	1	27	10.0
	Agaricus mushroom	2	3	25	16.7
	Maitake mushroom	3	3	24	20.0
"Koji" fermentation	Hoelen	1	2	27	10.0
	Agaricus mushroom	2	4	24	20.0
	Maitake mushroom	3	5	22	26.7
Far infrared rays roasting + "Koji" fermentation	Hoelen	1	3	26	13.3
	Agaricus mushroom	3	5	22	26.7
	Maitake mushroom	5	7	18	40.0
Far infrared rays roasting + "Koji" fermentation + oily agent-making	Hoelen	1	4	25	16.7
	Agaricus mushroom	4	7	19	36.7
	Maitake mushroom	6	8	16	46.7

The number in the parenthesis shows the number of total person tested.

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Table 4 Treatment effects of various mushrooms on lung cancer patients

Kind of treatment	Specimen	Lung cancer (100 persons)			Effective ratio (%)
		Effective	Slightly effective	Not effective	
No treatment	Hoelen	0	1	19	5.0
	Agaricus mushroom	0	2	18	10.0
	Maitake mushroom	1	1	18	10.0
Polysaccharide lytic enzyme fermentation	Hoelen	1	1	18	10.0
	Agaricus mushroom	1	2	17	15.0
	Maitake mushroom	3	4	13	35.0
"Koji" fermentation	Hoelen	1	2	17	15.0
	Agaricus mushroom	1	3	16	20.0
	Maitake mushroom	3	5	12	40.0
Far infrared rays roasting + "Koji" fermentation	Hoelen	2	1	17	15.0
	Agaricus mushroom	3	3	14	20.0
	Maitake mushroom	4	5	11	45.0
Far infrared rays roasting + "Koji" fermentation + oily agent-making	Hoelen	2	2	16	20.0
	Agaricus mushroom	3	4	13	35.0
	Maitake mushroom	5	5	10	50.0

The number in the parenthesis shows the number of total person tested.

From the above-mentioned Table 1, it can be understood that the MDA value which is an index of the formed amount of lipid peroxide becomes greater, in the same kind of mushrooms, when the far infrared rays roasting is applied to than the untreated case, when the polysaccharide lytic enzyme fermentation is applied to than the case where the far infrared rays roasting is applied, when the "Koji" fermentation is applied to than the polysaccharide lytic enzyme fermentation, when the both of the far infrared rays roasting and "Koji" fermentation are applied to than the case where "Koji" fermentation is applied, and when the three of the far infrared rays roasting, "Koji" fermentation and oily agent-making procedure are applied to than the case where the both of the far infrared rays roasting and "Koji" fermentation are applied, respectively.

Such a tendency is common irrespective of the kinds of the mushrooms to be applied to the tests except for the case of PORIA (BUKURYO) between the far infrared rays roasting and the polysaccharide lytic enzyme fermentation, and the case of matsutake mushroom (MATSUTAKE, Tricholoma matsutake) between the polysaccharide lytic enzyme fermentation and the "Koji" fermentation.

Substantially the same (correctly the value of one of the treatment methods is larger about 1% than that of the other treatment method) MDA value can be obtained when the far infrared rays roasting is applied to and when the

polysaccharide lytic enzyme fermentation is applied to with regard to PORIA (BUKURYO), and when the polysaccharide lytic enzyme fermentation is applied to and when the "Koji" fermentation is applied to with regard to matsutake mushroom (MATSUTAKE, Tricholoma matsutake).

On the other hand, from Tables 2 to 4 showing the clinical test results of administering PORIA (BUKURYO), agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, black maitake mushroom (Kuromaitake) (snow country maitake mushroom (Yukiguni maitake)), POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES)) to which the above-mentioned respective treatments were applied, to breast cancer patients, stomach cancer patients and lung cancer patients, it can be understood that effective ratios in the respective cases of breast cancer patients, stomach cancer patients and lung cancer patients becomes higher, in the same kind of mushrooms, when the polysaccharide lytic enzyme fermentation is applied to than the untreated case, when "Koji" fermentation is applied to than the case where the polysaccharide lytic enzyme fermentation is applied, when the both of the far infrared rays roasting and "Koji" fermentation are applied to than the case where "Koji" fermentation is applied, and when the three of the far infrared rays roasting, "Koji" fermentation and oily agent-making procedure are applied to than the case where the both of the far infrared rays roasting and "Koji" fermentation are applied, respectively.

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For example, from Table 2, in PORIA (BUKURYO), it can be understood that the effective ratio is increased when the far infrared rays roasting + "Koji" fermentation + oily agent-making procedure are applied to as compared with the untreated case from 7.5% to 22.0%, i.e., about three times. In agaricus mushroom (AGARIKUSUTAKE), the effective ratio is increased from 7.5% to 35%, i.e., about 4.9 times. In maitake mushroom (MAITAKE), the effective ratio is marvelously increased in manifesting property of an antitumor effect from 10.0% to 57.5%, i.e., 5.75 times. Such a tendency is the same as in Tables 3 and 4.

From the above results, it is shown that the treatment method which is successively combined the far infrared rays roasting step, the "Koji" fermentation step, and the oily agent-making step can be effectively used as an increasing method of manifesting property of the antitumor activity of mushrooms, i.e., as the antitumor activity strengthening method.

Table 5 Amount of free ß-glucan in the respective kinds of mushrooms of untreated and to which the far infrared rays roasting and the "Koji" fermentation treatment are applied

Kind of treatment	Specimen	Amount of ß-glucan
Untreated	Agaricus mushroom (AGARIKUSUTAKE)	9.0 g/100 g
	Maitake mushroom (MAITAKE, black)	24.4 g/100 g
Far infrared rays roasting step + "Koji" fermentation step	Agaricus mushroom (AGARIKUSUTAKE)	14.2 g/100 g
	Maitake mushroom (MAITAKE, black)	30.4 g/100 g

On the other hand, amounts of free ß-glucan as an effective ingredient showing an antitumor activity were measured in the case where the treatment to enlarge manifestation of the above-mentioned antitumor effect and the case of untreated. The mushrooms used were agaricus mushroom (AGARIKUSUTAKE) and maitake mushroom (MAITAKE, black maitake mushroom (Kuromaitake)) which showed large effective ratios in Tables 2 to 4. Incidentally, the analyses were carried out at the Food Analysis Center at Suita, Osaka, Japan. The results are shown in Table 5.

From the results of Table 5, it can be understood that amounts of free ß-glucan were increased when the far infrared rays roasting + "Koji" fermentation treatment were applied to in either of agaricus mushroom (AGARIKUSUTAKE) and maitake mushroom (MAITAKE). With regard to maitake mushroom (MAITAKE), other than the black maitake mushroom (Kuromaitake), the same effects can be obtained also in

POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES)). On the other hand, in the clinical test results in Tables 2 to 4, it is shown that the antitumor effects are higher in the case of applying the far infrared rays roasting treatment + "Koji" fermentation treatment than the case of untreated.

Thus, by examining the results of Tables 2 to 4 and those of Table 5 in combination, it can be considered that among ß-glucans effective for antitumor activity of mushrooms, particularly when an amount of free ß-glucan is a large, the antitumor effects are remarkably manifested.

The present inventor has progressed the above-mentioned series of studies by setting forth the hypothesis as mentioned above that, in the non-treated mushrooms, ß-glucans having antitumor action are in an unmovable state by binding to each other to form a chain (a simile expression) which is so-called non-activated state, and when mushrooms are internally administered, the chain cannot be cut by a gastric juice of a usual person whereby the antitumor action would not be shown than expected. However, as mentioned above, it can be found that increase in an amount of free ß-glucan markedly affects to manifestation of the antitumor action and the results which could be expected by the present inventors hypothesis can be obtained.

Also, the composition produced by the far infrared rays roasting step, the fermentation step and the oily agent-making step which constitute the antitumor activity

strengthening method as mentioned above is, as shown in Table 5, etc., a composition in which an amount of free ß-glucan is increased and shows the antitumor activity effectively by internal administration. As compared with the composition containing mushrooms (untreated mushrooms) to which the above-mentioned antitumor activity strengthening method is not applied, its antitumor activity is markedly strengthened.

Also, from Table 1, it can be understood that the order of the height of the MDA value in the mushrooms are changed between various kinds of treatments including untreated ones. In the order shown in Table 1, with regard to three kinds of PORIA (BUKURYO), Matsutake mushroom (MATSUTAKE) and Shiitake mushroom (SHIITAKE), they show a possibility of changing the order, i.e., effectiveness of the antitumor activity in the three treatment methods of the untreated, "Koji" fermentation and far infrared rays roasting + "Koji" fermentation.

For example, it can be understood that in the case of the untreated one, the antitumor activity becomes high in the order of Shiitake mushroom (SHIITAKE), Matsutake mushroom (MATSUTAKE) and PORIA (BUKURYO), whereas the far infrared rays roasting and the "Koji" fermentation are applied, the antitumor activity becomes high in the order of PORIA (BUKURYO), Matsutake mushroom (MATSUTAKE) and Shiitake mushroom (SHIITAKE). It is understood that, with applying the above-mentioned antitumor activity

strengthening method according to the present invention, it is possible to raise an evaluated order for untreated mushrooms which had been evaluated to be lower in the antitumor activity.

In the field of Chinese medicine using a natural crude drug, there is a scarce species which cannot ensure an amount of yield stably even when it is a mushroom having high antitumor activity. However, when the above-mentioned method of the present invention is used, in place of such a scarce species, it is possible to use mushrooms which can ensure a sufficient amount of yield or can cultivate. According to this, stabilization of supply and low price of the product can be established whereby many persons can effectively enjoy the antitumor effects of mushrooms.

Incidentally, in the above-mentioned explanation, as a best embodiment, only the treatment in which three kinds of the far infrared rays roasting treatment, the fermentation treatment and the oily agent-making treatment are applied is explained. However, only the far infrared rays roasting treatment is applied, or only the polysaccharide lytic enzyme fermentation treatment such as hemicellulase, etc. is applied, or only the fermentation treatment by microorganisms such as "Koji", etc., or only the far infrared rays roasting and the fermentation treatment are applied, a manifestation of the antitumor effects are strengthened as compared with the non-treated case.

Thus, when there are any reasons that the oily agent-making cannot suitably carried out due to the conditions of equipments, the treatments of the far infrared rays roasting and the fermentation by microorganism can be carried out in combination, the antitumor activity can be markedly effectively shown than that of the non-treated case.

Moreover, in the treatment in which to the above-mentioned mushrooms are applied three kinds of treatments of the far infrared rays roasting, the fermentation treatment and the oily agent-making treatment, or to the mushrooms are applied two kinds of treatments of the far infrared rays roasting and the fermentation treatment, in addition to the fermentation treatment using microorganisms such as "Koji" or yeast bacteria, there may be used a fermentation treatment using a polysaccharide lytic enzyme.

In the oily agent-making step in the above-mentioned antitumor activity strengthening method, a sesame paste oil obtained from a roasted sesame is used, but other than sesame, plants oil obtained by roasting soy bean, corn, a safflower, an evening primrose, rice bran, rapeseed, etc. by far infrared rays, and then squeezing and pressing may be used.

Also, in the composition containing mushrooms to which the above-mentioned antitumor activity strengthening method is applied, in addition to the materials obtained by treating mushrooms by the above-mentioned method, any

component which does not participate any bad effects on showing the antitumor activity, such as a vitamin agent, etc., may be further added.

As a composition having such a constitution, a preparation having an active oxygen suppressing effect which has been developed by the present inventor may be formulated. The antitumor activity strengthening treated-crude drug containing compound having such a constitution may be actually provided in an agent form which can be internally administered easily by encapsulating them in a gelatin capsule, etc.

Moreover, by using a suitable excipient, a binder, etc., it may be a tablet by tabletting, or may be in a preparation form such as a granule or a pill without any problem. Furthermore, whereas the present invention is a method of strengthening antitumor activity by an internal administration, it can be considered that an injection preparation prepared by using mushrooms to which the antitumor activity strengthening method of the present invention is applied can be expected to have an effect of enlarging manifestation of the antitumor effect as compared with the case where the antitumor activity strengthening method of the present invention is not applied to.

Incidentally, in the above-mentioned explanation, an explanation was made about the case where mushrooms such as agaricus mushroom (AGARIKUSUTAKE), but it is not necessary to restrict the mushrooms only to the above-mentioned

mushrooms, and any mushrooms than the above may be used so long as it contains ß-glucan. For example, it may be shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes), enokitake mushroom (ENOKITAKE, Flammulina velutipes), etc.

Moreover, a crude drug containing a polysaccharide having an antitumor activity other than ß-glucan may be used. Furthermore, the present invention can be used as a method for strengthening manifestation of the antitumor effect containing a polysaccharide having an antitumor activity such as ß-glucan in a natural or cultivated products derived from plants widely including mushrooms.

(Embodiment 2)

In the present embodiment, an evaluating method of effectiveness on antitumor activity for a crude drug, and an antitumor effectivity evaluating method of treated by crude drug are explained.

To the experiment system in which lipid peroxide is formed by irradiating ultraviolet rays explained in the above-mentioned embodiment 1 is added a crude drug for evaluating antitumor activity, e.g., mushrooms, and a formed amount of lipid peroxide is examined. When the mushrooms are added with increasing their amounts, a mushroom which has a tendency of markedly increasing the formed amount of lipid peroxide shows a potent antitumor activity when it is administered.

That is, when the increased ratio of the formed

amount of lipid peroxide is large based on the increased ratio of the concentration of the mushroom added, the mushroom can be evaluated as it shows potent antitumor effect when it is internally administered. Thus, a plural kinds of mushrooms are introduced in the above-mentioned experimental system, and when the increased ratio of the formed amount of lipid peroxide based on the ratio of the concentration of the mushroom added is compared to each other, the order of effectiveness on showing antitumor effect by internal administration of the plural kinds of mushrooms can be determined.

In the following, the above-mentioned both of the evaluating methods in the embodiment 2 of the invention are explained based on the above-mentioned examples. When the MDA values with respect to the respective treatment methods in Table 1 mentioned above are compared to each other, it can be understood either of the cases in which any other treatment such as far infrared rays roasting, etc. is carried out showed a larger MDA value as compared with the case of non-treatment.

When either of the far infrared rays roasting, or the "Koji" fermentation is applied, the MDA value is larger when the mushrooms to which the "Koji" fermentation is applied. When the "Koji" fermentation and the far infrared rays roasting are used in combination, the MDA value is larger than the case where either one of the far infrared rays roasting, or the "Koji" fermentation is applied singly.

Moreover, the MDA value when three of the far infrared rays roasting, the "Koji" fermentation and the oily agent-making treatments are applied is larger than that of the case where two of the far infrared rays roasting and the "Koji" fermentation are applied.

On the other hand, from Tables 2, 3 and 4 mentioned above in which clinical tests were carried out with regard to breast cancer, stomach cancer and lung cancer in the cases of untreated, the polysaccharide lytic enzyme fermentation treatment, the "Koji" fermentation treatment, the far infrared rays roasting treatment + the "Koji" fermentation treatment, and the far infrared rays roasting treatment + the "Koji" fermentation treatment + the oily agent-making treatment, respectively, with regard to PORIA (BUKURYO), agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, black maitake mushroom (Kuromaitake) (snow country maitake mushroom (Yukiguni maitake)) and POLYPORUS (CHOREIMAITAKE, Polyporus umbellatus FRIES) were each used) among the above-mentioned mushrooms, it can be understood that, in the same mushroom, the clinical effect becomes high as the treatment showing high MDA value shown in Table 1 is applied.

From such results, height of the clinical effect, i.e., effectiveness of the antitumor effect can be judged by using the MDA value as an index, and it can be said that the treatment which makes the formed amount of the MDA value high is effective for showing the antitumor effect.

That is, as the MDA value is large, which shows an amount of lipid peroxide formed by irradiating ultraviolet rays to docosahexaenoic acid, the treatment can be evaluated as it has high effectivity in antitumor activity of mushrooms.

Also, when the results of Table 1 and those of Tables 2 to 4 are compared, in the same treatment method to mushrooms, the order of height of the MDA value is in agreement with the order of height of effective ratio in clinical effects. That is, to the system which forms lipid peroxide by irradiating ultraviolet rays to the above-mentioned docosahexaenoic acid is added a different mushroom to which the same treatment is applied with the same concentration, and the formed amount of the lipid peroxide is shown with a MDA value (amount) in the same manner as in the above-mentioned embodiment, it can be said that the mushroom which makes the formed amount of MDA high is effective for showing the antitumor effect.

Accordingly, the antitumor activity of the respective kinds of mushrooms can be expected as the mushrooms having a large MDA value which shows an amount of lipid peroxide formed by irradiating ultraviolet rays to docosahexaenoic acid have high antitumor activity at the time of internal administration. That is, without effecting clinical tests, effectiveness of the antitumor activity of the respective kinds of mushrooms by internal administration can be expected.

Incidentally, in the above explanation, an explanation was made in the case where it is applied to mushrooms such as agaricus mushroom (AGARIKUSUTAKE), etc., but as the mushrooms to be applied, it is not limited only to the above-mentioned mushrooms. The present invention can be applied to mushrooms other than the above so long as it contains ß-glucan.

Moreover, the present invention can be applied to mushrooms containing polysaccharides having antitumor activity other than ß-glucan. Furthermore, the present invention may be applied to widely those which are crude drugs such as a natural or cultivated products derived from plants other than mushrooms and contain polysaccharides having antitumor activity such as ß-glucan.

Also, in the above-mentioned explanation, docosahexaenoic acid was used, but a fatty acid other than docosahexaenoic acid can be used, which is, for example, an unsaturated fatty acid in which an amount of lipid peroxide formed by irradiation of ultraviolet rays by addition of the above-mentioned mushrooms. Furthermore, such unsaturated fatty acid derived from living body in which an amount of lipid peroxide formed by irradiation of ultraviolet rays by addition of the above-mentioned mushrooms may be used in the range in which the above-mentioned evaluation method can be applied, even when it is not derived from a living body, i.e., a synthetic fatty acid.

Also, by adding mushrooms containing a carcinostatic polysaccharide to the system which forms lipid peroxide by irradiating ultraviolet rays to docosahexaenoic acid as explained above with various kinds of concentrations, and by catching the amounts of the formed lipid peroxides with the respective concentrations, an average and suitable administration dose in comply with the progressed stage of the malignant tumor such as cancer can be easily expected without carrying out clinical test about the respective mushrooms to be used.

That is, the relationship between the concentration of the mushroom and the MDA value in the respective kinds of mushrooms is grasped with the above-mentioned manner, and the MDA value and the effectiveness thereof at the respective progress stage of cancer are previously confirmed by the clinical test in a certain kind of mushrooms. By effecting the above, if the concentration of said mushroom is calculated back so as to obtain an MDA value which had been confirmed to be effective in the respective progress stage of tumor such as cancer, etc., an average internal administration dose can be easily expected from the concentration of the mushroom.

Also, in the present invention, the antitumor activity of a crude drug can be compared through a common index such as the MDA value so that the antitumor activity of the various kinds of crude drugs which had been compared by carrying out clinical tests can be realized to compare

with each other without effecting clinical tests, i.e., without exerting danger by some chance to a patient.

That is, the meanings that the present inventor has found the MDA value as a parameter which is capable of using as a common measure for the respective kinds of crude drugs, and mutual evaluation of the antitumor activity is realized by correlating the MDA value and the antitumor activity are significantly large in the point that mutual evaluation of a number of crude drugs can be carried out simply and easily.

According to the antitumor activity strengthening method of crude drugs of the present invention, effectivity in the antitumor activity of the crude drugs such as mushrooms at internal administration can be heightened as compared with the case in which such a method is not applied.

According to the antitumor activity strengthening method of crude drugs of the present invention, even in crude drugs such as mushrooms, etc. which are not admitted as effective when the method of the present invention is not applied, they can be applied to as an antitumor agent by heightening effectivity at internal administration.

The antitumor activity strengthened crude drug composition containing crude drugs such as mushrooms to which the antitumor activity strengthening method of the present invention is applied has high antitumor effectivity at internal administration as compared with the composition

containing crude drugs to which such a method is not applied.

According to the evaluating method of antitumor effectivity of treatment by the crude drugs according to the present invention, effectivity in view of the antitumor activity by the various kinds of treatment methods applied to the crude drugs can be simply evaluated within a short term.

According to the evaluating method of antitumor effectivity of the crude drugs according to the present invention, effectivity of the crude drugs at internal administration can be expected without effecting clinical tests. Thus, as compared with the conventional method in which the results can be obtained by carrying out a long term of animal experiments or clinical tests, a search of an antitumor effective substance by screening a tremendous number of natural products can be carried out simply as compared with the conventional manner.

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What is claimed is:

1. An antitumor activity strengthening method applied to a crude drug containing polysaccharides having antitumor activity, which comprises a roasting step of roasting the crude drug by far infrared rays and a fermentation step of fermenting by adding microorganisms to strengthen the antitumor activity of the crude drug than the case of not effecting the roasting and the fermentation.

2. The antitumor activity strengthening method of a crude drug according to Claim 1, wherein

a mushroom which contains ß-glucan is used as the crude drug.

3. The antitumor activity strengthening method of a crude drug according to Claim 2, wherein

at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

4. The antitumor activity strengthening method of a crude drug according to Claim 3, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

5. The antitumor activity strengthening method of a crude drug according to Claim 1, wherein

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subsequent to the fermentation step, a step of making an oily agent in which the fermentated crude drug is enclosed with an oily component obtained from plants such as sesame roasted by far infrared rays whereby strengthening the antitumor activity of the crude drug than the case of not effecting the roasting, the fermentation and the oily agent-making steps.

6. The antitumor activity strengthening method of a crude drug according to Claim 5, wherein

a mushrooms which contains ß-glucan is used as the crude drug.

7. The antitumor activity strengthening method of a crude drug according to Claim 6, wherein

at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

8. The antitumor activity strengthening method of a crude drug according to Claim 7, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

9. An antitumor activity strengthened crude drug-containing composition which comprises a crude drug to which the antitumor activity strengthening method

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comprising a roasting step of roasting the crude drug by far infrared rays and a fermentation step of fermenting by adding microorganism is applied whereby the antitumor activity of said crude drug is strengthened than the case of not effecting the roasting and the fermentation.

10. The antitumor activity strengthened crude drug-containing composition according to Claim 9, wherein

a mushrooms which contains ß-glucan is used as said crude drug.

11. The antitumor activity strengthened crude drug-containing composition according to Claim 10, wherein

at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

12. The antitumor activity strengthened crude drug-containing composition according to Claim 11, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

13. The antitumor activity strengthened crude drug-containing composition according to Claim 9, wherein

subsequent to the fermentation step, a step of making an oily agent in which the crude drug is enclosed with an oily component obtained from plants such as sesame roasted

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by far infrared rays whereby strengthening the antitumor activity of the crude drug than the case of not effecting the roasting, the fermentation and the oily agent-making steps.

14. The antitumor activity strengthened crude drug-containing composition according to Claim 13, wherein

a mushroom which contains ß-glucan is used as said crude drug.

15. The antitumor activity strengthened crude drug-containing composition according to Claim 14, wherein

at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

16. The antitumor activity strengthened crude drug-containing composition according to Claim 15, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

17. An evaluating method of effectiveness on an antitumor activity of a treatment to be applied to a crude drug containing polysaccharides having an antitumor activity, which comprises adding the crude drug to which the treatment is applied to a system which forms lipid peroxide by irradiating ultraviolet rays to an unsaturated fatty

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acid such as docosahexaenoic acid and evaluating an effect of the treatment for strengthening the antitumor activity of the crude drug to be large as an increased ratio of a formed amount of the lipid peroxide based on an increased ratio of the concentration of the crude drug by the treatment being large.

18. The evaluating method of effectiveness on an antitumor activity of a treatment to be applied to a crude drug according to Claim 17, wherein

a mushroom which contains ß-glucan is used as said crude drug.

19. The evaluating method of effectiveness on an antitumor activity of a treatment to be applied to a crude drug according to Claim 18, wherein

at least one mushroom selected from the group consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

20. The evaluating method of effectiveness on an antitumor activity of a treatment to be applied to a crude drug according to Claim 19, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

21. An evaluating method of effectiveness of the crude

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drug for evaluating effectiveness of an antitumor effect shown by the crude drug containing polysaccharides having an antitumor activity without carrying out clinical tests, which comprises adding the crude drug to a system which forms lipid peroxide by irradiating ultraviolet rays to an unsaturated fatty acid such as docosahexaenoic acid and evaluating and expecting an effect of the antitumor activity of the crude drug by its internal administration to be large as an increased ratio of a formed amount of the lipid peroxide based on a ratio of the concentration of the crude drug being large.

22. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 21, wherein

said crude drug to which the antitumor activity strengthening method comprising a roasting step of roasting the crude drug by far infrared rays and a fermentation step of fermenting by adding microorganism is applied whereby the antitumor activity of said crude drug is strengthened than the case of not effect the roasting and the fermentation.

23. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 22, wherein

a mushroom which contains ß-glucan is used as the crude drug.

24. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 23, wherein

at least one mushroom selected from the group

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consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

25. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 24, wherein

the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

26. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 21, wherein

said crude drug to which the antitumor activity strengthening method comprising a step of making an oily agent in which the fermentated crude drug is enclosed with an oily component obtained from plants such as sesame roasted by far infrared rays subsequent to the fermentation step, whereby strengthening the antitumor activity of the crude drug than the case of not effecting the roasting, the fermentation and the oily agent-making steps.

27. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 26, wherein

s mushroom which contains ß-glucan is used as the crude drug.

28. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 27, wherein

at least one mushroom selected from the group

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consisting of agaricus mushroom (AGARIKUSUTAKE), maitake mushroom (MAITAKE, Grifola frondosu), shiitake mushroom (SHIITAKE, Cortinellus Shiitake), matsutake mushroom (MATSUTAKE, Tricholoma matsutake), shimejitake mushroom (SHIMEJITAKE, Lyophyllum decastes) and enokitake mushroom (ENOKITAKE, Flammulina velutipes) is used as the mushroom.

29. The evaluating method of effectiveness on an antitumor activity of a crude drug according to Claim 28, wherein the agaricus mushroom (AGARIKUSUTAKE) is used, then raw agaricus mushroom is roasted by far infrared rays.

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