

USE OF EXTRACT COMPOSITION OF FRUITING BODY OF ANTRODIA

FIELD OF THE INVENTION

[0001] The invention relates to use of extract of fruiting body of Antrodia,
5 particularly in the treatment of low number of red blood cells and/or
thrombocytopenia.

BACKGROUND OF THE INVENTION

[0002] Chemotherapeutic agents play a very important role in cancer treatment.
However, although the chemotherapeutic agents kill cancer cells, they also harm to
10 many normal cells and cause many side effects. In general, cancer cells are cells with
rapid growth and division rates. The cytotoxic mechanism of the chemotherapeutic
agents is usually associated with inhibition of cell division. However, the growth and
division rates of many cells in the normal tissue are also rapid, and thus the
chemotherapeutic agents affect physiological functions of these cells. Examples of
15 the cells usually suffered from the impact of the chemotherapeutic agents are bone
marrow cells, gastrointestinal epithelial cells, cells of the reproductive system, and
follicle cells.

[0003] Among the above mentioned cells usually suffered from the impact of the
chemotherapeutic agents, bone marrow cells are vital cells, which are a major
20 hematopoietic tissue with rapid growth and division rates. In accordance with
different sensitivity of the bone marrow cells for the chemotherapeutic agents, almost
all of varying degrees of "bone marrow suppression" side effects happen in the
chemotherapeutic agent treatment, with the symptoms of low numbers of white blood
cells, red blood cells and platelets. Low number of white blood cells makes patients
25 vulnerable to infection; low number of red blood cells may cause anemia, followed by
weakness, fatigue and other symptoms; while low number of platelets

(thrombocytopenia) makes patients suffering from coagulation dysfunction.

[0004] Currently, in response to the clinical embodiments of the bone marrow suppression, different treatment mechanisms are applied. For low number of white blood cells, granulocyte-macrophage colony-stimulating factor (GM-CSF) is used; for
 5 low number of red blood cells, ferralia is used, if necessary, transfusing blood or erythropoietin (EPO) is used; for low number of platelets, transfusing platelets is used. However, the aforementioned treatments involving blood products have safety and supply considerations. Erythropoietin is expensive. The use of granulocyte-macrophage colony-stimulating factor also causes many side effects,
 10 including bone pain, muscle aches, headache, fatigue, nausea, vomiting, fever, insomnia; elevated alkaline phosphatase, liver function index, and uric acid found in biochemical tests; and in addition, affecting musculoskeletal system, skin, respiratory system, cardiovascular system, and kidney functions.

[0005] Therefore, a pharmaceutical agent is still needed to be developed to deal with
 15 the side effects of bone marrow suppression.

[0006] Antrodia is a Taiwan endemic fungus, belonging to Basidiomycotina, Hymenomycetes, Aphyllophorales, Polyporaceae, and Antrodia. Antrodia is a perennial fungus, and grows only in the inner wall of hollow trunk of Taiwan endemic cattle camphor (*Cinnamomum kanehirai*). The fruiting body of Antrodia is annual to
 20 perennial, bright red, orange or pale cinnamon color, and with camphor aroma.

[0007] Antrodia is early regarded as hangover medicine by Taiwan Aborigines, with the effect of relieving a hangover, containing triterpenoids, polysaccharides and β -glucans. After extensive research, Antrodia has been proved to have effects on anti-hepatitis B virus, anti-liver cancer, immune regulation, anti-inflammatory,
 25 anti-oxidation, anti-angiogenesis, lowering blood pressure, lowering blood fat, neuroprotection, anti-bacteria, whitening, and other functions.

[0008] Antrodia is a fungus and the growth cycle includes stages of hypha,

mycelium, fruiting body, and sporophyte. The cultivation costs, ingredients, and ingredient contents of different stages are different. The hypha and mycelium can be cultured in liquid fermentation or solid fermentation. The liquid fermentation has the advantages of low cost and short incubation time; while the mycelium having similar shape of fruiting body can be obtained in solid fermentation. However, the triterpenoid contents in hypha and mycelium are very low and the pharmaceutical effects thereof are far less than those in fruiting body. The fruiting body of Antrodia could be only obtained by collecting from the field in the past, and was expensive and harmful to the ecological environment. Nowadays, wood segment cultivation has been developed and the fruiting body can be cultured by wood segments of cattle camphor (host of Antrodia), and Antrodia obtained in such cultivation is regarded as the most similar one with the Antrodia collected from the field and with the best pharmaceutical effects.

[0009] Because the fruiting body of Antrodia contains various physiologically active ingredients, exploring the applications of Antrodia to broaden the applications thereof remains to be exerted.

SUMMARY OF THE INVENTION

[0010] The invention provides use of an extract composition of fruiting body of Antrodia in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia, wherein the extract composition of fruiting body of Antrodia comprises a supercritical carbon dioxide fluid extract of fruiting body of Antrodia.

[0011] The use according to the invention is preferably in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia complicated with low number of white blood cells, or in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia complicated with body weight loss.

[0012] The invention also provides a pharmaceutical composition for treating low number of red blood cells and/or thrombocytopenia, which comprises pharmaceutically effective amount of an extract composition of fruiting body of Antrodia, wherein the extract composition of fruiting body of Antrodia comprises a supercritical carbon dioxide fluid extract of fruiting body of Antrodia.

[0013] The pharmaceutical composition according to the invention is preferably for treating low number of red blood cells and/or thrombocytopenia complicated with low number of white blood cells, or treating low number of red blood cells and/or thrombocytopenia complicated with body weight loss.

[0014] In another aspect, the extract composition of fruiting body of Antrodia further comprises a sesame extract.

[0015] The present invention is described in detail in the following sections. Other characteristics, purposes and advantages of the present invention can be found in the detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 shows results of administrating different concentrations of the extract composition of fruiting body of Antrodia (SR4) to THP-1 cells for 24 hours, and monitoring the effects on granulocyte-macrophage colony-stimulating factor secretion.

[0017] FIG. 2 shows results of administrating different concentrations of the extract composition of fruiting body of Antrodia (SR4s) to THP-1 cells for 24 hours, and monitoring the effects on granulocyte-macrophage colony-stimulating factor secretion.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The invention provides use of an extract composition of fruiting body of *Antrodia* in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia, wherein the extract composition of fruiting body of Antrodia comprises a supercritical carbon dioxide fluid extract of fruiting body of

Antrodia. The invention also provides a pharmaceutical composition for treating low number of red blood cells and/or thrombocytopenia, which comprises pharmaceutically effective amount of an extract composition of fruiting body of Antrodia, wherein the extract composition of fruiting body of Antrodia comprises a supercritical carbon dioxide fluid extract of fruiting body of Antrodia and optionally a pharmaceutical acceptable carrier or excipient.

[0019] The present invention can be more readily understood by reference to the following detailed description of various embodiments of the invention, the examples, and the chemical drawings and tables with their relevant descriptions. It is to be understood that unless otherwise specifically indicated by the claims, the invention is not limited to specific preparation methods, carriers or formulations, or to particular modes of formulating the extract of the invention into products or compositions intended for topical, oral or parenteral administration, because as one of ordinary skill in the relevant arts is well aware, such things can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0020] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meaning:

[0021] Often, ranges are expressed herein as from "about" one particular value and/or to "about" another particular value. When such a range is expressed, an embodiment includes the range from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the word "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to and independently of the other endpoint.

[0022] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where

said event or circumstance occurs and instances where it does not. For example, the phrase "optionally comprising an agent" means that the agent may or may not exist.

[0023] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context
5 clearly dictates otherwise. Thus, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular.

[0024] The term "subject" as used herein denotes any animal, preferably a mammal, and more preferably a human. The examples of subjects include humans, non-human primates, rodents, guinea pigs, rabbits, sheep, pigs, goats, cows, horses, dogs and cats.

[0025] The term "effective amount" of an active ingredient as provided herein means
10 a sufficient amount of the ingredient to provide the desired regulation of a desired function. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the disease state, physical conditions, age, sex, species and weight of the subject, the specific identity and formulation of the
15 composition, etc. Dosage regimens may be adjusted to induce the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. Thus, it is not possible to specify an exact "effective amount." However, an appropriate effective amount can be determined by one of ordinary skill
20 in the art using only routine experimentation.

[0026] The term "treating" or "treatment" as used herein denotes reversing, alleviating, inhibiting the progress of, or improving the disorder, disease or condition to which such term applies, or one or more symptoms of such disorder, disease or condition.

[0027] The term "carrier" or "excipient" as used herein refers to any substance, not
25 itself a therapeutic agent, used as a carrier and/or diluent and/or adjuvant, or vehicle for delivery of a therapeutic agent to a subject or added to a formulation to improve its

handling or storage properties or to permit or facilitate formation of a dose unit of the composition into a discrete article such as a capsule or tablet suitable for oral administration. Suitable carriers or excipients are well known to persons of ordinary skill in the art of manufacturing pharmaceutical formulations or food products.

5 Carriers or excipients can include, by way of illustration and not limitation, buffers, diluents, disintegrants, binding agents, adhesives, wetting agents, polymers, lubricants, glidants, substances added to mask or counteract a disagreeable taste or odor, flavors, dyes, fragrances, and substances added to improve appearance of the composition. Acceptable carriers or excipients include citrate buffer, phosphate buffer, acetate
10 buffer, bicarbonate buffer, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, magnesium carbonate, talc, gelatin, acacia gum, sodium alginate, pectin, dextrin, mannitol, sorbitol, lactose, sucrose, starches, gelatin, cellulosic materials (such as cellulose esters of alkanolic acids and cellulose alkyl esters), low melting wax cocoa butter, amino acids, urea, alcohols,
15 ascorbic acid, phospholipids, proteins (for example, serum albumin), ethylenediamine tetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), sodium chloride or other salts, liposomes, mannitol, sorbitol, glycerol or powder, polymers (such as polyvinyl-pyrrolidone, polyvinyl alcohol, and polyethylene glycols), and other pharmaceutically acceptable materials. The carrier should not destroy the
20 pharmacological activity of the therapeutic agent and should be non-toxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

[0028] Preferably, the extract composition of fruiting body of Antrodia is comprised in a composition. According to the invention, the composition is preferably a food composition or a pharmaceutical composition.

25 [0029] The extract composition of fruiting body of Antrodia can be added to a conventional food composition (i.e. the edible food or drink or precursors thereof) in the manufacturing process of the food composition. Almost all food compositions

can be supplemented with the extract composition of fruiting body of Antrodia of the invention. The food compositions that can be supplemented with the extract composition of fruiting body of Antrodia of the invention include, but are not limited to, candies, baked goods, ice creams, dairy products, sweet and flavor snacks, snack bars, meal replacement products, fast foods, soups, pastas, noodles, canned foods, frozen foods, dried foods, refrigerated foods, oils and fats, baby foods, or soft foods painted on breads, or mixtures thereof.

[0030] The pharmaceutical composition of the invention is preferably administered topically or systemically by any method known in the art, including, but not limited to, intramuscular, intradermal, intravenous, subcutaneous, intraperitoneal, intranasal, oral, mucosal or external routes. The appropriate route, formulation and administration schedule can be determined by those skilled in the art. In the present invention, the pharmaceutical composition can be formulated in various ways, according to the corresponding route of administration, such as a liquid solution, a suspension, an emulsion, a syrup, a tablet, a pill, a capsule, a sustained release formulation, a powder, a granule, an ampoule, an injection, an infusion, a kit, an ointment, a lotion, a liniment, a cream or a combination thereof. If necessary, it may be sterilized or mixed with any pharmaceutically acceptable carrier or excipient, many of which are known to one of ordinary skill in the art.

[0031] As used herein, the term "an extract composition of fruiting body of Antrodia" refers to a composition comprising an extract of fruiting body of Antrodia, particularly, comprising a supercritical carbon dioxide fluid extract of fruiting body of Antrodia.

[0032] As used herein, the term "Antrodia" (*Antrodia cinnamomea*) is a Taiwan endemic and perennial fungus, belonging to Basidiomycotina, Hymenomycetes, Aphyllophorales, Polyporaceae, and Antrodia, with scientific names of *Antrodia cinnamomea*, *Ganoderma comphoratum*, *Antrodia camphorata*, or *Taiwanofungus*

camphoratus.

[0033] As used herein, the term "a fruiting body of Antrodia" refers to a multi-cellular structure for sexual sporulation, with sporulation structures such as asci and basidia. The fruiting body is a sexual stage in growth cycle of Antrodia, and is
5 annual to perennial, bright red, orange or pale cinnamon, with camphor aroma. The fruiting body of Antrodia according to the present invention can be collected from cattle camphor trees or cultured by a wood segment derived from cattle camphor trees; preferably, cultured by a wood segment derived from cattle camphor trees.

[0034] The supercritical carbon dioxide fluid extract of fruiting body of Antrodia
10 according to the invention refers to an extract obtained by extracting the fruiting body of Antrodia with supercritical carbon dioxide fluid. The supercritical fluid refers to a homogenous fluid state finally obtained when the properties of gas and liquid get similar at a temperature higher than the critical temperature and a pressure higher than the critical pressure. Similar to gas, the supercritical fluid has compressibility, and
15 similar to fluid, has the fluidity, and can be used for extraction; moreover, a commercial supercritical fluid extraction equipment is available, for example, NATEX, SEPAREX, UHDE and TAIWAN SUPERCRITICAL TECHNOLOGY Co., Ltd. provide commercial supercritical fluid extraction equipment, of which the specifications are generally indicated by the capacity of the extraction tank, such as
20 500 g to 2000 kg for selection, so that a suitable supercritical fluid extraction equipment can be selected according to requirements.

[0035] In one embodiment of the invention, the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is obtained by extracting the fruiting body of Antrodia at a pressure of about 150 bar to about 1000 bar; preferably about 200 bar to
25 about 800 bar; more preferably about 350 bar to about 600 bar.

[0036] In one embodiment of the invention, the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is obtained by extracting the fruiting body of

Antrodia at a temperature of about 25°C to about 80°C; preferably about 35°C to about 70°C; more preferably about 40°C to about 65°C.

[0037] In one preferred embodiment of the invention, the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is extracted with a flux of carbon dioxide supercritical fluid of from about 20 kg/h to about 70 kg/h; more preferably from about 30 kg/h to about 60 kg/h; still more preferably from about 35 kg/h to about 55 kg/h.

[0038] In one preferred embodiment of the invention, the extraction time for the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is from about 40 minutes to about 120 minutes; more preferably from about 50 minutes to about 100 minutes.

[0039] In one preferred embodiment of the invention, the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is extracted in the existence of a co-solvent such as about 1% to about 10% of 95% ethanol.

[0040] According to the invention, in the process of preparing the supercritical carbon dioxide fluid extract of fruiting body of Antrodia, the weight ratio of fruiting body of Antrodia to supercritical carbon dioxide fluid is from about 10:1 to about 1:10, preferably, from about 5:1 to about 1:5; more preferably, from about 3:1, to about 1:3.

[0041] In one preferred embodiment of the invention, the fruiting body of Antrodia is first cut into small pieces; preferably, further blended the small pieces into powder. The manner of cutting and/or blending is well-known to artisans skilled in this field.

[0042] Preferably, the process of supercritical carbon dioxide fluid extracting further comprises a concentrating step. In one preferred embodiment of the invention, the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is obtained by extracting the fruiting body of Antrodia with supercritical carbon dioxide fluid, concentrating with a simulated moving bed and C18 solid adsorbent, and collecting a portion with Henry constant of more than 2.8. The simulated moving bed according

to the invention is for isolating and concentrating active ingredients of Antrodia according to polarity (Henry constant, H) of the active ingredients. The embodiment thereof is as described in the paragraphs of "concentrating step" of ROC Patent No. I487531, and the contents are incorporated by reference herein.

5 [0043] According to the present invention, the low number of red blood cells and/or thrombocytopenia refers to low number of red blood cells and/or thrombocytopenia caused by any reasons, such as being congenital or acquired, or caused by other diseases, or drugs. In one preferred embodiment of the invention, the low number of red blood cells and/or thrombocytopenia is caused by a drug; more preferably, the drug
10 is for treating tumor and has a side effect of bone marrow suppression, such as a chemotherapeutic agent for treating cancer. As used herein, the term "a chemotherapeutic agent" refers to an agent for inhibiting cancer cell division. The term "bone marrow suppression" refers to a side effect of affecting normal physiological conditions of bone marrow cells caused by chemotherapeutic agent
15 administration, such as lowering the numbers of white blood cells, red blood cells and/or platelets.

[0044] In one embodiment of the invention, the tumor includes but is not limited to malignant lymphoma, Wilm's tumor, head and neck squamous cell carcinoma, bone cancer, hypercalcemia associated with bone metastasis lesions and testicular cancer.

20 [0045] In one embodiment of the invention, the drug includes but is not limited to chlorambucil, cytarabine, dactinomycin, bleomycin, pilcamycin, etoposide, nitrosourea, and cyclophosphamid; preferably cyclophosphamid.

[0046] In one preferred embodiment of the invention, the extract composition of fruiting body of Antrodia further comprises a sesame extract. As used herein, the
25 term "sesame," also called as benne, belonging to Pedaliaceae family and Sesamum, with the scientific name of *Sesamum indicum*; preferably refers to seeds of sesame plants. As used herein, the term "a sesame extract" refers to a mixture obtained by

extracting active ingredients from sesame seeds. Examples of the extraction methods include, but are not limited to solvent extraction, supercritical fluid extraction, thermal extraction, and solid phase extraction; preferably supercritical fluid extraction; more preferably carbon dioxide supercritical fluid extraction. In one preferred embodiment of the present invention, the sesame extract comprises sesamin. As used herein, the term "sesamin" refers to a lignan compound of fat-soluble antioxidants.

[0047] According to the invention, the sesame and Antrodia can be extracted separately and then combined as the extract composition of fruiting body of Antrodia. For example, the sesame extract and the supercritical carbon dioxide fluid extract of fruiting body of Antrodia are prepared separately, and optionally added with carriers/excipients to obtain the extract composition of fruiting body of Antrodia. In one preferred embodiment of the invention, the weight ratio of the sesame extract to the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is from about 1:3 to about 3:1; preferably, the ratio is from about 1:2 to about 2:1. Alternatively, the sesame and the fruiting body of Antrodia can be combined and co-extracted with supercritical carbon dioxide fluid, and optionally added with carriers/excipients to obtain the extract composition of fruiting body of Antrodia. In one preferred embodiment of the invention, the weight ratio of the sesame to the fruiting body of Antrodia is from about 1:3 to about 3:1; preferably, the ratio is from about 1:2 to about 2:1.

[0048] Preferably, the extract composition of fruiting body of Antrodia according to the invention is for the treatment of low number of red blood cells and/or thrombocytopenia complicated with low number of white blood cells to comprehensively treat the side effects of bone marrow suppression, and to improve the side effects of conventional granulocyte-macrophage colony-stimulating factor treatment, such as reduced number of platelets and hemoglobin in spite of increased the numbers of inner peripheral blood neutrophils, monocytes and lymphocytes.

[0049] In one embodiment of the invention, the treatment of low number of red blood cells and/or thrombocytopenia includes but is not limited to increasing the total number of white blood cells, increasing the number of granulocytes, increasing the number of neutrophils, increasing the number of lymphocytes, increasing the number of monocytes and promoting granulocyte-macrophage colony-stimulating factor secretion.

[0050] In another aspect, the extract composition of fruiting body of Antrodia according to the invention effects on the treatment of low number of red blood cells and/or thrombocytopenia complicated with body weight loss. Because almost all cancer patients combine body weight loss, the extract composition of fruiting body of Antrodia according to the invention is able to treat body weight loss along with treating bone marrow suppression, and to widely assist the treatment of chemotherapeutic agents.

[0051] In one BALB/c mice animal model of the invention, the effects of the extract composition of fruiting body of Antrodia on the side effects of bone marrow suppression induced by cyclophosphamide (CTX) are monitored. On day 0, the mice are intraperitoneally administered with 300 mg/kg of a chemotherapeutic agent, CTX, for inducing bone marrow suppression in the experimental animals. The extract composition of fruiting body of Antrodia is orally administered (PO) from day 1 to day 4. The results show that the toxicity of CTX injection causes the number of red blood cells to drop on day 4, while orally administering the extract composition of fruiting body of Antrodia enhances the number of red blood cells up to 1.19 times, and the number of red blood cells is continually enhanced up to 1.23 times on day 7. On the other hand, the toxicity of CTX injection causes the number of platelets to drop significantly on day 7; compared to day 0, CTX injection causes about 50% decline in the number of platelets. Orally administering different concentrations of the extract composition of fruiting body of Antrodia has the ability to protect the number of

platelets.

[0052] For the low number of white blood cells, when the mice are administered a single dose of CTX (300 mg/kg), the number of white blood cells of the mice significantly decreases to a minimum value on day 4; the number of white blood cells begins to increase on day 7. Analyzing the data of day 4 and day 7, the number of white blood cells of the mice orally administrated with the extract composition of fruiting body of Antrodia also decreases to a minimum value on day 4 (about 86.7% to 91.5%). After administrated with CTX on day 0, the number of white blood cells of the mice in each group begins to increase on day 7. The number of white blood cells of the CTX group increases about three times. Administrating the extract composition of fruiting body of Antrodia significantly enhances the number of white blood cells.

[0053] The effects of the extract composition of fruiting body of Antrodia on sub-populations of granulocytes, neutrophils, monocytes and lymphocytes are further analyzed. The results show that, injecting a single dose of CTX (300 mg/kg) on day 0 results in decreases of granulocytes and neutrophils to a minimum values on day 4. The number of granulocytes begins to increase on day 7. The extract composition of fruiting body of Antrodia has the ability to significantly improve the number of granulocytes in 2 times. In another aspect, the extract composition of fruiting body of Antrodia has the ability to significantly improve the number of neutrophils in 2.93 times on day 7. Moreover, injecting a single dose of CTX (300 mg/kg) results in significant decreases of lymphocytes and monocytes on day 4. Meanwhile, orally administrating the extract composition of fruiting body of Antrodia improves the number of lymphocytes in 2.49 times on day 7. As to monocytes, the extract composition of fruiting body of Antrodia has the ability to improve the numbers of monocytes on day 7.

[0054] Regarding body weight loss, compared to a blank group, injecting a single

dose of CTX (300 mg/kg) results in body weight loss on day 3 (approximately about 10% of the decline). The mice receiving oral administration of the extract composition of fruiting body of Antrodia are observed to have the rise in body weight. Therefore, the extract composition of fruiting body of Antrodia has the ability to treat
5 body weight loss.

[0055] For the assessment of improving granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion, LPS-induced granulocyte-macrophage colony-stimulating factor secretion in monocytes is as a positive control group. With this test platform, the effect of the extract composition of fruiting body of Antrodia on GM-CSF
10 secretion is determined. The absorbance values at OD450 measured by ELISA are converted to the percentage based on 100% of GM-CSF amount secreted by THP-1 cell line itself. In the positive control group, the GM-CSF secreted by the LPS-induced THP-1 cells has an average increase of about 1.6 times. When the THP-1 cells treated with different concentrations of the extract composition of fruiting
15 body of Antrodia, the amount of GM-CSF generated correspondingly increases when the concentration of the extract composition of fruiting body of Antrodia increases. It shows that the amount of GM-CSF and the concentration of the extract composition of fruiting body of Antrodia have dose-dependent relationship, and the high concentration of 100 µg/mL of the extract composition of fruiting body of Antrodia
20 induces 1.3 times of GM-CSF secretion.

[0056] The following examples are provided to aid those skilled in the art in practicing the present invention.

EXAMPLES

Extract composition of fruiting body of Antrodia

25 Supercritical carbon dioxide fluid extract of fruiting body of Antrodia (SR4)

[0057] 105g of fruiting body of *Antrodia cinnamomea* is cut into small pieces with

an edge length of about 5 cm and placed in an extraction vessel of a supercritical extraction equipment. An internal pressure of the extraction vessel is set at 350 bar, with an internal temperature set at 50°C. 95% ethanol is used as a cosolvent, with a feed flow rate set at 3 ml/min. The weight ratio of the fruiting body of *Antrodia* to the supercritical carbon dioxide fluid is in a range of 20:80 to 40:60, and a flow rate of the supercritical carbon dioxide fluid is 35-55 kg/h. Since the extract of *Antrodia* is in a cream form containing 95% ethanol, an oven with a temperature of 30-35°C is used to remove ethanol from the cream-form extract of *Antrodia*, such that the following concentration step can be readily carried out.

[0058] During the concentrating step, a simulated moving bed is used as a continuously fed purification platform for separating the extract of *Antrodia cinnamomea* into several classes having different Henry constant. In the present embodiment, the stationary phase (SP) of the simulated moving bed is C18 column, and the mobile phase (MP) is a mixture of water and methanol, with said water containing 0.05% acetic acid. The extract of *Antrodia* is dissolved in the mobile phase. A weight ratio of water to methanol is preferably set in a range of 20:80 to 40:60, particularly at 22:78, so as to make the selectivity factor larger than 1.3. Hence, the component A and component B with different polarities can be sufficiently separated from each other.

[0059] The simulated moving bed of the present embodiment includes at least three separation zones, which sequentially are zone α , zone β and zone γ . A first outlet O_α (extract outlet) is arranged at the rear end of the zone α . A second outlet O_γ (raffinate outlet) is arranged at the rear end of the zone γ . An inlet I (feed inlet) is arranged between the zone β and the zone γ .

[0060] Each of the three zones α , β and γ is composed of two columns c, with the columns c in these three zones communicated with each other. The stationary phase

is filled in the column c. Particularly, spaces are formed between particles of the stationary phase for the mobile phase to flow therethrough. The stationary phase flows in a same direction through the columns of zone α , zone β and zone γ , sequentially. The inlet I is switched between different positions relative to the three separation zones at an inlet-switching interval T_{sw} by an inlet-switching device. The stationary phase thus moves in an opposite direction relative to the flowing direction of the mobile phase.

[0061] Based on the Triangle Theory of simulated moving bed, the desired separated components include the component A with Henry constant above 2.8 and the component B with Henry constant below 2.8. Within the three separation zones α , β and γ , the net flux F_A of component A and the net flux F_B of component B meet the criteria listed in Table 1, such that the component A moves toward the zone α and the component B moves toward the zone γ . Besides, the flow-rate ratio "n" of the three zones α , β and γ (flow-rate ratio n_α , n_β and n_γ of the zones α , β and γ , respectively) correlates to the polarity of the desired separated components (i.e. the Henry constant H_A of component A and the Henry constant H_B of component B). Each of the flow-rate ratios n_α , n_β and n_γ should meet the criteria listed in Table 1. Accordingly, the Henry constant H of each component is calculated according to Equation I, wherein T_0 is retention time of an unretained compound flowing through the column ($T_0 = 1.71$), T_r is retention time of the desired separated component, and ε is porosity of the column used in the embodiment.

$$H = \frac{T_r - T_0}{T_0} \times \frac{\varepsilon}{1 - \varepsilon} \quad (\text{Equation I})$$

Table 1: The criteria of net flux F , flow-rate ratio n and Henry constant H in each separation zone of in the present embodiment.

Zone	α	β	γ
Net flux F	$F_B > 0 ; F_A > 0$	$F_B > 0 ; F_A < 0$	$F_B > 0 ; F_A < 0$
Flow-rate ratio n	$n_\alpha > H_A > H_B$	$H_A > n_\beta > H_B$	$H_A > n_\gamma > H_B$

[0062] A liquid pump (HITACHI L-2130) is used to apply a pressure to the simulated moving bed of the embodiment, so as to make the mobile phase in the columns of each separation zone flow in the same direction. The inlet-switching interval T_{sw} is set to simulate the condition where the stationary phase moves in the opposite direction relative to the flowing direction of the mobile phase. The separation factors are set as Table 2 for the separation of the extract of Antrodia. The component A with Henry constant above 2.8 and the component B with Henry constant below 2.8 are respectively obtained from the first outlet O_a and the second outlet O_b , which are the supercritical carbon dioxide fluid extract of the fruiting body of Antrodia (SR4).

Extract composition of fruiting body of Antrodia (SR4s) comprising supercritical carbon dioxide fluid extract of fruiting body of Antrodia and sesame extract

[0063] 250g of sesame is placed in an extraction vessel of a supercritical extraction equipment. An internal pressure of the extraction vessel is set at 350 bar, with an internal temperature set at 50°C. 95% ethanol is used as a cosolvent, with a feed flow rate set at 3 ml/min. The weight ratio of the sesame to the supercritical carbon dioxide fluid is in a range of 50:91. Since the sesame extract is in a cream form containing 95% ethanol, an oven with a temperature of 30-35°C is used to remove ethanol from the cream-form sesame extract.

[0064] The above prepared sesame extract and supercritical carbon dioxide fluid extract of fruiting body of Antrodia are formulated according to the weight ratio of 7:9 to obtain the extract composition of fruiting body of Antrodia (SR4s).

Pharmaceutical effects of extract composition of fruiting body of Antrodia

Materials and methods

[Animal model]

[0065] A chemotherapeutic agent, cyclophosphamide (CTX), is administrated to

male BALB/c mice to enhance bone marrow suppression induced by the chemotherapeutic agent for monitoring if test articles improve the bone marrow suppression induced by the chemotherapeutic agent.

[Methods]

5 [0066] The animals are orally administrated with the test articles for analyzing the number changes of the white blood cells and sub-populations of granulocytes, neutrophils, monocytes and lymphocytes, red blood cells and platelets.

[Test articles]

[0067] The above prepared supercritical carbon dioxide fluid extract of fruiting body
10 of Antrodia (SR4) and the extract composition of fruiting body of Antrodia (SR4s).

[Designs]

Test articles and preparation thereof

[0068] CTX (Sigma Chemical Co. CAS Number: 6500-19-2 (St Louis, MO, USA)) and G-CSF (Filgrastim, Cat. 08502A, Kirin Brewery Co. Ltd., Japan) are
15 commercially available. CTX and normal saline are formulated to an intraperitoneal injection (IP) formulation with a final concentration of 45 mg/mL and administrated to each mouse at a dose of 300 mg/kg. The test articles of SR4s and SR4 are dissolved in DMSO (dimethyl sulfoxide, purchased from sigma) at a concentration of 125
20 mg/mL, and further diluted to a concentration of 1.25 mg/mL with water as an oral formulation, with the dose of 10 mg/Kg.

[Animal feeding and grouping]

[0069] The experimental animals are 6 to 8-week-old male BALB/c mice purchased from Nova Lok Biological Technology Co., rearing in $25 \pm 2^{\circ}\text{C}$ with humidity range of 40-70%, 12 hour light/dark cycle, and water *ad libitum*. After one week of
25 acclimation period, the mice are weighted and grouped randomly for no difference in the average body weight between the groups. The mice are divided into twelve groups and five in each group.

Chemotherapeutic agent CTX and test articles administration

[0070] On day 0, the mice are injected intraperitoneally with a chemotherapeutic agent, CTX, at the dose of 300 mg/kg for inducing bone marrow suppression. SR4s, SR4 are orally administered to the experiment groups on day 1 to day 4. During the test, the body weight of the mice are weighted and recorded every three days to observe changes in body weight of mice; at day 0, 4, and 7 day, blood samples are collected and observed.

Hematology analysis

[0071] The blood samples of bare handed animals are collect according to a lancet, facial vein method by puncturing a needle into the region behind the mandibula of the mice, and collecting the blood sample with a centrifuge tube. When enough amount of blood is collected, the wound is pressed with sterile gauze for 10-30 sec to stanch bleeding. The peripheral blood of mice collected is mixed well and 20 μ L of blood is five-fold diluted and analyzed with XT-1800i hematology analyzer (SYSMEX) for quantitative detection of blood cells.

Results

SR4s and SR4 treating the low number of red blood cells and thrombocytopenia induced by CTX

[0072] As shown in Tables 1 and 2, the toxicity of CTX injection causes the number of red blood cells to drop on day 4, while orally administrating SR4s enhances the number of red blood cells up to 1.19 times, and orally administrating SR4 enhances the number of red blood cells up to 1.18 times. On day 7, the number of red blood cells is continually enhanced up, while orally administrating SR4s enhances the number of red blood cells up to 1.17 times, and orally administrating SR4 enhances the number of red blood cells up to 1.23 times. The effect of injecting a single dose of CTX (300 mg/kg) on the number of platelets is shown in Table 2. The toxicity of CTX injection causes the number of platelets to drop significantly on day 7; compared to day 0, CTX

injection causes about 50% decline in the number of platelets. Orally administrating different concentrations of SR4s and SR4 has the ability to protect the number of platelets, especially SR4.

Table 1: Results of hematology analysis on day 4

Day 4 average±SD	White blood cells%	Sub-populations				Red blood cell %	Platelet %
		granulocyte %	neutrophil %	lymphocyte %	monocyte %		
blank	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CTX	16.21	17.25	24.35	14.53	9.91	92.21	50.95
CTX+SR4	20.03	17.77	6.03	15.39	0.18	108.40	493.56
CTX+SR4s	12.03	23.68	11.51	8.83	10.04	110.14	83.94

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Table 2: Results of hematology analysis on day 7

Day 7 average±SD	White blood cells%	Sub-populations				Red blood cell %	Platelet %
		granulocyte %	neutrophil %	lymphoc yte %	monocyte %		
blank	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CTX	48.04	93.68	113.84	26.38	108.17	99.61	61.71
CTX+SR4	119.41	300.16	334.10	65.66	182.44	121.81	715.28
CTX+SR4s	70.40	186.14	226.49	26.19	156.10	116.57	215.96

SR4s and SR4 treating the low number of white blood cells and sub-populations of granulocytes, neutrophils, monocytes and lymphocytes induced by CTX

[0073] As shown in Tables 1 and 2, injecting a single dose of CTX (300 mg/kg) results in the decreases of white blood cells to a minimum values on day 4. The number of white blood cells begins to increase on day 7. Analyzing the data of day 4 and day 7, the number of white blood cells of the mice orally administrated with SR4s

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and SR also decreases to a minimum value on day 4 (about 86.7% to 91.5%). After administrated with CTX on day 0, the number of white blood cells of the mice in each group begins to increase on day 7. The number of white blood cells of the CTX group increases about three times. Administrating SR4s and SR4 significantly enhances the number of white blood cells (7 times and 5 times, respectively).

[0074] Further analyzing the effects of SR4s and SR4 on sub-populations of granulocytes, neutrophils, monocytes and lymphocytes. As shown in Tables 1 and 2, injecting a single dose of CTX (300 mg/kg) on day 0 results in decreases of granulocytes and neutrophils to a minimum values on day 4. The number of granulocytes begins to increase on day 7. SR4s has the ability to significantly improve the number of granulocytes in 2 times. In another aspect, orally administrating SR4 has the ability to significantly improve the number of neutrophils in 2.93 times on day 7; while orally administrating SR4s has the ability to significantly improve the number of neutrophils in 1.99 times on day 7.

[0075] Moreover, injecting a single dose of CTX (300 mg/kg) results in the significant decreases of lymphocytes and monocytes on day 4. Meanwhile, orally administrating SR4 improves the number of lymphocytes in 2.49 times on day 7. As to monocytes, SR4s has the ability to improve the number of monocytes on day 7.

SR4s and SR4 treating body weight loss induced by CTX

[0076] Regarding body weight loss, compared to the blank group, injecting a single dose of CTX (300 mg/kg) results in body weight loss on day 3 (approximately about 10% of the decline). The mice receiving oral administration of SR4 are observed to rise in body weight. Therefore, the extract composition of fruiting body of Antrodia has the ability to treat body weight loss.

Extract composition of fruiting body of Antrodia enhancing granulocyte-macrophage colony-stimulating factor secretion

Materials and methods

[Materials]

Cell line: THP-1 cell line [ATCC-TIB-202]

Agents for cell culture and assay: RPMI-1640 medium, fetal calf serum (FCS), 2-mercaptoethanol, lipopolysaccharide (LPS, purchased from Sigma), Human
5 GM-CSF ELISA kit (Biolegend)

[Methods]

THP-1 cell culture

[0077] THP-1 cells is a suspension human monocytic leukemia cell line, growing in RPMI medium containing 10% FCS and 2-mercaptoethanol at 37°C and 5% CO₂.

10 The medium is changed every 2 to 3 days. When the cell culture bottles are covered approximately 80% by the cells, the cells are subcultured. Vi-cell cell counter is used for counting the number of cells, and an appropriate number of cells are collected for assay.

[Test articles]

15 [0078] SR4 and SR4s are both dissolved in DMSO at a concentration of 125 mg/ml and stored at 4°C. The test articles are diluted to a desired concentration when assay.

LPS (10 mg/ml) is dissolved in 1x PBS and stored at -20°C. LPS is diluted to a desired concentration when assay.

THP-1 cells as a platform for assaying GM-CSF

20 [0079] 4×10^5 cells/well of THP-1 cells are inoculated on a 12-well plate and cultured for 6 hours. The cells are divided into a control group (0 µg/ml), LPS group (1 µg/ml) and experiment groups (treated with 0.01, 0.1, 1, 10, and 100 µg/ml of test articles). Each assay is performed in triplicate and cultured at 37°C, 5% CO₂ for 24 hours. After culturing, the cells are centrifuged at 1000 rpm and 4°C for 5 minutes

to collect the supernatant. The amount of GM-CSF secreted by the cells is assayed with GM-CSF ELISA kit.

Human GM-CSF ELISA assay

[0080] Human GM-CSF ELISA kit (Biolegend) is applied for assaying GM-CSF.

5 100 μ l/well of capturing antibodies are coated on a 96-well plate and incubated at 4°C for 12 to 16 hours, and then warmed to room temperature. The coated 96-well plate is washed with a washing buffer and blocked at 200 μ l/well at room temperature for 1 hour and then washed with the washing buffer again. A standard solution of 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 pg/ml are prepared. The test articles and the series
10 diluted standard solution are added onto the 96-well plate and incubated at room temperature for 2 hours. After washing, detecting antibodies are added and incubated at room temperature for 1 hour. After washing, 100 μ l/well of substrate solution (coloring agent A+B) is added in dark at room temperature for 30 minutes and a stop solution was added to terminate the reaction. The absorbance values at OD450 is
15 measured with the ELISA reader, and the values are converted using R-squared value of the standard curve by the interpolation method to calculate the concentration of GM-CSF.

Results

[0081] LPS-induced GM-CSF secretion in monocytes is as the positive control
20 group. With this test platform, the effects of SR4s and SR4 on GM-CSF secretion are determined and shown in FIGs. 1 and 2. The absorbance values at OD450 measured by ELISA are converted to the percentage based on 100% of GM-CSF amount secreted by THP-1 cell line itself. In the positive control group, the GM-CSF secreted by the LPS-induced THP-1 cells has an average increase of about 1.6 times.
25 When the THP-1 cells treated with different concentrations of SR4s, the amount of GM-CSF generated correspondingly increases when the concentration of SR4s increases. It shows that the amount of GM-CSF and the concentration of SR4s have

dose-dependent relationship, and the high concentration of 100 µg/mL of SR4s induces 1.3 times of GM-CSF secretion.

[0082] While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives thereto and modifications and
5 variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are regarded as falling within the scope of the present invention.

CLAIMS

What is claimed is:

1. Use of an extract composition of fruiting body of Antrodia in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia, wherein the extract composition of fruiting body of Antrodia
5 comprises a supercritical carbon dioxide fluid extract of fruiting body of Antrodia.

2. The use according to claim 1, wherein the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is obtained by extracting the fruiting body of Antrodia at a pressure of about 150 bar to about 1000 bar and at a temperature of about
10 25°C to about 80°C.

3. The use according to claim 2, wherein the supercritical carbon dioxide fluid extract of fruiting body of Antrodia is obtained by extracting the fruiting body of Antrodia with supercritical carbon dioxide fluid, concentrating with a simulated moving bed and C18 solid adsorbent, and collecting a portion with Henry constant of
15 more than 2.8.

4. The use according to claim 1, which is in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia caused by a drug.

5. The use according to claim 4, wherein the drug is for treating tumor and has
20 a side effect of bone marrow suppression.

6. The use according to claim 5, wherein the tumor is selected from the group consisting of malignant lymphoma, Wilm's tumor, head and neck squamous cell carcinoma, bone cancer, hypercalcemia associated with bone metastasis lesions and testicular cancer.

7. The use according to claim 5, wherein the drug is selected from the group consisting of chlorambucil, cytarabine, dactinomycin, bleomycin, pilcamycin, etoposide, nitrosourea, and cyclophosphamid.

8. The use according to claim 1, wherein the extract composition of fruiting
5 body of Antrodia further comprises a sesame extract.

9. The use according to any of claims 1 to 8, which is in the manufacture of a medicament of the treatment of low number of red blood cells and/or thrombocytopenia complicated with low number of white blood cells.

10. The use according to claim 9, which is in the manufacture of a medicament
10 of increasing the total number of white blood cells, increasing the number of granulocytes, increasing the number of neutrophils, increasing the number of lymphocytes, increasing the number of monocytes and/or promoting granulocyte-macrophage colony-stimulating factor secretion.

11. The use according to any of claims 1 to 8, which is in the manufacture of a
15 medicament of the treatment of low number of red blood cells and/or thrombocytopenia complicated with body weight loss.