

A sanguisorba tannins extract, and preparation method and use thereof

Technical Field

The present invention belongs to pharmaceutical field, and relates to a sanguisorba tannins extract, and preparation method and use thereof.

Background

Bone marrow inhibition refers to the decrease of activity of blood cell precursor in bone marrow. Both white cells and red cells in blood originate from hemopoietic stem cells in bone marrow. Blood cells in blood always need to be continually replenished due to their short life. In order to achieve the purpose of timely supplements, stem cells as the precursor of blood cells must rapidly divide. Chemotherapy, radiotherapy and various other antitumor therapies are directed to rapidly dividing cells, which always result in the inhibition of normal bone marrow cells, causing bone marrow hematopoiesis functional disturbance of patients, and further decreasing red cells, white cells and platelets of patients, thus leading to the phenomena such as anemia, bleeding and declining in immune function.

Leucopenia is a common hematological disorder, and means a situation of white cells in peripheral blood continuously less than $4 \times 10^9/L$. White cells are used for defending bacteria. With white cells decreasing, antibacterial ability in the body will be weakened, thus enabling people easily vulnerable. Especially, immunosuppressant used in radiotherapy, chemotherapy, infected, rheumatism, systemic lupus erythematosus and anti-TB drug rifampicin used in infectious diseases can all result in the decrease of white cells in peripheral blood, thus weakening antibacterial ability in the body and causing side effects such as poor immune function. Currently, leucopenia mainly comprises two types, i.e., unexplained leucopenia and secondary leucopenia. The former is common, while the latter is always caused by bone marrow injury which may be the result of radiotherapy, chemotherapy, infection, immune factors, etc.

Although leucopenia and bone marrow inhibition both present a significant phenomenon of white cells decrease, they also have some differences in utilizing of

drugs. Currently, some drugs with the function of increasing white cells have no function of protecting bone marrow. For example, rhG-CSF has a strong effect on increasing white cells, but a non-ignored negative influence on bone marrow cells. Hence, it is necessary to find out a drug which can increase white cells concentrations and as well effectively protect bone marrow cells.

Sanguisorba originates from the dried roots of *Rosaceae* plants, *Sanguisorba officinalis* L. or *Sanguisorba officinalis* L. var. *longifolia* (Bert.) Yu et Li, with the functions of cooling blood and hemostasis, detoxification and sore healing. Garden Burnet Root (scientific name: *Radix Sanguisorbae*) comprises a large amount of tannins (about 20%), and further comprises compounds such as saponins and flavones. Currently, most researches focus on Sanguisorba focus on Sanguisorba saponins. It is generally believed that Sanguisorba saponins are active ingredients in Sanguisorba to increase white cells and protect bone marrow. Domestic fundamental research on Sanguisorba increasing white cells believed that Sanguisorba saponins are main activity parts to enhance the proliferation in vitro of bone marrow cells of mice; moreover, Sanguisorba saponins also can increase the numbers of white cells, red cells and platelets of bone marrow inhibition of mice. However, Sanguisorba tannins and Sanguisorba flavones can't promote bone marrow cells proliferation, instead cause bone marrow inhibition in high concentrations. (Gao Xiaoping *et al.* Screen of active parts for Sanguisorba hematopoietic function, Chinese Journal of Natural Medicines, Volume 4(2), 2006).

Currently, researches on efficacy of sanguisorba tannins are relatively rare. It is reported in foreign literatures that sanguisorba tannins have anti-cancer functions [Bastow kF. Bort LD. Fukushrma Y *et al.* Inhibition of DNA topoisomerases by sanguim H-6, a cytotoxic dimeric ella-gitan-ton from sanguisorba officinalis. Planta Med, 1993; 59(3): 240-245.]. There are some reports about the sanguisorba tannins extract, for example, sanguisorba tannins extract containing 43.86% tannins is obtained through extraction with 70% hydrous acetone (Gao Xiaoping *et al.* Chinese Journal of Natural Medicines, Volume 4(2), 2006). Extracted by using acetone but without separation, the sanguisorba tannins extract obtained by the above process not only contains tannins, but also contains saponins, which comprises 15% saponins by detection. Wang Manli *et al.* separated sanguisorba tannins from sanguisorba saponins by using an aluminium oxide column, and can finally achieve the separation (Journal of Guizhou Institute of Technology, Volume 22(2), 1993).

As yet, no report related to applying sanguisorba tannins into bone marrow protection and leucopenia therapy has been known.

Summary of the Invention

The present invention aims to provide a sanguisorba tannins extract, and further aims to provide new uses of said sanguisorba tannins extract and compounds contained in Sanguisorba tannins.

The present invention provides a sanguisorba tannins extract, wherein said extract comprises 35%-100%(w/w) of tannins, less than 10% (w/w) of total saponins, and less than 5% (w/w) of sanguisorbin I.

Preferably, said extract contains 50%-100% (w/w) of tannins, more preferably 55%-100% (w/w) of tannins.

Preferably, said extract comprises less than 7.2% (w/w) of total saponins, and less than 4.9% (w/w) of sanguisorbin I. Preferably, said extract comprises less than 0.001% (w/w) of total saponins, and less than 0.0001% (w/w) of sanguisorbin I.

Further, sanguisorba tannins comprise 0.05% to 3.0% by weight of sanguin, 1.0% to 5.0% by weight of catechin, and 0.05% to 1.5% by weight of proanthocyanidin B2, all calculated based on said extract. Further preferably, said sanguisorba tannins comprise 1.0% to 2.0% by weight of sanguin, 2.0% to 4.0% by weight of catechin, and 0.05% to 0.4% by weight of proanthocyanidin B2, all calculated based on said extract.

Further, said extract contains 62%-100% (w/w) of tannins.

Said extract originates from dried roots of *Sanguisorba officinalis* L or *Sanguisorba officinalis* L. var.*longifolia* (Bert.) Yu et Li of Rosaceae.

Said extract can be prepared by the following steps:

1) extraction: grinding Garden Burnet Root (scientific name: *Radix Sanguisorbae*) into coarse powders, extracting it with water, alcohol, hydrous ethanol, acetone or hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: treating the concentrated solution obtained in step 1) with common tannins separation method, thus obtaining the sanguisorba tannins extract.

Said common tannins separation method comprises chromatography method, protein precipitation method, solvent method or the combination thereof.

Further, said chromatography method is adsorption chromatography. Preferably in said chromatography method the absorption is carried out with gel and macroporous adsorption resin.

Further, in said protein precipitation method the precipitation is carried out with gelatin.

Further, in said solvent method, aqueous solution containing the concentrated solution is defatted and then extracted by ethyl acetate so as to obtain the sanguisorba tannins extract;

alternatively, the concentrated solution is dissolved in ethanol and ethyl acetate, and the extract is precipitated through adding ethyl ether or petroleum ether.

Moreover, said extract is prepared by the following steps:

1) extraction: grinding Garden Burnet Root (scientific name: *Radix Sanguisorbae*) into coarse powders, extracting it with water, alcohol, 10%-90% hydrous ethanol, acetone or 50%-90% hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: adsorbing and purifying the concentrated solution with macroporous adsorption resin, wherein it is eluted with water into colorless, then with 10% ethanol, finally with 60% ethanol, and collecting the elution with 60% ethanol, thus obtaining the sanguisorba tannins extract after drying;

alternatively, defatting the concentrated solution and then extracting it with ethyl acetate, thus obtaining the sanguisorba tannins extract.

Preferably, the concentration of said hydrous ethanol is 70%; the concentration of said hydrous acetone is 70%.

Said macroporous adsorption resin is non-polarity resin or low-polarity resin. Preferably, said macroporous adsorption resin is DA-201 macroporous adsorption resin, D-101 macroporous adsorption resin, LSA-20 macroporous adsorption resin, HP-10 macroporous adsorption resin or AB-8 macroporous adsorption resin.

The present invention also provides a process for preparing said sanguisorba tannins extract, the process comprising:

1) extraction: grinding Garden Burnet Root (scientific name: *Radix Sanguisorbae*) into coarse powders, extracting it with water, alcohol, hydrous ethanol, acetone or hydrous acetone, and obtaining a concentrated solution after filtration and concentration ;

2) purification: treating the concentrated solution with common tannins separation method, thus obtaining the sanguisorba tannins extract.

Said common tannins separation method comprises chromatography method, protein precipitation method, solvent method or the combination thereof.

Further, said chromatography method is adsorption chromatography, preferably in said chromatography method the absorption is carried out with gel and macroporous adsorption resin.

Further, in said protein precipitation method the precipitation is carried out with gelatin.

Further, in said solvent method, aqueous solution containing the concentrated solution is defatted and then extracted by ethyl acetate so as to obtain the sanguisorba tannins extract;

alternatively, the concentrated solution is dissolved in ethanol and ethyl acetate, and the extract is precipitated through adding ethyl ether or petroleum ether.

Moreover, said extract is prepared by the following steps:

1) extraction: grinding Garden Burnet Root (scientific name: *Radix Sanguisorbae*) into coarse powders, extracting it with water, alcohol, 10%-90% hydrous ethanol, acetone or 50%-90% hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: adsorbing and purifying the concentrated solution with macroporous adsorption resin, wherein it is eluted with water into colorless, then with 10% ethanol, finally with 60% ethanol, and collecting the elution with 60% ethanol, thus obtaining the sanguisorba tannins extract after drying;

alternatively, defatting the concentrated solution and then extracting it with ethyl acetate, thus obtaining the sanguisorba tannins extract.

Preferably, the concentration of said hydrous ethanol is 70%; the concentration

of said hydrous acetone is 70%.

Said macroporous adsorption resin belongs to non-polarity resin or low-polarity resin. Preferably, said macroporous adsorption resin is DA-201 macroporous adsorption resin, D-101 macroporous adsorption resin, LSA-20 macroporous adsorption resin, HP-10 macroporous adsorption resin or AB-8 macroporous adsorption resin.

The present invention also provides the use of said sanguisorba tannins extract for manufacturing medicaments for protecting bone marrow.

Said medicaments are used to prevent or/and treat bone marrow inhibition.

Further, said medicaments are used to prevent or/and treat normal bone marrow cells inhibition caused by radiotherapy or chemotherapy.

Moreover, said medicaments are used to prevent or/and treat anemia, leucopenia, or thrombocytopenia caused by radiotherapy or chemotherapy, preferably to prevent or/and treat leucopenia, caused by radiotherapy or chemotherapy.

The present invention further provides the use of said sanguisorba tannins extract for manufacturing medicaments for the treatment of leucopenia.

The present invention further provides the use of sanguin and proanthocyanidin B2 for manufacturing medicaments for protecting bone marrow.

Said medicaments are used to prevent or/and treat bone marrow inhibition.

Further, said medicaments are used to prevent or/and treat normal bone marrow cells inhibition caused by radiotherapy or chemotherapy.

Moreover, said medicaments are used to prevent or/and treat anemia, leucopenia, or thrombocytopenia caused by radiotherapy or chemotherapy, preferably to prevent or/and treat leucopenia, caused by radiotherapy or chemotherapy.

The present invention further provides the use of sanguin, proanthocyanidin B2 for manufacturing medicaments for the treatment of leucopenia.

The present invention also provides a medicinal composition for preventing or treating bone marrow inhibition, leucopenia, comprises an effective amount of sanguin, proanthocyanidin B2 or said sanguisorba tannins extract as active ingredients, and is prepared to common formulation by adding pharmaceutically acceptable supplementary materials or assisted ingredients.

Said formulation is an oral formulation.

The present invention further provides the use of tumor chemotherapeutics and said sanguisorba tannins extract for manufacturing medicaments for the combination treatment of anti-tumor.

Said tumor chemotherapeutics are alkylating agent.

Further, said tumor chemotherapeutics are cyclophosphamides.

The present invention also provides a medicinal composition for anti-tumor, wherein it is an formulation prepared from an effective amount of tumor chemotherapeutics, the sanguisorba tannins extract according to any one of claims 1-16 as active ingredient, and pharmaceutically acceptable adjuvant.

Said tumor chemotherapeutics are alkylating agent.

Further, said tumor chemotherapeutics are cyclophosphamides.

The active ingredient in the sanguisorba tannins extract according to the present invention is sanguisorba tannins, which can protect bone marrow hematopoietic stem cells, have remarkable protective effects on the inhibition of mouse bone marrow DNA caused by chemical substances and radiotherapy, thus providing a new choice for relieving inhibition of normal bone marrow cells caused by radiotherapy and chemotherapy. In addition, sanguisorba tannins can also effectively increase white cells. The sanguisorba tannins extract according to the present invention, when used in combination with chemotherapeutics, can not only improve the curative effect of anti-tumor, but protect bone marrow cells and whole blood cells, reduce or avoid the damages to bone marrow cells and whole blood cells caused by chemotherapeutics. Therefore, before, after, or during the treatment of cancer with radiotherapy or chemotherapy, the sanguisorba tannins extract according to the present invention can be used in combination with radiotherapy and chemotherapeutics, in order to reduce

or avoid the injury to bone marrow cells and white cells of patients caused by radiotherapy or chemotherapy.

Specific Embodiments of the Invention

1. Measurement of the content of Sanguisorba tannins in the present invention.

The content of tannins is measured according to Appendix XB of *Chinese Pharmacopoeia* (Edition 2010, Volume I).

The experiment should be performed away from light.

Preparation of reference solutions. 50 mg of gallic acid as control substance is weighed out accurately and added into a 100ml brown volumetric flask. Water is added to dissolve the gallic acid and dilute it until the solution reaches the graduation line. 5ml of the solution is weighed out accurately and placed into a 50ml brown volumetric flask. Water is added to dilute the solution until it reaches the graduation line. The solution is then shaken well, thus obtaining the control solution with 0.05 mg gallic acid per 1 ml solution.

Preparation of standard curve. 0.5ml, 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml of control solutions are weighed out accurately and added into six 25ml brown volumetric flasks respectively. 1ml of phosphomolybdium tungstic acid solution is added to each of the above flasks, and then 11.50ml, 11ml, 10ml, 9ml, 8ml and 7ml of water are added therein respectively. Each solution is diluted with 29% sodium carbonate solution to reach the graduation line and shaken well. After the solution is stood for 30 min, the absorbency at a wavelength of 760nm is determined according to UV-VIS spectrophotometry (Appendix VA) with the corresponding reagent as the blank test. Then, the standard curve can be obtained with the absorbency as Y-axis against the concentration as X-axis.

Preparation of sample solutions. 0.1g of extract powders are weighed out accurately and added into a 250-ml brown volumetric flask. 150ml of water is added in the flask. The solution is stood for night and then treated by ultrasonic for 10 min. After being cooled, it is diluted with water to reach the graduation line. The solution is shaken well and stood so as to enable solids to be precipitated from the solution. The solution is filtered and 50ml of preliminary filtrate is abandoned, whereas 20ml of residual filtrate is weighed out accurately and then transferred into a 100ml brown

volumetric flask. Then, the obtained solution is diluted with water to reach the graduation line and shaken well, thus obtaining the sample solution.

Measurement

Total phenols. 2ml of sample solution is weighed out accurately and added into a 25ml brown volumetric flask. According to the method provided in the part of “preparation of standard curve”, after “1ml of phosphomolybdenum tungstic acid solution is added”, 10ml of water is added. The absorbancy is determined in a conventional manner, and the weight (mg) of gallic acid in the sample solution can be read out from the standard curve. Thus the content of total phenols can be obtained by calculation.

Non-adsorbed polyphenols. 25ml of sample solution is weighed out accurately and then added to a 100ml conical flask filled with 0.6g of casein. The flask is sealed with a cover and placed in a water bath with a temperature of 30°C for 1h. The flask is constantly shaken, and then removed from the water bath and cooled. The solution is shaken well and filtered, and the preliminary filtration is abandoned. 2ml of residual filtration is weighed out accurately, and then transferred into a 25ml brown volumetric flask. According to the method provided in the part of “preparation of standard curve”, after “1ml of phosphomolybdenum tungstic acid solution is added”, 10ml of water is added. The absorbancy is determined in a conventional manner, and the weight (mg) of gallic acid in the sample solution can be read out from the standard curve. Thus the content of the non-adsorbed phenols can be obtained by calculation.

The content of tannins is calculated based on the following formula: the amount of tannins = the amount of total phenols – the amount of non-adsorbed polyphenols.

2. Method for measuring the content of sanguin, catechin and proanthocyanidin B2

See, LIU Haiying, Component analysis and Function research on Sanguisorba polyphenols, Shaanxi Normal University, 2009. Details are as follows.

2.1 Instruments and Experimental materials

2.1.1 Instruments

Agilent 1200 high performance liquid chromatography (Agilent 1200 diode array detector; Quaternary pump online degassing systems; Agilent 1200 chromatography workstation) (Agilent Technologies);

Mettler AE240 one over hundred-thousand electronic analytical balance (Mettler, Germany);

FA1104 one over ten-thousand electronic analytical balance (Shanghai analytical balance Electronic Instrument Co., Ltd.);

KQ3200-type ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd)

2.1.2 Reagent

Proanthocyanidin B2 (Purity: at least 99% by HPLC)

Catechin (Purity: at least 99% by HPLC)

Sanguin (Purity: at least 99% by HPLC)

Methanol is HPLC grade;

Water produced by redistilling (self-made);

All other reagents used herein are analytical grade.

2.1.3 Sanguisorba tannins extract

The sanguisorba tannins extract is prepared according to the following examples.

2.2 Preparation of sample solution

1g of sanguisorba tannins extract is weighed out accurately, and transferred to a 100ml volumetric flask. 90ml of water is added, and the solution is treated by ultrasonic for 30min (with 250W of power and 20KHz of frequency rate). After treatment, the solution is cooled and diluted with water to reach the graduation line, and then filtered.

2.3 Chromatography conditions

2.3.1 The selection of detection wavelength

In order to maximize information from the chromatogram and display the whole

components of sanguisorba as many as possible, the components should be generally separated from each other using binary gradient elution of methanol-phosphate buffered saline at a column temperature of 30°C. All the chromatogram-spectrum information having chromatogram components falling into the spectrum region at the range of 195nm-400nm are collected with a diode array detector (DAD). Based on the absorption status of peaks in the chromatogram at each wavelength observed, it is found that the absorption of each peak is higher at 265nm, and the baseline of spectra is smooth. Therefore, the detection wavelength is finally decided as 265nm.

2.3.2 Chromatography conditions

In the experiment, several mobile phases are adopted in order to determine the optimal mobile phase. See Table 1.

Table 1 Mobile phase and Elution procedure

time (min)	methanol (%)	0.05% phosphate buffered saline (%)
0	5	95
80	45	55
90	55	45
91	5	95
100	5	95

2.3.3 Durability

Several sanguisorba tannins extracts from one batch are tested by using three chromatographic columns from different manufacturers under different brands respectively. Resolution and numbers of chromatographic peaks are used to evaluate the durability of instruments.

In the selected chromatographic conditions, the chromatogram and the resolution of each peak obtained from the annular column is superior to those from other two chromatographic columns. Therefore, the annular column is adopted.

In view of the foregoing, chromatography conditions are determined as follows:

Annular chromatographic column (4.6mm*250mm, 5 μ m); DAD detector; Gradient elution procedure with methanol-0.05% phosphate buffered saline: 0 min→80min, Methanol 5%→45%, 0.05% Phosphate buffered saline 95%→55%;

80min→90min, Methanol 45%→55%, 0.05% Phosphate buffered saline 45%→95%;
91min→100min, Methanol 5%→5%, 0.05% Phosphate buffered saline 95%→95%;
Detection wavelength: 265nm; Column temperature: 30°C; Flow rate: 1.0ml/min;
Run time: 100min.

2.3.4 Specialization

Sample solution is prepared in accordance with the above Item 2, and the detection is conducted as in accordance with the chromatographic method in the following Item “3”. Chromatographic peaks, its reserved time and peak areas within 90min are recorded. 10μl of water solution and 10μl of mixed control solution are accurately weighted out respectively, and then injected into a HPLC system. The chromatogram is recorded in 90min, and the chromatogram of each control substance is also recorded. Results show that no interference is observed under this condition.

2.3.5 Measurement of content

3. Measurement of total saponins and sanguisorbin I in the sanguisorba tannins extract

The measurement method is as follows.

1) Measurement of the content of total saponins

Preparation of control solution. 50mg of sanguisorbin I is added in a 10ml volumetric flask. Then about 9ml of anhydrous ethanol is added to dissolve the sanguisorbin I by ultrasonic. After being cooled, the solution is diluted to reach the graduation line with anhydrous ethanol, thus obtaining the control solution (0.5mg per 1ml).

Preparation of Standard curve. 0, 200μl, 300μl, 400μl, 500μl, 600μl, 800μl, and 1000μl of the control solution is sucked into eight of 10ml test tubes with cover respectively. 1ml of 8% newly-prepared Vanilin-ethanol solution (obtained by diluting 8g of Vanilin with anhydrous ethanol to 100ml) and 10ml of 70% sulfuric acid (obtained by slowly adding 70ml of sulfuric acid into 30ml of water) are accurately added in each tube, mixed well and sealed. Each tube is placed in a water bath with a temperature of 60°C for 15 min, then cooled in ice water for 2min, and mixed well. The absorbency at a wavelength of 530nm is determined according to the

spectrophotometry (Appendix VIB) with the colorimetric solution with control substance as the blank test. Then, the standard curve can be obtained with the absorbency as Y-axis against the concentration as X-axis.

Preparation of sample solution. About 5g of sanguisorba tannins extract is ground, and weighed out accurately and put in a 250ml volumetric flask. About 200ml of anhydrous ethanol is added and the solution is extracted by ultrasonic for 30min, and then filtered. The filtrate is collected in a 250ml volumetric flask, and diluted to reach the graduation line with anhydrous ethanol.

Measurement

Accurately 1ml of sample solution is weighed out and transferred to a 10ml test tube with a cover. The absorbance is determined according to the method in the part of preparation of standard curve, and calculated in the regression equation.

2) Measurement of the content of sanguisorbin I

Chromatography conditions. Chromatographic column: Agilent-Zorbax (4.6mm*250mm, 5 μ m); mobile phase, acetonitrile: water (32:68); flow rate: 1.0ml/min; column temperature: 30°C; evaporative light-scattering detector.

Preparation of control solution. 25mg of sanguisorbin I is added in a 25ml volumetric flask. Then about 20ml of anhydrous ethanol is added to dissolve the sanguisorbin I by ultrasonic. After being cooled, the solution is diluted to reach the graduation line with anhydrous ethanol, thus obtaining the control solution (1mg per 1ml).

Preparation of standard curve. 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml of the above control solutions are sucked into five of 5ml volumetric flasks respectively. Each solution is diluted to reach the graduation line with anhydrous ethanol. Accurately 10 μ l of each solution is used for measurement under the above chromatography conditions. A standard curve is obtained with the natural logarithm of peak area as Y-axis and the natural logarithm of sample amount as X-axis.

Preparation of sample solution. About 5g of sanguisorba tannins extract is ground, and weighed out accurately and transferred in a 100ml volumetric flask. About 90ml of anhydrous ethanol is added and the solution is extracted by ultrasonic

for 30min. After being cooled, the solution is diluted to reach the graduation line with anhydrous ethanol, shaken well, and then filtered through a filtering head with micropores of 0.45 μm , thus obtaining the sample solution.

Measurement

Accurately 10 μl of sample solution is weighed out, measured under the above chromatography conditions, and calculated in the regression equation.

Example 1 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then flash extracted with 70% acetone for 2 times, each for 2min. The solvent amount is 10 times of the powders in the first extraction, and 8 times in the second extraction. Then, the filtrates are merged and concentrated under reduced pressure. The concentrate is extracted and defatted by ethyl ether with a volume ratio of 1:1 until the ethyl ether layer is colorless, and then the mother liquor is further extracted by ethyl acetate with a volume ratio of 1:1 to enrich total phenols for 6 times. The ethyl acetate solution is merged and concentrated at a low temperature 45°C under reduced pressure to a proper amount, and dried at 45°C under reduced pressure. Thus the sanguisorba tannins extract can be obtained.

Results: the extract contains 62% (w/w) of tannins, 1.32% (w/w) of sanguin, 2.61% (w/w) of catechin, 0.08% (w/w) of proanthocyanidin B2, 6.2% (w/w) of sanguisorba saponins, and 3.7% (w/w) of sanguisorbin I.

Example 2 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then reflux extracted with 70% acetone for 3 times, each for 2h. The solvent amount is 10 times of the powders in the first extraction, 8 times in the second extraction, and 6 times in the third extraction. Then, the filtrates are merged and concentrated under reduced pressure. The concentrate is extracted and defatted by ethyl ether with a volume ratio of 1:1 until the ethyl ether layer is colorless, and then the mother liquor is further extracted by ethyl acetate with a volume ratio of 1:1 to enrich total phenols for 6 times. The ethyl acetate solution is merged and concentrated at a low temperature 45°C under reduced pressure to a proper amount, and dried at 45°C under reduced pressure. Thus the sanguisorba tannins extract can be obtained.

Results: the extract contains 65% (w/w) of tannins, 1.41% (w/w) of sanguin, 2.65% (w/w) of catechin, 0.09% (w/w) of proanthocyanidin B2, 5.4% (w/w) of sanguisorba saponins, and 3.1% (w/w) of sanguisorbin I.

Example 3 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then reflux extracted with 70% ethanol for 3 times, each for 2h. The solvent amount is 10 times of the powders in the first extraction, 8 times in the second extraction, and 6 times in the third extraction. Then, the filtrates are merged and concentrated under reduced pressure. The concentrate is extracted and defatted by ethyl ether with a volume ratio of 1:1 until the ethyl ether layer is colorless. The concentrate is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of twice of the column volume. The 60% ethanol eluent is collected, and concentrated at 55°C under reduced pressure to a proper amount. After spray drying, the sanguisorba tannins extract can be obtained.

Results: the extract contains 73% (w/w) of tannins, 1.52% (w/w) of sanguin, 2.88% (w/w) of catechin, 0.11% (w/w) of proanthocyanidin B2, 3.2% (w/w) of sanguisorba saponins, and 1.7% (w/w) of sanguisorbin I.

Example 4 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then reflux extracted with 70% ethanol for 3 times, each for 2h. The solvent amount is 10 times of the powders in the first extraction, 8 times in the second extraction, and 6 times in the third extraction. Then, the filtrates are merged and concentrated under reduced pressure. The precipitate is removed, and the supernate is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of twice of the column volume. The 60% ethanol eluent is collected, and concentrated through film to a proper amount. After spray drying, the sanguisorba tannins extract can be obtained.

Results: the extract contains 79% (w/w) of tannins, 1.72% (w/w) of sanguin, 3.24% (w/w) of catechin, 0.13% (w/w) of proanthocyanidin B2, 2.4% (w/w) of

sanguisorba saponins, and 1.3% (w/w) of sanguisorbin I.

Example 5 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then decocted with water for 3 times, each for 2h. The water amount is 10 times of the powders in the first decoction, 8 times in the second decoction, and 6 times in the third decoction. Then, the filtrates are merged and concentrated under reduced pressure to a proper amount. The precipitation is removed. The solution is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of twice of the column volume. The 60% ethanol eluent is collected and concentrated at 55°C under reduced pressure to a proper amount. After spray drying, the sanguisorba tannins extract can be obtained.

Results: the extract contains 61% (w/w) of tannins, 1.22% (w/w) of sanguin, 2.43% (w/w) of catechin, 0.07% (w/w) of proanthocyanidin B2, 6.9% (w/w) of sanguisorba saponins, and 4.3% (w/w) of sanguisorbin I.

Example 6 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then decocted with water for 3 times, each for 2h. The water amount is 10 times of the powders in the first decoction, 8 times in the second decoction, and 6 times in the third decoction. Then, the filtrates are merged and concentrated under reduced pressure to a proper amount. The precipitation is removed. The solution is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of twice of the column volume. The 60% ethanol eluent is collected and concentrated through film to a proper amount. After spray drying, the sanguisorba tannins extract can be obtained.

Results: the extract contains 67% (w/w) of tannins, 1.34% (w/w) of sanguin, 2.59% (w/w) of catechin, 0.08% (w/w) of proanthocyanidin B2, 5.5% (w/w) of sanguisorba saponins, and 3.4% (w/w) of sanguisorbin I.

Example 7 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then flash extracted with 70% acetone for 2 times, each for 2min. The solvent amount is 10 times of the powders in the first extraction, and 8 times in the second extraction. Then, the filtrates are merged and concentrated through film to a proper amount. The solution is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of twice of the column volume. The 60% ethanol eluent is collected and concentrated through film to a proper amount. After spray drying, the sanguisorba tannins extract can be obtained.

Results: the extract contains 78% (w/w) of tannins, 1.69% (w/w) of sanguin, 3.35% (w/w) of catechin, 0.13% (w/w) of proanthocyanidin B2, 3.1% (w/w) of sanguisorba saponins, and 1.4% (w/w) of sanguisorbin I.

Example 8 Preparation of sanguisorba tannins extract of the present invention

Coarse powders of Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is warmly immersed in water in an amount of 12 times of the powders twice, each 3h. After filtration, the filtrates are merged, concentrated to 0.5g crude per ml and is placed in a centrifuge. It is centrifuged for 10min at the speed of 4000 rpm, and the supernate is collected. The supernate is slowly fed to D-101 macroporous resin with a diameter height ratio of 1:5 at 2BV/h. After a static adsorption for 30min, it is washed with 2BV water at 2BV/h, and then washed with 1BV 10% ethanol at 2BV/h, finally eluted with 2BV 60% ethanol at 1BV/h. The eluent is collected, concentrated and dried.

Results: the extract contains 55% (w/w) of tannins, 7.2% (w/w) of sanguisorba saponins, and 4.9% (w/w) of sanguisorbin I. The yield of tannins in the present invention is up to 13%.

For ten batches of sanguisorba tannins prepared according to Example 8, the contents of sanguin, catechin, and proanthocyanidin B2 therein are determined. Results are shown as follows.

Table 2 Content determination of ten batches of sanguisorba tannins extracts

No.	Content (%)		
	Sanguin	Catechins	Proanthocyanidin B2
110503	1.62	3.01	0.12

110511	1.62	2.89	0.09
110516	1.54	3.11	0.15
110522	1.47	2.76	0.14
110527	1.75	3.24	0.14
110601	1.56	3.22	0.11
110606	1.66	2.97	0.09
110610	1.72	3.00	0.10
110615	1.49	2.86	0.11
110619	1.69	3.12	0.11
Average	1.61	3.02	0.12

The average content of sanguin, catechin, and proanthocyanidin B2 is 1.61% (w/w), 3.02% (w/w), and 0.12% (w/w) respectively.

Example 9 Preparation of sanguisorba tannins extract of the present invention

The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then decocted with water for 2 times, each for 1.5h. The water amount is 10 times of the powders in the first decoction, and 8 times in the second decoction. Then, the filtrates are merged and concentrated under reduced pressure to a proper amount. The precipitation is removed. The solution is separated by macroporous resin, wherein it is firstly eluted by water to colorless, then by 10% ethanol with an amount of twice of the column volume, finally by 60% ethanol with an amount of 3 times of the column volume. The 60% ethanol eluent is collected and concentrated under reduced pressure to a proper amount. After the concentrated solution is spray dried, it is dissolved in water, and then the obtained solution is stood for 1h, and the precipitation is filtered. The filtrate is placed in an evaporating dish, into which gelatin solution is sprayed slowly. After standing for 1h, the precipitation is collected, and taken out after freeze dried for 12h. The precipitation is then ground into fine powders, and resolved with 90% acetone for 2h. The obtained solution is fully distilled at 45°C under reduced pressure to recover acetone, and the concentrate is poured out and freeze dried for 12h. Thus highly purified sanguisorba tannins with the tannins content of 98.5% can be obtained.

1. The content of sanguin, catechin, and proanthocyanidin B2 is 2.29% (w/w), 4.85% (w/w), and 0.38% (w/w), respectively.
2. Determination of the content of Tannins

The content of tannins is determined according to Appendix XB of *Chinese Pharmacopoeia* (2010 Edition, Volume I):

The experiment should be performed away from light.

Preparation of control solution. 48.30 mg of gallic acid as control substance is weighed out accurately and added into a 100ml brown volumetric flask. Water is added to dissolve the gallic acid and dilute it until the solution reaches the graduation line. 5ml of the solution is weighed out accurately, and added into a 50ml brown volumetric flask. Water is added to dilute the solution until it reaches the graduation line. The solution is then shaken well, thus obtaining the control solution with 0.0483mg gallic acid per 1ml solution.

Preparation of standard curve. 0.5ml, 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml of control solutions are weighed out accurately and added into six 25ml brown volumetric flasks respectively. 1ml of phosphomolybdenum tungstic acid solution is added to each of the above flasks, and then 11.50ml, 11ml, 10ml, 9ml, 8ml and 7ml of water are added therein respectively. Each solution is then diluted with 29% sodium carbonate solution to reach the graduation line and shaken well. After the obtained solution is stood for 30 min, the absorbency at a wavelength of 760nm is determined according to UV-VIS spectrophotometry (Appendix VA) with the corresponding reagent as the blank test. Then, the standard curve can be obtained with the absorbency as Y-axis against the concentration as X-axis.

Concentration (μg/ml)	Absorbance (A)	Regression Equation
0.966	0.154	$Y = 0.118 X + 0.046 (R^2 = 0.999)$
1.932	0.276	
3.864	0.511	
5.796	0.731	
7.728	0.961	
9.66	1.184	

Preparation of sample solution. 0.101g, 0.105g and 0.997g of extract powders are weighed out accurately, and then added into three 250ml brown volumetric flasks respectively. 150ml of water are added in the three flasks respectively. Each solution is stood for night and then treated by ultrasonic for 10 min. After being cooled, it is diluted with water to reach the graduation line. The solution is shaken well and stood

so as to enable solids to precipitate from the solution. The solution is filtered, and 50ml of preliminary filtrate is abandoned, whereas 20ml of residual filtrate is weighed out accurately and then transferred into a 100ml brown volumetric flask. Then, the obtained solution is diluted with water to the graduation line and shaken well, thus obtaining the sample solution.

Measurement

Total phenols. 2ml of sample solution is weighed out accurately and added into a 25ml brown volumetric flask. According to the method provided in the part of “preparation of standard curve”, after “1ml of phosphomolybdium tungstic acid solution is added”, 10ml of water is added. The absorbance is determined in known manner, and the weight (mg) of gallic acid in the sample solution can be read out from the standard curve. Thus the content of total phenols can be obtained by calculation.

Non-adsorbed polyphenols. 25ml of sample solution is weighed out accurately and then transferred to a 100ml conical flask filled with 0.6g of casein. The flask is sealed with a cover and placed in a water bath with a temperature of 30°C for 1h. The flask is constantly shaken, and then removed from the water bath and cooled. The solution is shaken well and filtered, and the preliminary filtration is abandoned. 2ml of residual filtration is weighed out accurately, and then transferred into a 25ml brown volumetric flask. According to the method provided in the part of “preparation of standard curve”, after “1ml of phosphomolybdium tungstic acid solution is added”, 10ml of water is added. The absorbance is determined in known manner, and the weight (mg) of gallic acid in the sample solution can be read out from the standard curve. Thus the content of non-adsorbed phenols can be obtained by calculation.

The content of tannins can be calculated as follows: the amount of tannins = the amount of total phenols – the amount of non-adsorbed polyphenols.

Sample	Sample Weight (g)	Total phenols (%)	Non-adsorbed polyphenols (%)	Content of Tannins (%)	Average (%)
1	0.101	98.9	0.6	98.3	98.5
2	0.105	98.7	0.3	98.4	
3	0.997	99.3	0.5	98.8	

3. Determination of total saponins and sanguisorbin I in the sanguisorba tannins extract

Three batches of tannins extract are determined, with no peak of sanguisorbin I can be observed with HPLC analysis. The absorbance of total saponins determined by UV spectrophotometry is 0.001. It can be concluded that the content of total saponins is less than 0.001% and the content of sanguisorbin I is less than 0.0001%. Therefore, when the content of tannins are more than or equal to 98%, the content of total saponins and sanguisorbin I is substantially zero.

No.	total saponins (%)	sanguisorbin I (%)
20100609	< 0.001	< 0.0001
20100613	< 0.001	< 0.0001
20100617	< 0.001	< 0.0001

In combination with the results of the contents of sanguiin, catechin, and proanthocyanidin B2 in Examples 1-9, it can be found that the contents of sanguiin, catechin, and proanthocyanidin B2 in the sanguisorba tannins extract of the present invention are relatively stable. Specifically, said extract contains 0.05%-3.0% (w/w) of sanguiin, 1.0%-5.0% (w/w) of catechin, and 0.05%-1.5% (w/w) of proanthocyanidin B2. Preferably, said extract contains 1.0%-2.0% (w/w) of sanguiin, 2.0%-4.0% (w/w) of catechin, and 0.05%-4% (w/w) of proanthocyanidin B2.

Example 10 Optimized process of purifying sanguisorba tannins with macroporous resin

1 Type of resin

1.1 Experimental method

15g of dried macroporous resin is weighted out and transferred in a 100ml conical flask sealed with a cover. 50ml of 2% hydrochloric acid is added for immersion of 3h, and then the solution is washed with water to a neutral pH value. Subsequently, 50 ml of 2% NaOH solution is added for immersion of 3h, and the resin is activated through adding 60ml of 95% alcohol. 50ml of the sanguisorba tannins extract solution (0.33g/ml) is weighted out and placed in a conical flask containing activated macroporous resin sealed with a cover, enabling macroporous resin fully soaked in the solution for 24h. The volume of the residual liquid in the conical flask is determined, thus obtaining the adsorption amount of macroporous resin. The macroporous resin is removed from the conical flask and then immersed in 95% ethanol and then treated by ultrasonic for 1h. The content of tannins in the eluent is

determined, thus obtaining the elution efficiency based on the contents of tannins before and after adsorption respectively.

Maximum adsorbance = (the content of tannins before adsorption - the content of tannins after adsorption)/mass of macroporous resin.

Elution efficiency = the content of tannins in the eluent/(the content of tannins before adsorption - the content of tannins after adsorption).

Based on the maximum adsorbance and the elution efficiency of DA-201, D-101, LSA-20, HP-10 or AB-8 macroporous resins, a suitable type of resin is selected.

1.2 Results

Table 3 shows the data from the experiment.

Table 3 Selection of resin

resin type	maximum absorbance (g/g)	elution efficiency (%)
DA-201	0.106	72.41
D-101	0.120	76.52
LSA-20	0.097	62.76
HP-10	0.102	68.45
AB-8	0.104	64.33

From the above experiment it can be confirmed that macroporous resin is of good adsorption and elution capability for sanguisorba tannins. Therefore, macroporous resin can be selected for purifying sanguisorba tannins, wherein in the present invention macroporous resin is preferably D-101.

2 The leakage curve of D-101 macroporous resin

20g of pretreated D-101 macroporous resin is packaged in column, which has a column volume of 30.4cm³ and a diameter height ratio of 1:4. 60ml of sanguisorba tannins extract solutions (0.33g crude per ml) as sample is fed in a dynamic manner at a controlled flow speed of 0.77ml/min constantly. The effluent from the column bottom is collected for 11 bottles, each of 5ml. For each effluent the content of tannins therein is determined respectively. Thus the leakage curve of macroporous resin can be obtained, and a leakage curve with the content of tannins as Y-axis and the volume

as X-axis can be drawn.

1ml of the effluent is transferred accurately from each bottle, and diluted to 100ml with water. 2ml of the diluted solution is transferred accurately into a 25ml brown volumetric flask. 1ml of phosphomolybdenum tungstic acid solution is added to the flask, and then 10ml of water is added therein. The solution is then diluted with 29% sodium carbonate solution to reach the graduation line. After the solution is stood for 30 min, the absorbance is determined. Then, the weight (mg) of gallic acid in the sample solution can be read out from the standard curve.

Table 4 Data of the leakage curve of D-101 macroporous resin

Volume (ml)	Absorbance (ABS)
5	0.065
10	0.067
15	0.067
20	0.071
25	0.098
30	0.161
35	0.436
40	0.865
45	1.326
50	1.345
55	1.351

In summary, the solution starts to leak out at 30ml. The optimized sample volume is 30ml, with a concentration of 0.33g crude per ml and an amount of 20g macroporous resin. Therefore, the optimized ratio of the amount of raw material to that of macroporous resin is 1:2.

3 The elution curve

60ml of sanguisorba tannins extract solution (0.33g crude per ml) is used. 20g of D-101 macroporous resin is packaged in column, which has a column volume of 30.4cm³ and a diameter height ratio of 1:4. The flow rate for feeding sample is kept constant. After adsorption of 1h till saturation, the solution is eluted with a column volume of water, 10% ethanol, 30% ethanol, 50% ethanol, 70% ethanol and 90% ethanol respectively at a constant flow speed. The eluent is collected and the content of tannins therein is determined. The influences of different eluent concentrations on

macroporous resin are compared with each other.

0.5ml of the eluent is diluted to 100ml, from which accurately 2ml of sample solution is weighted out and placed in a 25ml brown volumetric flask. 1ml of phosphomolybdium tungstic acid solution is added to the flask, and then 10ml of water is added therein. The solution is then diluted with 29% sodium carbonate solution to reach the graduation line. After the solution is stood for 30 min, the absorbency is determined. Then, the weight (mg) of gallic acid in the sample solution can be read out from the standard curve, thus obtaining the elution curve by calculation. The experimental data are shown in Table 5.

Table 5 Influence of different eluents on the elution curve

Eluent	Absorbance (ABS)
Water	0.153
10% ethanol	0.118
30% ethanol	0.556
50% ethanol	0.954
70% ethanol	0.192
90% ethanol	0.040

With the elution curve and the actual production cost taken into consideration, 60% ethanol is selected for eluting tannins.

4 Optimizing process parameters by uniform design

The content of tannins, the conversion rate and the value of overall desirability (OD value) are used to evaluate the influences of five levels of each of the diameter height ratio, the concentration of sample solution, the flow rate and the amount of 60% ethanol on the purification effect of tannins by a $U_5(5^4)$ uniform design table. The factors and levels adopted in the uniform design are shown in Table 6, and the experimental data are shown in Table 7.

Table 6 Factors and levels of the uniform design table

Factor No.	A diameter height ratio	B 60% ethanol	C flow rate	D concentration of sample solution
1	1:5	2	1	1
2	1:6	3	2	0.2

3	1:3	2.5	2.5	0.8
4	1:7	1.5	3	0.6
5	1:4	1	1.5	0.4

Table 7 Experimental data of the uniform design

No.	Tannins contents (%)	conversion rate (%)	OD value
1	53.44	68.50	0.99
2	47.25	42.00	0.73
3	53.16	28.95	0.64
4	46.22	31.25	0.62
5	54.47	39.23	0.76

By analyzing the results with spss17.0 software, the main output result displays a selected x_3 , $R^2=0.85$, and variance analysis reveals $F=16.192$, $P=0.026$. Stepwise regression is statistically suitable.

Anova^b

Model		sum of squares	df	mean square	F	Sig.
1	Regression	.074	1	.074	16.912	.026 ^a
	Residual	.013	3	.004		
	Total	.087	4			

a. predicting variable: (constant), x_3

b. dependent variable: y

Equation coefficients, partial regression coefficient t of $x_3=-4.112$, unilateral $P=0.026$, reserved. That is, the regression equation is $y=1.092-0.172x_3$. Since x_3 in the regression equation is minus and there is no x_1 , x_2 and x_4 , the value of x_3 should be the minimum in the test scope. According to the actual conditions, $x_1=1:5$, $x_2=2$ and $x_4=0.5$. The approximate evaluation at the optimal point is $x_3=1$. Therefore, the optimized parameters are as follows: diameter height ratio is 1:5, concentration of sample solution is 0.5, flow rate is 1BV/h, and the amount of 60% ethanol is 2 times of the column volume.

Coefficients ^a										
Model		Non-standard coefficients		standard coefficients	t	Sig.	95.0% confidence intervals of B		Multicollinearity statistic	
		B	standard error	trial version			Low level	Upon level	tolerance	VIF
1	(constant)	1.092	.089		12.308	.001	.810	1.374		
	x3	-.172	.042	-.922	-4.112	.026	-.305	-.039	1.000	1.000

a. dependent variable: y

5 Verification test of process for purifying sanguisorba tannins by macroporous resin

According to the above optimized process, the OD value is predicted as 0.92, which is verified as follows.

Table 8 verification for the process (HPLC)

No.	Tannins contents(%)	conversion rate (%)	OD value
1	57.18	64.92	0.91
2	55.24	66.36	0.92
3	58.75	65.22	0.93

The results show that the purification process results are relatively stable.

6 Summary

According to the above experimental results, the purification conditions for sanguisorba are as follows. A sanguisorba extract solution, after being concentrated to 0.5g crude per ml, is placed in a centrifuge. It is centrifuged for 10min at the speed of 4000 rpm, and the supernate is collected. The supernate is slowly fed to D-101 resin column with a diameter height ratio of 1:5 at 2BV/h. After a static adsorption for 30min, it is washed with 2BV water at 2BV/h, and then washed with 1BV 10% ethanol at 2BV/h, finally eluted with 2BV 60% ethanol at 1BV/h. The eluent is collected, concentrated and dried.

In practice, other macroporous resins can be selected for purifying sanguisorba tannins. The yield of sanguisorba tannins extract reported in prior arts is very low,

averagely less than 10%. However, the extraction efficiency in the present invention is higher than 13%.

The beneficial effect of the present invention is proved by the following pharmacodynamics tests.

Test 1 Protective effect of the sanguisorba tannins in the present invention on bone marrow

1. Sanguisorba tannins extract

Sanguisorba tannins extract with high purify prepared according to Example 9 is used, wherein the extract comprises 98.5% of tannins.

2. The pharmaceutical effect of Sanguisorba tannins against bone marrow inhibition caused by chemical substances

2.1 Experimental materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; High purity sanguisorba tannins (containing more than 98.5% of tannins); Cyclophosphamide for Injection; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); and Normal saline.

2.2 Experimental method

2.2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 60 mice are randomly divided into 6 groups by gender (see Table 9 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for administration of an equivalent volume of normal saline to the blank group, other groups are injected intraperitoneally (ip) with Cyclophosphamide

at 100mg/kg·d for 3 days based on the mouse body weight of the day. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 9 Grouping of animals and dosage of drug administration

Group Name	Administration Dosage	n
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF(30μg/kg·d)	10
Sanguisorba tannins Low-dose group	2.5mg/kg·d	10
Sanguisorba tannins moderate-dose group	5mg/kg·d	10
Sanguisorba tannins high-dose group	10mg/kg·d	10

2.2.2 Evaluation index and data treating

Blood is collected from orbits of mice at 7th day after modeling, and detected for parameters of peripheral blood routine by the Automatic Blood Cell Counter, including white blood cell (WBC) count, red blood cell (RBC), Hemoglobin (HGB), and platelet (PLT). Meanwhile, the spleens of mice are taken out and weighted, and the spleen coefficient is calculated. After executing the above treated mice, left-side intact femurs of mice are taken out and the soft tissue thereof are removed. The whole bone marrow is flushed into a test tube with 10ml of 0.005mol/L CaCl₂. The tube is stood in a fridge at 4°C for 30min, and centrifuged at 2500r/min for 15min. Supernate is removed, and the precipitate is sufficiently mixed with 5ml of 0.2mol/L HClO₄. After being heated at 90°C for 15min, the tube is cooled and then centrifuged at 3500r/min for 10min. Supernate is used to determine the OD value at a wavelength of 260nm by ELIASA.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

2.2.3 Result analysis

Table 10 shows the influence of sanguisorba tannins on the decrease of white blood cells, red blood cells and platelet of peripheral blood of mice caused by

cyclophosphamide and spleen coefficient. Intraperitoneal injection of cyclophosphamide into the abdomens of mice will enable significant decreases of white blood cells, red blood cells and platelet in peripheral blood of mice, and also significant decreases of spleen coefficient and DNA contents in bone marrow. Thus a statistical difference presents compared with Blank group. Compared with Model group, it can be found that sanguisorba tannins high-dose group, sanguisorba tannins moderate-dose group and sanguisorba tannins low-dose group and rhG-CSF can significantly increase the counts of white blood cells ($P<0.05$), and improve spleen coefficient. Sanguisorba tannins high-dose group can significantly increase the counts of red blood cells and platelet in peripheral blood, while Sanguisorba tannins and rhG-CSF do not affect Hemoglobin contents obviously. Sanguisorba tannins high-dose group can significantly improve DNA contents in bone marrow ($P<0.05$), while rhG-CSF has a harmful effect on DNA contents in bone marrow.

Table 10 Pharmaceutical study on sanguisorba tannins
against bone marrow inhibition caused by chemical substances
(mean±S.D., n=10)

Groups	Dosage (mg/kg)	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/L)	PLT ($10^9/L$)	spleen coefficient (mg/10g)	DNA contents in bone marrow (μg)
Blank group	-	6.18±0.44	4.77±0.92	147.40±14.92	544.90±71.92	32.18±4.01	121.87±8.11
Model group	-	2.44±0.29	3.69±0.51	125.09±10.97	273.63±92.14	20.56±2.99	67.85±5.63
Positive group	30 $\mu g/kg \cdot d$	6.75±0.46 ^Δ	4.82±0.86	135.88±16.64	467.35±69.25	30.90±4.20 ^Δ	56.29±3.67
Sanguisorba tannins Low- dose group	2.5	3.87±0.39	4.11±0.55	135.89±4.03	337.87±46.13	26.16±3.01	87.22±3.26
Sanguisorba tannins moderate-dose group	5	4.55±0.47	4.27±0.75	133.83±12.63	368.33±54.72	29.86±4.23	94.28±7.98
Sanguisorba tannins high- dose group	10	4.92±0.74 ^Δ	4.65±0.70	135.80±14.79	386.74±50.54	32.44±4.20 ^Δ	106.32±8.93 ^Δ

Δ:compared with Model group $P<0.05$

It can be known from the above experiments that rhG-CSF can strongly improve white blood cells, enabling the count of white cells obviously greater than that in

normal group ($P < 0.05$). However, rhG-CSF cannot protect bone marrow, and even accelerate cell division in bone marrow through stimulation, thus further aggravating the load of hemopoietic stem cells. This is one reason to cause some side effects such as bone pain. As one of main cells used for adjusting bone marrow granulopoiesis, RhG-CSF has a significantly beneficial treatment effect for leucopenia, erythropenia and thrombocytopenia caused by radiotherapy and/or chemotherapy. The mechanism thereof is as follows. RhG-CSF selectively influences on CFU-C, and directly accelerates cell proliferation and differentiation, and improves the function of granulopoietic cell terminal differentiation, thus rapidly increasing white blood cells.

The sanguisorba tannins of the present invention has a significantly protection ($P < 0.05$) against the bone marrow inhibition of mice caused by cyclophosphamide, including obvious decrease of white blood cells, red blood cells, hemoglobin and platelet in peripheral blood and the decrease of DNA contents in bone marrow. It also exhibits a dose-dependent relationship: High>Moderate>Low. The sanguisorba tannins of the present invention comprises greater than 98.5% tannins and have a significantly beneficial treatment effect on bone marrow inhibition, leucopenia, erythropenia and thrombocytopenia caused by chemical substances.

3 The pharmaceutical effect of sanguisorba tannins against bone marrow inhibition caused by radiation

3.1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; Sanguisorba tannins (containing more than 98.5% of tannins); An external ray machine of Co-60 rays; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75 μ g); and Normal saline.

3.2 Experimental method

3.2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the

observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 60 mice are randomly divided into 6 groups by gender (see §2), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for normal group, other groups all are placed in an isolated cycloid plastic box, defining distance based on the box cover as center. A total body irradiation 7.5Gy with Co-60 rays at dose rate of 82.6cGy/min is conducted with the irradiation distance of 80 cm. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

3.2.2 Evaluation index and data processing

The count of White blood cells WBC is used as the observation index in the Experiment.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

3.3 Result analysis

Table 11 shows the influence of sanguisorba tannins against the bone marrow inhibition caused by Co rays.

Table 10 Influence on sanguisorba tannins against bone marrow inhibition caused by Co rays
(mean±S.D., n=10)

Group	Dosage (mg/kg)	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/L)	PLT ($10^9/L$)	Spleen coefficient (mg/10g)	DNA contents in bone marrow (μg)
Blank group	-	6.92±0.38	4.83±0.42	144.32±12.082	532.41±48.06	33.26±5.25	132.21±6.43
Model Group	-	2.13±0.14	3.72±0.39	122.11±12.38	269.03±52.31	19.39±4.82	77.45±6.43
Positive group	30 $\mu g/kg \cdot d$	7.81±0.32 ^Δ	4.01±0.73	136.12±13.51	402.29±59.12	31.07±3.98 ^Δ	47.11±3.09
Sanguisorba tannins Low- dose group	2.5	4.12±0.23	4.23±0.54	137.90±10.12	397.12±60.36	24.26±4.03	91.34±5.79
Sanguisorba tannins Moderate-	5	5.21±0.43	4.45±0.54	137.23±7.89	421.36±51.22	27.45±3.54	102.54±6.98

dose group							
Sanguisorba tannins high- dose group	10	6.01±0.42 ^Δ	4.77±0.54	139.02±12.43	413.57±32.68	29.43±2.46 ^Δ	124.34±7.86 ^Δ

Δ: P<0.05, compared with Model group;

Sanguisorba tannins can also increase the numbers of red blood cells and plates (P<0.05).

It can be seen from the above experiment that rhG-CSF can strongly improve white blood cells, enabling the amount of white cells obviously greater than that in normal group (P<0.05). However as mentioned in §2, DNA content in bone marrow of rhG-CSF group is decreased.

The sanguisorba tannins of the present invention has a significantly protection (P<0.05) against the decrease of DNA contents in bone marrow of mice and the decrease of white blood cells caused by chemical substances. It also exhibits a dose-dependent relationship: High>Moderate>Low. The sanguisorba tannins produced by the present invention comprise greater than 98.5% tannins and have a significantly beneficial treatment effect on bone marrow inhibition, leucopenia, erythropenia and thrombocytopenia caused by chemical substances.

The Sanguisorba tannins of the present invention has a significantly protection (P<0.05) against the decrease of DNA contents in bone marrow of mice, and the decrease of white blood cells caused by Chemical substances, exhibiting a dose-dependent relationship: High>Moderate>Low. The sanguisorba tannins of the present invention comprises greater than 98.5% tannins. Test proves that the Sanguisorba tannins has a significantly beneficial treatment effect on bone marrow inhibition, leucopenia, erythropenia and thrombocytopenia caused by radiation.

Test 2 Pharmacological effect of sanguisorba tannins in the present invention and the components thereof

It is reported that Sanguisorba tannins contain sanguiin, catechin, proanthocyanidin B2, galocatechin, epicatechin gallate, epicatechin and galocatechin gallate (LIU Haiying, Component analysis and Function research on Sanguisorba polyphenols, Shaanxi Normal University, 2009). Therefore, the following experiment is performed in order to reveal the main active substances in the sanguisorba tannins of the present invention, and the activity thereof is compared with single compound.

1 Pharmaceutical effect of sanguisorba tannins and its components on bone marrow inhibition caused by chemical substances

1.1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; Cyclophosphamide for Injection; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); Normal saline; High purity sanguisorba tannins (containing more than 98.5% of tannins, prepared according to Example 9); sanguin (HPLC>99%); catechin (HPLC>99%); proanthocyanidin B2 (HPLC>99%); galocatechin (HPLC>99%); epicatechin gallate (HPLC>99%); epicatechin (HPLC>99%); and galocatechin gallate (HPLC>99%). All the above single compounds are purchased from Chengdu Biopurify Phytochemicals Ltd.

1.2 Experimental method

1.2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 110 mice are randomly divided into 11 groups by gender (see Table 12 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for administration of an equivalent volume of normal saline to the blank group, other groups are injected intraperitoneally (ip) with cyclophosphamide at 100mg/kg*d for 3 days based on the mouse body weight of the day. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 12 Grouping of animals and dosage of drug administration

Group Name	Administration Dosage	n
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF(30μg/kg•d)	10

Sanguisorba tannins group	10mg/kg·d	10
Sanguin group	10mg/kg·d	10
Catechin group	10mg/kg·d	10
Gallocatechin group	10mg/kg·d	10
Epicatechin gallate group	10mg/kg·d	10
Proanthocyanidin B2 group	10mg/kg·d	10
Epicatechin group	10mg/kg·d	10
Gallocatechin gallate group	10mg/kg·d	10

1.2.2 Evaluation index and data processing

The count of White blood cells WBC is used as the observation index in the Experiment.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

1.2.3 Result analysis

Table 13 The Pharmacological effect of Sanguisorba tannins and its components on bone marrow inhibition caused by chemical substances
(mean±S.D., n=10)

Group	Dose (mg/kg)	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/L)	PLT ($10^9/L$)	Spleen Coefficient (mg/10g)	DNA contents in bone marrow (μg)
Blank group	-	6.09±0.33	4.49±0.87	145.40±12.87	535.09±68.78	30.69±3.19	124.46±7.99
Model group	-	2.37±0.28	3.78±0.68	126.99±11.69	288.27±87.96	19.79±4.38	69.19±7.68
Positive group	30 $\mu g/kg \cdot d$	7.78±0.53	4.21±0.79	131.19±14.89	400.53±78.28	32.19±3.98	58.29±4.79
Sanguisorba tannins group	10	5.88±0.79 ^{A☆}	4.62±0.65	134.13±11.69	392.19±44.92	34.12±3.95 ^A	121.21±6.89 ^{A☆}
Sanguin group	10	4.92±0.22 ^A	4.42±0.22	132.31±8.29	377.22±39.31	32.16±2.01 ^A	115.29±2.91 ^A
Catechin group	10	4.21±0.12 ^A	4.36±0.39	131.32±10.01	344.29±49.14	29.41±3.44 ^A	101.13±3.78 ^A
Gallocatechin group	10	3.66±0.19	3.91±0.19	128.19±9.09	315.19±58.31	25.09±3.01	87.08±4.98
Epicatechin gallate group	10	3.71±0.34	4.11±0.28	128.01±8.02	321.33±51.09	26.09±5.02	90.03±3.98
Proanthocyanidin B2 group	10	4.17±0.28 ^A	4.51±0.25	129.98±9.02	331.32±46.78	28.99±4.22 ^A	99.61±4.02 ^A

Epicatechin group	10	3.59±0.34	3.99±0.22	127.89±7.09	312.09±44.02	25.78±3.99	84.04±5.02
Gallocatechin gallate group	10	3.60±0.30	4.02±0.42	128.98±8.09	320.09±51.02	24.49±4.03	82.09±4.12

Δ: P<0.05, compared with Model group;

☆: P<0.05, comparing sanguisorba tannins group with catechin group;

Sanguisorba tannins increase red blood cells and plates (P<0.05).

Experimental results show that the components, such as sanguin, catechin, gallocatechin and epicatechin gallate etc., contained in the sanguisorba tannins can treat the bone marrow inhibition of mice caused by cyclophosphamide; especially, sanguin, catechin and proanthocyanidin B2 have a significant effect (P<0.05), wherein among them sanguin is strongest, catechin is followed, and proanthocyanidin B2 is least. Moreover, given the same dosage, the effect of sanguisorba tannins is stronger than the component of sanguin or catechin, revealing a synergistic effect exists among these components.

2 Pharmaceutical effect of sanguisorba tannins and its components on bone marrow inhibition caused by radioactivity

2.1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; An external ray machine of Co-60 rays; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); Normal saline; High purity sanguisorba tannins (containing more than 98.5% of tannins, prepared according to Example 9); sanguin (HPLC>99%); catechin (HPLC>99%); proanthocyanidin B2 (HPLC>99%); gallocatechin (HPLC>99%); epicatechin gallate (HPLC>99%); epicatechin (HPLC>99%); and gallocatechin gallate (HPLC>99%).

2.2 Experimental method

2.2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 110 mice are randomly divided into 11 groups by gender (see §1), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for normal group, other groups all are placed in an isolated cycloid plastic box, defining distance based on the box cover as center. A total body irradiation 7.5Gy with Co-60 rays at dose rate of 82.6cGy/min is conducted with the irradiation distance of 80 cm. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

2.2.2 Evaluation index and data processing

The same as Test 1.

2.3 Result analysis

Table 14 The pharmaceutical effect of Sanguisorba tannins and its components on bone marrow inhibition caused by radiation
(mean±S.D., n=10)

Group	Dose (mg/kg)	WBC (10 ⁹ /L)	RBC (10 ¹² /L)	HGB (g/L)	PLT (10 ⁹ /L)	Spleen Coefficient (mg/10g)	DNA contents in bone marrow (μg)
Blank group	-	7.72±0.27	4.65±0.37	141.78±11.17	517.16±46.98	31.39±4.98	138.12±7.01
Model group	-	2.61±0.21	3.66±0.41	124.21±11.49	273.27±46.37	18.98±4.66	73.78±7.78
Positive group	30μg/kg•d	8.69±0.28	4.17±0.49	133.19±14.19	407.68±49.56	29.09±4.02	45.98±4.03
Sanguisorba tannins group	10	6.11±0.38 Δ ☆	4.75±0.49	139.28±11.34	411.37±31.76	30.12±3.01 ^Δ	130.22±8.29 ^Δ ☆
Sanguin group	10	5.66±0.21 Δ	4.49±0.45	134.15±11.29	401.41±55.22	28.42±4.65 ^Δ	121.79±5.45 ^Δ
Catechin group	10	4.28±0.29 Δ	4.22±0.74	131.65±9.89	387.34±44.87	27.55±5.01	105.18±6.67 ^Δ
Gallocatechin group	10	3.61±0.19	4.02±0.33	128.32±10.09	331.98±41.32	23.09±3.98	91.97±4.09
Epicatechin Gallate group	10	3.47±0.18	3.98±0.51	129.31±8.12	348.29±49.25	22.89±5.02	92.19±5.09
Proanthocyanidin	10	4.81±0.31	4.29±0.48	130.97±11.09	409.09±51.09	29.58±4.18 ^Δ	109.79±4.86 ^Δ

B2 group		Δ					
Epicatechin group	10	3.65 ± 0.29	3.81 ± 0.44	126.68 ± 10.14	305.21 ± 54.69	24.01 ± 5.49	90.78 ± 5.11
Gallocatechin gallate group	10	3.59 ± 0.26	3.85 ± 0.41	128.25 ± 11.01	301.78 ± 49.78	24.99 ± 5.01	91.09 ± 7.01

Δ : $P < 0.05$, compared with Model group;

☆: $P < 0.05$, comparing sanguisorba tannins group with catechin group;

Sanguisorba tannins can also increase red blood cells and plates etc. ($P < 0.05$).

Experimental results show that the components, such as sanguin, catechin, galocatechin and epicatechin gallate etc., contained in the sanguisorba tannins can treat the bone marrow inhibition of mice caused by radiation; especially, sanguin, catechin and proanthocyanidin B2 have a significant effect ($P < 0.05$), wherein among them sanguin is strongest, proanthocyanidin B2 is followed, and catechin is least. Moreover, given the same dosage, the effect of sanguisorba tannins is stronger than the component of sanguin, catechin or proanthocyanidin B2, revealing a synergistic effect exists among these components.

In summary, the main active ingredients of sanguisorba tannins against bone marrow inhibition and the decrease of white blood cells, red blood cells and the platelets caused by chemical damages and radiation damages are sanguin, proanthocyanidin B2 and catechin, wherein sanguin has a stronger function for two model therapies, while proanthocyanidin B2 and catechin have their own different focus. That is, catechin has a better protection for chemical damages, while proanthocyanidin B2 directs to the protection for physical trauma. The foregoing also reveals that a synergistic effect exists among the components of sanguisorba tannins.

The above experiments sufficiently prove that sanguisorba tannins are able to function against the bone marrow inhibition caused by chemical damages and radiation damages, and the decrease of white blood cells, red blood cells and platelets. Therefore, it can be predicted that due to the protection against bone marrow inhibition, sanguisorba tannins is very optimistic in clinical application in the combination treatment of anti-tumor.

Since in prior arts it is reported that sanguisorba saponins are active substances for increasing white blood cells. Inventors cautiously conducted a series of experiments to further study material basic of sanguisorba for increasing white blood cells.

Test 3 Material basic for sanguisorba tannins' function of increasing white blood cells in the present invention

1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; High purity sanguisorba tannins (containing more than 98.5% of tannins, prepared according to Example 9); Cyclophosphamide for Injection; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); Normal saline.

2. Experimental method

2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 70 mice are randomly divided into 7 groups by gender (see Table 15 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for administration of an equivalent volume of normal saline to the blank group, other groups are injected intraperitoneally (ip) with cyclophosphamide at 100mg/kg·d for 3 days based on the mouse body weight of the day. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 15 Grouping of animals and dosage of drug administration

Group Name	Administration Dosage	<i>n</i>
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF(30μg/kg·d)	10
High-dose of sanguisorba tannins extract	sanguisorba tannins extract (10mg/kg·d)	10
Low-dose of sanguisorba tannins extract	sanguisorba tannins extract (2.5mg/kg·d)	10

High-dose of sanguisorba tannins extract, tannins removed	sanguisorba tannins extract (10mg/kg·d), tannins removed by Gelatin	10
Low-dose of sanguisorba tannins extract, tannins removed	sanguisorba tannins extract (2.5mg/kg·d), tannins removed by Gelatin	10

2.3 Evaluation index and data processing

The count of White blood cells WBC is used as the observation index in the Experiment.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

3. Result analysis

By analyzing the experimental results of white blood cells WBC in Table 16, it can be seen that: (1) There are significant differences between blank group and model group ($P<0.05$), which indicates the modeling for animal models of mice leukopenia caused by cyclophosphamide is successful; (2) There are significant differences among high-dose group of sanguisorba tannins, low-dose group of sanguisorba tannins, positive group and model group ($P<0.05$), which indicates sanguisorba tannins have a beneficial treatment effect on mice leukopenia caused by cyclophosphamide; there are no significant differences between other drug administration groups and model groups ($P>0.05$), which indicates other drug administration groups have no treatment effect on leukopenia caused by cyclophosphamide; (3) The efficacy strength of sanguisorba tannins exhibits an obvious dose-dependent relationship: high-dose>low-dose ($P<0.05$).

Table 16 Experimental results about white blood cells WBC
(mean±S.D., n=10)

Group Name	WBC	n
Blank group	6.22±0.53	10
Model group	2.26±0.15	10
Positive group	7.54±0.57 ^Δ	10
High-dose group of sanguisorba tannins extract	5.69±0.35 ^{Δ, ☆}	10
Low-dose group of sanguisorba tannins extract	4.30±0.24 ^{Δ, ☆}	10

High-dose of sanguisorba tannins extract, tannins removed	2.55±0.21	10
Low-dose of sanguisorba tannins extract, tannins removed	2.42±0.19	10

Δ: P<0.05, compared with model group

☆: P<0.05, compared with groups with tannins removed

Test 4 Material basic for the function of improving white cells of Sanguisorba tannins extract in the present invention.

1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; Sanguisorba tannins (prepared according to Example 8, containing 55% of tannins, 1.61% of sanguin, 3.02% of catechin, and 0.12% of proanthocyanidin B2, 7.2% of sanguisorba saponins, and 4.9% of sanguisorbin, all calculated by weight); Cyclophosphamide for Injection; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); Normal saline.

2. Experimental method

2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 70 mice are randomly divided into 7 groups by gender (see Table 17 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for administration of an equivalent volume of normal saline to the blank group, other groups are injected intraperitoneally (ip) with cyclophosphamide at 100mg/kg*d for 3 days based on the mouse body weight of the day. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 17 Grouping of animals and dosage of drug administration

Group Name	Administration Dosage	<i>n</i>
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF(30 μ g/kg·d)	10
High-dose of sanguisorba tannins extract	sanguisorba tannins extract (10mg/kg·d)	10
Low-dose of sanguisorba tannins extract	sanguisorba tannins extract (2.5mg/kg·d)	10
High-dose of sanguisorba tannins extract, tannins removed	sanguisorba tannins extract (10mg/kg·d), tannins removed by Gelatin	10
Low-dose of sanguisorba tannins extract, tannins removed	sanguisorba tannins extract (2.5mg/kg·d), tannins removed by Gelatin	10

2.3 Evaluation index and data processing

The count of White blood cells WBC is used as the observation index in the Experiment.

Experimental data are presented as mean $\pm$ S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

3. Result analysis

By analyzing the experimental results of white blood cells WBC in Table 18, it can be seen that: (1) There are significant differences between blank group and model group ($P<0.05$), which indicates the modeling for animal models of mice leukopenia caused by cyclophosphamide is successful; (2) There are significant differences among high-dose group of sanguisorba tannins, low-dose group of sanguisorba tannins, positive group and model group ($P<0.05$), which indicates sanguisorba tannins have a beneficial treatment effect on mice leukopenia caused by cyclophosphamide; there are no significant differences between other drug administration groups and model groups ($P>0.05$), which indicates other drug administration groups have no treatment effect on leukopenia caused by cyclophosphamide; (3) The efficacy strength of sanguisorba tannins exhibits an obvious dose-dependent relationship: high-dose $>$ low-dose ($P<0.05$).

Table 16 Experimental results about white blood cells WBC
(mean±S.D., n=10)

Group Name	WBC	n
Blank group	6.65±0.39	10
Model group	2.54±0.33	10
Positive group	7.82±0.45 ^Δ	10
High-dose group of sanguisorba tannins extract	5.36±0.44 ^{Δ, ☆}	10
Low-dose group of sanguisorba tannins extract	4.17±0.37 ^{Δ, ☆}	10
High-dose of sanguisorba tannins extract, tannins removed	2.62±0.32	10
Low-dose of sanguisorba tannins extract, tannins removed	2.57±0.25	10

Δ: P<0.05, compared with model group

☆: P<0.05, compared with group with tannins removed

4. Conclusion

Tests 3 and 4 indicate that sanguisorba tannins extract of the present invention has a significant treatment effect on mice leukopenia caused by cyclophosphamide (P<0.05); moreover, after removing tannins by Gelatin, sanguisorba tannins extract has no treatment effect, which further proves that sanguisorba tannins are active ingredients for treating leucopenia.

Test 5 Sanguisorba saponins' function of increasing white blood cells and protecting bone marrow

1 Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; Sanguisorba tannins extract (98.18%); Cyclophosphamide for Injection; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); and Normal saline.

Preparation of sanguisorba saponins. The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then reflux extracted with 70% ethanol for 3 times, each for 1.5h. The solution is concentrated to a proper amount (no alcohol flavor) at 70°C under reduced pressure. The concentrate is transferred to a separating funnel, defatted with water-saturated ether twice. The mother liquor is treated at 70°C under reduced pressure to recover the residual ether, then dissolved in water, and extracted twice with water-saturated n-butanol. The extract is then condensed at 80°C under reduced pressure, and solids therein are dissolved through adding ethanol with a certain concentration. The solution is transferred to a beaker, with an appropriate amount of water added to adjust the pH value within the range of 10-14. After standing and being centrifuged, the precipitation is removed. After vacuum drying for 2 hours at 70°C, it is transferred to a triangular flask with a cover, and extracted by ultrasonic through adding anhydrous ethanol. After filtration, the filtrate is condensed at 70°C under reduced pressure to recover ethanol, till to just form precipitation. Then, it is transferred to an evaporation dish, where ethanol is volatilized in a water bath at 80°C. After vacuum drying for 12 hours at 70°C, the residual is taken out and ground. Sanguisorba saponins: 98.18%.

2 Experimental method

2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 60 mice are randomly divided into 6 groups by gender (see Table 9 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for administration of an equivalent volume of normal saline to the blank group, other groups are injected intraperitoneally (ip) with Cyclophosphamide at 100mg/kg*d for 3 days based on the mouse body weight of the day. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 19 Grouping of animals and dosage of drug administration

Group Name	Administration Dosage	n
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF(30μg/kg•d)	10

Low-dose group of Sanguisorba tannins	0.5mg/kg·d	10
Moderate-dose group of Sanguisorba tannins	1mg/kg·d	10
High-dose group of Sanguisorba tannins	2mg/kg·d	10

2.2 Evaluation index and data processing

Blood is collected from orbits of mice at 7th day after modeling, and detected for parameters of peripheral blood routine by the Automatic Blood Cell Counter, including white blood cell (WBC) count, red blood cell (RBC), Hemoglobin (HGB), and platelet (PLT). Meanwhile, the spleens of mice are taken out and weighted, and the spleen coefficient is calculated. After executing the above treated mice, left-side intact femurs of mice are taken out and the soft tissue thereof are removed. The whole bone marrow is flushed into a test tube with 10ml of 0.005mol/L CaCl_2 . The tube is stood in a fridge at 4°C for 30min, and centrifuged at 2500r/min for 15min. Supernate is removed, and the precipitate is sufficiently mixed with 5ml of 0.2mol/L HClO_4 . After being heated at 90°C for 15min, the tube is cooled and then centrifuged at 3500r/min for 10min. Supernate is used to determine the OD value at a wavelength of 260nm by ELIASA.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

2.2.3 Result analysis

Table 20 shows the influence of sanguisorba saponins extract on the decrease of white blood cells, red blood cells and platelet in peripheral blood of mice caused by cyclophosphamide and the spleen coefficient. Compared with the model group, high-dose group, moderate-dose group and low-dose group of Sanguisorba saponins extract have no significant influence on increasing white blood cells ($P>0.05$), and also no treatment effect on bone marrow inhibition caused by cyclophosphamide ($P>0.05$).

Table 20 Pharmaceutical effect of sanguisorba saponins extract
(mean±S.D., n=10)

Groups	Dose (mg/kg)	WBC ($10^9/\text{L}$)	RBC ($10^{12}/\text{L}$)	HGB (g/L)	PLT ($10^9/\text{L}$)	Spleen Coefficient	DNA Contents in
--------	-----------------	----------------------------	-------------------------------	--------------	----------------------------	-----------------------	--------------------

						(mg/10g)	bone marrow (μ g)
Blank group	-	6.34 $\pm$ 0.25	5.22 $\pm$ 0.45	151.22 $\pm$ 12.43	567.11 $\pm$ 54.60	31.43 $\pm$ 3.77	126.09 $\pm$ 9.43
Model group	-	2.38 $\pm$ 0.32	2.99 $\pm$ 0.37	122.22 $\pm$ 9.88	265.43 $\pm$ 25.09	19.87 $\pm$ 3.01	72.54 $\pm$ 3.75
Positive group	30 μ g/kg·d	7.08 $\pm$ 0.54	3.72 $\pm$ 0.42	136.32 $\pm$ 17.83	455.71 $\pm$ 39.67	29.43 $\pm$ 2.78	61.33 $\pm$ 4.52
Low-dose group of Sanguisorba tannins extract	0.5	2.43 $\pm$ 0.22 Δ	3.09 $\pm$ 0.31	126.64 $\pm$ 7.43	243.09 $\pm$ 56.22	18.57 $\pm$ 2.83 Δ	67.83 $\pm$ 5.21 Δ
Moderate-dose group of Sanguisorba tannins extract	1	2.46 $\pm$ 0.26 Δ	3.22 $\pm$ 0.28	123.36 $\pm$ 11.54	247.65 $\pm$ 38.77	19.82 $\pm$ 3.03 Δ	74.95 $\pm$ 5.05 Δ
High-dose group of Sanguisorba tannins extract	2	2.57 $\pm$ 0.32 Δ	3.54 $\pm$ 0.55	122.96 $\pm$ 10.62	254.01 $\pm$ 57.52	22.37 $\pm$ 2.95 Δ	76.47 $\pm$ 7.33 Δ

Δ : $P > 0.05$, compared with the model group

It is generally believed that: Sanguisorba saponins are the active ingredients of sanguisorba for increasing white blood cells and protecting bone marrow; Sanguisorba saponins also can increase the numbers of white blood cells and platelets in bone marrow inhibition of mice; However, sanguisorba tannins and sanguisorba flavones cannot promote bone marrow cells proliferation, instead cause bone marrow inhibition under high concentrations. During the preparation of sanguisorba extract, it is certainly intended to obtain sanguisorba saponins. However, Inventors unexpectedly and surprisingly find from the above experiments that the active ingredient in sanguisorba for increasing white blood cells is sanguisorba tannins, but not sanguisorba saponins.

Test 6 Influence of Sanguisorba tannins and Sanguisorba saponins on tumor

1 Experimental Materials

C57 mice (half ♀ and half ♂, 18-22g, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); Cyclophosphamide for Injection; Sanguisorba tannins extract (containing more than 98% of tannins, prepared according to Example 9); and Normal saline.

Lewis lung cancer cell suspension: Mice inoculated with lung cancer cells for 7 days are executed, and ascites are extracted under aseptic condition and centrifuged. After removing supernate, cancer cells in the lower layer are taken out and diluted into 250mg/ml suspension with normal saline.

Preparation of sanguisorba saponins: The Garden Burnet Root (scientific name: *Radix Sanguisorbae*) is ground into coarse powders and then reflux extracted with 70% ethanol for 3 times, each for 1.5h. The solution is concentrated to a proper amount (no alcohol flavor) at 70°C under reduced pressure. The concentrate is transferred to a separating funnel, defatted with water-saturated ether twice. The mother liquor is treated at 70°C under reduced pressure to recover the residual ether, then dissolved in water, and extracted twice with water-saturated n-butanol. The extract is then condensed at 80°C under reduced pressure, and solids therein are dissolved through adding ethanol with a certain concentration. The solution is transferred to a beaker, with an appropriate amount of water added to adjust the pH value within the range of 10-14. After standing and being centrifuged, the precipitation is removed. After vacuum drying for 2 hours at 70°C, it is transferred to a triangular flask with a cover, and extracted by ultrasonic through adding anhydrous ethanol. After filtration, the filtrate is condensed at 70°C under reduced pressure to recover ethanol, till to just form precipitation. Then, it is transferred to an evaporation dish, where ethanol is volatilized in a water bath at 80°C. After vacuum drying for 12 hours at 70°C, the residual is taken out and ground. The content of Sanguisorba saponins is 98.18%.

2. Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 60 of C57 mice are randomly divided into 6 groups by weigh, including model group, cyclophosphamide group, sanguisorba tannins extract group, sanguisorba tannins extract + cyclophosphamide group, sanguisorba saponins group, and sanguisorba saponins + cyclophosphamide group (see Table 21 for details). 0.2ml of Lewis lung cancer cell suspension is inoculated in animals of each group by subcutaneous injection through armpits, and modeling is carried out for once. Except for administration to cyclophosphamide group through intraperitoneal injected (ip), animals of each group are administrated as 10g mice weight /0.2ml dosage by

intragastric (ig), and the model group is administrated corresponding normal saline for 14 days.

Table 21 Grouping of animals and dosage of drug administration

Group name	Administration dosage	<i>n</i>
Model group	A equivalent volume of distilled water	10
Cyclophosphamide group	20 mg/kg·d	10
Sanguisorba tannins extract group	10 mg/kg·d	10
Sanguisorba tannins extract + Cyclophosphamide group	10mg/kg·d +20mg/kg·d	10
Sanguisorba saponins group	10mg/kg·d	10
Sanguisorba saponins + Cyclophosphamide group	10mg/kg·d +20mg/kg·d	10

3. Evaluation index and data processing

The animals of each group are executed at 15th day, and the axillary tumors are cut and weighted. The tumor inhibition rate is recorded. Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

Tumor inhibition rate (%) = {Model group average (tumor weight /body weight)- experiment group average (tumor weight /body weight)}/model group average (tumor weight /body weight)*100%.

4. Result analysis

Table 22 Influence of Sanguisorba tannins and Sanguisorba saponins on tumor

Group Name	Dosage (mg/kg)	Tumor weight /body weight	Tumor Inhibition Rate (%)
Model Group	-	0.0976	-
Cyclophosphamide group	20 mg/kg·d	0.0415	57.48 ^Δ
Sanguisorba tannins extract group	10 mg/kg·d	0.0758	22.34
Sanguisorba tannins extract+ Cyclophosphamide group	10mg/kg·d +20mg/kg·d	0.0294	69.88 ^Δ
Sanguisorba saponins group	10mg/kg·d	0.1063	-8.91
Sanguisorba saponins+	10mg/kg·d	0.0568	41.80 ^Δ

Cyclophosphamide group	+20mg/kg·d		
------------------------	------------	--	--

Δ: P<0.05, compared with Model group

Although it is reported that sanguisorba saponins can be used for anti-tumor, according to the above experiment results, sanguisorba saponins group has no function of tumor inhibition, instead a side effect of stimulating tumor growth. Moreover, after sanguisorba saponins are used in combination with cyclophosphamide, the tumor inhibition rate is lower than that of cyclophosphamide alone, which indicates that sanguisorba saponins rival the therapy effect of chemical medicines on tumor. However, after using sanguisorba tannins, the therapy effect becomes stronger, and bone marrow cells and whole blood cells are protected also, thus reducing or avoiding the damages to bone marrow cells and whole blood cells caused by chemotherapeutics. Therefore, the content of sanguisorba saponins in the sanguisorba tannins extract of the present invention should be controlled, in order to prevent the side effects of sanguisorba saponins. According to the results of the above examples and tests, the content of total saponins in sanguisorba tannins extract is less than 10% (w/w), and the content of sanguisorbin I is less than 5% (w/w). Preferably, the content of total saponins in sanguisorba tannins extract is less than 0.001% (w/w), and the content of sanguisorbin I is less than 0.0001% (w/w).

Test 7 Pharmaceutical effect of sanguisorba tannins extract of the present invention on bone marrow protection

1. Experimental Materials

KM mice (half ♀ and half ♂, 18-22g, clean grade, Certification No. SCXK (Sichuan) 2004-15, purchased from Institute of Experimental Animal of Medical Scientific Academy in Sichuan, China); Automatic Blood Cell Counter (MEK-6318, manufactured by Nihon Kohden Corporation); Electronic Analytical Balance (BP211D, manufactured by German Sartorius Group); ELASA; An external ray machine of Co-60 rays; Recombinant human granulocyte-colony stimulating factor (rhG-CSF, 75μg); Normal saline; Sanguisorba tannins extracts of Examples 1-8 (which include 55%-79% (w/w) of tannins and less than 10%(w/w) of sanguisorba saponins).

2. Experimental method

2.1 Grouping and drug administration

The experimental animals used in the present invention are housed in the observation room for animals of Chengdu University of TCM, and maintained under the environment with normal food and water feeding for 3 days before the experiment. 110 mice are randomly divided into 11 groups by gender (see Table 23 for details), prophylactic intragastric (ig) administration of the drug for 3d before modeling. At the 4th day, except for normal group, other groups all are placed in an isolated cycloid plastic box, defining distance based on the box cover as center. A total body irradiation 7.5Gy with Co-60 rays at dose rate of 82.6cGy/min is conducted with the irradiation distance of 80 cm. Except for positive group with chemical drugs, intragastric (ig) administration for 6 days from modeling, and conducting subcutaneous (sc) with rhG-CSF from 7th to 9th day.

Table 23 Grouping and drug administration

Group name	Administration dosage	<i>n</i>
Blank group	A equivalent volume of distilled water	10
Model group	A equivalent volume of distilled water	10
Positive group	rhG-CSF (30μg/kg•d)	10
Example 1	10mg/kg·d	10
Example 2	10mg/kg·d	10
Example 3	10mg/kg·d	10
Example 4	10mg/kg·d	10
Example 5	10mg/kg·d	10
Example 6	10mg/kg·d	10
Example 7	10mg/kg·d	10
Example 8	10mg/kg·d	10

2.2 Evaluation index and data processing

Blood is collected from orbits of mice at 7th day after modeling, and detected for parameters of peripheral blood routine by the Automatic Blood Cell Counter, including white blood cell (WBC) count, red blood cell (RBC), Hemoglobin (HGB), and platelet (PLT). Meanwhile, the spleens of mice are taken out and weighted, and the spleen coefficient is calculated. After executing the above treated mice, left-side intact femurs of mice are taken out and the soft tissue thereof are removed. The whole bone marrow is flushed into a test tube with 10ml of 0.005mol/L CaCl₂. The tube is stood in a fridge at 4°C for 30min, and centrifuged at 2500r/min for 15min. Supernate is removed, and the precipitate is sufficiently mixed with 5ml of 0.2mol/L HClO₄.

After being heated at 90°C for 15min, the tube is cooled and then centrifuged at 3500r/min for 10min. Supernate is used to determine the OD value at a wavelength of 260nm by ELIASA.

Experimental data are presented as mean±S.D. With SPSS software, statistical test is performed with group t and single factor F, the level of test $\alpha=0.05$.

2.3 Result analysis

Table 24 shows the influence of sanguisorba tannins on the bone marrow inhibition caused by Co rays.

Table 24 Comparison on pharmaceutical effect of Sanguisorba tannins prepared by different methods (mean±S.D., n=10)

Group	Dosage (mg/kg)	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/L)	PLT ($10^9/L$)	Spleen Coefficient (mg/10g)	DNA content in bone marrow (μg)
Blank group	-	6.55±0.32	5.21±0.37	157.43±13.76	521.27±43.45	35.19±3.98	129.48±7.65
Model group	-	2.62±0.22	3.89±0.24	121.39±10.11	276.89±34.33	21.26±2.56	69.92±5.58
Positive group	30 $\mu g/kg \cdot d$	7.43±0.27 ^Δ	3.76±0.46	133.58±11.88	423.25±34.56	34.21±2.29 ^Δ	52.29±5.69
Example 1	10	4.44±0.33 ^Δ	4.02±0.25	137.76±14.54	433.23±38.87	28.27±3.32 ^Δ	99.67±6.59 ^Δ
Example 2	10	4.72±0.26 ^Δ	4.12±0.23	132.84±8.65	398.52±41.65	29.32±2.79 ^Δ	96.47±6.55 ^Δ
Example 3	10	5.45±0.31 ^Δ	4.18±0.43	136.48±15.07	402.64±38.57	31.21±3.09 ^Δ	109.76±8.53 ^Δ
Example 4	10	5.63±0.38 ^Δ	3.98±0.26	131.55±7.69	399.07±35.11	32.45±3.14 ^Δ	115.54±8.12 ^Δ
Example 5	10	4.25±0.37 ^Δ	3.87±0.34	138.27±7.55	421.09±30.98	28.38±3.45 ^Δ	97.56±6.27 ^Δ
Example 6	10	4.64±0.29 ^Δ	4.07±0.28	130.29±9.42	387.43±29.75	29.57±2.67 ^Δ	99.89±5.29 ^Δ
Example 7	10	5.75±0.43 ^Δ	4.22±0.18	135.69±12.38	409.28±21.98	32.87±3.34 ^Δ	121.55±9.60 ^Δ
Example 8	10	4.25±0.28 ^Δ	3.54±0.32	127.34±13.09	394.76±24.55	28.65±3.07 ^Δ	102.37±8.44 ^Δ

Δ: P<0.05, compared with model group

It can be known from the experimental results that the sanguisorba tannins extract of the present invention has a significant protection against the decrease of DNA content in bone marrow and the decrease of white blood cells caused by chemical substances (P<0.05). Moreover, with the increase of sanguisorba tannins content, the contents of sanguin, catechins, and proanthocyanidin B2 will increase accordingly, and thus the pharmaceutical effect reflects an upward trend.

Test 8 Side effects of sanguisorba tannins extract

1. Test on acute toxicity

KM mice are randomly divided into a blank group and a drug administration group, 20 animals each group. Normal saline was given to the blank group through intragastric administration, while the largest volume of drug with the highest concentration (around 2g extract /ml) with which animals can endure is given to the animals in the drug administration group through intragastric administration (ig). Observation for 2 weeks. By calculation, the largest tolerant quantity of mice against the sanguisorba tannins extract (Prepared according to Example 9) is 80g/kg.

2. Test on different drug administration methods

KM mice are randomly divided into an intramuscular injection group, an intragastric administration group, an intraperitoneal group, a subcutaneous injection group and an intravenous administration group, 10 animals each group. Except for the blank group, the equivalent volume of dosage 10mg/kg of sanguisorba tannins extract (Prepared according to Example 9) is added into other groups respectively for 14 days. After the animals are executed, the histomorphological change of the animals by anatomy are observed and pictured.

Results show other drug administration methods of tannins have strong side effects as follows:

Intraperitoneal (ip): adhesive lesion of muscle or tissue;

Intramuscular injection (im): extravasated blood in muscle;

Subcutaneous injection (sc): congestion in subcutaneous tissues;

Intravenous injection (iv): mice was dead immediately.

However, the method of intragastric (ig) administration proves no side effect, which indicates sanguisorba tannins can be adopted for intragastric administration.

The above experiments on pharmaceutical effect can prove that, the sanguisorba tannins extract of the present invention contains 35%-100%w/w of tannins, less than 10% (w/w) of total saponins, and less than 5% (w/w) of sanguisorbin I. The activity ingredient in the extract is sanguisorba tannins, which has an efficacy increasing with the increase of the content of sanguisorba tannins. Used with the same dosage, high

purity sanguisorba tannins are superior to sanguin and catechins etc., and can protect bone marrow hematopoietic stem cells and have a remarkable protective effect on the inhibition of mice bone marrow DNA caused by chemical substances and radiotherapy and increase the white blood cells. When used in combination with chemotherapeutic drug, sanguisorba tannins can improve the efficacy of anti-tumor, protect bone marrow cells and whole blood cells, reduce or avoid the damages to bone marrow cells and whole blood cells caused by chemotherapeutics. It can be prepared through a simple process with high yield. Therefore, it is a new choice for treating and preventing normal bone marrow cells inhibition caused by radiotherapy and chemotherapy.

Industrial application

The sanguisorba tannins extract of the present invention can protect bone marrow hematopoietic stem cells, and have remarkable protective effects on the inhibition of mice bone marrow DNA caused by chemical substances and radiotherapy. In addition, the sanguisorba tannins extract of the present invention can increase white blood cells significantly. The product provides a new choice for treating and preventing normal bone marrow cells inhibition caused by radiotherapy and chemotherapy with an excellent clinical application and industrial prospects.

Claims

1. Use of sanguisorba tannins extract for preparing medicaments for protecting bone marrow,

wherein said sanguisorba tannins extract comprises 35%-100% (w/w) of tannins, less than 10% (w/w) of total saponins, and less than 5% (w/w) of sanguisorbin I.

2. The use according to claim 1, wherein said medicaments are used to prevent and/or treat bone marrow inhibition.

3. The use according to claim 1 or 2, wherein said medicaments are used to prevent and/or treat normal bone marrow cell inhibition caused by radiotherapy or chemotherapy.

4. The use according to claim 3, wherein said medicaments are used to prevent and/or treat anemia, leucopenia or thrombocytopenia, preferably leucopenia, caused by radiotherapy or chemotherapy.

5. Use of sanguisorba tannins extract for preparing medicaments for the treatment of leucopenia,

wherein said sanguisorba tannins extract comprises 35%-100% (w/w) of tannins, less than 10% (w/w) of total saponins, and less than 5% (w/w) of sanguisorbin I.

6. A pharmaceutical composition for preventing or treating bone marrow inhibition or leucopenia, wherein comprises an effective amount of sanguisorba tannins extract and is prepared to common formulation by adding pharmaceutically acceptable adjuvants or ancillary components,

wherein said sanguisorba tannins extract comprises 35%-100% (w/w) of tannins, less than 10% (w/w) of total saponins, and less than 5% (w/w) of sanguisorbin I.

7. The medical composition according to claim 6, wherein said formulation is an oral formulation.

8. The use according to claim 1 or 5, wherein said extract contains 50%-100% (w/w) of tannins.

9. The use according to claim 8, wherein said extract contains 55%-100% (w/w) of tannins.

10. The use according to claim 1 or 5 or 8, wherein said extract comprises less than 7.2% (w/w) of total saponins, and less than 4.9% (w/w) of sanguisorbin I.

11. The use according to claim 10, wherein said extract comprises less than 0.001% (w/w) of total saponins, and less than 0.0001% (w/w) of sanguisorbin I.

12. The use according to claim 1 or 5 or 8, wherein said sanguisorba tannins comprises 0.05% to 3.0% by weight of sanguiin, 1.0% to 5.0% by weight of catechin, and 0.05% to 1.5% by weight of proanthocyanidin B2, all calculated based on said extract.

13. The use according to claim 12, wherein said sanguisorba tannins comprises 1.0% to 2.0% by weight of sanguiin, 2.0% to 4.0% by weight of catechin, and 0.05% to 0.4% by weight of proanthocyanidin B2, all calculated based on said extract.

14. The use according to any one of claims 1 or 5, 8 to 13, wherein said extract originates from dried roots of *Sanguisorba officinalis* L or *Sanguisorba officinalis* L. var.*longifolia* (Bert.) Yu et Li of Rosaceae.

15. The use according to any one of claims 1 or 5, 8 to 13, wherein said extract is prepared through the following steps:

1) extraction: grinding Garden Burnet Root into coarse powders, extracting it with water, alcohol, hydrous ethanol, acetone or hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: treating the concentrated solution obtained in step 1) with common tannins separation method, thus obtaining the sanguisorba tannins extract.

16. The use according to claim 15, wherein said common tannins separation method comprises chromatography method, protein precipitation method, solvent method or the combination thereof.

17. The use according to claim 16, wherein in said chromatography method, the absorption is carried out with gel and macro-porous adsorption resin.

18. The use according to claim 16, wherein in said protein precipitation method the precipitation is carried out with gelatin.

19. The use according to claim 16, wherein in said solvent method, aqueous solution containing the concentrated solution is defatted and then extracted by ethyl acetate so as to obtain the sanguisorba tannins extract;

alternatively, the concentrated solution is dissolved in ethanol and ethyl acetate, and the extract is precipitated through adding ethyl ether or petroleum ether.

20. The use according to any one of claims 15 to 19, wherein said extract is prepared through the following steps:

1) extraction: grinding Garden Burnet Root into coarse powders, extracting it with water, alcohol, 10%-90% hydrous ethanol, acetone or 50%-90% hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: adsorbing and purifying the concentrated solution with macroporous adsorption resin, wherein it is eluted with water into colorless, then with 10% ethanol, finally with 60% ethanol, and collecting the elution with 60% ethanol, thus obtaining the sanguisorba tannins extract after drying;

alternatively, defatting the concentrated solution and then extracting it with ethyl acetate, thus obtaining the sanguisorba tannins extract.

21. The use according to claim 20, wherein said macroporous adsorption resin is non-polar resin or low-polar resin.

22. The use according to claim 21, wherein said macroporous adsorption resin is DA-201 macroporous adsorption resin, D-101 macroporous adsorption resin, LSA-20 macroporous adsorption resin, HP-10 macroporous adsorption resin or AB-8 macroporous adsorption resin.

23. A process for preparing the extract of claim 1 or 5, wherein comprising the following steps:

1) extraction: grinding Garden Burnet Root into coarse powders, extracting it with water, alcohol, hydrous ethanol, acetone or hydrous acetone, and obtaining a concentrated solution after filtration and concentration ;

2) purification: treating the concentrated solution with common tannins separation method, thus obtaining the sanguisorba tannins extract.

24. The process according to claim 23, wherein said common tannins separation method comprises chromatography method, protein precipitation method, solvent method or the combination thereof.

25. The process according to claim 24, wherein in said chromatography method the absorption is carried out with gel and macro-porous adsorption resin.

26. The process according to claim 24, wherein in said protein precipitation method the precipitation is carried out with gelatin.

27. The process according to claim 24, wherein in said solvent method, aqueous solution containing the concentrated solution is defatted and then extracted by ethyl acetate so as to obtain the sanguisorba tannins extract;

alternatively, the concentrated solution is dissolved in ethanol and ethyl acetate, and the extract is precipitated through adding ethyl ether or petroleum ether.

28. The process according to any one of claims 23 to 27, wherein said extract is prepared through the following steps:

1) extraction: grinding Garden Burnet Root into coarse powders, extracting it with water, alcohol, 10%-90% hydrous ethanol, acetone or 50%-90% hydrous acetone, and obtaining a concentrated solution after filtration and concentration;

2) purification: adsorbing and purifying the concentrated solution with macroporous adsorption resin, wherein it is eluted with water into colorless, then with 10% ethanol, finally with 60% ethanol, and collecting the elution with 60% ethanol, thus obtaining the sanguisorba tannins extract after drying;

alternatively, defatting the concentrated solution and then extracting it with ethyl acetate, thus obtaining the sanguisorba tannins extract.

29. The process according to claim 28, wherein said macroporous adsorption resin is DA-201 macroporous adsorption resin, D-101 macroporous adsorption resin, LSA-20 macroporous adsorption resin, HP-10 macroporous adsorption resin or AB-8 macroporous adsorption resin.