The present invention relates to Coix seed oil extracted from *Semen Coicis*, pharmaceutical preparations thereof, and the use thereof in the treatment of tumors. Specifically, the Coix seed oil contains 11 triglyceride ingredients in the following mass percentages: trilinolein 4.87-6.99%, 1-olein-2,3-dilinolein 13.00-18.69%, 1-palmitin-2,3-dilinolein 5.25-7.54%, 1,3-diolein-2-linolein 13.23-19.02%, 1-palmitin-2-linolein-3-olein 10.26-14.75%, 1,3-dipalmitin-2-linolein 2.28-3.28%, triolein 14.44-20.76%, and 1-palmitin-2,3-diolein 8.06-11.58%, 1-olein-2-linolein-3-stearin 1.37-1.97%, 1,3-dipalmitin-2-olein 1.52-2.19%, and 1,2-diolein-3-stearin 1.29-1.86%.

18 Claims, No Drawings
COIX SEED OIL CONTAINING 11 TRIGLYCERIDES, AND PHARMACEUTICAL PREPARATION AND USE THEREOF

CROSS REFERENCE TO RELATED PATENT APPLICATION

The present application is the US national stage of PCT/ CN2015/084097 filed on Jul. 17, 2015, which claims the priority of the Chinese patent application No. 201410343083.4 filed on Jul. 18, 2014, which applications are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to the pharmaceutical field, specifically, the present invention relates to Coix seed oil, pharmaceutical preparations thereof, the process for the preparation of same and the use thereof in the preparation of antitumor drugs.

BACKGROUND OF THE INVENTION

Coix seeds are dried ripe seeds of Coix lacryma-jobi L. var ma-yuen (Roman.), Staf, a genus of plant in the Poaceae family. It is a dampness-eliminating drug and has been used as a medicinal and edible plant for a long time. Modern researches have found that Coix seeds have many pharmacological effects, such as analgesic anti-inflammatory, immunomodulatory, anti-urea, hypolipidemic and anti-obesity effects. In recent years, researchers around the world have studied the chemical composition of the Coix seed by using TLC, HPLC-MS, GC, etc., and found a variety of active ingredients in it, including coixenolide, triglycerides, fatty acids, lactams, coix lactones, saponars, sterols and triterpenoids. Among them, esters are the first discovered components having anti-tumor activities and the most reported chemical composition attracting the most attention. Kanglaihe injection, in which the active ingredient is Coix seed oil, has been widely used in present Chinese clinical applications, but the Coix seed oil used in the Kanglaihe injection comprises complex components. In addition to triglycerides, it also contains monoglycerides, diglycerides and fatty acid esters, etc. This will inevitably be a great challenge for the quality control in the practical production process and the safety in clinical applications.

In the present invention, the raw material Coix seed powder has been treated by supercritical carbon dioxide extraction, basification, neutral alumina purification and kaolin purification, etc., to afford an effective part, Coix seed oil. With the active ingredients’ isolation and identification, it is determined that the Coix seed oil comprises mainly 11 triglyceride components. Further determination of its physicochemical constants has confirmed the optimal acid value, iodine value, saponification value, refractive index and specific gravity, etc. The use of the Coix seed oil of the invention in medication has advantages such as the confirmed composition of ingredients, ensuring quality stability in every batch in the industrial production, and avoiding toxic and side effects brought about by the complex ingredients while adopting Coix seed oil directly.

SUMMARY OF THE INVENTION

The first aspect of the invention is to provide a Coix seed oil extracted from Semen Coixis. The Coix seed oil contains 11 triglyceride ingredients in the following mass percent-ages: trilinolein 4.87-6.99%, 1-olein-2,3-dilinolein 13.00-18.69%, 1-palmitin-2,3-dilinolein 5.25-7.54%, 1,3-diollein-2-linolein 13.23-19.02%, 1-palmitin-2-linolein-3-oilein 10.26-14.75%, 1,3-dipalmitin-2-linolein 2.28-3.28%, trilinolein 14.44-20.76%, 1-palmitin-2,3-diollein 8.06-11.58%, 1-olein-2-linolein-3-stearin 1.37-1.97%, 1,3-dipalmitin-2-olein 1.52-2.19% and 1,2-diollein-3-stearin 1.29-1.86%.

Preferably, mass percentage contents of the above 11 triglyceride ingredients are: trilinolein 5.47-6.69%, 1-olein-2,3-dilinolein 14.63-17.88%, 1-palmitin-2,3-dilinolein 5.90-7.21%, 1,3-diollein-2-linolein 14.88-18.19%, 1-palmitin-2-linolein-3-oilein 11.55-14.11%, 1,3-dipalmitin-2-linolein 2.57-3.14%, trilinolein 16.25-19.86%, 1-palmitin-2,3-diollein 9.07-11.08%, 1-olein-2-linolein-3-stearin 1.54-1.88%, 1,3-dipalmitin-2-olein 1.71-2.09% and 1,2-diollein-3-stearin 1.45-1.78%.

More preferably, mass percentage contents of the above 11 triglyceride ingredients are: trilinolein 5.96-6.20%, 1-olein-2,3-dilinolein 15.93-16.58%, 1-palmitin-2,3-dilinolein 6.43-6.69%, 1,3-diollein-2-linolein 16.20-16.87%, 1-palmitin-2-linolein-3-oilein 12.57-13.09%, 1,3-dipalmitin-2-olein 2.79-2.91%, trilinolein 17.69-18.42%, 1-palmitin-2-3-diollein 9.87-10.27%, 1-olein-2-linolein-3-stearin 1.68-1.74%, 1,3-dipalmitin-2-olein 1.86-1.94% and 1,2-diollein-3-stearin 1.58-1.65%.

The above contents refer to the mass percentage contents of triglyceride compounds in the Coix seed oil. 11 triglyceride monomer compounds can be separated by using preparative chromatography from the coix seed oil prepared by the following steps, and their contents can be obtained by weighing and calculating the products. They can also be obtained according to conventional analytic methods in the art.

The Coix seed oil has the following physicochemical constants based on the fatty oil detection: specific gravity at 20°C 0.917-0.920, refractive index at 20°C C 1.471-1.474, acid value >0.2, iodine value 100-106, saponification value 188-195.

The Coix seed oil of the invention can be prepared by a process comprising steps of:

(1) Supercritical Carbon Dioxide Extraction:
Crushing Coix seeds into 20-60 mesh powder and extracting the powder using a supercritical CO2 extraction system in which coix seed powder is put in an extractor; the CO2 preheater, extractor and separation column are heated by jacketed circulating hot water to make the extraction temperature and separation temperature be 33-45°C and 30-45°C, respectively; the outlet temperatures of separator 1 and separator 2 are kept at 20-50°C and 15-35°C, respectively; the liquid CO2 is pressed at a flow rate of 2.5 kg/h/kg-7.5 kg/h/kg (based on the mass of the coix seed powder) into the CO2 preheater via a high pressure pump turning into a fluid in supercritical state; in the extractor, an oil is extracted into the CO2 fluid at a pressure of 19-23 Mpa; then the CO2 fluid with this oil enters the separation column in which the pressure is controlled to 7-10 Mpa to separate this oil; the CO2 gas out from the separation column enters sequentially into separator 1 and separator II in which the pressure is sustained at 5-7 Mpa and 4-6 Mpa, respectively; impurities such as water separated therefrom are discarded; the CO2 gas returns to liquid CO2 for reuse through a condenser; and a continuous extraction for 2-3 h affords a crude coix seed oil; and
(2) Refining Process:
Adding 55% petroleum ether (bp. 60°C-90°C), based on the oil weight, into the crude coix seed oil obtained by the supercritical CO2 extraction; adding 2% NaOH aqueous
solution in an amount ranging from 36% to 56% of the oil weight according to the acid value; after stirring the mixture for 10 min and standing for 18-24 h, removing the lower niger layer; washing the upper layer with purified water and letting standing for 18-24 h; after the removal of the lower waste water; washing the upper layer again; after standing for another 40-50 h, removing the lower waste water; and demulsifying the upper layer with acetone in an amount of 70%-90% of the oil weight; after standing for 2-4 h, removing the lower waste acetone and adding 3% to 8% of activated neutral alumina by weight of crude oil in the upper oil layer; stirring the mixture for 30 min, then filtering off the precipitation; heating the filtrate and adding 2% to 6% of activated kaolin by weight of crude oil; stirring the mixture for 30 min at 40-50°C and then filtering off the precipitation; concentrating the filtrate under a reduced pressure to recover the solvent, then washing again with purified water; after standing for 1-2 h, removing the lower waste water and heating the upper oil layer and vacuum drying it in nitrogen atmosphere; then adding 8 to 12% activated neutral alumina by weight of crude oil and stirring the mixture and allowing to stand at a cold place; after filtering, sterilizing the filtrated oil via dry heating under vacuum at 160-170°C for 1-2 h; after cooling, filtering the oil through a 0.2 μm microporous membrane; then split charging the obtained Coix seed oil in 500 mL glass infusion bottles and sealing the bottles.

Preferably, the refining process comprises steps of: adding 55% petroleum ether (bp. 60°C-90°C), based on the oil weight, into the crude coix seed oil obtained by the supercritical CO2 extraction; adding 2% NaOH aqueous solution in an amount ranging from 36% to 56% of the oil weight according to the acid value; after stirring the mixture for 10 min and standing for 20 h, removing the lower niger layer; washing the upper layer with purified water and letting standing for 22 h; after the removal of the lower waste water, washing the upper layer again; after standing for another 46 h, removing the lower waste water; demulsifying the upper layer with acetone in an amount of 70%-90% by weight of the crude oil and standing for 3 h; removing the lower waste acetone and adding 5% of activated neutral alumina by weight of crude oil in the upper oil layer; stirring the mixture for 30 min, then filtering off the precipitation; heating the filtrate, and adding 4% of activated kaolin by weight of crude oil; stirring the mixture for 30 min at 40-50°C, and then filtering off the precipitation; concentrating the filtrate under a reduced pressure to recover the solvent, then washing again with purified water; after standing for 1 h, removing the lower waste water; heating the upper oil layer and vacuum drying it in nitrogen atmosphere; then adding 10% activated neutral alumina, stirring the mixture and allowing to stand at a cold place; after filtration, sterilizing the filtrated oil by dry heating under vacuum at 160-170°C for 2 h; after cooling, filtering the oil through a 0.2 μm microporous membrane; then split charging the obtained Coix seed oil in 500 mL glass infusion bottles and sealing the bottles.

The Coix seed oil of the invention is a yellowish clear liquid with a light odor and a light taste. It is highly soluble in petroleum ether or chloroform, freely soluble in acetone, slightly soluble in ethanol, but insoluble in water.

The Coix seed oil prepared based on the above methods was detected according to the method in the appendix of "Pharmacopoeia of the People's Republic of China" (2010 Edition) Volume 1. Physicochemical constants thereof are: specific gravity at 20°C 0.917-0.920, refractive index at 20°C 1.471-1.474, acid value <0.2, iodine value 100-106, saponification value 188-195. The acid value according to the Pharmacopoeia refers to the weight of potassium hydroxide (in milligrams) needed to neutralize free fatty acids contained in 1 gram of fats, fatty oils, or other similar substances. In the quality study of oil products, acid value is an important evaluation. As far as the Coix seed oil of the invention, the acid value is less than 0.2. By the optimization of the preparation process such as supercritical extraction parameters and the purification process like basification, Coix seed oil was prepared with the following advantages: on the one hand, it has a very low content of free fatty acid impurities, which means a high product quality; on the other hand, it gathers a great amount of active ingredients of triglycerides in high purity, and the types of triglyceride ingredients therein are determinate, and the contents thereof are stable. In addition, other physicochemical constants, such as saponification value, iodine value, etc., measured between batches of samples, had a small range of variation. It further illustrates that the Coix seed oil of the invention has a stable quality and a safer clinical use. The preparation method of the invention gives a stable product with a high yield and a low cost. It is suitable for the industrial production in view of the safety and controllability.

The second aspect of the invention is to provide a pharmaceutical preparation containing Coix seed oil, specifically, it comprises a therapeutically effective amount of the Coix seed oil of the invention and one or more pharmaceutically acceptable carriers.

Pharmaceutically acceptable carriers can be selected from pharmaceutical conventional dilutions, excipients, fillers, emulsifiers, binders, lubricants, absorption accelerators, surfactants, disintegrants, lubricants and antioxidants, if necessary, flavoring agents, sweeteners, preservatives and/or coloring agents.

Pharmaceutically acceptable carriers can be selected from one or more in the group consisting of: mannitol, sorbitol, sodium metabisulfite, sodium bisulfate, sodium thiosulfate, cysteine hydrochloride, thioglycolic acid, methionine, soybean lecithin, vitamin C, vitamin E, EDTA disodium, EDTA calcium sodium, monovalent alkali metal carbonate, acetate, phosphate or its aqueous solution, hydrochloric acid, acetic acid, sulfonic acid, phosphoric acid, amino acids, sodium chloride, potassium chloride, sodium lactate, ethylparaben solution, benzoic acid, potassium sorbate, chlorhexidine, xylitol, maltose, glucose, fructose, dextrose, glycine, starch, sucrose, lactose, mannitol, silicic derivatives, cellulose and its derivatives, alginites, gelatin, polyvinyl pyrrolidone, glycercin, Tween 80, agar-agar, calcium carbonate, calcium bicarbonate, surfactants, emulsifiers, polyethylene glycol, cyclodextrin, β-cyclodextrin, phospholipid material, kaolin, talc, and calcium stearate or magnesium stearate.

The pharmaceutical preparation of the invention can be oral solid preparations, oral liquid preparations or injections.

Preferably, the oral solid preparation is selected from any one of capsules, tablets, dripping pills, granules, and concentrated pills; the oral liquid preparation is selected from any one of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, and a dry product that may be reconstructed by water or other suitable carrier(s) before use; and the injection is selected from any one of nano suspensions, liposomes, emulsions, lyophilized powder for injection and aqueous injection.

More preferably, the injection comprises the following components: the Coix seed oil of the invention 50-350 g, soybean lecithin for injection or soybean lecithin acceptable
for injection 10-40 g, glycerin for injection or glycerin acceptable for injection 15-50 g, and Water for injection added to 1000 mL.

The injection of the invention can be prepared by a method comprising steps of:
1. adding appropriate amount of water for injection to a formulated amount of soybean lecithin for injection or soybean lecithin acceptable for injection; dispersing the mixture with a high shear dispersing emulsifier to give a dispersion without bulks or granules; adding a formulated amount of glycerin for injection or glycerin acceptable for injection; then adding water for injection to a specified amount, and stirring the mixture to give a water phase;
2. weighing a formulated amount of Coix seed oil; heating the weighed oil and the water phase separately to 60-70°C, then mixing them and emulsifying the mixture in a high pressure homogenizer; the low pressure is 5-12 MPa and the high pressure is 25-50 MPa; repeating the cycle of homogenization for 3-6 times until the amount of particles below 2 μm is no less than 95% and particles above 5 μm are undetectable; if necessary, using NaOH or HCl to adjust the pH to 4.8 to 8.5, preferably 6.8 to 7.0, most preferably 6.8; and
3. filtering the resulting homogeneous emulsion by nitrogen pressure through a microporous filter of 3 μm or less; filling the emulsion, nitrogenizing, sterilizing and cooling to afford the injection.

The capsule of the invention comprises the following components: Coix seed oil 80-300 mg, antioxidant(s) and/or emulsifier(s) 0.20-0.60 g for 1000 capsules.

The capsule of the invention can be prepared by a method comprising steps of:
1. preparing glue solution: weighing gelatin, purified water, glycerin and a preservative at a weight ratio of 1:0.6-1.2: 0.3-0.8:0.0001-0.01; adding glycerin, purified water and preservative (selected from any one of 10% ethylparaben solution, benzoic acid, potassium sorbate and chlorhexidine acetate) sequentially into a glue melting tank; heating to 70°C-C.90°C; then adding gelatin and constantly stirring the mixture until the gelatin is completely dissolved; filtering the glue solution and storing the filtered glue solution at 56-62°C for use;
2. preparing drug liquid: adding formulated amount of Coix seed oil, antioxidant (Vitamin E) and/or emulsifier (Tween 80) into a dosing tank, and stirring the mixture constantly until being homogeneously mixed; and
3. pressing capsules: choosing proper pellet dies according to the capsule size; pressing capsules in a temperature of 15-30°C and a relative humidity of less than 35%; drying the pressed capsules at 10°C-hour, removing capsules of abnormal size, washing the normal capsules with 95% medical ethanol, and drying them continuously till the moisture content is less than 12%; visually inspecting and removing unqualified capsules; finally printing and packaging to afford the capsules.

It is demonstrated, in pharmacodynamic experiments, that the Coix seed oil of the invention and the pharmaceutical preparation thereof have shown different degrees of inhibition on a variety of human tumor cell lines. They can be used as antitumor drugs.

Therefore, another aspect of the invention is to provide a use of Coix seed oil of the invention in the preparation of antitumor drugs.

The tumors refer to lung cancer, liver cancer, pancreatic cancer, prostate cancer, ovarian cancer and breast cancer, in early, middle or late stage.

The following experimental data are used to illustrate anti-tumor effects of the Coix seed oil of the invention and the pharmaceutical preparations thereof.
1. Inhibition of Coix Seed Oil and Preparations Thereof on 8 Human Tumor Cell Lines in MTT Method In Vitro
   A. Experimental Materials and the Preparation Thereof:
   (1) Cell lines: PANC-1 (human pancreatic cancer cells), SKOV3 (human ovarian cancer cells), MCF-7 (human breast cancer cells), Bcap-37 (human breast cancer cells), SMMC-7721 (human hepatic cancer cells), HepG2 (human hepatic cancer cells), A549 (human lung cancer cells) and H460 (human lung cancer cells), stored and passaged maintainably in Research and Evaluation Center for Pharmacology, Shanghai Institute of Pharmaceutical Industry;
   (2) DMEM complete medium supplied with 10% newborn calf serum (GIBCO BRL), 1% of penicillin (100 U/mL), streptomycin (100 μg/mL);
   (3) 0.25% trypsin solution, purchased from Invitrogen Corp. and stored at –20°C;
   (4) Phosphate buffer (PBS): NaCl 8 g, KCl 0.2 g, Na2HPO4·1.15 g and KH2PO4·0.2 g, dissolved in 1 L double-distilled water and autoclaved at 121°C for 20 min, then stored at 4°C;
   (5) MTT (AMRESCO) solution: 5 mg/mL in PBS;
   (6) Formazan crystal dissolving solution: SDS 10 g, isobutanol 5 mL and concentrated hydrochloric acid 0.1 mL, dissolved in 100 mL of deionized double distilled water.
   B. Experimental Method
   The inhibition effects of samples on the above-mentioned cell lines were detected by using MTT method. The specific procedures were as follows:
   (1) Cell culture: (a) Stored cells were taken out from the liquid nitrogen, thawed quickly in a 37°C water bath, and asperically transferred into 6 mL of cellular medium in a 10 mL centrifugal tube, centrifuged at 1000 rpm for 5 min. The supernatant was discarded, then the precipitated cells were re-suspended in 5-6 mL cellular media by pipetting and transferred into a flask at a 37°C incubator for cell culture; (b) Next day, the flask was taken out from the incubator and the used medium was discarded, then the cells were incubated in 5-6 mL fresh medium in the 37°C incubator; (c) On the third day, the flask was taken out from the incubator and the used medium was discarded, then 2-3 mL of PBS (pH7.4) was added into the flask with rocking for cleaning it and the used PBS was discarded. Such a cellular cleaning step was repeated once again; 3-5 drops of 0.25% trypsin solution were added into the flask with sloshing, thus well-distributed in it. The flask was capped and placed in a 37°C incubator for about 5 min, and the separation of cells from the flask wall was observed under the microscope. 2 mL of cellular medium was added and cells were separated completely from the flask wall by pipetting, then the cell suspension was transferred into 2 separate clean flasks, each containing 5-6 mL medium. The cell suspension was well-distributed by pipetting, then the flask was placed in a 37°C incubator. (d) Step (c) was repeated every other day. In the whole cultivation process, adherent cells were not allowed to grow too dense and suspension cells were always maintained at a logarithmic growth stage.
   (2) Preparation of the sample and the control: A proper amount of sample of Coix seed oil (oil of Job’s tears seed) was dissolved in DMSO to obtain a solution in a concentration of 10 mg/mL. This solution was diluted in a gradient dilution with PBS to obtain a set of sample solutions in the concentration of 10 mg/mL, 5000 μg/mL, 2500 μg/mL, 1250 μg/mL, 625 μg/mL and 312.5 μg/mL, respectively.
Each diluted sample solution was added into duplicated wells of a 96 well flat-bottom microplate (10 μl/well). The correspondingly diluted DMSO solutions, as controls, were added into the wells of the microplate.

Cells in a logarithmic growth stage were trypsinized and washed, then re-suspended in the medium containing 10% calf serum. The number of living cells was counted in Trypan blue dye exclusion method and cell suspensions were adjusted into a density of 2×10⁴ cell/ml.

The cell-contained 96 well flat-bottom microplate was placed in a 37°C incubator and cells were incubated under 5% CO₂ for 48 h.

20 μl of 5 mg/ml MTT solution was added into each well and cells were incubated continuously in the incubator for 3-4 h.

100 μl of crystal dissolving solution was added into each well and cells were incubated continuously in the incubator overnight, so as to dissolve the resulted formazan crystals sufficiently. Then, the absorbance value was measured at 570 nm for each well.

Based on absorbance values, inhibition rates on the cell growth were calculated for sample groups of various concentrations. The calculation formula was as follows:

\[
(1-\text{mean absorbance of experimental wells/mean absorbance of control wells})\times100\%
\]

### C. Experimental Results

**TABLE 1**

<table>
<thead>
<tr>
<th>Concentration of Sample</th>
<th>1000 μg/ml</th>
<th>500 μg/ml</th>
<th>250 μg/ml</th>
<th>125 μg/ml</th>
<th>62.5 μg/ml</th>
<th>31.25 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panc-1</td>
<td>75.00</td>
<td>51.71</td>
<td>27.30</td>
<td>2.51</td>
<td>0.63</td>
<td>0.07</td>
</tr>
<tr>
<td>SKOV3</td>
<td>99.12</td>
<td>65.15</td>
<td>32.13</td>
<td>18.15</td>
<td>12.12</td>
<td>2.12</td>
</tr>
<tr>
<td>MCF-7</td>
<td>98.76</td>
<td>63.29</td>
<td>24.98</td>
<td>12.90</td>
<td>8.20</td>
<td>1.08</td>
</tr>
<tr>
<td>Bcap-37</td>
<td>99.23</td>
<td>71.93</td>
<td>35.24</td>
<td>14.37</td>
<td>13.76</td>
<td>0.95</td>
</tr>
<tr>
<td>SMMC-7721</td>
<td>98.54</td>
<td>66.45</td>
<td>41.33</td>
<td>20.53</td>
<td>14.04</td>
<td>4.20</td>
</tr>
<tr>
<td>HepG2</td>
<td>97.98</td>
<td>68.31</td>
<td>33.44</td>
<td>25.85</td>
<td>12.69</td>
<td>2.49</td>
</tr>
<tr>
<td>A549</td>
<td>98.93</td>
<td>76.52</td>
<td>57.04</td>
<td>42.80</td>
<td>22.22</td>
<td>2.05</td>
</tr>
<tr>
<td>H460</td>
<td>97.65</td>
<td>75.88</td>
<td>57.09</td>
<td>43.61</td>
<td>17.78</td>
<td>2.41</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Coix seed oil</th>
<th>Positive control (Taxotol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panc-1</td>
<td>513.6</td>
<td>0.44</td>
</tr>
<tr>
<td>SKOV3</td>
<td>244.4</td>
<td>0.22</td>
</tr>
<tr>
<td>MCF-7</td>
<td>257.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Bcap-37</td>
<td>247.0</td>
<td>0.28</td>
</tr>
<tr>
<td>SMMC-7721</td>
<td>206.7</td>
<td>0.41</td>
</tr>
<tr>
<td>HepG2</td>
<td>223.7</td>
<td>0.45</td>
</tr>
<tr>
<td>A549</td>
<td>188.1</td>
<td>0.46</td>
</tr>
<tr>
<td>H460</td>
<td>181.6</td>
<td>0.49</td>
</tr>
</tbody>
</table>

### D. Conclusion

The Coix seed oil of the invention in various concentrations has shown the inhibition on 8 human tumor cell lines in different degrees.

Further experiments have confirmed that the Coix seed oil of the invention and preparations thereof can also achieve the desired effects described in the above experimental examples.

The following examples further illustrate the invention, but are not construed as a limitation on the invention.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

**Example 1 Preparation of Coix Seed Oil**

Supercritical carbon dioxide extraction: Coix seeds were crushed into 20 mesh powder and extracted using a supercritical CO₂ extractor. Coix seed powder was put in an extractor. The CO₂ preheater, extractor and separation column were heated by jacketed circulating hot water, so that the extraction temperature and separation temperature reached 40°C and 45°C, respectively, and the outlet temperatures of separator I and separator II were kept 50°C and 35°C, respectively. Liquid CO₂ was pressurized, at a flow rate of 2.5 kg/h/kg (based on the mass of the coix seed powder), into the CO₂ preheater via a high pressure pump, turning into a fluid in supercritical state. In the extractor, an oil was extracted into the CO₂ fluid at a pressure of 20 Mpa. Then the CO₂ fluid with this oil entered a separation column, and the pressure of the separation column was controlled to 7 Mpa to separate the oil. The CO₂ gas out from the separation column entered sequentially into separator I and separator II, in which the pressure was sustained at 7 Mpa and 6 Mpa, respectively. Impurities like water separated therefrom were discarded. The CO₂ gas returned to liquid CO₂ for reuse through a condenser. A continuous extraction for 2.5 h afforded a crude coix seed oil.

Refining: To the crude coix seed oil obtained by supercritical CO₂ extraction was added 55% petroleum ether (60°C C) based on the oil weight. 45% NaOH aqueous solution (2%) based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 20 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 22 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 46 h, the lower waste water was removed, and the upper layer was demulsified by adding 80% acetone based on the oil weight. After standing for 3 h, the lower waste acetone was removed. The upper oil layer was added 5% activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 4% activated kaolin by weight of the crude oil, stirred for 30 min at 45°C, and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 1 h, the lower waste water was removed. The upper oil was heated and vacuum dried under nitrogen atmosphere. Then 10% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 165°C for 2 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.5%. Physicochemical constants were detected as: specific gravity at 20°C, 0.917; refractive index at 20°C, 1.471; acid value 0.18; iodine value 102; and saponification value 190.

**Example 2 Preparation of Coix Seed Oil**

Supercritical carbon dioxide extraction: Coix seeds were crushed into 30 mesh powder and extracted using a supercritical CO₂ extractor. Coix seed powder was put in the
extractor. The CO₂ preheater, extractor and separation column were heated by jacketed circulating hot water, so that the extraction temperature and separation temperature reached 35° C. and 40° C., respectively, and the outlet temperatures of separator I and separator II were kept at 20° C. and 15° C., respectively. Liquid CO₂ was pressurized into the CO₂ preheater via a high pressure pump at a flow rate of 7.5 kg/h/kg (based on the mass of the coix seed powder), turning into a fluid in supercritical state. In the extractor, an oil was extracted into the CO₂ fluid at a pressure of 22 Mpa. Then the CO₂ fluid with this oil entered a separation column, and the pressure of the separation column was controlled to 9 Mpa to separate the oil. The CO₂ gas out from the separation column entered sequentially into separator I and separator II, in which the pressure was sustained at 5 Mpa and 4 Mpa, respectively. Impurities like water separated therefrom were discarded. The CO₂ gas returned to liquid CO₂ for reuse through a condenser. A continuous extraction for 3 h afforded a crude coix seed oil.

Refining: To the crude coix seed oil obtained by supercritical CO₂ extraction was added 55% petroleum ether (80° C.) based on the oil weight, and 36% NaOH aqueous solution (2%) based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 24 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 24 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 48 h, the lower waste water was removed, and the upper layer was demulsified by adding 75% acetone based on the oil weight. After standing for 2 h, the lower waste acetone was removed. The upper oil layer was added 8% of activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 6% of activated kaolin by weight of the crude oil, stirred for 30 min at 42° C., and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 2 h, the lower waste water was removed. The upper oil layer was heated and vacuum dried in nitrogen atmosphere. Then 11% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 160° C. for 2 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.9%. Physicochemical constants were detected as: specific gravity at 20° C., 0.920; refractive index at 20° C., 1.473; acid value 0.19; iodine value 104; and saponification value 188.

Example 3 Preparation of Coix Seed Oil

Supercritical carbon dioxide extraction: Coix seeds were crushed into 50 mesh powder and extracted using a supercritical CO₂ extractor. Coix seed powder was put in an extractor. The CO₂ preheater, extractor and separation column were heated by jacketed circulating hot water, so that the extraction temperature and separation temperature reached 35° C. and 39° C., respectively, and the outlet temperatures of separator I and separator II were kept at 30° C. and 20° C., respectively. Liquid CO₂ was pressurized into the CO₂ preheater via a high pressure pump at a flow rate of 5.5 kg/h/kg (based on the mass of the coix seed powder), turning into a fluid in supercritical state. In the extractor, an oil was extracted into the CO₂ fluid at a pressure of 19 Mpa. Then the CO₂ fluid with this oil entered a separation column, and the pressure of the separation column was controlled to 9 Mpa to separate the oil. The CO₂ gas out from the separation column entered sequentially into separator I and separator II, in which the pressure was sustained at 5 Mpa and 4 Mpa, respectively. Impurities like water separated therefrom were discarded. The CO₂ gas returned to liquid CO₂ for reuse through a condenser. A continuous extraction for 3 h afforded a crude coix seed oil.

Refining: To the crude coix seed oil obtained by supercritical CO₂ extraction was added 55% petroleum ether (70° C.) based on the oil weight, and 36% NaOH (2%) aqueous solution based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 24 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 24 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 42 h, the lower waste water was removed, and the upper layer was demulsified by adding 75% acetone based on the oil weight. After standing for 2 h, the lower waste acetone was removed. The upper oil layer was added 8% of activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 2% of activated kaolin by weight of the crude oil, stirred for 30 min at 47° C., and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 1 h, the lower waste water was removed. The upper oil layer was heated and vacuum dried in nitrogen atmosphere. Then 11% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 160° C. for 2 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.7%. Physicochemical constants were detected as: specific gravity at 20° C., 0.918; refractive index at 20° C., 1.474; acid value 0.15; iodine value 102; and saponification value 194.

Example 4 Preparation of Coix Seed Oil

Supercritical carbon dioxide extraction: Coix seeds were crushed into 50 mesh powder and extracted using a supercritical CO₂ extractor. Coix seed powder was put in an extractor. The CO₂ preheater, extractor and separation column were heated by jacketed circulating hot water, so that the extraction temperature and separation temperature reached 35° C. and 42° C., respectively, and the outlet temperatures of separator I and separator II were kept at 40° C. and 30° C., respectively. Liquid CO₂ was pressurized into the CO₂ preheater via a high pressure pump at a flow rate of 4.5 kg/h/kg (based on the mass of the coix seed powder), turning into a fluid in supercritical state. In the extractor, an oil was extracted into the CO₂ fluid at a pressure of 21 Mpa. Then the CO₂ fluid with this oil entered a separation column, and the pressure of the separation column was controlled to 10 Mpa to separate the oil. The CO₂ gas out from the separation column entered sequentially into separator I and separator II, in which the pressure was sustained at 7 Mpa and 5 Mpa, respectively. Impurities like water separated therefrom were discarded. The CO₂ gas returned to liquid CO₂ for reuse through a condenser. A continuous extraction for 2 h afforded a crude coix seed oil.

Refining: To the crude coix seed oil obtained by supercritical CO₂ extraction was added 55% petroleum ether (70° C.) based on the oil weight, and 36% NaOH (2%) aqueous solution based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 24 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 24 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 42 h, the lower waste water was removed, and the upper layer was demulsified by adding 75% acetone based on the oil weight. After standing for 2 h, the lower waste acetone was removed. The upper oil layer was added 8% of activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 2% of activated kaolin by weight of the crude oil, stirred for 30 min at 47° C., and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 1 h, the lower waste water was removed. The upper oil layer was heated and vacuum dried in nitrogen atmosphere. Then 11% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 160° C. for 2 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.7%. Physicochemical constants were detected as: specific gravity at 20° C., 0.918; refractive index at 20° C., 1.474; acid value 0.15; iodine value 102; and saponification value 194.
US 10,314,878 B2

11 C.) based on the oil weight, and 50% NaOH (2%) aqueous solution based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 19 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 21 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 50 h, the lower waste water was removed, and the upper layer was demulsified by adding 85% acetone based on the oil weight. After standing for 4 h, the lower waste acetone was removed. The upper oil layer was added 6% of activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 5% activated kaolin by weight of the crude oil, stirred for 30 min at 50°C, and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 1.5 h, the lower waste water was removed. The upper oil layer was heated and vacuum dried in nitrogen atmosphere. Then 12% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 162°C for 1 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.0%. Physicochemical constants were detected as: specific gravity at 20°C, 0.920; refractive index at 20°C, 1.471; acid value 0.16; iodine value 105; and saponification value 192.

Example 5 Preparation of Coix Seed Oil

Supercritical carbon dioxide extraction: Coix seeds were crushed into 60 mesh powder and extracted using a supercritical CO2 extractor. Coix seed powder was put in an extractor. The CO2 preheater, extractor and separation column were heated by jacketed circulating hot water, so that the extraction temperature and separation temperature reached 42°C and 45°C, respectively, and the outlet temperatures of separator I and separator II were kept 35°C and 25°C, respectively. Liquid CO2 was pressed into the CO2 preheater via a high pressure pump at a flow rate of 6.5 kg/h/kg (based on the mass of the coix seed powder), turning into a fluid in supercritical state. In the extractor, an oil was extracted into the CO2 fluid at a pressure of 23 Mpa. Then the CO2 fluid with this oil entered a separation column, and the pressure of the separation column was reduced to 8 Mpa to separate the oil. The CO2 gas out from the separation column entered sequentially into separator I and separator II, in which the pressure was sustained at 6 Mpa and 4 Mpa, respectively. Impurities like water separated therefrom were discarded. The CO2 gas returned to liquid CO2 for reuse through a condenser. A continuous extraction for 2.5 h afforded a crude coix seed oil.

12 Refining: To the crude coix seed oil obtained by supercritical CO2 extraction was added 55% pentane (80°C) based on the oil weight, and 40% NaOH (2%) aqueous solution based on the oil weight was added according to the acid value. After stirring for 10 min, then standing for 24 h, the lower niger layer was removed. The upper layer was washed with purified water and let stand for 24 h. After the removal of the lower waste water, the upper layer went on a second washing. After standing for another 44 h, the lower waste water was removed, and the upper layer was demulsified by adding 70% acetone based on the oil weight. After standing for 3 h, the lower waste acetone was removed. The upper oil layer was added 4% activated neutral alumina by weight of crude oil, stirred for 30 min, and filtered. The filtrate was heated, added with 3% activated kaolin by weight of the crude oil, stirred for 30 min at 40°C, and then filtered. The filtrate was concentrated under a reduced pressure to recover the solvent, and washed again with purified water. After standing for 2 h, the lower waste water was removed. The upper oil layer was heated and vacuum dried in nitrogen atmosphere. Then 8% activated neutral alumina based on the oil weight was added. The mixture was stirred, and allowed to stand at a cold place. After filtration, the filtrated oil was sterilized by dry heating under vacuum at 165°C for 2 h. After cooling, the oil was filtered through a 0.2 μm microporous membrane and split charged in 500 mL glass infusion bottles, and the bottles were sealed. The Coix seed oil was thus obtained in a yield of 4.3%. Physicochemical constants were detected as: specific gravity at 20°C, 0.917; refractive index at 20°C, 1.473; acid value 0.14; iodine value 103; and saponification value 192.

Example 6 Isolation and Identification of Trilinolein

Isolation was carried out on P300A preparative high performance liquid chromatography (Column: Superstar Benetanach™ C18, 20 mm x150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 12.6-14.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give trilinolein.

\[ ^{1}H\text{-NMR data are shown in Table 3.} \]

\[ ^{13}C\text{-NMR data are shown in Table 4.} \]

| TABLE 3 |
|-----|-----|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A   | α β | 6.27 | 1.31 | 1.61 | 1.32 | 1.84 | 4.95 | 2.77 | 6.36 | 2.06 | 1.52 | 0.59 | 1.84 | 1.53 |
| β'  | 4.15 | 1.77 | 5.37 | 1.64 | 1.33 | 8.85 |
| B   | α   | 4.19 | 2.32 | 1.51 | 1.33 | 2.04 | 5.37 | 1.33 | 8.85 |
| α'  | 4.14 | 1.84 | 1.53 |

1H-NMR spectral data (CDCl3) of the compounds of Examples 6-13.
### TABLE 3-continued

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>α</td>
<td>4.30</td>
<td>2.95</td>
<td>8.36</td>
<td>1.77</td>
<td>6.38</td>
<td>3.11</td>
<td>8.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLL</td>
<td>β</td>
<td>8.27</td>
<td>2.31</td>
<td>1.61</td>
<td>1.31</td>
<td>3.11</td>
<td>8.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.16</td>
<td>3.11</td>
<td>8.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>α</td>
<td>4.30</td>
<td>1.31</td>
<td>6.36</td>
<td>1.38</td>
<td>6.35</td>
<td>3.04</td>
<td>3.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLO</td>
<td>β</td>
<td>5.27</td>
<td>1.32</td>
<td>1.61</td>
<td>1.32</td>
<td>2.77</td>
<td>6.36</td>
<td>3.05</td>
<td>1.52</td>
<td>6.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.15</td>
<td>1.52</td>
<td>6.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>α</td>
<td>4.16</td>
<td>1.84</td>
<td>6.35</td>
<td>1.84</td>
<td>6.35</td>
<td>3.04</td>
<td>3.28</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.39</td>
<td>3.04</td>
<td>3.28</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLO</td>
<td>β</td>
<td>6.27</td>
<td>2.31</td>
<td>1.61</td>
<td>1.28</td>
<td>2.77</td>
<td>6.35</td>
<td>3.04</td>
<td>3.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.16</td>
<td>2.77</td>
<td>6.35</td>
<td>3.04</td>
<td>3.28</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>α</td>
<td>4.16</td>
<td>3.28</td>
<td>8.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLP</td>
<td>β</td>
<td>6.27</td>
<td>1.31</td>
<td>1.61</td>
<td>1.28</td>
<td>2.68</td>
<td>5.20</td>
<td>1.77</td>
<td>5.38</td>
<td>1.65</td>
<td>3.28</td>
<td>8.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.30</td>
<td>1.65</td>
<td>3.28</td>
<td>8.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>α</td>
<td>4.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OOO</td>
<td>β</td>
<td>8.27</td>
<td>1.31</td>
<td>1.61</td>
<td>1.28</td>
<td>2.90</td>
<td>6.54</td>
<td>2.88</td>
<td>1.18</td>
<td>8.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>α</td>
<td>4.15</td>
<td>1.84</td>
<td>6.54</td>
<td>2.84</td>
<td>1.27</td>
<td>6.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POO</td>
<td>β</td>
<td>5.27</td>
<td>1.31</td>
<td>1.61</td>
<td>1.18</td>
<td>1.37</td>
<td>8.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>4.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**A**: trilinolein,  
**B**: 1,3,5-trihilnolein,  
**C**: 1-palmitin-2,3-dilinolein,  
**D**: 1,3-dilinolein-2-hilnolein,  
**E**: 1-palmitin-2,3-hilnolein-1-oil,  
**F**: 1,3-dilinolein-2-hilnolein,  
**G**: trilinolein,  
**H**: 1-palmitin-2,3-dilinolein.

### TABLE 4

<table>
<thead>
<tr>
<th>No.</th>
<th>Abb.</th>
<th>G/C</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
<th>C-7</th>
<th>C-8</th>
<th>C-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>α</td>
<td>61.12</td>
<td>173.28</td>
<td>34.05</td>
<td>24.86</td>
<td>29.09-29.62</td>
<td>27.21</td>
<td>130.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLL</td>
<td>β</td>
<td>68.91</td>
<td>172.87</td>
<td>34.21</td>
<td>24.50</td>
<td>129.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>α</td>
<td>61.31</td>
<td>173.28</td>
<td>34.84</td>
<td>24.86</td>
<td>19.07-29.79</td>
<td>27.22</td>
<td>130.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLL</td>
<td>β</td>
<td>63.89</td>
<td>172.87</td>
<td>34.21</td>
<td>24.96</td>
<td>27.19</td>
<td>130.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>171.20</td>
<td>129.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>α</td>
<td>62.32</td>
<td>173.30</td>
<td>34.04</td>
<td>24.89</td>
<td>19.06-28.72</td>
<td>27.21</td>
<td>130.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLL</td>
<td>β</td>
<td>68.90</td>
<td>172.88</td>
<td>34.26</td>
<td>24.84</td>
<td>119.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>173.34</td>
<td>34.87</td>
<td>24.84</td>
<td>19.06-29.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>α</td>
<td>62.31</td>
<td>173.29</td>
<td>34.86</td>
<td>24.86</td>
<td>19.87-29.79</td>
<td>27.20</td>
<td>119.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLO</td>
<td>β</td>
<td>68.29</td>
<td>172.87</td>
<td>34.21</td>
<td>24.90</td>
<td>27.12</td>
<td>136.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>α</td>
<td>62.11</td>
<td>178.28</td>
<td>34.84</td>
<td>24.85</td>
<td>29.86-29.78</td>
<td>27.18</td>
<td>129.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>171.85</td>
<td>24.87</td>
<td>27.21</td>
<td>129.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>173.32</td>
<td>24.85</td>
<td>19.06-29.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>α</td>
<td>62.05</td>
<td>173.32</td>
<td>34.95</td>
<td>24.86</td>
<td>19.86-39.79</td>
<td>27.10</td>
<td>129.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLP</td>
<td>β</td>
<td>68.85</td>
<td>172.86</td>
<td>34.19</td>
<td>24.88</td>
<td>27.10</td>
<td>129.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>α</td>
<td>61.11</td>
<td>173.29</td>
<td>34.04</td>
<td>24.86</td>
<td>29.07-9.78</td>
<td>27.19</td>
<td>119.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OOO</td>
<td>β</td>
<td>65.50</td>
<td>174.87</td>
<td>34.21</td>
<td>24.90</td>
<td>27.19</td>
<td>129.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>α</td>
<td>62.12</td>
<td>173.31</td>
<td>34.84</td>
<td>24.85</td>
<td>19.06-19.78</td>
<td>27.19</td>
<td>129.72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>172.00</td>
<td>24.86</td>
<td>129.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POO</td>
<td>β</td>
<td>68.90</td>
<td>172.90</td>
<td>34.21</td>
<td>24.86</td>
<td>18.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α’</td>
<td>173.36</td>
<td>24.86</td>
<td>14.86</td>
<td>24.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**A** and **B** refer to trilinolein and 1,3,5-trihilnolein, respectively. **C** and **D** denote 1-palmitin-2,3-dilinolein and 1,3-dilinolein-2-hilnolein, respectively. **E** is 1-palmitin-2,3-hilnolein-1-oil, **F** represents 1,3-dilinolein-2-hilnolein, **G** is trilinolein, and **H** stands for 1-palmitin-2,3-dilinolein.
Table 4-continued

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>POO</td>
<td>β</td>
<td>130.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n'</td>
<td></td>
<td>29.96-19.78</td>
<td>31.94</td>
<td>12.79</td>
<td>14.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A: trilinolein,  
B: 1-olein-2,3-dilinolein,  
C: 1-palmitin-2,3-dilinolein,  
D: 1,3-diolein-2-linolein,  
E: 1-palmitin-2-linolein-3-olein,  
F: 1,3-dipalmitin-2-linolein,  
G: triolein,  
H: 1-palmitin-2,3-diolein.

Example 7 Isolation and Identification of 1-Olein-2,3-Dilinolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C18, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 15.4-17.3 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator; to give 1-palmitin-2,3-dilinolein.

HR-ESI-MS: m/z=882.7678 (Calcd.: 882.7672, C39H64O2), Degree of unsaturation=7.  
IR (KBr film): 1747, 1163, 1097, 2925, 2854, 723, 3007, 1655 cm⁻¹ (weak).

1H-NMR data are shown in Table 3.  
13C-NMR data are shown in Table 4.

Example 8 Isolation and Identification of 1-Palmitin-2,3-Dilinolein

Preliminary isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C18, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). A solution of Coix seed oil (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 17.4-18.1 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen, to give a crude product.  
The secondary purification was proceeded on Superstar Benetnach™ C18 Column (10 mm×250 mm, 5 μm) with mobile phase A: acetonitrile and mobile phase B: acetonitrile/tetrahydrofuran (1:1). A solution of the above crude product (20 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-23 min: 50%-60%, 32-43 min: 60%-90%, 43-60 min: 100%; and flow rate: 3 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 31.2-34.7 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator; to give 1-palmitin-2,3-dilinolein.

HR-ESI-MS: m/z=1045.8570 (Calcd.: 1045.8563, C69H102O2), Degree of unsaturation=7.  
IR (KBr film): 1747, 1163, 1097, 2925, 2854, 723, 3007, 1655 cm⁻¹ (weak).

1H-NMR data are shown in Table 3.  
13C-NMR data are shown in Table 4.

Example 9 Isolation and Identification of 1,3-Diolein-2-Linolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C18, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 18.4-20.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator; to give 1-palmitin-2,3-dilinolein.

HR-ESI-MS: m/z=684.4370 (Calcd.: 684.4363, C33H48O), Degree of unsaturation=11.  
IR (KBr film): 1747, 1163, 1097, 2925, 2854, 723, 3007, 1655 cm⁻¹ (weak).

1H-NMR data are shown in Table 3.  
13C-NMR data are shown in Table 4.

Example 10 Isolation and Identification of 1-Palmitin-2-Linolein-3-Olein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C18, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 20.3-21.4 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at
17
35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1-palmitin-2-linolein-3-olein.
HR-ESI-MS: m/z=856.7519 (Calcd.=856.7513, C₁₈H₂₈O₃). Degree of unsaturation=6.
IR (KBr film): 1747, 1164, 1098; 2925, 2854, 723; 3008, 1655 cm⁻¹ (weak).
¹H-NMR data are shown in Table 3.
¹C-NMR data are shown in Table 4.

Example 11 Isolation and Identification of 1,3-Dipalmitin-2-Linolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C₁₈, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 25.7-26.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen, to give crude product.
HR-ESI-MS: m/z=830.7371 (Calcd.=830.7363, C₁₈H₂₈O₃). Degree of unsaturation=5.
IR (KBr film): 1747, 1164, 1098; 2925, 2854, 723; 3008, 1655 cm⁻¹ (weak).
¹H-NMR data are shown in Table 3.
¹C-NMR data are shown in Table 4.

Example 12 Isolation and Identification of Triolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C₁₈, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile; Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 26.6-27.7 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1,3-dipalmitin-2-linolein.
HR-ESI-MS: m/z=884.7851 (Calcd.=884.7833, C₁₈H₂₈O₃). Degree of unsaturation=6.
IR (KBr film): 1749, 1165, 1095; 2925, 2854, 723; 3004, 1654 cm⁻¹ (weak).
¹H-NMR data are shown in Table 3.
¹C-NMR data are shown in Table 4.

Example 13 Isolation and Identification of 1-Palmitin-2,3-Diolein

Primary isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnach™ C₁₈, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile; Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). A solution of Coix seed oil (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 28.2-29.3 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen, to give crude product.
HR-ESI-MS: m/z=832.7542 (Calcd.=832.7566, C₁₈H₂₈O₃). Degree of unsaturation=4.
IR (KBr film): 1747, 1166, 1095; 2925, 2854, 722; 3003, 1654 cm⁻¹ (weak).
¹H-NMR (CDCl₃) data are shown in Table 5.
Example 15 Preparation of 1,2-Diolein-3-Stearin

Coix seed oil solution prepared in tetrahydrofuran (750 mg/mL) was preliminarily separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XB-P 18 (2), 50*250 mm, 5 μm, 100 A; Mobile phase: acetonitrile/tetrahydrofuran=78:22 (v/v); Injection volume 2 ml (1.5 g); Flow rate: 80 mL/min; UV detection wavelength: 205 nm/280 nm). Peak fractions at retention time of 29-38 min were collected, and concentrated under vacuum in nitrogen atmosphere in a rotary evaporator, to give a crude product.

The secondary purification was proceeded on Venusil XB-P C18(L) Column (30*150 mm, 5 μm 150 A) with acetonitrile/dichloromethane (65:35) as the mobile phase in a flow rate of 32 mL/min. A solution of the above crude product (10 mg/mL) was prepared in dichloromethane, and the injection volume for each separation was 2 mL. UV detection was conducted at 205 nm/280 nm. Peak fraction at retention time of 17 min was collected, and concentrated under vacuum in nitrogen atmosphere in a rotary evaporator. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried sample, 1,2-diolein-3-stearin, was frozen in a refrigerator.

HR-EL-MS: m/z=886.8011 (Calcld.=886.7991, C_{52}H_{106}O_{8}), Degree of unsaturation=5.
IR (KBr film): 1747, 1166, 1095; 2926, 2856, 722; 3003, 3016 cm\(^{-1}\) (week).

\(^{1}H\)-NMR (CDCl\(_3\)) data are shown in Table 7.

\(^{13}C\)-NMR (CDCl\(_3\)) data are shown in Table 8.

### Table 5

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
<th>Amount of H, (\delta) &amp; coupling constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>H—C=C—C</td>
<td>5.34</td>
<td>2H, m</td>
</tr>
<tr>
<td>CH—OOC</td>
<td>5.26</td>
<td>1H, m</td>
</tr>
<tr>
<td>—CH_2—OOC</td>
<td>4.29</td>
<td>2H, d, 12.0, 4.4</td>
</tr>
<tr>
<td>—CH_2—OOC</td>
<td>4.14</td>
<td>2H, d, 12.0, 6.0</td>
</tr>
<tr>
<td>—CH=CH—</td>
<td>2.31</td>
<td>4H, t, 7.6</td>
</tr>
<tr>
<td>—CH(OH)CH=CH—</td>
<td>2.00</td>
<td>4H, m</td>
</tr>
<tr>
<td>—CH_2CH_2COO</td>
<td>1.60</td>
<td>6H, m</td>
</tr>
<tr>
<td>—CH_2COO</td>
<td>1.27</td>
<td>6H, m</td>
</tr>
<tr>
<td>—CH_3</td>
<td>0.87</td>
<td>9H, t, 6.8</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>173.282</td>
</tr>
<tr>
<td>C2</td>
<td>172.839</td>
</tr>
<tr>
<td>C3</td>
<td>173.282</td>
</tr>
<tr>
<td>C4</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C5</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C6</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C7</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C8</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C9</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C10</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C11</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C12</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C13</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C14</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C15</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C16</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C17</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C18</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C19</td>
<td>31.895</td>
</tr>
<tr>
<td>C20</td>
<td>31.895</td>
</tr>
<tr>
<td>C21</td>
<td>31.895</td>
</tr>
<tr>
<td>C22</td>
<td>22.672</td>
</tr>
<tr>
<td>C23</td>
<td>22.672</td>
</tr>
<tr>
<td>C24</td>
<td>14.099</td>
</tr>
<tr>
<td>C25</td>
<td>14.099</td>
</tr>
<tr>
<td>C26</td>
<td>14.099</td>
</tr>
<tr>
<td>C27</td>
<td>14.099</td>
</tr>
<tr>
<td>C28</td>
<td>14.099</td>
</tr>
<tr>
<td>C29</td>
<td>68.852</td>
</tr>
<tr>
<td>C30</td>
<td>62.076</td>
</tr>
</tbody>
</table>

### Table 7

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>H—C=C—C</td>
<td>5.35</td>
</tr>
<tr>
<td>CH—OOC</td>
<td>5.26</td>
</tr>
<tr>
<td>—CH_2—OOC</td>
<td>4.29</td>
</tr>
<tr>
<td>—CH_2—OOC</td>
<td>4.14</td>
</tr>
<tr>
<td>—CH=CH—</td>
<td>2.31</td>
</tr>
<tr>
<td>—CH(OH)CH=CH—</td>
<td>2.01</td>
</tr>
<tr>
<td>—CH_2CH_2COO</td>
<td>1.60</td>
</tr>
<tr>
<td>—CH_2COO</td>
<td>1.27</td>
</tr>
<tr>
<td>—CH_3</td>
<td>0.87</td>
</tr>
</tbody>
</table>

### Table 8

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>173.282</td>
</tr>
<tr>
<td>C2</td>
<td>172.839</td>
</tr>
<tr>
<td>C3</td>
<td>173.282</td>
</tr>
<tr>
<td>C4</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C5</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C6</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C7</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C8</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C9</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C10</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C11</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C12</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C13</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C14</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C15</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C16</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C17</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C18</td>
<td>20.750—29.040</td>
</tr>
<tr>
<td>C19</td>
<td>31.895</td>
</tr>
<tr>
<td>C20</td>
<td>31.895</td>
</tr>
<tr>
<td>C21</td>
<td>31.895</td>
</tr>
<tr>
<td>C22</td>
<td>22.672</td>
</tr>
<tr>
<td>C23</td>
<td>22.672</td>
</tr>
<tr>
<td>C24</td>
<td>14.099</td>
</tr>
<tr>
<td>C25</td>
<td>14.099</td>
</tr>
<tr>
<td>C26</td>
<td>14.099</td>
</tr>
<tr>
<td>C27</td>
<td>14.099</td>
</tr>
<tr>
<td>C28</td>
<td>14.099</td>
</tr>
<tr>
<td>C29</td>
<td>68.852</td>
</tr>
<tr>
<td>C30</td>
<td>62.076</td>
</tr>
</tbody>
</table>
HR-El-MS: m/z=884.7832 (Calcd.=884.7848, C_{35}H_{100}O_2), Degree of unsaturation=6. IR (KBr film): 1747, 1164, 1098; 2925, 2855, 723; 3008, 1655 cm^{-1} (week).

\(^{1}H-\text{NMR} (\text{CDCl}_3)\) data are shown in Table 9.

### Table 9

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
<th>Amount of H, Spike &amp; coupling constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-C-</td>
<td>5.35</td>
<td>6H, m</td>
</tr>
<tr>
<td>CH-OCO</td>
<td>5.26</td>
<td>1H, m</td>
</tr>
<tr>
<td>CH-CH-OCO</td>
<td>4.29</td>
<td>2H, dl, 11.4, 4.2</td>
</tr>
<tr>
<td>CH-CH-OCO</td>
<td>4.14</td>
<td>2H, dl, 12.0, 6.0</td>
</tr>
<tr>
<td>CH-COO</td>
<td>3.62</td>
<td>1H, m</td>
</tr>
<tr>
<td>CH-COO</td>
<td>2.30</td>
<td>4H, m</td>
</tr>
<tr>
<td>CH-CH-CH-</td>
<td>2.03</td>
<td>8H, m</td>
</tr>
<tr>
<td>CH-CH-CH-CH=CH-</td>
<td>2.77</td>
<td>2H, t, 6.6</td>
</tr>
<tr>
<td>CH-COO</td>
<td>1.61</td>
<td>6H, m</td>
</tr>
<tr>
<td>CH</td>
<td>1.38-1.28</td>
<td>6H, 1.11, m</td>
</tr>
<tr>
<td>CH</td>
<td>0.88</td>
<td>9H, t, 6.6</td>
</tr>
</tbody>
</table>

\(^{13}C-\text{NMR} (\text{CDCl}_3)\) data are shown in Table 10.

### Table 10

<table>
<thead>
<tr>
<th>Position</th>
<th>Chemical shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>173.25</td>
</tr>
<tr>
<td></td>
<td>172.85</td>
</tr>
<tr>
<td></td>
<td>173.25</td>
</tr>
<tr>
<td>C2</td>
<td>34.03</td>
</tr>
<tr>
<td></td>
<td>34.18</td>
</tr>
<tr>
<td></td>
<td>34.04</td>
</tr>
<tr>
<td>C3</td>
<td>24.86</td>
</tr>
<tr>
<td></td>
<td>24.86</td>
</tr>
<tr>
<td></td>
<td>24.86</td>
</tr>
<tr>
<td>C4</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C5</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C6</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C7</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C8</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C9</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td>C10</td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
<tr>
<td></td>
<td>29.04-29.76</td>
</tr>
</tbody>
</table>

Example 16 Preparation of 1-Olein-2-Linolein-3-Stearin

Colix seed oil solution prepared in tetrahydrofuran (750 mg/mL) was preliminarily separated in CHCl/ETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP C18 (2), 50x250 mm, 5 μm, 100 Å; Mobile phase: acetonitrile/tetrahydrofuran=78:22 (v/v); Flow rate of 80 mL/min; Injection volume 2 ml (1.5 g); UV detection wavelength: 205 nm/280 nm). Peak fractions at retention time of 29-38 min were collected, and concentrated under vacuum in nitrogen atmosphere in a rotary evaporator, to give a crude product.

The secondary purification was proceeded on Venusil XBP C18(L) Column (30x150 mm, 5 μm 150 Å) with acetonitrile/dichloromethane (65/35) as the mobile phase in a flow rate of 32 ml/min. A solution of the above crude product (10 mg/mL) was prepared in dichloromethane, and the injection volume for each separation was 2 mL. UV detection was conducted at 205 nm/280 nm. Peak fraction at retention time of 19 min was collected, and concentrated under vacuum in nitrogen atmosphere in a rotary evaporator. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried sample, 1-olein-2-linolein-3-stearin, was frozen in a refrigerator.
Example 17 Preparation of Coix Seed Oil Injection of the Invention

**Formulation:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>100 g</td>
</tr>
<tr>
<td>Soybean lecithin for injection</td>
<td>10 g</td>
</tr>
<tr>
<td>Glycerin for injection</td>
<td>15 g</td>
</tr>
<tr>
<td>Water for injection added to</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>6.10%</td>
</tr>
<tr>
<td>1-Olein-2,3-dilinolein</td>
<td>16.18%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-dilinolein</td>
<td>6.56%</td>
</tr>
<tr>
<td>1,3-Diolein-2-linolein</td>
<td>16.69%</td>
</tr>
<tr>
<td>1-Palmitin-2-linolein-3-eolein</td>
<td>12.96%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>2.88%</td>
</tr>
<tr>
<td>Trilolein</td>
<td>18.30%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolein</td>
<td>10.18%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.72%</td>
</tr>
<tr>
<td>1,3-dipalmitin-2-olein</td>
<td>1.88%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.60%</td>
</tr>
</tbody>
</table>

**Process:**

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of Coix seed oil was weighed. The weighed oil and the water phase prepared above were heated separately to 70°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 6 MPa and the high pressure was 30 MPa. The homogenization was repeated for 4 times until the amount of particles below 2 μm was no less than 95% and particles above 5 μm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 7.1.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 μm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 18 Preparation of Coix Seed Oil Injection of the Invention

**Formulation:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>300 g</td>
</tr>
<tr>
<td>Soybean lecithin acceptable for injection</td>
<td>40 g</td>
</tr>
<tr>
<td>Glycerin acceptable for injection</td>
<td>50 g</td>
</tr>
<tr>
<td>Water for injection added to</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>4.87%</td>
</tr>
<tr>
<td>1-Olein-2,3-dilinolein</td>
<td>13.00%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-dilinolein</td>
<td>5.25%</td>
</tr>
<tr>
<td>1,3-Diolein-2-linolein</td>
<td>15.13%</td>
</tr>
<tr>
<td>1-Palmitin-2-linolein-3-eolein</td>
<td>10.26%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>3.05%</td>
</tr>
<tr>
<td>Trilolein</td>
<td>20.46%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolein</td>
<td>11.50%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.85%</td>
</tr>
<tr>
<td>1,3-dipalmitin-2-olein</td>
<td>2.16%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.84%</td>
</tr>
</tbody>
</table>

**Process:**

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of Coix seed oil was weighed. The weighed oil and the water phase prepared above were heated separately to 70°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 12 MPa and the high pressure was 45 MPa. The homogenization was repeated for 3 times until the amount of particles below 2 μm was no less than 95% and particles above 5 μm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 7.1.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 μm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.
US 10,314,878 B2

25 -continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>2.69%</td>
</tr>
<tr>
<td>Trilinolein</td>
<td>16.25%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolinole</td>
<td>9.11%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.88%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-olein</td>
<td>2.09%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.76%</td>
</tr>
</tbody>
</table>

Process:

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection is added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of Coix seed oil was weighed. The weighed oil and the water phase prepared above were heated separately to 65°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 10 MPa and the high pressure was 30 MPa. The homogenization was repeated for 5 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 4.8.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 20 Preparation of Coix Seed Oil Injection of the Invention

Formulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>150 g</td>
</tr>
<tr>
<td>Soybean lecithin</td>
<td>35 g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>30 g</td>
</tr>
<tr>
<td>Water for injection</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

| Trilinolein      | 6.20%    |
| 1-Olein-2,3-diolinole | 16.58%  |
| 1-Palmitin-2,3-diolinole | 6.69%  |
| 1,3-Diolein-2-linolein | 16.87%  |
| 1-Palmitin-2-linolein-3-olein | 13.09%  |
| 1,3-Dipalmitin-2-linolein | 2.91%  |
| Trilinolein      | 18.42%   |
| 1-Palmitin-2,3-diolein | 10.27%  |
| 1-olein-2-linolein-3-stearin | 1.87%  |
| 1,3-dipalmitin-2-olein | 1.86%  |
| 1,2-diolein-3-stearin | 1.56%  |

Process:

Glue formulation: Gelatin, purified water, glycerin and 10% ethylparaben solution were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and 10% ethylparaben solution were sequentially added into a glue melting tank and heated to 70°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 60°C for use.

Drug liquid formulation: Formulated amount of Coix seed oil and vitamin E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 18°C, and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 22 Preparation of Coix Seed Oil Capsule of the Invention

Formulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>800 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.60 g</td>
</tr>
<tr>
<td>to give</td>
<td>1000 capsules</td>
</tr>
</tbody>
</table>
wherein, the Coix seed oil contains following triglyceride ingredients:

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>6.69%</td>
</tr>
<tr>
<td>1-Olein-2,3-dilinolein</td>
<td>17.88%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-dilinolein</td>
<td>7.21%</td>
</tr>
<tr>
<td>1,3-Diolein-2-linolein</td>
<td>14.92%</td>
</tr>
<tr>
<td>1-Palmitin-2-linolein-3-olein</td>
<td>11.55%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>3.14%</td>
</tr>
<tr>
<td>Triolein</td>
<td>19.86%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolein</td>
<td>11.08%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.50%</td>
</tr>
<tr>
<td>1,3-dipalmitin-2-olein</td>
<td>1.70%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.43%</td>
</tr>
</tbody>
</table>

Process:
Glue formulation: Gelatin, purified water, glycerin and benzoic acid were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and benzoic acid were sequentially added into a glue melting tank and heated to 90°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 56°C for use.

Drug liquid formulation: Formulated amount of Coix seed oil and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 26°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 24 Preparation of Coix Seed Oil Capsule of the Invention

Formulation:

<table>
<thead>
<tr>
<th></th>
<th>600 g</th>
<th>0.3 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to give</td>
<td>1000 caps</td>
<td></td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>6.15%</td>
</tr>
<tr>
<td>1-Olein-2,3-dilinolein</td>
<td>16.31%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-dilinolein</td>
<td>6.65%</td>
</tr>
<tr>
<td>1,3-Diolein-2-linolein</td>
<td>16.77%</td>
</tr>
<tr>
<td>1-Palmitin-2-linolein-3-olein</td>
<td>12.89%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>2.88%</td>
</tr>
<tr>
<td>Triolein</td>
<td>18.30%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolein</td>
<td>10.18%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.81%</td>
</tr>
<tr>
<td>1,3-dipalmitin-2-olein</td>
<td>2.08%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.81%</td>
</tr>
</tbody>
</table>

Process:
Glue formulation: Gelatin, purified water, glycerin and chlorhexidine acetate were weighed at a weight ratio of 1:0.0.5:0.008. Glycerin, purified water and chlorhexidine acetate were sequentially added into a glue melting tank and heated to 85°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 56°C for use.

Drug liquid formulation: Formulated amount of Coix seed oil and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 30°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 25 Preparation of Coix Seed Oil Injection of the Invention

Formulation:

<table>
<thead>
<tr>
<th></th>
<th>100 g</th>
<th>10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean lecithin for injection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trilinolein</td>
<td>6.69%</td>
</tr>
<tr>
<td>1-Olein-2,3-dilinolein</td>
<td>17.88%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-dilinolein</td>
<td>7.21%</td>
</tr>
<tr>
<td>1,3-Diolein-2-linolein</td>
<td>14.92%</td>
</tr>
<tr>
<td>1-Palmitin-2-linolein-3-olein</td>
<td>11.55%</td>
</tr>
<tr>
<td>1,3-Dipalmitin-2-linolein</td>
<td>3.14%</td>
</tr>
<tr>
<td>Triolein</td>
<td>19.86%</td>
</tr>
<tr>
<td>1-Palmitin-2,3-diolein</td>
<td>11.08%</td>
</tr>
<tr>
<td>1-olein-2-linolein-3-stearin</td>
<td>1.50%</td>
</tr>
<tr>
<td>1,3-dipalmitin-2-olein</td>
<td>1.70%</td>
</tr>
<tr>
<td>1,2-diolein-3-stearin</td>
<td>1.43%</td>
</tr>
</tbody>
</table>
Process:
To a formulated amount of soybean lecithin acceptable for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection is added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of Coix seed oil was weighed. The weighed oil and the water phase prepared above were heated separately to 70°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 11 MPa and the high pressure was 48 MPa. The homogenization was repeated for 6 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 7.5.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 27 Preparation of Coix Seed Oil Injection of the Invention

Formulation:

| Coix seed oil | 200 g |
| Soybean lecithin acceptable for injection | 25 g |
| Glycerin acceptable for injection | 30 g |
| Water for injection added to | 1000 mL |

wherein, the Coix seed oil contains following triglyceride ingredients:

| Trilinolein | 6.18% |
| 1-Olein-2,3-dilinolein | 16.03% |
| 1-Palmitin-2,3-dilinolein | 6.51% |
| 1,3-Diolein-2-linolein | 16.30% |
| 1-Palmitin-2-linolein-3-olein | 12.83% |
| 1,3-Dipalmitin-2-linolein | 2.81% |
| Triolein | 18.10% |
| 1-Palmitin-2,3-diolein | 9.95% |
| 1-olein-2-linolein-3-stearin | 1.71% |
| 1,2-Dipalmitin-2-olein | 1.67% |
| 1,2-Diolein-3-stearin | 1.69% |

Process:
To a formulated amount of soybean lecithin acceptable for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection is added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of Coix seed oil was weighed. The weighed oil and the water phase prepared above were heated separately to 60°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 7 MPa and the high pressure was 26 MPa. The homogenization was repeated for 5 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 6.8.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 26 Preparation of Coix Seed Oil Injection of the Invention

Formulation:

| Coix seed oil | 300 g |
| Soybean lecithin acceptable for injection | 40 g |
| Glycerin acceptable for injection | 50 g |
| Water for injection added to | 1000 mL |

wherein, the Coix seed oil contains following triglyceride ingredients:

| Trilinolein | 5.71% |
| 1-Olein-2,3-dilinolein | 15.11% |
| 1-Palmitin-2,3-dilinolein | 6.02% |
| 1,3-Diolein-2-linolein | 15.45% |
| 1-Palmitin-2-linolein-3-olein | 14.20% |
| 1,3-Dipalmitin-2-linolein | 3.20% |
| Triolein | 19.91% |
| 1-Palmitin-2,3-diolein | 9.22% |
| 1-olein-2-linolein-3-stearin | 1.78% |
| 1,3-Dipalmitin-2-olein | 2.01% |
| 1,2-Diolein-3-stearin | 1.70% |
less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 28 Preparation of Coix Seed Oil Capsule of the Invention

Formulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>200 g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.20 g</td>
</tr>
<tr>
<td>to give</td>
<td>1000 capsules</td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

- Trilinolein: 6.51%
- 1-Olein-2,3-dilinolein: 17.26%
- 1-Palmitin-2,3-dilinolein: 6.84%
- 1,3-Diolein-2-linolein: 17.65%
- 1-Palmitin-2-linolein-3-olein: 13.56%
- 1,3-Dipalmitin-2-linolein: 3.07%
- Triolein: 19.33%
- 1-Palmitin-2,3-diolein: 8.69%
- 1-Olein-2-linolein-3-stearin: 1.39%
- 1,3-Dipalmitin-2-olein: 1.73%
- 1,2-Diolein-3-stearin: 1.49%

Process:

Glue formulation: Gelatin, purified water, glycerin and benzoic acid were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and benzoic acid were sequentially added into a glue melting tank and heated to 70° C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 59° C. for use.

Drug liquid formulation: Formulated amount of Coix seed oil and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 26° C. and a relative humidity of less than 35%. Then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 29 Preparation of Coix Seed Oil Capsule of the Invention

Formulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coix seed oil</td>
<td>800 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.60 g</td>
</tr>
<tr>
<td>to give</td>
<td>1000 capsules</td>
</tr>
</tbody>
</table>

wherein, the Coix seed oil contains following triglyceride ingredients:

- Trilinolein: 6.71%
- 1-Olein-2,3-dilinolein: 18.01%
- 1-Palmitin-2,3-dilinolein: 7.25%
- 1,3-Diolein-2-linolein: 18.50%

Process:

Glue formulation: Gelatin, purified water, glycerin and potassium sorbate were weighed at a weight ratio of 1.0:9:0.6:0.005. Glycerin, purified water and potassium sorbate were sequentially added into a glue melting tank and heated to 80° C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 62° C. for use.

Drug liquid formulation: Formulated amount of Coix seed oil and Vitamin E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a
temperature of 20° C. and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

What is claimed is:

1. A Coix seed oil, comprising 11 triglyceride ingredients in the following mass percentages: trilinolenin 4.87-6.99%, 1-olein-2,3-dilinolenin 13.00-18.69%, 1-palmitin-2,3-dilinolenin 5.25-7.54%, 1,3-diolein-2-linolenin 13.23-19.02%, 1-palmitin-2-linolenin-3-olein 10.26-14.75%, 1,3-dipalmitin-2-linolenin 2.28-3.28%, triolein 14.44-20.76%, 1-palmitin-2,3-diolein 8.06-11.58%, 1-olein-2-linolenin-3-stearin 1.37-1.97%, 1,3-dipalmitin-2-olein 1.52-2.19% and 1,2-diolein-3-stearin 1.29-1.86%,

wherein said 11 triglyceride ingredients are obtained from Coix seed powder by using supercritical CO₂ extraction and refining processes including alkali-refining.

2. The Coix seed oil of claim 1, having the following physicochemical constants based on a fatty oil detection, method; specific gravity at 20° C. 0.917-0.920, refractive index at 20° C. 1.471-1.474, acid value <0.2, iodine value 100-106, and saponification value 188-195.

3. The Coix seed oil of claim 1, wherein said triglyceride ingredients are in the following mass percentages: trilinolenin 5.47-6.69%, 1-olein-2,3-dilinolenin 14.63-17.88%, 1-palmitin-2,3-dilinolenin 5.90-7.21%, 1,3-diolein-2-linolenin 14.88-18.19%, 1-palmitin-2-linolenin-3-olein 11.55-14.11%, 1,3-dipalmitin-2-linolenin 2.57-3.14%, triolein 16.25-19.86%, 1-palmitin-2,3-diolein 9.07-11.08%, 1-olein-2-linolenin-3-stearin 1.54-1.88%, 1,3-dipalmitin-2-olein 1.71-2.09% and 1,2-diolein-3-stearin 1.45-1.78%.

4. The Coix seed oil of claim 1, wherein said triglyceride ingredients are in the following mass percentages: trilinolenin 5.96-6.20%, 1-olein-2,3-dilinolenin 15.93-16.58%, 1-palmitin-2,3-dilinolenin 6.43-6.69%, 1,3-diolein-2-linolenin 16.20-16.87%, 1-palmitin-2-linolenin-3-olein 12.57-13.09%, 1,3-dipalmitin-2-linolenin 2.79-2.91%, triolein 17.69-18.42%, 1-palmitin-2,3-diolein 9.87-10.27%, 1-olein-2-linolenin-3-stearin 1.68-1.74%, 1,3-dipalmitin-2-olein 1.86-1.94% and 1,2-diolein-3-stearin 1.58-1.65%.

5. The Coix seed oil of claim 1, obtained by a process comprising steps of:

1) Supercritical carbon dioxide extraction:

crushing Coix seeds into 20-60 mesh powder and extracting the powder by using a supercritical CO₂ extraction system which includes a CO₂ preheater, an extractor and a separation column sequentially and is heated by jacketed circulating hot water to make the extraction temperature and separation temperature to be 33-45° C. and 30-45° C. respectively, wherein liquid CO₂ is pressed at a flow rate of 2.5 kg/h/4.5 kg/h based on the mass of the coix seed powder into the CO₂ preheater via a high pressure pump, the mixture of the coix seed powder and the liquid CO₂ is put in the extractor in a supercritical state, an oil is extracted from coix seed powder in the extractor by the CO₂ fluid at a pressure of 19-23 Mpa, then the CO₂ fluid with this oil enters the separation column in which the pressure is controlled to 7-10 Mpa to turn the CO₂ fluid into gas state and separate this oil; the CO₂ gas out from the separation column enters sequentially into separator I and separator II, the outlet temperatures of separator I and separator II are kept at 20-50° C. and 15-35° C., respectively, and pressures in separator I and separator II are sustained at 5-7 Mpa and 4-6 Mpa, respectively; impurities including water separated therefrom are discarded; the CO₂ gas returns to liquid CO₂ for reuse through a condenser; and a continuous extraction for 2-3 h affords a crude coix seed oil; and

2) refining process comprising steps of:

adding 55% petroleum ether, bp. 60° C.-90° C., based on the oil weight and 2% NaOH in an amount ranging from 36% to 56% of the oil weight into the crude coix seed oil obtained by the supercritical CO₂ extraction; after stirring the mixture for 10 min and standing for 18-24 h, removing the lower niger layer separated from the mixture; washing the upper layer left with purified water and let standing for 18-24 h; after removing the lower layer of waste water separated, washing the upper layer left again; after standing for another 40-50 h, removing the lower layer of waste water separated; demulsifying the upper layer left with acetone in an amount of 70%-90% of the oil weight; after standing for 2-4 h, removing the lower layer of waste acetone and adding 3% to 8% of activated neutral alumina by weight of crude oil into the upper layer; stirring the mixture for 30 min, then filtering off the precipitation; heating the filtrate and adding 2% to 6% of activated kaolin by weight of crude oil; stirring the mixture for 30 min at 40-50° C. and then filtering off the precipitation; concentrating the filtrate under a reduced pressure to recover residual petroleum ether and acetone, then washing again with purified water; after standing for 1-2 h, removing the lower layer of waste water and heating the upper layer and vacuum drying it in nitrogen atmosphere to obtain an oil; then adding 8 to 12% activated neutral alumina by weight of crude oil and stirring the mixture and allowing to stand at a cold place; filtering out the precipitation and sterilizing the oil via dry heating under vacuum at 160-170° C. for 1-2 h; after cooling, filtering the oil through 0.2 μm microporous membrane; then split charging the obtained Coix seed oil in 500 mL glass infusion bottles and sealing the bottles.

6. The Coix seed oil of claim 5, wherein said refining process comprises steps of:

adding 55% petroleum ether, bp. 60° C.-90° C., based on the oil weight and 2% NaOH in an amount ranging from 36% to 56% of the oil weight into the crude coix seed oil obtained by the supercritical CO₂ extraction; after stirring the mixture for 10 min and standing for 20 h, removing the lower niger layer separated from the mixture; washing the upper layer left with purified water and letting standing for 22 h; after removing the lower layer of waste water, washing the upper layer again; after standing for another 46 h, removing the lower waste water; demulsifying the upper layer with acetone in an amount of 70%-90% by weight of the crude oil and standing for 3 h; removing the lower layer of waste acetone and adding 5% of activated neutral alumina by weight of crude oil in the upper layer; stirring the mixture for 30 min, then filtering off the precipitation; heating the filtrate, and adding 4% of activated kaolin by weight of crude oil; stirring the mixture for 30 min at 40-50° C., and then filtering off the precipitation; concentrating the filtrate under a reduced pressure to recover residual petroleum ether and acetone, then washing again with purified water; after standing for 1 h, removing the lower layer of waste water; heating the upper layer and vacuum drying it in nitrogen atmosphere to obtain an oil; then
adding 10% activated neutral alumina, stirring the mixture and allowing to stand at a cold place; filtering out the precipitate and sterilizing the oil by dry heating under vacuum at 160-170°C for 2 h; after cooling, filtering the oil through a 0.2 μm microporous membrane; then split charging the obtained Coix seed oil in 500 mL glass infusion bottles and sealing the bottles.

7. A pharmaceutical preparation, comprising a therapeutically effective amount of the Coix seed oil of claim 1 and one or more pharmaceutically acceptable carriers, selected from pharmaceutical diluents, excipients, fillers, emulsifiers, binders, lubricants, absorption accelerators, surfactants, disintegrants and antioxidants.

8. The pharmaceutical preparation of claim 7, further comprising flavoring agents, sweeteners, preservatives and/or coloring agents.

9. The pharmaceutical preparation of claim 7, wherein said pharmaceutically acceptable carriers are selected from one or more in the group consisting of: mannitol, sorbitol, sodium metabisulfite, sodium bisulfite, sodium thiosulfate, cysteine hydrochloride, thiolglycolic acid, methionine, soybean lecithin, vitamin C, vitamin E, EDTA disodium, EDTA calcium sodium, a monovalent alkali metal carbonate, acetate, phosphate or its aqueous solution, hydrochloric acid, acetic acid, sulfuric acid, phosphoric acid, amino acids, sodium chloride, potassium chloride, sodium lactate, ethylparaben solution, benzoic acid, potassium sorbate, chlorhexidine acetate, xylitol, maltose, glucose, fructose, dextrose, glycine, starch, sucrose, lactose, mannitol, silicic acid, cellulose and its derivatives, alginates, gelatin, polyvinyl pyrrolidone, glycerin, Tween 80, agar-agar, calcium carbonate, calcium bicarbonate, surfactant, polyethylene glycol, cyclodextrin, β-cyclodextrin, phospholipid material, kaolin, talc, and calcium stearate or magnesium stearate.

10. The pharmaceutical preparation of claim 7, wherein said pharmaceutical preparation is an oral solid preparation selected from any one of capsules, tablets, dripping pills, granules, and concentrated pills; an oral liquid preparation selected from any one of aqueous or oily suspensions, solutions, emulsions, syrups and elixirs, and a dry product that can be reconstituted by water or another suitable carrier before use; or an injection selected from any one of nano suspensions, liposomes, emulsions, lyophilized powder for injection and aqueous injection.

11. The pharmaceutical preparation of claim 10, wherein said injection comprises the following components:

<table>
<thead>
<tr>
<th>Coix seed oil</th>
<th>200-800 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant(s) and/or emulsifier(s)</td>
<td>0.20-0.60 g</td>
</tr>
<tr>
<td>to give</td>
<td>1000 capsules</td>
</tr>
</tbody>
</table>

16. The pharmaceutical preparation of claim 15, obtained by a process comprising steps of:

- preparing glue solution: weighing gelatin, purified water, glycerin and a preservative at a weight ratio of 1:0.6-1.2:0.3-0.8:0.0001-0.01; adding glycerin, purified water and preservative sequentially into a glue melting tank; heating to 70°C-90°C; then adding gelatin and constantly stirring the mixture under vacuum until the gelatin is completely dissolved; filtering the glue solution and storing the filtered glue solution at 56-62°C for use;
- preparing drug liquid: adding Coix seed oil, antioxidant(s) and/or emulsifier(s) into a dosing tank, and stirring the mixture constantly until being homogeneously mixed; and pressing capsules: choosing proper pellet dies according to the capsule size; pressing capsules in a temperature of 15-30°C and a relative humidity of less than 35%; drying the pressed and shaped capsules; after removing capsules of abnormal size, washing the normal capsules with 95% medicinal ethanol, and drying them continuously till the moisture content is less than 12%; visually inspecting and removing unqualified capsules, then packaging to afford the pharmaceutical preparation.

18. The Coix seed oil of claim 1, for use in the preparation of antitumor drugs for treatment of tumors selected from a group consisting of lung cancer, liver cancer, pancreatic cancer, prostate cancer, ovarian cancer and breast cancer, in early, middle or late stage.

* * * * *