Title: PLANT CELL CULTURE OF ACER TEGMENTOSUM AND USE THEREOF

Abstract: Provided are a method of preparing a plant cell culture of Acer tegmentosum, a method of preparing an extract from plant cell culture of Acer tegmentosum, a pharmaceutical composition for the prevention, improvement and treatment of a liver disease, and a food composition for treating a hepatotoxicity and protecting and improving a liver function including the extract of plant cell culture.
Invention Title
PLANT CELL CULTURE OF *ACER TEGMENTOSUM* AND USE THEREOF

[Technical Field]
The present invention relates to a method of preparing a plant cell culture of *Acer tegmentosum*, a method of preparing an extract from the plant cell culture of *Acer tegmentosum*, a pharmaceutical composition for prevention, improvement and treatment of a liver disease, and a food composition for ameliorating a hepatotoxicity and protecting and improving a liver function including the extract of plant cell culture.

[Background Art]
The tree of *Acer tegmentosum* Maxim is a species of maple genus and is found in Korea and north-eastern China. The tree has a wide leaf, soft and fragile stem and thick bark. The tree is not toxic and capable of being used as an herbal medicine for any person without side effect.


As described above, the various efficacies of *Acer tegmentosum* and its extract have been known, and have been studied for the medicine and dietary supplement. However, because of the limited amount of plant source and the low content of the active ingredients in the plant, *Acer tegmentosum* has a difficulty in being developed as the industrial-scale use.

Usually, a method of obtaining *Acer tegmentosum* and its extract is to cultivate the tree and to extract the active ingredients from the tree. The method can be affected by the weather, and geographical and political condition. In particular, the active ingredients have been obtained from the tree in a low productivity. Because the active
ingredients involves very complicated pathway of plant and have complicated structure, they cannot be synthesized in chemical method. Thus, the method of preparing the active ingredients of plant using the plant cell culture has been considered as a good alternative for production of useful secondary metabolites in industrial field. However, the method of cell culture of *Acer tegmentosum* and the industrial application thereof have not been reported as yet.

[Disclosure]

[Technical Problem]

To resolve the problems of the prior arts, an embodiment of present invention is to provide a method of preparing an extract from a plant cell culture of *Acer tegmentosum* by inducing a callus from a tissue of *Acer tegmentosum*, in order to obtain the increased amount of secondary metabolites in a large scale.

Another embodiment is to provide a plant cell culture of *Acer tegmentosum* and an extract of the cell culture.

Further embodiment is to provide a method of preparing a secondary metabolite which is produced by the plant cell derived from *Acer tegmentosum*.

Further embodiment is to provide a plant cell of *Acer tegmentosum*, preferably a plant cell deposited under KCTC accession number of KCTC12257BP.

Further embodiment is to provide a pharmaceutical composition for prevention, improvement or treatment of a liver disease, including an extract from a plant cell culture of *Acer tegmentosum*.

Further embodiment is to provide a food composition for protection or improvement of a liver function or a liver disease, including an extract from a plant cell culture of *Acer tegmentosum*.

Further embodiment is to provide a pharmaceutical composition for prevention, improvement or treatment of a liver disease including a salidroside as an active ingredient.

Further embodiment is to provide a food composition for prevention or improvement of a liver function or a liver disease including a salidroside as an active ingredient.
[Technical Solution]

To achieve the object of the present invention, an embodiment of the present invention is to provide a method of preparing an extract from a plant cell culture of *Acer tegmentosum*, comprising:

(a) inducing a callus from a tissue of *Acer tegmentosum* inoculated in callus-inducing medium;

(b) preparing a suspension-cultured cell line capable of being cultured in liquid culture medium from the induced callus; and

(c) culturing the prepared suspension-cultured cell line in a suspension culture to form a cell culture and obtaining an extract from the cell culture.

The extract can be obtained by extracting the cell culture solution with at least a solvent selected from the group consisting of water, C<sub>1</sub>-C<sub>5</sub> lower alcohol, chloroform, dichloromethane, benzene, acetone, hexane, ethyl acetate and a mixture thereof. In a further embodiment, the solvent extract can be further extracted by the liquid-liquid extraction method using the organic solvent to produce the distributed extract. The solvent-extract or the distributed extract can be further processed with the column chromatography to produce the fractional extract. The extract can contain salidroside at a concentration of 0.1% to 99.9 wt% in the fractional extract.

In another embodiment of the present invention, provided is a method of preparing a secondary metabolite using a plant cell culture, including (a) inducing a callus from a tissue of *Acer tegmentosum* inoculated in callus-inducing medium; (b) preparing a suspension-cultured cell line capable of being cultured in liquid culture medium from the induced callus; and (c) culturing the prepared suspension-cultured cell line in a suspension culture to produce the cell culture and obtaining an extract from the cell culture, to produce an extract from a plant cell culture of *Acer tegmentosum*, and a step of recovering the secondary metabolite from the cell culture. Specifically, the secondary metabolite can be contained at a high concentration in the solvent extract, the distributed extract and the fractional extract. The example of secondary metabolite is salidroside, preferably.

By using the plant cell derived from plant *Acer tegmentosum* to produce the salidroside with pharmaceutical efficacy, the present invention provides a method of
preparing the plant cell culture of plant *Acer tegmentosum*, an extract thereof, and salidroside in a large scale, thereby contributing the industrial production of pharmaceutical materials for protection of liver function and treatment of hepatotoxicity, or of nutritional food materials for protection and improvement of liver function.

Hereinafter, the method of preparing an extract of plant cell culture in the present invention will be described in detail at each step.

A. Callus induction from a tissue of plant *Acer tegmentosum*

All the live tissues of plant can be induced to callus to prepare the plant cell, and include all live tissues such as young seeding, leaf, stem, root, flower, seed, undifferentiated germ, young seeding germinated from sterile seed, and cambium of each plant organ, but not limited thereto.

The methods of induction and proliferation of the callus are performed according to the conventional method known in the art. For example, the callus can be induced from the plant *Acer tegmentosum*, preferably young seedling, leaf or stem by thoroughly sterilizing the surface of plant tissue, with water and sterilizers such hypochlorite, wetting agent, surfactant of Tween or Triton, antibiotics and/or antifungal agent. Generally, the sterilized plant tissue can be put on the solid medium for callus induction and proliferation, and be cultured under the sterile condition for about 1 to 12 weeks, until the undifferentiated lump (callus) becomes similar to the plant cell. After establishment of callus culture, dedifferentiated cells can be transferred to new medium and then cultured continuously.

In an embodiment of the present invention, the medium for the callus induction and proliferation includes Linsmeier-Skoog(LS), Murashige and Skoog's(MS), B5(Gamborg) and Schenk and Hildebrandt(SH) as listed in Table 1, or preferably LS medium or B5 medium.

The medium for the callus induction and proliferation may further include at least an additive selected from the group consisting of a regulator of plant growth, a solidifying agent, an anti-browning agent and an anti-oxidant. The solidifying agent includes agar, hydrogen, gelatin, Gelrite (trademark) and Phytagel (trademark). The medium further includes the anti-browning agent of medium and an agent of preventing
callus necrosis, for examples XAD resins, polyvinylpolypyrrolidone (PVPP) and an activated carbon. These additional components may serve as an absorbent for phenol-based compounds secreted by the plant cells under stressful condition during induction into suspension-cultured cell lines, in order to promote plant cell growth and contribute to stable induction of the suspension-cultured cell lines.

The preferable temperature for induction and proliferation of callus cannot be limited particularly, but for example, can be 20 to 30°C, and preferably, 22 to 27°C. The pH ranges for induction and proliferation of callus cannot be limited but for example, can be pH 5 to 7, and preferably pH 5 to 6. The suitable time for induction and proliferation of callus cannot be limited but for example, can be about 1 to 12 weeks, and preferably 7 to 9 weeks. To induce the callus, it is possible to use the light condition by controlling the light intensity and the irradiation time using an artificial light and to use the dark condition without using the light, preferably the dark condition.

B. Preparation of plant cell from callus

When the callus grows sufficiently, the suspension-cultured cell lines can be induced from the callus. Specifically, the callus is transferred to the suspension culture using a liquid medium. The cell culture obtained for 2 to 3 weeks can be transferred to the same medium and be serially sub-cultured.

The suspension-cultured cell lines having a high growth rate and a high productivity of secondary metabolite can be selected. The suspension-cultured cell line capable of being cultured in liquid culture medium from callus can be prepared by any method of producing the plant cell from the callus, but not limited particularly.

In the present invention, the suspension-cultured cell line induced from Acer tegmentosum was named as SYGB-5 and deposited under the KCTC accession number of KCTC 12257BP at the Korean Collection for Type Cultures located in 52 Eoeundong, Yuseong-gu, Daejeon, Korea, on August 7, 2012, with the receipt no. KCTC 12257BP.

An embodiment of the present invention provides a plant cell derived from plant Acer tegmentosum, and preferably a plant cell line deposited under the KCTC accession number of KCTC 12257BP.
C. Plant cell culture

The method of the present invention includes the steps of preparing the suspension culture capable of being cultured in liquid culture medium from callus, and culturing the suspended cell line in the liquid medium.

The medium used for the suspension culture of plant cell includes nutrients and factors for maintaining the growth of plant cell, such as carbon sources, nitrogen sources, salts, vitamins and all commonly used components for plant cell. The mediums includes Schenk and Hildebrandt medium (SH medium), Anderson rhododendron medium, CHU(N6) medium, CLC/Ipomoea medium, Chee & Pool (C2D) vitis medium, De Greef & Jacobs medium, DKW/JUGLANS medium, Eriksson(er) medium, Gamborg B5 medium(B5 medium, Gresshof & Doy (DBM2) medium, Hellers medium, Kao Michayluk medium, Knudson Orchid medium, Lindemann Orchid medium, Litvay medium, Linsmaier & Skoog medium(LS medium), McCowns woody plant medium, Murashige & Skoog medium(MS medium), Murashige & Miller medium, Nitsch medium, NLN medium, Orchimax medium, Quoirin & Lepoivre medium, Rugini Olive medium, Schenk & Hildebrandt medium, S-Medium, Vacin and Went medium, White medium and Westvaco WV3 medium and modified mediums thereof, but not limited thereto.

The medium for the suspension culture of plant cell can further include the additives or be removed by a part of the medium components as necessary. The additives can be a plant growth regulator which includes any natural or synthetic material for plant growth, cell division, tissue differentiation, flowering, seed formation and maturation.

The examples of the plant growth regulator include a-naphtalene acetic acid(NAA), 2,4-D (2,4-dichlorophenoxo acetic acid), p-Chlorophenoxyacetic acid, indol-3-acetic acid, 3-indolbutyric acid), b-naphthoxyacetic acid, zeatin, 6-benzyaminopurine (BA), kinetin, isophtynyliminopurine (2iP), giberellin(GA_3; giberelic acid), and thidiazuron(TDZ), but not limited thereto. Preferably, the plant growth regulator is a-naphtalene acetic acid, 2,4-D, BA or kinetin. The plant growth regulator can be added with a proper amount, based on the kind of plant cell, and for
example 0.01mg/l to 10mg/l, preferably 0.1mg/l to 10mg/l.

Preferably, the plant cell derived from plant *Acer tegmentosum* can be cultured in the suspension culture. The suspension culture means the culture of plant cells which are dispersed in the liquid nutritional solution, and includes the cells at various aggregation stages. Generally, the suspension culture can be induced from the sufficiently-proliferated callus by transferring the callus to the liquid nutritional medium, culturing for 1 to 4 weeks and inoculating the cell culture at the same medium in the serial sub-culture.

The medium for suspension culture can be made by excluding the solidifying agent from the medium used for the induction and proliferation of callus, or can be different medium from that of the induction and proliferation of callus. In an embodiment of the present invention, the medium for suspension culture may be selected from the group consisting of LS medium, MS medium, B5 medium, SH medium, and preferably LS medium or B5 medium.

To induce the suspension culture, the liquid medium can be added by 10 to 40g/l, or preferably 20 to 30g/l of callus, at a culturing temperature of 20 to 30°C or preferably 22 to 26°C under the dark condition.

All culture method used generally in this art can be applied for the present invention. The examples of the culture methods include batch culture process, continuous culture process, fed-batch culture process, semi-continuous batch culture process, immobilized culture process, two-phase culture process and the like. The method can be selected according to the characteristics of plant cell.

In accordance with the present invention, in the step of culturing the plant cell line in the liquid culture medium, the production of secondary metabolite can be increased by adding at least one selected from the group consisting of an elicitor of secondary metabolite and a precursor of secondary metabolite to the medium. Specifically, the plant cell can be cultured with addition of at least one selected from the group consisting of an elicitor of secondary metabolite and a precursor of secondary metabolite at an initial stage or a middle stage of culture. Because the biochemical pathways of producing the secondary metabolite are various according to the kinds of metabolites, an elicitor must be selected suitably and the treatment condition must be
optimized to increase the production of metabolite.

Alternatively, the plant cell can be cultured in the nutritional medium including at least one selected from the group consisting of an elicitor of secondary metabolite and a precursor of secondary metabolite. The nutritional medium cannot be limited particularly, so long as it can be used for culturing the plant cell. The nutritional medium is the same as or different from that of callus induction and/or suspension culture. Usually, the conditions of growth and the production of secondary metabolite are different for the plant cell, the medium for producing the secondary metabolite can be optimized separately. However, the same medium for the cell growth and the production of secondary metabolite can be used for some plant cell.

The elicitors are classified into a biotic elicitor and an abiotic elicitor. Depending on the kinds and the properties of elicitors, the added amount can be different. The kinds of plant cell affect the result of elicitor addition. Thus, the added amount of elicitor can be selected properly on the basis of the kinds of elicitors and the plant cells. The examples of applicable elicitors are listed in the following Table 1.

<table>
<thead>
<tr>
<th>Biotic elicitor</th>
<th>Abiotic elicitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal extracts</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>Bacterial extracts</td>
<td>Elaidic acid</td>
</tr>
<tr>
<td>Yeast extracts</td>
<td>Cyclic AMP</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Dibutyryl Cyclic AMP</td>
</tr>
<tr>
<td>Lichenan</td>
<td>Methyl jasmonate</td>
</tr>
<tr>
<td>Glucosannan</td>
<td>Cis-jasmonate</td>
</tr>
<tr>
<td>Pleuran</td>
<td>Miconanol</td>
</tr>
<tr>
<td>Glucan</td>
<td>Vanadyl sulfate</td>
</tr>
<tr>
<td>Carboxymethylglucan</td>
<td>Ferulic acid</td>
</tr>
<tr>
<td>Sulfoethylglucan</td>
<td>Vanadyl sulfate</td>
</tr>
<tr>
<td>Hydroxymethylglucan</td>
<td>Uniconanol</td>
</tr>
<tr>
<td>Mannan</td>
<td>Paclorbutrazol</td>
</tr>
<tr>
<td>Xylan</td>
<td>Spermine</td>
</tr>
<tr>
<td>Mannobiose</td>
<td>Spemidine</td>
</tr>
<tr>
<td>Mannotriose</td>
<td>Putresmine</td>
</tr>
<tr>
<td>Mannopentaose</td>
<td>Cadavirine</td>
</tr>
<tr>
<td>Mannotetraose</td>
<td>Protamine sulfate</td>
</tr>
<tr>
<td>Cellulysin</td>
<td>Fenpropemorph</td>
</tr>
<tr>
<td>Multifect XL</td>
<td>Prochloraz</td>
</tr>
<tr>
<td>Multifect CL</td>
<td>Naptifeme</td>
</tr>
<tr>
<td>Resinase</td>
<td>EDU</td>
</tr>
<tr>
<td>Pulpxyme</td>
<td>HTA</td>
</tr>
<tr>
<td>SP43</td>
<td>MPTA</td>
</tr>
</tbody>
</table>

Table 1
The examples of biotic elicitors are fungal extracts, Bacterial extracts, Yeast extracts, Chitosan, Lichenan, Glucomannan, Pleuran, Glucan, Carboxymethylglucan, Sulfoethylglucan, Hydroxymethylglucan, Mannan, Xylan, Mannobiose, Mannotriose, Mannopentaose, Mannotetraose, Cellulysin, Multifect XL, Multifect CL, Resinase, Pulpxyme, SP43 l, Pectinol, Rapidase, and Chitinase, but not limited thereto.

The examples of abiotic elicitors are Arachidonic acid, Elaidic acid, Cyclic AMP, Dibutylr Cyclic AMP, Methyl jasnone, Cis-jasmone, Miconazol, Ferulic acid, Vanadyl sulfate, Uniconazol, Paclobutrazol, Spermine, Spermidine, Putrescine, Cadaverine, Protamine sulfate, Fenpropemorph, Prochloraz, Naptifme, EDU, HTA, MPTA, Glutathione, EGTA, AMO-1618, Triton X-100, Benzoic acid, Salicylic acid, Propyl gallate, Sesamol, Chlorocholine chloride, 3,4-dichlorophenoxytriethyl-(amine), Hydroquinone, Chloroethylphosphonic acid, Diethylidithiocarbamic acid, Nordihydroguaiaretic acid, Dithiothreitol, Sodium metabisulfite, Potassium metabisulfite, d-amino-DL-phenylalanine, Ancymidol, Triadimefon, Phosphon D, Thiourea, Dextran sulfate, Carragenan, Tyramine, Sodium acetate, Potassium acetate, Ammonium acetate, Mevalonic acid, Farnesyl acetate, Geranylgeraniol acetate, Tryptamine, Menthol, alpha.-Pinene, Trans-cinnamic acid, Cambrene A, Verticillene, Verticillol, Camphor, Quercetin, and Levulinic acid, but not limited thereto.

In another embodiment, to achieve a high concentration of culture and a high volumetric productivity of secondary metabolite, a carbon source as a hyperosmotic agent can be added to the culture at a concentration of 3 (w/v)% to 10 (w/v)%, or preferably 3 (w/v)% to 6 (w/v)%, so as to increase the osmotic pressure. When the added concentration of carbon source is lower than 3 (w/v)%, the effect of osmotic
pressure is reduced. When the concentration is higher than 10%, the excessively high osmotic pressure causes the cell necrosis. The hyperosmotic agent can be any one used for the plant cell culture as a carbon source, but preferably be at least one selected from the group consisting of sucrose, lactose, fructose and glucose.

To increase the volumetric productivity of the secondary metabolite, the high inoculation concentration of plant cell can be employed for the present invention, compare to the general inoculation concentration. Preferably, the plant cell can be inoculated at a concentration of 2 g/l to 10 g/l and cultured for 7 days or longer, or inoculated at a concentration of 5 g/l to 10 g/l or cultured for 14 days. When the concentration is smaller than 2 g/l, the long culture time and delayed cell growth can decrease he production of secondary metabolite. When the concentration is higher than 10 g/l, the excessive cell growth can reduce the capability of cell to produce the secondary metabolite.

D. Production of an extract of plant cell culture

In another embodiment, provided is a method of preparing an extract from a plant cell culture of *Acer tegmentosum*, including (a) inducing a callus from a tissue of *Acer tegmentosum* inoculated in callus-inducing medium; (b) preparing a suspension-cultured cell line capable of being cultured in liquid culture medium from the induced callus; (c) culturing the prepared suspension-cultured cell line in a suspension culture to produce the suspension culture and (d) obtaining an extract from the suspension culture.

According to the methods of extracting and purifying the plant cell culture which have been known to those skilled in the art can be applied to an embodiment of the present invention.

The cell culture can be extracted with hot water, alcohol or organic solvent. Salidroside, salidroside derivatives and other secondary metabolites can be extracted together, depending on the kinds of solvents. The extract can be used for various pharmaceutical or food compositions.

When the plant cell is cultured in a liquid medium including the elicitor or the precursor of the secondary metabolite, the secondary metabolites of interest including salidroside can be obtained at a high concentration.
The dry weight of salidroside can be included at a concentration of 0.01 wt% to 30 wt%, for example, 0.02 to 20 wt% or 0.3 to 15 wt% in the dried cell culture.

The methods of separating and purifying the plant cell culture which has been known to those skilled in the art, such as the solvent extraction method, the recrystallization method, the chromatography method and the like can be applied for the cell culture of the present invention. Preferably, the cell culture can be extracted by at least one method selected from the group consisting of the solvent extraction method, the liquid-liquid extraction method using an organic solvent, and the fractional extraction method using column chromatography. To obtain the product with a high concentration, the extract can be concentrated under the reduced pressure, or can be purified by an additional purification step. The extract can be formulated to the powder by using an additional processing step of the distillation under reduced pressure, the freeze-drying or the spray-drying.

The extract can be obtained by extracting the cell culture with at least a solvent selected from the group consisting of water, C1-C5 lower alcohol, chloroform, dichloromethane, benzene, acetone, hexane, ethyl acetate and a mixture thereof. In a further embodiment, the solvent extract can be further extracted by the liquid-liquid extraction method using the organic solvent to produce the distributed extract. The solvent extract or the distributed extract can be further processed with the column chromatograph to produce the fractional extract. The salidroside is contained at a concentration of 0.1% to 99.9 wt% in the fractional extract.

In an embodiment of the present invention, provided is a plant cell culture of Acer tegmentosum and an extract of the plant cell culture. The extract includes the solvent-extract, the distributed extract and the fractional extract.

In the cell culture of the present invention, the concentration of salidroside is 1,600 times as much as the natural plant of Acer tegmentosum. In accordance with the present invention, the cell culture of plant Acer tegmentosum with excellent protection and improvement of liver function can be prepared in a large scale. The salidroside, the plant cell culture and the extract thereof can be used for a pharmaceutical composition or a functional food composition for preventing, improving or treating the hepatotoxicity and for protection and improvement of liver function or a liver disease.
In another embodiment, provide is a pharmaceutical composition for prevention, improvement or treatment of a liver disease, including an extract from a plant cell culture of *Acer tegmentosum*. The active ingredient of the present invention can be contained at an amount of 0.1 to 99 wt% per total weight of the composition.

The liver disease includes a hepatocirrhosis, hepatitis, alcoholic fatty liver and a liver cancer.

The pharmaceutical composition further includes a pharmaceutically-acceptable excipients, diluents and/or carriers.

In further embodiment, provided is a food composition for protection or improvement of a liver function, comprising an extract from a plant cell culture of *Acer tegmentosum*. The active ingredient of the present invention can be contained at an amount of 0.1 to 99 wt% per total weight of the composition.

The examples of food composition include a functional food, beverage, gum, tea, nutritional supplement, and dietary supplement. The food composition further includes various nutrients, vitamins, electrolytes, a synthetic and natural flavoring agent, a colorant and a thickening agent such as cheese, chocolate, or etc., pectic acid and its salt, alginic acid or salt thereof, organic acid, protective colloid, thickening agent, pH adjusting agent, a stabilizer, a preservative, glycerin, alcohol, carbon dioxide and the like.

The pharmaceutical composition of the present invention may be formulated for oral administration, for example, granules, subtilized granules, powders, hard capsules, soft capsules, syrup, emulsion, suspension, or solution, or may be administered for parenteral administration, for example, injections for intravenous administration, intramuscular administration, or subcutaneous administration, drip infusions, suppositories, percutaneous absorbent, transmucosal absorption preparations, nasal drops, ear drops, instillation, inhalants, creams, ointments, and cataplasm (*Remington's Pharmaceutical Science; Mack Publishing Company, Easton PA*). Preparations made as pharmaceutical compositions in a form of powder may be dissolved upon use and administered as injections or drip infusions. Particularly, the medicament of the present invention may sometimes be preferably applied parenterally and topically as external preparation such as creams, paste ointments, solution, gel, spray, lotion, and suspension.
A dose of the pharmaceutical composition of the present invention is not particularly limited. A dose may generally be 10 to 10,000 mg per day for an adult as the weight of the compound of the present invention. It is preferred to increase or decrease the above dose appropriately depending on the age, pathological conditions, and symptoms of a patient. The above dose may be administered once a day or 2 to 3 times a day as divided portions with appropriate intervals, or intermittent administration for every several days may be applied.

[Advantageous Effects]

As described above, the present invention provides a method of preparing the cell culture *Acer tegmentosum*, thereby being capable of producing the extract of plant cell culture and the secondary metabolites in a large scale without being affected by the weather, geographical and political conditions. In addition, the extract of the plant cell culture obtained according to the present invention can be used for a pharmaceutical composition for prevention, improvement and treatment of a liver disease, and a food composition for ameliorating a hepatotoxicity and protecting and improving a liver function.

[Description of Drawings]

FIG. 1 is a photograph showing the callus culture derived from plant *Acer tegmentosum* according to Example 1-1.

FIG. 2 shows the variation of the cell dry weight during culture of the suspension-cultured cells of *Acer tegmentosum* obtained in Example 1-2 in four (4) kinds of liquid culture medium.

FIG. 3 shows the analysis of High Performance Liquid Chromatography (HPLC) of the cell culture of *Acer tegmentosum* in Example 2 and salidroside standard sample ((A) for the result of HPLC analysis of the cell culture of *Acer tegmentosum* and (B) for result of HPLC analysis of salidroside standard sample at a concentration of 1 mg/ml).

FIG. 4 shows the analysis result of Liquid Chromatography-Mass Spectroscopy
(LC-MS) of the cell culture of plant *Acer tegmentosum* in Example 2 and salidroside standard sample ((A) for the result of LC-MS analysis of the cell culture of plant *Acer tegmentosum* and (B) for result of LC-MS analysis of salidroside standard sample at a concentration of 1 mg/ml).

**Mode for Invention**

The present invention is further explained in more detail with reference to the following examples. These examples, however, should not be interpreted as limiting the scope of the present invention in any manner.

**Example 1: Establishment of cell culture of plant *Acer tegmentosum***

Example 1-1: Callus induction from *Acer tegmentosum*

The leaves and stems were collected from *Acer tegmentosum* grown naturally in Korea, cut into about 5 cm, surface washed by dishwashing detergent, and rinsed with tap water.

The prepared sample was immersed in 95% ethanol solution for 1 minute, and then sterilized by continuously shaking it in 1% Sodium Hypochlorite solution for 30 minutes, and rinsed three times with sterilized distilled water.

The sterilized sample was cut with a scalpel into slices having 1 cm width x 1 cm length, and inoculated on four kinds of medium (LS, MS, B5, and SH) which were prepared by adding 30g/L sucrose, 8g/L agar, 2 mg/L NAA, 0.02 mg/L BA, 1 g/L casein hydrolysate, 1 g/L 2-Morpholinoethanesulfonic acid(MES), and adjusting the pH to 5.7, as indicated in Table 2, and then cultivated in the dark at a temperature of 24°C for 8 weeks to induce the callus. The composition of culture medium for culturing the plant cell is summarized in Table 2.

<table>
<thead>
<tr>
<th>Component(mg/L)</th>
<th>LS</th>
<th>MS</th>
<th>B5</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂</td>
<td>332.02</td>
<td>332.02</td>
<td>113.23</td>
<td>151</td>
</tr>
<tr>
<td>KH₃PO₄</td>
<td>170</td>
<td>170</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>-</td>
<td>-</td>
<td>130.44</td>
<td>-</td>
</tr>
</tbody>
</table>
The induced callus was transferred to new medium at an interval of 4 weeks. The results of the callus induction and proliferation from *Acer tegmentosum* using the four kinds of media are shown in Table 3.

As shown in Table 3, the rates of callus induction in the LS medium and B5 medium were the highest, but callus proliferation in a sub-culture on B5 medium was fastest. The optimum mediums were summarized for callus induction and proliferation of *Acer tegmentosum* in Table 3.

### Table 3

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus induction</th>
<th>Callus proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>MS</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>B5</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>SH</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

#### Example 1-2: Suspension culture

The suspension culture was induced by using callus tissue cultured on the B5 medium for 4 weeks according to the method of EXAMPLE 1-1.

Two grams of callus were finely chopped up with a scalpel and added to a 250
ml flask containing 50ml of 4 kinds of liquid culture medium, and then was cultured by shaking at 110rpm at 24°C in the dark.

The suspension culture was sampled often and the amount of remaining sugar was measured with the refractometer. As a result, when the remaining sugar decreased to about 1%(w/v), it was transferred to a new sub-culture medium.

During the first 2-3 sub-cultures, 0.5 - 1%(w/v) of PVPP, XAD resin or 0.5 %(w/v) activated carbon were added to the medium to prevent medium browning and cell oxidation, but were not added in subsequent sub-cultures.

Among plant cell lines of *Acer tegmentosum* obtained from suspension cultures, the plant cell lines with fastest growth rate and the highest production rate were selected and named SYGB-5 (*Acer tegmentosum* Maxim. SYGB-5), and then deposited on August 7, 2012 at the Korean Collection for Type Cultures (KCTC) which is located at 52 Eoeun-dong, Yuseong-gu, Daejeon, Republic of Korea as KCTC accession number of KCTC 10822BP.

Sixty(60) mL of cell suspension culture obtained by sub-culturing for 2-3 months was inoculated into a 500 mL flask containing 180 ml liquid solution of four kinds of medium and cultured at 150 rpm, and the remaining sugar and cell dry weight were measured every other day. The cell dry weight was obtained by filtering sampled plant cell culture through Watman no. 4 filter paper using a Buchner funnel, and oven drying at a temperature of 60°C for 24 hours.

The cell dry weight obtained by culturing the plant cell in 4 kinds of culture mediums is shown in FIG. 2. As a result, LS medium was most preferable for fast growth. In LS medium, the suspension culture of *Acer tegmentosum* grew to a dry cell weight of 18.9 g/L in only 10 days, which was 3.8 times as high as the amount of inoculated cells.

**Example 2: Production of salidroside from the cell culture of plant *Acer tegmentosum***

The *Acer tegmentosum* cell lines obtained in Example 1-2 were cultured in LS medium and after 14 days, the cultured cells were collected for extracting the salidroside as follows:

5 ml sample obtained from the cell culture was centrifuged to remove the
medium solution, sufficiently oven dried at a temperature of 40°C for 24 hours, and then were ground up using a mortar. 250 mg of the cell powder was initially extracted by shaking for 24 hours in 5 ml of methanol. The initial extract was centrifuged to collect the methanol fraction.

The analysis of salidroside was carried out with High Performance Liquid Chromatography (HPLC) under the analysis condition where Capcell Pak C18 (Shiseido, 4.6 x 250 mm) column was used an a mixture of water and methanol (19:1, v/v) containing 0.05% trifluoroacetic acid as a mobile phase were eluted at a flow rate of 1ml/min, and the absorbance at 214nm were measured.

FIG. 3 shows the analysis of HPLC of the plant cell culture of *Acer tegmentosum* in Example 2 and salidroside standard sample. (A) is for the result of HPLC analysis of the cell culture of plant *Acer tegmentosum* and (B) is for result of HPLC analysis of salidroside standard sample at a concentration of 1 mg/ml.

To perform the molecular weight comparison of a standard sample of salidroside and salidroside separated from the suspension-cultured cell line of *Acer tegmentosum*, the liquid chromatography- mass spectroscopy (LC-MS) were carried out. The apparatuses for LS- MS analysis are LCQ-Deca system (Finnigan™, USA) and the analysis conditions of LS- MS are shown in Table 4.

<table>
<thead>
<tr>
<th>Item</th>
<th>Numerical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion source</td>
<td>ESI mode</td>
</tr>
<tr>
<td>Sheath gas flow rate</td>
<td>80 arb</td>
</tr>
<tr>
<td>Aux gas flow rate</td>
<td>20 arb</td>
</tr>
<tr>
<td>Spray voltage</td>
<td>5.00 kV</td>
</tr>
<tr>
<td>Capillary current</td>
<td>270 °C</td>
</tr>
<tr>
<td>Capillary voltage</td>
<td>15 V</td>
</tr>
<tr>
<td>Tube lens offset</td>
<td>50 V</td>
</tr>
</tbody>
</table>

FIG. 4 shows the analysis of LC-MS of the plant cell culture of *Acer tegmentosum* in Example 2 and salidroside standard sample. (A) is for the result of LC-MS analysis of the plant cell culture of *Acer tegmentosum* and (B) is for result of LC-MS analysis of salidroside standard sample at a concentration of 1 mg/ml.

The salidroside production by the plant cell culture was confirmed by HPLC analysis and LC-MS analysis.
Example 3: Salidroside production with addition of an elicitor and a precursor

50 mL of the cell culture obtained in Example 1-2 were inoculated and cultured for 2 weeks on 200 mL of LS liquid medium containing 2 mg/L of NAA and 0.5 mg/L of BA, with the addition of various elicitors and precursors listed in Table 4.

After completion of the cell culture, the cell culture was recovered and analyzed according to the same method of Example 2 to compare the content of salidroside.

The productivity of salidroside was measured according to the following quantitative analysis method. The concentration of salidroside was calculated from the peak area of salidroside of HPLC obtained according the same method of Example 2, by using the standard curve of 0.05 to 1 mg/L salidroside standard sample, and the dry weight of salidroside (%DW) was calculated from the concentration of salidroside. %DW means the ratio of wt% salidroside contained in 1 g of cell dry weight. The final volumetric productivity (mg/L) was calculated by multiplying the calculated %DW with the cell concentration (g/L, DW) of culture.

The productivities of salidroside were summarized in Table 5. As shown in Table 5, the addition of elicitor and precursor increased the productivity of salidroside. The precursor of Tyrosol was very effective. When methyl jasmonate and tyrosol were added together, the productivity of salidroside increased more.

Table 5

<table>
<thead>
<tr>
<th>Added material</th>
<th>Added amount</th>
<th>Productivity of salidroside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%DW</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>5 μM</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>10 μM</td>
<td>0.01</td>
</tr>
<tr>
<td>Methyl jasmonate</td>
<td>50 μM</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>100 μM</td>
<td>0.07</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.5 mM</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>2 mM</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>4 mM</td>
<td>0.05</td>
</tr>
<tr>
<td>Tyrosol</td>
<td>0.5 mM</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>2 mM</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>4 mM</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>6 mM</td>
<td>4.42</td>
</tr>
<tr>
<td>tyrosol 6 mM + methyl jasmonate 100 µM</td>
<td>8.26</td>
<td>834.66</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>tyrosol 8 mM + methyl jasmonate 100 µM</td>
<td>8.39</td>
<td>923.17</td>
</tr>
</tbody>
</table>

**Example 4**: Liver protection effect of the culture extract of plant *Acer tegmentosum* and extracted salidroside

Example 4-1: the extract of plant *Acer tegmentosum* and salidroside separation

According to the methods of Example 1-2 and Example 3, the cells of *Acer tegmentosum* were cultured for 2 weeks, collected and dried to obtain the dried powder of cell culture. 1 kg of cell culture powder was extracted with addition of 20 L of 70% ethanol for 24 hours, and filtered to recover the ethanol fraction. The recovered ethanol fraction was freeze-dried to 250 g of cell culture powder. The content of salidroside in cell culture powder was analyzed with HPLC and determined as 9.8% DCW.

200 g of cell culture powder were suspended in 1 L of distilled water, and the distribution extraction was performed with the addition of 1 L of butanol at three times to produce the concentrated butanol fraction. The butanol fraction was purified with a silica gel column chromatography by using 1 L of a mixture of chloroform and methanol (9:1) and then a mixture of chloroform and methanol (6:4) as a mobile phase to obtain a fraction including salidroside. The obtained fraction was concentrated under the reduced pressure to produce the colorless powder, and then the powder was confirmed as salidroside by analyzing according to the same method of Example 2.

**Example 4-2**: Treatment of test animal

The animal model was 4-week male mice (Sprague-Dawley) purchased from SAMTAKO BIO KOREA Co., Ltd and raised at the constant condition (temperature 21.4 °C±0.05 °C, humidity 61±1%, 12 hours of light and darkness cycle). The diet was water and feed (AIN-93G Purified Rodent Diet) and was fed to the animal sufficiently for 1 week. The mice with body weight of 150±10 g were selected for 10 mice per a test group. After taming for 1 week, the animals were orally administered by the cell culture (300 mg/kg) obtained in Example 4-1 and salidroside (30 mg/kg, 100 mg/kg) dissolved
in very small amount of DMSO (dimethyl sulfoxide) and diluted in PBS and 1% of olive oil, before 2 days, 1 day and 2 hours and before 2 days, 1 day, and 2 hours of administering D-galactosamine. Then, D-galactosamine (700 mg/kg) was administered abdominally to induce the hepatotoxicity.

After 24 hours, the test animals were starved for 12 hours, anesthetized with ether and the blood was collected from carotid. The collected blood was centrifuged at 1,500 rpm for 15 minutes, the serum was recovered and stored at -80°C for use.

**Example 4-3: Test of Liver protection effect by using the blood analysis**

The collected blood was test for AST (aspartate aminotransferase), ALT (alanine aminotransferase) and LDH (lactate dehydrogenase) which was known as an indicator for liver function. The analysis of AST and ALT activities were performed with a test kit (Asan, Korea) prepared according to the Reitman-Frankel method (1957). That is, the substrate solution was reacted at 37°C for 5 minutes and then was reacted with the serum for 30 minutes for ALT test, and 60 minutes for AST. The obtained solution was added by 19.8 mg/100 ml of 2,4-dinitrophenylhydrazine and 0.4N-NaOH solution, left at a room temperature and absorbance was measured at 505 nm. The activity was represented as Karmen unit (IU/L) per 1 ml of serum according to the standard curve. The analysis of LDH activity was performed with a test kit (Sigma-Aldrich, St. Louis, USA) prepared according to the Wroblewskin and LaDue method. That is, the substrate solution was mixed with 2,4-dinitrophenylhydrazine at a ratio of 1:1 and reacted at 37°C for 5 minutes. Then, the mixture was added to the sample, left at 37°C for 10 minutes and stop with addition of HCl. The absorbance was measured at 570 nm and analyzed for the activity using the standard curve.

The results were represented as mean ± standard error. The statistical analysis were carried out with using SPSS(version 12.0) and t-test to confirm the variation and mean. The analysis results are confirmed Duncan test and determined as significant, when the p-value is lower than 0.05. The analysis results of AST, ALT and LDH are shown in Table 6 showing the effect of the active ingredients treated before eliciting the hepatotoxicity in the test animals.

Table 6
The AST and ALT activities of control were 60.1±3.2 IU/L and 35.6±2.0 IU/L, the activities of GalN test group which had a hepatotoxicity induced by D-galactosamine were increased significantly as 180.0±1.5 IU/L and 101.0±3.5 IU/L. The increased activities were 150.7±1.8 IU/L and 95.6±0.9 IU/L in the test group of extract of cell culture (300 mg/kg). The increased activities were reduced to 132.1±0.9 IU/L and 90.2±0.1 IU/L in the test group of salidroside (30 mg/kg) and were reduced significantly to 99.5±1.4 IU/L and 42.9±0.7 IU/L in the test group of salidroside (100 mg/kg).

LDH activity was 1041.2±11.2 IU/L in a control group. LDH activity was increased as 2317.0±13.1 IU/L in the GalN test group due to the hepatotoxicity, but reduced to 2017.2±19.1 IU/L in the test group of extract of cell culture (300 mg/kg), to 1880.3±23.3 IU/L in the test group of salidroside (30 mg/kg), and 1320.0±19.6 IU/L in the test group of salidroside (100 mg/kg), which was reduced significantly as being equal to the LDH activity of the control group.

**Example 5:** Test of the hepatotoxicity treatment effect of plant cell culture.

**Example 5-1:** Treatment of test animal

The test animal tamed according to the method of Example 4-2 was administered abdmominally by D-galactosamine (400 mg/kg) to induce the chronic hepatotoxicity on initial day and after three days. The control group was administered
by the same volume of PBS, instead of D-galactosamine. After the test group of drug was administered by D-galactosamine in 24 hours, the group was orally administered daily for 5 days by the cell culture (300 mg/kg) obtained in Example 4-1 and salidroside (30 mg/kg, 100 mg/kg) dissolved in very small amount of DMSO and diluted in PBS and 1% of olive oil.

On final day, the test animals were starved for 12 hours, anesthetized with ether and the blood was collected from carotid. The collected blood was centrifuged at 1,500 rpm for 15 minutes; the serum was recovered and stored at -80°C for use.

Example 5-2: The hepatotoxicity treatment by using blood analysis

According to the same method of Example 4-3, the collected blood was test for AST (aspartate aminotransferase), ALT (alanine aminotransferase) and LDH (lactate dehydrogenase) to represent the result in Table 7.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.5±4.2</td>
<td>34.9±1.1</td>
<td>1030.2±11.9</td>
</tr>
<tr>
<td>GalN</td>
<td>201.0±3.3</td>
<td>99.8±2.0</td>
<td>2510.0±16.7</td>
</tr>
<tr>
<td>Extract of cell culture</td>
<td>162.4±3.2</td>
<td>90.2±1.5</td>
<td>2401.8±14.4</td>
</tr>
<tr>
<td>(300 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salidroside(30 mg/kg)</td>
<td>105.3±2.2</td>
<td>84.7±1.0</td>
<td>1895.1±10.8</td>
</tr>
<tr>
<td>Salidroside(100 mg/kg)</td>
<td>101.6±1.0</td>
<td>80.3±1.4</td>
<td>1540.6±17.1</td>
</tr>
</tbody>
</table>

*<p < 0.05, **<p < 0.01 indicate a significant difference between the control group and the GalN treated group.

*<p < 0.05, **<p < 0.01 indicate a significant difference between the GalN group and the medicine treated group.

The AST and ALT activities of control were 62.5±4.2 IU/L and 34.9±1.1 IU/L, the activities of GalN test group which had a hepatotoxicity induced by D-galactosamine were increased significantly as 201.0±3.3 IU/L and 99.8±2.0 IU/L. The increased activities were 162.4±3.2 IU/L and 90.2±1.5 IU/L in the test group of extract of cell culture (300 mg/kg). The increased activities were reduced to 105.3±2.2 IU/L and 64.7±1.0 IU/L in the test group of salidroside (30 mg/kg) and were reduced significantly to 101.6±1.0 IU/L and 50.3±1.4 IU/L in the test group of salidroside (100 mg/kg).
mg/kg).

LDH activity was 1030.2±11.9 IU/L in a control group. LDH activity was increased as 2510.0±16.7 IU/L in the GalN test group due to the hepatotoxicity, but reduced to 2401.8±14.4 IU/L in the test group of extract of cell culture (300 mg/kg), to 1895.1±10.8 IU/L in the test group of salidroside (30 mg/kg), and 1540.6±17.1 IU/L in the test group of salidroside (100 mg/kg), which was reduced significantly as being equal to the LDH activity of the control group.
**INTERNATIONAL FORM**

**RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT**

issued pursuant to Rule 7.1

**TO:** Sanyang Genexbio Corporation  
31 Jongno 33-gil, Jongno-gu, Seoul 110-725  
Republic of Korea

<table>
<thead>
<tr>
<th>I. IDENTIFICATION OF THE MICROORGANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification reference given by the DEPOSITOR:</td>
</tr>
<tr>
<td>SYGB-5 (plant cell line)</td>
</tr>
<tr>
<td>Accession number given by the INTERNATIONAL DEPOSATORY AUTHORITY:</td>
</tr>
<tr>
<td>KCTC 12257BP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>The microorganism identified under I above was accompanied by:</td>
</tr>
<tr>
<td>[x J a scientific description</td>
</tr>
<tr>
<td>[ ] a proposed taxonomic designation</td>
</tr>
<tr>
<td>(Mark with a cross where applicable)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. RECEIPT AND ACCEPTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>This International Depositary Authority accepts the microorganism identified under I above, which was received by it on <strong>August 7, 2012</strong>.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV. RECEIPT OF REQUEST FOR CONVERSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>V. INTERNATIONAL DEPOSATORY AUTHORITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name: Korean Collection for Type Cultures</td>
</tr>
</tbody>
</table>
| Address: Korea Research Institute of Bioscience and Biotechnology (KRIIBB)  
125 Gwahak-ro, Yuseong-gu, Daejeon 305-806  
Republic of Korea |
| Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s): |
| BAE, Kyung Sook, Director  
Date: **August 31, 2012** |

Form BP/4 (KCTC Form 17)
[CLAIMS]

[Claim 1]

A method of preparing an extract from a plant cell culture of *Acer tegmentosum*, comprising:

(a) inducing a callus from a tissue of *Acer tegmentosum* inoculated in callus-inducing medium;

(b) preparing a suspension-cultured cell line capable of being cultured in liquid culture medium from the induced callus; and

(c) culturing the suspension-cultured cell line in a suspension culture to form a cell culture and obtaining the extract from the cell culture.

[Claim 2]

The method of preparing an extract according to claim 1, wherein further comprises adding in step (b) at least one selected from the group consisting of an elicitor of secondary metabolite and a precursor of secondary metabolite, to increase the production of a secondary metabolite.

[Claim 3]

The method of preparing an extract according to claim 2, wherein the elicitor of secondary metabolite is at least a biotic elicitor selected from the group consisting of fungi, bacteria, and yeast, and a lysate, an extract, a fraction and a filtrate thereof.

[Claim 4]

The method of preparing an extract according to claim 3, wherein the elicitor of secondary metabolite is at least one selected from the group polysaccharide, glycoprotein, inactivated enzyme, pleuran, xanthan, chitosan and glucan which are obtained from at least one selected from the group of fungi, bacteria and yeast.

[Claim 5]

The method of preparing an extract according to claim 2, wherein the elicitor of secondary metabolite is at least an abiotic elicitor selected from the group consisting of metal salts and a regulator of plant metabolism.
[Claim 6]

The method of preparing an extract according to claim 5, wherein the metal salt is at least one selected from the group consisting of silver nitrate, cadmium chloride and cobalt chloride.

[Claim 7]

The method of preparing an extract according to claim 5, wherein the regulator of plant metabolism is at least one selected from the group consisting of salicylic acid, jasmonic acid and methyl jasmonate.

[Claim 8]

The method of preparing an extract according to claim 5, wherein further comprises adding in the step (b) at least one hyperosmotic agent selected from the group consisting of sucrose, lactose, fructose and glucose.

[Claim 9]

The method of preparing an extract according to claim 1, wherein the plant cell of Acer tegmentosum is SYGB-5 deposited under KCTC accession number of KCTC12257BP.

[Claim 10]

The method of preparing an extract according to claim 1, wherein the extract obtained in step (c) is at least one selected from a solvent extract obtained by extracting the culture with a solvent, a distributed extract obtained by using liquid-liquid extraction of an organic solvent, and a fractional extract obtained by using a chromatography.

[Claim 11]

The method of preparing an extract according to claim 10, wherein the solvent extract is obtained by extracting with at least a solvent selected from the group consisting of water, C1-C5 lower alcohol, chloroform, dichloromethane, benzene, acetone, hexane, ethyl acetate and a mixture thereof.
The method of preparing an extract according to claim 10, wherein the distributed extract is obtained by distributing with at least an organic solvent selected from the group consisting of butanol, chloroform, dichloromethane, benzene, hexane and ethyl acetate.

[Claim 13]

The method of preparing an extract according to claim 10, wherein the fractional extract is obtained by sequentially performing a liquid-liquid extraction and a column chromatography for the solvent extract.

[Claim 14]

A plant cell of *Acer tegmentosum* which is deposited under KCTC accession number of KCTC12257BP.

[Claim 15]

A pharmaceutical composition for prevention, improvement or treatment of a liver disease, comprising an extract from a plant cell culture of *Acer tegmentosum*.

[Claim 16]

The pharmaceutical composition according to claim 15, wherein the liver disease is a hepatocirrhosis, a hepatitis, an alcoholic fatty liver and a liver cancer.

[Claim 17]

The pharmaceutical composition according to claim 15, wherein the extract comprises 0.1 to 99.9 wt% of salidroside.

[Claim 18]

The pharmaceutical composition according to claim 15, wherein the extract is at least one selected from a solvent extract obtained by extracting the culture solution with a solvent, a distributed extract obtained by using liquid-liquid extraction of an organic solvent, and a fractional extract obtained by using a chromatography.

[Claim 19]

The pharmaceutical composition according to claim 18, wherein the solvent extract is obtained by extracting with at least a solvent selected from the group...
consisting of water, C_{i-C_5} lower alcohol, chloroform, dichloromethane, benzene, acetone, hexane, ethyl acetate and a mixture thereof.

[Claim 20]

The pharmaceutical composition according to claim 18, wherein the distributed extract is obtained by distributing with at least an organic solvent selected from the group consisting of butanol, chloroform, dichloromethane, benzene, hexane and ethylacetate.

[Claim 21]

The pharmaceutical composition according to claim 18, wherein the fractional extract is obtained by sequentially performing a liquid-liquid extraction and a column chromatography for the solvent extract.

[Claim 22]

A food composition for protection or improvement of a liver function, comprising an extract from a plant cell culture of *Acer tegmentosum*. 

[Claim 23]

The food composition according to claim 22, wherein the food is a functional food, beverage, gum, tea, nutritional supplement, or dietary supplement

[Claim 24]

The food composition according to claim 22, wherein the extract comprises 0.1 to 99.9 wt% of salidroside.

[Claim 25]

The food composition according to claim 22, wherein the extract is at least one selected from a solvent extract obtained by extracting the culture solution with a solvent, a distributed extract obtained by using liquid-liquid distribution of an organic solvent, and a fractional extract obtained by using a chromatography.

[Claim 26]

The food composition according to claim 25, wherein the solvent extract is obtained by extracting with at least a solvent selected from the group consisting of water,
C1-C5 lower alcohol, chloroform, dichloromethane, benzene, acetone, hexane, ethyl acetate and a mixture thereof.

[Claim 27]

The food composition according to claim 25, wherein the fractional extract is obtained by sequentially performing a distribution extraction and a column chromatography for the solvent extract.

[Claim 28]

A pharmaceutical composition for prevention, improvement or treatment of a liver disease, comprising a salidroside.

[Claim 29]

The pharmaceutical composition according to claim 28, wherein the liver disease is a hepatocirrhosis, a hepatitis, an alcoholic fatty liver and a liver cancer.

[Claim 30]

A food composition comprising for protection or improvement of a liver function or a liver disease, comprising a salidroside.

[Claim 31]

The food composition according to claim 31, wherein the food is a functional food, beverage, gum, tea, nutritional supplement, or dietary supplement.
[DRAWINGS]

[Figure 1]
[Figure 2]

Day 0, 4, 8, and 12.

DCW (g/L)
0 5 10 15 20

Days
0 4 8 12

LS
MS
B5
SH