Title: METHOD FOR ISOLATING CHLOROCENIC ACID AND 1,4-DI-O-CAFFEYOYL-D-(+)-QUINIC ACID

Abstract: Disclosed is a method for isolating chlorogenic acid and 1,4-D-O-cafeoyl-D-(+)-quinic acid by suspension-culturing embryogenic cells of Eleutheroceoccus senticosus Max in a liquid medium for somatic embryogenesis to prepare an Eleutheroceoccus senticosus Max seedling, producing the seedling on a large scale through continuous culture, and isolating chlorogenic acid and 1,4-D-O-cafeoyl-D-(+)-quinic acid from the extract of the Eleutheroceoccus senticosus Max. Consequently, the extract of the Eleutheroceoccus senticosus Max seedling. According to the present invention, the extract of the Eleutheroceoccus senticosus Max seedling and the compounds of the Formulas I and II are excellent in anti-oxidation and anti-liperoxidation activities and harmless to the human body because they are derived from Eleutheroceoccus senticosus Max seedling obtained by the present invention method is applicable to various additives for beverages, cosmetics, tea stuff and foods as well as medical substances.
METHOD FOR ISOLATING CHLOROGENIC ACID AND 1,4-D-O-CAFFEOLYL-D-(+)-QUINIC ACID

BACKGROUND OF THE INVENTION

5 Field of the Invention

The present invention relates to a method for isolating chlorogenic acid and 1,4-D-O-caffeoyl-D-(+)-quinic acid by suspension-culturing embryogenic cells of Eleutherococcus senticosus Max in a liquid medium for somatic embryogenesis to prepare an Eleutherococcus senticosus Max seedling, producing the seedling on a large scale through continuous culture, and isolating chlorogenic acid and 1,4-D-O-caffeoyl-D-(+)-quinic acid from the extract of the Eleutherococcus senticosus Max seedling. More particularly, the invention relates to a composition for medical substances, cosmetics and food additives that contains the extract of the Eleutherococcus senticosus Max seedling.

15 Description of the Prior Art

Eleutherococcus senticosus Max stands high in valuation for medicinal purposes. Many studies have been made on the ingredients of the natural Eleutherococcus senticosus Max in many countries including Russia and found out that Eleutherococcus senticosus Max has a great deal of medicinal benefits. Namely, Eleutherococcus senticosus Max proves efficacious as wild ginseng owing to its valuable ingredients such as β-sitosterol, eleutheroside A-G, and stigmasterol as well as aconthic acid whose anti-inflammatory activity is five times stronger than aspirin. More specifically, the medicinal benefits of Eleutherococcus senticosus Max are proved in the improvement of stamina, the prolongation of life, the maintenance of health condition, anti-inflammatory, analgesic and antipyretic activities, and the treatment of hypertension, diabetes, cancer and neuralgia. In particular, Eleutherococcus senticosus Max is a specific remedy marvelously effective
for fatigue as published in Russia and frequently used in the meals of young students as well as athletes and metal workers (See. “Cultivation and Use of *Eleutherococcus Senticosus* Max”, Cho, Sun-Haeng). In Korea, *Eleutherococcus senticosus* Max was first discovered in Duk-Yoo mountain by the team of the medical botany research in the Honam Agricultural Experimental Station in 1992 and known to be growing naturally in a remote mountain that is more than 1,000 meters above sea level. The species of *Eleutherococcus senticosus* Max is endangered because of over-timbering and now designated as a preserved species. The Korean *Eleutherococcus senticosus* Max is reported to contain physiologically active eleutheroside E 1.7 to 5.5 times as much as that of *Acanthopanax chisanense* including *Acanthopanax seoulense*, *Acanthopanax sieboldianum* and *Acanthopanax koreanum* (Park, Ho-Ki, Doctorial Thesis of Cheon-Buk National University, Korea, 1997). However, *Eleutherococcus senticosus* Max is very difficult to cultivate in general Korean farm because of the inappropriate weather conditions for seed formation and cutting. Furthermore, *Eleutherococcus senticosus* Max, which is a shrub, requires a relative long cultivation term in order to obtain root bark or the like for medicinal use purposes and hardly survives after removal of the bark. Thus *Eleutherococcus senticosus* Max cannot be produced in a repeating manner.

Recently, the ginseng has been successfully cultured on a large scale in a culture tube to extract saponin and thereby commercialize the culture as a dry powdery healthful food without a separate processing. Also, many companies have attempted to culture *Taxus baccata* Pendula Cell of *Eleutherococcus senticosus* Max and extract the anti-cancer component, taxol.

The inventors found that the suspension culture of the embryogenic cells of *Eleutherococcus senticosus* Max can be conducted in an MS medium to produce an *Eleutherococcus senticosus* Max seedling (Korean Patent Application No. 99-4108) and
that the somatic cells thus obtained can be cultured in a bio-reactor for about 10 days to produce *Eleutherococcus senticosus* Max seedlings with sturdy seed leaves and roots, which seedlings are now commercially available as a healthful and medicinal food. The inventors have contrived the present invention knowing that the effective component isolated from the seedling extract has anti-oxidation and anti-lipoperoxidation activities.

Since it is demonstrated that the ageing and geriatric diseases result from active oxygen species, many researchers have attempted to obtain an anti-oxidant that is known to regulate active oxygen species. More specifically, such studies have been made on anti-oxidative enzymes such as superoxide dismutase, peroxidase, catalase or glutathione, and naturally occurring low molecular weight anti-oxidants such as tocopherol, ascorbate or carotenoid. However, some reports reveal that the consumers tend to avoid synthetic anti-oxidants (Branen, 1975) and that the use of synthetic anti-oxidants causes cancer. Furthermore, the natural anti-oxidants those have been developed and reported are inferior in efficacy and expense to synthetic anti-oxidants. So, there is an increasing need of a novel natural anti-oxidant excellent in pharmacological efficacy and harmless to the human body.

**SUMMARY OF THE INVENTION**

It is, therefore, an object of the present invention to provide an anti-oxidant or an anti-lipoperoxidant containing an extract of the *Eleutherococcus senticosus* Max seedling, which is obtained by preparing the extract of the *Eleutherococcus senticosus* Max through continuous culture on a large scale, isolating a fraction with high anti-oxidation activity from the extract, and isolating and identifying a compound with high pharmacological activity from the fraction, the extract containing the compound isolated and identified.

To achieve the above object of the present invention, there is provided a novel
compound prepared by a method, in which the embryogenic cells of *Eleutherococcus senticosus* Max is suspension-cultured in an MS medium to produce an *Eleutherococcus senticosus* Max seedling (Korean Patent Application No. 99-4108) and the somatic cells thus obtained are cultured in a bio-reactor for about 10 days to produce *Eleutherococcus senticosus* Max seedlings with sturdy seed leaves and roots, which seedlings are subjected to extraction with an organic solvent such as distilled water, methanol or ethanol and continuous fractionation with various solvents to obtain the extract fraction containing the novel compound with high pharmacological activities.

The continuous fractionation method as used in the present invention in order to obtain an extract fraction comprises the steps of: (1) adding distilled water and hexane to an extract containing distilled water and alcohol so as to obtain a water fraction and a n-hexane fraction; (2) adding ethyl acetate to the water fraction to obtain a second water fraction and an ethyl acetate fraction; and (3) adding n-butanol to the second water fraction to finally obtain a third water fraction and a butanol fraction.

Subsequently, the individual fractions obtained by the continuous fractionation method, i.e., hexane fraction, ethyl acetate fraction, butanol fraction and water fraction are analyzed in regard to anti-oxidation and anti-lipoperoxidation activities caused by the fractionation in order to select a fraction with the highest activities. According to the present invention, the ethyl acetate fraction and the butanol fraction are superior in both anti-oxidation and anti-lipoperoxidation activities to the other fractions, as shown in Table 1.

The ethyl acetate fraction and the butanol fraction that show relatively high anti-oxidation and anti-lipoperoxidation activities are sequentially subjected to a silica gel column chromatography using a mixed solvent of chloroform and methanol as a developing solvent to obtain two active fractions, which are then purified on an ODS
(octadecylsilylated, C18) and Sephadex LH-20 column chromatography using a mixture of distilled water and methanol as a developing solvent.

The two isolated compounds that exhibit high anti-oxidation and anti-lipoperoxidation activities are chlorogenic acid which is a phenylpropanoid compound represented by the Formula I, and 1,4-D-O-caffeoyl-D-(+)-quinic acid as a novel compound represented by the following Formula II:

![Formula I](image1)

![Formula II](image2)

The compounds represented by the Formulas I and II are soluble in both ethanol and methanol, especially, in water. The compound represented by the Formula I is a pale powder of which the molecular weight is 354[M]+ and the Rf value is 0.60 on the thin layer chromatograph (TLC) using ODS (RP-18F254s, Merck Inc.) as an adsorbent and
a mixed solvent of methanol and distilled water (4:6) as a developing solvent. The compound represented by the Formula II is a light yellow powder that has a molecular weight of 516[M]+, an optical rotation of $[\alpha]_D^{22} +67$ and a mass spectrum (FAB-MS, m/z) as shown in FIG. 8. The Rf value of this compound represented by the Formula II is 0.32 on the thin layer chromatograph (TLC) using silica gel (Kieselgel 60F$_{254}$, Merck Inc.) as an adsorbent and a mixed solvent of ethyl acetate, methanol and distilled water (7:2:7:0.3) as a developing solvent.


The anti-oxidation activity of the phenylpropanoid compounds was measured by a method using a free radical, 1,1-diphenyl-2-picrylhydrazyl (hereinafter, referred to as “DPPH”) (See. Blois, Nature, 188:1199, 1958; and Choi, et al., Kor. J. Pharmacogn,

As a result, the phenylpropanoid compounds of the present invention showed high efficacies in both anti-oxidation and anti-lipoperoxidation activities.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a $^1$H-NMR spectrum of the compound represented by the Formula I (i.e., chlorogenic acid) isolated from the extract of an *Eleutherococcus senticosus* Max seedling;

FIG. 2 is a $^{13}$C-NMR spectrum of the compound represented by the Formula I;

FIG. 3 is a $^1$H-NMR spectrum of the compound represented by the Formula II (i.e., 1,4-D-O-caffeoyl-D-(+)-quinic acid) isolated from the extract of an *Eleutherococcus senticosus* Max seedling;

FIG. 4 is a $^{13}$C-NMR spectrum of the compound represented by the Formula II;

FIG. 5 is a HMBC spectrum of the compound represented by the Formula II;

FIG. 6 is a COSY spectrum of the compound represented by the Formula II;

FIG. 7 is a DEPT spectrum of the compound represented by the Formula II;

FIG. 8 is a mass spectrum of the compound represented by the Formula II; and

FIG. 9 is shows the measurement results of the compound represented by the Formula II in regard to the optical rotation.

**DETAILED DESCRIPTION OF THE INVENTION**

Hereinafter, the present invention will be described in detail by way of the following examples and experimental examples, which are not intended to limit the scope of the present invention. Accordingly, the derivatives including inorganic or organic
salts and esters of the present invention compounds, and isomers thereof are equivalents of the present invention compounds and included in the scope of the present invention.

**EXAMPLE 1:** Organic solvent extracts of the extract of an *Eleutherococcus senticosus* Max seedling and pharmacological activity of the same

The extract of an *Eleutherococcus senticosus* Max seedling was fractioned with a proper solvent to measure the pharmacological activities. First, 113 g of the *Eleutherococcus senticosus* Max seedling dried in the shadow was pulverized with a homogenizer (PH-91, AMT Inc.) and then subjected to cold maceration with 100% methanol for one week to obtain 48.4 g of a methanol extract. The methanol extract was mixed with 952 ml of distilled water and 1000 ml of hexane, and separated into a water fraction and a hexane fraction. The water fraction was mixed with 1000 ml of ethyl acetate and separated into an ethyl acetate fraction and a second water fraction, which was then mixed with 500 ml of butanol and finally separated into a butanol fraction and a third water fraction. After concentration under vacuum, there were obtained 1.072 g of the hexane fraction, 2.184 g of the ethyl acetate fraction, 4.326 g of the butanol fraction and 35.9 g of the water fraction.

The individual fractions thus obtained were analyzed in regard to the anti-oxidation and anti-lipoperoxidation activities according to the same procedures as described in Examples 4 and 5, which will be described later. The results are presented in Table 1. The unit of the anti-oxidation activity, $\text{RC}_{50}(\mu g/mL)$ is the concentration of the fraction that reduces the concentration of the control using DPPH by 50%.

<TABLE 1>
Pharmacological Activities of *Eleutherococcus senticosus* Max Seedling Extract by Solvent Fractions.

<table>
<thead>
<tr>
<th>Solvent Fraction</th>
<th>Extract (g)</th>
<th>Anti-oxidation Activity (RC$_{50}$, µg/mL)</th>
<th>Anti-lipoperoxidation Activity (RC$_{50}$, µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane Fraction</td>
<td>1.072</td>
<td>&gt; 50</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Ethyl Acetate Fraction</td>
<td>2.184</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Butanol Fraction</td>
<td>4.326</td>
<td>6</td>
<td>49</td>
</tr>
<tr>
<td>Water Fraction</td>
<td>35.9</td>
<td>12</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

As is apparent from the results of Table 1, the ethyl acetate fraction and the butanol fraction showed high pharmacological activities.

EXAMPLE 2: Isolation and purification of active substance from *Eleutherococcus senticosus* Max seedling extract.

Among the solvent fractions obtained from the *Eleutherococcus senticosus* Max seedling extract in Example 1, the fractions of high anti-oxidation activity were isolated and purified to obtain active substances. First, 4 g of the butanol fraction was dissolved in methanol and then added to a glass column (ϕ 5 x 70 cm, 7734 Merck Inc.) filled with silica gel so as to elute the active substance with a mixed solvent of toluene, acetone and methanol. This procedure yielded 0.7 g of a fraction having an anti-oxidation activity. Subsequently, the active fraction obtained by the primary silica gel column chromatography was purified on an intermediate pressure column in the following procedures. First, ODS was filled in a glass column (ϕ 1.6 x 100 cm) and the active substance was eluted with a concentration gradient ranging from 10% methanol to 95% methanol using an HPLC pump, thus obtaining 238 mg of the active fraction. The active fraction thus obtained was added to a glass column (ϕ 1.0 x 50 cm) filled with sephadex (Sephadex LH-20, Pharmacia Biotech Inc.) and eluted with 60% methanol to obtain 106 mg of the compound represented by the Formula I.

Subsequently, another active substance was isolated and purified from the ethyl
acetate fraction with high anti-oxidation activity according to the following procedures.
First, 2.1 g of the ethyl acetate fraction was dissolved in methanol and then added to a
glass column (ϕ 3 x 70 cm, 7734 Merck Inc.) filled with silica gel so as to elute the active
substance with a mixed solvent of ethyl acetate, methanol and distilled water (7 : 2.7 : 0.3).
This procedure yielded 187 mg of a fraction having an anti-oxidation activity.
Subsequently, the active fraction obtained by the primary silica gel column
chromatography was purified on a glass column (ϕ 1.0 x 50 cm) filled with sephadex
(Sephadex LH-20, Pharmacia Biotech Inc.) and eluted with 40% methanol to obtain 66 mg
of the active fraction. This active fraction was then finally purified by the HPLC
according to the following procedures. First, the active fraction was added to an HPLC
(LC-10A Series, Shimadzu) with an ODS-C18 column (column ϕ 10 x 250 mm, YMC
Inc.) and eluted with 50% methanol containing 0.1% acetic acid at UV 285 nm and 1.5
ml/min to obtain 28 mg of the compound represented by the Formula II. The contents of
the compounds obtained from the Eleutherococcus senticosus Max seedling extract were
measured as 0.051% and 0.014%, respectively, but actually higher than these values.

The compounds represented by the Formulas I and II as obtained on the column
chromatography were analyzed in regard to anti-oxidation and anti-lipoperoxidation
activities according to the procedures as described in Examples 4 and 5. The results are
presented in Table 2.
<TABLE 2>

Comparison of Anti-oxidation and Anti-lipoperoxidation Activities between Two Compounds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Anti-oxidation Activity (RC50: μg/ml)</th>
<th>Anti-lipoperoxidation Activity (RC50: μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>12</td>
<td>98</td>
</tr>
<tr>
<td>Compound of Formula I</td>
<td>1.2</td>
<td>8.2</td>
</tr>
<tr>
<td>Compound of Formula II</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>12</td>
<td>5.2</td>
</tr>
</tbody>
</table>

**EXAMPLE 3**: NMR identification of compounds of Formulas I and II

The compound of the Formulas I and II were analyzed by the hydrogen and carbon nuclear magnetic resonance (NMR) spectroscopy according to the following procedures.

The compound of the Formula I was subjected to the $^1$H-NMR spectroscopy using deuterium methanol (MeOH- $d_4$) as a solvent and tetramethylsilane (TMS) as a standard material. The results are presented in FIG. 1.

$^1$H-NMR(400MHz, MeOH-$d_4$) ppm: δ 7.67(1H, d, J=15.9, H-7'), 7.05(1H, d, J=2.0, H-2'), 6.94(1H, dd, J=8.2, 2.0, H-6'), 6.78(1H, d, J=8.2, H-5'), 6.29(1H, d, J=15.9, H-8'), 5.38(1H, d, dd, J=11.2, 6.2, 5.0, H-5), 4.11(1H, dd, J=6.2, 3.0 H-3), 3.67(1H, dd, J=10.0, 3.7, H-6), 2.15(1H, dd, J=14.7, 3.1, H-2), 2.07(1H, dd, J=14.7, 2.1, H-6ax), 2.01(1H, dd, J=14.7, 3.1, H-2eq).

The compound of the Formula I was also subjected to the $^{13}$C-NMR spectroscopy using deuterium methanol (MeOH- $d_4$) as a solvent and tetramethylsilane (TMS) as a standard material. The results are presented in FIG. 2.

$^{13}$C-NMR(400MHz, MeOH-$d_4$) ppm: 181.0(s, C-7), 169.2(s, C-9'), 149.5(s, C-4'), 146.9(d, C-7', 3'), 127.9(s, C-1'), 123.0(s, C-6'), 116.6(d, C-8'), 115.6(d, C-2'), 115.2(d, C-5'), 77.8(s, C-1), 75.3(d, C-4), 73.2(d, C-5), 72.7(d, C-3), 40.7(C-6), 39.3(C-2).

The compound of the Formula II was subjected to the $^1$H-NMR spectroscopy
using deuterium methanol (MeOH-\textsubscript{d4}) as a solvent and tetramethylsilane (TMS) as a standard material. The results are presented in FIG. 3.

$^1$H-NMR(500MHz, MeOH-\textsubscript{d4}) ppm: 8 7.59(1H, d, J=15.9Mz, H-7\textsuperscript{"}), 7.56(1H, d, J=15.9Mz, H-7\textsuperscript{'}), 7.02(2H, s, H-2\textsuperscript{"}, 2\textsuperscript{'}), 6.94(1H, dd, J=8.0, 2.0Hz, H-6\textsuperscript{"}), 6.93(1H, dd, J=8.0, 2.0Hz, H-6\textsuperscript{'}), 6.78(1H, d, J=8.1Mz, H-5\textsuperscript{"}), 6.76(1H, d, J=8.1Mz, H-5\textsuperscript{'}), 6.33(1H, d, J=15.9Mz, H-8\textsuperscript{"}), 6.30(1H, d, J=15.9Mz, H-8\textsuperscript{'}), 5.44(1H, ddd, J=4.3, 3.0, 4.3Mz, H-3), 4.22(1H, ddd, J=9.6, 3.4, 4.3Mz, H-5), 3.71(1H, dd, J=3.5, 9.5Mz, H-4), 2.69(2H, m, H-2eq, 6eq), 2.25(1H, dd, J=3.5, 15.6Mz, H-6ax), 2.0(1H, m, H-2ax).

The compound of the Formula II was also subjected to the $^{13}$C-NMR spectroscopy using deuterium methanol (MeOH-\textsubscript{d4}) as a solvent and tetramethylsilane (TMS) as a standard material. The results are presented in FIG. 4.

$^{13}$C-NMR(500MHz, MeOH-\textsubscript{d4}) ppm: 180.3(s, COOH), 169.2(s, C-9\textsuperscript{"}), 168.4(s, C-9\textsuperscript{'}), 149.8(s, C-4\textsuperscript{"}), 149.4(s, C-4\textsuperscript{'}), 146.9(d, C-3\textsuperscript{"}, 3\textsuperscript{'}), 146.8(s, C-7\textsuperscript{"}), 146.2(s, C-7\textsuperscript{'}), 128.3(s, C-1\textsuperscript{"}), 127.9(s, C-1\textsuperscript{'}), 122.9(s, C-6\textsuperscript{"}), 122.8(s, C-6\textsuperscript{'}), 117.0(s, C-5\textsuperscript{"}), 116.6(s, C-5\textsuperscript{'}), 115.6(d, C-2\textsuperscript{"}, 2\textsuperscript{'}), 115.2(s, C-8\textsuperscript{"}), 115.1(s, C-8\textsuperscript{'}), 84.1(s, C-1), 74.7(s, C-4), 71.9(s, C-5), 71.2(s, C-3), 38.5(s, C-2), 36.9(s, C-6).

The HMBC(Heteronuclear Multiple Bond Connectivity), COSY(Correlated Spectroscopy) and DEPT(Distortionless Enhancement through Polarization Transfer) spectrum of the compound represented by the Formula II were represented in FIG. 5, 6 and 7, respectively.

**EXAMPLE 4:** Evaluation of anti-oxidation activity

The compounds of the Formulas I and II were evaluated in regard to the anti-oxidation activity by a measurement method using DPPH. First, 4 ml of methanol was added to a glass test tube and mixed with the individual samples varying in concentration ranging from 0.1 to 30 \textmu l. After adding 1 ml of the 0.15 mM DPPH solution, the
mixture was kept at the room temperature for 30 minutes and measured in regard to the absorbance at 517 nm. Here, the unit of the anti-oxidation activity, RC<sub>50</sub> (µg/ml) is the concentration of the compound that reduces the concentration of the control by 50%.

As seen from the results of FIG. 2, the compounds of the Formula I and II exhibited a very high anti-oxidation activity. The α-tocopherol was used as a control.

**EXAMPLE 5: Evaluation of anti-lipoperoxidation activity**

The compounds of the Formulas I and II were evaluated in regard to the anti-lipoperoxidation activity with the microsomes derived from the liver of the mouse (See. Ohkawa, et al., Anal. Biochem., 95:351, 1979). First, the microsomes were isolated from the liver of the mouse and suspended in 100 ml of a Tris-HCl buffer solution (pH 7.4). 0.3% of the microsome fraction was mixed with the individual compound samples varying in concentration. After adding a 500 µM FeSO<sub>4</sub>7H<sub>2</sub>O, the mixture was incubated at 37 °C for 30 minutes and mixed with 20% TCA (3M trichloroacetic acid: 2.5M ammonium chloride = 1:1) to suspend the reaction. Meanwhile, malondialdehyde (MDA) produced depending on the concentration of the compound was reacted with thiobarbituric acid (TBA). The production yield of this TBA reaction can be measured to obtain the anti-lipoperoxidation activity (%) according to the Equation 1:

<Equation 1>

\[
\text{Anti-lipoperoxidation activity (\%)} = 1 - \frac{(T-B)}{C-B} \times 100
\]

Here, T is the absorbance (at 530 nm) of the sample causing peroxidation with the compound added; C is the absorbance (at 530 nm) of the sample causing peroxidation without the compound added; and B is the absorbance (at 530 nm) of the control not causing peroxidation.

As is apparent from the results of Table 2, the compounds of the Formula I and II showed the anti-lipoperoxidation activity. Particularly, the compound of the Formula II
was very excellent in the anti-lipoperoxidation activity. The α-tocopherol was used as a control.

According to the present invention, the extract of the *Eleutherococcus senticosus* Max seedling and the compounds of the Formulas I and II are excellent in anti-oxidation and anti-lipoperoxidation activities and harmless to the human body because they are derived from *Eleutherococcus senticosus* Max. Consequently, the extract of the *Eleutherococcus senticosus* Max seedling obtained by the present invention method is applicable to various additives for beverages, cosmetics, tea stuff and foods as well as medicines.
WHAT IS CLAIMED IS:

1. A composition for medical substances, cosmetics and food additives, containing an extract comprising as an effective component a compound represented by the Formula I and/or II and extracted from an Eleutherococcus senticosus Max seedling:

   ![Chemical Structure](image)

2. 1,4-D-O-caffeoyl-D-(+)-quinic acid represented by the Formula II and isolated from the extract of Eleutherococcus senticosus Max according to claim 1.

3. A method for isolating a compound represented by the Formula II, in which a pharmacological active substance is extracted from an extract of Eleutherococcus senticosus Max, the method comprising the steps of:

   (a) adding water and hexane to the extract to obtain a hexane fraction;

   (b) adding ethyl acetate to the water fraction to obtain a second water fraction and an ethyl acetate fraction;

   (c) adding butanol to the second water fraction to obtain a third water fraction and a butanol fraction; and

   (d) isolating the compound of the Formula II from the ethyl acetate fraction and the butanol fraction by a chromatography.
4. The method of claim 3, wherein the chromatography includes a series of a silica gel column chromatography and an intermediate pressure column chromatography comprising Sephadex-filled column chromatography and ODS-filled column chromatography.

5. The method as claimed in claim 3, further comprising the steps of:
   
   (e) dissolving the ethyl acetate fraction in methanol and sequentially eluting the active substance of the ethyl acetate fraction on a glass column filled with silica gel using a mixed solvent of ethyl acetate, methanol and distilled water to obtain an active fraction;

   (f) eluting the active fraction on a glass column filled with Sephadex using 40% methanol to obtain a second active fraction; and

   (g) eluting the second active fraction on a high performance liquid chromatograph (HPLC) equipped with an ODS-C18 column using 50% methanol containing 0.1% acetic acid at a UV wavelength of 285 nm and a flux of 1.5 ml/min to obtain the compound represented by the Formula II.
Fig. 6
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<td>+0.0001deg</td>
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<td>RSD</td>
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Fig. 9
# INTERNATIONAL SEARCH REPORT

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC** A61K 31/21

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/21, A61K 35/78, A23L 1/29, A61K 7/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, NPS, PAJ, CA on line

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>WO 9426282 A1 (Omega Pharmaceuticals, Inc., USA) 24 November 1994 (24. 11. 94), abstract; claims</td>
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<td>X</td>
<td>Sugiyara, Haruo et al. Effects of aqueous extracts from Eleutheroceus on the oxidative enzyme activities in mouse skeletal muscle. In: Gifu Yakka Daigaku Kio (38), 38-48, 1989, see entire document</td>
<td>1</td>
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</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  
  "A" document defining the general state of the art which is not considered to be of particular relevance
  
  "E" earlier application or patent but published on or after the international filing date
  
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  "O" document referring to an oral disclosure, use, exhibition or other means
  
  "P" document published prior to the international filing date but later than the priority date claimed

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  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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  "&" document member of the same patent family

Date of the actual completion of the international search
23 NOVEMBER 2000 (23.11.2000)

Date of mailing of the international search report
28 NOVEMBER 2000 (28.11.2000)

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Telephone No. 82-42-481-5604

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