Title: LOWER ALCOHOL INSOLUBLE EXTRACT OF THE YOUNG BRANCH OF HOVENIA DULCIS THUNB., POLYSACCHARIDES ISOLATED THEREFROM AND AN ANTIPATHOTOXIC COMPOSITION CONTAINING SAME

1~4 year-old young branch (1,000 g)

hot water extract (HDBRHW) (12.2 g, 1.2%)

HPLC-degrade MeOH

MeOH insoluble fraction (HDBRHW-1) (11.57 g, 1.1%)

MeOH soluble fraction (HDBRHW-2) (0.63 g, 0.06%)

Abstract: A pharmaceutical composition and health care food comprise a lower alcohol-insoluble extract of the young branch of *Hovenia dulcis* var. or a polysaccharide isolated therefrom having potent atoprotective and antihepatotoxicity activities.
LOWER ALCOHOL INSOLUBLE EXTRACT OF THE YOUNG BRANCH OF HOVENIA DULCIS THUNB., POLYSACCHARIDES ISOLATED THEREFROM AND AN ANTIHEPATOTOXIC COMPOSITION CONTAINING SAME

FIELD OF THE INVENTION

The present invention relates to a lower alcohol-insoluble extract of the young branch of *Hovenia dulcis* Thunb., polysaccharides isolated therefrom having hepatoprotective activity, and an antihepatotoxic composition containing same.

BACKGROUND OF THE INVENTION

Hepatitis afflicts an increasing number of the world population, and due to the lack of therapeutically effective drugs, it usually progresses to chronic hepatitis, liver cirrhosis or cancer. Various types of hepatitis may develop when a person is exposed to, e.g., stress, excessive consumption of alcohol, and hepatotoxic substances.

Exemplary hepatotoxic substances are carbon tetrachloride (CCl₄), D-galactosamine, lipopolysaccharide (LPS), bromobenzene and aldehydes such as acetaldehyde which is an intermediate in the metabolic pathway of alcohol. Accordingly, there have been many attempts to find new drugs which can protect the liver from such hepatotoxic substances or restore the liver function damaged thereby.

For example, triterpene glycoside components isolated from the seed and fruit of *Hovenia dulcis* Thunb. are known to inhibit the release of histamine and the absorption of alcohol in human body (Yoshikawa, K. T. et al., *Chem. Pharm. Bull. Tokyo* 43(3):532-534, 1995); and the fruit of *Hovenia dulcis* Thunb. have been found to inhibit liver damage inducible carbon tetrachloride or D-galactosamine/lipopolysaccharide (Hase K. et al., *Chem. Pharm. Bull. Tokyo* 20(4):381-385, 1997). The above and other prior art literatures describe pharmacological activities of an extracts of the fruit or seed of *Hovenia dulcis* Thunb. However, there have been no reports directed to the use of an extract of the young branch of *Hovenia dulcis* Thunb. as a source of new pharmacologically active agents.

*Hovenia dulcis* Thunb. is a rare deciduous plant species belonging to the Rhamnaceae family and is indigenous to Korea, distributed in the southern parts of Kangwon Province. *Hovenia dulcis* Thunb. var. *koreana* NAKAI is distinctly different from *Hovenia dulcis* Thunb. var *tomentella* Makino and *Hovenia dulcis* Thunb
in that while both the Chinese and Japanese species exhibit bright green petals, the petal of the Korean species is white, and also in that the size of fruit-peduncle and the seed shape are not the same. Therefore, *Hovenia dulcis* Thunb. var. Koreana NAKAI has been classified as a different species (Uehara K. I., (1960) *JUMOKUDAITSUSETSU*, Yumei Press. 2nd Ed. pp1072-1074), and its fruit and seed have been used for quenching thirst and treating nausea (Chung, B. S. et al., (1998) *DOHAEHYANGYAKDAISAJEON (Plant part)*, Youngrim Press, pp291-292).

**SUMMARY OF THE INVENTION**

Accordingly, it is an object of the present invention to provide a pharmacologically active substance extracted from the young branch of *Hovenia dulcis* Thunb.

Another object of the present invention is to provide a method for isolating said substance from the young branch of *Hovenia dulcis* Thunb.

An additional object of the present invention is to provide a pharmaceutical composition for inhibiting alcohol dehydrogenase and lactic acid dehydrogenase, comprising a pharmaceutically acceptable carrier and the above-described active substance.

A further object of the present invention is to provide a pharmaceutical composition for preventing or inhibiting a liver disease, comprising a pharmacologically acceptable carrier and the above-described active substance.

A still further object of the present invention is to provide a health care food comprising the above-described active substance.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The above and other objects and features of the present invention will become apparent from the following description of the invention, when taken in conjunction with the accompanying drawings, which respectively show:

Fig. 1: a schematic procedure for preparing a methanol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb.;

Fig. 2: UV and RI scans of polysaccharide fractions isolated from the methanol-insoluble fraction when subjected to an ion exchange column chromatography;

Figs. 3 to 7: SEC peaks and MALLS spectra of polysaccharide fractions 1 to 5, respectively, to measure respective molecular weight;
Figs. 8 to 12: a GC spectrum of polysaccharide fractions 1 to 5, respectively, to analyze sugar chains thereof;

Fig. 13: LDH release inhibitory activity of the methanol-insoluble fraction on the liver tissue damaged by carbon tetrachloride;

1: control (no treatment)
2: CCl₄ 4 mM
3: CCl₄ 4 mM + methanol-insoluble fraction 200 µg/ml
4: CCl₄ 4 mM + methanol-soluble fraction 200 µg/ml

Fig. 14: \textit{in vitro} protein synthesis activity of the methanol-insoluble fraction on the liver tissue damaged by carbon tetrachloride;

1: control (no treatment)
2: CCl₄ 4 mM
3: CCl₄ 4 mM + methanol-insoluble fraction 200 µg/ml
4: CCl₄ 4 mM + methanol-soluble fraction 200 µg/ml

Fig. 15: LDH release inhibitory activity of the methanol-insoluble fraction on the liver tissue damaged by galactosamine/LPS;

1: control (no treatment)
2: D-galactosamine 4 mM
3: D-galactosamine 4 mM + methanol-insoluble fraction 200 µg/ml
4: D-galactosamine 4 mM + methanol-soluble fraction 200 µg/ml

Fig. 16: \textit{in vitro} protein synthesis activity of the methanol-insoluble fraction on the liver tissue damaged by galactosamine/LPS;

1: control (no treatment)
2: D-galactosamine 4 mM
3: D-galactosamine 4 mM + methanol-insoluble fraction 200 µg/ml
4: D-galactosamine 4 mM + methanol-soluble fraction 200 µg/ml

Fig. 17: LDH release inhibitory activity of the methanol-insoluble fraction on the liver tissue damaged by bromobenzene;

1: control (no treatment)
2: bromobenzene 4 mM
3: bromobenzene 4 mM + methanol-insoluble fraction 200 µg/ml
4: bromobenzene 4 mM + methanol-soluble fraction 200 µg/ml

Fig. 18: \textit{in vitro} protein synthesis activity of the methanol-insoluble fraction on the liver tissue damaged by bromobenzene;

1: control (no treatment)
2: bromobenzene 4 mM
3: bromobenzene 4 mM + methanol-insoluble fraction 200 µg/ml
4: bromobenzene 4 mM + methanol-soluble fraction 200 μg/ml

Fig. 19: blood alcohol concentration-lowering activity of the methanol-insoluble fraction;

Fig. 20: alcohol dehydrogenase inhibitory activity of the methanol-insoluble fraction; and

Figs. 21 to 25: in vitro protein synthesis activity of polysaccharide fractions 1 to 5 isolated from the methanol-insoluble fraction on the liver tissue damaged by carbon tetrachloride.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a lower alcohol-insoluble fraction obtained from the young branch of Hovenia dulcis Thunb. having hepatoprotective and antihepatotoxic activities, which is prepared by the steps of: extracting the dried young branch of Hovenia dulcis Thunb. with hot-water under high pressure to obtain a hot-water extract; and treating the hot-water extract with a lower alcohol to obtain a lower alcohol insoluble fraction.

In accordance with another aspect of the present invention, there is also provided polysaccharide fractions having potent hepatoprotective activity, which is isolated from said lower alcohol-insoluble fraction.

The inventive lower alcohol-insoluble fraction of the young branch of Hovenia dulcis Thunb. can be prepared by a simple procedure. First, a hot-water extract of the dried young branch of Hovenia dulcis Thunb. is dried and obtained using a high pressure extraction procedure, and the hot-water extract thus obtained is treated with a lower alcohol to obtain said fraction. Preferably, Hovenia dulcis var. latifolia Nakai, Hovenia dulcis var. tomentella Makino or Hovenia dulcis var. latifolia Nakai can be used in the present invention. It is preferable to use one to four-year old of Hovenia dulcis Thunb.

For example, the young branch of Hovenia dulcis Thunb. is sliced and dried in shades. Then, an appropriate amount of water ranging from 1 to 5 fold, preferably about 3 fold volume of young branch slices is added to the dried young branch slices and the mixture is kept at a temperature ranging from 100 to 150°C, preferably 100 to 120°C, under a pressure ranging from 1 to 3 atm., preferably 1.5 atm., for a period ranging from 15 min to 48 hrs, preferably 30 min to 12 hrs. Then the mixture is cooled to room temperature, filtered and the filtrate is lyophilized pursuant to a conventional lyophilizing method, to obtain a hot-water extract.

In the second step, the hot-water extract is further dried at room temperature,
evaporated under a reduced pressure, and subjected to three or four cycles of reflux extraction with a lower alcohol, e.g., methanol, ethanol, butanol or a mixture thereof, preferably 100% methanol, for 30 min to 3 hrs, preferably about 1 hr. The reflux extracts are combined and centrifuged to remove lower alcohol-soluble components therefrom, to obtain the intended lower alcohol-insoluble fraction. Further, the reflux extracts are filtrated, and the unfilterable insoluble fraction is extracted with an appropriate amount of a lower alcohol, e.g., methanol, ethanol, butanol or a mixture thereof, preferably 100% ethanol, ranging from 2 to 8 fold, preferably 4 fold volume of the insoluble fraction and the resulting precipitate was isolated. The precipitate is dissolved in distilled water and lyophilized pursuant to a conventional lyophilizing method, to obtain a lower alcohol-insoluble extract.

The lower alcohol-insoluble fraction contains polysaccharides having high hepatoprotective activity, which can be isolated by the following procedure.

The lower alcohol-insoluble fraction is dissolved in distilled water, charged to an ion exchange column, eluted stepwise using solutions having increasing NaCl concentrations from 0 to 5 M, dialyzed, concentrated, and lyophilized. In carrying out the ion exchange chromatography, either a cation exchange resin or an anion exchange resin may be used. Examples of ion exchange resins that can be used for this purpose are: strong acidic cation exchange resins such as AG 50W-x8, Amberlite IR-120, and Dowex 50W-x8; weak acidic cation exchange resins such as Amberlite IRC-50, Bio-Rex 70, Duolite-436; weak basic cation exchange resins such as Amberlite IRA-67, and Dowex 3-x4A; strong basic cation exchange resins such as AG 2x8, Amberlite IRA-400, and Dowex 2-x8; modified cellulose cation exchange resins such as CM-Celluose and SE-Cellulose; and anion exchange resins such as DEAE Cellulose; cationic sephadex-type resins such as G-25 and G-50 bead type cross-linked dextran resins; and modified bead-type ion exchange resins made from agarose such as Cepharose CL, Biogel A Cepharose resin, Fractogels and Toyopearl. The preferred resins are Toyopearl DEAE type exchange resins, and the more preferred are Toyopearl DEAE-650C type exchange resins.

Several polysaccharide fractions are obtained by above isolation process according to the concentration of sodium chloride. The polysaccharide fraction eluted at 0 M NaCl has 2 peaks having molecular weights of 68,040 and 36,440 Da, respectively, as determined by MALLS and are composed of mannose, glucose, galactose, arabinose and xylose. The polysaccharide fraction eluted at 0.1 M NaCl has 3 peaks having molecular weights of 22,500, 233,300 and 200,700 Da, respectively, as determined by MALLS and are composed of mannose, glucose, rhamnose and arabinose.
The polysaccharide fraction eluted at 0.2 M NaCl has 1 peak having a molecular weight of 32,840 Da as determined by MALLS and is composed of mannose, rhamnose, arabinose and xylose. The polysaccharide fraction eluted at 0.3 M NaCl has 2 peaks having molecular weights of 88,610 and 79,190 Da, respectively, as determined by MALLS and are composed of mannose, glucose and arabinose. The polysaccharide fraction eluted at 3 M NaCl has 1 peak having a molecular weight of 216,500 Da as determined by MALLS and is composed of mannose, galactose, arabinose and xylose.

The present invention includes all polysaccharides having a molecular weight of 20 kDa or more which eluted as above.

Various experiments clearly show that the lower alcohol-insoluble fraction and the polysaccharides isolated therefrom inhibit alcohol dehydrogenase and lactate dehydrogenase activities and possess high antihepatotoxicity and hepatoprotective activities. Accordingly, the lower alcohol-insoluble fraction and the polysaccharides isolated therefrom of the present invention can be advantageously used for preventing and treating liver diseases.

Thus, the lower alcohol-insoluble fraction and the polysaccharide of the present invention may be employed as a pharmaceutical agent for preventing or treating liver toxicity and liver diseases such as hepatitis, fatty liver, liver cirrhosis, hangover and fatigue.

Accordingly, the present invention provides a pharmaceutical composition for inhibiting alcohol dehydrogenase and lactic acid dehydrogenase, which comprises the lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb. or the polysaccharide isolated therefrom as an active ingredient, in combination with pharmaceutically acceptable excipients, carriers or diluents. Additionally, the present invention provides a health care food comprising the extract or the polysaccharide described above.

Further, the present invention also provides a pharmaceutical composition for treating and preventing liver disease, which comprises the lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb. or the polysaccharide isolated therefrom as an active ingredient, in combination with pharmaceutically acceptable excipients, carriers or diluents. Additionally, the present invention provides a health care food comprising the extract or the polysaccharide described above.

The inventive pharmaceutical formulation may be prepared in accordance with any of the conventional procedures. In preparing the formulation, the active ingredient is preferably admixed or diluted with a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material acting as a vehicle, excipient or
medium for the active ingredient. Thus, the formulation may be in the form of a tablet, pill, powder, sachet, elixir, suspension, emulsion, solution, syrup, aerosol, soft and hard gelatin capsule, sterile injectable solution, sterile packaged powder and the like.

Examples of suitable carriers, excipients, or diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, alginates, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methylhydroxybenzoates, propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulation may additionally include fillers, anti-agglutinating agents, lubricating agents, wetting agents, flavoring agents, emulsifiers, preservatives and the like. The composition of the invention may be formulated so as to provide a quick, sustained or delayed release of the active ingredient after it is administrated to a patient, by employing any one of the procedures well known in the art.

The pharmaceutical formulation of the present invention can be administered via various routes including oral, transdermal, subcutaneous, intravenous and intramuscular introduction. For treating a human patient, a typical daily dose of the above-mentioned fraction or polysaccharide isolated from the young branch of *Hovenia dulcis* Thumb. may range from about 0.01 to 10 g/kg body weight, preferably 1 to 5 g/kg body weight, and can be administered in a single dose or in divided doses. However, it should be understood that the amount of the active ingredient actually administered ought to be determined in light of various relevant factors including the condition to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom; and, therefore, the above dose should not be intended to limit the scope of the invention in any way.

The above-mentioned lower alcohol-insoluble fraction or the polysaccharide isolated therefrom can be added to food or beverage for preventing various liver diseases and hangover. The amount of said fraction or polysaccharide that may be added to food or beverage for the purpose of preventing liver diseases may generally range from about 0.1 to 15 w/w %, preferably 1 to 10 w/w % based on the total weight of food, and 1 to 30 g, preferably 3 to 10 g based on 100 ml of beverage.

The health care beverage composition of the present invention may contain other components, e.g., deodorants and natural carbohydrates as in conventional beverages. Examples of such natural carbohydrates are monosaccharides such as glucose and fructose; disaccharides such as maltose and sucrose; conventional sugars such as dextrin and cyclodextrin; and sugar alcohols such as xylitol, sorbitol and erythritol. As the deodorant, a natural deodorant such as taumatin, levaudioside A, and glycyrrhizin, or a synthetic deodorant such as saccharin and aspartam may be used.
The amount of the above-described natural carbohydrate is generally in the range of about 1 to 20 g, preferably 5 to 12 g based on 100 ml of beverage.

Other components that may be added to the inventive food or beverage composition are various nutrients, vitamins, minerals, synthetic flavoring agents, coloring agents pectic acid and its salt, alginic acid and its salt, organic acids, protective colloidal adhesives, pH controlling agents, stabilizers, preservatives, glycerin, alcohol, carbonizing agents, fruit juices and vegetable juices.

The following Reference Examples, Test Examples and Formulation Examples are intended to further illustrate the present invention without limiting its scope.

Example 1: Preparation of a hot water extract of the young branch of *Hovenia dulcis* Thunb.

Dried slices (1.0 kg) of the young branch of *Hovenia dulcis* Thunb. collected at the Korean National Arboretum site located at Jikdong-ri, Sohol-myun, Pochun-kun Kyunggi-do, were extracted with 5 l of hot-water using an extractor (Habdong Machinery Co., HD5-A 190 model, Korea) at 120°C for 3 hrs under a high pressure (1.5 atm) to obtain a hot water extract. The resulting extract was filtered through a Whatman filter paper No. 2 and the filtrate (12.2 g) was lyophilized using a vacuum freeze dryer (Samwon Cold Engineering, SFDSM24L model, Korea).

Example 2: Preparation of a lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb.

(2-1) Isolation of a lower alcohol-insoluble fraction 1

The hot water extract (12.2 g) obtained in Example 1 was subjected to three cycles of reflux-extraction, each with 3 l of HPLC grade pure methanol for 1 hour. The extracts were combined and centrifuged for 10~20 min at 4,000 rpm, to obtain a methanol-soluble fraction (dry weight 0.63 g, yield: 0.06% w/w) and a methanol-insoluble fraction (dry weight 11.57 g, yield: 1.1% w/w) (Fig. 1).

(2-2) Isolation of a lower alcohol-insoluble fraction 2

The hot water extract (12.2 g) obtained in Example 1 was subjected to three cycles of reflux-extraction, each with 3 l of HPLC grade pure methanol for 1 hour.
The extracts were combined and filtered through a Whatman filter paper No. 2 to isolate the methanol-insoluble fraction from the methanol-soluble fraction. The unfiltered methanol insoluble fraction was mixed with a 4-fold volume of ethanol and the ethanol-insoluble precipitate was isolated. The precipitate was dissolved in 100 ml distilled water and lyophilized using a vacuum freeze dryer (Samwon Cold Engineering, SFDSM24L model, Korea) to obtain an ethanol insoluble fraction (dry weight 11.50 g, yield: 1.1% w/w).

**Example 3: Isolation of polysaccharide fractions having hepatoprotective activity**

To isolate hepatoprotective active compounds from the methanol-insoluble fraction obtained in Example 2, 1 g of the methanol-insoluble fraction was dissolved in 100 ml of distilled water at room temperature. 160 ml of a Toyopearl® DEAE-650C column (4.0×30 cm; Tosoh, Japan) equilibrated with desalted water was charged to a liquid chromatography column (35 cm in length, 2.5 cm in diameter, Glass Econo-column, BioRad Laboratories, USA) installed in a lower pressure chromatography system (Biologic LP, BioRad Laboratories, USA) equipped with UV (254 nm) and RI detectors (RI monitor: RI-10 model, EYELA, Japan) and the column was equilibrated with 500 ml of desalted water. 100 ml of the methanol insoluble fraction solution was loaded on the DEAE-650C resins and eluted successively with 500 ml of 0, 0.1, 0.2, 0.3 and 3 M NaCl solutions at a flow rate of 2 ml/min. As shown in Fig 2, all fractions contained components detectable by UV, but fractions eluted at 0.3 M or more of NaCl concentration were detected only by RI. To remove sodium chloride from the eluted fractions, each fraction was subjected to three cycles of dialysis using a dialysis membrane permeable at a molecular weight of 1,200 Da or below (average flat diameter 32 mm, volume 100 ml/ft, benzoyl dialysis tubing, Sigma, USA) with distilled water for 4 hrs, concentrated, and then lyophilized to obtain polysaccharide fractions.

**Example 4: Characterization of the polysaccharide fractions**

The polysaccharide fractions obtained in Example 3 by eluting with 0, 0.1, 0.2, 0.3 and 3 M NaCl were designated fractions 1 to 5, respectively. The properties of the polysaccharide fractions were analyzed as follows.

(4-1) **Determination of a molecular weight by gel permeation chromatography**

The molecular weight measurement was conducted by gel permeation
chromatography (GPC) using a HPSEC (high performance size-exclusion chromatography) apparatus, equipped with a pump (spectra system, p2000 model), a guard column (TSK PWH, Tosoh Company), an RI-detector (Shodex SE71 model), SEC (size exclusion chromatography) columns (TSK gel 3000pw, 4000pw, 5000pw (7.8×500 mm, Tosoh Company)), and a MALLS (multi angle laser light dispersion, Dawn DSP-F, Wyatt Technology Co.) detector, using a 0.02% sodium azide developing solvent containing 0.15 M NaNO₃ at a flow rate of 0.5 ml/min.

As the result shown in Figs. 2 to 7, the fraction 1 was composed of 2 peaks having the molecular weight of 68,040 and 36,440 Da, respectively; the fraction 2, 3 peaks having the molecular weight of 22,500, 233,300 and 200,700 Da, respectively; the fraction 3, 1 peak having the molecular weight of 32,840 Da; the fraction 4, 2 peaks having the molecular weight of 88,610 and 79190 Da, respectively; and the fraction 5, 1 peak having the molecular weight of 216,500 Da.

(4-2) Analysis for sugar chains

For each fraction, the total sugar content was determined by the phenol-sulfuric acid method (Dubois, M. et al.; Anal. Chem. 28:350-356, 1956), and the result thus obtained is shown in Table 1.

<Table 1>

<table>
<thead>
<tr>
<th>Constituting component</th>
<th>Amount (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction 1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>1,514</td>
</tr>
</tbody>
</table>

To examine whether the above main-peak components of fractions contained sugar chains, a methylation analysis was conducted according to the method described by Hakomori et al. (J. Biochem. Tokyo 55:205-209, 1964) and Waeghe, T. J. et al. (Carbohydrate Research 123:281-304, 1983).

500 µg of a sample was methylated, and then, the methylated product was collected using an ethanol-adsorbed C₁₈ cartridge column (Sep-Pak). Acidic sugar moieties of the methylated product were reduced using LiB(C₂H₅)₂D (Super-Deuprize, 1 ml, room temperature, Aldrich Company) in THF and the reduction product was recovered using a C₁₈ cartridge column (Sep-Pak). Subsequently, the treated sample was subjected sequentially to: hydrolysis at 121 ℃, for 2 hours in 1.0 M TFA; reduction
by NaBD₄; and acetylation. The resulting partially methylated alditol acetate was analyzed by GLC and GC-EIMS, and the peak areas were measured with an FID (flame ionization detector).

GC analyses were conducted (Varian CP-3800 model, set-up condition; detector: FID, column: SP-2380 (30 cm×0.25 mm×0.2 μm) at column temperature: 230°C, injector temperature: 250°C, detector temperature: 250°C, mobile phase: N₂ gas (1.0 ml/min)) to identify the sugar components of the above fractions and the relative amounts of mannose, glucose, rhamnose, arabinose and xylose present in each fraction were determined. The result is shown in Table 2 and Figs 8 to 12.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mannose</th>
<th>Glucose</th>
<th>Galactose</th>
<th>Rhamnose</th>
<th>Arabinose</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>1</td>
<td>3.14</td>
<td>0.88</td>
<td>-</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>1</td>
<td>0.92</td>
<td>-</td>
<td>1.4</td>
<td>2.14</td>
<td>-</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0.69</td>
<td>1.23</td>
<td>0.61</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>1</td>
<td>0.31</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>Fraction 5</td>
<td>1</td>
<td>-</td>
<td>6.84</td>
<td>-</td>
<td>1.5</td>
<td>1.45</td>
</tr>
</tbody>
</table>

As the results in Table 2 and Figs. 8 to 12 show, each fraction was composed of mannose, glucose, galactose, rhamnose, arabinose or xylose. The amount of each sugar component was calculated by setting the amount of mannose as a standard value of 1. The fraction 1 consisted of mannose, glucose, galactose, arabinose and xylose; the fraction 2, mannose, glucose, rhamnose and arabinose; the fraction 3, mannose, rhamnose, arabinose and xylose; the fraction 4, mannose, glucose and arabinose; and the fraction 5, mannose, galactose, arabinose and xylose.

From the results of Examples (4-1) and (4-2), it has been confirmed that the fraction 1 contains two polysaccharides having molecular weights of 68,000 and 36,440 Da, respectively, which consist of mannose, glucose, galactose, arabinose and xylose; the fraction 2, three polysaccharides having molecular weights of 22,500, 233,300 and 200,700 Da, respectively, which consist of mannose, glucose, rhamnose and arabinose; the fraction 3, a polysaccharide having a molecular weight of 32,840 Da which consists of mannose, rhamnose, arabinose and xylose; the fraction 4, two polysaccharides having molecular weights of 88,610 and 79,190 Da, respectively, which consist of mannose, glucose and arabinose; and the fraction 5, a polysaccharide having a molecular weight...
of 216,500 Da which consists of mannose, galactose, arabinose and xylose.

**Test Example 1: Hepatoprotective activity of the lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb.**

To examine hepatoprotective activity of the lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb., *in vitro* hepatoprotective activity using a Dynamic Liver Slice Culture method and the activity for restoring the liver function damaged by carbon tetrachloride (Chang, I. M., et al., Drug and chemical toxicology 6(5):443-453, 1983) were measured as follows.

**1-1) Preparation of experimental animal**

BALB/c mice and Sprague-Dawley (SD) rats were used for the following experiments. They were allowed to freely ingest feed (Purina Korea) and water, and kept in a cage under the condition of 21~24°C, 40~80% of relative humidity and a daily lighting cycle of 12 hrs light/12 hrs dark.

**1-2) Pretreatment of *in vitro* hepatotoxicity experiment using a liver tissue culture method**

Livers taken from five-week old Sprague-Dawley rats were sliced to obtain disc shaped samples each having a diameter of about 0.8 mm and a thickness of 200 μm (wet weight: 18~22 mg), using a tissue cutter (Brendel/Vitron Co., USA). The sliced samples were subjected to surface cultivation under an atmosphere of O₂/CO₂=95%/5% in a thermodynamic organ tissue cultivator (Sanyo Co., Japan). Then, the cultured tissue samples were treated with the methanol-insoluble fraction, the methanol-soluble fraction and distilled water, respectively. After 1 hour, carbon tetrachloride (4 mM), bromobenzene (1 mM) or D-galactosamine (500 μM) was added to the sample and kept for 5 hrs to induce liver damage while still maintaining an atmosphere of O₂/CO₂=95%/5%.

Thereafter, the liver detoxification efficacy of the methanol-insoluble fraction was evaluated by determining the amounts of LDH released and protein synthesized in the liver according to the method described by Bonney et al. ("*Some Characteristics and Function of Adult Rat Liver Primary Culture, in Gene Expression and Carcinogenesis in Cultured Liver*" Gerschenson, E and Thompson, E. B. (Eds), Academic Press, New York, 24-45, 1975).
(1-3) Measurement of the amount of LDH released in liver tissue

To determine hepatoprotective and antihapatotoxicity activities of the methanol-insoluble fraction for the liver damaged by a hepatotoxic substance, e.g., carbon tetrachloride, D-galactosamine and bromobenzene, the amount of LDH (lactic acid dehydrogenase) released into the culture solution of liver tissue was determined with a 340-UV spectrometer (Sigma Co.).

As the results in Figs. 13, 15 and 17 show, the methanol-insoluble fraction obtained from the young branch of *Hovenia dulcis* Thunb. significantly reduced the amount of LDH released in the liver tissue damaged by carbon tetrachloride, D-galactosamine or bromobenzene as compared with the control. Accordingly, it has been confirmed that the lower alcohol-insoluble fraction of the present invention has an antifatigue activity, because it effectively prevents the release of LDH which is closely related to general fatigue caused by stress.

(1-4) Measurement of the amount of protein synthesized in liver tissue

The amount of protein synthesized in liver tissue damaged by carbon tetrachloride, D-galactosamine or bromobenzene was measured according to the method described by Bonney et al. ("Some Characteristics and Function of Adult Rat Liver Primary Culture, in Gene Expression and Carcinogenesis in Cultured Liver" Gerschenson, E and Thompson, E. B. (Eds), Academic Press, New York, 24-45, 1975).

To determine the amount of the synthesized protein having healing activity in the liver tissue damaged by a hepatotoxic substance, the liver tissue was labeled with the $^3$H-Leucine (5 μCi/plate, 0.38 mM) isotope for 2 hrs. The labeled tissue was dissolved in 20% trichloroacetic acid (TCA) and centrifuged at 2,000× g for 10 min to obtain a precipitate. Then, the precipitate was washed twice with 10% TCA and digested with 1 N sodium hydroxide to measure the radioactivity.

As shown in Figs 14, 16 and 18, the methanol-insoluble fraction obtained from the young branch of *Hovenia dulcis* Thunb. exhibited liver detoxification activity by increasing the amount of protein synthesized in the liver tissue damaged by carbon tetrachloride, D-galactosamine or bromobenzene as compared with the control. Accordingly, it has been confirmed that the lower alcohol-insoluble fraction of the present invention can be effectively used for treating fatty liver.

(1-5) Measurement of blood alcohol concentration
40% (w/v) alcohol was orally administered to rats at a dose of 10 ml/kg. After 1 hr, the methanol-insoluble fraction and the methanol-soluble fraction were each orally administered thereto. Four hrs after the administration of alcohol, blood was taken from rat’s tail vein or heart and subjected to blood alcohol measurement using a kit (Sigma 332, UV, Endpoint method, USA).

As shown in Fig. 19, the methanol-insoluble fraction of the present invention significantly lowered the blood alcohol concentration to one half level of the control.

(1-6) Measurement of alcohol dehydrogenase activity

To examine the mechanism for hangover relieving activity of the lower alcohol-insoluble fraction, 40% (w/v) alcohol was orally administered to rats at a dose of 10 ml/kg. After 1 hr, 0.3 ml (500 mg/ml) each of the methanol-insoluble fraction and the methanol-soluble fraction was orally administered thereto. Four hrs after the administration of alcohol, the alcohol dehydrogenase activity in liver was measured as follows.

Rats were sacrificed with carbon dioxide, and the liver was extracted therefrom. The extracted liver was washed with saline, measured its weight, soaked in 10-fold volume of 0.1 M potassium phosphate buffer (pH 0.74) containing 0.154 M KCl, and homogenized with a teflone-glass homogenizer. After the homogenized liver was subjected to centrifugation at 4°C, 9,000× g for 30 min to obtain a supernatant, the supernatant was subjected to super-centrifugation at 4°C, 110,000× g for 1 hr and the supernatant thus obtained was used as a cytoplasmic protein containing fraction.

The alcohol dehydrogenase activity was measured by the gas chromatography head space analytic method using HP 5890 gas chromatography (Hewlett Packard Company in USA) equipped with an FID (flame ionization detector). Principally, the alcohol dehydrogenase activity was determined by monitoring the amount of NAD reduced under the presence of excessive alcohol as absorbance variation. 2–3 mg of the cytoplasmic protein was added to the reaction mixture comprising 55 mM sodium pyrophosphate buffer (pH 7.4), 20 mM ethanol and 0.2 mM NAD. The absorbance variation was recorded at 340 nm for 3 min, and the activity of alcohol dehydrogenase was calculated from the slope of the observed absorbance variation.

As the result shown in Fig. 20, it has been confirmed that the alcohol dehydrogenase activity increases in the rat administered with the lower alcohol-insoluble fraction. Accordingly, it has been confirmed that the lower alcohol-insoluble fraction of the present invention has hangover relieving activity.
**Test Example 2: In vitro detoxification effect of the polysaccharide fractions isolated from the methanol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb.**

(2-1) Cultivation of liver cell

Liver cells were isolated according to the collagenase perfusion method (Berry, M. N. et al., *J. Cell Biol.* 43:506, 1969) using Hank’s balanced salt solution as a perfusion solution. The isolated liver cells were dissolved in a hormonal AB medium (Sigma), and stained with a trypane blue dye to examine their viability. The liver cells showing 90% or more viability were diluted at a concentration of $2 \times 10^5$ cells/ml, and 1 ml of diluted solution was added to each well of a 24-well plate which was pretreated with a rat’s tail collagen. Then, Williams’ E medium (Sigma) containing 10% bovine serum albumin, 1% penicillin-streptomycin and 4 mM L-glutamine were added to each well, and the well plate was incubated at 37°C for 24 hrs under 5% CO$_2$/95% O$_2$. After 4 hrs incubation, the culture solution was replaced with a fresh medium to remove the cells unbonded to the well. The well plate was further incubated for 20 hrs to form a complete monolayer of cells.

(2-2) The activity of restoring the liver damaged by carbon tetrachloride

The monolayer cultured liver cells were treated with 10 $\mu$l of 4 mM carbon tetrachloride (CCl$_4$) dissolved in DMSO to induce hepatotoxicity. 10 $\mu$l each of polysaccharide fractions 1 to 5 purified from the young branch of *Hovenia dulcis* Thunb. was added thereto at a concentration of 1~10 $\mu$g/ml and then, the cells were incubated for 1.5 hrs. Since lactate dehydrogenase (LDH) is released into the blood stream when the liver is damaged, the hepatoprotective activity was determined by measuring the amount of LDH released in the liver using a hematobiochemistry analyzer (Chiron/Diagnostics Express Plus, USA). Silybin having the hepatoprotective activity to LDH release caused by liver damage was used as a positive control.

As the results in Figs. 21 to 25 show, polysaccharide fractions 1 to 5 of the present invention significantly inhibited the release of LDH from the liver damaged by carbon tetrachloride, similar to the same extent as silybin (Figs 21 to 25).

The lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb. and the polysaccharide isolated therefrom can be used in preparing a
pharmaceutically effective powder, tablet, capsule, injection or liquid composition according to any one of the known conventional methods, as exemplified below.

**Formulation Example 1**

2 g of the dried lower methanol-insoluble fraction obtained in Example 2 or the polysaccharide isolated therefrom was mixed with 1 g of lactose to obtain a powder preparation, which was filled and sealed in a sealed package.

**Formulation Example 2**

100 mg of the dried methanol-insoluble fraction obtained in Example 2 or the polysaccharide isolated therefrom, 100 mg of corn starch, 100 mg of lactose, and 2 mg of magnesium stearate were mixed and tabletted to obtain a tablet preparation.

**Formulation Example 3**

100 mg of the dried methanol-insoluble fraction obtained in Example 2 or the polysaccharide isolated therefrom, 100 mg of the corn starch, 100 mg of lactose, 2 mg of magnesium stearate were mixed and filled in a gelatin capsule to obtain a capsule preparation.

**Formulation Example 4**

100 mg of the dried methanol-insoluble fraction obtained in Example 2 or the polysaccharide isolated therefrom, distilled water, and an appropriate amount of a pH controller were mixed to obtain an injection formulation, which was filled in a 2 ml ample and sterilized according to a conventional injection preparation method, to obtain an injection preparation.

An exemplary health care food was prepared by the following method.

**Preparation of health care food**

A scorched dried meal mixture of brown rice, barley, glutinous rice and Job’s tear was pulverized and sieved to obtain a grain powder of 60 mesh or less. Also, a mixture of black bean, black sesame and wild sesame was steamed, dried, scorched,
pulverized and sieved to obtain a seed powder of 60 mesh or less.

The dried methanol-insoluble fraction obtained in Example 2 or the polysaccharide isolated therefrom was pulverized and sieved to obtain a powder of 60 mesh or less, which were mixed with the grain and seed a powder in the following proportions to prepare a granule type health food.

Grains : brown rice 30 w%, Job's tear 15 w%, barley 20 w%,
Seeds : wild sesame 7 w%, black bean 8 w%, black sesame 7 w%,
Dried powder of methanol-insoluble fraction or polysaccharide isolated therefrom : 3 w%,
Shiitake mushroom 0.5 w%, rehmania root 0.5 w%

An exemplary health care beverages were prepared by the following method.

**Preparation of health care beverage 1**

1~10% dried powder of the methanol-insoluble fraction obtained from Example 2 or the polysaccharide isolated therefrom, 5~10% sugar, 0.05~0.3% citric acid, 0.005~0.02% caramel and 0.1~1% vitamin C were mixed, and 79~94% distilled water was added thereto to make a syrup. The syrup thus obtained was sterilized at 85~98°C for 20~180 sec, and mixed with cold water at a ratio of 1:4. Then, 0.5~0.82% carbonic acid gas was injected to the mixture to obtain a carbonated beverage.

**Preparation of health care beverage 2**

1~10% dried powder of the methanol-insoluble fraction obtained from Example 2 or the polysaccharide isolated therefrom, 0.5% liquid fructose, 2% oligosaccharide, 2% sugar, 0.5% salt and 75% water were mixed, sterilized for a second, and packaged into a container.

While the embodiments of the subject invention have been described and illustrated, it is obvious that various changes and modifications can be made therein without departing from the spirit of the present invention which should be limited only by the scope of the appended claims.
What is claimed is:

1. A lower alcohol-insoluble fraction of the young branch of *Hovenia dulcis* Thunb. having hepatoprotective and antihepatotoxicity activities, which is prepared by the steps of: extracting the dried young branch of *Hovenia dulcis* Thunb. with hot-water to obtain a hot-water extract; drying the hot-water extract; and subjecting the dried hot-water extract to a lower alcohol extraction treatment to obtain the lower alcohol-insoluble fraction.

2. The lower alcohol-insoluble fraction of claim 1, wherein the *Hovenia dulcis* Thunb. is *Hovenia dulcis* var. *latifolia* Nakai., *Hovenia dulcis* var. *tomentella* Makino or *Hovenia dulcis* var. *latifolia* Nakai.

3. A process for preparing the lower alcohol-insoluble fraction of claim 1, comprising the steps of: extracting the dried young branch of *Hovenia dulcis* Thunb. with hot-water to obtain a hot-water extract; drying the hot-water extract; and subjecting the dried hot-water extract to a lower alcohol extraction treatment to obtain the lower alcohol-insoluble fraction.

4. The process of claim 3, wherein the lower alcohol is selected from the group consisting of methanol, ethanol, butanol and a mixture thereof.

5. The process of claim 5, wherein the lower alcohol is methanol.

6. A polysaccharide fraction having hepatoprotective and antihepatotoxicity activities, which is isolated from the lower alcohol-insoluble fraction of claim 1.

7. The polysaccharide fraction of claim 6, which contains two polysaccharides composed of mannose, glucose, galactose, arabinose and xylose, and having molecular weights of 68,040 and 36,440 Da, respectively, as determined by MALLS.

8. The polysaccharide fraction of claim 6, which contains three polysaccharides composed of mannose, glucose, rhamnose and arabinose, and having molecular weights of 22,500, 233,300 and 200,700 Da, respectively, as determined by MALLS.

9. The polysaccharide fraction of claim 6, which contains a polysaccharide composed of mannose, rhamnose, arabinose, and xylose, and having a molecular weight
of 32,840 Da, as determined by MALLS.

10. The polysaccharide fraction of claim 6, which contains two polysaccharides composed of mannose, glucose and arabinose, and having molecular weights of 88,610 and 79,190 Da, respectively, as determined by MALLS.

11. The polysaccharide fraction of claim 6, which contains a polysaccharide composed of mannose, galactose, arabinose and xylose, and having a molecular weight of 216,500 Da, as determined by MALLS.

12. A process for preparing the polysaccharide fraction of claim 6, comprising the steps of subjecting the lower alcohol-insoluble fraction of claim 1 to ion exchange column chromatography using aqueous NaCl eluting solution to obtain the polysaccharide fraction.

13. The process of claim 12, wherein the ion exchange column chromatography is conducted using a cation exchange resin or an anion exchange resin.

14. The process of claim 13, wherein the ion exchange column chromatography is conducted using a modified bead-type ion exchange resin selected from the group consisting of Cepharose CL, Biogel A Cepharose resin, Fractogels and Toyopearl resin.

15. The process of claim 14, wherein the ion exchange column chromatography is conducted using the Toyopearl resin.

16. A composition for inhibiting alcohol dehydrogenase, comprising the lower alcohol-insoluble fraction of claim 1 or the polysaccharide fraction of claim 6, and a pharmaceutically acceptable carrier.

17. A composition for inhibiting lactic acid dehydrogenase, comprising the lower alcohol-insoluble fraction of claim 1 or the polysaccharide fraction of claim 6, and a pharmaceutically acceptable carrier.

18. A pharmaceutical composition for preventing or treating a liver disease, comprising the lower alcohol-insoluble fraction of claim 1 or the polysaccharide fraction of claim 6, and a pharmaceutically acceptable carrier.
19. The pharmaceutical composition of claim 18, wherein the liver disease is hepatitis or liver cirrhosis.

20. A health care food comprising the lower alcohol-insoluble fraction of claim 1 or the polysaccharide fraction of claim 4, and a sitologically acceptable additive.

21. The health care food of claim 20, wherein the food is of a beverage type.
Fig. 1

1~4 year-old young branch (1,000 g)

hot water extract (HDBRHW) (12.2 g, 1.2%)

HPLC-degrade MeOH

MeOH insoluble fraction (HDBRHW-1) (11.57 g, 1.1%)

MeOH soluble fraction (HDBRHW-2) (0.63 g, 0.06%)

Fig. 2
Fig. 3
Multi-angle laser light scattering detector

Fig. 4
Multi-angle laser light scattering detector

Detector: AUX1
3/13

**Fig. 5**

Multi-angle laser light scattering detector

Differential Refractive Index detector

**Fig. 6**

Multi-angle laser light scattering detector

Differential Refractive Index detector

Detector: AUX1

MW: 32,840

MW: 79,190

MW: 88,610
Fig. 7

Multi-angle laser light scattering detector

Differential Refractive Index detector

Fig. 8

mVolts

Minutes
Fig. 11

Fig. 12
Fig. 19

![Bar chart of Blood alcohol concentration (%) for control, HDBRHW-1, and HDBRHW-2.]

Fig. 20

![Bar chart of Absorption at 340/minute/mg protein for samples 1, 2, and 3.]

Fig. 21

Fig. 22
**Fig. 23**

![Bar chart showing LDH activity (%)](image)

**Fig. 24**

![Bar chart showing LDH activity (%)](image)
Fig. 25

LDH activity (%) vs Concentration

- CCl₄ + Fraction 5
- DMSO + Fraction 5
- Silybin
**INTERNATIONAL SEARCH REPORT**

<table>
<thead>
<tr>
<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
</tr>
<tr>
<td>1. ☐ Claims Nos.:</td>
<td>because they relate to subject matter not required to be searched by this Authority, namely:</td>
</tr>
<tr>
<td>2. ☐ Claims Nos.:</td>
<td>because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
</tr>
<tr>
<td>3. ☐ Claims Nos.:</td>
<td>because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Box No. III</th>
<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
</tr>
<tr>
<td></td>
<td>1) Claims 16-19 are directed to a pharmaceutical composition.</td>
</tr>
<tr>
<td></td>
<td>2) Claims 20-21 are directed to a health care food.</td>
</tr>
<tr>
<td></td>
<td>Since the abovementioned groups of claims do not share any of the technical features identified, a technical relationship between the inventions does not exist.</td>
</tr>
<tr>
<td></td>
<td>Accordingly the claims do not relate to one invention or to a single inventive concept, a priori.</td>
</tr>
<tr>
<td>1. ☐</td>
<td>As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
</tr>
<tr>
<td>2. ✗</td>
<td>As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addition fee.</td>
</tr>
<tr>
<td>3. ☐</td>
<td>As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
</tr>
<tr>
<td>4. ☐</td>
<td>No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:</td>
</tr>
</tbody>
</table>

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)
## INTERNATIONAL SEARCH REPORT

### A. CLASSIFICATION OF SUBJECT MATTER

**IPC7** A61K 35/78

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 35/78, A23L 1/00, A23L 2/38

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 practicable, search terms used)

PubMed 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>KR 2002-0064151 A (LIFETREE BIOTECH CO., LTD.), 07 August 2002</td>
<td>1-6, 12-21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>WO 02/24678 A1 (LEE, HY et al.), 28 March 2002</td>
<td>1-21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>JP 04-282318 A (SUNTORY LTD.), 07 October 1992</td>
<td>1-21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>CN 1416838 A (LL B), 14 May 2003</td>
<td>1-21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>CN 1058907 A (YU, Z), 26 February 1992</td>
<td>1-21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>KR 1997-0073403 A (HANSOL PAPER CO., LTD.), 10 December 1997</td>
<td>20, 21</td>
</tr>
<tr>
<td></td>
<td>See entire document</td>
<td></td>
</tr>
</tbody>
</table>

[X] Further documents are listed in the continuation of Box C.  
[X] Sec 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
  * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
  * "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

**T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

**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

**Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

**&** document member of the same patent family

Date of the actual completion of the international search  
09 FEBRUARY 2004 (09.02.2004)

Date of mailing of the international search report  
10 FEBRUARY 2004 (10.02.2004)

**Name and mailing address of the ISA/KR**

Korean Intellectual Property Office  
920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Form PCT/ISA/210 (second sheet) (January 2004)
## INTERNATIONAL SEARCH REPORT

<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>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>AN, SW et al. 'Comparison of hepatic detoxification activity and reducing serum alcohol concentration of Hovenia dulcis Thunb and Alnus japonica Steud' In; Korean J. Medicinal Crop Sci. 1999; 7(4): 263-8</td>
<td>1-21</td>
</tr>
<tr>
<td>A</td>
<td>YOSHIKAWA, M et al. 'Bioactive constituents of chinese natural medicines. III. Absolute stereostructures of new dihydroflavonols, hovenitins 1, 2, 3, isolated from hoveniae semen seu fructus, the seed and fruit of Hovenia dulcis THUNB. (Rhamnaceae): inhibitory effect on alcohol-induced muscular relaxation and hepatoprotective activity' In; Yakugaku Zasshi, 1997; 117(2): 108-18</td>
<td>1-21</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1355654 A1</td>
</tr>
<tr>
<td>WO 02/24678 A1</td>
<td>28/03/2002</td>
<td>WO 02/25753 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 0074561 A5</td>
</tr>
<tr>
<td>CN 1416838 A</td>
<td>14/05/2003</td>
<td>NONE</td>
</tr>
<tr>
<td>CN 1058907 A</td>
<td>26/02/1992</td>
<td>NONE</td>
</tr>
<tr>
<td>KR 1997-0073403 A</td>
<td>10/12/1997</td>
<td>NONE</td>
</tr>
<tr>
<td>KR 2001-0069022 A</td>
<td>23/07/2001</td>
<td>NONE</td>
</tr>
</tbody>
</table>