Title: ACTIVE FRACTION HAVING ANTI-CANCER AND ANTI-METASTASIS ISOLATED FROM ACANTHOPANAX SPECIES AND FRUITS

Abstract: Provided is a composition that contains, as an active ingredient, an extract or polysaccharides separated from plants belonging to *Acanthopanax* genus, for an anticancer drug, or its adjuvant having an effect on the enhancement of hemotopoiisis, defense of bone marrow, inhibition of radiation side effects, etc., and a method of preparing the extract from the *Acanthopanax* genus plants.

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ACTIVE FRACTION HAVING ANTI-CANCER AND ANTI-METASTASIS ISOLATED FROM ACANTHOPANAX SPECIES AND FRUITS

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TECHNICAL FIELD

The present invention relates to a composition containing, as an active ingredient, an extract obtained from plants belonging to Acanthopanax genus and a process of preparation of the extract, and more particularly a composition containing polysaccharides extracted from the leaves, stems, roots and/or fruits of plants belonging to Acanthopanax genus, specifically Acanthopanax sessiliflorum and Acanthopanax senticosus, which can be used as an anticancer drug and a cancer metastasis inhibitor by activation of immune cells, such as natural killer (NK) T cells, NK cells, etc., and also used as an adjuvant for anticancer drugs by the hemopoiesis enhancement effect, bone marrow defense effect, radiation sensitivity defense effect, etc., and a process for preparation of the same.

BACKGROUND OF THE INVENTION

20% of American deaths every year have been reported to be caused by cancer-related diseases. For treatment of these cancers, chemotherapy is usually used but few anticancer drugs have been known to be effective.

Most of deaths caused by cancers stem from the metastasis of cancer rather than the first occurrence of cancer itself. (Fifler, 1991 cancer metastasis. Br. Med. Bull. 47, 157-177). By many experiments and clinical tests, it was confirmed that the natural immune ability plays an important role in inhibiting the cancer metastasis and
destroying a cancer itself (Schantz et al., cancer immunol. Immunother. 25., 141-148, 1987).

NKT (Natural Killer T) cells are a specialized population of α/β T cells that coexpress receptors of the NK lineage and have the unique potential to secrete very rapidly the large amount of cytokines, providing early help for effector cells and regulating the Th1 or Th2 differentiation of some immune responses (Annu. Rev. Immunol. 1997. 15; Albert Bendelae et al. p535). Moreover, they also eliminate infectious germs or bacteria such as cancer cells, parasites, listerias, tuberculoses, and so on (Seki et al., Clin. Immunol., 28, p1069, 1996).

NK cells, LAK cells and macrophages, in addition to the NKT cells, are known to be cells that can effectively inhibit infection by cancer cells, viruses, and bacteria. More particularly, the effective activation of NK cells, LAK cells, and macrophages has been known to block the cancer cell growth and its metastasis. Also, the activation of NK cells by immune stimulators has been reported to inhibit the cancer cell proliferation caused by cancer metastasis (Herberman, 1984 J. invest. Dermatol. 83, 137-140).

These anticancer, anti-metastasis and anti-virus functions of the immune system are mediated by the secretion of various kinds of cytokines, activating the immune system. Especially, gamma interferon, tumor necrosis factor-alpha, etc. are the representative cytokines associated with the anticancer, anti-metastasis and anti-virus functions.

Gamma interferon, which is mainly generated by T cells, serves to control the immune reaction and also activate T and B cells, neutrophils, NK cells, and macrophages to make them attack cancer cells. Therefore, Gamma interferon is used as a treatment for chronic bone marrow leukemia and kidney cancer. Moreover, Gamma interferon has a strong inhibitory effect on DNA synthesis and cell proliferation, so that it is also clinically applied to not only cancer treatment but also to treat virus infection, multi-resistance bacteria and fungi infection, by suppressing the proliferation of
microorganisms.

Tumor necrosis factor-alpha is mainly generated by macrophages and is involved in various immune reactions such as the inflammatory reaction, and especially shows a very strong toxicity to cancer cells. At present, TNF-alpha is on the verge of approval as a skin cancer treatment in Japan, pending clinical test results.

However, using cytokines directly for anticancer therapy brings unexpected side effects, such as an inflammatory reaction, emesis and so on. Therefore, many trials are being made to find materials that can wholly activate the immune system, rather than using only a particular cytokine.

Regretfully, few natural products are known to activate immune cells including NK cells: for example, a lectin from *Viscum coloratum* extraction, which is partially used as a substitute therapy for a cancer treatment, and polysaccharides belonging to beta-glucan series obtained from a mushroom.

Meanwhile, *Acanthopanax* genus plants are deciduous broadleaf shrubs belonging to *Araliaceae* family and representatively include *Acanthopanax sessiliflorum* and *Acanthopanax senticosus* (so-called, "Siberian Ginseng"), having small thorns on each stem. *Acanthopanax* genus plants are mainly distributed in Northeast Asia and South Africa. The representative pharmaceutical effects of *Acanthopanax* genus plants are known to be sedative effects, anti-stress effects, smooth muscle-relaxing effects, anti-inflammatory effects, anaphylaxis-inhibiting effects, etc. Also, *Acanthopanax* genus plants are known to have excellent effects for treating neuralgia, paralysis, diabetes, hypertension, hypotension and insomnia. Major components, providing these effects, are known to be saponin-based materials and acanthoic acid. *Acanthopanax senticosus* contains acanthoic acid, having effects on inhibition of thrombocyte aggregation, inhibition of inflammation, inhibition of oxygen radicals, etc. (Kang, H. S, Cell Immuno. Vol. 170, No. 2. 1996). Moreover, *Acanthopanax senticosus* contains tripenoid-based glycosides (Eleutheroside A, B, C, D, E, F, G and Aconthoside A, B, C, D), other glycosides such as sterol, cumarin, lignin,
flavonoid and so on, to accelerate the secretion of many hormones by stimulating the pituitary gland, to express tonic effects and liver-protecting effects. As some examples of *Acanthopanax senticosus* applications, there are the treatment of acne and pimples (Korean Patent Laid-open No. 2000-9820), the treatment of impotence (Korean Patent Laid-open No. 2000-74868), the composition for anti-stress (Korean Patent No. 160402), the drug for nerve protection (Korean Patent Laid-open No. 2000-77717), and a material or additive for functional foods and teas.

Despite much research, however, there have been no reports relevant to using an extract or polysaccharides obtained from plants belonging to *Acanthopanax* genus as an anticancer drug or its adjuvant, by separating from them polysaccharides having the activity of immune enhancement and anti-metastasis.

**SUMMARY OF THE INVENTION**

The inventors of the present invention are the first to find that an extract or polysaccharides obtained from plants belonging to *Acanthopanax* genus activates immune cells, such as NKT cells, NK cells, macrophages, etc., and accelerates the secretion of cytokines, such as interferon-alpha, tumor necrosis factor-alpha, etc., thereby suppressing the proliferation and metastasis of cancer cells, accelerating the hematopoiesis, and also reducing the side effects of the existing anticancer drugs and radiation treatment. The present invention was accomplished on the basis of these findings.

Accordingly, an object of the present invention is to provide an extract, obtained from plants belonging to *Acanthopanax* genus, more particularly, the leaves, stems, roots and/or fruits of *Acanthopanax sessiliflorum* and *Acanthopanax senticosus*, which has cancer inhibition effect, hematopoiesis enhancement effect, bone marrow defense effect, radiation sensitivity defense effect, etc.

Another object of the present invention is to provide a composition containing...
the above extract or polysaccharides as an active ingredient.

A further object of the present invention is to provide novel uses of the above composition, such as an anticancer drug for inhibiting cancer cells and reducing the metastasis of cancer cells, a cancer metastasis inhibitor, a hematopoiesis enhancer, an inhibitor of radiation side effects, an inhibitor of anticancer drug side effects, an autoimmune disease treatment drug, etc.

Another object of the present invention is to provide a process of obtaining the extract or polysaccharides from leaves, stems, roots and/or fruits of plants belonging to *Acanthopanax* genus and preparing the composition.

The composition according to the present invention contains an extract as an active ingredient, which can be obtained by treating the leaves, stems, roots and/or fruits of plants belonging to *Acanthopanax* genus (hereinafter, referred to as "*Acanthopanax* genus plants" or sometimes as "*Acanthopanax*") under a special condition ("hydrothermal treatment"). *Acanthopanax* genus plants which can be preferably used in the present invention include *Acanthopanax sessiliflorum* and *Acanthopanax senticosus*.

The form of the composition according to the present invention is not particularly limited so far as the effect of the above extract can be exhibited, and includes, for example, a solid phase, suspension phase, emulsion phase, liquid phase and so on. Also, the amount of the above extract in the composition is not particularly limited so far as it functions as an active ingredient for the particular purpose.

In accordance with the present invention, in order to obtain an extract or polysaccharides from the leaves, stems, roots and/or fruits of *Acanthopanax* genus plants, these are first heated in 5 – 20 volumes of water at 50 – 180°C for 0.5 – 20 hours, preferably at 80 – 120°C for 1 – 10 hours, more preferably around 100°C for 3 – 4 hours. The extract solution obtained thus contains low molecular weight materials such as alkaloids, flavonoids and terpenoids, and high molecular weight materials such as polysaccharides, protein and tannin. In order to separate the polysaccharides, 3 – 4
volumes of ethanol is added to the extract solution to precipitate the polysaccharides. Then, the precipitant is separated to obtain a crude polysaccharide fraction.

In a preferred embodiment, a supernatant of the extract solution obtained after hydrothermal treatment is adjusted to alkali pH and then loaded onto an ion exchange resin, and is precipitated with ethanol to separate polysaccharides, following dialysis and the adsorption of another ion exchange resin. Elution is performed with 0.5 M NaCl, and then the eluate is dialyzed and freeze-dried to obtain the polysaccharide fraction having excellent immune enhancement activity and anticancer activity.

In below, the present invention will be described in more detail.

Acanthopanax genus plants, which can be used in the present invention, include plants of Acanthopanax gracilistylus type: Acanthopanax gracilistylus, Acanthopanax chilsanensis, Acanthopanax seoulensis, Acanthopanax rufinerve, and Acanthopanax pedunculus, plants of Acanthopanax senticosus type: Acanthopanax senticosus, Acanthopanax senticosus var. Koreanus, Acanthopanax senticosus var. inermis, and Acanthopanax divaricatus, plants of Acanthopanax koreanum type: Acanthopanax koreanum, and plants of Acanthopanax sieboldianum type: Acanthopanax sieboldianum, and Acanthopanax sessiliflorum which can be used alone or in the combination of two or more.

In order to obtain an extract or polysaccharides from Acanthopanax genus plants, for example, the stems of Acanthopanax are added 10 – 15 equivalents of distilled water, based upon the weight of dried stems, and heated for 3 – 4 hours. In this hydrothermal treatment, the stems are desirably used in the form of powder. An extract solution is separated from slurry using gauze or a continuous centrifugal separator, and the remnants existing in the extract solution are removed by the centrifugation (7000 rpm, 20 min.) to obtain only a supernatant. pH of the supernatant is in the range of 4.5 – 6. Accordingly, pH of the supernatant is adjusted to 8 using NaOH, and then NaCl is added to a concentration of 0.5 M. The supernatant treated thus is loaded onto an anion exchange resin column. The anion exchange resin should be previously equilibrated
with 0.5 M NaCl/10 mM Tris-HCl buffer (pH 8.0) to adsorb only pigments and impurities but not polysaccharides. Until the loaded solution is completely eluted, the anion exchange resin column is sufficiently washed with the buffer solution. 3 volumes of 95% ethanol is added to eluate, to efficiently precipitate only polysaccharides, thereby concentrating the polysaccharides. To collect the polysaccharides, centrifugation (5000 rpm, 15 min) and dialysis are performed. Then, the solution is adjusted to pH 8 and again loaded in another anion exchange resin column, which is then sufficiently washed with a buffer, and elution is performed with 0.5 M NaCl. 3 volumes of ethanol is added to the eluate to precipitate polysaccharides, and then centrifugation for collecting the polysaccharides and dialysis for removing the remained ethanol are performed, followed by freeze drying to obtain the polysaccharide fraction.

The analysis of the extract shows that the polysaccharides of *Acanthopanax sessiliflorum* have a neutral sugar content of 69.81% (average), an uronic acid content of 30.07% (average), a KDO-like material content of 0.07% (average), and a protein content of 0.01% (average); the polysaccharides of fruits of *Acanthopanax sessiliflorum* have a neutral sugar content of 37.43% (average), an uronic acid content of 62.49% (average), a KDO-like material content of 0.07% (average), and a protein content of 0.01% (average); and the polysaccharides of *Acanthopanax senticosus* have a neutral sugar content of 66.23% (average), an uronic acid content of 33.15% (average), a KDO-like material content of 0.23% (average), and a protein content of 0.39% (average).

Meanwhile, the composition and content of sugars in polysaccharides vary depending upon the kind and part of *Acanthopanax* genus plants, the separation process, etc. Particularly, the content of ingredients in polysaccharides is 20 to 80% neutral sugar, 20 to 75% uronic acid, and 0.0001 to 2% protein. The sugar composition includes rhamnose, 2-methyl fucose, fucose, arabinose, 2-methy xylose, xylose, raffinose, aceric acid, mannose, glucose, galactose, galacturonic acid, glucuronic acid, DHA (3-deoxy-D-lyxo-2-heptulosaric acid), and KDO (2-keto-3-deoxyoctulosonic acid).

For *Acanthopanax sessiliflorum*, the molecular weights of polysaccharides were
in the range of approximately 6,000 to 250,000 Da and the main peak was detected at 140,000 Da. For the fruits of *Acanthopanax sessiliflorum*, the molecular weights of polysaccharides were in the range of approximately 3,000 to 758,000 Da and the main peak was detected at 130,000 Da. For *Acanthopanax senticosus*, the molecular weight of polysaccharides were in the range of approximately 1,600 to 758,000 Da and the main peak was detected at 150,000 Da.

The composition containing the extract or polysaccharides obtained thus can activate the overall immune system, such as NKT cells, NK cells, macrophages, T cells, B cells, etc., and also accelerate the secretion of cytokines having anticancer effects, thereby significantly inhibiting the proliferation and metastasis of cancer cells. Accordingly, the composition according to the present invention can be used as an anticancer drug and anti-metastasis drug. Also, the composition can be used as an adjuvant for anticancer drugs or radiation treatment by inhibiting the side effects thereof, e.g., the decrease of leucocytes.

The composition according to the present invention can be formulated by itself or together with carrier, conventionally acceptable in the pharmaceutical field, in conventional drug forms such as oral administration drugs, for example, pill, capsule, liquid form, suspension form, etc., and injection drugs, for example, liquid solution, suspension solution, emulsion solution, etc. In order to prevent the drug from being degraded by the gastric acids in oral administration, it may be co-administered with antacid agents or formulated with an enteric coating.

The dosage of the polysaccharides administered to the human body must be properly determined in consideration of the absorption rate of active ingredients, the inactivation rate, the excretion rate, patient’s age, gender and current condition, etc., and is generally 100 µg – 6000 mg, preferably 20 – 5000 mg per day for adults.

The composition according the present invention may further contain an anticancer drug(s) and/or an adjuvant(s) therefor, or be co-administered together them. The kind of the anticancer drugs is not particularly limited, and representative examples
of the anticancer drugs includes Taxol® and Cisplatin®.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 is a graph showing the inhibition of cancer metastasis to lung by polysaccharides extracted from *Acanthopanax* genus plant, in accordance with EXAMPLE 6;

FIG. 2 is a graph showing the anticancer effect on solid cancer by polysaccharides, extracted from *Acanthopanax* genus plant, in accordance with EXAMPLE 7;

FIG. 3 is a graph showing the effect of promoting proliferation of bone marrow cells by oral administration of polysaccharides, extracted from *Acanthopanax* genus plant, in accordance with EXAMPLE 8;

FIG. 4 is a graph showing the effect of promoting proliferation of bone marrow cells through Peyer’s patch by polysaccharides, extracted from *Acanthopanax* genus plant, in accordance with EXAMPLE 9;

FIG. 5 is a graph showing the effect of reducing hematopoiesis inhibition in splenocytes, by co-administration of an anticancer drug and the polysaccharides of *Acanthopanax* genus plant, in accordance with EXAMPLE 10;

FIG. 6 is a graph showing the effect of reducing hematopoiesis inhibition in bone marrow cells, by co-administration of an anticancer drug and the polysaccharides of *Acanthopanax* genus plant, in accordance with EXAMPLE 10;

FIG. 7 is a graph showing the effect of protecting splenocytes against irradiation by the polysaccharides in accordance with EXAMPLE 11;

FIG. 8 is a graph showing the effect of protecting bone marrow cells against irradiation by the polysaccharides of *Acanthopanax* genus plant, in accordance with EXAMPLE 11;

FIGS. 9A, 9B, 10A, 10B and 10C are graphs showing the effect of inhibiting
side effects of an anticancer drug by co-administration the anticancer drug and the polysaccharides of *Acanthopanax* genus plant, in accordance with EXAMPLE 12;

FIG. 11 contains graphs showing the activation effect on immune cells by the polysaccharides of *Acanthopanax* genus plant in accordance with EXAMPLE 13;

FIG. 12 contains graphs showing the effect of promoting anticancer cytokine release by the polysaccharides of *Acanthopanax* genus plant in accordance with EXAMPLE 13; and

FIG. 13 is a graph showing the synergistic anticancer effects by co-administration of an anticancer drug and the polysaccharides of *Acanthopanax* genus plant, in accordance with EXAMPLE 14.

**DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

The present invention is described in more detail with reference to the following examples. However, the scope of the present invention is not limited to these.

**EXAMPLE 1**: Separation of polysaccharides from the stems of *Acanthopanax sessiliflorum*

500 g of the *Acanthopanax sessiliflorum* stems in the form of powder was added into 5 L of water and then heated at 100°C for 3 hours in order to extract active ingredients therefrom. After this hydrothermal treatment, slurry was separated from an extract solution using gauze. The extract solution was centrifuged at 5000 rpm for 20 minutes to obtain a supernatant. The pH of the supernatant was in the range of 4.5 – 6. The supernatant was adjusted to pH 8, using NaOH, and 0.5 M NaCl/10 mM Tris-HCl (pH 8.0). This supernatant was loaded in an anion exchange resin column (PA312 strong basic anion exchange resin; Samyang Corporation). This anion exchange resin column was previously equilibrated with 0.5 M NaCl/10 mM Tris-HCl (pH 8) buffer before
loading, so as to adsorb only coloring materials and impurities but not polysaccharides. Until the loaded solution was fully eluted, the column was sufficiently washed with the buffer (0.5 M NaCl/10 mM Tris-HCl). 3 volumes of 95% ethanol was added to the passed-through solution to efficiently precipitate only the polysaccharides. The precipitated polysaccharides were centrifuged at the rate of 5000 rpm for 15 minutes, dialyzed and then loaded in an anion exchange resin column (Q sepharose; Pharmacia biotech.) at the rate of 15 ml/min. The column was sufficiently washed with the buffer (10 mM Tris-HCl, pH 8) and then elution was performed with 0.5 M NaCl/10 mM Tris-HCl buffer. Ethanol was added into the resulting solution to precipitate the polysaccharides. These polysaccharides were separated, dialyzed and then freeze dried to obtain 1 g (0.2%) of *Acanthopanax sessiliflorum* polysaccharide fraction.

**EXAMPLE 2:** Separation of polysaccharides from the fruits of *Acanthopanax sessiliflorum*

500 g of the *Acanthopanax sessiliflorum* fruits, mechanically pulverized, was added to 10 equivalents of distilled water, and heated for about 4 hours. After this hydrothermal treatment, slurry was separated from an extract solution using gauze. The extract solution was centrifuged at the rate of 5000 rpm for 20 minutes to obtain a supernatant. The pH of the supernatant was in the range of 4.5 - 6. The supernatant was adjusted to pH 8, using NaOH, and 0.5 M NaCl/10 mM Tris-HCl (pH 8.0). This supernatant was loaded onto an anion exchange resin column (PA312 strong basic anion exchange resin; Samyang Corporation). This anion exchange resin column was previously equilibrated with 0.5 M NaCl/10 mM Tris-HCl buffer (pH 8) before loading, so as to adsorb only coloring materials and impurities but not polysaccharides. Until the loaded solution was fully eluted, the column was sufficiently washed with the buffer (0.5 M NaCl/10 mM Tris-HCl). 3 volumes of 95% ethanol was added to the passed-through solution to efficiently precipitate only the polysaccharides. The precipitated polysaccharides were centrifuged at 5000 rpm for 15 minutes, dialyzed and then loaded.
in an anion exchange resin column (Q sepharose; Pharmacia biotech. Corporation) at
the rate of 15 ml/min. The column was sufficiently washed with the buffer (10 mM Tris-
HCl, pH 8) and elution was performed with 0.5 M NaCl/10 mM Tris-HCl buffer.
Ethanol was added into the resulting solution to precipitate the polysaccharides. This
polysaccharides were separated, dialyzed and then freeze dried to obtain 1.2 g (0.24%)
of *Acanthopanax sessiliflorum* polysaccharide fraction.

**EXAMPLE 3:** Separation of polysaccharides from the stems of *Acanthopanax
senticosus*

500 g of the *Acanthopanax sessiliflorus* stems, mechanically pulverized, was
added to 10 equivalents of distilled water, and heated for about 4 hours. After this
hydrothermal treatment, slurry was separated from an extract solution using gauze. The
extract solution was centrifuged at 5000 rpm for 20 minutes to obtain a supernatant. The
pH of the supernatant was in the range of 4.5 – 6. The supernatant was adjusted to pH 8,
using NaOH, and 0.5 M NaCl/10 mM Tris-HCl (pH 8.0). This supernatant was loaded
onto an anion exchange resin column (PA312 strong basic anion exchange resin;
Samyang Corporation). This anion exchange resin column was previously equilibrated
with a 0.5 M NaCl/10 mM Tris-HCl buffer (pH 8) before loading, so as to adsorb only
coloring materials and impurities but not polysaccharides. Until the loaded solution was
fully eluted, the column was sufficiently washed with the buffer (0.5 M NaCl/10 mM
Tris-HCl). 3 volumes of 95% ethanol was added to the passed-through solution to
efficiently precipitate only the polysaccharides. The precipitated polysaccharides were
centrifuged at 5000 rpm for 15 minutes, dialyzed and then loaded in an anion exchange
resin column (Q sepharose; Pharmacia biotech.) at the rate of 15 ml/min. The column
was sufficiently washed with the buffer (10 mM Tris-HCl, pH 8) and then elution was
performed with 0.5 M NaCl/10 mM Tris-HCl buffer. Ethanol was added into the
resulting solution to precipitate the polysaccharides. This polysaccharides were
separated, dialyzed and then freeze dried to obtain 1.5 g (0.3%) of *Acanthopanax
**sesiliflorum** polysaccharide fraction.

**EXAMPLE 4:** Preparation of liquid solution for injection

Each solution, having passed through the anion exchange resin column (Q-Sepharose) in EXAMPLES 1, 2 and 3, was loaded in a column which was filled with silica gels to remove pyrogen materials therefrom. 3 volumes of ethanol was added to the eluate to precipitate polysaccharides. The polysaccharides was collected by centrifugation and washed two times with 95% ethanol to remove impurities. The polysaccharides obtained thus were dissolved in triple distilled water and the resulting solution was dialyzed under the condition of the molecular weight cutoff of 6,000 to remove ethanol. For asepsis, the resulting solution was passed through 0.2 μm filtering system to prepare an ingredient solution without pyrogen materials. The aseptic ingredient solution was meristeded in a 3 ml vial by a certain amount and then freeze-dried. The dried ingredient was dissolved in saline solution to prepare a liquid solution for injection, which was used in EXAMPLES as below.

**EXAMPLE 5:** Analysis of the sugar composition of polysaccharides and identification of molecular weight

The content of neutral sugar was measured by the phenol-sulfuric acid method (Dubois et al Anal. Chem., 28, 350, 1956) using galactose as a standard material. The content of uronic acid was measured by the m-hydroxybiphenyl method (Blumenkrantz et al, Anal. biochem., 54, 484, 1973) using D-galacturonic acid as a standard material. The content of KDO (2-keto-3-deoxyoctulosonic acid) was measured by the tiobarbituric acid method. The content of protein was measured by the Bio-Rad protein assay using bovine serum albumin as a standard material. For analysis, the extracts were dissolved at the concentration of 1 – 10 mg/ml and refined.

The analysis of the polysaccharides of *Acanthopanax sesiliflorum* showed a neutral sugar content of 51.30%, an uronic acid content of 46.90%, a KDO-like material
content of 1.83%, and a protein content of 0 – 0.01%. The analysis of the polysaccharides of *Acanthopanax sessiliflorum* fruits showed a neutral sugar content of 37.43%, an uronic acid content of 62.49%, a KDO-like material content of 0.07%, and a protein content of 0.01%. And, the analysis of the polysaccharides of *Acanthopanax senticosus* showed a neutral sugar content of 48.03%, an uronic acid content of 49.83%, a KDO-like material content of 2.14%, and a protein content of 0.01%.

*Acanthopanax* genus plants other than *Acanthopanax sessiliflorum* and *Acanthopanax senticosus* showed the composition and content of polysaccharides different from those of the above, depending the kind and part of the plants and the refining process; however, the content of neutral sugar can be defined in the range of 20 to 80%, the content of uronic acid in the range of 20 to 75%, and the content of protein in the range of 0.0001 to 2%.

Furthermore, the analysis of constituent sugars showed rhamnose of 1 – 10% (3.8 – 7.5%), 2-methyl fucose of 0.1 – 5% (0.38 – 2.1%), fucose of 0.1 – 2% (0.3 – 1.23%), 2-methyl xylose of 0.1 – 2% (0.55 – 0.73%), arabinose of 1 – 20% (6.3 – 16.5%), xylose of 0.1 – 5% (1 – 1.5%), raffinose of 0.05 – 1% (0.24 – 0.33%), aceric acid of 0.05 – 1% (0.39 – 0.49%), mannose of 0.1 – 10% (0.4 – 5.4%), glucose of 0.5 – 10% (1.3 – 6.8%), galactose of 10 – 40% (15 – 27%), galacturonic acid and glucuronic acid of 30 – 60% (46 – 50%), and KDO and DHA, i.e., KDO like materials of 0.5 – 5% (1.8 – 2.2%), in which the values in parentheses show the values for *Acanthopanax sessiliflorum* and *Acanthopanax senticosus*.

The molecular weight was measured by HPLC with GS-520HQ, GS-320HQ and GS-220HQ (Shodex Asaipack GS series, Showa Denko Corporation) being connected in series. According to the measurement, the polysaccharides of *Acanthopanax sessiliflorum* have molecular weights of approximately 6,000 to 250,000 Da and the main peak of 140,000 Da, and the polysaccharides of *Acanthopanax sessiliflorum* fruit have molecular weights of approximately 3,000 to 758,000 Da and the main peak of 130,000 Da, and the polysaccharides of *Acanthopanax senticosus* have
molecular weights of 1,600 to 758,000 Da and the main peak of 150,000 Da.

EXAMPLE 6: Prophylaxis effect versus cancer metastasis to lung

Colon 26-M.3.1 cancer cells were cultured on Eagles MEM in a 100 mm cell culture petri dish. The polysaccharides, obtained in each of EXAMPLES 1, 2 and 3, were intravenously administered to Balb/c mice (6 – 7 weeks age, female) at the amount of 10 mg/kg, and after 2 days, colon 26-M3.1 cancer cells, being lung metastatic cancer cells, were injected at 1.35 x 10^5 cells/head (Yoo et al, 1994. Vaccine 12, 175-180). After 1 day, the polysaccharides dissolved in PBS (phosphate buffered saline, pH 7.3) and filtered by a 0.2 µm filter, were again injected. After 14 days, the lungs of mice were harvested and then fixed in Bouin's solution to count the number of cancer cells having metastasized to the lungs.

The results showed that while the number of cancer cells having colonized the lungs of mice in a group with the polysaccharides not administered was 175 on the average, the number of cancer cells having colonized the lungs of mice in a group with the polysaccharides administered was 5 in the average, which means that the polysaccharides of *Acanthopanax sessiliflorum* fruit can inhibit the metastasis of cancer cells by 97.1%. Also, the polysaccharides from the *Acanthopanax sessiliflorum* and *Acanthopanax senticosus* exhibited the inhibition of cancer metastasis by 92% and 90%, respectively. Accordingly, the polysaccharides from the *Acanthopanax* genus plants can be said to have the excellent prophylaxis ability versus cancer metastasis (refer to FIG. 1).

EXAMPLE 7: Anticancer effect on solid cancers

Sarcoma 180 cell, being a non-epithelial cancer cell line, was abdominally administered at 1x10^6 cells per mouse. 3 days after injection, the polysaccharides obtained in EXAMPLES 1, 2 and 3 were abdominally administered at the concentration of 10 mg/kg 10 times every day, and 4 weeks after administration, the size of tumor was
compared with that of the control group with cancer cells not administered. The above process was repeated for UV2237P cell being an epithelial cancer cell line. The results showed that, in the case of Sarcoma 180 cells, the polysaccharides of *Acanthopanax sessiliflorum* (EXAMPLE 1) inhibited tumor growth by 65%, the polysaccharides of *Acanthopanax sessiliflorum* fruit (EXAMPLE 2) inhibited tumor growth by 70%, and the polysaccharides of *Acanthopanax senticosus* inhibited tumor growth by 63%; and in the case of UV2237P cell, the polysaccharides of *Acanthopanax sessiliflorum* (EXAMPLE 1) inhibited tumor growth by 70%, the polysaccharides of *Acanthopanax sessiliflorum* fruit (EXAMPLE 2) inhibited tumor growth by 88%, and the polysaccharides of *Acanthopanax senticosus* inhibited tumor growth by 82% (refer to FIG. 2).

**EXAMPLE 8:** Effect of promoting bone marrow cell proliferation by oral administration of polysaccharides

Polysaccharides, obtained each in EXAMPLES 1, 2 and 3 and dissolved in distilled water, were orally administered to C3H/HeN mouse (6 weeks, female) in the amount of 250 mg/kg per day for 10 days. Eleven days after the first administration, Peyer’s patch was aseptically separated from the small intestines of mice and then put into RPMI 1640 culture medium. The cell suspension obtained thus was filtered, washed and then adjusted to 2.0 x 10⁶ cells/ml using RPMI 1640 medium (with 10% FBS). The cell solution obtained thus was divided in a 96 well plate by 200 µl and cultivated in an incubator at 37°C and 5% CO₂ for 5 days. A supernatant was separated from the solution to be used for measurement of the activation of proliferation of bone marrow cells by the polysaccharides.

After 5 days, bone marrow cells were harvested from the femur of the same species of mouse, filtered and washed in the same manner as above, and then adjusted to the concentration of 2.5 x 10⁵ cells/ml. 100 µl of the solution obtained thus, 50 µl of the cultivated supernatant obtained by reaction of Peyer’s patch cells and
polysaccharides, and 50 µl of RPMI 1640 culture medium (with 10% FBS) were divided in 96 well plate, respectively, and then cultivated in an incubator at 37°C and 5% CO₂ for 6 days. 15 hours before completion of cultivation, 20 µl of AlamarBlue was added to each well, and the extent of development was measured at the excitation wavelength of 544 nm and the emission of 590 nm using a fluoromètre, or using an ELISA reader (570 nm) (refer to FIG. 3).

The result showed significant proliferation of bone marrow cells in the polysaccharides-administered mice group, compared with that in the control group, which means that the proliferation of bone marrow cells through Peyer’s patch can be caused by the oral administration of polysaccharides.

**EXAMPLE 9: Effect of promoting bone marrow cell proliferation through Peyer’s patch**

Peyer’s patch was aseptically separated from the small intestines of C3H/HeN mice (6 weeks age, female) and put in RPMI 1640 medium. The tissue was pressed with the sterilized metal mesh (100 mesh) to discharge lymphocytes. The cell suspension solution obtained thus was washed and then adjusted to 2.0 x 10⁶ cells/ml using RPM 1640 medium (with 10% FBS). The cell solution was divided in 96 well plate by 180 µl. Polysaccharides obtained each in EXAMPLES 1, 2 and 3 were dissolved in PBS to a concentration of 1 mg/ml, filtered by a 0.2 µm filter, and then divided in each well by 20 µl and cultivated in an incubator at 37°C and 5% CO₂ for 5 days. From the growth media, a supernatant was separated and used for measurement of the proliferation activity of bone marrow cells.

After 5 days, bone marrow cells were harvested from the femur of the same species of mouse, filtered and washed in the same manner as above, and then adjusted to a concentration of 2.5 x 10⁵ cells/ml. 100 µl of the solution obtained thus, 50 µl of the cultivated supernatant obtained from the reaction of Peyer’s patch cells and polysaccharides, and 50 µl of RPMI 1640 culture medium (with 10% FBS) were divided in a 96 well plate, respectively, mixed and then cultivated in the incubator of
37°C and 5% CO₂ for 6 days. 15 hours before completion of cultivation, 20 μl of AlamarBlue was added to each well, and the extent of development was measured at the excitation wavelength of 544 nm and the emission wavelength of 590 nm using a fluorometer, or using ELISA reader (570 nm).

The result showed the significant proliferation of bone marrow cells in the polysaccharides-treated group, compared with that in the control group (refer to FIG. 4).

EXAMPLE 10: Effect of reducing hematopoiesis inhibition induced by anticancer drugs

Generally, anticancer drugs (e.g. cyclophosphamide) work as immune inhibitors in vivo, thereby decreasing the number of cells in the immune system. Accordingly, it is very important to reduce the side effects occurring after administration of anticancer drugs. In the present experiment, the number of immune cells was measured upon co-administration of the polysaccharides of *Acanthopanax* genus plants with cyclophosphamide, which was expected to reduce the side effects occurring after administration of cyclophosphamide.

*Acanthopanax sessiliflorum* polysaccharides (EXAMPLE 1) were dissolved in PBS and filtered by a 0.2 μm filter to prepare a liquid solution for injection in the amounts of 10 mg/kg and 20 mg/kg, respectively. Balb/c mice (female, 8 weeks) were divided into a group where the liquid solution is abdominally administered to five mice 2 days before administration of an anticancer drug, and a group where the liquid solution is abdominally administered to five mice 1 day after administration of an anticancer drug, and the control group. Cyclophosphamide (anticancer drug) was dissolved in PBS and filtered by a 2 μm filter, which was abdominally administered at the concentration of 250 mg/kg. 7 days after administration of cyclophosphamide, the mice were killed to separate the spleen and femur. Then, the number of splenocytes and bone marrow cells was counted using tryphan blue, respectively.
A. Variation of the number of splenocytes

In the mice group (control group) where cyclophosphamide was administered alone, the number of splenocytes decreased by 69.1% compared to a normal mouse. Meanwhile, in the mice group where 20 mg/kg of the Acanthopanax sessiliflorum polysaccharides was administered 2 days before administration of cyclophosphamide, the number of splenocytes was 22.7% higher than in the control group. Also, in the mice group where 20 mg/kg of the polysaccharides was administered 1 day after administration of cyclophosphamide, the number of splenocytes decreased only by 21.5%, compared with a of the normal mouse, which means that the Acanthopanax sessiliflorum polysaccharides can reduce the side effects of the anticancer drug by 47.6% (refer to FIG. 5).

B. Variation of the number of bone marrow cells

The experiment regarding bone marrow cells also showed results similar to the above. While the number of bone marrow cells in the anticancer drug administration group (control group) decreased by 51.7% compared to a normal mouse, the number of bone marrow cells nearly recovered in the mice group where the Acanthopanax sessiliflorum polysaccharides were administered at the concentration of 20 mg/kg after administration of anticancer drug. Moreover, in the mice group where the polysaccharides were administered 2 days before administration of anticancer drug, the number of bone marrow cells were 25.9% higher than that of the control group, which means that the Acanthopanax sessiliflorum polysaccharides can reduce the side effects of the anticancer drug (refer to FIG. 6).

EXAMPLE 11: Effect of reducing hematopoiesis inhibition caused by radiation

Radiation therapy, usually accompanying administration of anticancer drugs in cancer treatment, destroys bone marrow cells and has a negative effect on the generation
and division processes of normal immune cells, thereby deteriorating the functions of hematopoiesis and immune response. In the present experiments, it was examined whether the co-administration of the *Acanthopanax* polysaccharides can reduce the deterioration of immune function in the procedure of radiation treatment.

*Acanthopanax sessiliflorum* polysaccharides (EXAMPLE 1) were dissolved in PBS and filtered by a 0.2 μm filter to prepare a liquid solution for injection in the amounts of 10 mg/kg and 20 mg/kg, respectively. Balb/c mice (female, 8 weeks age) were divided into a group where the liquid solution is abdominally administered to five mice 2 days before irradiation, and a group where the injection solution is abdominally administered to five mice 1 day after irradiation, and the control group. Irradiation was performed by irradiating 4.5 Gy of cobalt (60Co) gamma ray to the whole body of mouse. 5 days and 9 days after irradiation, respectively, the mice were killed to separate the spleen and femur. Then, the numbers of splenocytes and bone marrow cells were counted using tryphan blue.

**A. Variation of the number of splenocytes**

While the number of splenocytes was $6.2 \times 10^5$ cells/ml in a normal mouse, the number of splenocytes was $2.5 \times 10^4$ cells/ml in the mice group where mice were killed 5 days after irradiation, which is 96% less than that of a normal mouse. However, the number of splenocytes increased by 7.2% in the mice group where *Acanthopanax sessiliflorum* polysaccharides were administered at the concentration of 10 mg/kg 2 days before irradiation, compared with the control group. Moreover, in the mice group where mice were killed 9 days after irradiation, the number of splenocytes was 28% higher than the control group. Accordingly, the *Acanthopanax* polysaccharides can be said to have an effect of protecting the immune system against irradiation (refer to FIG. 7).

**B. Variation of the number of bone marrow cells**
5 days after irradiation, the number of bone marrow cells decreased by 96.03% compared with that of a normal mouse. However, in the mice group where *Acanthopanax sessiliflorum* polysaccharides were administered 1 day before irradiation, the number of cells was 6.3% higher than that of the control group. Also, 9 days after irradiation, in the mice group where the polysaccharides were administered at the concentration of 10 mg/kg, the number of cells was 19% higher than that of the control group. This means that the *Acanthopanax* polysaccharides have an effect of protecting and recovering the bone marrow cells of mice exposed to radiation (FIG. 8).

**EXAMPLE 12:** Inhibition of side effects by co-administration of polysaccharides and anticancer drug

Cancer cell line B16-BL16 was intradermally injected to C57BL/6 mice in the amount of $1 \times 10^5$ cells per mouse. *Acanthopanax* polysaccharides, obtained in EXAMPLES 1, 2 and 3, respectively, were dissolved in PBS, filtered by a 0.2 μm filter, and then intravenously injected 4 times at intervals of 3 days, from 1 day after injection of cancer cells, at a concentration of 200 μg per mouse. Cisplatin, an anticancer drug, was intravenously injected at a concentration of 50 μg and 20 μg per mouse, respectively, 4 times at intervals of 3 days, from 1 day after injection of cancer cells. The mice were divided into a mice group where an anticancer drug was administered alone (control group), and a mice group where the *Acanthopanax* polysaccharides were co-administered with the anticancer drug, and the tumor size and body weight of mice were measured.

The tumor size was not remarkably reduced either in the mice group where 20 μg of Cisplatin was administered alone or in the mice group where 20 μg of Cisplatin and polysaccharides were co-administered. However, in the mice group where 50 μg of Cisplatin was administered alone, a remarkable reduction of tumor size was observed but serious side effects, such as the loss of body weight and the decrease of mobility, were also observed. Meanwhile, in the mice group where Cisplatin and the
Acanthopanax polysaccharides were co-administered, the tumor sizes decreased by about 60% (for polysaccharides of EXAMPLE 1), 90% (for polysaccharides of EXAMPLE 2) and 70% (for polysaccharides of EXAMPLE 1) and the inhibition of side effects was observed, i.e., recovery of body weight and mobility. This is anticipated to be caused by the contribution of Acanthopanax polysaccharides to the stimulation of hematopoiesis and activation of the immune system (FIG. 9 and 10).

EXAMPLE 13: Activation of immune cells and generation of anticancer cytokines

Acanthopanax polysaccharides were abnormally administered to C57/BL6 mice at the concentration of 10 mg/kg, and the splenocytes of mice were harvested every hour. By FACS analysis, the extent of the initial immune activity in splenocytes was measured by comparing the immune activation factor CD69. As seen in FIG. 11, the Acanthopanax polysaccharides-administered group exhibited immune system activation associated with NK cells, NKT cells and T cells, compared with the non-administered group (control group). The extent of activation of each immune cell in both groups is described in Table 1, below.

| Table 1 |
|---------|----------|
| NK cells (%) | Cell mean | NKT cells (%) | Cell mean | T cells (%) | Cell mean |
| Control group | 46 | 9.49 | Control group | 78.43 | 11.96 | Control group | 46.75 | 9.44 |
| Acanthopanax group | 95.5 | 22.67 | Acanthopanax group | 88.24 | 31.51 | Acanthopanax group | 55.6 | 17.15 |

In order to measure the amount of cytokine secreted from each immune cell, Acanthopanax polysaccharides were abnormally administered to C57/BL6 mice in the amount of 10 mg/kg and, after 16 hours, splenocytes were harvested. The amount of cytokine, secreted from each immune cell, was measured using a fluorescent-labeled receptor to each immune cell and a cytokine antibody by the intracellular staining.
method.

As seen in FIG. 12, the expression extent of GM-CSF in NK cells of the spleen was 35.6% (cell distribution %) in the control group but 57.39% in the *Acanthopanax* group, and also 8.84 (mean value) in the control group but 10.65 in the *Acanthopanax* group.

The expression extent of GM-CSF in NKT cells of liver was 64.2% (cell distribution %) in the control group but 84.5% in the *Acanthopanax* group, and also 21.9 (mean value) in the control group but 29 in the *Acanthopanax* group.

Concerning the expression extent of IL-12 in macrophages and dendritic cells of liver, the amount of IL-12 cytokine expressed in macrophages and dendritic cells increased, compared with that of the control group. Cells where the expression of IL-12 increased were 32.87% in the control group and 54.45% in the *Acanthopanax* group; therefore, the mean value increased from 9.89 to 12.05.

Concerning the expression extent of IFN-r in NK cells of spleen, the amount of IFN-r cytokine expressed in splenocytes increased, compared with that of the control group. Cells where the expression of IFN-r increased were 47.38% in the control group and 68.91% in the *Acanthopanax* group; therefore, the mean value increased from 4.21 to 5.38 (refer to FIG. 12).

**EXAMPLE 14:** Synergistic anticancer effects upon co-administration of anticancer drug and polysaccharide

Effects of an anticancer drug (Taxol®)-alone administration, polysaccharides-alone administration, and an anticancer drug and polysaccharides-coadministration were evaluated by measuring tumor sizes in athyomic nude mouse (male) as an experimental subject. The anticancer drug was administered, at an amount of 12.5 mg/kg, 7 times at intervals of 3 days from 6 days after injection of tumor cells. *Acanthopanax* polysaccharides were abdominally administered, at an amount of 10 mg/kg, one time 2 days before injection of tumor cells, and at intervals of 3 days after injection of tumor.
cells. The cancer cell line used was PC-3, hypodermically injected in the amount of 2x10^6 cells per mouse. The tumor size was measured from 14 to 31 days after injection of tumor cells. The tumor size in the mice group where anticancer drug and polysaccharides were co-administered was less than in the mice group where anticancer drug was administered alone. This shows that the co-administration of anticancer drug and polysaccharides induces the activation of immune system to exhibit a synergistic effect on the anticancer activity (refer to FIG. 13).

**EXAMPLE 15: Preparation of tablet**

<table>
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<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tr>
<td>Corn starch</td>
<td>34 g</td>
</tr>
<tr>
<td>Crystalline cellulose</td>
<td>10 g</td>
</tr>
<tr>
<td>Brewer’s yeast</td>
<td>40 g</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1 g</td>
</tr>
<tr>
<td><strong>Polysaccharide fraction of EXAMPLE 1</strong></td>
<td>15 g</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100 g</td>
</tr>
</tbody>
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Ingredients of the above recipe were evenly mixed and then compressed by a tableting machine so that tablets could be made at 500 mg/tablet.

**EXAMPLE 16: Preparation of powder drug**

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<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>45 g</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>5 g</td>
</tr>
<tr>
<td><strong>Polysaccharide fraction of EXAMPLE 1</strong></td>
<td>50 g</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100 g</td>
</tr>
</tbody>
</table>

Ingredients of the above recipe were evenly mixed and then filled in a capsule by an assembly machine so that capsules could be made at 500 mg/capsule.
EFFECT OF THE INVENTION

An extract or polysaccharide fraction according to the present invention, extracted from plants belonging to *Acanthopanax* genus, has an effect of inhibiting cancer cell metastasis, whereby it can be used as a drug for cancer treatment, an drug for prophylaxis and treatment of cancer metastasis, and an adjuvant for anticancer drugs and radiation treatment.

Accordingly, a composition according to the present invention, containing the above extract or polysaccharide fraction, has an effect on the overall activation of the immune system, including NKT cells, NK cells, macrophages, T cells, B cells, etc., and the enhancement of cytokine secretion associated with anticancer activity, thereby significantly inhibiting the proliferation and metastasis of cancer cells, so that it can be used as an drug having anticancer and anti-metastasis effects, and an adjuvant for general anticancer drugs and radiation treatment by reducing their side effects, such as the decrease of leucocytes.

As the present invention may be embodied in several forms without departing from the spirit or essential characteristics thereof, it should also be understood that the above-described examples are not limited by any of the details of the foregoing description, unless otherwise specified, but rather should be construed broadly within its spirit and scope as defined in the appended claims, and therefore all changes and modifications that fall within the meets and bounds of the claims, or equivalences of such meets and bounds are therefore intended to be embraced by the appended claims.
WHAT IS CLAIMED IS:

1. A composition for anticancer drug and/or its adjuvant, containing an extract obtained from plants belonging to *Acanthopanax* genus as an active ingredient.

2. The composition according to claim 1, wherein the plant is one or more selected from the group consisting of plants of *Acanthopanax gracillistylus* type: *Acanthopanax gracillistylus*, *Acanthopanax chilsanensis*, *Acanthopanax seoulensis*, *Acanthopanax rufinerve* and *Acanthopanax pedunculus*, plants of *Acanthopanax senticosus* type: *Acanthopanax senticosus*, *Acanthopanax senticosus var. Koreanus*, *Acanthopanax senticosus var. inermis* and *Acanthopanax divaricatus*, plants of *Acanthopanax koreanum* type: *Acanthopanax koreanum*, and plants of *Acanthopanax sieboldianum* type: *Acanthopanax sieboldianum* and *Acanthopanax sessiliflorum*, and the extract was obtained the leaves, stems, roots and/or fruits of them.

3. The composition according to claim 2, wherein the *Acanthopanax* genus plant is *Acanthopanax sessiliflorum* or *Acanthopanax senticosus*.

4. The composition according to claim 1, wherein the extract comprises polysaccharides.

5. The composition according to claim 1, wherein the extract is obtained by heating the *Acanthopanax* genus plant in water (hydrothermal treatment).

6. The composition according to claim 4, wherein the polysaccharides comprise glycoproteins including neutral sugar, uronic acid and protein.

7. The composition according to claim 6, wherein the sugar composition of the polysaccharides includes rhamnose, 2-methyl fucose, fucose, arabinose, 2-methy xylose, xylose, raffinose, aceric acid, mannose, glucose, galactose, galacturonic acid, glucuronic acid, DHA (3-deoxy-D-lyxo-2-heptulosonic acid), and KDO (2-keto-3-deoxyoctulosonic acid).

8. The composition according to one of claims 1 to 7, wherein the composition
has an effect of inhibiting the proliferation and/or metastasis of cancer cells.

9. The composition according to claim 8, wherein the composition has a therapeutic and/or prophylactic effect on solid cancers of epithelial or non-epithelial origin.

10. The composition according to one of claims 1 to 7, wherein the composition has a therapeutic and/or prophylactic effect on cancer.

11. The composition according to one of claims 1 to 7, wherein the composition is effective for at least one of the hematopoiesis enhancement, bone marrow defense, immune enhancement, radiation treatment aid, anti-virus, and anti-germ activities.

12. The composition according to claim 11, wherein the composition has the activity of hematopoiesis enhancement.

13. The composition according to claim 11, wherein the composition has the activity of inhibiting the side effects of general anticancer drugs, including the decrease of leucocytes and thrombocytes.

14. The composition according to claim 11, wherein the composition has the activity of protecting bone marrow and spleen from radiation.

15. The composition according to claim 11, wherein the composition has the activity of enhancing of the immune system including NKT cells, NK cells, macrophages, T cells and B cells.

16. The composition according to claim 11, wherein the composition activates NKT cells, NK cells, macrophages, T cells and B cells to secrete tumor necrosis factors, gamma interferon and interleukin 1, being anticancer material secreted from these cells, thereby having anticancer, anti-microbial and/or anti-virus activities.

17. The composition according to one of claims 1 to 7, wherein the composition further contains an anticancer drug(s) and/or an adjuvant(s) therefor.

18. The composition according to claim 17, wherein the anticancer drug is Taxol,
Cisplatin, cyclophosphamide, or two or more of them.

19. The composition according to one of claims 1 to 7 and 17, wherein the composition further contains a pharmaceutically acceptable carrier or excipient.

20. The composition according to claim 19, wherein the composition contains the pharmaceutically acceptable carrier or excipient of the form of drink or food.

21. A method of co-administering the composition according to one of claims 1 to 7 together with an anticancer drug(s), or performing administration of the composition together with radiation therapy, for the purpose of cancer treatment.

22. The method according to claim 21, wherein the anticancer drug is Taxol, Cisplain, cyclophosphamide, or two or more of them.

23. A drug comprising the composition according to one of claims 1 to 7 and a pharmaceutically acceptable carrier or excipient, for the purpose of hemotopoiesis enhancement, bone marrow defense, immune enhancement, anticancer adjuvant, radiation treatment adjuvant, virus and microbial pathogen protection, cancer prophylaxis, and/or cancer treatment.

24. The drug according to claim 23, wherein the drug further contains an antacid.

25. The drug according to claim 24, wherein the drug is in the form of one of tablet, powder, hard or soft capsule, suspension, solution for injection, emulsion, and non-oral administration form for single or multiple dosage.

26. The drug according to one of claims 23 to 25, wherein the content of the active ingredient is in the range of 0.1 – 6,000 mg/day, based upon the average body weight of an adult.

27. A process of obtaining an extract from plants belonging to Acanthopanax genus (herein, referred to as Acanthopanax), comprising,

a) a step of heating the leaves, stems, roots and/or fruits of Acanthopanax in water (hydrothermal treatment) to obtain a crude extract solution;

b) optionally, a step of concentrating the crude extract solution;

c) a step of precipitating polysaccharides, contained in the crude extract
solution, using an organic solvent to separate the polysaccharides; and
d) a step of removing the organic solution to obtain a polysaccharide fraction.

28. The process according to claim 27, wherein a step is further included, prior to
the step c) and/or d), that the product obtained in the preceding step is purified
by anion exchange chromatography and dialyzed.

29. The process according to claim 27, wherein the hydrothermal treatment is
performed in the range of 50 – 180°C for 0.5 – 20 hours.

30. The process according to claim 27, wherein the organic solvent is a low
molecular weight alcohol or acetone.

31. The process according to claim 30, wherein the low molecular weight alcohol
is ethanol, methanol, isopropyl alcohol or propanol.

32. A composition containing an extract, obtained by the process according to one
of claims 27 to 31.

33. The composition according to claim 32, wherein the extract has a neutral sugar
content of 20 – 80%, an uronic acid content of 20 – 75%, and a protein content
of 0.0001 – 2%.

34. The composition according to claim 32, wherein the extract is polysaccharides
obtained from Acanthopanax sessiliflorum and/or Acanthopanax senticosus,
and has a neutral sugar content of 40 – 80%, an uronic acid content of 20 –
60%, a KDO-like material content of 0.5 – 5%, and a protein content of 0.0001
– 1%.

35. The composition according to claim 34, wherein the molecular weight of the
polysaccharides obtained from Acanthopanax sessiliflorum is in the range of
6,000 to 250,000 Da.

36. The composition according to claim 34, wherein the molecular weight of the
polysaccharides obtained from Acanthopanax senticosus is in the range of
1,600 to 758,000 Da.

37. The composition according to claim 32, wherein the extract is polysaccharides
obtained from the fruits of *Acanthopanax sessiliflorum*, and has a neutral sugar content of 20\% – 40\%, an uronic acid content of 55\% – 75\%, and a protein content of 0.0001\% – 1\%.

38. The composition according to claim 37, wherein the molecular weight of the polysaccharides is in the range of 3,000 to 758,000 Da.
FIG. 10A

![Graph showing body weight changes over time with different treatment groups.]

**Acanthopanax sessiliflorum** polysaccharides

FIG. 10B

![Graph showing body weight changes over time with different treatment groups.]

**Acanthopanax sessiliflorum** fruit polysaccharides
FIG. 10C

![Graph showing body weight changes over different treatments and days.]

_**Acanthopanax senticosus** polysaccharides_

FIG. 11

![Histograms illustrating the counts of untreated and treated samples.]

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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)
A61K35/78

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

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<td>Wang JZ et al. 'Biochemical and morphological alterations of macrophages and spleen cells produced by antitumor polysaccharide from Acanthopanax obovatus roots' In; Planta Med. 1993; 59(1): 54-8</td>
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<td>Shen ML. et al. 'Immunopharmacological effects of polysaccharides from Acanthopanax senticosus on experimental animals' In; Int. J. Immunopharmacol. 1991; 13(5): 549-54</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
05 SEPTEMBER 2003 (05.09.2003)

Date of mailing of the international search report
05 SEPTEMBER 2003 (05.09.2003)

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Facsimile No. 82-42-472-7140

Authorized officer
YEON, Ho Sup
Telephone No. 82-42-481-5627

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