

METHOD FOR PREPARING AN EXTRACT OF *HEVEA* LATEX AND COMPOSITION THEREOF

TECHNICAL FIELD

5 The present invention relates generally to the field of cancer including tumor therapy. More particularly, the present invention relates to the prevention and treatment of cancers and cancer metastasis by using an extract of *Hevea* latex that is rich in low-molecular weight carbohydrates. The present invention also relates to pharmaceutical compositions for use in treating cancers, and to anti-cancer functional food compositions.

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BACKGROUND

Throughout history, mankind has been troubled by cancers, which result from the inability of the body to control the growth and the spreading of certain malignant cells. According to the World Health Organization, cancers as a group is a major noncommunicable disease among the top four: cardiovascular disease, cancers, diabetes, and chronic lung diseases. The American Cancer Society reported that, in 2012, the number of diagnosed new cancer cases worldwide was 14.1 million, with 8.2 million deaths. It has been predicted that by 2030 the number of new cancer cases may reach 21.7 million, with 13 million deaths (IARC. 2012. Global Cancer Facts & Figures, 3rd edition, produced by the American Cancer Society in partnership with the International Agency for Research on Cancer).

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Different strategies exist for cancer chemotherapy. Traditional brute-force cytotoxic cancer chemotherapeutic agents often involve considerable side effects to normal cells. Even with monoclonal antibody targeting, some level of undesirable side effects is to be expected.

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More recently, cancer has been found to be closely related to biosignaling pathways that control the proliferation and destruction of body cells. Chemical agents that manipulate such pathways may be useful as anti-cancer agents. Small molecule targeted therapy drugs are generally inhibitors of catalytic or binding sites on mutated, overexpressed, or otherwise critical proteins within the cancer cells but only work for certain types of cancers. Other modern anti-cancer agents may work by acting as signals to stimulate the immune system of the patients.

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Many natural product-based anti-cancer treatments and medications are known in the prior arts. These active therapeutic agents range from small biomolecules to large macromolecules. An example of small anti-cancer biomolecules found in plant extracts, as disclosed in US patents number 6,432,452 (Anti-cancer compounds), is the angeloyl-substituted ingenane, a macrocyclic diterpene which competitively blocks the carcinogenic effects of phorbol ester, itself another macrocyclic diterpene. At the other end of the spectrum, peptide allergens in *Hevea* has been used in pharmaceutical preparations to induce immune response in the treatment of diseases including cancer. Examples are disclosed in PCT application numbers PCT/EP2003/011190 (Modular antigen transporter molecules for modulating immune reactions, associated constructs, methods and uses thereof), and PCT/EP2013/000291 (Pharmaceutical composition comprising a polymeric carrier cargo complex and at least one protein or peptide antigen), and also in European Patent application numbers EP2012/000418 (Negatively charged nucleic acid comprising complexes for immunostimulation), and EP2012/000420 (Pharmaceutical composition comprising a polymeric carrier cargo complex and at least one protein or peptide antigen).

In between the small molecules with molecular weights less than a thousand Dalton (hereinafter referred to as “kD”) and macromolecules with molecular weights of over 10 kD, are biochemical anti-cancer agents with molecular weight of a few kD, as shown in European Patent publication number 0 589 074 (Carbohydrate complexes for destruction of resistant cancer cells). Some researchers believe that these low-molecular weight carbohydrates stop cancer cells growth and metastasis by interfering with cell-cell interaction mediated by cell surface carbohydrate binding molecules, as disclosed in US Patent 5,834,442 (Method for inhibiting cancer metastasis by oral administration of soluble modified citrus pectin), and US Patent 5,895,784 (Method for treatment of cancer by oral administration of modified pectin).

It is known in the prior art that certain animal-derived low-molecular weight carbohydrates, have anti-cancer effect. Examples include hyaluronan (Ween, M.P., K. Hummitzsch, R.J. Rodgers, M.K. Oehler, and C. Ricciardelli. 2011. Versican induces a pro-metastatic ovarian cancer cell behavior which can be inhibited by small hyaluronan

- oligosaccharides. *Clinical and Experimental Metastasis* **28**; 113—125), maltohexose sulphate (Parish, C.R., C. Freeman, K.J. Brown, D.J. Francis, and W.B. Cowden. 1999. Identification of Sulfated Oligosaccharide-based Inhibitors of Tumor Growth and Metastasis Using Novel *in Vitro* Assays for Angiogenesis and Heparanase Activity. *Cancer Research* 59:3433-3441) and heparinomimetic phosphomannopentaose sulphate (PI-88) (Demir, M' O. Iqbal, D.A. Hoppensteadt, P. Piccolo, S. Ahmad, C.L. Schultz, R.J. Linhardt, and J. Fareed. 2001. Anticoagulant and Antiprotease Profiles of a Novel Natural Heparinomimetic Mannopentaose
- 10 Phosphate Sulfate (PI-88). *Clinical and Applied Thrombosis/Hemostasis* 7:131-140). Such anticancer effect may result from a combination of direct cytotoxicity, enhancement of chemotherapeutic agents' efficacy, and blockage of carcinogenesis. The antiproliferative effects have been shown for different types of human cancer, including ovarian cancer, colon cancer, prostate cancer (Khasraw, M., N. Pavlakis, S. McCowatt, C. Underhill, S. Begbie, P. de Souza, A. Boyce, F. Pamis, V. Lim, R. Harvie, and G. Marx. 2010. Multicentre phase I/II study of PI-88, a heparanase inhibitor in combination with docetaxel in patients with metastatic castrate-resistant prostate cancer. *Annals of Oncology* **21**:1302-1307), and astrogloma (Camby, I., C. Decaestecker, L. Gordower, R. DeDecker, Y. Kacem, A. Lemmers, H.C. Siebert, N.V. Bovin, P. Wesseling, A. Danguy, I. Salmon, H.J. Gabius, R. Kiss. 2001. Distinct differences in binding capacity to saccharide epitopes in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas, and glioblastomas. *Journal of Neuropathology and Experimental Neurology* 60:75—84). Nevertheless, animal-derived low-molecular weight carbohydrates are relatively expensive to manufacture in large scale and have not been used commercially in anti-cancer formulations.
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- As disclosed in US Patent 8,722,107 (Modified pectins, compositions and methods related thereto), some plant-derived low-molecular weight carbohydrates, especially modified pectins, have been shown to have anti-cancer effect as well. Examples include low-molecular weight carbohydrates from the fruit of the apple tree. (Li, Q., Z. Zhou, J. Jing, T. Yang, S. Duan, Z. Wang, Q. Mei, and L. Liu. 2013. Oligosaccharide from apple induces apoptosis and cell cycle arrest in HT29 human colon cancer cells. *Int. J. Biol. Macromol.*
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57:245-254) and modified pectin from the fruits of the citrus trees (US Patents 5,834,442 and 5,895,784 as cited above). The disadvantages of using plant-derived low-molecular weight carbohydrates, especially modified pectin, in anti-cancer formulations comprise the cost of raw materials (for example, apple or lemon) that are commodity goods and the multi-step extraction and modification of pectin into low-molecular weight carbohydrates. Unavoidable contaminations of trace organic solvents in the final low-molecular weight carbohydrate products may also pose long-term health issues.

Instead of using raw materials based on edible plants or on fruits like citrus or apples, the inventor surprisingly found that a more economically advantageous raw material for an anti-cancer extract is the serum that is obtained when acidified *Hevea* latex is calendered. It was also surprisingly found that this starting material, in conjunction with the extraction method disclosed herein, produces more uniform and reproducible products compared to other methods. Although it is highly unlikely that the chemical composition of the *Hevea* extract according to the present invention is the same as that of the so-called "low-molecular weight" carbohydrates cited in the prior arts due to differences both in the starting materials and in the extraction processes and conditions, the *Hevea* extract according to the present invention has been found to be active against cancer. Additionally, a relatively safe alcohol, such as ethanol, is used in the final precipitation step according to the present invention to eliminate any long-term health problems.

Although fractions of natural rubber latex obtained from the soft bark of the *Hevea brasiliensis* tree have been shown to possess some anti-cancer property (Lam, K.L., K.L. Yang, E. Sunderasan, and M.T. Ong. 2012. Latex C-serum from *Hevea brasiliensis* induces non-apoptotic cell death in hepatocellular carcinoma cell line (HepG2). *Cell Prolif.* **45(6)**: 577-585; Lee, Y.K., L.K. Lay, M.S. Mahsufi, T.S. Guan, S. Elumalai, and O.M. Thong. 2012. Anti-proliferation effect of *Hevea brasiliensis* latex B-serum on human breast epithelial cells. *Pak. J. Pharm. Sci.* 25(3):645-650; Sunderasan, E., N. Abd. Rahman, K.L. Lam, K.L. Yang, and M.T. Ong. 2013. Cell viability assay guided fractionation of natural rubber latex sera. *J. Rubb. Res.* 16(3):195-202), it is not economical to use natural rubber latex *per se* as starting material due to the inefficient non-utilization of the solid rubber fraction of the latex.

It is well-known that when fresh *Hevea* latex is coagulated with acid, such as formic acid, and then calendered (squeeze milled), the liquid released from the milling process (hereinafter referred to as the “serum”) contains excess, unreacted, formic acid as well as many biomolecules that are present originally in the rubber latex. This serum can serve as an inexpensive source of starting material for biomolecules of high added values, especially those with therapeutic properties. The present invention utilizes this serum as the starting material for preparing a mixture of low-molecular weight carbohydrates.

- Unfortunately, in addition to containing carbohydrates, the *Hevea* latex serum comprises many other biomolecules, including allergenic proteins such as Hev b1, Hev b2, Hev b4, Hev b5, and Hev b6.02. (Chen, Z., R. Cremer, A. Posch, M. Raulf-Heimsoth, H-P. Rihs, and X. Baur. 1997. "On the allergenicity of Hev b 1 among health care workers and patients with spina bifida allergic to natural rubber latex". *Journal of Allergy and Clinical Immunology* 100(5):684-93; Barre, A., R. Culeirier, C. Granier, L. Selman, W.J. Peumans, E.J.M. Van Damme, F. Bienvenu, J. Bienvenu, and P. Rouge. 2009. ^MMapping of IgE-binding epitopes on the major latex allergen Hev b 2 and the cross-reacting 1, 3p-glucanase fruit allergens as a molecular basis for the latex-fruit syndrome". *Molecular Immunology* 46(8-9):1595-604; Kolarich, D., F. Altmann, and E. Sunderasan. 2006. "Structural analysis of the glycoprotein allergen Hev b 4 from natural rubber latex by mass spectrometryⁿ. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1760(4):715-20**; Beezhold, D.H., V.L. Hickey, J.E. Slater, and G.L. Sussman. 1999. "Human IgE-binding epitopes of the latex allergen Hev b 5". *Journal of Allergy and Clinical Immunology* **103(6):1166-72**; Reyes-López, C.A., A. Hernández Santoyo, M. Pedraza-Escalona, G. Mendoza, A. Hernández-Arana, and A. Rodríguez-Romero. 2004. ^MInsights into a conformational epitope of Hev b 6.02 (hevein)*'. *Biochemical and Biophysical Research Communications* 314(1):123-30; Pedraza-Escalona, M., B. Becerril-Luján, C. Agundis, L. Dominguez-Ramirez, A. Pereyra, L. Riaño-Umbarila, and A. Rodriguez-Romero. 2009. ^{*}'Analysis of B-cell epitopes from the allergen Hev b 6.02 revealed by using blocking antibodies^f. *Molecular Immunology* 46(4):668-76.) The present invention accordingly needs to provide steps to remove proteinaceous substances, including enzymes with known allergenic epitopes.

It is an objective of the present invention, therefore, to provide a method for preparing a Hevea latex extract that has anti-cancer activities.

5 It is also an objective of the present invention to provide a method for preparing a *Hevea* latex extract that has anti-metastatic activities on proliferating cancer cells.

It is also an objective of the present invention to provide a method for preparing low-molecular weight carbohydrates from a relatively inexpensive biological starting material.

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It is also an objective of the present invention to provide a method for preparing a *Hevea* latex extract that is enriched with low-molecular weight carbohydrates.

It is also an objective of the present invention to provide a method for preparing an extract
15 of *Hevea* latex such that the low-molecular weight carbohydrates in the final product have a relatively uniform and reproducible molecular composition.

It is also an objective of the present invention to provide a method for preparing an extract
20 of *Hevea* latex such that the final product is devoid of proteinaceous substances, especially allergen proteins.

It is also an objective of the present invention to provide a method for preparing a *Hevea* latex extract that is safe to be used in pharmaceutical and food compositions. Safety in this case comprises the absence of acute, sub-acute, and chronic toxicities as determined by
25 relevant protocols.

It is also an objective of the present invention to provide a simple, efficient, and relatively inexpensive method for preparing a *Hevea* latex extract that has anti-cancer and anti-metastatic activities.

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It is also an objective of the present invention to provide a method for preparing and extract of *Hevea* latex such that the method is easily scalable for commercialization.

It is also an objective of the present invention to utilize the anti-cancer activities of the *Hevea* latex extract and to produce a product that can be used therapeutically as an anti-cancer medication and prophylactically as an anti-cancer food supplement.

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SUMMARY OF INVENTION

The invention herein described is directed to provide an extract of *Hevea* latex that is rich in low-molecular weight carbohydrates and having anti-cancer activity.

10 One aspect of the disclosed invention is directed towards a method for preparing an extract of *Hevea* latex comprising the steps of: obtaining the serum released when acidified *Hevea* latex is calendered; optionally removing debris and micro-organisms from the serum; removing proteins; removing quebrachitol and small polar molecules; optionally dissolving the remaining solid powder in water; further removing proteins by treatment with acid;
 15 optionally neutralizing the excess acid; precipitating with solvent; collecting the precipitate; and optionally freeze drying the precipitate. Debris and micro-organisms are removed by technique comprising filtration. Proteins are removed by biochemical techniques comprising ultrafiltration and/or spray drying. Quebrachitol and small polar molecules are removed by extracting with a solvent such as methanol and collection of the
 20 remaining undissolved solid powder. Any leftover proteins are further removed by treatment with acid such as trichloroacetic acid. The remaining mixture is optionally adjusted to neutral pH and the final product is separated by precipitating with a cold organic solvent such as ethanol. The precipitate is collected by centrifugation and optionally freeze dried.

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Another aspect of the invention is directed towards a method for treating or preventing cancer growth and/or metastasis in a subject comprising administering to the subject an extract of *Hevea* latex, which is prepared by the process according to the present invention, in an effective amount to induce death of the cancer cells.

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In another aspect of the invention, a method for inhibiting tumor cell proliferation in a patient is described. The method comprises administering to the patient *Hevea* latex

extract according to the present invention in an effective amount to achieve a favorable change in one or more of the following markers: decreasing CD31 endothelial cell marker; decreasing VEGF angiogenic marker; decreasing COX-2 inflammatory marker; decreasing EGFR proliferation marker; increasing apoptosis of cancer cells; and decreasing galectin-3 metastasis marker.

Another aspect of the invention is a composition comprising an extract of *Hevea* latex according to the present invention and one or more pharmaceutically acceptable substances. Yet another aspect of the invention is a composition comprising an extract of *Hevea* latex according to the present invention and one or more edible ingredients. The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one, " but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

These, and other, embodiments of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating various embodiments of the invention and numerous specific details thereof is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such substitutions, modifications, additions and/or rearrangements.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The present invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 is a flow diagram that illustrates the origin of the raw material, *Hevea* latex serum.

Figure 2 is a flow diagram that illustrates the steps in preparing an extract from *Hevea* latex serum according to the present invention.

Figure 3 is a flow diagram that illustrates the best mode embodiment of the invention.

Figure 4 is a Fourier transform infrared (FT-IR) spectrum that illustrates a possible composition of the extract from *Hevea* latex according to the best mode embodiment of the invention.

Figure 5 is the liquid chromatography - mass spectrometry (LC-MS) molecular mass profile of the extract of *Hevea* latex according to the best mode embodiment of the invention.

Figure 6 illustrates the size measurement of a tumor in a nude mouse using a vernier calliper.

Figure 7 shows a time course of relative tumor volume in nude mice that received the extract of *Hevea* latex, according to the best mode embodiment of the invention, to illustrate the anti-cancer property. (Dashed line) Experimental group that received daily oral gavage of 60 mg *Hevea* latex extract per kg body weight for 28 days. (Solid line) Control group that received water instead of the *Hevea* latex extract.

Figure 8 illustrates how the dorsal skin fold chamber is surgically attached to a nude mouse in order to visualize angiogenesis. The diameter of the dorsal skin fold chamber is approximately 7 millimeter.

Figure 9 illustrates the suppression of angiogenesis in nude mice with cancer implant in the dorsal skin fold chamber. The mouse was first anaesthetized. A fluorescein isothiocyanate-dextran (FITC-dextran) solution was then transfused into a jugular vein in order to label the blood vessels. The dorsal skin fold chamber, along with the skin with a cancer implant, was surgically removed and observed using a technique called “intravital

confocal fluorescence video microscopy.” (Left) No cancer control. (Middle) With cancer implant. (Right) With cancer implant and extract of *Hevea* latex according to the best mode of the invention.

5 **Figure 10** illustrates the suppression of angiogenesis in nude mice with cancer implant by daily oral gavage of 60 milligram (hereinafter referred to as “mg”) *Hevea* latex extract per kilogram (hereinafter referred to as “kg”) body weight compared to controls which received water. Visualization by immunohistochemistry was performed using CD31 endothelial cell marker. (Left) No cancer control. (Middle) With cancer implant. (Right)
10 With cancer implant and extract of *Hevea* latex according to the best mode of the invention. Bar = 100 micrometer.

Figure 11 illustrates the suppression of angiogenesis in nude mice with cancer implant by daily oral gavage of 60 mg *Hevea* latex extract per kg body weight compared to controls
15 which received water. Visualization by immunohistochemistry was performed using VEGF angiogenic marker. (Left) No cancer control. (Middle) With cancer implant. (Right) With cancer implant and extract of *Hevea* latex according to the best mode of the invention. Bar = 100 micrometer.

20 **Figure 12** illustrates the reduction of inflammation in nude mice with cancer implant by daily oral gavage of 60 mg *Hevea* latex extract per kg body weight compared to controls which received water. Visualization by immunohistochemistry was performed using COX-2 inflammatory marker. (Left) No cancer control. (Middle) With cancer implant. (Right) With cancer implant and extract of *Hevea* latex according to the best mode of the
25 invention. Bar = 100 micrometer.

Figure 13 illustrates the reduction of cell divisions in nude mice with cancer implant by daily oral gavage of 60 mg *Hevea* latex extract per kg body weight compared to controls which received water. Visualization by immunohistochemistry was performed using EGFR proliferation marker. (Left) No cancer control. (Middle) With cancer implant. (Right) With
30 cancer implant and extract of *Hevea* latex according to the best mode of the invention. Bar = 100 micrometer.

Figure 14 illustrates the increase in apoptosis in nude mice with cancer implant by daily oral gavage of 60 mg *Hevea* latex extract per kg body weight compared to controls which received water. Visualization was performed using terminal deoxynucleotidyl transferase (TdT)- mediated deoxyuridine triphosphate nucleotide (dUTP) nick end labelling (TUNEL) method. (Left) No cancer control. (Middle) With cancer implant. (Right) With cancer implant and extract of *Hevea* latex according to the best mode of the invention. Bar = 100 micrometer.

Figure 15 shows the results of an experimental tail vein metastasis assay. Mice were injected through their tail veins with 1×10^5 melanoma B16-F1 cells. Bottom row figures show dissected lungs of these mice that were daily fed with 60 mg/kg *Hevea* latex extract for 2 weeks. Top row figures show lungs from the control mice that were fed with water. Black spots show colonies of metastasized melanoma cells.

Figure 16 shows the results of an immunohistochemistry staining assay for galectin- 3, a metastasis marker. (Left) No cancer control. (Middle) Mice were injected through their tail veins with 1×10^5 melanoma B16-F1 cells and fed with water instead of *Hevea* latex extract. (Right) Mice were injected through their tail veins with 1×10^5 melanoma B16-F1 cells and then fed with 60 mg/kg *Hevea* latex extract daily for two weeks. Bar = 100 micrometer.

The figures and written description are not intended to limit the scope of the disclosed method for preparing an extract of *Hevea* latex in any manner. Rather, the figures and written description are provided to illustrate the disclosed method for preparing an extract of *Hevea* latex to a person having ordinary skill in the art by reference to particular embodiments of the invention. The invention will now be described with such particularity as to enable any person skilled in the pertinent art to practice the invention without extensive experimentation.

DETAILED DESCRIPTION

Referring to **Figure 1**, the well-known process for preparing *Hevea* serum starts with fresh latex **101** that is collected from rubber trees. An acid, usually formic acid, is added to

coagulate the latex. The coagulation with acid **102** converts the rubber isoprene polymer particles in the latex into coagula that start to separate from the liquid. This separation is expedited by squeeze milling, or calendering **103**, that produces solid rubber, sheet rubber **104**, as an output. The liquid released from this squeeze milling step is called the *Hevea* latex serum **105**. The process according to the present invention uses this serum **105** as the starting material.

Referring to **Figure 2**, the present invention provides a method for preparing an extract of *Hevea* latex. The method according to the invention, comprises the steps of: obtaining the serum **205** released when acidified *Hevea* latex is calendered (This serum is identical to *Hevea* latex serum **105** in **Figure 1**.); optionally removing debris and micro-organisms **206**; removing proteins **207**; removing quebrachitol and small polar molecules **208**; optionally dissolving the remaining solid powder in water **209**; further removing proteins by treatment with acid **210**; optionally neutralizing the excess acid **211**; precipitating with solvent **212**; collecting the precipitate **213**; and optionally freeze drying the precipitate **214**, to produce the *Hevea* latex extract **215**.

Steps **206-214**, inclusive, comprise conventional biochemical and biophysical techniques that are separately known to persons skilled in the art.

Logically, steps **207**, **208**, and **210** do not need to be practiced in this particular sequence. Persons skilled in the art should be able to rearrange, substitute, and alter the details of these three steps. The best mode for carrying out the invention, nevertheless, will illustrate that arranging steps **207**, **208**, and **210** in this particular sequence has a practical and logistical advantage when the method is practiced.

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Detailed descriptions for successive steps in **Figure 2** are given below:

Step **206** is optionally needed if the latex serum is contaminated by debris and micro-organisms such as bacteria, which depends on how clean the *Hevea* Latex has been treated. One example of the removal technique is filtration through a filter with pore size small enough to exclude debris and micro-organisms, typically smaller than 0.5 micrometer and preferably 0.2 micrometer. Although smaller pore sizes can exclude even finer debris and particles, the filtration process gets slower as the pore size gets smaller. Since the inventor

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did not find any negative effect from possible contaminations of ultra-fine debris and nanoparticles, 0.2 micrometer filter pore size seems to be the best trade-off.

Step **207** comprises removal of proteins, especially protein allergens that are naturally found in *Hevea* latex. This step comprises a combination of biochemical techniques such as enzymatic digestion, ultrafiltration, and spray drying at elevated temperatures. An example of step 207 is to destroy proteins by any commercial proteolytic enzyme that is cost-effective and retains its biological activity in the environment of the *Hevea* latex serum. Another example of step **207** is to filter the liquid through an ultrafiltration membrane with a cutoff molecular mass of at least 3 kD and preferably 10 kD. Another example of step **207** is to spray dry the liquid at a temperature above 60 degree Celsius, preferably above 100 degree Celsius and most preferably between 100 and 120 degree Celsius. In case ultrafiltration and spray drying are used in tandem, any protein that gets through the ultrafiltration membrane should then be denatured and deprived of its biological activity by the high temperature spray drying.

Step **208** comprises the removal of quebrachitol and small polar molecules from the serum. The importance of this step will be apparent when the *Hevea* latex extract according to the present invention is combined with one or more pharmaceutically acceptable substances in order to form a composition to be used as anti-cancer medication. The importance will also be apparent when the *Hevea* latex extract is combined with one or more edible ingredients in order to form a composition to be used as anti-cancer functional food. In order for the medication, or the functional food, to carry out the method for treating cancer growth and/or metastasis to achieve the death of cancer cells, or the method for inhibiting tumor cell proliferation in a patient to achieve favorable changes in one or more suitable markers or indicators for angiogenesis (decreasing VEGF), inflammatory (decreasing COX-2), proliferation (decreasing EGFR), apoptosis, and metastasis (decreasing galectin-3), an effective amount of the medication or the functional food has to be consumed by the cancer patient. Compliance of the patient depends, *inter alia*, on the lack of undesirable side effect such as diarrhoea that is known to be caused by quebrachitol. Accordingly, the main purpose of step **208** is to remove this undesirable compound. Many other small polar molecules, including nucleotides, if present, would be removed in this step as well.

An example of step **208** is an extraction by a suitable mixture of polar solvents such as methanol, which tend to dissolve polar molecules, including quebrachitol, leaving other less polar molecules undissolved. The methanol concentration should be at least 70 per cent, preferably 100 per cent. The extraction may be performed repeatedly, preferably
5 twice more, to decrease the concentration of quebrachitol adequately for further uses.

Step **210** comprises further removal of proteins by precipitating with acid in order to ensure more complete protein removal, especially small proteins and peptides that might have survived step **207**. An example of step **210** is precipitation with 1 to 50 per cent,
10 preferably from 2 to 10 per cent, and most preferably between 2 and 3 per cent of trichloroacetic acid at temperature above 4 degree Celsius, preferably between 16 and 30 degree Celsius, and most preferably between 20 and 25 degree Celsius. The time it takes to precipitate out proteins depends, *inter alia*, on the concentration of the acid used and the temperature at which the precipitation takes place. Practically, the precipitation is visible to
15 the naked eyes in about 0.1 minute (almost immediately) after the acid is added. The optimum incubation time for the precipitating reaction can be determined by a person skilled in the art of biochemistry. Nevertheless, it is wise to allow ample time, preferably 24 hours, for the precipitate to fully form.

20 Step **209** serves as a transition from step **208** to step **210**. In this step, the solid powder that remains undissolved from step **208** is optionally dissolved in a small volume of water in order to aid the precipitation reaction in step **210**.

Step **211** serves to neutralize any excess acid from step **210**. Neutralization is normally
25 canied out with addition of sodium hydroxide. This step is considered optional since the amount of the added base depends on the amount of the leftover acid.

Steps **212** and **213** involve separation of the final product, which is devoid of proteins but rich in low-molecular weight carbohydrates, for example, by precipitation at low
30 temperature with a suitable mixture of solvents such as ethanol and water. The concentration of ethanol should be at least 70 per cent, preferably 100 per cent. Precipitation is carried out below 4 degree Celsius, preferably below -10 degree Celsius,

and most preferably at -20 degree Celsius. Although precipitation is visible in about 5 minutes, it is recommended to allow ample time of 24 hours for a maximum precipitation. Collection of the final product may be achieved, for example, by at least 3,000 x g centrifugation for at least 5 minutes, preferably for 10 minutes. This precipitate is
5 optionally freeze dried in step **214**. The final product is the anti-cancer *Hevea* latex extract **215**. If the extract is to be used right away, step **214** can be omitted.

The above description reveals that the method according to the present invention automatically enriches the latex extract with low-molecular weight carbohydrates without
10 any explicit digestion step for two reasons. Firstly, utilizing the serum **105**, or equivalently **205**, as raw material carries an inherent advantage of having carbohydrates that are originally present in the fresh *Hevea* latex **101** at least partially digested down to low-molecular weight carbohydrates in the acid coagulation step **102**. In other words, compared to fresh *Hevea* latex, the raw material used according to the present invention is
15 already partially enriched in low-molecular weight carbohydrates. Secondly, if spray drying at an elevated temperature is used in the removal of protein step **207**, the high temperature may contribute to breaking down some of the carbohydrates. Finally, the further removal of proteins by acid step **210** also helps to further hydrolyze any remaining carbohydrates into low-molecular weight carbohydrates. In practice, the time, temperature,
20 and concentrations of reactants in each step must be controlled to ensure that the resulting extract **215** has a uniform and reproducible composition of low-molecular weight carbohydrates. The inventor has unexpectedly discovered that the method according to the best mode of the present invention gives the most uniform and best reproducibility in the composition of low molecular weight carbohydrates compared to other methods and
25 embodiments. Again, it should be emphasized that the exact chemical nature of the low molecular weight carbohydrates according to the present invention is expected to be different from that of the so-called "low molecular weight" carbohydrates according to the prior arts, due to differences in the starting materials and in the extraction processes.

30 Another advantage of the present invention is that, if spray drying at elevated temperature is utilized in the removal of protein step **207**, any protein that is not physically

removed in other steps will be denatured by heat and will lose its three-dimensional disposition of any possible antigenic epitope, rendering such protein non-allergenic.

5 The following example is included to demonstrate preferred embodiment or the best mode of the invention. It should be appreciated by persons having ordinary skill in the art that the techniques disclosed in the example which follows represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred mode and best mode for its practice. However, those skilled in the art should, in light of the present disclosure, appreciate that many changes
10 can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention. In other words, the best mode, as well as other exemplary embodiments of the present invention must not be construed as any limitation of the invention.

15 Best Mode for Carrying Out the Invention

The best mode of carrying out the invention known at present is to use the method according to the Example below.

20 **Figure 3** serves to illustrate the best mode for carrying out the present invention. The best mode starts with obtaining the serum **305**, which is released when acidified *Hevea* latex is calendered. The starting material **305** refer to the same latex serum **105** in **Figure 1** and **205** in **Figure 2**. The final product **315** corresponds to the same product **215** in **Figure 2**.

25 In this best mode, the filtration step **306** to remove debris and micro-organisms comprises filtration through a membrane with a pore diameter of 0.2 micrometer.

Again in this best mode, the protein removal step **307** consists of ultrafiltration **316** through an ultrafiltration membrane with a cutoff molecular weight of 10 kD, followed by
30 spray drying **317** at a temperature of 110 degree Celsius. Since ultrafiltration and spray drying are used in tandem, any protein that passes through the ultrafiltration membrane would then be denatured and deprived of its biological activity by the high temperature

spray drying. This allows the use of ultrafiltration membrane with 10 kD cutoff molecular weight instead of 3 kD membrane, which is more expensive and produces slower ultrafiltration rate at the same pressure. Any allergenic protein with molecular weight between 3 and 10 kD, such as Hev b 6.02, which is a 4.72 kD polypeptide, which would
5 pass through the 10 kD ultrafiltration membrane, would be deactivated by the high temperature in the spray drying process. The inventor has found that the spray drying should be performed at a temperature above 60 degree Celsius, preferably above 100 degree Celsius, and most preferably between 100 and 120 degree Celsius. Although higher spray dry temperatures may allow for faster drying, better protein denaturation, and
10 possibly production of more short-chain carbohydrates, they present a risk of altering the sugar structure and the color of the product.

According to the present invention, in order to remove small polar molecules, including
15 nucleotides and especially quebrachitol, the solid powder from the spray drying step 317 is then extracted **308** with a mixture of at least 70 per cent aqueous methanol, which is a relatively polar organic solvent that is also relatively inexpensive. In the best mode, the extraction is performed with absolute methanol at 1:6 weight to volume ratio, i.e. 1 weight of the solid powder to 6 volume of solvent. The extraction is repeated twice more, again
20 with methanol, at 1:4 weight to volume ratio, for a total of three times.

The remaining solid powder from the methanol extraction step **308** is dissolved in distilled water **309** at 1:8 weight to volume ratio. Trichloroacetic acid is added in step **310** to achieve a final concentration of 2.5 per cent. The mixture is stirred for 24 hours at 25
25 degree Celsius. The precipitate is removed and discarded by 3,000 x g centrifugation for 5 minutes. Although the function of this precipitation step is to remove proteins, macromolecules including nucleic acid polymers, if present, would be removed as well.

In the best mode, the supernatant from step **310** is then neutralized with sodium hydroxide
30 **311** and then precipitated **312** with absolute ethanol. The temperature is kept at -20 degree Celsius. After 24 hours, the precipitate is collected in step **313** at 3,000 x g centrifugation

for 5 minutes. The precipitate is freeze dried in step **314** to produce the *Hevea* latex extract **315** that can be kept in a deep freezer for an extended period, typically several months.

In the best mode embodiment disclosed above, the logistics for proteins and small polar molecules removal starts with ultrafiltration **316** followed by spray drying **317** then methanol extraction **308** and finally treatment with trichloroacetic acid **310**. This sequence ensures that the large volume of the raw material **305** that has been cleared of debris and micro-organisms by filtration **306** is reduced by spray drying **317** to solid powder so as to facilitate the steps of methanol extraction **308** and treatment with trichloroacetic acid **310**.

10 The sequence of steps disclosed in this best mode example is the most practical and effective way to prepare the *Hevea* latex extract according to the present invention known to the inventor at the time of filing the PCT application.

The yield of the best mode embodiment is approximately 0.17 per cent (weight of latex extract to serum volume). In an actual representative preparation using the best mode of the present invention, 9,650 litre of fresh *Hevea* latex **101** released 4,147 litre of serum **105** after calendering **103**, which served as the raw material **305** to the best mode embodiment of the present invention. After the ultrafiltration step **316**, the volume was reduced to 3,869 litres. The spray drying step **317** gave 102 kg of solid powder. The ethanol precipitation step **312** and the freeze drying step **314** gave 7 kg of the final product **315**.

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Described below are representative test results obtained from the product of the best mode embodiment of the present invention. These results are presented to show the preliminary characterisation and the preliminary safety test results of the *Hevea* latex extract according to the best mode of the present invention.

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(1) Preliminary Characterization of the Extract

Figure 4 shows a Fourier transform infrared (FT-IR) spectrum that illustrates a possible composition of the extract from *Hevea* latex. The horizontal axis represents the wavenumber from 4000 to well under 1000 cm^{-1} . The vertical axis represents per cent transmittance. Major absorption peaks comprise 3392.75, 1619.95, 1418.21, 847.55, and 526.14 cm^{-1} . Although the 1081.41 cm^{-1} absorption peak cannot be further resolved, it is

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fairly close to the theoretical 1078.21 cm^{-1} and 1079.77 cm^{-1} absorption peaks that result from stretching of the beta 1,3 glycosidic bond of beta-1, 3-glycan.

Figure 5 shows the liquid chromatography-mass spectrometry (LC-MS) molecular mass profile of the extract of *Hevea* latex. The horizontal axis of this particular histogram has been prepared to represent the mass of the molecular fragments from below 2000 to 6000 Dalton. The vertical axis represents the per cent of a particular fragment in the sample. The graph illustrates the size distribution of the molecular mass of the components in the extract, with centers around 3712.1 Dalton. Representative molecular masses around the center peak are labeled both above (3713.6, 3744.8, 3759.1, 3800.1, and 3824.6 Dalton) and below (3705.6, 3651.0, 3635.8, and 3554.5 Dalton) the peak. If this molecule was in fact a carbohydrate, the center molecular weight would correspond to a polymer of about 20 saccharide residues.

From **Figures 4** and **5**, and the fact that the method according to the present invention is designed to remove proteins, nucleic acids, and other polar molecules, it is likely that the *Hevea* extract 315 contains low-molecular weight carbohydrates of about 20 saccharide residues.

(2) Preliminary Safety Test Results

The extract from *Hevea* latex according to the present invention has been tested for acute toxicity, 6-week sub-acute toxicity, and 9-month chronic toxicity.

From an acute toxicity test performed according to OECD guideline No. 420, feeding of 1 gram *Hevea* latex extract per kg body weight of rats does not result in any change in body weight, alertness, breathing, muscular force, urine color and fecal appearance, compared to a negative control group.

When 6 male rats were fed daily with 1 gram *Hevea* latex extract per kg body weight for 6 weeks and compared to 6 male rat control group, no sub-acute toxicity was found with regard to food appetite, body weight, liver function, kidney function, white blood cell count, platelet count, hemoglobin and hematocrit, blood sugar level, blood lipids level,

fatty deposit under the skin and in internal organs such as liver, and the size of internal organs (liver, kidney, heart, lung, pancreas, adrenal gland, testis, prostate gland, and seminal vesicle). Furthermore, the *Hevea* latex extract seems to induce nitric oxide release from vascular lining that results in better compliance of blood vessels and restoration of endothelium dysfunction.

A 9-month chronic toxicity study (limit test) was performed according to the OECD-OCDC-452 guideline (revised from the 1981 version and adopted on 7 September 2009). Ninety-eight male and female 4-week post-weaning rats were divided into 4 groups. Rats in the experimental group were fed daily with 1 gram *Hevea* latex extract per kg body weight for 9 months. Rats in the control group were fed with distilled water at the same volume as the *Hevea* latex extract in the experimental group. In addition, two satellite groups (one experimental and one control) were also provided in order to monitor the reversibility of any toxicological changes that may be observed in the experimental group. Accordingly, rats in the experimental satellite group were fed daily with 1 gram *Hevea* latex extract per kg body weight for 9 months followed by distilled water for another 28 days while rats in the control satellite group were fed with distilled water for the whole duration of 9 months plus 28 days. No abnormality in appearance or behavior, such as food appetite, was observed. No abnormality was detected by gross necropsy with respect to body weight, the weight of internal organs (liver, kidney, adrenal gland, left atrium, right atrium, ventricles, spleen, lung, testis, epididymis, prostate gland, and seminal vesicle), subdermal fatty deposit, and fatty deposit in internal organs (epididymis, prostate, mesentery, and retroperitoneal organs). Histological comparisons of internal organs (liver, kidney, stomach, intestines, and adrenal gland) showed no significant difference between the experimental and the control groups. Hematological tests showed no significant difference between experimental and control groups with respect to white blood cell count (WBC), per cent hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), per cent lymphocytes (LYMPH), and platelet concentration (Pit). No adverse effect was detected with respect to the blood serum levels of glucose, lipids [triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and LDL/HDL ratio], urea nitrogen (BUN), creatinine,

total protein, albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). In short, no toxicity was found in this 9-month chronic toxicity study.

5 **Industrial Applicability of the Invention**

In the following experiments, the *Hevea* extract according to the present invention was shown to be endowed with (1) anti-cancer activity and (2) anti-metastasis activity. Owing to these activities, the present invention is applicable to industries comprising (1) the pharmaceutical industry and (2) the food industry.

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(1) Preliminary Results for Anti-Cancer Activity

Three sets of experiments have been performed to assess the anti-cancer activity of the *Hevea* latex extract according to the present invention: (a) volume change of tumor planted in nude mice, (b) effect on angiogenesis of tumor planted in nude mice, and (c) immunohistochemistry studies of markers or indicators related to angiogenesis, inflammation, proliferation, and apoptosis.

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(a) volume change of tumor planted in nude mice

Six to eight weeks old male BALB/C nude mice (athymic mice), weighing between 20 and 25 grams, were injected with 10^6 CaSki cells (cervical cancer cells of human papillomaviruses type 16 origin) and allowed to live normally for approximately one month until the tumor volume was approximately 100 to 120 cubic millimeter, as calculated from linear measurements using a vernier calliper shown in **Figure 6**. Mice in the experimental group received daily oral gavage of 60 milligram *Hevea* latex extract **315** per kg body weight for 28 days while mice in the control group received water. **Figure 7** shows the time course of relative tumor volume in the two groups of mice. While the volume of tumor in the control group increased about 7 times in 4 weeks, the volume of tumor in the group that received *Hevea* latex extract **315** stayed roughly unchanged.

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(b) effect on angiogenesis of tumor planted in nude mice

Six to eight weeks old male BALB/C nude mice, weighing between 20 and 25 grams, were fitted with 7 millimetre dorsal skin fold chambers as shown in **Figure 8** and implanted into

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the chamber with 2×10^6 CaSki cells. Mice in the experimental group received daily oral gavage of 60 milligram *Hevea* latex extract per kg body weight for 14 days while mice in the control group received water. On the 14th day, the animals were anaesthetised with 50 mg/kg sodium pentobarbital. Blood plasma was labelled with fluorescein isothiocyanate-dextran (FITC-dextran). The dorsal skin fold chambers were carefully removed along with the skin. Angiogenesis was then visualised and recorded by intravital confocal fluorescent video microscopy. The results in **Figure 9** qualitatively show that there was less angiogenesis in the experimental group. Quantitative image analysis for per cent capillary density using “Image Pro Plus” computer software (Media Cybernetics, Inc., Rockville, MD, USA) confirmed a statistically significant reduction of angiogenesis around cancer cells in the group that received *Hevea* latex extract according to the present invention.

(c) immunohistochemistry studies of markers or indicators related to angiogenesis, inflammation, proliferation, and apoptosis

When tumors from the above experiment were studied by immunohistochemistry, it was found that angiogenesis around the tumors was reduced in the group that received daily 60 milligram *Hevea* latex extract per kg body weight for 28 days, as shown by the expression of CD31 endothelial cell marker in **Figure 10**, and as shown by the presence of VEGF angiogenic marker in **Figure 11**. Furthermore, the *Hevea* latex extract also reduced the level of COX-2 inflammatory marker in **Figure 12** and EGFR proliferation marker in **Figure 13**. Finally, apoptosis of cancer cells were enhanced by the *Hevea* latex extract as shown in **Figure 14**.

(2) Preliminary Results for Anti-Metastatic Activity

The metastasis of melanoma cells injected into nude mice tail veins was assessed at two weeks after injection. At the gross tissue level, metastatic melanoma colonies can be seen by naked eyes as black spots against the reddish lung background. At the microscopic level, metastasized melanoma cells can be located by the immunohistochemistry staining assay of galectin-3, a metastasis marker. Both of these experiments confirm the existence

of anti- metastatic activity in the *Hevea* latex extract according to the present invention. The details of these experiments are as follow:

The anti-metastatic activity of the *Hevea* latex extract according to the present invention was shown in nude mice that had been injected into their tail veins with 10^5 melanoma B16-F1 cells. **Figure 15** shows the result of this experiment. In the experimental group (bottom row), which received 60 mg/kg *Hevea* latex extract daily for two weeks following the cancer cells injection, the number of metastasized melanoma black spots are significantly fewer than in the group which received water instead of the *Hevea* latex extract (top row).

At the microscopic level, the lungs of nude mice that had been injected into their tail veins with 10^5 melanoma B16-F1 cells were sectioned, stained for galectin-3, and observed under light microscopy as shown in **Figure 16**. (Galectin-3 staining in this figure appears as brown granules and tint that are superimposed on light blue cell outline and darker blue nuclei, which are somewhat difficult to differentiate in black-and-white photographs as shown.) In the experimental group (right), which received 60 mg/kg *Hevea* latex extract daily for two weeks following the cancer cells injection, the cumulative brownish tint of galectin-3 expression amounted to approximately 50 per cent of the expression in the untreated group (center) that received water instead of the *Hevea* latex extract. In the negative control group (left), where the nude mice had not been injected with any melanoma cell, the specimen showed a slight brownish tint of galectin-3 staining that corresponds to approximately 17 per cent of the galectin-3 expression in the untreated group (center). This preliminary result positively confirms the anti-metastatic activity of the *Hevea* latex extract according to the present invention.

In short, the preliminary results shown above lead to the conclusion that the *Hevea* extract according to the present invention has both anti-cancer and anti-metastasis activities. The reduction of angiogenesis and the enhancement of cancer cell apoptosis also suggest that the *Hevea* extract according to the present invention has both therapeutic as well as prophylactic values. It then follows that the present invention is applicable to industries comprising (1) the pharmaceutical industry and (2) the food industry.

(1) Application to the Pharmaceutical Industry

The invention is applicable to the pharmaceutical industry since the *Hevea* extract
 5 according to the present invention is useful as an ingredient of pharmaceutical
 compositions for treating cancers. The acute, sub-acute, and chronic toxicological studies
 in rats, as described in the above section, have shown that the *Hevea* latex extract
 according to the present invention is non-toxic. A medical composition for treating cancer
 can be made comprising an extract of *Hevea* latex according to the present invention and
 10 one or more pharmaceutically acceptable substances selected from the group consisting of
 additive, binder, carrier, diluent, excipient, filler, lubricant, solvent, and stabilizer.

Owing to the *Hevea* latex extract's anti-cancer and anti-metastatic activities in nude mice,
 it follows that we should expect the extract according to the present invention to function
 15 as an anti-cancer and anti-metastatic agent in a broad range of animals, including humans,
 and in a broad range of cancers, such as AIDS related cancer, acoustic neoma, adenocystic
 carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-
 part sarcoma, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell
 carcinoma (bcc), brain stem glioma, carcinoid cancers, childhood cancer, childhood soft
 20 tissue sarcoma, chondrosarcoma, choriocarcinoma, colorectal cancers, cutaneous T-Cell
 lymphoma, dermatofibrosarcoma-protuberans, desmoplastic small round cell cancer, ductal
 carcinoma, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra
 hepatic bile duct cancer, eye melanoma, retinoblastoma, fallopian tube cancer, fanconi
 anaemia, fibrosarcoma, gastric cancer, gastrointestinal cancers, gastrointestinal carcinoid
 25 cancer, genitourinary cancers, germ cell cancers, gestational trophoblastic disease,
 glioblastoma, glioma, gynecological cancers, hematological malignancies, hepatocellular
 cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus,
 hydatidiform mole, hypopharynx cancer, intra-ocular melanoma, isle T-cell cancer,
 Kaposi's sarcoma, Langerhan's cell histiocytosis, leiomyosarcoma, leukemias, li-
 30 fraumeni syndrome, liposarcoma, lymphedema, lymphoma, Hodgkin's lymphoma, non-
 Hodgkin^ lymphoma, male breast cancer, malignant rhabdoid cancer of kidney,
 medulloblastoma, melanoma, merkel cell cancer, mesothelioma, metastatic cancer,

multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasopharyngeal cancer, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small cell lung cancer (nsc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx
 5 cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral neuroectodermal cancers, polycythemia vera, rare cancers and associated disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, rothmund Thomson syndrome, sarcoma, schwannoma, sezary syndrome, small cell lung cancer (sclc), small intestine cancer, soft
 10 tissue sarcoma, squamous cell carcinoma (sec), synovial sarcoma, transitional cell cancer (bladder), transitional cell cancer (renal-pelvis/-ureter), trophoblastic cancer, uroplakins, uterine sarcoma, Waldenstrom's macroglobulinemia and Wilms' Cancer.

Alternatively, the anti-cancer and anti-metastasis activities of the *Hevea* latex extract
 15 according to the present invention should be applicable to cancers of different organs and organ systems, such as the anus, bladder, bone, bowel, brain, breast, central nervous system, cervix, colon, endocrine gland, ear, endothelial cells, esophagus, eye, gall bladder, head, intestine, kidney, larynx, leucocytes, lip, liver, lung, mouth, nasal cavity, neck, nose, oral cavity, ovary, pancreas, pharynx, pituitary, prostate, rectum, salivary gland, skin,
 20 spinal cord, stomach, testicles, thymus, thyroid, urethra, urinary system, uterus, vagina, and vulva.

In case the *Hevea* latex extract is to be used as an anti-cancer/ anti-metastasis agent, it may be prepared into the forms of aqueous solution, tablet, lozenge, powder, aqueous or oily
 25 suspension, emulsion, syrup, elixir. The route of administration may be enteral, intramuscular, intravenous, nasal, oral, parenteral, rectal, subcutaneous, sublingual, sublabial, transdermal and transmucosal.

Furthermore, the *Hevea* latex extract may be combined with a therapeutically effective amount or one or more chemotherapeutic agents such as alkylating agent, antimetabolite,
 30 anti-tumor antibiotic, kinase inhibitor, aromatase inhibitor, mitotic inhibitor, steroid hormone, and topoisomerase inhibitor.

(2) Application to the Food Industry

The present invention is also applicable to the functional food industry since the *Hevea* extract according to the present invention can also be useful as a food and drink supplement with anti-cancer and cancer-prevention activities. Practically, an extract of *Hevea* latex according to the present invention may be mixed with one or more edible ingredients selected from the group consisting of acid, acidity regulator, additive, anti caking agent, antifoaming agent, antioxidant, baking agent, binder, carrier, color retention agent, diluent, emulsifier, excipient, filler, flavour, flavour enhancer, flour treatment agent, food colouring agent, glazing agent, humectant, lubricant, preservative, solvent, stabilizer, sweetener, and thickener.

The foregoing description of preferred and other embodiments is not intended to limit or restrict the scope or applicability of the inventive concepts contained herein that were conceived by the Inventor. In exchange for disclosing the inventive concepts contained herein, the Inventor desires all patent rights afforded by the appended claims. Therefore, it is intended that the inventive concepts contained herein include all modifications and alterations to the full extent that they come within the scope of the following claims or the equivalents thereof.

Claims

1. A method for preparing an extract of *Hevea* latex from the serum that is obtained from calendered acidified *Hevea* latex, the method comprising the sequential steps of:
 - obtaining the serum released when acidified *Hevea* latex is calendered
 - removing debris and micro-organisms;
 - removing proteins;
 - removing quebrachitol and small polar molecules; optionally dissolving the
 - remaining solid powder in water; further removing proteins by treatment with
 - acid; optionally neutralizing the excess acid;
 - precipitating out carbohydrates with solvent; collecting the precipitate; and
 - optionally freeze drying the precipitate.
2. The method according to claim 1, wherein the technique for removal of debris and micro-organisms comprising filtration through a filter with a pore diameter of smaller than 0.5 micrometers, preferably 0.2 micrometers.
3. The method according to claim 1, wherein the technique for removal of proteins comprising at least one of ultrafiltration and spray drying.
4. The method according to claim 1, wherein the removal of quebrachitol and small polar molecules comprising extraction with a mixture of one or more organic solvents in water and collection of the remaining solid powder.
5. The method according to claim 1, wherein the further removal of proteins by acid treatment comprising precipitation of proteins with trichloroacetic acid.
6. The treatment according to claim 1, wherein the alkalinity of the supernatant after the acid treatment step is neutralized by adding an effective amount of a water soluble base, preferably sodium hydroxide.
7. The method according to claim 1, wherein the separation of *Hevea* latex extract comprising precipitating with a mixture of one or more organic solvents in water.

8. The method according to claim 3, wherein ultrafiltration is performed through an ultrafiltration membrane with a cutoff molecular mass of at least 3 kD, preferably 10 kD.
9. The method according to claim 3, wherein the spray drying is performed at a temperature above 60 degree Celsius, preferably above 100 degree Celsius, and most preferably between 100 and 120 degree Celsius.
10. The method according to claim 4, where the mixture of one or more organic solvents in water comprising at least 70 per cent methanol, preferably 100 per cent methanol.
11. The method according to claim 4, wherein the extraction is optionally repeated, preferably for a total of three times.
12. The method according to claims 5, wherein the concentration of trichloroacetic acid is from 1 to 50 per cent, preferably from 2 to 10 percent, and most preferably between 2 and 3 percent.
13. The method according to claim 5, wherein the acid treatment is performed for at least 0.1 minutes, preferably for 24 hours, at a temperature above 4 degree Celsius, preferably between 16 and 30 degree Celsius, and most preferably between 20 and 25 degree Celsius.
14. The method according to claim 5, wherein the precipitate formed by treatment with the acid is removed by at least 3,000 x g centrifugation for at least 5 minutes, preferably for 10 minutes.
15. The method according to claim 7, wherein the mixture of one or more organic solvents in water comprising at least 70 per cent ethanol, preferably 100 percent ethanol.
16. The method according to claim 7, wherein the precipitation step is performed at a temperature below 4 degree Celsius, preferably below -10 degree Celsius, and most preferably at -20 degree Celsius for at least 5 minutes, preferably for 24 hours.

17. The method according to claim 7, wherein the precipitation in the organic solvent is collected by at least 3,000 x g centrifugation for at least 5 minutes.
- 5 18. A composition comprising an extract of *Hevea* latex prepared according to any one of claims 1-17 inclusive, and one or more pharmaceutically acceptable substances selected from the group consisting of additive, binder, carrier, diluent, excipient, filler, lubricant, solvent, and stabilizer.
- 10 19. A composition comprising an extract of *Hevea* latex prepared according to any one of claims 1-17 inclusive, and one or more edible ingredients selected from the group consisting of acid, acidity regulator, additive, anti caking agent, antifoaming agent, antioxidant, baking agent, binder, carrier, color retention agent, diluent, emulsifier, excipient, filler, flavour, flavour enhancer, flour treatment agent, 15 food colouring agent, glazing agent, humectant, lubricant, preservative, solvent, stabilizer, sweetener, and thickener.