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(54) Title: COMPOSITION COMPRISING A MORUS ALBA EXTRACT, AND ITS COSMETIC USES

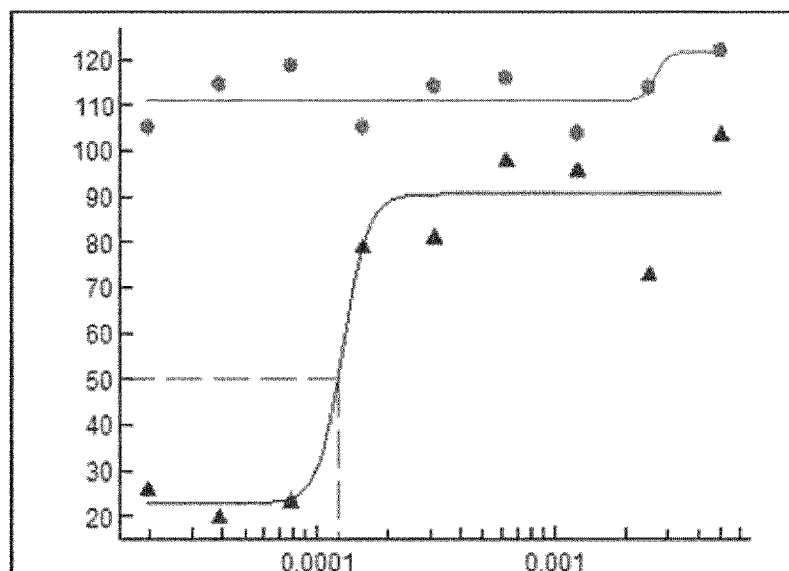


FIG.2

(57) Abstract: The present invention relates to a composition comprising, in a physiologically acceptable medium, at least mulberroside A and mulberroside E. It also relates to cosmetic uses of said composition, especially for depigmenting, lightening and/or bleaching keratin materials, in particular the skin.

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Composition comprising a *Morus alba* extract, and its cosmetic uses

The present invention concerns a composition, preferably a cosmetic composition, comprising, in a physiologically acceptable medium, at least mulberroside A and mulberroside E. It also relates to the use of such a composition for depigmenting, lightening and/or bleaching keratin materials, in particular the skin.

The human skin color is primarily determined by the nature and concentration of a pigment, melanin. There are two types of melanin in skin cells: eumelanin, a brown-black pigment, and pheomelanin, a yellow-red pigment. Melanin is synthesized by specific dendritic cells, called melanocytes, located in the basal layer of the epidermis.

Melanogenesis, i.e. the formation of melanin, takes place in special organelles, the melanosomes, which, loaded with melanin, are transferred to the neighboring epidermal cells (keratinocytes) via dendrites. The mechanism of melanogenesis is particularly complex and schematically involves the following main steps inside the melanosomes:

Tyrosine -> Dopa -> Dopaquinone -> Dopachrome -> Melanin.

Tyrosinase (monophenol dihydroxyl phenylalanine: oxygen oxidoreductase EC1.14.18.1) is the essential enzyme involved in this sequence of reactions. It catalyzes the reaction for conversion of tyrosine into dopa (dihydroxyphenylalanine) by means of its hydroxylase activity, and the reaction for conversion of dopa into dopaquinone by means of its oxidase activity. This tyrosinase acts only when it is in mature form under the action of certain biological factors.

The pigmentation of the facial skin and/or body, especially the skin pigmentation, is based on factors such as environmental factors related to seasons and the origin of the individual.

In addition, at different periods of their lives, some people see the appearance on their skin, and more in particular on the hands, of darker and/or more colored spots, which give the skin heterogeneity. These spots are in particular due to a high concentration of melanin in the keratinocytes located at the surface of the skin.

Thus, when the pigmentation process is altered, it can result in pigmentation defects, hypopigmentation, or conversely in an excess of pigmentation, hyperpigmentation. Among the benign pigmentary changes characterized by abnormal

accumulation (not tanning) of melanin, one can mention actinic lentigo (also called solar lentigo or more commonly "sunspots"), senile lentigo (commonly known as "age spots" or "senile scum", "cemetery daisies" or "graveyard flowers"), lentigines and freckles.

5 Many processes, preferably cosmetic processes, have therefore been developed to try to eliminate or reduce the presence of these benign pigmentary changes. Eliminating or reducing the presence of these alterations is usually based on the application of depigmenting treatments, which reduce melanin synthesis activity in melanocytes. The depigmenting molecules are distinct from anti-pigmenting molecules
10 that limit the action of the stress responsible for pigmentation due to ultraviolet radiation.

More specifically, a molecule is recognized as depigmenting if, in particular, it interferes with one of the steps in the biosynthesis of melanin, either by inhibiting one of the enzymes involved in melanogenesis, or by being inserted as a structural analogue of
15 one of the chemical compounds of the melanin synthesis, which may then be blocked and thus ensure depigmentation.

One of the main avenues explored to date is based on the inhibition of tyrosinase. The purpose of these treatments is to reduce or even stop the synthesis of the pigment.
20

Classical known depigmenting substances are hydroquinone and its derivatives, particularly its ethers such as monomethyl ether and hydroquinone monoethyl ether, kojic acid, arbutin, the iminophenols or ascorbic acid and its derivatives.

25 However, there are major drawbacks associated with the use of the above depigmenting agents. Indeed, depigmenting substances may especially be unstable, and/or show low efficiency at low concentration or toxic or allergenic properties.

In this regard, the Applicant has discovered, surprisingly and unexpectedly, that
30 specific mixtures of compounds present in white mulberries have good depigmenting activity, even at low concentration.

The present invention thus relates to a composition comprising, in a physiologically acceptable medium, at least mulberroside A and mulberroside E ("composition of the invention" or "composition according to the invention"). Preferably, such a composition is a
35 cosmetic composition.

The composition according to the invention can efficiently depigment and/or lighten, or even bleach, human skin. It is in particular intended to be applied to the skin of individuals bearing brownish pigmentation spots or senescence spots, or to the skin of individuals wishing to combat the appearance of a brownish color caused by melanogenesis. It may also make it possible to depigment and/or lighten bodily hairs, the eyelashes, head hair and/or also the lips.

The composition according to the invention, comprising a mixture of at least mulberrosides A and E, indeed shows a beneficial enhanced depigmenting efficacy compared to mulberroside A alone.

The present invention also relates to the cosmetic use of a composition of the invention, for depigmenting, lightening and/or bleaching keratin materials, in particular the skin.

The present invention further relates to a cosmetic non-therapeutic process for depigmenting, lightening and/or bleaching keratin materials, in particular the skin, comprising a step of applying a composition of the invention onto the keratin materials, in particular the skin.

The term "keratin materials" means skin, hair, eyelashes, and nails.

The term "skin" refers to the entire body skin, including the scalp, mucous membranes and semi-mucous membranes, and its annexes. Specifically, it is considered in the present invention the skin of the chest, neck and face, hands, armpits and especially facial skin.

Composition of the invention

The composition of the invention, preferably cosmetic composition, comprises, in a physiologically acceptable medium, at least mulberroside A and mulberroside E.

Preferably, the composition of the invention comprises, in a physiologically acceptable medium, an extract which comprises at least mulberroside A and mulberroside E. The extract which comprises at least mulberroside A and mulberroside E may be a vegetal extract, or a synthetic extract.

By vegetal extract, it is meant an extract from plants and/or trees.

By synthetic extract, it is meant a formulation made up of (i) at least mulberroside A and mulberroside E, and (ii) a vehicle, which may be aqueous or non-aqueous, and which is compatible with an application onto keratin materials.

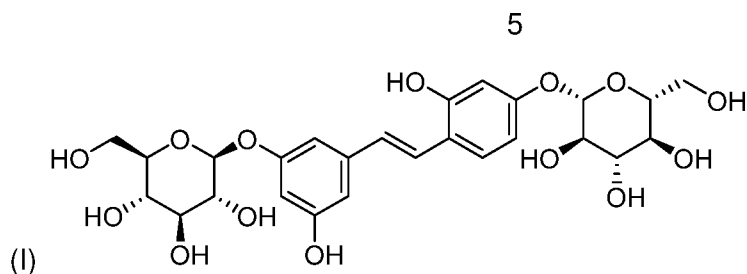
Preferably, the extract which comprises at least mulberroside A and mulberroside E is a vegetal extract. Preferably, the vegetal extract is an extract from white mulberries (also called *Morus alba*), particularly white mulberry twigs.

Thus, preferably, the composition of the invention comprises a *Morus alba* extract. More preferably, the composition of the invention comprises a *Morus alba* twig extract.

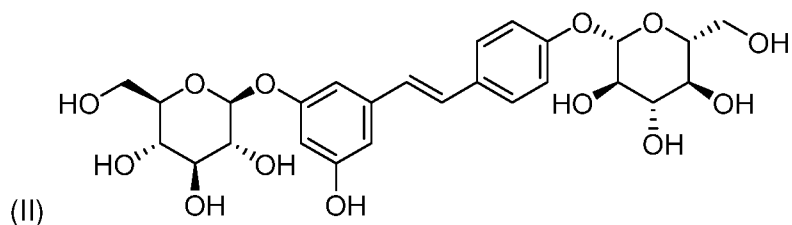
White mulberry, also called *Morus alba*, grows as a dense, round-topped, perennial shrub or tree, reaching heights around 50 feet (15 m). The thin bark is shallowly furrowed and has long, narrow ridges. White mulberry leaves are alternate, simple, ovate, 2 to 4 inches (6-10 cm) long and 1 to 2 inches (3-6 cm) wide, with margins varying from coarsely serrate to deeply lobed and serrate. Leaves exude a milky juice when broken. Staminate and pistillate flowers develop in separate catkins. Plants are typically dioecious and occasionally monoecious. White mulberry fruits are cylindrical drupes, 0.5 to 1.0 inches (1.5-2.5 cm) long. Fruits may be black, purple, or white. The ovoid nutlet has a thin, soft shell, and the seed has a "hard bony coat".

China is the largest producer of white mulberry and silk in the world. Preferably, the white mulberry comes from China, especially from Chongqing city. Especially, the twigs are collected from Chongqing city, in the Western part of China (Sichuan province). The Sichuan province is the one with the largest mulberry area in China, situated on the upper Yangtze River. The climate shows an average temperature of 16-18°C, a frostless season of from 240 to 330 days, and an annual rainfall of from 1000 to 1250 mm.

Mulberroside A belongs to stilbenoids; it is the diglucoside of oxyresveratrol. Its chemical name is (2S,3R,4S,5S,6R)-2-[3-hydroxy-4-[(E)-2-[3-hydroxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]ethenyl]phenoxy]-6-(hydroxymethyl)oxane-3,4,5-triol, and it is the "E" diastereoisomer. It has the following formula (I):



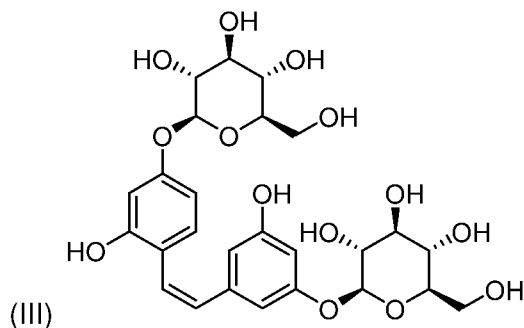
Mulberroside E also belongs to stilbenoids. Its chemical name is 4-[(1E)-2-[3-(β-D-Glucopyranosyloxy)-5-hydroxyphenyl]ethenyl]phenyl-β-D-glucopyranoside. It has the following formula (II):



Preferably, the extract comprises at least 15% by weight of the total weight of the extract, of mulberroside A, preferably at least 17% by weight. Preferably, the extract comprises less than 50% by weight of the total weight of the extract, of mulberroside A, preferably less than 45% by weight, preferably less than 40% by weight. Preferably, the extract comprises from 15% to 40% by weight of the total weight of the extract, of mulberroside A. Preferably, the extract comprises from 15% to 25% by weight, or from 25% to 40% by weight, of the total weight of the extract, of mulberroside A.

Preferably, the extract comprises at least 3% by weight of the total weight of the extract, of mulberroside E, preferably at least 4% by weight. Preferably, the extract comprises less than 15% by weight of the total weight of the extract, of mulberroside E, preferably less than 10% by weight, preferably less than 8% by weight. Preferably, the extract comprises from 4% to 8% by weight of the total weight of the extract, of mulberroside E.

Preferably, the composition of the invention further comprises cis-mulberroside A. Cis-mulberroside A is the "Z" diastereoisomer of mulberroside A ("cis"). Its chemical name is 3-[(1Z)-2-[4-(β-D-Glucopyranosyloxy)-2-hydroxyphenyl]ethenyl]-5-hydroxyphenyl-β-D-glucopyranoside. It has the following formula (III):



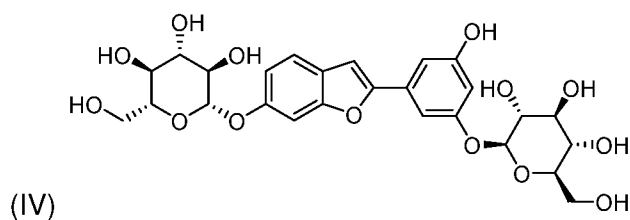
Preferably, the extract further comprises cis-mulberroside A.

Preferably, the extract comprises at least 0,2% by weight of the total weight of the extract, of cis-mulberroside A, preferably at least 0,4% by weight, preferably at least 0,5% by weight. Preferably, the extract comprises less than 5% by weight of the total weight of the extract, of cis-mulberroside A, preferably less than 4% by weight, preferably less than 2% by weight. Preferably, the extract comprises from 0,5% to 2% by weight of cis-mulberroside A.

Preferably, the composition of the invention further comprises at least one compound chosen from mulberroside F, cis-oxyresveratrol-3'-O- β -glucopyranoside, trans-oxyresveratrol-3-O- β -glucopyranoside, oxyresveratrol, and their mixtures.

Preferably, the extract further comprises at least one compound chosen from mulberroside F, cis-oxyresveratrol-3'-O- β -glucopyranoside, trans-oxyresveratrol-3-O- β -glucopyranoside, oxyresveratrol, and their mixtures.

Preferably, the composition of the invention or the extract further comprises mulberroside F. Its chemical name is 3-[6-(β -D-Glucopyranosyloxy)-2-benzofuranyl]-5-hydroxyphenyl β -D-glucopyranoside. It corresponds to the following formula (IV):

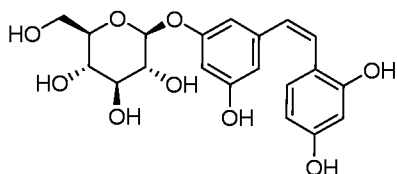


Preferably, the extract comprises at least 0,4% by weight of the total weight of the extract, of mulberroside F, preferably at least 0,5% by weight. Preferably, the extract comprises less than 5% by weight of the total weight of the extract, of mulberroside F, preferably less than 3% by weight, preferably less than 2% by weight. Preferably, the

extract comprises from 0,5% to 2% by weight of the total weight of the extract, of mulberroside F.

Preferably, the composition of the invention or the extract further comprises cis-oxyresveratrol-3'-O- β -glucopyranoside. Its chemical name is 3-[(1Z)-2-(2,4-Dihydroxyphenyl)ethenyl]-5-hydroxyphenyl β -D-glucopyranoside.

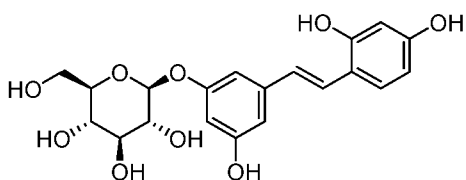
It corresponds to the following formula (V):



(V)

Preferably, the extract comprises at least 0,4% by weight of the total weight of the extract, of cis-oxyresveratrol-3'-O- β -glucopyranoside, preferably at least 0,5% by weight. Preferably, the extract comprises less than 5% by weight of the total weight of the extract, of cis-oxyresveratrol-3'-O- β -glucopyranoside, preferably less than 3% by weight, preferably less than 2% by weight. Preferably, the extract comprises from 0,5% to 2% by weight of the total weight of the extract, of cis-oxyresveratrol-3'-O- β -glucopyranoside.

Preferably, the composition of the invention or the extract further comprises trans-oxyresveratrol-3'-O- β -glucopyranoside. Its chemical name is 3-[(1E)-2-(2,4-Dihydroxyphenyl)ethenyl]-5-hydroxyphenyl β -D-glucopyranoside. It corresponds to the following formula (VI):

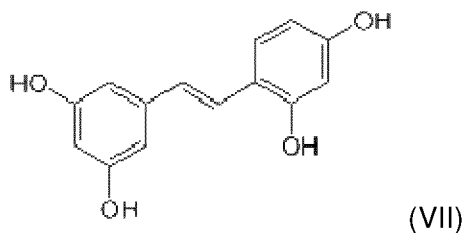


(VI)

Preferably, the extract comprises at least 0,4% by weight of the total weight of the extract, of trans-oxyresveratrol-3'-O- β -glucopyranoside, preferably at least 0,5% by weight. Preferably, the extract comprises less than 5% by weight of the total weight of the extract, of trans-oxyresveratrol-3'-O- β -glucopyranoside, preferably less than 3% by weight, preferably less than 2% by weight. Preferably, the extract comprises from 0,5% to

2% by weight of the total weight of the extract, of trans-oxyresveratrol-3'-O- β -glucopyranoside.

Preferably, the composition of the invention or the extract further comprises oxyresveratrol. Oxyresveratrol has the chemical name 4-[(E)-2-(3,5-dihydroxyphenyl)ethenyl]benzene-1,3-diol, and corresponds to the following formula (VII):



Preferably, the extract comprises at least 0,1% by weight of the total weight of the extract, of oxyresveratrol, preferably at least 0,2% by weight. Preferably, the extract comprises less than 3% by weight of the total weight of the extract, of oxyresveratrol, preferably less than 2% by weight, preferably less than 1% by weight. Preferably, the extract comprises from 0,3% to 1% by weight of the total weight of the extract, of oxyresveratrol.

More preferably, the extract comprises:

- at least 0,4%, preferably 0,5% to 2% by weight of mulberroside F, and/or
- at least 0,4%, preferably 0,5% to 2% by weight of cis-oxyresveratrol-3'-O- β -glucopyranoside, and/or
- at least 0,4%, preferably 0,5% to 2% by weight of trans-oxyresveratrol-3-O- β -glucopyranoside, and/or
- at least 0,2%, preferably 0,3% to 1% by weight of oxyresveratrol.

Preferably, the composition of the invention comprises from 0,1% to 0,5% by weight preferably from 0,2% to 0,5% by weight of the total weight of the composition, of the extract.

Preparation of the extract

The composition of the invention preferably comprises a vegetal extract which comprises at least mulberroside A and mulberroside E.

More preferably, the composition of the invention comprises a *Morus alba* extract, more preferably a *Morus alba* twig extract.

Such a *Morus alba* twig extract may be prepared as described in example 1.

Preferably, the *Morus alba* twig extract is prepared as follows:

Step 1:

Mulberry twigs and branches of *Morus alba* are mixed with a solution of ethanol, preferably 70% aqueous ethanol, and extracted, to obtain a liquid extract.

Preferably, the weight ratio (biomass: ethanol solution) is 1:5. Preferably, the extraction is performed by heating the mixture at a temperature of around 70°C, during a few hours such as 3h.

Preferably, at the end of step 1, the extract is filtered.

Step 1 may be repeated many times, for example 2 or 3 times.

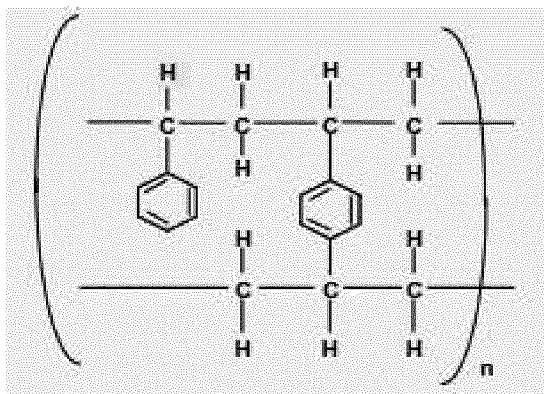
Step 2:

The liquid extract obtained from step 1 is heated at a temperature of from 50°C to 60°C, in order to obtain an aqueous solution that comprises 18 to 20% (in weight/volume) of the total solids extract.

Preferably, the liquid extract obtained from step 2 is diluted with demineralized water, and then filtered using diatomaceous earth to afford a clear liquid extract.

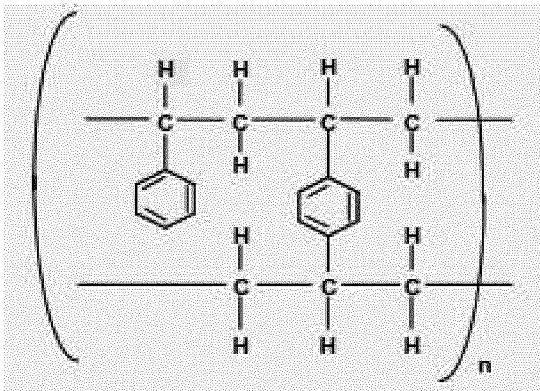
Step 3:

The liquid extract obtained at the end of step 2 is dissolved in a solution of ethanol, preferably 1-20% aqueous ethanol, and adsorbed over a macroporous polymeric resin made up of styrene-divinylbenzene copolymer or of the following structure:



Preferably, the macroporous polymeric resin made up of styrene-divinylbenzene copolymer is Diaion HP-20.

Preferably, the macroporous polymeric resin made up of the following structure is Amberlite XAD 1600 N:



Elution may be made with demineralized water and/or using a gradient mixture containing aqueous alcohol.

The eluate is collected.

5 **Step 4:**

The eluate of step 3 is concentrated to evacuate water and ethanol, and dried to obtain a first *Morus alba* twig extract (extract B). Said first extract contains <30% Mulberrosides and oligosaccharides <40%.

Step 5:

10 Optionally, the first fraction from step 3 is again adsorbed over the macroporous resin as in step 3, and the eluate is collected. Said eluate is concentrated and dried to obtain a second *Morus alba* twig extract (extract A). Said second extract contains contains <40% Mulberrosides and oligosaccharides <50%.

15 Physiologically acceptable medium

The composition of the invention also comprises a physiologically acceptable medium. The term "physiologically acceptable medium" is intended to mean a medium that is compatible with human keratin materials, such as the skin of the body or of the face, the lips, the mucous membranes, the eyelashes, the nails, the scalp and/or the hair.

20 The composition used according to the invention may then comprise any adjuvant commonly used in the cosmetics field. Mention may be made in particular of an aqueous phase, which comprises water and optionally organic solvents; and/or an oily phase, which comprises at least one oil and/or wax. Mention may also be made of pigments, fillers, dyes, surfactants, emulsifiers; cosmetic or dermatological active agents, UV-screening agents (mineral or organic, anti-UVA and/or anti-UVB), polymers, hydrophilic or
25 lipophilic gelling agents, thickeners, preservatives, fragrances, bactericides, ceramides, odor absorbers and antioxidants.

The composition according to the invention preferably comprises an aqueous medium comprising water and possibly an organic solvent soluble in water at 25°C, chosen for example from among linear or branched alkanols, in C2-C4, such as ethanol and isopropanol, propanol, butanol; polyols particularly with 2 to 20 carbon atoms, preferably 2 to 6 carbon atoms such as glycerol, diglycerol, propyleneglycol, glycol isoprene, dipropyleneglycol, butylene glycol, hexylene glycol, 1,3-propanediol, pentylene glycol, polyethyleneglycols with 2 to 200 ethylene oxide motifs, and mixtures thereof.

The composition generally comprises from 10% to 99% by weight of water with respect to the total weight of the composition and preferably from 30% to 80%.

The quantity of organic solvents can range for example from 0% to 30% by weight, preferably from 0.5% to 25% by weight, better from 5% to 20% by weight, even better from 10% to 20% by weight relative to the total weight of the composition.

The composition according to the invention may also comprise an oily phase.

When the composition used according to the invention comprises an oily phase, this oily phase preferably contains at least one oil, particularly a cosmetic oil. It may further contain other fats.

By way of oils suitable for use in the composition according to the invention, mention may be made for example of hydrocarbon oils of animal origin; hydrocarbon oils of plant origin; esters and synthetic esters, in particular fatty acids, such as oils having formulas R_1COOR_2 and R_1OR_2 wherein R_1 is the remainder of a fatty acid comprising from 8 to 29 carbon atoms, and R_2 is a hydrocarbon chain, branched or not, containing from 3 to 30 carbon atoms; hydroxylated esters; polyol esters; linear or branched hydrocarbons, with inorganic or synthetic origin, hydrocarbon oils with branched chain containing 10 to 20 carbon atoms, vaseline, polydecenes, hydrogenated polyisobutene; natural or synthetic essential oils; fatty alcohols having 8 to 26 carbon atoms; partially hydrocarbon and/or silicone fluorinated oils such as those described in the document JP-A-2-295912; silicone oils such as polymethylsiloxanes (PDMS), optionally volatile; or mixtures thereof.

The other fats suitable for being present in the oil phase are for example fatty acids comprising 8 to 30 carbon atoms; waxes; silicone resins; and silicon elastomers and mixtures thereof.

These fats may be chosen in varied ways by those skilled in the art in order to prepare a composition having the sought properties, for example consistency or texture properties.

The quantity of oil phase may range for example 0.1% to 25%, and for example from 10% to 20% by weight with respect to the total weight of the composition.

5 The composition according to the invention may be in any galenical form normally used in the cosmetics field, and in particular in the form of an aqueous or aqueous-alcoholic solution, optionally gelled, a dispersion of the lotion type, optionally a two-phase dispersion, an oil-in-water or water-in-oil or multiple (W/O/W or O/W/O for example) emulsion, an aqueous gel, a dispersion of oil in an aqueous phase by means of spherules,
10 these spherules possibly being polymer nanoparticles such as nanospheres and nanocapsules, or, better still, lipid vesicles of ionic and/or nonionic type; aqueous or oily gel. These compositions are prepared according to the usual methods.

 The composition used according to the invention may constitute a skincare composition, and in particular a cleansing, protecting, treating or care cream for the face,
15 the hands, the feet, the major anatomical folds or the body (for example day creams, night creams, makeup-removing creams, foundation creams or anti-sun creams); a fluid foundation, a makeup-removing milk, a protective or care body milk or an anti-sun milk; a skincare lotion, gel or mousse, such as a cleansing lotion.

20 The invention also relates to the cosmetic use of a composition of the invention, for depigmenting, lightening and/or bleaching keratin materials, in particular the skin.

 The invention further relates to a cosmetic non-therapeutic process for depigmenting, lightening and/or bleaching keratin materials, in particular the skin,
25 comprising a step of applying a composition of the invention onto the keratin materials, in particular the skin.

 We will now give concrete examples illustrating the invention, but that are in no way restrictive.

30 All percentages given in the examples are given by mass, unless specified otherwise, and the temperature is ambient (20°C) and expressed in degrees Celsius unless specified otherwise, and the pressure is atmospheric pressure, unless specified otherwise.

35 The following examples are illustrated by the enclosed figures which are as follows:

Efficacy of mulberroside A, extract A or extract B, corresponds to the line with triangles;

Viability of cells corresponds to the line with filled circles; and

EC50 and EC80 are dotted lines.

5 In easting (x-axis) are the concentrations of mulberroside A, extract A or extract B, which are tested; in northing (y-axis) are the percentages (viability and efficacy).

Figure 1 corresponds to the results obtained with mulberroside A.

10 Figure 2 corresponds to the results obtained with Morus alba extract B of the invention.

Figure 3 corresponds to the results obtained with Morus alba extract A of the invention.

15 **Example 1: Preparation of two *Morus alba* extracts according to the invention**

Two *Morus alba* extracts, i.e. extract A and extract B, were obtained by the following process:

- 20 1) White mulberries (*Morus alba*) were cultivated in China (Chongqing region). 1 kg of mulberry twigs and branches of the Chinese biomass are mixed with 70% aqueous ethanol, in a respective weight ratio of 1:5, and extracted at 70°C, during 3h, to obtain a liquid extract. The extract is filtered, and the spent biomass is re-extracted for two successive times according to the same process, in order to obtain a combined liquid extract;
- 25 2) The combined liquid extract obtained from step 1 is heated at 50°C-60°C to strip the ethanol and the inventors obtain an aqueous solution that comprises 18 to 20 % (in weight/volume) of the total solids extract.
- 30 3) The aqueous liquid extract obtained from step 2 is diluted to 20 L with demineralized water, and then filtered using diatomaceous earth (200g) to afford a clear liquid extract;
- 35 4) the clear liquid extract from step 3 is dissolved in 1-20% aqueous ethanol and adsorbed over macroporous resin Diaion HP-20 or Amberlite XAD 1600 N, by allowing the resin and mixture (sample) to remain undisturbed for 1-4h, and further packed in a glass column with a sintered glass disc and eluted with 5 bed volumes of demineralized water to remove proteins 1-15%. The column is further

eluted using a gradient mixture containing aqueous alcohol and the eluate from 50% aqueous alcohol is collected;

- 5) Said eluate obtained from step 4 is concentrated to evacuate water and ethanol, and dried to obtain extract B, which contains <30% Mulberrosides and oligosaccharides <40%. The inventors obtain a yellow to brown powder that represents extract B.

- 6) First fraction from step 4 (before water wash, 1-20% aqueous ethanol) is again adsorbed over fresh macroporous resin Diaion HP-20, by allowing the resin and mixture (sample) to remain undisturbed for 1-4h, and further packed in a glass column with a sintered glass disc and eluted with 5 bed volumes of demineralized water to remove proteins 1-15%. The column is further eluted using a gradient mixture containing aqueous alcohol and the eluate from 50% aqueous alcohol is collected;

- 7) Said eluate obtained from step 6 is concentrated and dried to obtain extract A, which contains <40% Mulberrosides and oligosaccharides <50%. Said extract A is an aqueous liquid extract, which is totally stripped of ethanol and which has a better concentration of mulberroside A.

The yield of such a process is 1-2%.

Chemical composition of *Morus alba* Extract

The chemical composition of each extract was determined, and the results are as follows:

Parameter	Extract B	Extract A
Mulberroside A	19.60%	36.63%
Mulberroside E	6.18%	6.61%
Cis mulberroside A	0.93%	1.13%
Mulberroside F	0.90%	less than 1%
Cis-Oxyresveratrol-3'-O- β -Glucopyranoside	0.93%	less than 1%
trans-Oxyresveratrol-3-O- β -Glucopyranoside	1.03%	less than 1%
Oxyresveratrol	0.62%	less than 1%
Total Protein	3.49%	less than 1%

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Total Sugar	30.28%	47.01%
Water content	5.33%	6.71%
Total fats	2.77%	less than 1%
Total Composition	72.06%	83.63%

Specification

Thus, the specification for each extract is :

Parameter	Extract B	Extract A
Mulberroside A	15-25%	25-40%
Mulberroside E	4-8%	4-8%
Cis mulberroside A	0.5-2%	0.5-2%
Mulberroside F	0.5-2%	less than 1%
Cis-Oxyresveratrol-3'-O- β -Glucopyranoside	0.5-2%	less than 1%
trans-Oxyresveratrol-3-O- β -Glucopyranoside	0.5-2%	less than 1%
Oxyresveratrol	0.3-1%	less than 1%
Total Protein	2-5%	less than 1%
Total Sugar	20-35%	40-55%
Water content	Not More Than 8%	Not More Than 8%
Total fats	Not More Than 4%	less than 1%

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Example 2: Evaluation of Melanin Inhibition of two *Morus alba* extracts of the invention, in Indian Melanocyte Model (IMM)

10 **Method**

Normal Human Epidermal Melanocytes from Indian origin (Lonza, NHEM-Indian) of passage 3-6, were maintained as per the supplier's protocol. 40000 melanocytes/well of 24 wells were plated on day 1. Cells were treated with 8-10 concentrations of *Morus alba* extract A or B (as disclosed in example 1) in fresh media on day 2. Each plate contains vehicle control (water, DMSO or ethanol) and positive reference Lucinol at 50 μ M. After 3 days of incubation, a first viability assay (CACEIN-AM) followed by melanin content assay (Absorbance at 405nm) was performed.

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Data Analysis

Morus alba extract A or B was tested in duplicate wells of 24 well plates and 3-4 such experiments were repeated. Maximum depigmentation of Lucinol was set to 100% to calculate % Whitening and % Inhibition of extract. Using XLfit software, EC50 (efficacious concentration at 50% inhibition) was calculated based on Lucinol normalization. EC80 (viability at 80%) was calculated based on vehicle control.

Results and Discussion

The results are presented in the table below:

Sample	Concentration
Mulberroside A	0,0001% to 0,01%
<i>Morus alba</i> extract B	0,0001% to 0,01%
<i>Morus alba</i> extract A	0,0001% to 0,01%

Observations and Inferences

Mulberroside A solubilized in DMSO was tested at concentration 0,001%. The results are in Figure 1.

At 0,001%, it shows a viability of the cells which is 100% and an efficacy in terms of inhibition of the synthesis of melanin which is 10% inhibition.

Morus alba extract B of the invention, solubilized in DMSO, was tested at concentration 0,001%. The results are in Figure 2.

At 0,001%, it shows a viability of the cells which is 100% and an efficacy in terms of inhibition of the synthesis of melanin which is 90% inhibition.

Morus alba extract A of the invention, solubilized in DMSO, was tested at concentration 0,001%. The results are in Figure 3.

At 0,001%, it shows a viability of the cells which is 100% and an efficacy in terms of inhibition of the synthesis of melanin which is 80% inhibition.

It results from the above that contrary to mulberroside A, both extracts A and B of the invention are the only ones to present an active depigmenting activity 80% and 90% inhibition of melanin synthesis at 0,001%, respectively.

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Example 3: Comparison of the EC50 of the extracts according to the invention to an extract of the prior art

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The EC50 of the extracts A and B of the invention (as described in example 1, and results of Figures 3 and 2, respectively) were compared to the EC50 of the extract of prior art document US2006/0216253.

To this purpose, the EC50 values were calculated based on the data of Tables 2 and 3 of US2006/0216253 (Concentration & Melanin production) and compared to the EC50 of extracts A and B. Results are below:

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EXTRACTS	CONCENTRATION	EC50 (%)
Extract A of the invention	0,0001% to 0,01%	0.00024
Extract B of the invention	0,0001% to 0,01%	0.00014
Comparative extract from prior art US2006/0216253 Mulberroside A 0.5%-5% Oxyresveratrol 0.5%-5%	0,0001% to 0,01%	0.02164

Based on these EC50 values, extracts A and B according to the invention are much superior to the prior art *Morus alba* extract.

20

CLAIMS

- 5
1. Composition comprising, in a physiologically acceptable medium, at least mulberroside A and mulberroside E.
2. Composition according to claim 1, wherein it comprises an extract comprising at least mulberroside A and mulberroside E.
- 10
3. Composition according to claim 2, wherein the extract comprises at least 15% by weight of the total weight of the extract, of mulberroside A, preferably at least 17% by weight, preferably from 15% to 40% by weight of mulberroside A.
- 15
4. Composition according to claim 2 or 3, wherein the extract comprises at least 3% by weight of the total weight of the extract, of mulberroside E, preferably at least 4% by weight, preferably from 4% to 8% by weight of mulberroside E.
- 20
5. Composition according to any one of claims 1 to 4, wherein it further comprises cis-mulberroside A.
- 25
6. Composition according to any one of claims 2 to 5, wherein the extract comprises at least 0,2% by weight of the total weight of the extract, of cis-mulberroside A, preferably at least 0,4% by weight, preferably from 0,5% to 2% by weight of cis-mulberroside A.
- 30
7. Composition according to any one of the preceding claims, wherein it further comprises at least one compound chosen from mulberroside F, cis-oxyresveratrol-3'-O- β -glucopyranoside, trans-oxyresveratrol-3-O- β -glucopyranoside, oxyresveratrol, and their mixtures.
- 35
8. Composition according to any one of claims 2 to 7, wherein the extract comprises at least one compound chosen from mulberroside F, cis-oxyresveratrol-3'-O- β -glucopyranoside, trans-oxyresveratrol-3-O- β -glucopyranoside, oxyresveratrol, and their mixtures.
9. Composition according to claim 8, wherein the extract comprises, by weight of the total weight of the extract:
- at least 0,4%, preferably 0,5% to 2% by weight of mulberroside F, and/or

- at least 0,4%, preferably 0,5% to 2% by weight of cis-oxyresveratrol-3'-O- β -glucopyranoside, and/or
- at least 0,4%, preferably 0,5% to 2% by weight of trans-oxyresveratrol-3-O- β -glucopyranoside, and/or
- 5 - at least 0,2%, preferably 0,3% to 1% by weight of oxyresveratrol.

10. Composition according to any one of the preceding claims, wherein it comprises a *Morus alba* extract.

10 11. Composition according to any one of the preceding claims, wherein it comprises a *Morus alba* twig extract.

12. Composition according to any one of the preceding claims, wherein the physiologically acceptable medium comprises an aqueous phase and/or an oily
15 phase.

13. Cosmetic use of the composition according to any one of the preceding claims, for depigmenting, lightening and/or bleaching keratin materials, in particular the skin.

20 14. Cosmetic non-therapeutic process for depigmenting, lightening and/or bleaching keratin materials, in particular the skin, comprising a step of applying a composition according to any one of claims 1 to 12 onto the keratin materials, in particular the skin.

25 15. Process for preparing a *Morus alba* twig extract, which comprises the following steps:

Step 1:

30 Mulberry twigs and branches of *Morus alba* are mixed with a solution of ethanol, preferably 70% aqueous ethanol, and extracted, to obtain a liquid extract;

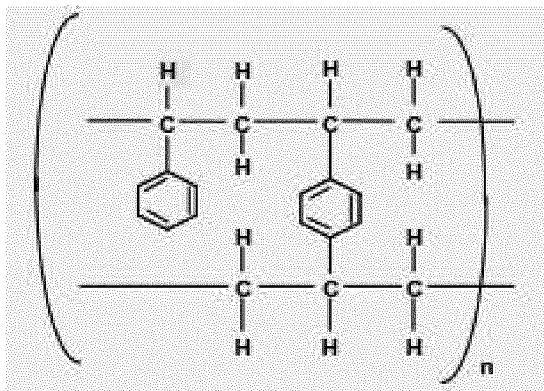
Step 1 may be repeated many times, for example 2 or 3 times;

Step 2:

35 The liquid extract obtained from step 1 is heated at a temperature of from 50°C to 60°C, in order to obtain an aqueous solution that comprises 18 to 20% (in weight/volume) of the total solids extract, and preferably diluted with demineralized water and then filtered using diatomaceous earth to afford a clear liquid extract;

Step 3:

The liquid extract obtained at the end of step 2 is dissolved in a solution of ethanol, preferably 1-20% aqueous ethanol, and adsorbed over a macroporous polymeric resin made up of styrene-divinylbenzene copolymer or of the following structure:



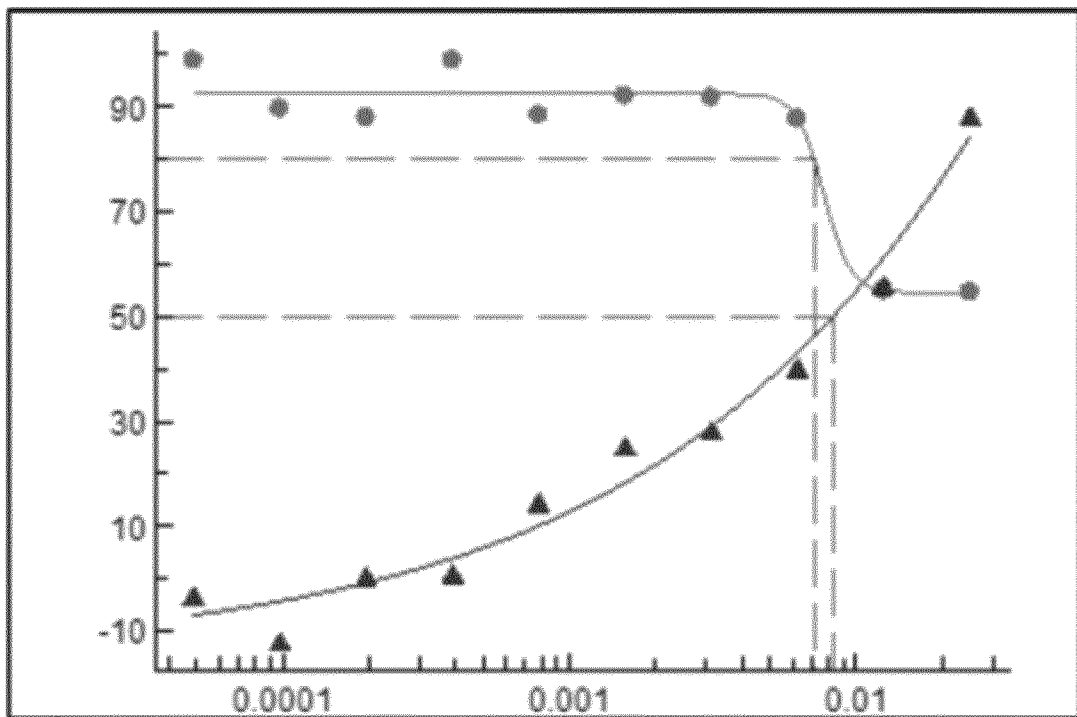
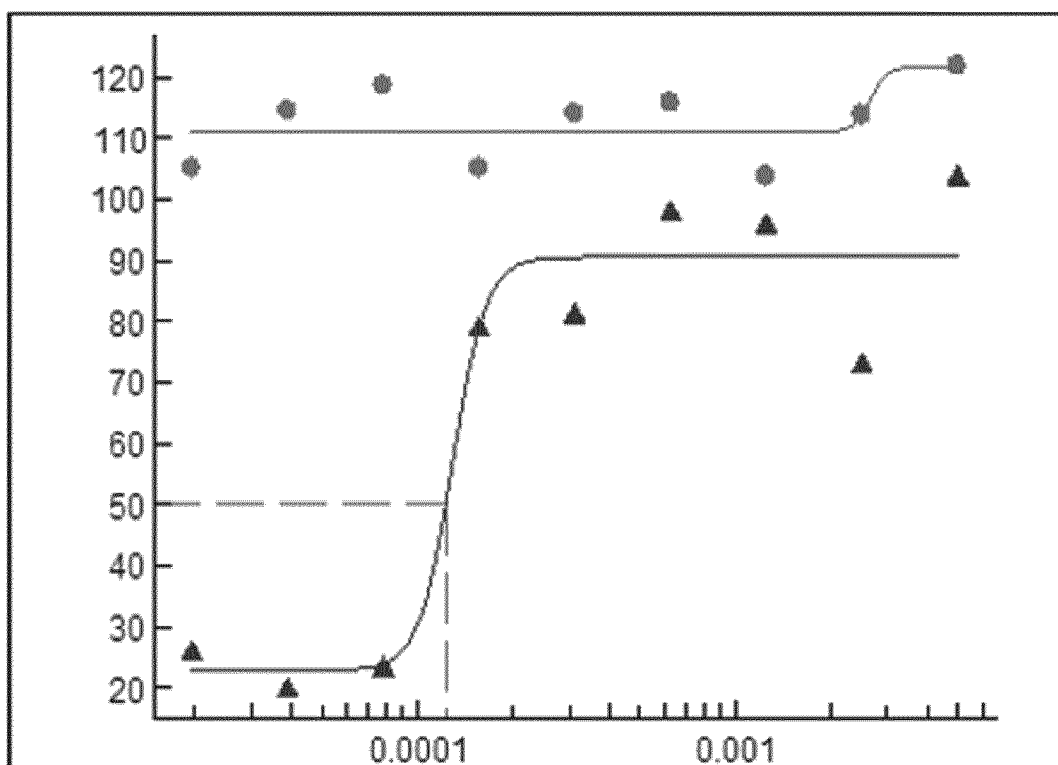
- 5 and eluted preferably with demineralized water and/or using a gradient mixture containing aqueous alcohol, and the eluate is collected;

Step 4: The eluate of step 3 is concentrated to evacuate water and ethanol, and dried to obtain a first *Morus alba* twig extract;

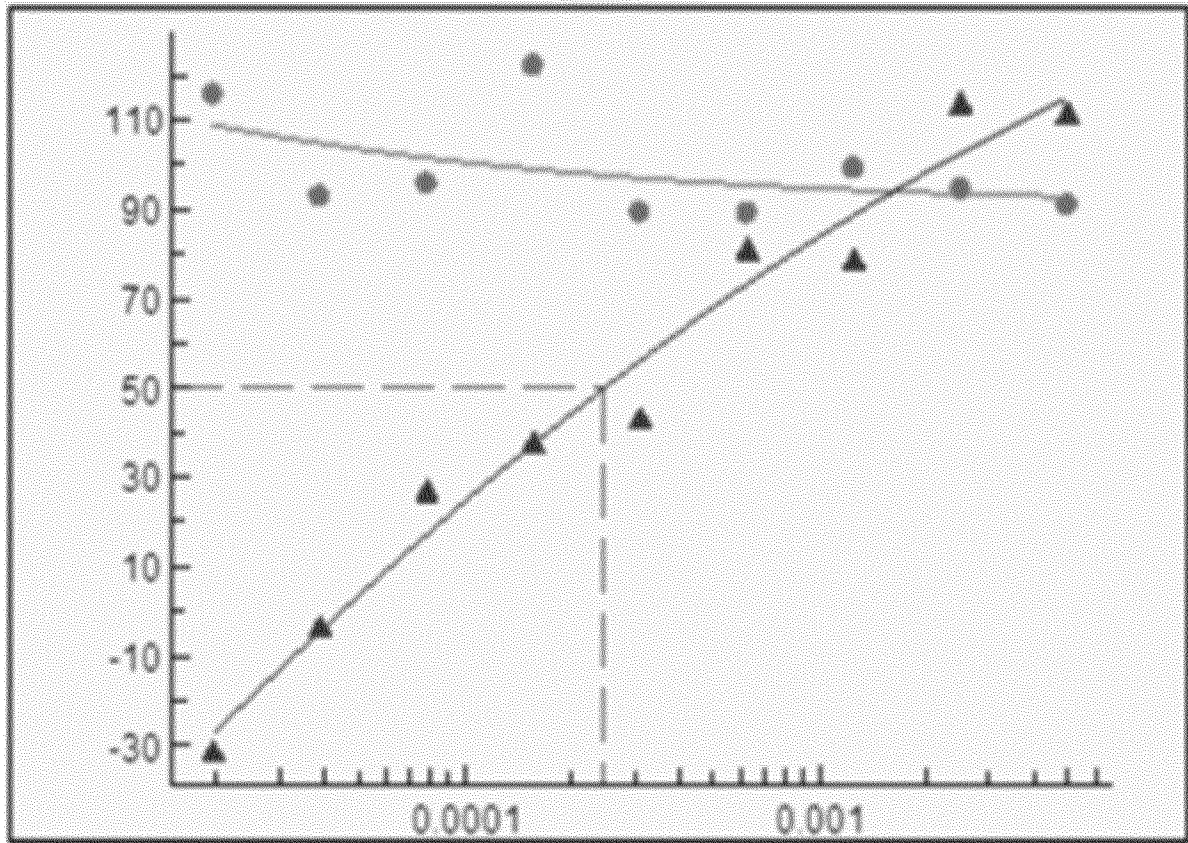
Step 5:

- 10 Optionally, the first fraction from step 3 is again adsorbed over the macroporous resin as in step 3, and the eluate is collected, concentrated and dried to obtain a second *Morus alba* twig extract.

1/2

FIG.1FIG.2

2/2

**FIG.3**

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/059417

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/49 A61K8/9783 A61Q19/02
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/216253 A1 (KIM JEONG-HA [US] ET AL) 28 September 2006 (2006-09-28)	1-14
A	paragraphs [0007], [0013] - [0017] table 2 examples	15
X	----- JEONG-KEUN KIM ET AL: "Biotransformation of mulberroside A from Morus alba results in enhancement of tyrosinase inhibition", JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY ; OFFICIAL JOURNAL OF THE SOCIETY FOR INDUSTRIAL MICROBIOLOGY, SPRINGER, BERLIN, DE, vol. 37, no. 6, 22 April 2010 (2010-04-22), pages 631-637, XP019809550, ISSN: 1476-5535	1-3,5, 7-14
A	page 631 - page 632 ----- -/--	4,6,15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

3 July 2020

Date of mailing of the international search report

15/07/2020

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/059417

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Y. HANO ET AL: "Sequential glucosylation determined by NMR in the biosynthesis of mulberroside D, a cis-oxyresveratrol diglucoside, in Morus alba L. cell cultures :", CMLS CELLULAR AND MOLECULAR LIFE SCIENCES., vol. 53, no. 3, 1 March 1997 (1997-03-01), pages 237-241, XP055711069, DE ISSN: 1420-682X, DOI: 10.1007/PL000000597 abstract figure 1; table 1 page 238, right-hand column -----	1-15
A	DATABASE GNPD [Online] MINTEL; 17 October 2018 (2018-10-17), anonymous: "Mulberry & Oat Milk Facial Foam", XP055710498, retrieved from www.gnpd.com Database accession no. 6041143 abstract -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2020/059417

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US 2006216253	A1	28-09-2006	NONE
