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(54) Titre : UNE METHODE DE PREPARATION D'UNE COMPOSITION RENFERMANT UN MELANGE DE COMPOSE
ANTIMICROBIEN OBTENU EN PULVERISANT UNE MATIERE DE BOIS OU EN EXTRAYANT UNE MATIERE DE
BOIS

(54) Title: A METHOD TO PREPARE A COMPOSITION COMPRISING AN ANTI-MICROBIAL COMPOUND MIXTURE
OBTAINED BY PULVERIZING WOOD MATERIAL AND/OR EXTRACTING WOOD MATERIAL

(57) **Abrégé/Abstract:**

The invention relates to a mixture to be included in a composition or its semi- finished product, which composition or its semi-finished product contains at least one anti-micro-organistic agent; a possible solvent system with either hydrophobic or hydrophilic nature, selected according to the target of usage; as well as possible surface active agent. The mixture is obtained by pulverizing wood or plant material and/or by extracting the possibly pulverized wood or plant material, so that said mixture contains at least two different polyphenolic compounds selected from the following group: lignans, stilbenes, juvabionones and flavonoids; said mixture further containing oligomers of said polyphenolic compounds, so that the mixture also must have the following properties: the mixture has an anti-micro-organistic effect in the composition or in the semi-finished product; the cytotoxicity of the polyphenols contained in the mixture is at least 10 times lower with respect to BHT; the mixture does not irritate the skin in a so-called single patch test with contents of 0.1 wt%.

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(54) Title: METHOD FOR MINIMIZING THE CYTOTOXICITY OF THE EFFECTIVE AGENT INHIBITING THE GROWTH OF MICRO-ORGANISMS AND FOR MAXIMIZING THE EFFECT

(57) Abstract: The invention relates to a mixture to be included in a composition or its semi-finished product, which composition or its semi-finished product contains at least one anti-micro-organistic agent; a possible solvent system with either hydrophobic or hydrophilic nature, selected according to the target of usage; as well as possible surface active agent. The mixture is obtained by pulverizing wood or plant material and/or by extracting the possibly pulverized wood or plant material, so that said mixture contains at least two different polyphenolic compounds selected from the following group: lignans, stilbenes, juvabionones and flavonoids; said mixture further containing oligomers of said polyphenolic compounds, so that the mixture also must have the following properties: the mixture has an anti-micro-organistic effect in the composition or in the semi-finished product; the cytotoxicity of the polyphenols contained in the mixture is at least 10 times lower with respect to BHT; the mixture does not irritate the skin in a so-called single patch test with contents of 0.1 wt%.



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A Method to Prepare a Composition Comprising an Anti-Microbial Compound Mixture Obtained by Pulverizing Wood Material and/or Extracting Wood Material

5 The invention relates to a method according to the disclosure herein for minimizing the cytotoxicity of the effective agent inhibiting the growth of micro-organisms and for maximizing its effect in a cosmetic composition, food industry composition, animal feed composition, for producing a technical composition or a packing material composition.

10 In cosmetic and food industry compositions, in commercial solvents and detergents as well as in industrially employed solvents, it is often necessary to add anti-microbial agents, such as agents inhibiting the growth of microbes, and microbicidal agents. Anti-micro-organistic agents may themselves be cytotoxic, skin irritative or otherwise harmful to the system when getting into contact with the skin. Moreover, the currently used anti-micro-organistic agents are without exception synthetic, which means that their production is a complicated process.

15 The object of the invention is to eliminate the above described drawbacks of the prior art.

Thus, the principal object of the invention is to realize such compositions or semi-finished compositions used in the production of said compositions, where the anti-micro-organistic agent contained by the compositions or their semi-finished composition is obtained from the nature, and where the undesirable properties are minimal. Undesirable properties are
20 for example cytotoxicity, other physiological toxicity, skin irritation and tendency to form or induce free radicals on the skin, for instance as the substance is decomposed owing to the effect of sunlight. For instance phenoxyethanol (derivative of benzoic acid) is a relatively wide-scale synthetic microbicide and preservative, used generally in foodstuffs and cosmetic compositions, because it is effective against both yeasts, fungi and Gram-
25 negative bacteria. However, its use is restricted, because it has been found to be physiologically toxic, allergenic and skin irritative, in addition to which it decomposes owing in sunlight, thus forming free radicals. The object of the invention is to reduce the use of generally used synthetic substances inhibiting microbial growth on a wide scale, or to completely replace said substances, particularly in cosmetic compositions and food industry compositions, by a corresponding substance obtained from natural sources, which substance causes
30 minimal irritation to mammal skin and has a minimal cytotoxicity.

Here the term 'substances inhibiting microbial growth on a wide scale' particularly refers to a substance that inhibits the growth of both Gram-positive bacteria and Gram-negative bacteria as well as yeasts and fungi.

A further object of the invention is to reduce the harmful properties of another substance inhibiting microbial growth contained in a composition, such as a synthetic substance inhibiting microbial growth on a narrow scale, by a mixture with a good availability and low production expenses. One of the most significant harmful properties is the cytotoxicity of a substance inhibiting microbial growth on a narrow scale.

Yet another object of the invention is to realize cosmetic industry compositions or food industry compositions or semi-finished compositions used for manufacturing these compositions, which composition or semi-finished composition contains as few anti-micro-organistic agents as possible.

10 General description of the invention

More precisely, the invention relates to a method for producing a cosmetic composition, food industry composition, animal feed composition, technical composition or packing material composition, said composition containing a widely anti-micro-organistically effective agent, a carrier agent such as solvent, with either hydrophobic or hydrophilic nature, selected according to the target of usage, as well as a possible surface active agent. Hence:

- in the composition, there is included carrier agent, possible auxiliary agents and possible surface active agent conventionally used in a cosmetic composition, food industry composition, animal feed composition, technical composition or packing material composition,
- in the composition, there is included a compound mixture with an anti-micro-organistic effect on a wide scale, which is obtained by pulverizing wood material from preferably two different wood species and/or by extracting the possibly pulverized wood material from two different wood species, so that said mixture contains at least two different compounds selected from among the following group: lignans according to general formulas IA and IB (Appendices 8 & 9), stilbenes according to general formula II (Appendix 9), juvabiones according to general formula III (Appendix 10), flavonoids according to general formula IV (Appendix 10) and betulin and its derivatives (betulinic acid, betulonic acid or betulonic acid), said mixture also containing oligomers of said polyphenolic compounds providing, however, that the compound mixture contains lignans, particularly 7-hydroxymatairesinol or secoisolariciresinol roughly 50-99.9 wt%; stilbenes, particularly pinosylvin or its ester or ether derivatives roughly 0-70 wt%; oligomers of lignans, stilbenes, juvabiones or flavonoids roughly 1-31 wt%; providing, however, that the mixture contains at least one compound, selected from among the following group: 7-hydroxymatairesinol, secoisolariciresinol, cycloisolariciresinol, anhydrosecoisolariciresinol, α -conidendrin, α -conidendric acid, isohydroxymatairesinol and stilbenes according to formula II, as well as their ether and ester de-

rivatives and stereoisomers. In the composition, there is included said compound mixture 0.1–5 wt% of the total weight of said composition, providing, however, that with said content, the cytotoxicity of said compounds dissolved in ethanol with a HaCat cell culture after 24 incubations is lower than the cytotoxicity of 0.02 - 0.1 wt% BHT dissolved in ethanol in the same incubation conditions, preferably lower than the cytotoxicity of 0.01 - 0.05 wt% BHT dissolved in ethanol in the same incubation conditions, in addition to which the mixture within said content range has wide-scale anti-microbial effects against Gram- bacteria, Gram+ bacteria, yeast and fungi in the composition.

Advantageously the compound mixture obtained from two different wood species, such as pine and spruce species and contains 7-hydroxymatairesinol or secoisolariciresinol 50 - 99.9 wt%, stilbene, particularly pinosylvins 0.1–70 wt%, conidendrin 3–6 wt%, lariciresinol 4–7 wt%, liovile 2–5 wt%, or ester or ether derivatives of said stilbene or lignan compounds or their stereoisomers, and also oligomers of lignans, stilbenes, juvabiones or flavonoids 5 -8 wt%. However, owing to the relatively high cytotoxicity of stilbenes, a mixture of polyphenolic compounds obtained from pine and spruce species preferably contains pinosylvins roughly 0.1 – 20 wt%, if it is added in a foodstuff composition or cosmetic composition. Now the content of stilbenes or their ester or ether derivatives in the end product is roughly if 0.1 wt% at most, in case in the composition there is added 5 wt% mixed extract obtained from two different wood species, said mixed extract containing 10 wt% mixture of phenolic compounds.

Here the term stereoisomers refers to compound diastereomers and to mixtures of different diastereomers, to pure enantiomers and rasemic mixtures of enantiomers.

The invention is first of all based on the surprising discovery that when in a composition meant for a mammal or a composition that in general gets into skin contact with a mammal at some stage, the employed anti-micro-organistic effective agent is a mixture of phenolic compounds obtained from extracting and/or pulverizing natural wood material, which mixture is not essentially refined, said mixture has remarkably less harmful properties than the generally used wide-scale synthetic anti-micro-organistic agents currently used in compositions, such as previously mentioned phenoxyethanol. The phenolic compound mixture according to the invention does not cause skin irritation even with high contents, is physiologically well tolerated and has a low cytotoxicity.

Secondly, the invention is based on the surprising observation that sufficient attention has previously not been paid to the level of overall cytotoxicity of the effective agents in cosmetic materials, foodstuffs, animal feed and packing materials, but each effective agent to be included in the composition, such as anti micro-organistic agent, has been added to an

extent which its maximum cytotoxicity by any means has allowed. With this observation in mind, the applicant has attempted to achieve compositions where the overall cytotoxicity of the different components of the effective agent is brought as low as possible, while the effect of the effective agent remains the same.

- 5 In the present invention, the optimizing of the overall cytotoxicity is realized by using, instead of, or in addition to, the first agent inhibiting the growth of micro-organisms contained in the composition, a compound mixture that is obtained by extracting and/or pulverizing and that is not essentially refined. The mutual ratio between the phenolic compounds contained by said compound mixture, as well as the compounds contained by the mixture,
10 can change depending on the effects required in the target of usage. The mutual content of the compounds contained in the compound mixture, the compounds and the effect of the mixture, as well as its profile of influence are adjusted depending on the target of usage of the composition, so that by using the compound mixture, there can be achieved a desired anti-microbial effect, while the overall cytotoxicity of the compound mixture is as low as
15 possible and remains within certain limits in comparison with the cytotoxicity of BHT. Compound mixture using can possible second inhibiting the growth of micro-organisms agent harmful properties such as its own cytotoxicity, reduced remarkably target of usage, such as on the skin or hairs of a mammal, for instance by reducing the quantity of the second agent inhibiting the growth of micro-organisms from the conventionally applied
20 level.

The composition can be a cosmetic composition, a food industry composition, an animal feed composition, a technical composition, or a packing material.

- The term 'cosmetic composition' here refers to compositions meant for the treatment of the skin, teeth, hair and body hair of mammals. In these, the carrier agent is semisolid material
25 such as cream, gel or paste; solid material such as solid foam; heterogeneous material such as solid matter mixture; liquid material such as homogeneous solution or colloidal solution, such as dispersion or suspension, microemulsion, nanoemulsion, or a gaseous material such as aerosol or mist.

- Food industry compositions here refer to both individual foodstuffs and foodstuff products.
30 In this sense, food industry compositions are for example fruits, vegetables etc. individual foodstuffs, but also food industry products and semi-finished products, in the preservation of which there are needed anti-micro-organistic agents.

The packing material composition is a composition where the carrier agent is a packing material that is impregnated by a compound mixture according to the invention, in which there is spread the compound mixture according to the invention.

5 The carrier in a technical composition is a solvent used in cleaning, a cleaning device such as a cleaning cloth, solid material such as a powder used as a surface treatment agent; or the carrier agent is a solution meant for cleaning the body of a mammal. In the carrier, there is impregnated or otherwise included in the compound mixture according to the invention.

10 The manufacturing of a mixture containing phenolic compounds is simple, and the mixture is amply available from wood industry pulping processes, so that the replacing of the effective agent by said mixture also is economically beneficial. The compound mixture according to the invention also has several other advantageous properties that its individual components do not necessarily have, so that by using the mixture, new properties can be obtained in the manufactured compositions, or that the profile of influence of the anti-micro-
15 organistic agent can be adjusted.

By means with the compound mixture according to the invention, there also is achieved the advantage that by using said compound mixture, antioxidative and free radical capturing properties can be obtained in cosmetic or food industry compositions, so that these types of compounds do not need to be separately added in said compositions.

20 The compound mixture is advantageously an unrefined powder ground of wood material, or a compound mixture extracted from wood material in an extraction solution, said compound mixture forming a homogeneous solution, suspension or dispersion with the extraction solution. Even more preferably, the compound mixture is an unrefined lignan mixture and/or stilbene mixture placed in a liquid, said mixture containing at least two different
25 lignan and/or stilbene compounds. The mixture is advantageously obtained by extracting wood material from two different wood species in an alcohol-based solution. Preferably the wood material is obtained from wood knot material or stemwood material from adjacent to knots. Here the term unrefined compound mixture, raw extract, refers to a mixture that is obtained by pulverizing and/or extracting wood or plant material, which mixture has
30 thereafter not been subjected to any such chemical or physical cleaning operations by which one of the mixture compounds would be completely removed from the mixture. Typical cleaning operations in this sense are chromatography or crystallization of certain compounds from the solution. Instead, the mixture may be subjected to such chemical treatments by which the content of one of the mixture compounds is adjusted in relation to
35 other compounds contained in the mixture. This kind of operation is for example extraction

by which the mutual contents of the compounds contained in the solution are adjusted with respect to each other, but by which the compounds are not completely removed. The phenolic compounds contained in the mixture may also be transformed into simple derivatives such as their esters, or into completely other stilbene/lignan/flavonoid compounds, for example when the pH of the extract solution is changed. The mixture of phenolic compounds to be included in the composition according to the invention, which mixture has free radical capturing properties, contains preferably at least two different compounds selected from the following groups belonging to flavonoids, lignans, juvabiones, stilbenes or betulin or its derivatives:

10 Lignans:

- matairesinol, hydroxymatairesinol, oxomatairesinol, didemethyl matairesinol, didemethyl matairesinol, isohydroxymatairesinol, epi-isohydroxymatairesinol and their stereoisomers, among which particularly let us point out hydroxymatairesinol stereoisomers 7S, 8R, 8'R-hydroxymatairesinol and 7R, 8R, 8'R-allohydroxymatairesinol, and their stereoisomers and ester or ether derivatives,

- secoisolariciresinol, isolariciresinol, lariciresinol, pinoresinol, dimethyl secoisolariciresinol, 7-hydroxysecoisolariciresinol, cyclolariciresinol, cycloisolariciresinol and their stereoisomers as well as their ester or ether derivatives,

- nortrachelogenin and its stereoisomers and ester or ether derivatives,

20 - enterolactone and its stereoisomers and ester or ether derivatives,

- conidendrin, α -conidendrin and their stereoisomers as well as ester or ether derivatives,

-lignan A and its stereoisomers and ester or ether derivatives,

- liovile and its stereoisomers and ester or ether derivatives;

Juvabiones:

25 -juvabiones and their stereoisomers and ester or ether derivatives;

Stilbenes:

- pinosylvin, dihydropinosylvin, pinosylvin monomethyl ether, dihydropinosylvin monomethyl ether, resveratrol, astringin, isorhapontine, and their stereoisomers and ester or ether derivatives;

Flavonoids:

- pinosembrin, catechin, pinobanxin, kaempferol, dihydrokaempferol, taxifolin, naringenin, teracacidine, ketoteracacidine, isoteracacidine, melacacidine, isomelacacidine and their stereoisomers and ester or ether derivatives,

- betulin, betulinic acid, betulonic acid or betulonic acid and their stereoisomers and esterified forms,

- as well as the glycosidized forms of these compound, and their oligomers such as trimers and tetramers. These oligomers are here called oligolignans in case they are oligomers of lignans, stilbenes or juvabiones; free lignans and stilbenes are dimers, having 2 phenylpropane units coupled together by beta-beta bonds, and their oligolignans have 3 - 6 phenylpropane units (C_6C_3) coupled together by beta-beta bonds.

In this connection let us point out that compounds called lignans are generally (poly)phenolic compounds obtained from wood and plants, having 2 phenyl propane units coupled together by beta-beta bonds (IUPAC, 2000), but in this application it has been considered necessary to distinguish lignans, stilbenes and juvabiones from each other owing to their different microbiological effects.

Lignans proper here refers to compounds according to the general formula IA and IB (Appendix).

Now, in formula IA:

R1 or R2 denote, irrespective of each other, residue selected from the groups hydrogen, OH or =O,

or either one of the residues R1 or R2 denotes the oxygen atom -O- bound to carbons 9 and 9', and now forms with carbons 8, 8', 9, 9' a 5-membered oxygenous heterocyclic ring C,

R3 denotes hydrogen or residue selected from the group OH, =O, or it forms a bond to the carbon 6, so that the carbons 6, 1, 7, 8, 8', 7' form a cyclohexane ring that is condensed with the phenyl ring A, and possibly also a ring with C,

R4 denotes hydrogen or methyl,

R5 denotes hydrogen or residue selected from the groups OH and OCH_3 ,

R6 denotes hydrogen or hydroxy,

R7 and R8 denote, irrespective of each other, hydrogen or residue selected from the groups OH and OCH₃.

Advantageous lignans according to formula 1A are:

5 7-hydroxymatairesinol (R1 denotes group =O, R2 denotes the oxygen atom pertaining to the hetero ring C, R4= CH₃, R7= OCH₃, R3=R5=R8=OH, R6=H, R9=H),

matairesinol (R1 denotes group =O, R2 denotes the oxygen atom pertaining to the hetero ring C, R3=R6=R9=H; R4= CH₃, R7= OCH₃, R5=R8=OH),

oxomatairesinol, which differs from hydroxymatairesinol in that R3 denotes group =O,

10 didemethyl matairesinol, which differs from hydroxymatairesinol in that R4 and R3 denote hydrogen,

isohydroxymatairesinol,

alpha-conidendrin (R1 denotes group =O, R2 denotes the oxygen atom pertaining to the hetero ring C, R4= CH₃, R7= OCH₃, R8=OH, R6=H, R3 is a bond to the carbon 6, R9=H),

15 alpha-conidendric acid (R4= CH₃, R7= OCH₃, R5=R8=OH, R6 denotes group =O, R1=R2=OH, R3 is a bond to the carbon 6, so that the carbons 6, 1, 7, 8, 8', 7' form a cyclohexane ring D that is condensed with the phenyl ring A, R9=H),

liovile (R4= CH₃, R7= OCH₃, R3=R5=R6=R8=OH, R1=H, R2 denotes the oxygen atom pertaining to the hetero ring C, R3 denotes a bond to the carbon 6, R9=H),

secoisolariciresinol (R1=R2= OH, R3=H, R4= CH₃, R7= OCH₃, R5=R8=OH, R6=R9=H),

20 dimethyl secoisolariciresinol, which differs from secoisolariciresinol in that R5 and R8 are methoxies,

isolariciresinol (R1=R2=OH, R4= CH₃, R7= OCH₃, R5=R8=OH, R6=H, R3 is a bond to the carbon 6, so that the carbons 6, 1, 7, 8, 8', 7' form a cyclohexane ring D, which is condensed with the phenyl ring A, R9=H),

25 cyclolariciresinol (R1=R2= OH, R3= a bond to the carbon 6, so that the carbons 6, 1, 7, 8, 8', 7' form a cyclohexane ring D, which is condensed with the phenyl ring A, H, R4= CH₃, R7= OCH₃, R5=R8=OH, R6=R9=H),

nortrachelogenin (R1 denotes group =O, R2 denotes the oxygen atom pertaining to the hetero ring C, R3=R6=H, R4=CH₃, R7=OCH₃, R5=R8=OH, R9=OH),

In Formula IB:

R10 denotes hydrogen or hydroxy

- 5 R11 denotes hydroxy or oxygen, which is bound by a bond to the carbons 7 and 9', forming now an oxygenous non-aromatic 5-membered heterocyclic ring (tetrahydrofuran) F with the carbons 7, 8, 8', 9', which ring is condensed in the hetero ring E (tetrahydrofuran) at the carbons 8, 8',

R12 denotes hydrogen or methyl,

- 10 R13 denotes hydrogen or methoxy,

R14 denotes hydrogen or methoxy,

R30 denotes hydrogen or group =O.

Advantageous compounds according to formula IB are:

pinoresinol (R13=R14=R12=R10=R30=H, and R11 is oxygen in the hetero ring F),

- 15 isohydroxymatairesinol (R12=R13=R14=R10=H, R11=OH, R30 denotes group =O),

lariciresinol (R12=R13=R14=R10=R30=H, R11=OH), and

lignan A (R10=R11=OH, R12=R13=R14=R30=H).

Stilbenes in turn refer to compounds according to the general formula II (Appendix),

where R15 denotes hydrogen or hydroxy

- 20 R16 denotes residue selected from the groups H, OH, OCH₃,

R17 denotes residue selected from the groups OH or OCH₃,

R18 and R19 denote, irrespective of each other, hydrogen or hydroxy,

R20 denotes residue selected from the groups hydrogen, OGlu

- 25 A few advantageous stilbenes according to formula II are pinosylvin (R18=R19=R20=R15=H, R16=R17=OH), monomethyl ether of pinosylvin

(R18=R19=R20=R15=H, R16=OCH₃, R17=OH), dihydropinosylvin
 (R18=R19=R20=R15=H, R16=R17=OH, the phenyl elements bonding ethenyl residue is
 hydrated to ethyl), resveratrol (R16=R17=R19=OH, R18=R20=H), astringin
 (R15=R17=OH, R16=R19=H, R18=OH, R20=OGlu and isorhapontine (R16=R19=H,
 5 R17=OCH₃, R15, R18=OH, R20=OGlu).

Juvabiones refer to compounds according to formula III (Appendix).

Flavonoids refer to compounds according to general formula IV (Appendix), where

R21 denotes residue selected from the groups H, OH,

R22 denotes residue selected from the groups H, OH, =O,

10 R23 denotes residue selected from the groups H, OH

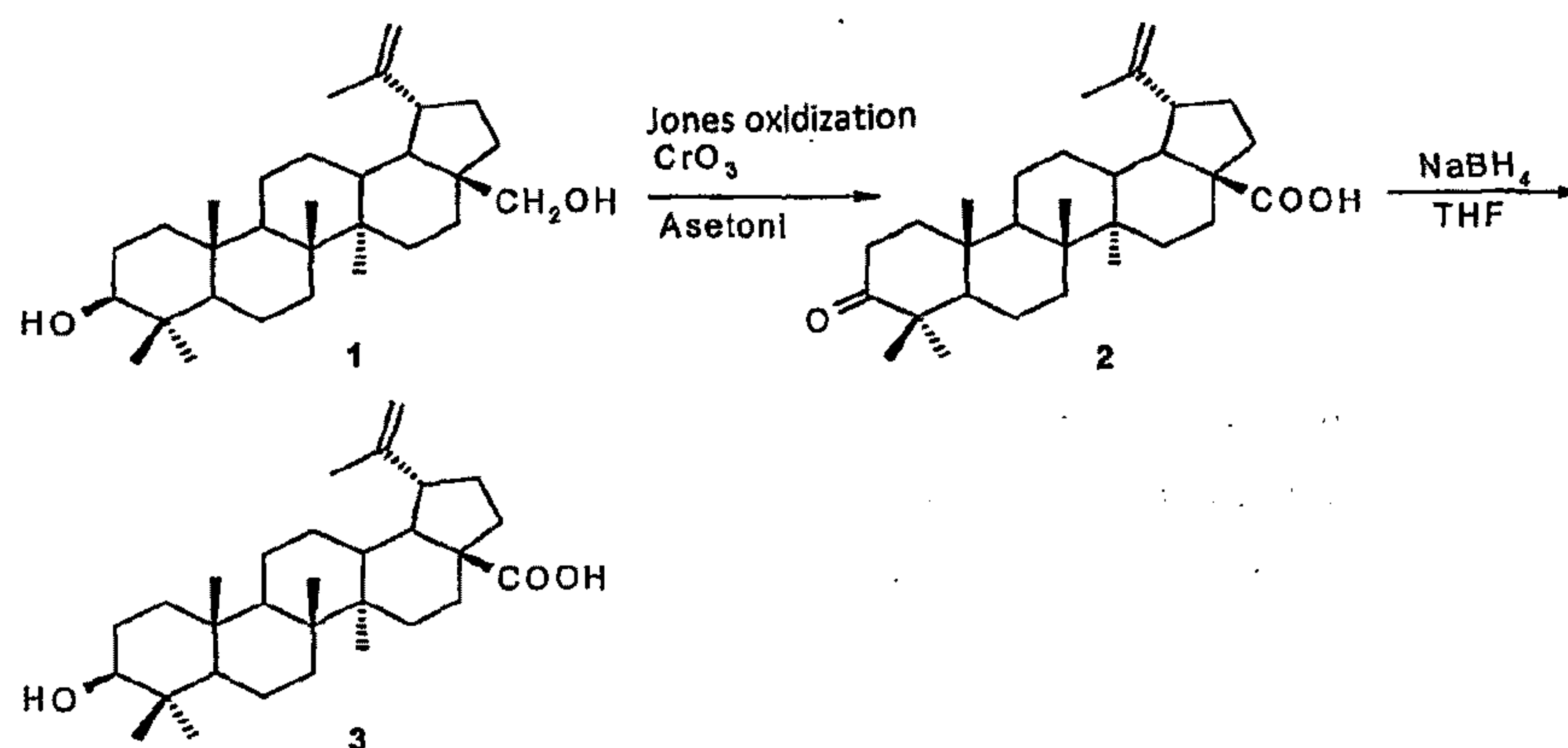
R24, R25, R26 denote, irrespective of each other, hydrogen or hydroxy,

R26 and R27 denote, irrespective of each other, hydrogen or hydroxy.

Among advantageous compounds according to formula IV are dihydromyricetin (R27=H,
 R21=R23=R24=R25=R26=R28=OH, R22 is oxo group), taxifolin (R24=R27=H,
 15 R21=R23=R25=R26=R28=OH, R22 is oxo group), dihydrokaempferol
 (R24=R26=R27=H, R21=R23=R25=R28=OH, R22 is oxo group), catechin
 (R24=R26=R27=H, R21=R23=R25=R26=R28=OH, R22 is hydrogen), naringenin
 (R23=R24=R26=R27=H, R21=R25=R28=OH, R22 is oxo group), kaempferol
 (R24=R26=R27=H, R21=R23=R25=R28=OH, R22 is oxo group), teracacidine
 20 (R21=R24=H, R22=R23=R25=R26=R27=R28=OH, ketoteracacidine (R21=R24=R26=H,
 R23=R25=R27=R28=OH, R22 is oxo group), isoteracacidine (R21=R24=R26=H,
 R22=R23=R25=R27=R28=OH), melacacidine (R21=R24=H,
 R22=R23=R25=R26=R27=R28=OH), isomelacacidine (R21=R24=H,
 R22=R23=R25=R26=R27=R28=OH), pinobaxin (R24=R25=R26=R27=H,
 25 R21=R23=R28=OH, R22 is oxo group) and pinosembrin (R23=R24=R25=R26=R27=H,
 R21=R28=OH, R22 is oxo group).

Betulin, by systematic name (IUPAC) lup-20(29)-ene-3 β ,28-diol, and its derivatives refer to compounds according to formula 1E. In the formula 1E, betulonic acid is compound 2, and betulinic acid is compound 3. Betulin is compound 1. Compounds 2 and 3 are obtained by oxidizing betulin 1 into compound 2, and by reducing compound 2 into compound 3 (US 6.280.778). Betuloinic acid is a derivative of betulonic acid.

Exemplary oligolignans are beta bound guaiacyl ethers of lignans and stilbenes (trimeric



so-called sesquilignans) and coumarates such as secoisolariciresinol guaiacyl glycerol ether, nortrachelogenin guaiacyl glycerol ether, hydroxymatairesinol guaiacyl glycerol ether, lariciresinol guaiacyl glycerol ether, liovile guaiacyl glycerol ethers, conidendrin guaiacyl glycerol ether, pinoresinol guaiacyl glycerol ether, lariciresinol coumarate and secoisolariciresinol coumarate (Willför et al, Holzforchnung, Vol 58, 3435 -354, 2004) and dilignans such as 5-5-bis-secoisolariciresinol, 5-5-bis-isolariciresinol, 5-5-bis-lariciresinol.

Depending on the pH, phenolic compounds occur in the compositions either as free, esterized or etherized forms, wherefore also said ester and ether derivatives belong within the scope of the invention.

Free radical here refers to a molecule or an atom that has unpaired electrons in its electron shell. Typical free radicals are oxygen radical, hydroxyl radical and peroxy radicals as well as superoxide radicals, but also for example lipid radicals. Further, for instance titanium oxide can be formed into a reactive free radical, in case it is tuned to a higher energy state owing to the effect of UV radiation. A compound is called free radical capturer, in case it is capable of inhibiting the creation of free radicals, or their function. In case the compound is capable of inhibiting the creation of particularly oxygenous free radicals and their effects, the compound is called antioxidant.

One of the most important properties of a compound mixture according to the invention is that the mixture of phenolic compounds present in the compositions inhibits on a wide

scale the growth of anti-microbial agents, when it is added in the composition for 0.1–5 wt%. Generally the amount of synthetic widely anti-microbial agents that can be added to compositions is only roughly 0.01–0.03 wt%, owing to their high cytotoxicity, in case the compositions get into contact with mammal skin at some stage of their usage life. As
5 for Gram-negative bacteria, a mixture of phenolic compounds has a growth inhibiting effect is at least against *E. coli*, *Ps. aeruginosa*, *Ps. putida*, and *Kl. pneumoniae*; as for Gram-positive bacteria, it has a growth-inhibiting effect at least against *S. aureus*; as for yeasts, it has a growth-inhibiting effect at least against *M. furfur* and *C. albicans*, and as for fungi, a growth-inhibiting effect at least against *A. niger*.

10 Thus, most of the compound mixtures according to the invention are relatively mild anti-micro-organistic agents, but this is compensated in that owing to their non-toxic nature, they can be used in remarkably larger quantities.

A particular surprising feature in the invention is that although it has been found out that several pure lignan or flavonoid compounds, or knot extracts obtained from trees contain-
15 ing abundantly such lignans or flavonoids, have a limited effect against the growth of micro-organisms, yeasts or fungi, it has not been verified that they should have a wide-scale anti-micro-organistic effect, and they are not effective for example against certain important Gram-positive bacteria such as *S. aureus* (cf. e.g. Välimaa et al., International J. of Microbiology, 115 (2007) 235-243). Moreover, it has earlier been shown that stilbene-bearing
20 raw extracts and solutions containing refined stilbenes are relatively cytotoxic (e.g. International J. Food Microbiology, 115 (2007) 235-243), which does not encourage a man skilled in the art to use these extracts in cosmetic products. The low cytotoxicity of the mixtures according to the invention, combined with low skin irritation and a wide-scale anti-micro-organistic effect, guarantees that the compound mixture according to the invention
25 can be used in sufficiently large quantities, particularly in cosmetic compositions, in order to ensure its anti-microbial effect. The wide-scale anti-micro-organistic effect of the mixtures containing phenolic compounds used in the invention, combined with their low cytotoxicity, is a surprising feature, because in the raw extracts obtained from knotty stemwood described in the prior art, their anti-micro-organistic effect has not been verified as particu-
30 larly wide-scale, not even for raw extracts containing stilbenes.

Further, one of the most important properties of the compound mixtures according to the invention is their minimal penetration to skin, wherefore skin irritation does not occur when using them, as opposite to the conventionally used anti-micro-organistic compounds. The applicant has studied the skin irritation of the compound mixtures according to the in-
35 vention in a so-called single patch test and found out that they do not irritate the skin with

0.1 wt% contents, and not even with 1 wt% contents; often even 5 wt% contents can be used without excessive skin irritation.

By using the microbial growth inhibiting compound mixture according to the invention, it is often possible to modify the harmful properties of other anti-micro-organistic agents, such as microbicidal and bacteriostatic agents contained in the composition, by reducing the amount of free radicals created during their decomposition, and hence the cytotoxicity of these agents.

Among others, the anti-micro-organistic compound mixture according to the invention can be used in cosmetic compositions and their semi-finished compositions, such as sun protection compositions and in semi-finished products used in the manufacturing of sun protection compositions. The mixture can also be used in so-called commercial solvents, such as surface treatment agents, solvents used in the cleaning branch solvents etc.

Description of preferred embodiments of invention and of compound mixtures used therein

- One of the advantageous uses of the compound mixture according to the invention is a semi-finished product used in the manufacturing of cosmetic compositions, which is formed of alcohol solvent used as the carrier, and of an anti-micro-organistic mixture according to the invention. Several examples of this embodiment are given in the description below.
- The compound mixture according to the invention can also be used for technical purposes in various solvents, in liquids used in the machining of metals with chip removal, in cleaning liquids, liquid surface treatment agents, wood preparation fluids, drilling fluids etc. During usage, these liquids may get into contact with human skin, and in that case the microbicidal and bacteriostatic compounds contained therein may cause various allergic reactions, cytotoxic exposure and skin damages. In these technical solutions, the phenolic compounds bearing mixture according to the invention can be used for replacing for example the skin irritative microbicides contained by said solutions, either partly or completely. The compounds contained in the compound mixture have anti-micro-organistic effects, but they do not, however, irritate the skin as much as synthetic anti-micro-organistic agents.
- Primarily the effective free radical capturing mechanism of the (poly)phenolic compounds contained in the compound mixture according to the invention appears to be based on the fact that mixture the phenolic compounds contained by the mixture go through an automatic oxidation-reduction reaction after neutralizing the free radicals. This automatic oxidation-reduction reaction ends in the formation of stable dimers. Polyphenol dimers can

thereafter be split back to polyphenols (regeneration). A secondary effect of the mixtures according to the invention lies in that they are capable of absorbing the UV radiation that creates free radicals, and inhibiting the action of enzymes creating free radicals and/or inhibiting the action of metal ions catalyzing the formation of free radicals, or breaking up hyperoxides. The phenolic compounds contained in the compound mixture according to the invention are well tolerated by mammals, their cytotoxicity is low and they do not themselves form nor induce free radicals.

The production method of a compound mixture according to the invention, and the phenolic compounds contained therein, as well as the mutual ratios of their quantities depend on the designed usage and on the availability of raw materials. The mixture according to the invention is often obtained from the material of two or more wood species. Thus for example wood processing industry generally uses both spruce and pine in the pulping process. The production of a mixture according to the invention can utilize knot wood or knotty stemwood parts that are less suitable in the pulping process. However, because the pulping process mainly uses spruce and to a lesser amount pine, it is often more advantageous to form a compound mixture of spruce, which is more disadvantageous for the use of the composition, because it has better availability than pine. For example, a compound mixture obtained from pine wood knot material in hydrophilic extraction has a remarkably abundant quantity of stilbenes, which are effective microbicides and effective compounds inhibiting microbial growth. As for a compound mixture obtained from spruce wood knot material in a hydrophilic extraction, it contains a remarkable quantity of 7-hydroxymatairesinol, as well as its isomers and derivatives, such as matairesinol. Although the microbicidal efficiency of 7-hydroxymatairesinol is lower than that of stilbenes, particularly that of pinosylvin and its derivatives, it is profitable to use knot extract obtained from spruce, which has better availability and thus generally lower price, in the microbicidal compound mixture.

The phenolic compound mixture according to the invention is produced either by pulverizing or by extracting wood material, or by combining said procedures. Because the wood material most widely available in Finland is pine or spruce, of which particularly the latter contains resin, the extraction is generally realized in two steps. Now resin compounds are extracted from the pulverized pine material, advantageously pulverized knot wood or knotty stemwood material, by a lipophilic extraction solvent, and successively the polyphenols are extracted by a hydrophilic extraction solution. The lipophilic organic extraction solution is for example hydrocarbon, such as lower alkane, for example hexane or heptane. Generally the hydrophilic organic extraction solution is an organic compound containing a carbonyl group, such as alcohol or ketone. Ketone can be used only in case the compound mixture is meant for technical usage. An advantageous ketone is a lower alkyl ketone such

as acetone. In case the compound mixture to be produced is a compound mixture in pulverized form, water is removed from the pulverized knotty stemwood material by freeze-drying.

Advantageously the alcohol is a monovalent, bivalent or trivalent lower alkyl alcohol, or a mixture of these. The monovalent lower alkyl is preferably ethanol, propanol, butanol, heptanol, octanol or decanol. A mixture of a lower alkyl alcohol and glycerol or glycol is an advantageous solvent agent when producing several skin care compositions or semi-finished products of skin care compositions, where the carrier agent is liquid. As for the lower alkylene glycol used as the hydrophilic extraction solution, it is preferably selected from a group comprising propylene glycol, butylene glycol, pentylene glycol and dipropylene glycol. Of these, the latter is particularly advantageous to be used in perfumes. In this kind of extract solution or in an extract concentrate obtained therefrom, the content of alkylene glycol is more than 7 wt%, preferably more than 10 wt%.

The composition of a compound mixture containing phenolic compounds, obtained from one and the same wood material by different extraction methods, fluctuates to some extent. The extraction method is selected according to the target of usage of the composition (for instance use in commercial solvents or cosmetic compositions), and according to the desired anti-micro-organistic properties of the compound mixture. For example from Table 2 to be described below, it can be observed the total quantities of polyphenolic lignan and stilbene compounds contained in pine knotwood and obtained by various extraction methods, as well as the mutual ratios of said polyphenolic compounds, fluctuated to some extent. Moreover, the obtained extract contained a certain amount of resin elements.

In case the extraction methods are changed, lignans can be transformed to other lignans. For example, 7-hydroxymatairesinol can in alkaline extraction conditions be transformed to alpha-conidenrin and further to alpha-conidendric acid or 7-hydroxymatairesinolic acid, and further to isohydroxymatairesinol. In acidic extraction conditions, 7-hydroxymatairesinol is transformed to isohydroxymatairesinol, and alpha-conidendric acid in turn is transformed to cyclolariciresinol, and secoisolariciresinol is in acidic conditions transformed to anhydrosecoisolariciresinol.

Hardwood species contain remarkable quantities of different flavonoids, biflavonoids and flavonoid glycosides, as is apparent from Table 3A below. Unrefined raw extracts containing flavonoids, obtained from hardwood species, have antioxidative and free radical capturing properties, wherefore they can be used for example together with raw extracts obtained from knotty pine or spruce stemwood for producing compound mixtures containing phenolic compounds according to the invention, as well as their intermediate products.

It has been found out that an unrefined extract solution obtained from knotty spruce stemwood material by hydrophilic extraction has, in its spectrum of influence, a similar wide-scale microbial growth-inhibiting effect as extracts obtained from knotty pine stemwood (cf. Table 4B). A raw extract extracted from spruce knotwood in alcohol contains mainly
5 lignans and oligolignans (cf. Table 3B below). Lignans contain mainly hydroxymatairesinol, secoisolariciresinol, conidendrin and oligolignans, as well as smaller amounts of other lignans such as liovile and lariciresinol. Although the microbial growth inhibiting effect of polyphenolic lignan compounds contained in a raw extract obtained from knotty spruce stemwood by alcohol is weaker than with raw extracts obtained from knotty pine stemwood (cf. Table 4B below), said extracts obtained from spruce stemwood chips by hydrophilic extraction can be used for producing cosmetic compositions according to the invention and their semi-finished products owing to their low cytotoxic effect and low skin irritation.

As can be observed for example from Table 4A below, extracts obtained from the bark of
15 different wood species by hydrophilic extraction contain various polyphenolic flavonoid compounds. In the present invention, these unrefined extracts mainly containing flavonoids can be used either as such or preferably together with hydrophilic extracts obtained from other the material of wood species, to be further used for producing various compositions according to the invention and their semi-finished products. One source of these advantageous flavonoids is the bark of birch (*Betula betula*). Compounds isolated from birch bark are enlisted in Table 4A and in Appendix 3 Scheme 2 illustrates compounds isolated from birch bark. As can be observed from Scheme 2 of Appendix 3, birch bark contains lignans, stilbenes, flavonoids, juvabionones and betuligenol and its derivatives, wherefore it can also be used as a raw material for producing an anti-micro-organistic compound mixture
20 according to the invention. Betuligenol (betulin) mentioned at the end of Scheme 2 of Appendix 3, as well as its derivatives betulinic acid, betuloinic acid or betulonic acid, can also be used in compound mixtures according to the invention, either as separately added therein in refined form, or together with an unrefined compound mixture obtained from birch bark extraction.

30 In Scheme 1 illustrated in Appendices 1 and 2, there are introduced polyphenolic compounds present in pine. As is apparent from Scheme 1 of Appendices 1 and 2, as well as from Table 4B, knotty pine stemwood and its bark contain several different stilbene compounds. Moreover, the percentual share of these stilbene compounds in the pine polyphenolic compounds present in knotty stemwood and bark is remarkably high in comparison
35 with the quantity of other polyphenolic compounds. Further, pine contains a remarkable quantity of various flavonoids.

Unrefined extracts obtained from knotty pine stemwood by hydrophilic extraction contain a remarkable quantity of stilbene compounds. The applicant has verified that these extracts inhibit the growth of micro-organisms (Gram-positive and negative bacteria, fungi and yeasts) in a wide scale. Thus, pine is a good source of the anti-micro-organistic compound mixtures according to the invention. It has also been discovered that stilbenes have anti-inflammatory properties. On the other hand, unrefined raw extracts obtained from spruce contain mainly lignans; the applicant has verified that said lignans have, by their spectrum of influence, a similar but weaker effect for inhibiting the growth of micro-organisms than stilbene-bearing raw extracts obtained from pine. Both raw extracts extracted from knotty pine stemwood and containing mainly stilbenes, as well as raw extracts extracted from knotty spruce stemwood and containing mainly lignans, are feasible when manufacturing different compositions that have a wide-scale anti-microbial effect and at the same time low skin irritation and low cytotoxicity in comparison with BHT.

From the pulping processes of wood processing industry, there is obtained remarkably more spruce than pine, which fact is in favor of a solution that the compound mixtures according to the invention comprise compound mixtures of unrefined extraction solutions obtained from spruce by hydrophilic extraction, or unrefined extraction solutions obtained from spruce and pine by hydrophilic extraction. In a suitable arrangement, the combined extraction solutions according to the invention contain for example 70 wt% (poly)phenolic compounds (lignans and oligolignans) extracted from knotty spruce stemwood, and 30 wt% (poly)phenolic compounds extracted from knotty pine stemwood, with stilbenes included. Other mixture ratios can also be applied, as long as it is taken care of that the compound mixture contained in the combined extraction solution obtained from pine and spruce has a sufficiently low cytotoxicity (the employed reference is BHT). The applicant has discovered that when the mutual mixing ratios of the raw extracts obtained from different wood species are selected so that the cytotoxicity of the compound mixture contained in the combined raw extract, when measured in ethanol for a HaCat cell culture after 24 incubations, is lower than the cytotoxicity of 0.02 -0.1 wt% BHT dissolved in ethanol in the same incubation conditions, preferably lower than the cytotoxicity of 0.01 -0.05 wt% BHT dissolved in ethanol in the same incubation conditions, the allowed quantity for the employed mixture is 0.1 -5 wt% of the total weight of the composition, in most cases 1 - 5 wt% of the total weight of the composition. This limit value of the content is remarkably higher than with most commercially available wide-scale, microbial growth inhibiting synthetic substances, which means that the compound mixtures according to the invention can be used in sufficient quantities for ensuring their wide-scale anti-micro-organistic effect.

In Tables 5A, 5B, 5C and 5D included in Appendix, there are represented antioxidative and free radical capturing effects of pure polyphenolic compounds and of unrefined poly-

phenolic compounds containing raw extracts obtained from wood bark and knotty stemwood.

Tables 5A and 5B represent the antioxidative effect of a few pure polyphenols and of unrefined polyphenolic compounds containing raw extracts, obtained from wood bark and knotty stemwood. Tables 5C and 5D in turn illustrate the peroxide radicals capturing capacity of a few pure polyphenols and of unrefined, polyphenolic compounds containing raw extracts, obtained from wood bark and knotty stemwood. In Tables 5A–5D, it can be observed that the antioxidative and free radical capturing properties of solutions containing pure polyphenol compounds, obtained from wood material in hydrophilic extraction, are often remarkably different from the corresponding properties of compound mixtures containing unrefined raw extract solutions and obtained from wood material in hydrophilic extraction, owing to the synergetic effects of the compounds contained in the compound mixtures in unrefined extracts. The compound mixtures according to the invention are obtained from these unrefined raw extract solutions.

15 Production of the compositions and their semi-finished products

A phenolic compounds containing mixture according to the invention can be included in cosmetic and food technology compositions and their semi-finished products in a way known as such, of which examples are also given below. Thus, in case a compound mixture is extracted for instance of wood material by an alcoholic solution into a raw extract, this raw extract can be made into a homogeneous mixture such as a homogeneous solution, or a colloidal dispersion with two or several phases such as gel, paste, emulsion, microemulsion, nanoemulsion suspension, dispersion or mist. In that case a phenolic compounds containing raw extract is included in a homogeneous solution by dissolving, and/or it is included by dispersing to the carrier agents of a colloidal mixture in a way known as such, so that the raw extract is admixed either in a phase containing a continuous carrier agent, or to a phase containing a carrier agent to be dispersed. For forming a carrier of carrier agents, there are used conventional auxiliary agents of the trade. Such agents are, among others, surface active agents, dispersing agents such as emulsifying agents, gel formers such as carbomers and methylcellulose.

The employed carrier agents are gel base formers such as water or alcohol, cream base and paste base formers such as paraffins, waxes, silicones, aqueous phase forming agents (water) or phase formers such as paraffin or stearic acid. The compositions can also be multiphase compositions, so that the carrier agent is formed of several aqueous and/or oil phases. With respect to the manufacturing of various compositions, we refer to the literature of the trade /1/, /2/, /3/ and to the examples to be given below.

In these homogeneous solutions and colloids, there can be admixed additives such as UV protective agents, antioxidants and vitamins, surface active agents, moisturizing agents, moisture maintaining agents, stabilizing agents, moisture absorbing agents, emollients, fats, lubricants, perfumes, viscosity regulators, colorants, antioxidants and narrow-scale anti-microbial agents etc., in a conventional way known as such, with respect to which we refer to the literature of the trade /1/, /2/, /3/.

In case the composition is a packing material composition, the carrier agent is a packing material, in which the compound mixture contained in the extraction solution is impregnated or spread on. Said packing material can be cardboard, corrugated board, plastic admixed cardboard or other packing material known from the prior art.

In case the composition is a technical composition, the carrier is a commercial solvent, cleaning device such as a cleaning cloth, solid substance such as a powder used as a surface treatment agent, or a solution meant for the cleaning of a mammal's body. In the carrier, there is impregnated or otherwise included the compound mixture according to the invention.

Surface active agents applicable in exemplary liquid compositions according to the invention, and in their semi-finished compositions are: tensides, lecithin, caprylic acid and monoglycerides and diglycerides of capric acids, polyglyceryl-3-di-isostearate/polyglyceryl-2 and polyhydroxystearate, alkyl glycoside/alkyl alcohol, cetearyl pyridium chloride, bent-salkonium chloride, ionogenic agents, cetearyl glycosides, lower alcoxilated glycosides and micelle-forming agents.

Perfumes can be selected for example from a group including phenyl ethyl glycol, eugenol, isoeugenol, geraniol, citronellol or linalool, or their esterized forms or their aldehydes.

Colorants can be selected for example from among the colorants accepted by FDA to be used in foodstuffs and cosmetic products.

In compositions according to the invention, as well as in their semi-finished products, it is also possible to add other effective agents as additives. Such effective agents to be used as additives are for example antioxidants. Among antioxidants, let us point out natural and synthetic vitamins such as vitamin A, B, C, D, E, provitamin B5, vitamin B3, L-ascorbic acid and vitamin E; further, there can be used antioxidants obtained from natural sources, such as antioxidants contained in green tea, antioxidants contained in flaxseed, antioxidants contained in horse chestnut, beta carotene, selenium, glutamine, ubiquinone (coenzyme Q10), glycolic acid, growth hormones and kinetin.

An advantageous botanical microbial growth inhibiting agent is betulinic acid, betuloinic acid or betulonic acid (US 6 280 778), derivatives of betuligenolin, and resvatrol obtained from spruce bark. These have been found to have an anti-microbial effect, and their cytotoxicity for healthy cells is low, and they enhance the dying of cancer cells. These can be added, either as pure compounds or as unrefined extract solutions obtained from wood bark, or as powders, in compositions and semi-finished products to be manufactured according to the invention.

Further, in compositions to be manufactured according to the invention, there can also be added pure flavonoids, lignans and stilbenes as well as their oligomers isolated from plants. In this application, the term 'oligomers' refers to homologs of a compound, i.e. to its dimers, trimers etc., where the included number of similar units is lower than in a polymer. Suitable botanical polyphenol compound sources are oilseeds, nuts, grain, fruits, berries and pulses.

Examples

Field tests

A) Cytotoxicity in comparison with BHT

Table 1 shows comparisons between the cytotoxicity of a compound mixture according to the invention and the cytotoxicity of an antioxidant (BHT) that is widely used in food and cosmetic industry.

Table 1

The cytotoxicity of sample extracts pulverized or alcohol-extracted of knotty spruce stem-wood chips, containing the compound mixture, or a powder containing the compound mixture, in comparison with the cytotoxicity of BHT (butylated hydroxytoluene). The composition of the individual compounds included in the compound mixture contained by the samples was in accordance with Table 3B. The cytotoxicity of the sample extracts and powders for human keratinocyte cells was measured. The employed measure of cytotoxicity was the total protein quantity created by the samples, when the samples were incubated together with human keratinocyte cells for a certain incubation time (24 h). For each sample, there was searched a limit value (EC20), by which 20% of the cultivated cells died.

Extraction solvent	sample	EC20 ppm 24 h
pentylene glycol	HMR-5	450

butylene glycol	HMR-4	600
glycerol	HMR	1.260
	HMR powder	160
with propylene glycol	HMR-3	620
ethanol	HMR extract	550
ethanol	BTH	5.50

From Table 1 it is observed that the most cytotoxic substance was BTH, which was 10–20 times more cytotoxic than HMR powder. HMR powder was obtained by pulverizing knotty spruce stemwood chips without other further cleaning, and said powder contained mainly 7-hydroxymatairesinol as well as, to a lesser degree, other polyphenolic lignans. Other knot extracts obtained from spruce knotwood chips by hydrophilic extraction with alcohol (ethanol, propylene glycol, pentylene glycol, butylene glycol, or glycerol) also contained lignan mixtures according to the invention, which included, as their principal component, 7-hydroxymatairesinol and also other phenolic lignans. With respect to HMR extract mixtures, BTH was 50–100 times more cytotoxic.

B) Anti-microbial effect

Test 1

The anti-micro-organistic effect of a few compound mixtures according to the invention against bacteria, yeast and fungi was examined:

- 15 - raw extracts extracted from knotty spruce stemwood chips by 4-glycol (sample 1), 5-glycol (sample 2) and 3), glycerine (sample 3) and glycerol (sample 4), containing 10 wt% of the compound mixture, the composition of the polyphenolic compounds of said raw extracts being in accordance with Table 3B (contained most 7-hydroxymatairesinol).
- 20 - combined raw extract (sample 5), extracted of knotty spruce stemwood chips and knotty pine stemwood by ethanol (pine) and by butylene glycol (spruce), containing roughly 10 wt% of the compound mixture obtained from pine and spruce. The sample mixture contained both a lignan mixture obtained from Norway spruce (*Picea abies*), the composition of which was in accordance with Table 3B, and a mixture of lignans and stilbenes obtained from pine (*Pinus sylvestris*), the composition of which roughly corresponded to the one illustrated in Table 2 (test 2, ethanol extraction).

The growth-inhibiting effect of the mixture samples was verified against the following micro-organisms:

- Staphylococcus aureus ATCC 6538
- Esterichia coli ATCC 8739
- Pseudamonas auriginosa ATCC 9027
- Psudomonas putida ATCC 49128
- 5 Klebsiella pneumoniae ATCC 10031
- Candida albicans ATCC 10231
- Malassezia furfur ATCC 96809 (yeast fungus)
- Aspergillus niger ATCC 16404

Performance of the study

- 10 In each of the 10 g sample batches taken from the samples, there were added different microbe cell suspensions, where the microbe population density was at least 5×10^6 microbes/ml. The sample batches were incubated at room temperature (22 °C) for 2, 4, 24 and 48 hours, 4 days, 7 days, 14 days and 28 days.

- 15 When reproductive microbes were not found in the samples anymore, each sample was cultivated for a 1 ml sample batch in a 100 ml Letheen broth base, and after concentration, possible bacterial growth was further checked on a culture base.

Results

- 20 All samples had an extremely good anti-microbial effect; after an incubation time of 4 hours, 24 hours, 48 hours, 7 days, 14 days and 28 days, microbial growth was not detected.

- 25 Both the raw extracts obtained from spruce material according to the invention by alcohol extraction, and the raw extracts obtained from pine material by alcohol extraction, as well as the raw extracts obtained from a combined spruce and pine material by alcohol extraction prevented the growth of anti-microbial agents on a wide scale. They prevented the growth of both Gram-negative bacteria E. coli, Ps. aeruginosa, Ps. putida, Kl. pneimoniae) and Gram-positive bacteria (S. aureus). In addition, they also efficiently prevent the growth of yeasts (M. furfur, C. albicans) and fungi (A. niger).

Test 2

Raw extract extracted from knotty spruce stemwood with pentylene glycol, containing a 3 wt% mixture of phenolic compounds, the composition of which was in accordance with Table 3B, was added in a sun protection cream for 0.3 wt% and 0.5 wt%, as well as known ethylhexyl glycerine (EH), efficient against Gram-positive bacteria. For the sake of comparison, to the same sun protection creams there was added a conventionally used, widely anti micro-organistic agent, phenoxyethanol (FE), which has a relatively low highest acceptable quantity of usage owing to its cytotoxicity and skin irritative properties.

10

Table 6		number of inoculation cycles						
Test		0	1	2	3	4	5	6
Test 1								
cream base		-	-	-	+Y	+M,Y	+M,Y	++M,Y
15	0.3 wt% EH							
1 wt% FE								
Test 2		-	-	-	-	-	-	-
Cream base								
0.5 wt% FE								
20	1 wt% EF							
Test 3		-	-	-	-	-	-	-
Cream base								
0.3 wt% EH								
3 wt% HMR-5								
25	Test 4	-	-	-	-	-	-	-
Cream base								
0.3 5-% EH								
3 wt% HMR-5								
30	Y= yeast growth							
M= Fungal growth								
- no microbial growth								
+ slight microbial growth								
++ moderate microbial growth								
35								

As is apparent from the above described test 2, when a 3 wt% raw extract obtained from knotty spruce stemwood was used in a sun protection cream instead of phenoxyethanol, it was possible to maintain a wide-scale anti-microbial effect. The effect was maintained, although the content ethylhexyl glycerine, inhibiting the growth of Gram-positive bacteria, was reduced, which shows that the raw extract from knotty spruce stemwood itself has a Gram-positive bacteria growth-inhibiting effect.

The sun protection cream used in the above described test was a cream according to example 2. When using phenoxyethanol, the highest acceptable content of which is 1 wt%, there was detected a growth of yeasts and fungi with an ethylhexyl glycerine content of 0.3% in the sun protection cream. On the other hand, with an ethylhexyl glycerine content of 0.5%, the growth of yeasts and fungi was inhibited. When phenoxyethanol was replaced by a lignan mixture HMR-5 obtained from knotty spruce stemwood with hydrophilic extraction (pentylene glycol), it was observed that the ethylhexyl glycerine content could be reduced to 0.3%, and yet the microbial growth in the cream was inhibited. Thus the alcohol extract (HMR-5) according to the invention, which contains a physiologically well-tolerated mixture, can be used to replace the anti-micro-organistic phenoxyethanol, the use of which is restricted owing to its physiological toxicity. The composition HMR-5 was in accordance with the composition according to Table 3B.

20 C) extraction of phenolic compounds from wood material

Table 2

Table 2 illustrates extracts obtained by different extraction methods from pulverized knotty stemwood material of pine (scots pine).

	Test 1	Test 2	Test 3	Test 4
Compound	% of peak	% of peak	% of peak	% of peak
PSMME	16	17	23 (14)	29 (16)
PS	15	20	19 (12)	20 (12)
NTG	16	30	30 (18)	33 (19)
Resin acids	18	16	12 (7)	10 (6)
Oxidized resin acids	35	17	17 (10)	8 (5)

25 In all tests, there were extracted pine chips composed of knotty stemwood. These were first extracted with hexane; in test 4, a technical hexane was used. After the extraction of lipo-

philic hexane, the samples were extracted with various hydrophilic solutions: in test 1 with acetone, in test 2 with ethanol (96%), in test 3 with acetone and in test 4 again with 96% ethanol. In tests 1 and 4, there were used chips were dead and live wood material were mixed; in tests 2 and 3, the employed wood material consisted of hand-picked dead knotty stemwood chips. After extraction, the quantities of the phenolic and resin compounds contained by the samples were analyzed by liquid-gas-chromatography. The contents of various compounds are given as a percentual area of the peak shown by each compound in relation to the area of all peaks. In tests 3 and 4, the weights of different compounds are given in parentheses with respect to the total weight of the solution.

Abbreviations of the compounds in the Table: PSMME: pinosylvin monomethyl ether (stilbene); PS: pinosylvin (stilbene); NTG: nortrachelogenin (lignan).

It has been discovered that raw extracts from pine knotwood are both microbial growth inhibiting on a wide scale, and also anti-inflammatory, obviously owing to the stilbene compounds contained therein, such as pinosylvin and its derivatives. Thus for example knot extracts containing unrefined phenolic compounds according to Table 2, from tests 1 and 3, can be used as such in commercial solvents. The pine knot extract obtained from examples 2 and 5, could in turn be used as such as a semi-finished product for manufacturing various cosmetic compositions, without further cleaning, in case the anti-micro-organistic compounds used in said products should be replaced by a mixture containing physiologically better tolerated phenolic compounds.

D) polyphenolic compounds contained by different wood species

Mixtures according to the invention can be isolated from wood material in general. An advantageous raw material source consists of branches, knotwood and knotty stemwood, but also other wood parts, such as stemwood, wood bark and needles can be used.

Table 3A

The principal components of unrefined extraction solutions obtained by hydrophilic extraction from knotty stemwood of various hardwood species.

Acacia crassicarpa		Populus grandidentata	
Flavonoids	54%	Flavonoids	31%
Melacasinine	24%	Dihydrokaempferol	13%
Isomelacasinine	18%	Catechin	9%
Biflavonoids	9%	Naringenin	7%
		Taxifolin	3%
		Flavonoid glycosides	34%

				Acacia mangium		Populus tremula	
				Flavonoids	36%	Flavonoids	21%
				Teracacidine	25%	Dihydrokaempferol	17%
5				Ketoteracacidine	3%	Naringenin	3%
				Biflavonoids	8%	Flavonoids glycosides	8%
				Fagus sylvatica		Populus tremuloides	
				Flavonoids	7%	Flavonoids	23%
10				Catechin	6%	Dihydrokaempferol	17%
						Naringenin	10%
				Eucalyptus globulus		Kaempferol	1%
				Tannins	19%	Taxifolin	1%
				Tannin monomers	5%	Flavonoid glycosides	24%
15				Ellagic acid	3%		
				Gallic acid	2%		

Table 3B

Polyphenolic compounds contained in a solution extracted from Norway spruce knotty stemwood by pentylene glycol, as defined by a gas-liquid chromatography. The raw extract contained 87 -93 wt% pentylene glycol (solvent) and 6.5 -7.5 wt% polyphenolic compounds (mainly lignans) extracted from spruce.

		Hydroxymatairesinol	70-80%
		Secoisolariciresinol	3-6%
		Conidendrin	4-7%
25		Lariciresinol	1-3%
		Liovile	2-5%
		Other lignans	5-8%

As is seen in Table 3B, spruce contains mainly lignans, of which the majority is hydroxymatairesinol. The other lignans mentioned in the Table are mainly oligolignans.

Table 4A

Principal components of unrefined extraction solutions obtained from the bark of different wood species by hydrophilic extraction (Extractives in stemwood and knots of Acasia and Aspen trees, Suvi Pietarinen, Abo Akademi, Turku 2005).

	Thuja occidentalis	Picea abies
	Sugars	Isorhapontine
	Catechin	Astringin
	Isorhapontine	Resveratrol-glycoside
5	Astringin	Tannins
	Tannins	
	Pinus banksiana	Abies lasiocarpa
	Sugars	Sugars
	Taxifolin	Resin acids
10	Isorhapontine	Tannins
	Dihydromyrcetin	
	Tannins	
	Betula pendula	Populus tremula
	Betuligenili glycoside	Undefined glycosides
15	Catechin	Tannins
	Sugars	
	Tannins	
	Pseudotsuga menziensii	Pinus mariana
	Sugars	Sugars
20	Taxifolin	Catechin
	Catechin	Tannins
	Tannins	

Table 4B

25	Principal components of unrefined extracts obtained from the knotty stemwood of a few pine and spruce species by hydrophilic extraction (Wilför et al., J. Agric. Food. Chem., 51, 26 (2003)), in percentages by weight of the total quantity of the components of the mixture extracted from the wood.		
30	Wood material	Compounds	wt%
	Picea abies		
		Lignans	53
	hydroxymatairesinol		41
35	secoisolariciresinol		3
	α -conidendrin		7

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	Oligolignans		12
	Abies sibirica		
	Lignans		33
5	secoisolariciresinol		21
	lariciresinol		7
	Oligolignans		31
	Juvabiones		3
10	Abies balsamea		
	Lignans		22
	secoisolariciresinol		18
	lariciresinol		9
	Oligolignans		19
15	Juvabiones		2
	Pinus sibirica		
	Lignans		26
	lariciresinol		19
20	isolariciresinol		3
	secolariciresinol		2
	Oligolignans		6
	Flavonoids		7
	pinosembrin		6
25	Stilbenes		25
	dihydropinosylvin monomethyl ether		15
	pinosylvin	3	
	dihydropinosylvin		2
30	Pinus contorta		
	Lignans		10
	nortrachelogenin		5
	liovile		3
	oligomers	3	
35	Flavonoids		20
	pinosembrin		15
	pinobanxin	7	
	Stilbenes		15
	Pinosylvin monomethyl ether		9
40	Pinosylvin	6	

Production of the compositions and their intermediate products

Example 1

Example 1 describes an anti-micro-organistic composition (semi-finished product), in which there is used an unrefined solution obtained by hydrophilic extraction from Norway knotty spruce stemwood material.

Intermediate product

Ethylhexyl glycerine, anti-micro-organistic compound

1 part

Raw extract obtained by pentylene glycol extraction from knotty stemwood chips of *Picea abies* (Norway spruce) 6 parts

5 The extract obtained from Norway spruce contained a mixture of phenolic compounds, where the composition of the phenolic compounds was in accordance with Table 3B. Ethylhexyl glycerine is efficient against Gram-negative bacteria, and it also has skin moisturizing and softening properties, wherefore it is used, among others, in deodorants for roughly 0.2 - 2 wt% solution of the total weight. Ethylhexyl glycerine is neither effective
10 against Gram-positive bacteria, nor yeasts or fungi, and for this purpose there is therefore generally used another compound that is widely inhibiting for microbial growth, such as phenoxyethanol, which has a synergic positive effect with ethylhexyl glycerine. Phenoxyethanol is toxic, allergenic and skin irritative, and its highest acceptable limit of usage is roughly 1 wt% of the total weight of the composition.

15 An intermediate product according to example 1, composed of ethylhexyl glycerine and a widely anti-micro-organistic raw extract obtained in hydrophilic extraction from knotty stemwood material, which contains the compound mixture according to the invention, can be used in the same targets of usage as the known ethylhexyl glycerine and phenoxyethanol combination. Typical targets of usage are sun protection products, deodorants and
20 cleaning cloths impregnated by the mixture.

Example 2

UVA/UVB Sun Protection Lotion, O/W type, with TINOSORB® M

25 Lotion with a very high SPF and excellent UVA protection due to the photostable UVA filter TINOSORB® M. This emulsion is smooth and spreads easily. SPF in vivo = 38, broadband.

30 This lotion includes phenonip (various parabens) as a narrow-scale anti-microbial agent. Parabens are effective against fungi and bacteria. By using extract from wood knots containing mixture of phenolic compounds in glycerine the amount of phenonip can be diminished.

Table 7

Composition				
	Trade name	Inci Name	Supplier	% w/w (as sup- plied)
Part A	Amphiosol K	Potassium Cetyl Phosphate	Roche	2.00
	Antaron WP-660	Tricontanyl PVP	ISP	1.00
	Myritol* 318	Caprylic/Capric Triglyceride	Cognis	5.00
	Crodamol AB	C12-15 Alkyl Benzoate	Croda	5.00
	Cetiol SN	Cetearyl Isononanoate	Cognis	5.00
	Cutina GMS	Glyceryl Stearate	Cognis	3.00
	Lanette 16	Cetyl Alcohol	Cognis	1.00
	Dow Corning 200 Fluid 350 cs	Dimethicone	Dow Corning	0.10
	TINOSORB™ OMC	Ethylhexyl Methoxycinnamate	Ciba Specialty Chemicals	5.00
Part B	Water	Water		q.s. to 100
		10 wt% Wood Knot Extract in Glycerine	Granula Ltd	3.00
Part C	SALCARE® SC80	Steareth-10 Allyl Eth- er/Acrylates Copolymer	Ciba Specialty Chemicals	0.50

*Trade-Mark

Part D	TINOSORB® M	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (and) Aqua (and) Decyl Glucoside (and) Propylene Glycol (and) Xanthan Gum	Ciba Specialty Chemicals	20.00
Part E	Phenonip	Phenoxyethanol (and) Methyl- paraben (and) Ethylparaben (and) Butylparaben (and) Pro- pylparaben (and) Isobutylpara- ben	Clariant	0.30
Part F	Sodium Hydrox- ide (Solution 10%)	Water (and) Sodium Hydroxide		q.s. to pH 7.00
Part G	Fragrance	Fragrance		q.s.

Technical Data

	pH value	7.00
5	Appearance	white lotion
	Viscosity (Brookfield DVIII+LV4/80rpm)	3000 mPas
	UVA/UVB ratio* / Critical Wavelength*	0.75 / 384 nm

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Example 3

Sun protection cream

Part 1

	Granlux GA12-45:	28%	
5	Concentrate containing inorganic UV protective agents (TiO ₂ and ZnO) protected by aluminum oxide, dispersed in dimethicone and surface active dispersing agent		
	Glycerine	4%	

Part 2

10	alpha-Bisaplol	1.5%
	Bytosperrum parkii Fruit	1.5%
	Cetiol CC	0.1%
	Oxynex K Fluido	0.1%
	Alkyl benzoate	4%
15		

Part 3

	Water	23.5%
	Aloe barnadensis	1%
	Multivitamin product	0.3%
20	Dinatrium EDTA	0.1%

Part 4

	Cyclomethicone	1.5%
	Ethylhexyl methoxycinnamate	5%
25	Organic UV protective agent	2%
	Homosalate	5%

Part 5

	Ethylhexyl glycerine	0.5%
30	HMR-5 (intermediate product of example 1)	3%

Parts 1–5 were mixed as a sun protection cream by conventional methods.

Example 4**Cleaning cloth containing anti-microbial compound mixture according to the invention**

- 5 This can be manufactured as is described in the US patent 6 287 582. The cloth contains a water-insoluble carrier and a cosmetic composition impregnated in the carrier, including a compound mixture according to the invention, pH regulator such as alpha- or beta-hydroxycarboxylic acid, silicone microemulsion and surface active agent. The pH of the cosmetic composition in water is no more than 6. The microemulsion is for example a
- 10 dimethiconol microemulsion.

In the Table below, there are given two exemplary compositions to be impregnated in a cleaning cloth, containing an extract mixture according to the invention.

Ingredient	Acceptable range (Percent by weight)
Dual chain quaternary (N-alkyl dimethyl Ethylbenzyl chloride or N-alkyldiemethyl Ethylbenzyl ammonium chloride)	0.0-2.0
Ortho phenyl phenol	0.0025 - 2.0
Paratertiary amyl phenol	0.0025 - 2.0
Extracted mixture in alcohol (Granula Ltd)	1.0 - 40.0
Tergitol 15-S-5	0.5 - 2.0
Citric acid	0.1 - 2.0
Emollient	0.1 - 3.0
Water	Up to 100
Ingredient	Specific composition formulation (Percent by weight)
Dual chain quaternary (N-alkyldimethylethylbenzyl chloride or N-alkyldimethylethylbenzyl	0-0.25

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ammonium chloride)	
Ortho phenyl phenol	0.0125
Paratertiary amyl phenol	0.0025
Extracted mixture in alcohol (Granula Ltd)	0.01-40.0
Tergitol 15-S-5	0.5
Citric acid	0.05
<i>Aloe vera</i> gel	1.0
Water	Up to 100

Example 5**5 Aftershave gel without alcohol**

	Trade name/INCI	p -%
	A	
10	Carbopol 940 /Carbomer	0.30
	Water dem. Aqua dem.	40.00
	B	
15	Cremophor CO 40 / PEG-40/ Hydrogenated castor oil	3.00
	Perfume	q.s.
	Menthol	0.10
	D-Panthenol 50 P/Panthenol,	0.10
	10% extracted mixture in propylene glycol	4.00*
20	Triethanolamine	0.40
	Water Aqua dem.	up to 100%

Production

- 25 Let phase A swell. Phase B is dissolved and mixed in phase A.
Viscosity: roughly 4 000 mPa s (Brookfield RVT), pH value roughly 7.

*a mixture of phenolic compounds obtained from knotty stemwood chips in propylene glycol extraction, contg. 10 wt% of the compound mixture and 90 wt% of propylene glycol.

Example 6**Rehydrating aftersun mist**

- 5 In the production of this aqueous mist-like composition, there was used a water component, emulgator (Luviquat®Mono CP) and PEG-40, a surface active silicone-based agent (Dow Corning 190 Surfactant), a moisturizer (D-panthenol), and a moisturizing agent Pro-
- 10 dew® 200. The growth of micro-organisms was inhibited by a mixture of phenolic compounds, obtained in pentylene glycol extraction from chips contained in knotty spruce stemwood in mixture, contg. 10 wt% of the compound mixture and 90 wt% of propylene glycol. This raw extract was used unrefined in the production of mist.

	Trade name/INCI	wt%
15	A	
	Deionized water	89.10
	Luviquat®Mono CP /Hydroxyethyl cetyl dimonium phosphate (1)	2.00
	D-Panthenol (1)	0.50
	10% extracted mixture in pentylene glycol (5)* ' 5.50	
20	Dow Corning 190 Surfactant /Dimethicone polyol (2)	0.50
	Prodew® 200 /Sodium lactate & Sodium PCA & Sorbitol &	
	Hydrolyzed Collagen & Proline (3)	2.00

- 25 * polyphenolic mixture obtained from wood chips contained in knotty stemwood in pentylene glycol extraction, contg. 10 wt% of the extract mixture and 90 wt% of propylene glycol.

	B	
	Cremophor® RH 40 /PEG-40, Hydrated castor oil (1)	0.30
30	Perfume	0.10

Production

- Combine ingredients of phase A and stir until solution is clear.
- 35 Combine ingredients of phase B. Melt hydrated castor oil and stir with perfume. Combine phase B with A and stir until mixture is clear.
- pH of the end product is 6.

Suppliers

1) BASF (2) Dow Corning (3) Ajinomoto (4) Nipa (5) Granula Ltd

5 **Example 7**

Body milk

10 The body milk according to Example 7 is an aqueous emulsion, in the manufacturing of which there are used emulsifying agents (Cremophor), auxiliary agent (glyceryl monostearate) as well as water and oil components, viscosity regulator (cetyl stearyl alcohol), emollients (Luvitol EHO) and conditioner (Luviquat PQ 11). The growth of micro-organisms was inhibited by a mixture of phenolic compounds obtained from knotty spruce stemwood in butylene glycol extraction, which mixture contained phenolic compounds 10 wt%, and
15 which raw extract was used unrefined for the production of body milk.

	Trade name/ INCI	wt%
	A	
20	Cremophor A 6/ Cetareth-6, Stearyl alcohol	1.00
	Cremophor A 25/ Cetareth-25	1.00
	Glyceryl monostearate	2.00
	Cetyl stearyl alcohol	2.00
	Paraffin oil/ Mineral oil	3.00
25	Luvitol EHO/ Cetyl stearyl octanoate	5.00
	B	
	10 wt% extracted mixture in butylene glycol*	5.00
	Luviquat PQ 11 (1) Polyquaternium-11	4.00
30	Water	77.00
	C	
	Perfume	q.s.
35	** polyphenolic mixture obtained from wood chips contained in knotty stemwood in butylene glycol extraction, contg. 10 wt% extract mixture and 90 wt% butylene glycol.	

Production

40 Mix phases A and B separately at roughly 80 °C. Mix phase B to phase A whilst homogenizing, and continue homogenizing for a while. Cool roughly at 80 °C, add phase C and homogenize again.

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Viscosity: roughly 3000mPas

pH: roughly 6

Example 8

5

Concentrated powder

	Trade name/ INCI	wt%
10	A	
	Talcum	72.00
	Magnesium stearate	10.00
	Calcium carbonate	2.00
	Sicovit White E 171/C.I. 77891/Titanium oxide	9.00
15	Sicovit Brown 70 E 172/Ferric oxides	1.00
	Powdered mixture	5.00
	B	
	Paraffin oil/ Mineral oil	0.50
20	Vaseline/ Petrolatum	0.50

Production

25 Mix ingredients of phase A and homogenize. Stir phase B to phase A and mix again.

Example 9**Cell-protective composition**

30	Trade name, compound /INCI	wt%	
	A		
	RonaCare™ Ectoin (1)	1.00	
	10% extracted mixture in pentyleneglycol (5)		3.00
35	Water, mineralized	up to 100%	
	B		
	Sistema SP30-C (2) /	saccharose distearate	2.70
	Sistema SP70-C (2) /	saccharose stearate	0.90
40	Cetiol OE	(3) / dicapryl ether	5.00
	Miglyol 812	(1) / kaprylic/kapric triglyceride	2.00
	Isopropyl palmitate	(3) / isopropyl palmitate	2.00
	Cegesoft C 24	(3) / ethylhexyl palmitate	
	Carbopol ETD 2001	(4) / carbomer	0.20
45	C		
	Sodium hydroxide, 10% solution (1) /sodium hydroxide q.s.		

Production

Heat phase A to 75° C, disperse phase B and heat to 75° C, add phase B to phase A, homogenize, adjust pH with sodium hydroxide, cool to room temperature by stirring simultaneously.

Note

pH (22° C): 6.50
Viscosity (21° C): 109 000 mPa.s (Brookfield RVT, spindle C, 5 rpm, Helipath)

Suppliers

(1) Merck KGaA/Rona®
(2) Sisterna C.V./ Dai-Ichi
(3) Cognis GmbH
(4) BF Goodrich GmbH
(5) Granula Ltd

Example 10

Night care cream

For producing this cream-like composition, there was used a water component, emulsifying agents (PEG-7), emollients (Luvitol EHO), wax components and fungicidal and moisturizing agents (jojoba oil). The growth of anti-microbial agents was prevented by jojoba oil and by a mixture of phenolic compounds, obtained from knotty spruce stemwood in glycerine extraction, which mixture contained phenolic compounds 10 wt%, and which raw extract was used unrefined for producing the cream.

	Trade name/ INCI	wt%
	A	
35	Cremophor WO 7 / PEG-7 Hydrated castor oil	6.00
	Luvitol EHO /Cetearyl octanoate	5.00
	Permulin 3220 /Microcrystalline wax	2.00
	Beeswax	0.50
	Cetiol SB 45	
40	/Shea Butter (Butyrospermum parkii)	0.50
	Jojoba oil/Jojoba (Buxus chinensis) oil	2.00
	Paraffin oil/Mineral oil	10.00
	B	
45	10% extracted mixture in glycerin	5.00
	Water	67.00

C	
Sodium ascorbyl sorbate	2.00
Perfume	q.s.

5 Production

Mix phases A and B separately to roughly 80 °C. Stir phase B to phase A whilst homogenizing, continue homogenizing for a while. Cool to roughly 40 °C, add C and homogenize again.

10

Example 11

NIGHT CARE CREAM

The night care cream according to example 11 was almost identical to night care cream of example 10, but instead of sodium ascorbyl sorbate of example 10, sodium ascorbyl phosphate was used in example 11 as an antioxidant agent.

	Trade name/ INCI	wt%
20	A	
	Cremophor WO 7 / PEG-7 Hydrogenated Castor Oil	6.00
	Luvitol EHO / Cetearyl Octanoate	5.00
	Permulin 3220 / Microcrystalline Wax	2.00
	Beeswax	0.50
25	Cetiol SB 45 / Shea Butter (<i>Butyrospermum parkii</i>)	0.50
	Jojoba Oil/Jojoba (<i>Buxus chinensis</i>) Oil	2.00
	Paraffin Oil/Mineral Oil	10.00
	B	
30	10% extracted mixture in glycerin	5.00
	Water	67.00
	C	
35	Sodium Ascorbyl Phosphate	2.00
	Perfume	q.s.

Production

40 Heat phases A and B separately to about 80° C. Stir phase B into phase A whilst homogenizing and continue homogenizing for a while. Cool to about 40° C, add phase C and homogenize again.

Example 12**SOFTCREAM WITH VITAMINE E**

For making softcream of example 12, there were used several emollients, oil and water components, preservatives, adjuvants and other additives including vitamins for antioxidant purposes. Growth of micro-organisms (bacteria, fungi, yeast) was inhibited by a mixture of 10 wt% raw extract (from spruce) including phenolic compounds in glycerin (10 wt% of mixture of compounds and 90 wt% of glycerin). This glycerin containing raw extract was used without further purification.

10	Trade name/ INCI	wt%
	A	
	Cremophor GO 32 / Polyglyceryl-3 Dioleate	0.75
15	Luvitol EHO / Cetearyl Octanoate	7.50
	Finsolv TN / Alkyl Benzoate	5.00
	Miglyol 812 / Caprylic/Capric Triglyceride	4.00
	Abil EM 90 / Cetyl Diethicone Copolyol	2.25
	Abil 350 / Dimethicone	1.50
20	Ascorbyl Palmitate, Citric Acid, Glyceryl Stearate, Propylene Glycol	0.20
	B	
	10% extracted mixture in Glycerin	0.75
25	Sodium Hydroxide	0.25
	D-Panthenol USP/Panthenol	1.50
	Sodium Chloride	1.50
	EDTA	0.1
	Preservative	q.s.
30	Water	69.80
	C	
	(-)-Alpha-Bisabolol nat./Bisabolol	0.10
	Vitamin A Palmitate 1 Mio./Retinyl Palmitate	0.10
35	Vitamin E Acetate/Tocopheryl Acetate	5.00
	Perfume	q.s.

Production

40 Heat phases A and B separately to about 80° C. Stir phase B into phase A whilst homogenizing. Cool to about 40° C, add phase C and homogenize again.

Viscosity: approx. 18 000 mPas

Example 13**MULTI-VITAMIN CREAM, TYP W/O Formula**

- 5 For manufacturing this W/O-type cream composition, water and oil were used as carrier agents, as an adjuvant emulgators (PEG-7, PEG-45, Claytone XL), and as an additive moisturizer (Jojoba oil,) perfume and vitamins (sodium ascorbyl phosphate and retinol). Growth of micro-organisms (bacteria, fungi, yeast) was inhibited by a mixture of 10 wt% extracted mixture (spruce) containing phenolic compounds extracted from spruce knots in-
- 10 to butylene glycol (10 wt% of mixture of phenolic compounds and 90 wt% of butylene glycol). This raw extract was used without further purification.

	Trade name/INCI	wt%	
15	A		
	Cremophor WO 7 /PEG-7 Hydrogenated Castor Oil	6.00	
	Paraffin Oil / Mineral Oil		10.00
	Vaseline/ Petrolatum		3.00
	Miglyol 812/ Caprylic/Capric Triglyceride		5.00
20	Elfacos ST 9/PEG-45/Dodecyl Glycol Copolymer		2.00
	Jojoba Oil/ Jojoba (Buxus Chinensis) Oil		5.00
	Claytone XL/ Quaternium-18 Bentonite	1.00	
	B		
25	10% Wood Knot Extract in Butylene Glycol		4.00
	EDTA		0.10
	Water		61.90
	C		
30	Sodium Ascorbyl Phosphate		1.00
	Retinol		1.00
	Perfume		q.s.

Production

- 35 Heat phases A and B separately to about 80° C. Stir phase B into phase A whilst homogenizing and continue homogenizing for a while. Cool to about 40° C, add phase C and homogenize again. Viscosity: approx. 14 000 mPa s (Haake Viscotester VT-02).

Example 14**GAI-45 TS High SPF Cream**

- 5 This composition was made of water phase, semi-composition of GranLux® GAI-45 TS which is an W/O-emulsion including UV-protective agents in a silicon emulsifier system and 10 wt% mixture of phenolic compounds originating to spruce knots and extracted into pentylene glycol (10 wt% of mixture of phenolic lignan compounds and 90 wt% of pentylene glycol). The end composition included also emollient and dispersive oil (isononyl isononanoate) and perfume.

	Trade name/INCI	Amount (%)	Manufacturer
	A		
15	GranLux® GAI-45 TS	25.0	Granula Ltd
	10% extracted mixture in pentylene Glycol	3.0	Granula Ltd
	B		
20	Water	10.0	
	C		
	Isononyl Isononanoate	22.0	Seppic
25	D		
	Water	39.0	
	Perfume	q.s.	

- 30 1) Mix A at room temperature.

- 2) Prepare B and add it to A. Mix ca 3-5 min until all water has been taken up. The water will go in by diffusion, hydrate the polar parts and form the liquid crystalline phase. The polar phase and the hydrophobic phase seem initially to be totally separated but the water phase will be taken up by time and mixing.

- 3) Add to C to A+B while mixing. Viscosity goes down.

- 4) Add D to C+A+B slowly (during ca 5 minutes) while processing well (Ystral speed 3-5) for totally 15 minutes.

SPF : well over 30 (SPF in vitro 49 +/- 3)

UVA: fulfills "Australian Standard"

Example 15**FLUID FOUNDATION using Granlux™ Melanin****Mimic™ TB concentrate**

5

For making this composition there was used a semi-composition Granlux™ Melanin Mimic™ which is a formula imitating the UV-protection of natural melanin. Growth of micro-organisms (bacteria, fungi, yeast) was inhibited by a mixture of 10 wt% mixture of phenolic compounds extracted from spruce knots in pentylene glycol (10 wt% of mixture of phenolic compounds and 90 wt% of pentylene glycol). This raw extract was used without further purification. Also betulonic acid originating to birch bark was used as an anti-microbial agent.

	Trade name/INCI	Amount (wt%)	Manufacturer
15	A		
	Magnesium aluminum silicate	0.70	
	(Veegum K)		Vanderbilt
			Xanthan gum
20	0.30		
	(Rodicare)		Rhône Poulenc
	10% extracted polyphenolic mixture		
	in pentylene glycol	6.00	Granula Ltd
	Glycerine	4.00	
25	Deionized water	q.s.	
Wet the Xanthan gum in water + glycerine + 10wt% polyphenolic mixture extracted from spruce knots into pentylene +10wt% Betulonic acid in propylene glycol. Homogenize with turboemulsifier and add Magnesium aluminum silicate while mixing, heat to 75 °C.			
30	B		
	Granlux™ Melanin Mimic™	27.50	Granula Ltd
	Limnantes alba;		
	Butyrospermum parkii	3.50	The Fanning Co
	(Fancol VB)		
35	Glyceryl stearate	0.80	Th. Goldschmidt
	(Tegin M)		
	Isopropyl myristate	4.00	
	Isohexadecane	10.00	ICI
40	(Arlamol HD)		
	Stearic acid	2.00	
	Dimethicone	1.00	Dow Corning
	(Dow Corning 200 Fluid 100 cs)		

Melt Phase B at 65° C, slowly homogenizing for ca 5 min, heat to 75° C.

B1

Talcum 1.00

5

Add Phase A to Phases B while homogenizing. As emulsion is formed, add Phases B1 and C slowly while continuing homogenizing.

C

10 Triethanolamine 1.50

D

PPG 25 Laureth 25 0.20 Vevy
(ADF Oleile)

15

At 40° C add Phase D while homogenizing. Cool to room temperature while mixing.

Characteristics:

pH: ca 7

20 Viscosity: 6.000

SPF: 21-24

Example 16**25 SOFT COLOURED CREAM (SCC/EM/98)**

	INCI(Trade name)	Amount (%)	Manufacturer
	A		
	Magnesium aluminum silicate	0.50	Vanderbilt
30	(Veegum K)		
	Xanthan gum	0.50	
	(Rodicare)		Rhône Poulenc
	Propylene glycol	6.00	
	10% Betulonic acid in Glycerine	4.00	Granula Ltd
35	Deionized water		up to 100%

Wet the Xanthan gum in water + betulonic acid in glycerine + propylene glycol. Homogenize with turboemulsifier and add Magnesium aluminum silicate while mixing, heat to 75° C.

40

B

Granlux™ EM-50 10.00 Granula Ltd

Limnanthes alba;

Butyrospermum parkii 3.50 The Fanning Co

45

(Fancol VB)

Glyceryl stearate 0.80 Th. Goldschmidt

(Tegin M)

Isopropyl myristate 4.00

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PCT/FI2009/050116

45

	Isohexadecane (Arlamol HD)	10.00	ICI
	Polydecene (Nexbase 2004 FG)	4.00	Fortum
5	Polyhydroxystearic acid (Arlacel P100)	0.50	ICI
Melt Phase B at 65° C, add Phase B1 slowly homogenizing for ca 5 min, heat to 75° C.			
10	B1		
	CI 77492 (Ariabel yellow)	1.40	Warner&Jenkinson
	CI 77491 + CI 77492 (Ariabel sienna)	0.30	Warner&Jenkinson
15	CI 77491 + CI 77492 CI 77499 (Ariabel umber)	0.30	Warner&Jenkinson
	CI 77891 (Titanium dioxide) (Kemira AFDC)	6.00	Kemira
20	Add Phase A to Phases B + B1 while homogenizing. As emulsion is formed, add Phase C while continuing homogenizing.		
	C		
	Talcum 1.00		
25	Aluminum starch octenylsuccinate (Dry-Flo PC)		3.00 National Starch
	D		
30	PPG 25 Laureth 25 (ADF Oleile)	0.20	Vevy
	Propylene glycol; Diazolidinyl urea; Methyl paraben; Propylparaben (Germaben II E)	1.00	ISP
35	At 40° C add Phase D whilst homogenizing. Cool to room temperature while mixing. Note: During the preparation the phase inversion temperature is clearly noticeable (PIT ca 40°) since the W/O system previously formed breaks into two phases: one liquid and one creamy. While continuing homogenization, the final emulsion (O/W) is easily obtained. The low value of PIT is not related to unstable behavior, in fact the formulation is still stable af-		
40	ter 4 months at 42° C.		
	Characteristics:		
	pH: ca 7		
45	Viscosity: 180.000 mPa s RVT Brookfield (5 rpm, 298 K, Helipath Stand T-D SPF: 21-23 in vitro, UVA/UVB = 0.77		

Example 17

A stick with UV-protection of SPF 15 was made from semi-composition of Granlux CCA-50, which includes mainly physical filter for UV-protection, beeswax and carnauba wax.

- 5 Large-scale protection against micro-organisms (bacteria, yeast, fungi) is achieved by a powdered compound mixture originating to pulverized spruce knots.

SPF 15 Stick

10

Hydrogenated Vegetable Oil (Cremeol HF-52)	15.0	Aarhus Olie
Vegetable Oil (Cremeol PS-6)	68.0	Aarhus Olie
Candelilla wax	6.0	
Powdered mixture	1.0	Oy Granula Ab, Ltd
15 Granlux CCA-50	10.0	Oy Granula Ab, Ltd

Heat ingredients to 75–80 ° C. Mix until uniform. Cool to 50 °C. Pour into moulds.

Characteristics:

SPF: 13–15 in vitro

- 20 UVA/UVB ratio 0.56

Example 18

A stick with UV-protection of SPF 30 was made from semi-composition of Granlux CCA-50, which includes mainly physical filter for UV protection, beeswax and carnauba wax.

- 25 Large-scale protection against micro-organisms (bacteria, yeast, fungi) is achieved by a powdered compound mixture originating to pulverized spruce knots.

Formula: SPF 30 Stick

30

Trade name/ INCI	wt%	
Beeswax (Cera alba)	12.0	
Caprylic Capric Triglycerides	12.5	
35 Macadamia nut oil	9.5	
Cetearyl alcohol	7.5	Henkel
Petrolatum	36.5	
Granlux CCA-50	20.0	Oy Granula Ab, Ltd
Powdered mixture	2.0	Oy Granula Ab, Ltd

40

Heat ingredients to 75–80 ° C. Mix until uniform. Pour into molds.

Characteristics:

SPF: 28–30 in vitro

Example 19**SUN PROTECTION GEL**

5	Trade name/ INCI	wt%
	A	
	Uvinul MC 80/Octyl Methoxycinnamate	8.00
	Uvinul N 539 T/Octocrylene	5.00
10	Uvinul M 40 /Benzophenone-3	2.00
	Parsol 1789/Butyl Methoxydibenzoylmethane	0.80
	Vitamin E Acetate/Tocopheryl Acetate	2.00
	Cremophor RH 410/PEG-40 Hydrogenated Castor Oil	1.00
	Perfume	q.s.
15	B	
	Pemulen TR-1	
	/ Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0.30
	Carbopol 940 / Carbomer	0.20
20	10% extracted mixture in Dioctyl Glycol	5.00 (Granula Ltd)
	Edeta BD /EDTA	0.20
	Water	75.30
	C	
25	Sodium Hydroxide	0.20

Production

30 Dissolve phase A. Stir phase B into phase A whilst homogenizing, then neutralize with phase C and homogenize again.

Viscosity: approx. 5 500 mPa s (Haake Viscotester VT-02)
pH-value: approx. 9.1

Example 20**SUNSCREEN FOAM**

- 5 Mixture of phenols was extracted from particles of knotwood of spruce into pentylene glycol. The amount of mixture of phenolic compounds in final composition was 0.05 wt%. The Sunscreen Foam also contains various organic compounds (Octyl Methoxycinnamate, Octyl Triazone and 4-Methylbenzylidene Camphor) having protective properties against UV-radiation.

10

	Trade name/ INCI	wt%
	A	
15	Cremophor A 25/Ceteareth-25	5.00
	Palmitic Acid	2.00
	Finsolv TN/Alkyl Benzoate	5.00
	Witconol APM/PPG-3 Myristyl Ether	5.00
	Uvinul MC 80/Octyl Methoxycinnamate	6.00
20	Uvinul T 150/Octyl Triazone	0.50
	Uvinul MBC 95/4-Methylbenzylidene Camphor	1.00
	B	
	10% Extracted mixture in Pentylene Glycol	5.00
25	Water	70.30
	C	
	Triethanolamine	0.20
30	D	
	Perfume	q.s.

Production

- 35 Heat phases A and B separately to about 80° C. Stir phase B into phase A whilst homogenizing. Stir in phase C and rehomogenize. Cool to about 40° C, add phase D and homogenize again.

- 40 Filling: 90% active ingredient
10% propane/butane 3.5 bar (20°C).

Example 21**Anti-scurf compositions**

Compound mixtures according to the invention inhibit microbial growth on a wide scale,
 5 so that they can be used for replacing several specific preservatives in cosmetic compositions, i.e. specifically agents designed individually against Gram-negative and Gram-positive bacteria, fungi and yeast. Moreover, the cytotoxicity of said mixtures is low, and the compounds contained therein do not penetrate the skin, wherefore their skin irritation low, as opposite to certain conventionally used agents inhibiting the growth of anti-
 10 microbial agents on a wide scale, such as phenoxyethanol.

The mixtures according to the invention are effective against the yeast *Malassezia furfur*, wherefore they can be used as both wide-scale preservatives and also for inhibiting the action of the scurf forming yeast *Malassezia furfur*. Generally with scurf shampoo compositions it is necessary to separately add both preservatives, such as wide-scale bacteriostat
 15 phenoxyethanol, and further also separately an agent inhibiting the growth of scurf forming yeast. Owing to their toxicity and skin penetration, generally used wide-scale preservatives cannot be used in quantities sufficiently large in order to ensure the inhibition of the growth of the scurf-forming yeast; whereas the mixtures according to the invention have a low skin penetration, so that they can be used in relatively large quantities in cosmetic
 20 compositions.

Example 21a**Oil shampoo**

25	Ingredient	Wt%''
	Sodium lauryl sulfate (Texapon N25)	50
	PPG- 5 Laureth-5 (Aetoxal B)	20
	Cocoamide DEA (Comperlan KD)	3
30	GranLux AOX-G3 Eco	
	(10 wt% Spruce knot extract in propylen-1.3-diol)	5
	Aqua and perfume	22

Oil shampoo has a clear continuous water phase with 0.5 wt% mixture of compounds originating to spruce knots, extracted with alcohol (propyle-1.3-diol). Spruce knot extract
 35 gives anti-scurf properties (against *M.furfur*) to oil shampoo. The amount of water can be

reduced substantially for example to 5 -20% if this oil shampoo is intended for large-scale consumers.

5 Example 21b

Combined hair conditioner and shampoo

	Ingredient	Wt%*
10	Coco-Betaine (Dehyton AB 30)	20%
	PEG-5 Cocoamide (Emulgin C4)	20%
	Cocoamid MEA (Comperlan KM)	4%
	Laurtrimonium Chloride (Dehyquat LT)	4%
	Granlux AOX-GL (10% Spruce knot extract in glycerine)	4%
15	Aqua and perfume	48%

Combined hair conditioner and shampoo has also a clear continuous water phase as a carrier agent with 0.4 wt% mixture of compounds originating to spruce knots, extracted with alcohol (glycerine). Spruce knot extract gives anti-scurf properties (against M.furfur) to hair conditioner and shampoo. The amount of water can be reduced substantially for example to 5 -20% if this hair conditioner and shampoo is intended for large-scale consumers.

Example 21c

25	Vitaminized protein shampoo	
	Ingredient	Wt%*
	Polysorbate 20 (Tween*20)	10
	Polysorbate 80 (Tween*80)	5
30	PEG-54 Hydrogenated Castor oil(Arlatone 289)	10
	PPG-15 Stearyl Ether (Arlamol E)	2
	Cocoamid DEA	5
	Cocoamidopropyl Betain (Tegobetain L7)	3
	Hydrolyzed collagen (Nutrilan L)	5
35	Vitamin F (Novarom)	2.5
	Granlux AOX (10% Spruce knot extract in butylene glycol)	5
	Aqua and perfume	27.5

Vitaminized protein shampoo is a turbid O/W emulsion/dispersion with 0.5 wt% mixture of compounds originating to spruce knots, extracted with alcohol (butylene glycol). Spruce knot extract gives anti-scurf properties (against M.furfur) to hair conditioner and shampoo. The amount of water can be reduced substantially for example to 5 -20% if this vitaminized shampoo is intended for large-scale consumers. This composition also con-

*Trade-mark

tains an amphoteric surfactant (cocoamidopropyl betain), foaming agent (Cocoamid DEA), and ionic surfactants (polysorbates).

Example 22

5

Animal feed

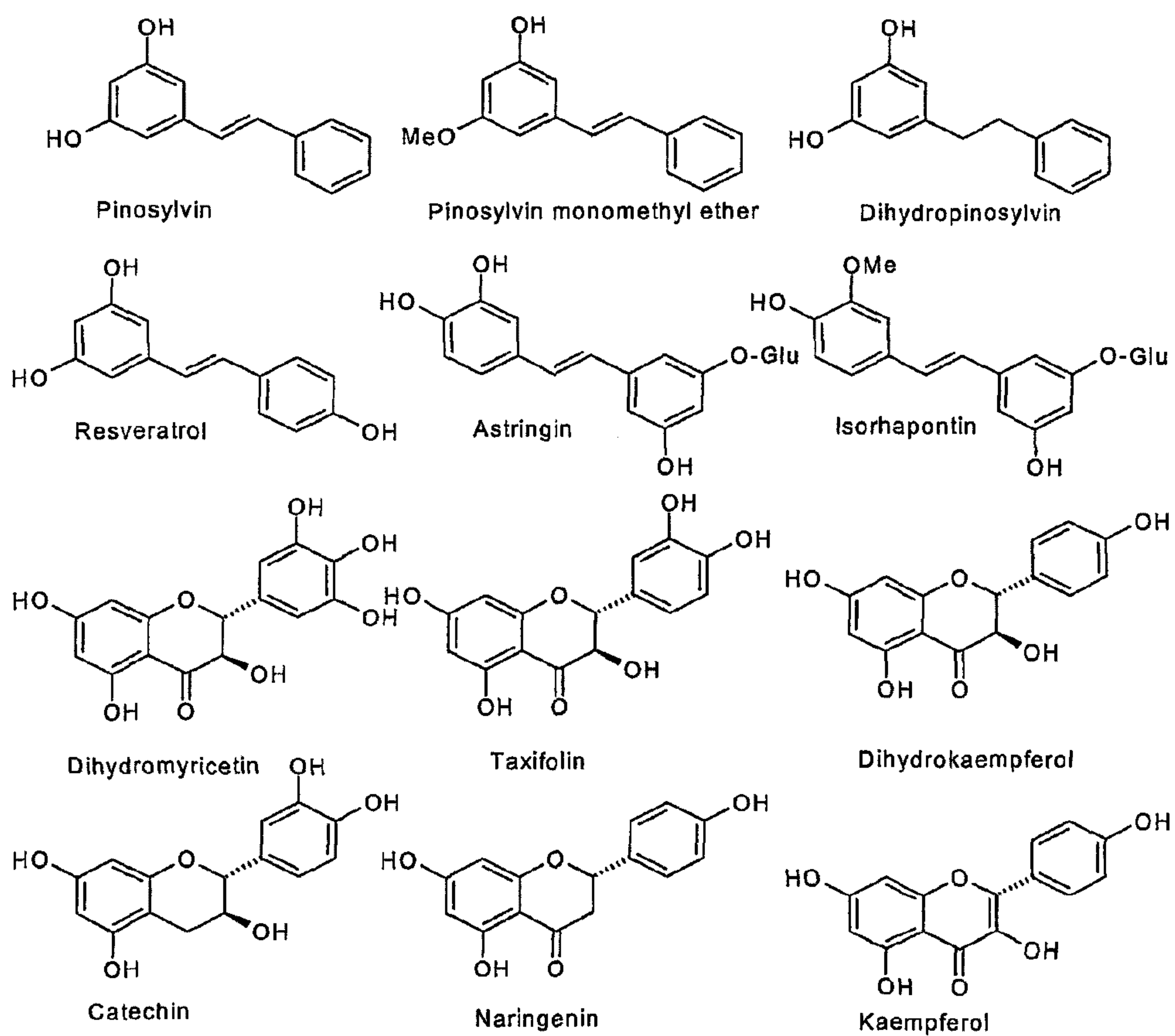
10 Into soybean feed, there was added by spraying a raw extract, with an alcohol content of 90-95 wt%, and compound mixture 5- 10 wt%. The compound mixture was obtained by extracting either a lignan mixture obtained from Norway spruce knotwood by extraction with alcohol, or mixed extract obtained from Norway spruce and pine knotwood by extrac-
tion with alcohol, which mixed extract included a compound mixture containing lignans and stilbenes.

15 In the examples, an extracted mixture or knot extract refers to a (poly)phenolic compounds containing compound mixture according to the invention, which mixture is obtained by extracting knotty stemwood chips in alcohol. A powdered mixture in turn refers to a corresponding pulverized mixture, which is obtained by pulverizing knotty stemwood chips. Mixed extracts of pine and spruce contained 1 part by volume of extracted mixture from pine, and 2 parts by volume of extracted mixture from spruce. Powdered mixtures con-
20 tained 1 part by weight pulverized pine and 2 parts by weight pulverized spruce.

Appendix 1

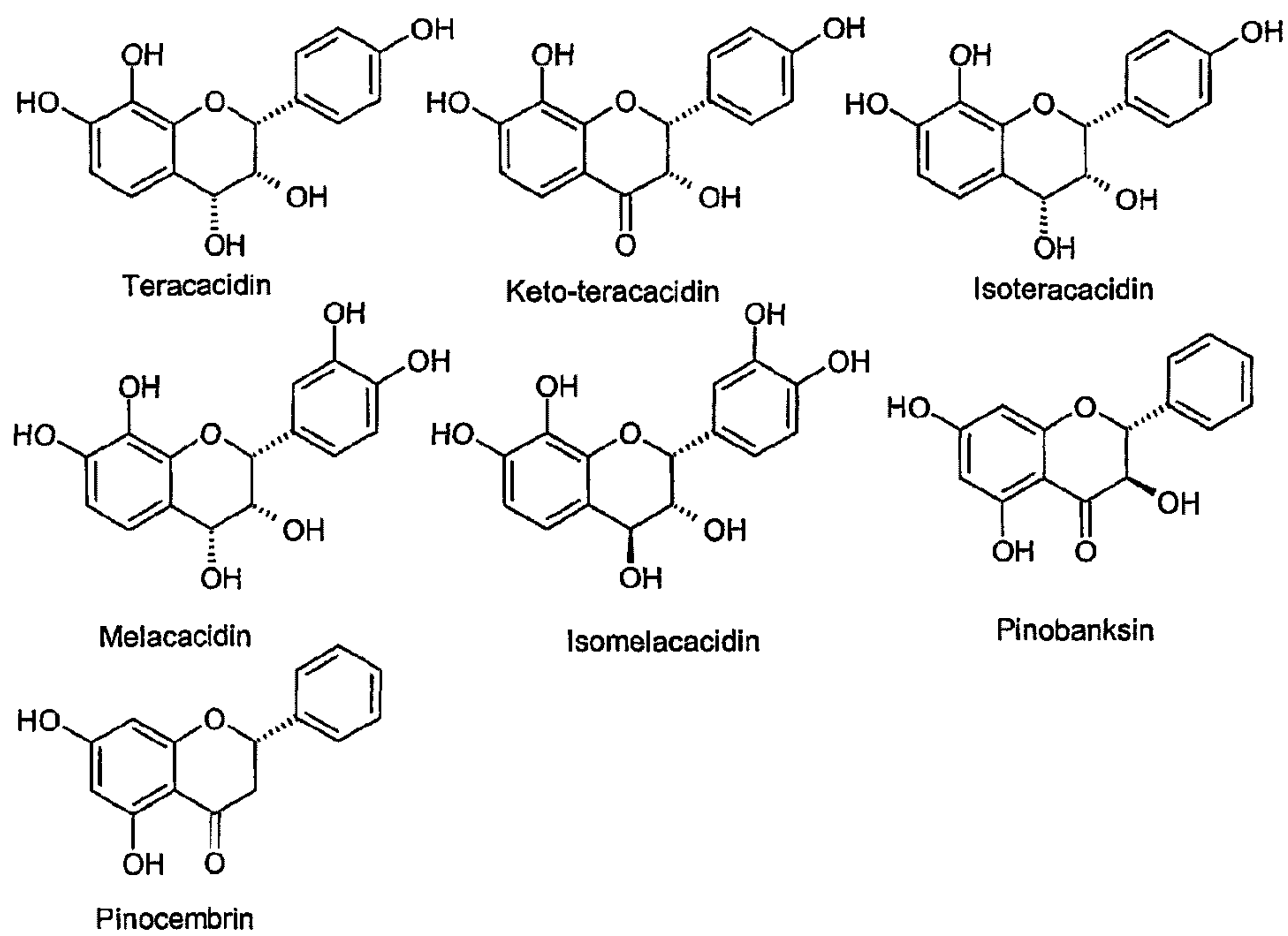
Scheme 1

Scheme 1 illustrates different phenolic compounds that can be obtained by a hydro-
5 philic extraction of wood material originated to knotty stemwood or bark of pine.



Scheme 1 continued

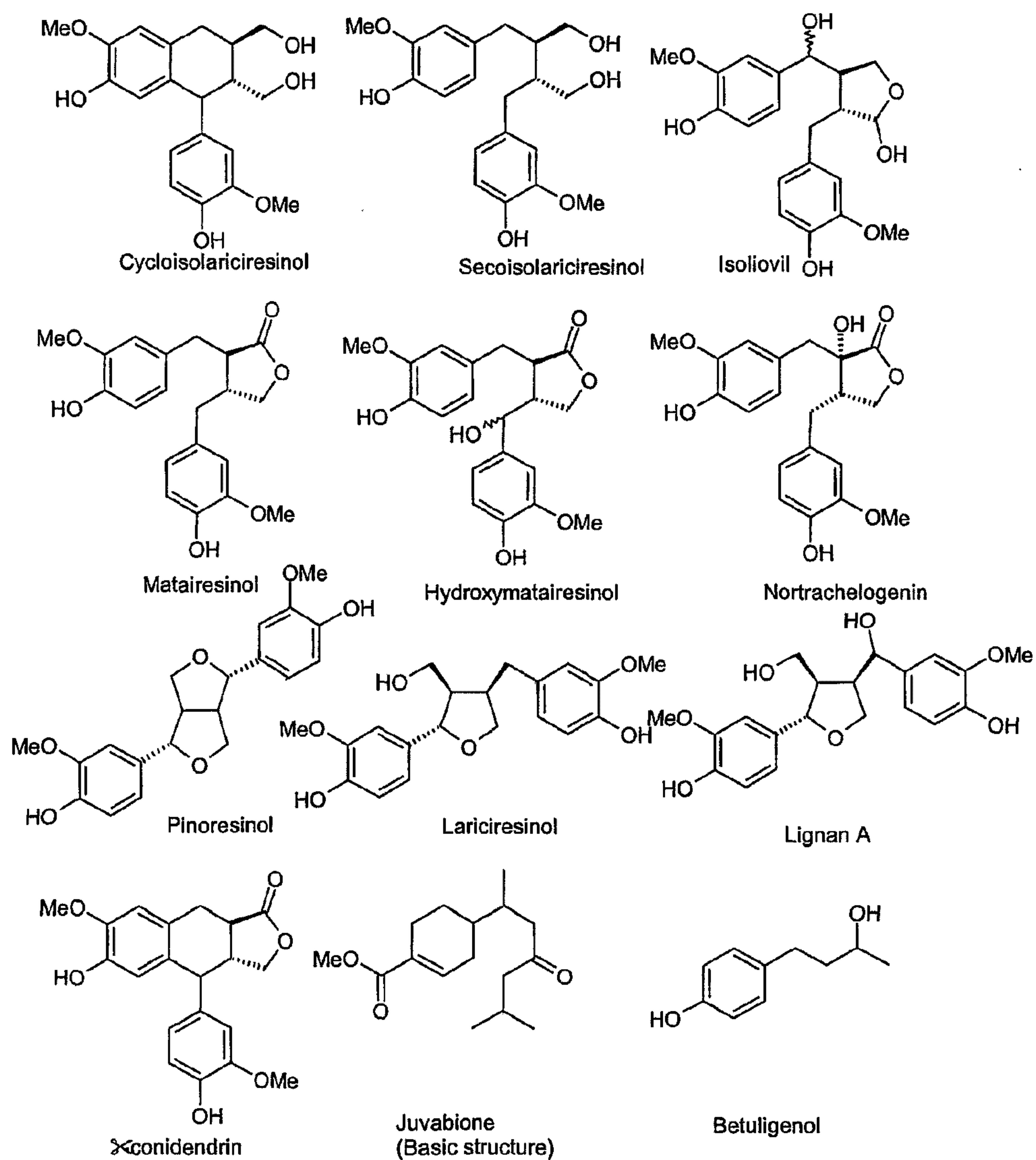
Appendix 2



Appendix 3

Scheme 2

Scheme 2 illustrates the different phenolic and polyphenolic compounds contained in the bark of *betula pendula* (birch tree).



Appendix 4

Table 5A

Antioxidative capacity of the lipids in polyphenols-bearing unrefined extracts obtained in hydrophilic extraction from knotty stemwood or wood bark, as expressed in terms of extract concentration EC50 $\mu\text{g/L}$, which inhibits 50% of the peroxidation of lipids.

	knotty stemwood obtained extracts	EC50 $\mu\text{g/L}$
10	Acacia crassicarpa	19
	Abies pectinata	21
	Picea glauca	24
	Acasia mangium	24
	Tsuga canadensis	27
15	Picea sitchensis	28
	Tsuga heterophylla	28
	Eukalyptus globulus	57
	Abies lasiocarpa	59
	Populus gradidentata	61
20	Pinus resinosa	61
	Fagus sylvatica	91
	Populus tremuloidis	135
	Pinus strobes	159
	Populus tremula	317
25	extracts obtained from wood bark	EC50 $\mu\text{g/L}$
	Picea abies	49
	Betula pendula	81
30	Pycnogenol	84
	Pseudotsugamenziensis	84
	Thuja occidentalis	131
	Pinus banksiana	143
	Populus tremula	213
35	Abies lasiocarpa	316

Appendix 5

Table 5B

Comparison of the antioxidative capacity of the lipids in pure polyphenol extracts
 5 isolated from certain wood materials, as expressed by the extract concentration
 EC50 $\mu\text{g/L}$, which inhibits 50 % of the peroxidation of the lipids.

	Compound	EC50 $\mu\text{g/L}$
	Cyclolariciresinol	17
10	Pinoresinol	20
	Melacacidine	36
	Secoisolariciresinol ¹	37
	Taxifolin ¹	46
	Pinosylvin	50
15	Teracacidine	50
	Nortrahelegenini ¹	53
	Hydroxymatairesinol ¹	58
	Matairesinol ¹	99
	Lariciresinol ¹	126
20	Dihydrokaempferol	488
	Pinosembrin	1135

1: Willför et al., J. Agric. Food. Chem, 51. 26 (2003).

Tables 5C and 5D further represent the free radicals capturing capacity of certain
 25 pure polyphenols and unrefined polyphenol extracts with respect to peroxide radi-
 cals.

Appendix 6

Table 5C.

5 Peroxide radicals /(mmol) capturing capacity of unrefined polyphenols-bearing ex-
tracts obtained in hydrophilic extraction from knotty stemwood or wood bark, as
expressed in capacity per gram of extract.

	extracts obtained from knotty stemwood	Wood material, capturing mmol/g	capacity
10	<i>Acacia crassicarpa</i>	21	
	<i>Eukalyptus globulus</i>	7.8	
	<i>Picea glauca</i>	7.8	
	<i>Abies pectinata</i>	6.8	
15	<i>Tsuga canadensis</i>	6.8	
	<i>Acasia mangium</i>	6.8	
	<i>Tsuga heterophylla</i>	5.8	
	<i>Larix laricina</i>	5.8	
	<i>Larix sibirica</i>	5.8	
20	<i>Picea mariana</i>	5.8	
	<i>Picea sitchensis</i>	4.9	
	<i>Pinus sylvestris</i>	4.9	
	<i>Thuja plicata</i>	3.9	
	<i>Populus gradidentata</i>	3.9	
25	<i>Fagus sylvatica</i>	2.9	
	<i>Abies lasiocarpa</i>	2.7	
	<i>Pinus resinosa</i>	2.7	
	<i>Pinus banksiana</i>	1.9	
	<i>Pinus strobus</i>	1.1	
30	<i>Populus tremuloidis</i>	0.39	
	<i>Populus tremula</i>	0.29	
	wood bark obtained extracts	mmol/g	
35	<i>Pseudotsuga menziensis</i>	4.9	

(Table 5C continued)

Appendix 7

	Pycnogenol	4.9
5	Pinus banksiana	3.1
	Betula pendula	2.9
	Picea abies	2.9
	Thuja occidentalis	1.9
	Abies lasiocarpa	0.58
10	Populus tremula	0.29

Table 5D

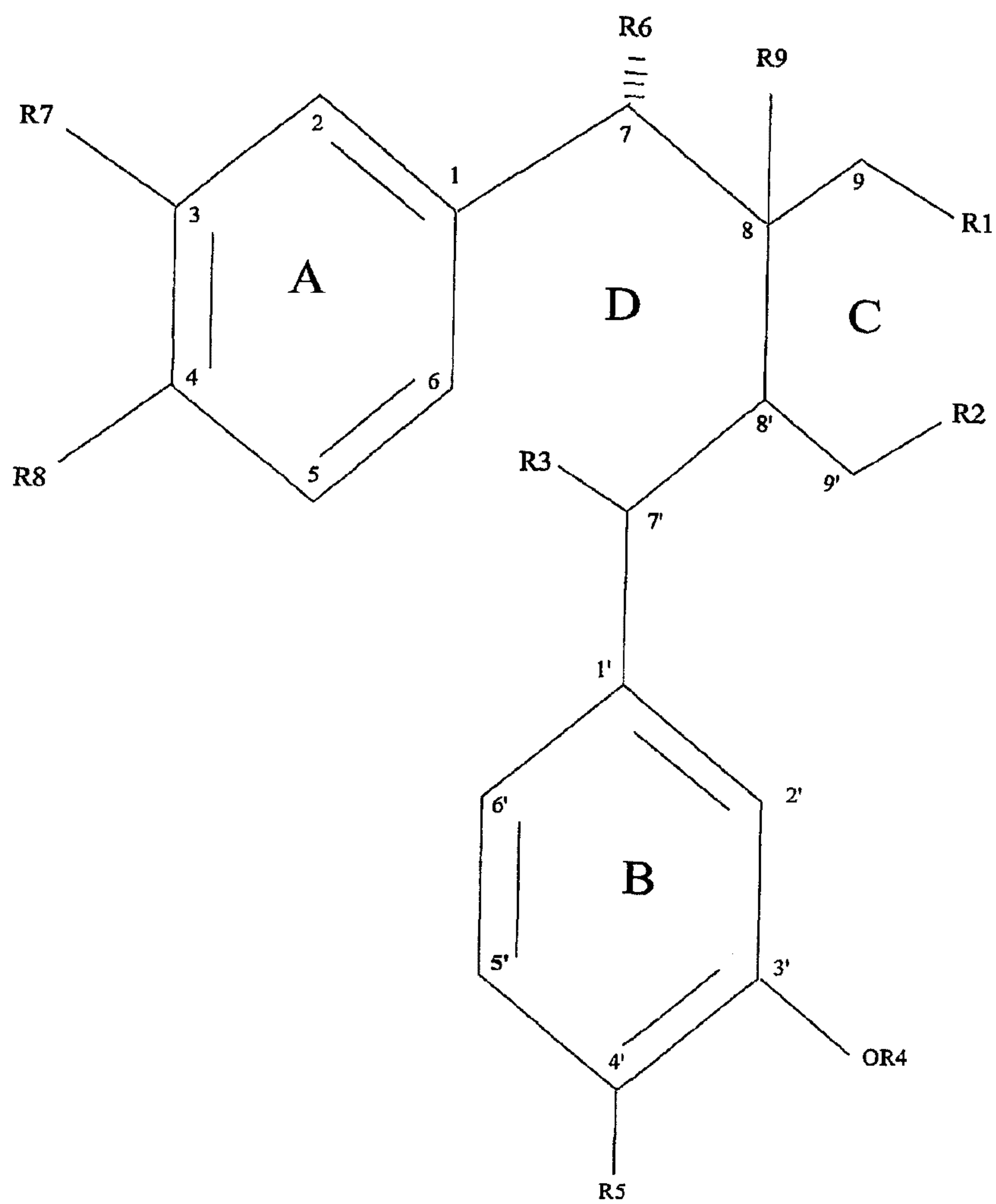
Comparison of the peroxide radicals (mmol) capturing capacity of certain pure polyphenol extracts isolated from wood material, as expressed per gram of extract.

Compound		capturing capacity
		mmol/g
20	Melacasinine	20
	Taxifolin ¹	16
	Cyclolariciresinol	12
	Secoisolariciresinol ¹	8.5
	Pinoresinol	7.8
25	Tetra casidin	7.8
	Nortrachelogenin ¹	5.9
	Hydroxymatairesinol ¹	5.6
	Matairesinol ¹	2.9
	Lariciresinol ¹	2.7
30	Dihydrokaempferol	0.78
	Pinosylvin	0.78
	Pinosembrin	0.49

1: Willför et al., J. Agric. Food. Chem., 51, 7600–7606 (2003).

Appendix 8

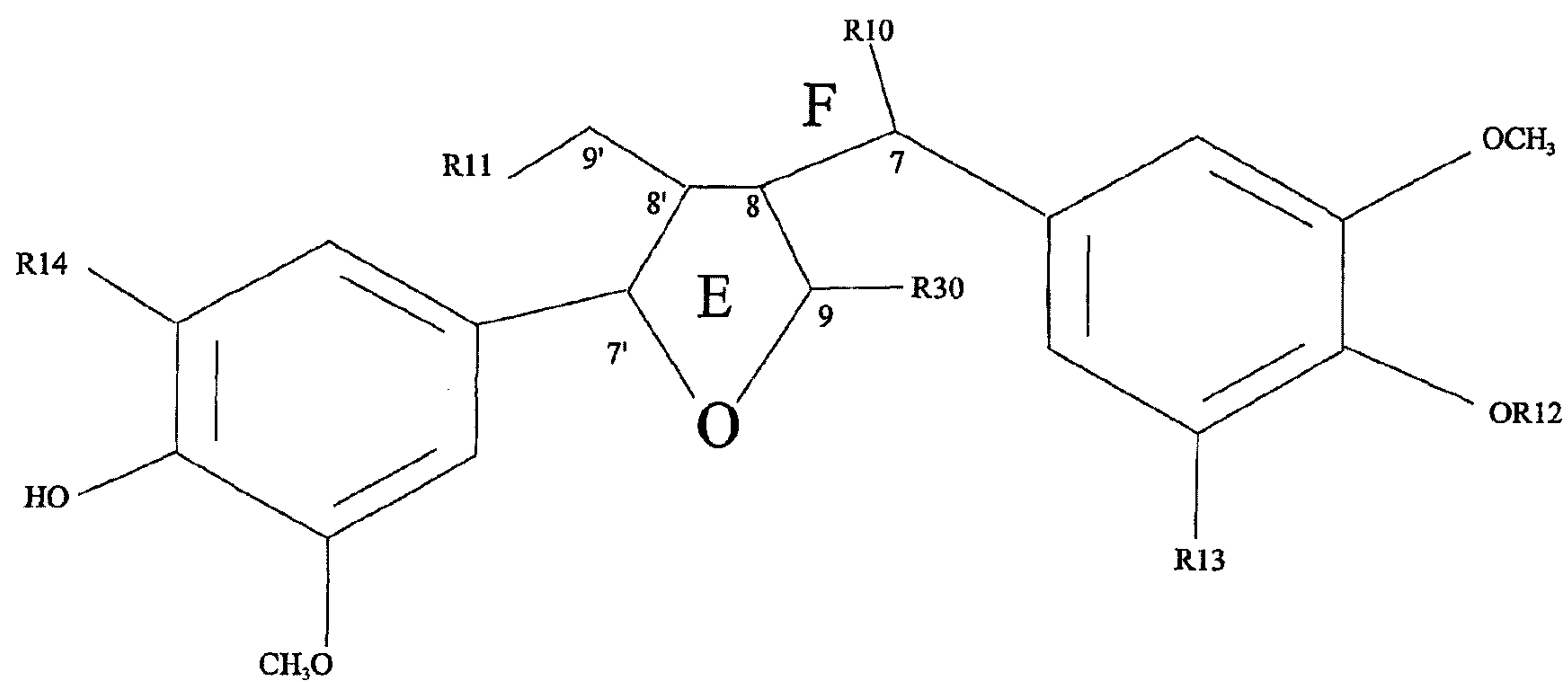
59



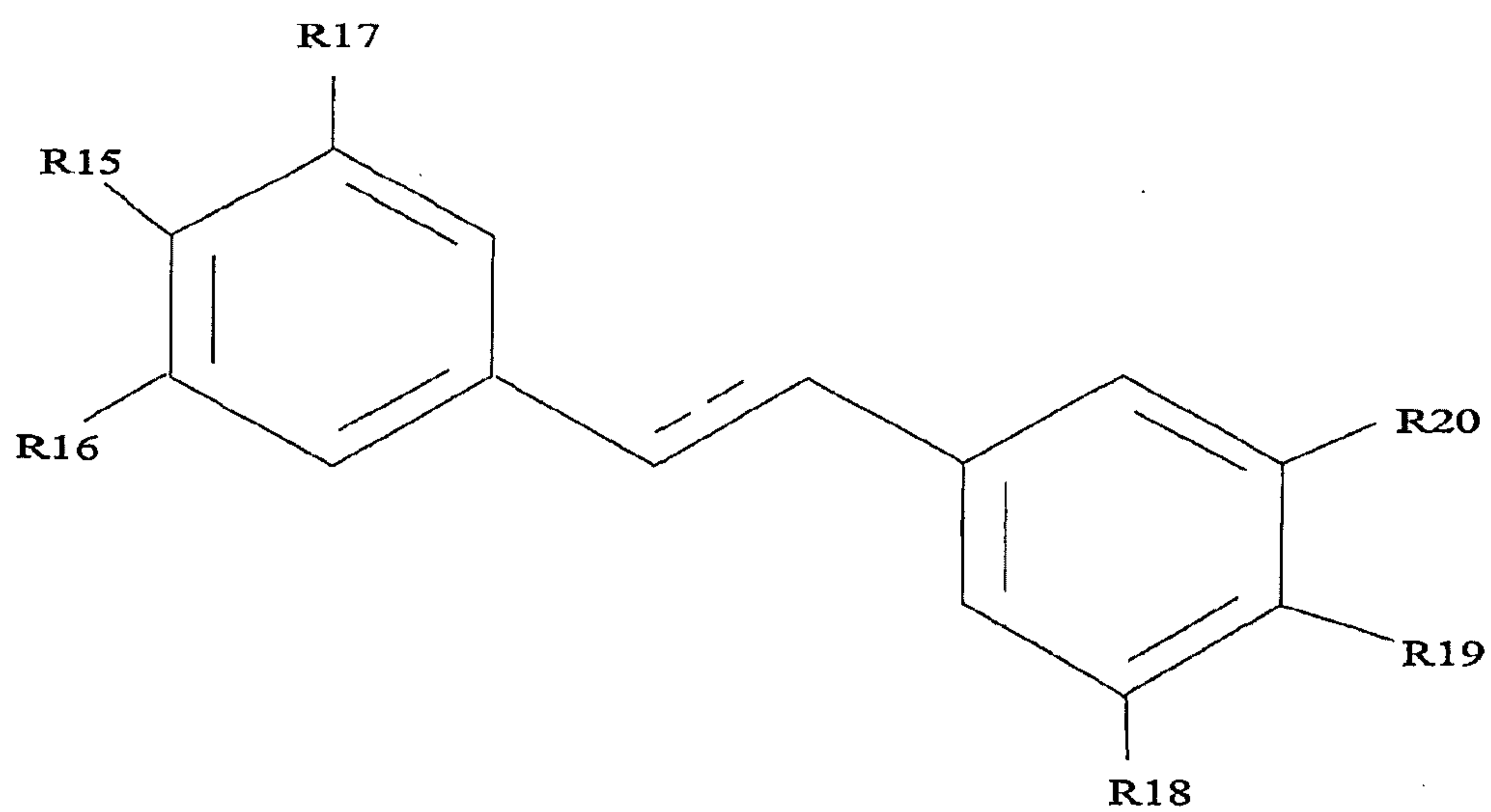
1A

Appendix 9

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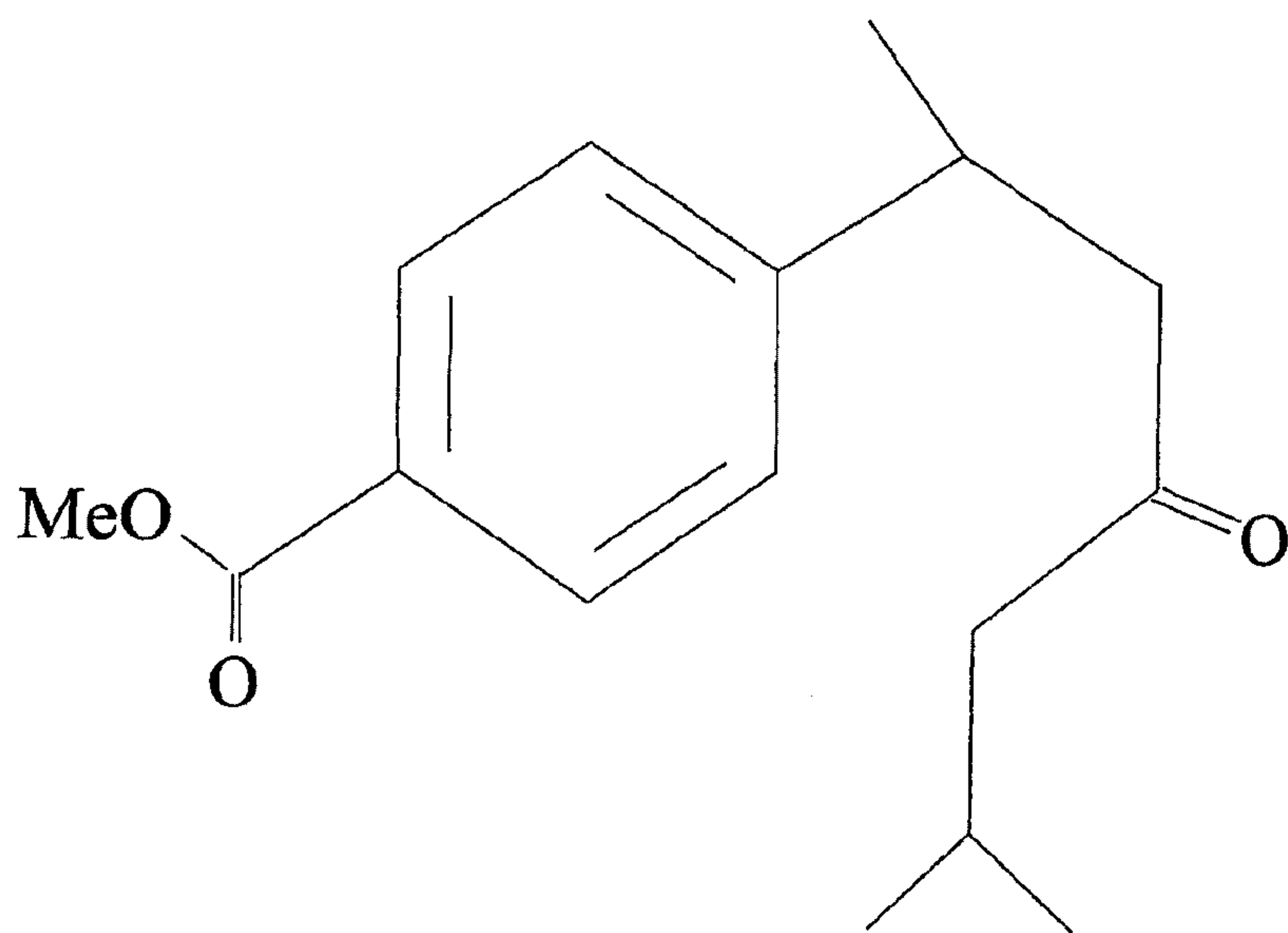
IB



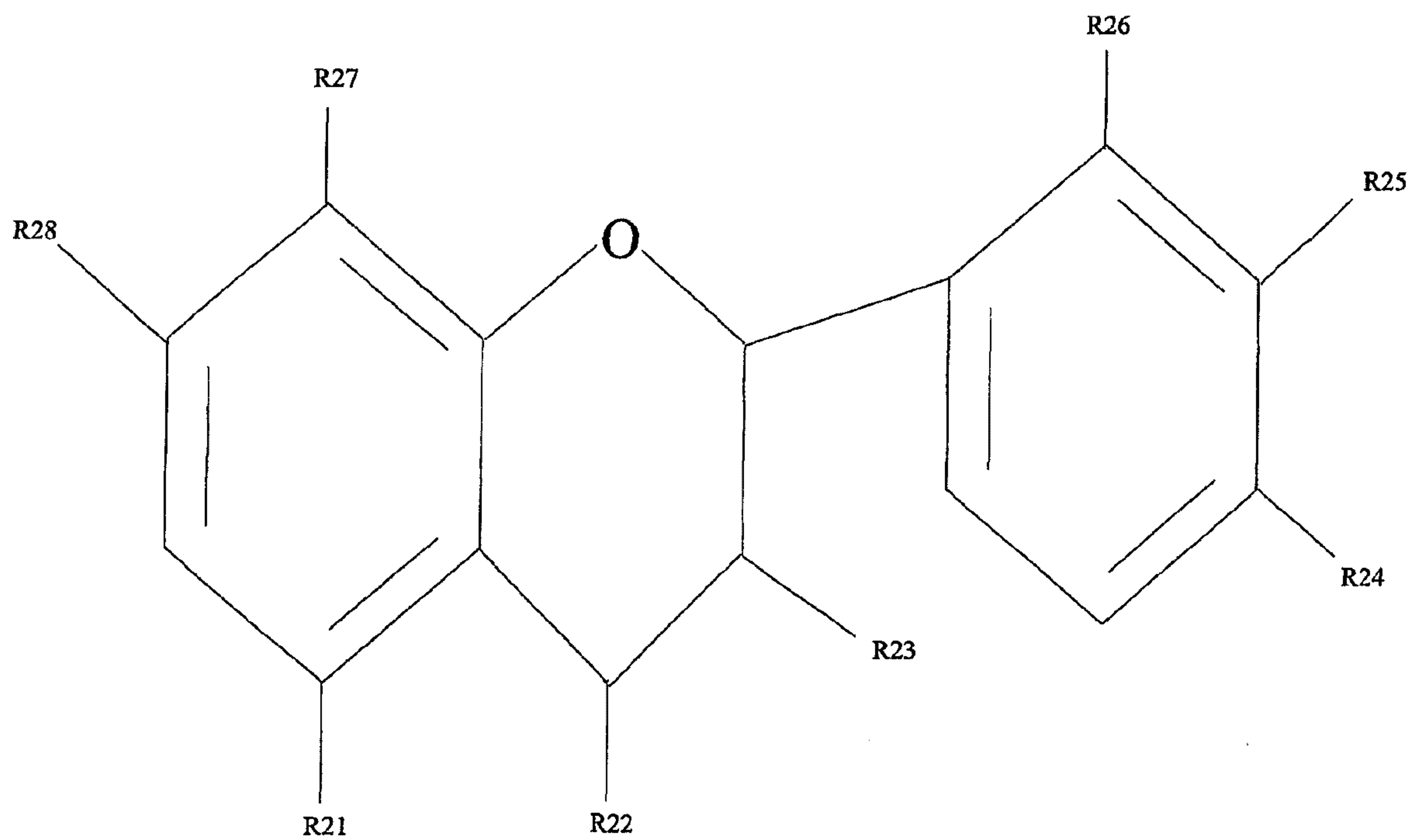
II

Appendix 10

61



III



IV

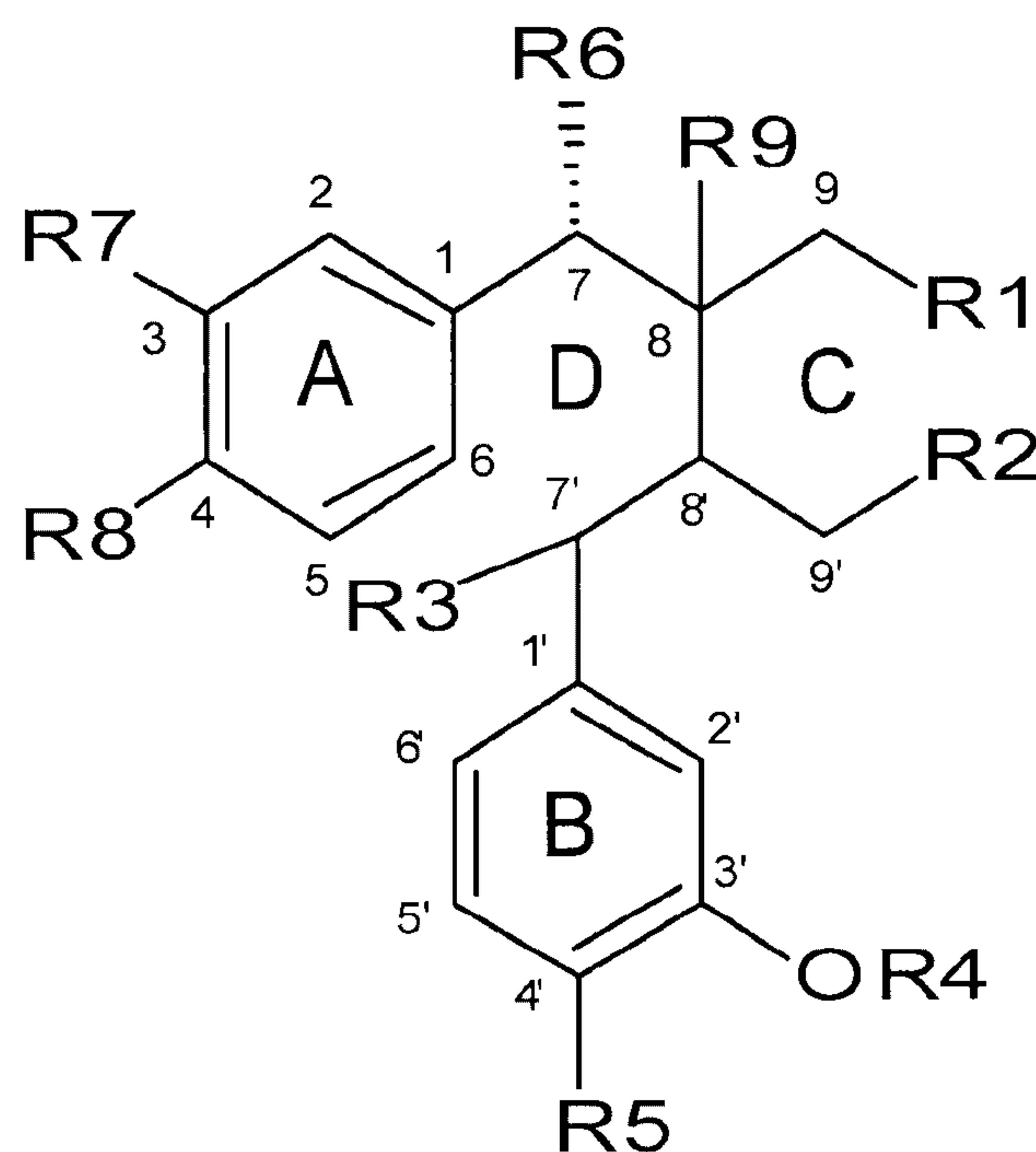
Claims

1. A method for production of a hair care composition comprising following steps:

- adding, in the hair care composition to be prepared, an aqueous carrier agent of hydrophobic or hydrophilic nature, surface active agents conventionally used in a hair care composition, perfumes and possible dispersing agents, and

- adding, in connection with the carrier, an anti-microbial compound mixture in the amount of 0.1– 5 wt% of the total weight of said hair care composition to be prepared, which compound mixture is obtained by pulverizing wood material and/or by extracting possibly pulverized wood material, so that said compound mixture contains at least two different compounds selected from

among the following groups: a lignan according to formula IA



IA

wherein

R1 or R2 is hydrogen, OH or =O,

or one of R1 and R2 is an oxygen atom -O- bound to carbons 9 and 9', and now forms with carbons 8, 8', 9, 9' a 5-membered oxygenous heterocyclic ring C,

R3 is hydrogen, OH, or =O, or it forms a bond to the carbon 6, so that the carbons 6, 1, 7, 8, 8', 7' form a cyclohexane ring that is condensed with the phenyl ring A, and possibly also a ring with C,

R4 denotes hydrogen or methyl,

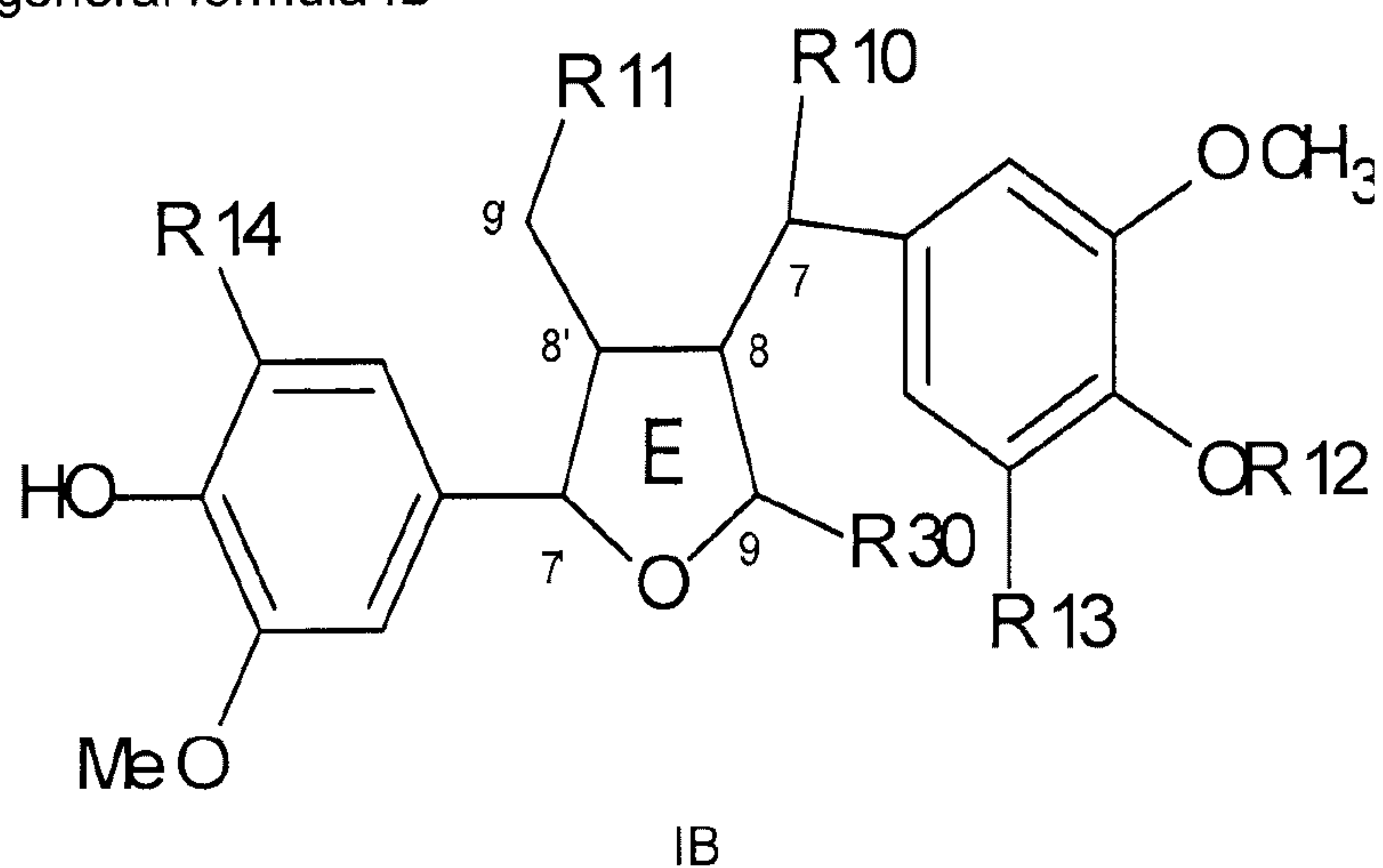
R5 denotes hydrogen or residue selected from the groups OH and OCH₃,

R6 denotes hydrogen or hydroxy,

R7 and R8 each independently is hydrogen, OH or OCH₃,

R9 denotes H or in case of nortrachelogenin R9 denotes OH,

or a lignan of general formula IB



wherein

R10 denotes hydrogen or hydroxyl

R11 denotes hydroxy or oxygen, which is bound by a bond to the carbons 7 and 9', forming now an oxygenous non-aromatic 5-membered heterocyclic ring (tetrahydrofuran) F with the carbons 7, 8, 8', 9', which ring is condensed with the hetero ring E (tetrahydrofuran) at the carbons 8, 8',

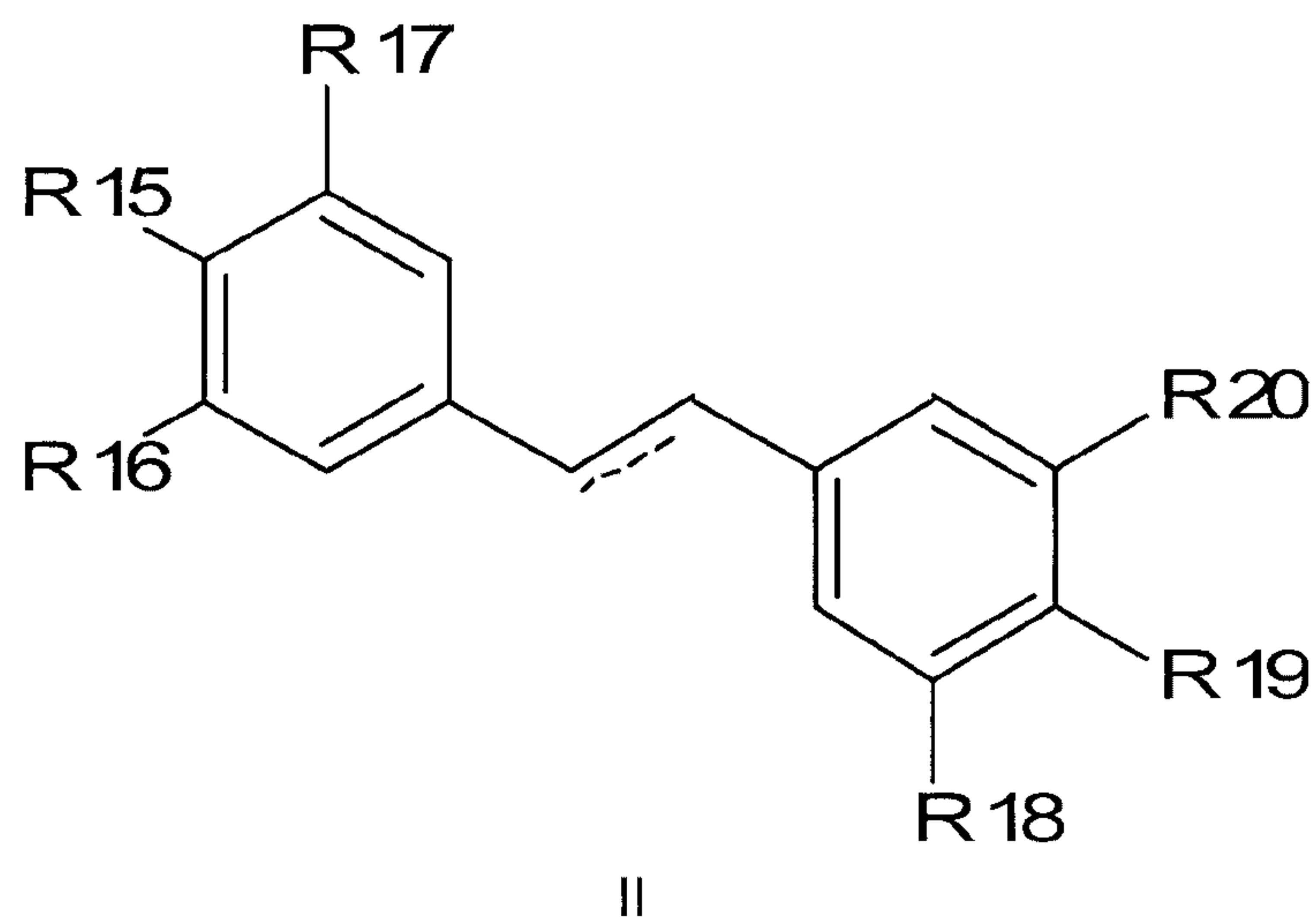
R12 denotes hydrogen or methyl,

R13 denotes hydrogen or methoxy,

R14 denotes hydrogen or methoxy,

R30 denotes hydrogen or =O;

a stilbene according to general formula II



wherein

R15 denotes hydrogen or hydroxy

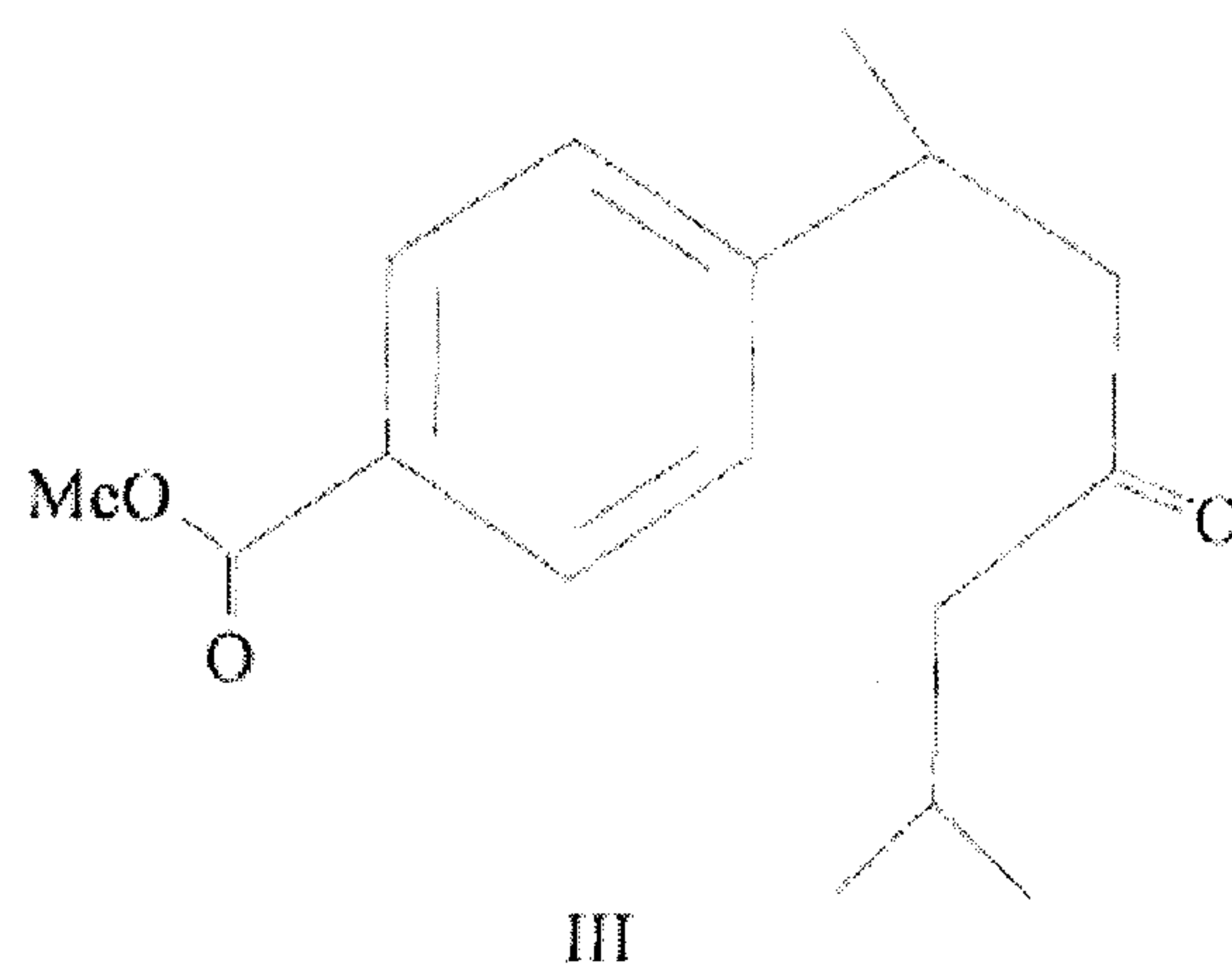
R16 is H, OH, or OCH₃,

R17 is OH or OCH₃,

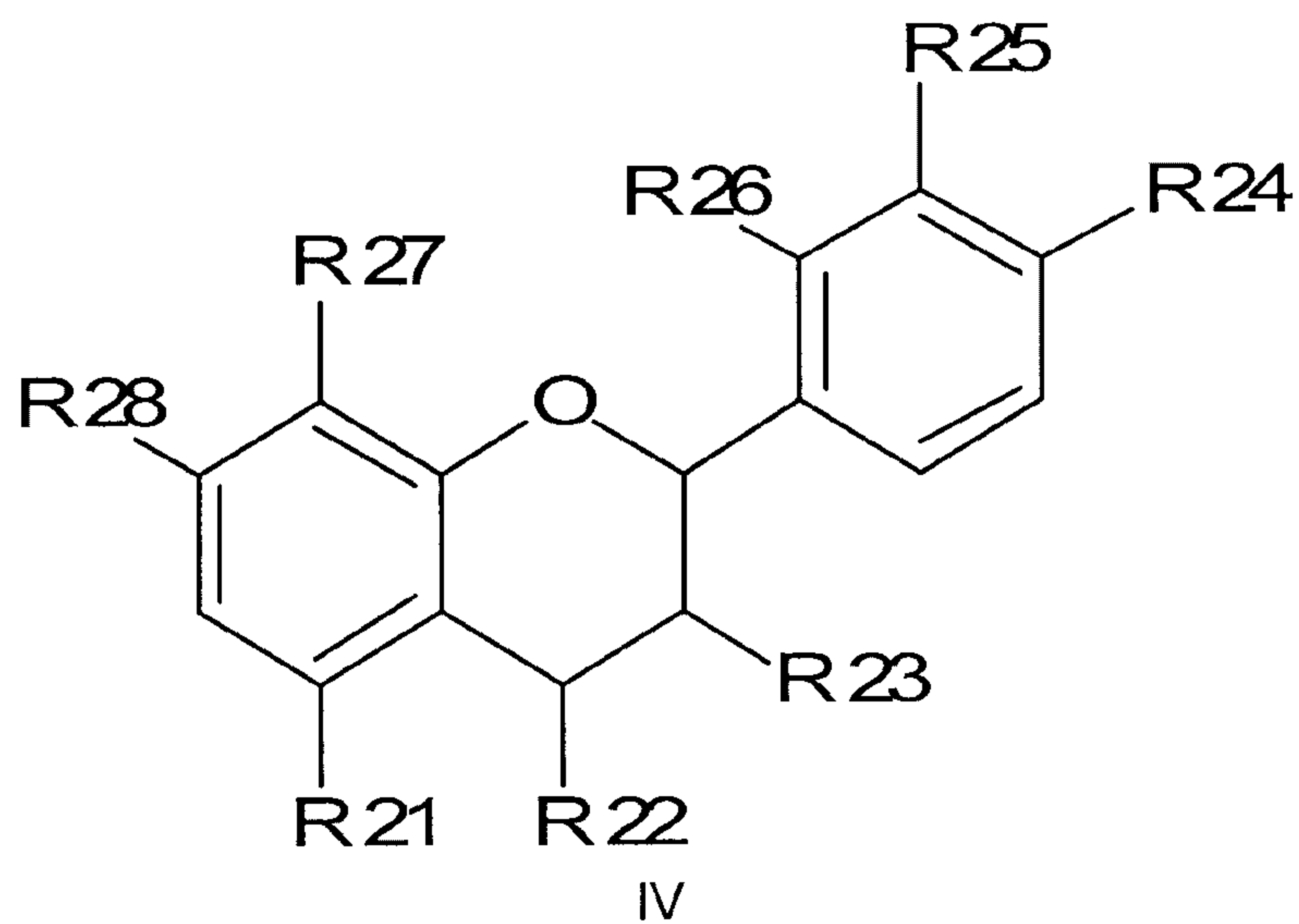
R18 and R19 each independently is hydrogen or hydroxy,

R20 is hydrogen or OGlu;

a juvabione according to general formula III,



a flavonoid according to general formula IV



wherein

R21 is H or OH,

R22 is H, OH or =O,

R23 is H or OH,

R24, R25, R26 each independently is hydrogen or hydroxy,

R28 and R27 each independently is hydrogen or hydroxy; and

betulin, betulonic acid, betulinic acid or betuloinic acid;

as well as ester or ether derivatives, as well as stereoisomers of above mentioned groups;

wherein said mixture further contains oligomers of said compounds of the compound mixture,

providing, however, that the compound mixture includes lignans A or B 50– 99.9 wt%, stilbenes of formula II 0 – 70 wt%, oligomers of lignans A or B 1 – 31 wt% or oligomers of stilbenes of formula II 1 – 31 wt% or oligomers of juvabionones of formula III 1 – 31 wt% or oligomers of flavonoids of formula IV 1 – 31 wt%,

further providing that the mixture contains at least one compound selected from the following group:

7- hydroxymatairesinol, conidendrin, conidendric acid, alpha- conidendrin, alpha- conidendric acid, isohydroxymatairesinol, cyclolariciresinol, secoisolariciresinol, anhydrosecoisolariciresinol and stilbenes as well as their ester or ether derivatives and stereoisomers, wherein in said method the content of the said compound mixture is kept in such that with said content,

- the compounds contained in the compound mixture do not irritate the skin in a so-called single patch test, and
- the compounds contained in the compound mixture are such that the cytotoxicity of said compounds, as measured in ethanol for a HaCat cell culture after 24 h incubations, is lower than the cytotoxicity of 0.02 - 0.1 wt% BHT dissolved in ethanol in the same incubation conditions, and
- the anti-microbial compound mixture also has a wide-scale anti-microbial effect against Gram- bacteria, Gram+ bacteria, yeast and fungi wherein said wide scale

anti-microbial effect means that the compound mixture has effects for inhibiting, from among Gram-negative bacteria, at least the growth of *E. coli*, *Ps. aeruginosa*, *Ps. putida*, and *Kl. pneumoniae*; from among Gram-positive bacteria at least the growth of *S. aureus*; from among yeasts at least the growth of scurf-causing yeast, *M. furfur* and *C. albicans*; and from among fungi at least the growth of *A. niger*.

2. The method as defined in claim 1 for production of the hair care composition as defined in claim 1, wherein the compounds contained in the compound mixture are such that the cytotoxicity of said compounds, as measured in ethanol for a HaCat cell culture after 24 h incubations, is lower than the cytotoxicity of 0.02 -0.05 wt% BHT dissolved in ethanol in the same incubation conditions.
3. The method as defined in any one of claims 1 to 2 for production of the hair care composition as defined in claim 1, wherein the lignan in the compound mixture is 7-hydroxymatairesinol or secoisolariciresinol.
4. The method as defined in any one of claims 1 to 3 for production of the hair care composition as defined in claim 1, wherein the stilbene in the compound mixture is pinosylvin or its ester or ether derivative.
5. The method as defined in any one of claims 1 to 4 for production of the hair care composition as defined in claim 1 which additionally contains a narrow-scale anti-microbial agent.
6. The method as defined in any one of claims 1 to 5 for production of the hair care composition as defined in claim 1, wherein in the compound mixture the oligomers are oligolignans.
7. The method as defined in any one of claims 1 to 6 for production of the hair care composition as defined in claim 1, wherein the method comprises obtaining the compound mixture by pulverizing wood material from at least two different wood species

and/or by extracting possibly pulverized wood material from at least two different wood species, so that the wood material is wood knot material or stemwood material from adjacent to knots.

8. The method as defined in claim 7 for production of the hair care composition as defined in claim 1, wherein an organic hydrophilic solvent is used in the extraction process.

9. The method as defined in claim 8 for production of the hair care composition as defined in claim 1, wherein the organic hydrophilic solvent is a hydrocarbon-carbonyl-based organic solvent.

10. The method as defined in claim 9 for production of the hair care composition as defined in claim 1, wherein the hydrocarbon-carbonyl-based organic solvent is a solution containing an alkyl carbonyl.

11. The method as defined in claim 10 for production of the hair care composition as defined in claim 1, wherein the alkyl carbonyl is an alcohol or ketone.

12. The method as defined in any one of claims 7 to 11 for production of the hair care composition as defined in claim 1, wherein in the method the compound mixture is obtained by extracting the wood material of pine and spruce, and that the compound mixture contains both stilbenes and lignans, 50 – 99.9% of the lignans being obtained from spruce knot material or spruce stemwood material adjacent to knots, and 0.1-50 wt% of the lignans is obtained from pine knot material or pine stemwood material adjacent to knots.

13. The method as defined in claim 5 for production of the hair care composition as defined in claim 1, wherein the method comprises adding the narrow-scale anti-microbial agent, wherein the narrow-scale anti-microbial agent is ethylhexyl glyceryl, betulin, betulonic acid, betulinic acid, betuloinic acid or their derivatives obtained from

birch bark, or resveratrol or its derivative obtained from spruce bark.

14. The method as defined in any one of claims 1 to 13 for production of the hair care composition as defined in claim 1, wherein the method further comprises adding an additive, an antioxidative agent or an antioxidant obtained from a plant.

15. The method as defined in claim 14 for production of the hair care composition as defined in claim 1, wherein the antioxidative agent is a natural or synthetic vitamin.

16. The method as defined in claim 15 for production of the hair care composition as defined in claim 1, wherein the natural or synthetic vitamin is vitamin A, B, C, D, E, provitamin B5, vitamin B3, L-ascorbic acid or vitamin E.

17. The method as defined in any one of claims 14 to 16 for production of the hair care composition as defined in claim 1, wherein the antioxidant obtained from the plant is an antioxidant contained in green tea, an antioxidant contained in flaxseed, an antioxidant contained in horse chestnut, a beta carotene, selenium, glutamine, ubiquinone, glycolic acid, growth hormones or kinetin.

18. The method as defined in any one of claims 1 to 17 for production of the hair care composition as defined in claim 1, wherein said compound mixture contains at least two different compounds, selected from the following groups:

lignans:

- matairesinol, hydroxymatairesinol, oxomatairesinol, didemethyl matairesinol, isohydroxymatairesinol, epi-isohydroxymatairesinol and their stereoisomers,
- secoisolariciresinol, isolariciresinol, lariciresinol, pinoresinol, dimethyl secoisolariciresinol, 7-hydroxysecoisolariciresinol, cyclolariciresinol, cycloisolariciresinol and their stereoisomers and ester or ether derivatives,
- nortrachelogenin and its stereoisomers and ester or ether derivatives,

- enterolactone and its stereoisomers and ester or ether derivatives,
- conidendrin, α -conidendrin and their stereoisomers and ester or ether derivatives;
- lignan A and its stereoisomers and ester or ether derivatives,
- liovile and its stereoisomers and ester or ether derivatives;

juvabiones:

- juvabiones and their stereoisomers and ester or ether derivatives;

stilbenes:

- pinosylvin, dihydropinosylvin, pinosylvin monomethyl ether, dihydropinosylvin monomethyl ether, resveratrol, astringin, isorhapontine, and their stereoisomers and ester or ether derivatives,

flavonoids:

- pinosembrin, catechin, pinobanxin, kaempferol, dihydrokaempferol, taxifolin, naringenin, teracacidine, ketoteracacidine, isoteracacidine, melacacidine, isomelacacidine and their stereoisomers and ester or ether derivatives,
- betulin, betulonic acid, betulinic acid, betuloinic acid or their ester derivative, as well as the glycosidized forms of these compounds and their oligomers.

19. The method as defined in claim 18, wherein the oligomers of the betulonic acid, betulinic acid, betuloinic acid, their ester derivative, or the glycosidized forms thereof are trimers and tetramers.

20. The method as defined in claim 18 or 19 for production of the hair care composition as defined in claim 1, wherein the lignans are selected from a group consisting of hydroxymatairesinol stereoisomers 7S, 8R; 8'R- hydroxymatairesinol and 7R, 8R, 8'R- allohydroxymatairesinol, and their stereoisomers and ester or ether derivatives.

21. The method as defined in claim 1 for production of the hair care composition as defined in claim 1, wherein the method comprises extracting the wood material with an organic lipophilic solvent, as well as with an organic hydrophilic solvent, and that the lipophilic and/or hydrophilic extraction solution forms part of the composition carrier agent.

22. The method as defined in claim 21 for production of the hair care composition as defined in claim 1, wherein the organic lipophilic solvent is hydrocarbon.

23. The method as defined in claim 22 for production of the hair care composition as defined in claim 1, wherein the hydrocarbon is a lower alkane.

24. The method as defined in claim 23 for production of the hair care composition as defined in claim 1, wherein the lower alkane is hexane or heptane.

25. The method as defined in any one of claims 21 to 24 for production of the hair care composition as defined in claim 1, wherein the organic hydrophilic solvent is an organic lower alkyl carbonyl compound.

26. The method as defined in claim 1 for production of the hair care composition as defined in claim 1, wherein the method comprises extracting the wood material with a monovalent or multivalent alcohol or their mixture.

27. The method as defined in claim 26 for production of the hair care composition as defined in claim 1, wherein the extraction solution is a multivalent lower alkyl alcohol.

28. The method as defined in claim 27 for production of the hair care composition as defined in claim 1, wherein the multivalent lower alkyl alcohol is lower alkyl diol or lower alkyl triol.

29. The method as defined in claim 28 for production of the hair care composition as defined in claim 1, wherein the lower alkyl diol is propyl glycol, butyl glycol, pentyl glycol or octyl glycol.

30. The method as defined in any one of claims 21 to 25 for production of the hair care composition as defined in claim 1, wherein in the method the extraction solution and the carrier present in the composition further contains a hydrocarbon- based solution, or a ketone, or an inorganic anion-cation solution or an ionogenic solution, or an ester of a trivalent alcohol.