The invention relates to the preparation of phenolics derivatives by enzymatic condensation of phenolics selected among pyrocatechol or its derivatives with the glucose moiety of sucrose. The production of said phenolics derivatives is achieved with a glucosyltransferase (EC 2.4.1.5). These O-β-glucosides of selected phenolics are new, have a solubility in water higher than that of their parent polyphenol and have useful applications in cosmetic and pharmaceutical compositions, such as antioxidative, antiviral, antibacterial, immune-stimulating, antiallergic, antihypertensive, anti-atherosclerotic, antiinflammatory, anticarcinogenic, antimitogenic, antineoplastic, anti-obesogenic, anti-thrombotic, and vasodilatory formulations, or in any other field of application.
Caffeic acid glucoside

4-methylcoumarin glucoside

Chlorogenic acid glucoside

Rosmarinic acid glucoside

Caffeic acid phenethyl ester glucoside

Esculetin glucoside

FIGURE 7
Nordalbergin glucoside

Chicoric acid glucoside

Echinacoside glucoside

FIGURE 7 (following)
Verbascoside O-α-D-glucoside

FIGURE 7 (following)
FIGURE 8
Hamamelitannin glucoside

FIGURE 8 (following)
Catechin glucoside

Epicatechin glucoside

Gallocatechin glucoside

Epigallocatechin glucoside

Epigallocatechin gallate glucoside

Epicatechin gallate glucoside

FIGURE 9
HO

Gossypetin glucoside

Homoorientin glucoside

Orientin glucoside

FIGURE 10 (following)
3,4-dihydroxybenzaldehyde glucoside

Maclurine glucoside

Pyrocatechol glucoside

Nordihydroguaiaretic acid glucoside

3,4 dihydroxybenzophenone glucoside

Anthracbin glucoside

3-hydroxydaidzein glucoside

Hydroxytyrosol glucoside

FIGURE 11
Erictyol chalcone glucoside

Maritimein glucoside

3,4-dihydroxyacetophenone glucoside

Salsolinol glucoside

FIGURE 11 (Following)
WATER SOLUBLE AND ACTIVABLE PHENOLCS DERIVATIVES WITH DERMOCOSMETIC AND THERAPEUTIC APPLICATIONS AND PROCESS FOR PREPARING SAID DERIVATIVES

FIELD OF THE INVENTION

[0001] The present invention relates to the preparation of phenolics derivatives, pharmacologic and cosmetic compositions comprising such phenolics derivatives, and their use for the beauty of the skin and for treating diseases.

BACKGROUND OF THE INVENTION

Phenolic Compounds and their Properties

[0002] Phenolic compounds (also called phenolics), or polyphenols, constitute one of the most numerous and widely-distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known. Polyphenols are products of the secondary metabolism of plants. The expression "phenolic compounds" embraces a considerable range of substances that possess an aromatic ring bearing one or more hydroxyl substituents. Most of the major classes of plant polyphenol are listed in Table 1, according to the number of carbon atoms of the basic skeleton. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins (HARBORNE JB, 1980).

The three important groups for humans are phenolic acids (C6-C1, C6-C2 and C6-C3), flavonoids (C6-C3-C6) and high-molecular weight polyphenols (more than 30 carbon atoms). Indeed, the phenolics, particularly polyphenols, exhibit a wide variety of beneficial biological activities in mammals, including antiviral, antibacterial, immune-stimulating, anti-inflammatory, anti-hypertensive, anti-tumor, anti-oxidant, and vasodilatory actions. They are powerful antioxidants in vitro.

TABLE 1

<table>
<thead>
<tr>
<th>NUMBER OF CARBON ATOMS</th>
<th>BASIC SKELETON</th>
<th>CLASS</th>
<th>EXAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 C6</td>
<td>Simple phenols</td>
<td>Catechol, hydroquinone</td>
<td></td>
</tr>
<tr>
<td>7 C6-C1</td>
<td>Phenolic acids</td>
<td>Gallic, salicylic</td>
<td></td>
</tr>
<tr>
<td>8 C6-C2</td>
<td>Acetophenones</td>
<td>3-Acetyl-6-phenylphenol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyrane derivatives</td>
<td>Methoxybenzaldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenylacetic acids</td>
<td>Tyrosol</td>
<td></td>
</tr>
<tr>
<td>9 C6-C3</td>
<td>Hydroxycinnamic acids</td>
<td>Caffeic, ferulic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenylpropenes</td>
<td>Methylstilbene, cinnamal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coumarins</td>
<td>Unibellifene, aucubinol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iso-coumarins</td>
<td>Bergapten</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chromones</td>
<td>Eugenin</td>
<td></td>
</tr>
<tr>
<td>10 C6-C4</td>
<td>Naphthoquinones</td>
<td>Juglone, plumbagin</td>
<td></td>
</tr>
<tr>
<td>13 C6-C1-C6</td>
<td>Xanthones</td>
<td>Mangiferin</td>
<td></td>
</tr>
<tr>
<td>14 C6-C2-C6</td>
<td>Stilbenes</td>
<td>Resveratrol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anthraquinones</td>
<td>Emodin</td>
<td></td>
</tr>
<tr>
<td>15 C6-C3-C6</td>
<td>Flavonoids</td>
<td>Quercetin, cyanidin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoflavonoids</td>
<td>Genistein</td>
<td></td>
</tr>
<tr>
<td>18 (C6-C3)2</td>
<td>Lignans</td>
<td>Pinocembrin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neo-lignans</td>
<td>Eugenin</td>
<td></td>
</tr>
<tr>
<td>30 (C6-C3-C6)2</td>
<td>Flavonoids</td>
<td>Amentoflavone</td>
<td></td>
</tr>
<tr>
<td>n (C6-C3)n</td>
<td>Lignins</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Catechol melanins</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among the phenolic acids, the most important constitutive carbon frameworks are the hydroxybenzoic (C6-C1) and hydroxycinnamic (C6-C3) structures. The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish, and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight. Hydroxybenzoic acids are components of complex structures such as hydrolyzable tannins (gallo-tannins in mangoes and ellagittannins in red fruits such as strawberries, raspberries and blackberries). The hydroxycinnamic acids are more common than are the hydroxybenzoic acid content of edible plants.
The flavonoids consist of a large group of low-molecular weight polyphenolic substances, benzo-γ-pyrone derivatives that are diverse in chemical structure; they represent the most common and widely distributed group of plant phenolics. The flavonoids common structure is that of diphenylpropenes (C6-C3-C6); its consists of two aromatic rings (cycles A and B) linked through three carbons that usually form an oxygenated heterocycle (cyclic C). FIG. 1 shows the basic structure and the system used for the carbon numbering of the flavonoid nucleus. Structural variations within the rings subdivide the flavonoids into several families: flavones, flavonols, flavanones, isoflavones, anthocyanidins and others. These flavonoids often occur as glycosides, glycosylation rendering the molecule more water-soluble and less reactive toward free radicals. The sugar most commonly involved in glycoside formation is glucose, although galactose, rhamnose, xylose and arabinose also occur, as well as disaccharides such as rutinose. The flavonoid variants are all related by a common biosynthetic pathway, incorporating precursors from both the shikimate and the acetate-malonate pathways (CROZIER A, BURNS J, AZIZ A A, STEWART A J, RABLI ASZH S, JENKINS G I, EDWARDS C A, LEAN M E J (2000) Antioxidant flavonoids from fruits, vegetables and beverages: measurements and bioavailability. Biol Res 33: 79-88). Further modifications occur at various stages, resulting in an alteration in the extent of hydroxylation, methylation, isoprenylation, dimerization and glycosylation (producing O- or C-glycosides). Phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. Indeed, excess levels of metal cations iron, zinc and copper in the human body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA; by forming complexes with these reactive metal ions, they can reduce their absorption and reactivity. It has to be underlined that though most flavonoids chelate Fe2+, there are large differences in the chelating activity. In particular, the dihydroflavonol taxifolin chelates more efficiently Fe2+ than the corresponding flavonol quercetin (VAN ACKER SAHE, VAN DEN BERG DJ, TROMP M N J L, GRIFFIOEN D H G, VAN BENNEKOM, VAN DER VLIEGH W J F, BAST A (1996) Structural aspects of antioxidant activity of flavonoids. Free Radic Biol Med 20: 331-342).

Flavonoids have ideal structural chemistry for free radical-scavenging activities (several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals and lipid peroxyl radicals by rapid donation of a hydrogen atom). One important finding from the studies of the relationship between the structural characteristics of flavonoids and their antiradical activity is that a catechol moiety (3',4'-dihydroxyphenol) on ring B is required for good scavenging activity. Recently, this statement was confirmed with nevertheless a modulation: in a study about the relationship between the structural characteristics of 29 flavonoids and their antiradical activity, it was indeed observed that the catechol structure in the B ring is not always a conditio sine qua non in achieving high free radical scavenging activity and that highly active flavonoids possess a 3',4'-dihydroxy B ring and/or a 3-OH group (AMIC D, DAVIDOVIC-AMIC D, BESLO D, TRINAJSTIC N (2003) Structure-radical scavenging activity relationships of flavonoids. Croatica Chem Acta 76: 55-61). Flavonoids have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis (RICE-EVANS C A, MILLER N J, PAGANGA G (1997) Antioxidant properties of phenolic compounds. Trends in Plant Science 2: 152-159). There are also reports of flavonoids inhibiting the activity of enzymes such as oxygenases.

Phenolics Derivatives and their Preparation

Due to their low aqueous solubility and/or high sensitivity toward oxidation, the use of phenolics in pharmaceutical or cosmetic preparations requires adapted and specific formulations. Since these formulations must also satisfy the constraints associated with their final usage, the compromise between acceptability, concentration and stability is often difficult to reach.

More water soluble and/or oxidation resistant forms of phenolics such as the glycosides are not always available in nature and may demand, when they exist, complex procedures of extraction and purification from the plant material. Both chemical and biochemical (enzymatic) approach have been attempted to increase water solubility and/or stability. As phenolic compounds have several free hydroxy groups, attempts for chemical modifications of phenolic compounds lead to unselective reactions, generating a panel of different molecules. Further steps of purification are then required to recover the desired product(s).

As far as the biochemical approach is concerned, three ways have been investigated to date to obtain phenolics glycosides, and basically flavonoids glycosides.

The first way relies on glycosyltransferases able to transfer the sugar moiety of a sugar nucleotide to an acceptor (in the case of UDP-glucose:glucosyltransferases (UGT), glucose is transferred from uridine 5'-diphosphoglucose). These enzymes, which contribute in the synthesis of secondary metabolism in plants, have broad acceptor substrate specificities (LIM E K, HIGGINS G S, BOWLES D J (2003) Regioselectivity of glucosylation of caffeic acid by UDP-glucose:glucosyltransferase is maintained in planta. Biochem J 373: 987-92; LIM E K, ASHFORD D A, HOU B, JACKSON R G, BOWLES D J (2004) Arabidopsis glucosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glycosides. Biotechnol. Bioeng. 87(5): 623-31). Nevertheless, this approach is impaired by the very high cost of the sugar nucleotides and the regeneration of the sugar nucleotide substrate, which is a way to decrease the substrate cost, is difficult to master at large scale.
The second way relies on saccharide-transferring enzymes able to transfer glucose from an α-glucosyl saccharide. Said enzymes are selected from the hydrolyases α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1), and from the transferase cyclodextrin-glucanotransferase (EC 2.4.1.19). Their substrates are amylose, dextrins, cyclodextrins, maltooligosaccharides and partial starch hydrolysates, all of them containing mainly or exclusively glucosyl residues linked each other through α 1→4 osidic bond. According to this approach, U.S. Pat. No. 5,565,435 states that α-glucosyl quercetin is obtained. It has to be underlined that the starch degrading enzymes link the glucosyl residue to the flavonoid through an α-osidic bond whereas the UDP-glucose:glucosyltransferase investigated by LIM et al. links the glucosyl residue to the flavonoid through a β-osidic bond. It has also to be underlined that in the conditions described in U.S. Pat. No. 5,565,435, the quercetin molecule could be solubilized by adjusting the pH at 8.5 and by maintaining the reaction medium at 60°C. The solubilisation of phenolics in alkaline media is due to the formation of phenolates; in these pH and temperature conditions, the stability of the substrate was achieved by operating under anaerobic conditions. It thus appear that this mode of preparation is highly difficult to control and to manage and that a simple mode of preparation should be valuable.

The third way involves glucosyltransfersases using sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) as glucosyl donor and producing glucans with the release of fructose. Several attempts have been achieved with this class of enzymes to try to get phenolics glucosides. First, the glucosyltransferase from Streptococcus sobrinus (reference by the authors strain 0715, serotype g) was proven to catalyze the synthesis of 4′-O-α-D-glucopyranosyl(−)-catechin in a strictly aqueous medium (catechin at 1 g/L in 100 mM phosphate buffer pH 6.0 containing 2% sucrose) (NAKAHARA K., KONTANI M., ONO H., KOMADA T., TANAKA T., OOSHIMA T., HAMADA S. (1995) Glucosyltransferase from Streptococcus sobrinus catalyzes glycosylation of catechin. Appl. Environ. Microbiol. 61(7): 2768-70). A similar enzyme, the glucosyltransferase from Streptococcus mutans GS-5 was proven to be less regioselective, as it catalyzes not only the synthesis of 4′-O-α-D-glucopyranosyl(−)-catechin but also the synthesis of 7-O-α-D-glucopyranosyl(−)-catechin and of the diglucosylated derivative 4,7-O-α-D-diglucopyranosyl(−)-catechin (MEULENBEKD G H., ZUIJHOF H., VAN VELDHUIZEN A., VAN DEN HEUVEL R H H., HARTMANS S (1999) Enhanced (+)-catechin transglucosylating activity of Streptococcus mutans GS-5 glucosyltransferase-D due to fructose removal. Appl Environ Microbiol. 65(9): 4141-7). Though several investigations regarding the acceptor specificity of Streptococcus mutans GS-5 glucosyltransferase lead the authors to infer that aromatic acceptors appear to require two adjacent aromatic hydroxyl groups (MEULENBEKD G H., HARTMANS S (2000) Transglycosylation by Streptococcus mutans GS-5 glucosyltransferase-D: acceptor specificity and engineering reaction conditions. Biotechnol Bioeng 70(4): 363-9), this statement was counteracted by the identification of glucosylation at position 7 in catechin (MEULENBEKD et al., 1999) and by the synthesis of non-pyrocatechol derivatives. Indeed, pinosylvin and resveratrol, respectively 3,5-di-hydroxy-trans-stilbene and 3,4′,5-trihydroxy-trans-stilbene, were glucosylated by a crude glucosyltransferase preparation produced by Streptococcus mutans to form respectively 3-O-α-D-glucopyranosyl-(E)-pinosylvin and 3-O-α-D-glucopyranosyl-(E)-resveratrol (SHIM H., HONG W., AHN Y. (2003) Enzymatic preparation of phenolic glucosides by Streptococcus mutans. Bull Korean Chem Soc 24(11): 1680-2). Very recently, it was shown that the flavonoids quercetin and myricetin and the flavone luteolin could be glucosylated by special glucosucrases, namely the Leuconostoc mesenteroides NRRL B-512F dextrantransferase (sucrose: 1,6-α-D-glucan 6-α-D-glucopyranosyltransferase, EC 2.4.1.5) and the Leuconostoc mesenteroides NRRL B-25192 alternansucrase (sucrose: 1,6(1,3)-α-D-glucan 6(3)-α-D-glucopyranosyltransferase, EC 2.4.1.140) (BERTRAND A., MOREL S., LEFOULON F., ROLLAND Y., MONSAN P., REMAUD-SIMEON M. (2006) Leuconostoc mesenteroides glucansucrase synthesis of flavonoid glucosides by acceptor reactions in aqueous-organic solvents. Carbohydr Res 341: 855-63). Conventionally, in the presence of sucrose, the former produces a glucan (dextran) in which 95% of the glucosidic bonds are α-(1→6) (skeleton of the polysaccharide) and 5% α-(1→3) (branching points), and the later a glucan (altan) in which the glucosidic bonds are alternatively α-(1→6) and α-(1→3). The obtained flavonoid derivatives were: luteolin-3′-O-α-D-glucopyranoside, luteolin-4′-O-α-D-glucopyranoside, quercetin-3′-O-α-D-glucopyranoside, quercetin-4′-O-α-D-glucopyranoside, quercetin-3′-4′-O-α-D-diglucopyranoside, myricetin-3′-O-α-D-glucopyranoside and myricetin-4′-O-α-D-glucopyranoside. This work demonstrates that yields of glucosides derivatives synthesis not only rely on the enzyme itself (the synthesis of luteolin-D-glucosides dropped down from 44% to 8% between dextrantransferase and alternansucrase), but also on slight chemical differences between two acceptors (no conversion was observed with the dextrantransferase on diosmetin and diosmin).

From the above significant (though not exhaustive) state of the art regarding the experimented ways to obtain glucosylated derivatives of polyphenols in general (and flavonoids in particular) in order to overcome the main conventional drawbacks of flavonoids (poor water solubility at physiological conditions, in particular at pH ranging from 5 to 7 and 30°C and high sensitivity to autoxidation in these biological conditions), it clearly appears that no precise guidelines can be deduced to set up the enzymatic production of a specific phenolics glycoside. On the contrary, it shows that there is no way for man of the art to predict which flavonoid can be glucosylated with which enzyme and in which conditions to obtain high glucoside concentrations (see summary in Table 2). Indeed, though attempts have been made in order to establish a relationship between the phenolic structures and the possibility of their use as glucosyl acceptor by glucosyltransferases, it still appears that the obtention of glucosylated phenolics strongly depends on the nature of the phenolic substance and on the enzyme used for the condensation reaction. This is particularly true with glucosyltransferases synthesizing conventionally α-glucans from sucrose (EC 2.4.1.5) for which only a very few number of polyphenolic structures have been successfully reported. Furthermore, in the case of the main glucosyltransferases studied, namely S. mutans GS-5 glucosyltransferase D and L. mesenteroides NRRL B-512F dextrantransferase, it has to be mentioned that the former synthesizes a water-soluble α-glucan in a primer-stimulated and dependent manner (HAHADA N., KURAMITSU H.K. (1989) Isolation and characterization of the Streptococcus mutans GTF gene involved in the primer-dependent soluble glucan synthesis. Infect Immun 56: 1999-2005) whereas the later does not (ROBYT J. F., WALSETH T. F. (1978) The mechanism of acceptor reactivities of Leuconostoc mesenteroides NRRL B 512F. Carbohydr Res 61: 433-45). These glucosyltransferases have distinct mechanism of action and consequently molecules that are acceptor for an enzyme are not necessarily acceptor for the other; in other words, as shown in the previously cited works, there is no justification to consider that the substances that act as
glucosyl acceptor in the case of *S. mutans* GS-5 glucosyl-transferase D act also as glucosyl acceptor in the case of *L. mesenteroides* NRRL B-512F dextrantransferase, and vice versa. [0016] All the more, prior art information shows that despite the interest and abundance of phenolics, few phenolics glycosides have been obtained by enzymatic reactions.

<table>
<thead>
<tr>
<th>POLYPHENOL</th>
<th>ENZYME ORIGIN</th>
<th>PRODUCT(S) AND REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (OH in 3 and 4)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Caffeoyl-3-O-β-glucoside - LIM et al. 2003</td>
</tr>
<tr>
<td>(OH in 2 and 3, respectively)</td>
<td></td>
<td>2-O- and 3-O-β-glucoside of α- and m-coumaric acids - LIM et al. 2003</td>
</tr>
<tr>
<td>Isoflavonic acid (OH in 3; OCH3 in 4)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>3-O-β-glucoside - LIM et al. 2003</td>
</tr>
<tr>
<td>(OH in 4), ferulic acid (OH in 4 and OCH3 in 3) and sinapic acid (OH in 4 and OCH3 in 3 and 5)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>No glucoside - LIM et al. 2003</td>
</tr>
<tr>
<td>Quercetin (flavonol; OH in 3, 5, 7, 3', and 4')</td>
<td><em>Arabidopsis thaliana</em></td>
<td>3-O-, 7-O-, 3',O-, 4'-O-monoglycosides and 3,7-di-O and 7-3'-di-O-glucosides LIM et al. 2003; LIM et al. 2004 Glucosides - LIM et al. 2003</td>
</tr>
<tr>
<td>Luteolin (flavone; OH in 5, 7, 3' and 4')</td>
<td><em>Arabidopsis thaliana</em></td>
<td>No glucoside - LIM et al. 2003</td>
</tr>
<tr>
<td>Eroditcyl (flavonone; OH in 5, 7, 3' and 4')</td>
<td><em>Arabidopsis thaliana</em></td>
<td>No glucoside - LIM et al. 2003</td>
</tr>
<tr>
<td>Catechin (flavonol; OH in 3, 5, 7, 3', and 4') and cyanidin (anthocyanin; OH in 5, 7, 3', 4')</td>
<td><em>Arabidopsis thaliana</em></td>
<td>No glucoside - LIM et al. 2003</td>
</tr>
</tbody>
</table>

Enzymes and substrates: Starch degrading enzymes (α-glucosidase, cyclodextrin glucoamylase or CGTase, α-amylase) and starch and/or starch hydrolytases

Quercetin (flavonol; OH in 5, 7, 3' and 4') α-glucosidase: pig liver, backwheat seed, Macor, Penicillium, Saccharomyces CGTase: Banillus, Klebsiella α-amylase: Aspergillus (OH glucosylated not mentioned)

Enzymes and substrates: Glycosyltransferases able to transfer the glucose moiety of sucrose

<table>
<thead>
<tr>
<th>POLYPHENOL</th>
<th>ENZYME ORIGIN</th>
<th>PRODUCT(S) AND REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin (flavonol; OH in 3, 5, 7, 3', and 4')</td>
<td><em>Streptococcus sobrinus</em></td>
<td>4'-O-α-D-glucopyranosyl- (+)-catechin (NAKAHARA et al. 1995)</td>
</tr>
<tr>
<td>Resveratrol (OH in 3, 5, 4' and pinoicin (OH in 3, 5)</td>
<td><em>Streptococcus mutans</em></td>
<td>3-O-α-D-glucopyranosyl-(E)-pinoicin and 3-O-α-D-glucopyranosyl-(E)-resveratrol (SHIM et al. 2003)</td>
</tr>
<tr>
<td>Catechin (flavonol; OH in 3, 5, 7, 3', and 4')</td>
<td><em>Streptococcus mutans</em> GS-5 (glucosyltransferase D)</td>
<td>4'-O-α-D-glucopyranosyl- (+)-catechin, 7-O-α-D-glucopyranosyl- (+)-catechin and 7-O-α-D- diglucopyranosyl- (+)-catechin (MEULENBIELD et al. 1998)</td>
</tr>
<tr>
<td>Catechol (OH in 1 and 2), 3-methoxy catechol (OCH3 in 3), 3-methylcatechol (CH3 in 3), 4-methylcatechol (CH3 in 4)</td>
<td><em>Streptococcus mutans</em> GS-5 (glucosyltransferase D)</td>
<td>Glucosides (MEULENBIELD and HARTMANS, 2000)</td>
</tr>
<tr>
<td>Phenol, 3-hydroxyphenol, benzylic alcohol, 2-hydroxybenzyl alcohol, 2-methoxybenzyl alcohol, 1-phenyl-1,2-ethanediol</td>
<td><em>Streptococcus mutans</em> GS-5 (glucosyltransferase D)</td>
<td>No glucoside (MEULENBIELD and HARTMANS, 2000)</td>
</tr>
<tr>
<td>Quercetin (flavonol; OH in 3, 5, 7, 3', and 4'), luteolin (flavone; OH in 5, 7, 3' and 4'), myricetin (flavonol; OH in 3, 5, 7, 3', 4' and 5')</td>
<td><em>L. mesenteroides NRRL B-512F</em></td>
<td>Glucosides (3' and 4' with luteolin and <em>L. mesenteroides</em> NRRL B-512F) (BERTRAND et al. 2006)</td>
</tr>
<tr>
<td>Diosmetin (flavonol; OH in 5 and 3', OCH3 in 4')</td>
<td><em>L. mesenteroides NRRL B-23192</em></td>
<td>No glucoside (BERTRAND et al. 2006)</td>
</tr>
</tbody>
</table>

Enzymes and substrates: Glycosyltransferases able to transfer the sugar moiety of a sugar nucleotide (e.g. UDP-glucose)
Another key point to consider in the enzymatic synthesis of phenolics glycosides, is the possibility to create phenolics derivatives that enable to recover the initial phenolics by an hydrolysis reaction in smooth conditions.

Indeed, for a given polyphenol, the advantageous properties that are presently known correspond to a specific structure and it has thus to be demonstrated that the valuable derivative with increased water solubility and stability properties can be converted into the saccharide part in one hand and the aglycone part in the other hand. One example of decrease of antioxidant activity due to glycosylation is given by MISHRA et al. (MISHRA B, PRIYADARSHINI K I, KUMAR M S, UNNIKRISHNAN M K, MOHAN A I 2005) Effect of O-glycosylation on the antioxidant activity and free radical reactions of a plant flavonoid, chrysoeriol. Bioorg Med Chem 11: 2677-85. Chrysoeriol and its glycoside (chrysoeriol-6-O-β-D-glucoside) are two flavonoids extracted from the tropical plant Coronopus didymus; chrysoeriol shows better protecting effect than the glycoside when tested for their ability to inhibit lipid peroxidation induced by gamma-radiation, Fe (III) and Fe (II). To date, this reversibility is only known for the α-glosyl quercetin obtained with starch degrading enzymes in vitro (U.S. Pat. No. 5,565,435). So, if the functionalization of phenolics as glycoside derivatives is a way (i) to facilitate their formulation in cosmetic, pharmaceutical or any other man made preparation due to a higher water solubility than that of the aglycone and (ii) to increase the stability of these phenolics in said formulas; both of them being universal properties of the glycosylated forms of polyphenols, these glycoside derivatives must be hydrolyzable in biological conditions.

There is therefore a need to create:

- new derivatives of valuable phenolic compounds with increased water solubility (in the same physico-chemical conditions (pH, salinity, temperature, ...)) and stability; and/or,
- new derivatives of valuable phenolic compounds that can be readily converted into their precursor, glucose and phenolic substance, in the place where they have to exert their biological activity and not during their storage in a commercial formula; and/or,
- new derivatives of valuable phenolic compounds that can be obtained through a process in which the synthesis and purification steps can be carried out in a reproducible manner and at any scale dependent on the market demand.

Owing to the fact that the protocatechuic structure (presence of two vicinal hydroxyl groups) is recognized as particularly important for the scavenging activity of polyphenols, the phenolic compounds that seem particularly efficient are those containing a catechol structure; among the phenolic compounds that are of particular interest, there are the following compounds:

- protocatechuic acid (3,4-dihydroxybenzoic acid, FIG. 2) and its esters derivatives; and/or,
- caffeic acid (3,4-dihydroxycinnamic acid, FIG. 3) and its esters derivatives, especially rosmarinic acid (3,4-dihydroxycinnamic acid (R)-1-carboxy-2-(3,4-di-hydroxyphenyl)ethyl ester), chlorogenic acid (3-O-(3,4-dihydroxycinnamoyl)-D-quinic acid), chioric acid, echinacoside, verbascoside and caffeic acid phenethyl ester, and its reduced form hydrocaffeic acid and its esters derivatives; and/or,
- special structures not closely related to protocatechuic acid or caffeic acid and containing the pyrocatechol ring: 3,4-dihydroxymandelic acid (FIG. 4) and its related substance 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylyglycol with a C2-C6 skeleton, and esculetin (6,7-dihydroxyecoumarine, FIG. 5) with a C6-C3 skeleton; and/or,

- the flavonones taxifolin (3,5,7,3',4'-pentahydrorxyflavonol, FIG. 6), fistizia (3,7,3',4'-tetrahydroyrxyflavonol), eriodictyol (5,7,3',4'-tetrahydroyrxyflavonol); and/or,

- the flavonones fisetine (3,7,3',4'-tetrahydroyrxyflavone) and rhamnetin (3,5',3'-tetrahydroyrxyflavone); and/or,

- the flavones cirsimil and 3',4',7-trihydroyrxyflavone and the isoflavone 3'-hydroydihizelion.

More detailed information on these phenolics of interest are included below.

- Protocatechuic acid (also named 3,4-dihydroxybenzoic acid, or (3,4-dihydroxybenzoic acid)) is found in many edible and medicinal plants, though most of the time at concentrations lower than derivatives of cinnamic acid. Though slightly less potent than caffeic acid, protocatechuic acid showed a time-dependent and dose-dependent inhibitory effect on T47D human breast cancer cell growth. It was also demonstrated that protocatechuic acid and caffeic acid interact directly with the aryl hydrocarbon receptor, inhibit nitric oxide synthase and have a pro-apoptotic effect (KAMP A, ALEXAKI V I, NOTAS G, NIFLI A P, NISTIKA A K A, HATZOGLOU A, BAKOGEORGIOU E, KOUMITZOGLOU E, BLEKAS G, BOSKOU D, GRAVANIS A, CASTANAS E 2004) Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res 6: R63-R74. LIU et al. (LIU K S, TSAO S M, YIN M C 2005) In vitro antibacterial activity of rosselle calyx and protocatechuic acid. Phytother Res 19(11): 942-5) demonstrated in vitro an inhibitory effect of protocatechuic acid on the growth of methicillin-resistant Staphylococcus aureus. Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumanntii. The data from inhibition zone and minimum inhibitory concentration (MIC) values showed that protocatechuic acid inhibited effectively the growth of all tested bacterial pathogens. Recent studies indicate that protocatechuic acid could be used as a protective agent against cardiovascular diseases and neoplasms (SZUMILO J 2005). Postepy Hig Med Dosw (Online) 59: 608-15). The mechanism of its action is mostly associated with antioxidant activity, including inhibition of generation as well as scavenging of free radicals and up-regulating enzymes which participate in their neutralization.

- It was also demonstrated that protocatechuic acid is a possible chemopreventive agent for colon carcinogenesis through the suppression of manifestation of intermediate biomarkers induced by azoxymethane (AOM)-induced colon carcinogenesis in rats (TANAKA T, KOJIMA T, SUZUI M, MORI H 1993) Chemoprevention of colon carcinogenesis by the natural product of a simple phenolic compound protocatechuic acid: suppressing effects on tumor development and biomarkers expression of colon tumorgenesis. Cancer Res. September 1: 53(17): 3908-13). Protocatechuic acid is therefore also a valuable active phenolic compound, but its bioavailability should be increased through functionalization to obtain more water soluble derivatives.
[0033] Caffeic acid (also named 3,4-Dihydroxycinnamic acid), a derivative of trans-cinnamic acid (trans-3-phenylacrylic acid) contains a -CH=CH-COOH group which ensures greater H-donating ability and subsequent radical stabilization than the carboxylate group in benzoic acids (RICE-EVANS C A, MILLER N J, PAGANDA G (1996) Structure—antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20(7): 933-56). In addition to its possible beneficial effects on human health (caffeic and 3-methoxycaffeic or ferulic acids react with nitrite in vitro and inhibit nitrosamine formation in vivo; they also inhibit tyrosine nitrification mediated by peroxynitrite), caffeic acid recently proved effective in protecting human skin from UVB-induced erythema (SVOBODOVA A, PSOTOVA J, WALTEROVA D (2003) Natural phenolics in the prevention of UV-induced skin damage. A review. Biomed Papers 147: 137-145). Caffeic acid is frequently encountered in the form of derivatives, with 1-carboxy-2-(3,4-dihydroxyphenyl)-ethanol to form rosmarinic acid, quinic acid to form chlorogenic acid and phenylethanol to form caffeic acid phenethyl ester.


[0036] Chlorogenic acid (also named 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate)) is the major soluble phenolic in Solanaceous species such as potato, tomato and eggplant. It also accumulates to substantial levels in apples, pears, plums and coffee. SAWA et al. (SAWA T, NAKAO M, AKAIKE T, ONO K, MAEDA H (1999) Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor prompted effect of vegetables. J Agric Food Chem 47: 397-402) observed that it removes particularly toxic reactive species by scavenging alkylperoxy radicals and may prevent carcinogenesis by reducing the DNA damage they cause.

[0037] Caffeic phenethyl ester (CAPE) is one of the major components of honeybee propolis, the resinous dark-colored material which is collected by honeybees from the buds of living plants mixed with bee wax and salivary secretions. CAPE is a potent and specific inhibitor of activation of members of the transcription factor NF-kB family and this may provide the molecular basis for its multiple immunomodulatory and antiinflammatory activities (YUN S Y, KIM S, BURGE T R, GRUNBERGER D, AGARWAL B B (1996) Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kB. Proc Natl Acad Sci USA 93: 9090-5). More recently, the role of CAPE as a potent antimetastatic agent which can markedly inhibit the metastatic and invasive capacity of malignant cells was evidenced (HONG Y J, PARK H J, CHUNG H J, MIN H Y, PARK E J, HONG J Y, LEE SK (2006) Inhibitory effects of caffeic acid phenethyl ester on cancer cell metastasis mediated by the down-regulation of matrix metalloproteinase expression in human HT1080 fibrosarcoma cells. J Nutri Biochem 17: 356-62).

[0038] Esculetin (or aesculetin, also named 6,7-Dihydroxycoumarin), a member of the family of the C6-C3 phenolics, has a coumarin structure is derived from trans-cinnamic acid via ortho-hydroxylation (for memory, caffeic acid is 3,4-dihydroxycinnamic acid), trans- cis isomerisation of the side chain double bond and lactonisation. Whereas the trans form is stable and can not cyclize, the cis form is very unstable and cyclization is thus favored. Glucose is a good leaving group which assists in the cis-trans transformation. A specific enzyme found in Methylobacterium (Leguminosae) specifically hydrolyses the cis-glucoside (β-glucosidase). Some of its properties are the inhibition of Ras-mediated cell proliferation and attenuation of vascular restenosis following angioplasty in rats (PAN S L, HUANG Y W, GUH J H, CHANG Y L, PENG C Y, TENG C M (2003) Esculetin inhibits Ras-mediated cell proliferation and attenuates vascular restenosis following angioplasty in rats. Biochem Pharmacol 65: 1897-1905) and the inhibition of mushroom tyrosinase (MASHIMOTO Y, ANDO H, MURAI A, SHIMOISHI Y, TADA M, TAKAHATA K (2003) Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of Emblica officinalis. Biotech Biochem Biochem 67(1): 631-3). It has to be mentioned that esculetin is frequently encountered as a glucoside, esculetin (esculetin-6-β-D-glucopyranoside), with a β-glucosidic linkage at position 6. The members of C6-C2 phenolics are basically found in the catecholamine metabolism and 3,4-dihydroxyphenyl related substances could have interesting properties (EISENHOFFER G, KOPIN I J, GOLDSTEIN D S (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Parmacol Rev 56(3): 331-49).
[0039] Taxifolin (or dihydroquercetin, or 3,5,7,3',4'-pentahydroxyflavone, or (2R,3S)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-chromanone) occurs in various barks (Larix sibirica Lebed, Pinus sibirica subsp. atlantica) and in Silybum marianum seeds (used for the preparation of the silymarin complex and containing silymarin flavonolignans which are biogenetically formed by oxidative addition of coniferyl alcohol to taxifolin. It has a chiral bond between cycle B and the two others cycles. Relating to the PP-vitamin group, it possesses a wide spectrum of biological activities (MIDDLETON E, KANDASWAMI C, THEOHARIDES TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol Rev 52(4): 673-752). It shows capillary-protecting, anti-inflammatory and gastro-protective action, decreases spasms of sleek muscles of the intestine, increases functions of the liver, possesses antiradical protective activity. Taxifolin has also been shown to have potential applications in reducing skin inflammation (BITTO T, ROY S, SEN C, SHIRAKAWA T, GOTOH A,UEDA M, ICHIHASHI M, PACKER L (2002) Flavonoids differentially regulate II/N-gamma-induced ICAM-1 expression in human keratinocytes: molecular mechanisms of action. FEBS Lett 520(1-3): 145-52). However Taxifolin is poorly soluble in aqueous solution (around 1 g/l), with prevents its usage for some cosmetic and therapeutic applications.

[0040] Glycosylation being recognized to render, in vegetal cells as well as in vitro, polyphenols more water-soluble and less reactive toward free radicals, if glucosides of these phenolics of particular interest exist, then they might represent polyphenol derivatives with increased water solubility and stability, and thus with increased added value.

[0041] It would also be useful to obtain derivatives from these phenolics which can be converted during their final usage in the metabolizable initial phenolics structure. This objective can be achieved by means of the present invention.

SUMMARY OF THE INVENTION

[0042] The present invention concerns a method for producing a phenolic compound O-α-glucoside comprising incubating sucrose and a glucansucrase from Leuconostoc species, preferably from Leuconostoc mesenteroides NRRL B-512F, preferably in buffered water at pH convenient for the enzymatic activity (well known by a skilled man) or in a buffered water at pH convenient for the enzymatic activity-cosolvent mixture, with a phenolic compound having the following formula:

\[ \text{(I)} \]

[0043] wherein R2 is H or OH; and
[0044] R1 is selected from the group consisting of
[0045] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH; and,

[0046] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH;

[0047] wherein R5 is OH or OCH₃; R6 is H or OH, R9 is H or OH, R10 is H, OCH₃ or C₆H₄O₃, and R11 is H, OH or CH₃O in, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C₆H₄O₃ then R11 is H;

[0048] wherein R5 is OH or OCH₃; R6 is H or OH, R9 is H or OH, R10 is H, OCH₃ or C₆H₄O₃, and R11 is H, OH or CH₃O in, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C₆H₄O₃ then R11 is H;

[0049] \( -\text{(CH₂)}_n\text{—COOR or —(CH₂)}_n\text{—CONHR, with n being an integer from 0 to 2;} \)

[0050] \( -(\text{CR₁₂—CH})\text{—COOR or -(CR₁₂—CH)}_n\text{—CONHR, R12 being H or a C})_n\text{—linear, branched or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferentially methyl or phenyl;} \)

[0051] \( -\text{(CH₂)}_n\text{—OR or —(CH₂)}_n\text{—NHR with n being an integer from 0 to 2;} \)

[0052] \( -\text{(CH₂)}_n\text{—COR or —(CH—CH)}_n\text{—COR with n being an integer from 0 to 2;} \)

[0053] H;
In a first embodiment, R1 of the phenolic compound is

In a second embodiment, R1 of the phenolic compound is

In a third embodiment, R1 of the phenolic compound is

preferably a hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi- or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heterotatom;

wherein R is H or a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C1-C10 hydrocarbon group, optionally interrupted by at least one heterotatom, wherein said hydrocarbon group comprises an alkyl, an alkeny1, or an alkenyl, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C2-C6)aryl, an (C2-C6)heterocycle, an (C2-C6)alkoxy, an (C2-C6)acyl, an (C1-C6)alkanol, a carboxylic group (—COOH), an (C2-C8)ester, an (C1-C8)amine, an amino group (—NH2), an amide (—CONH2), an (C1-C6)imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), an aldehyde, an (C1-C6)halogenoalkyl, a thiol (—SH), a (C1-C6)thioalkyl, a (C1-C6)sulfoxide, and a combination thereof.

preferably the buffered water at a pH convenient for the enzymatic activity used either without cosolvent or in a mixture with a cosolvent consisting of sodium or potassium acetate buffer at a concentration ranging from 20 to 500 mM in water but any other buffering substance without any negative effect on the enzymatic activity can be used. Preferably, the buffered water at a pH convenient for the enzymatic activity-cosolvent mixture consists in mixture of water, preferably a buffered water as previously described, and dimethyl sulfoxide (DMSO) with a ratio of less than 35% of DMSO (volume/volume), preferably between 15-25%, more preferably about 15%.

wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH. In particular, the phenolic compound can be selected from the group consisting of the taxifolin, the eriodictyol, the dihydorobinetin and the fustin.

wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH. In particular, the phenolic compound can be selected from the group consisting of the taxifolin, the eriodictyol, the dihydorobinetin and the fustin.

wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH. In particular, the phenolic compound can be selected from the group consisting of the catechin, the epicatechin, the catechin gallate, the epicatechin gallate, the galloallocatechin, the epigalloallocatechin, the galloallocatechin gallate and the epigalloallocatechin gallate.

wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH. In particular, the phenolic compound can be selected from the group consisting of the catechin, the epicatechin, the catechin gallate, the epicatechin gallate, the galloallocatechin, the epigalloallocatechin, the galloallocatechin gallate and the epigalloallocatechin gallate.

wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3, or C3H7O2, and R11 is H, OH or C3H7O2, with the proviso that R10 and R11 cannot be both H when R5 and R6 are both OH and that when R10 is C3H7O2 then R11 is H. In particular, the phenolic compound can be selected from the group consisting of the rhamnetin, the fisetin, the robinetin, the gossypetin, the orientin, the homoeriodictyol and the cirsilloid.

In a fourth embodiment, R1 of the phenolic compound is —(CH2)n—COOR or —(CH2)n—CONH2 with n being an integer from 0 to 2. In particular, the phenolic compound can be selected from the group consisting of the homoproteocatechuic acid, the dihydrocaffeic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitannin (2,5-di-O-galloyl-hamamelose) and the protocatechuic acid.

In a fifth embodiment, R1 of the phenolic compound is —(CR12—CH)—COOR or —(CR12—CH)—CONHR, R12 being H or a C1-C6 linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl. In
particular, the phenolic compound can be selected from the group consisting of the caffeic acid, the rosmarinic acid, the esculetin, the 4-methylesculetin, the nordalbergin (6,7-dihydroxyphenylcoumarin), the chlorogenic acid, the caffeic acid phenethyl ester, the chicoric acid (dicaffeyl tartaric acid), the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1→3)-O-(beta-D-glucopyranosyl-(1→6))-4-(3-(3,4-dihydroxyphenyl)-2-propenoate), beta-D-glucopyranoside) and the verbascoside.

In a sixth embodiment, R1 of the phenolic compound is \(-\text{CH}_2\)_n-OR or \(-\text{CH}=-\text{CH}\)_n-NHR with n being an integer from 0 to 2, for instance the phenolic compound is the hydroxytyrosol.

In a seventh embodiment, R1 of the phenolic compound is \(-\text{CH}_2\)_n-COR or \(-\text{CH}=-\text{CH}\)_n-COR with n being an integer from 0 to 2. In particular, the phenolic compound can be selected from the group consisting of the maclurine, the 3,4-dihydroxybenzaldehyde, the 3,4-dihydroxybenzophenone, the butein (2',3,4,4'-tetrahydroxychalcone), the 3,4-dihydroxyacetophenone, the marein (2',3',4,4'-pentahydroxy-4'-glucosylchalcone), and the eriodictyolchalcone (2',4',6',3,4-pentahydroxychalcone).

In an eighth embodiment, R1 of the phenolic compound is selected from the group consisting of

\[
\begin{align*}
\text{(I)} & \quad \text{HO} \quad \text{HO} \quad \text{HO} \\
\text{(II)} & \quad \text{R1} \quad \text{R2} \quad \text{OA} \quad \text{OB}
\end{align*}
\]

Preferably, the phenolic compound can be selected from the group consisting of the anthrarobin and the salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline).

The present invention also concerns the phenolic compounds O-\(\alpha\)-glucosides obtainable by the method of the invention. Consequently, the present invention concerns a phenolic compound O-\(\alpha\)-glucoside having the following formula:

\[
\begin{align*}
\text{(II)} & \quad \text{R1} \quad \text{R2} \quad \text{OA} \quad \text{OB}
\end{align*}
\]

Wherein

A and B, identical or different, are H or a -\(\alpha\)-glucosyl residue, with the proviso that at least one of A and B is a -\(\alpha\)-glucosyl residue;
R2 is H or OH; and,
R1 is selected from the group consisting of

![Diagram 1]

wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH; and,

![Diagram 2]

wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH, and at least one among R7 and R8 is OH;

wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H11O2, and R11 is H, OH or C6H11O2, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H11O2, then R11 is H;

![Diagram 3]

—(CH2)n—COOR or —(CH2)n—CONHR, with n being an integer from 0 to 2;

—(CR12—CH)—COOR or —(CR12—CH)—CONHR, R12 being H or a C1-C6 linear, branched or cyclic alkyl or alkylen, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl;

![Diagram 4]

—(CH2)n—OR or —(CH2)n—NHR with n being an integer from 0 to 2;

—(CH2)n—COR or —(CH═CH)n—COR with n being an integer from 0 to 2;

H;

![Diagram 5]

a C1-C10 hydrocarbon group which forms with the represented ring of formula (1) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom;

wherein R is H or a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C1-C10 hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkynyl, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C2-C8)aryl, an (C2-C8)heterocycle, an(C1-C3)alkoxy, an(C5-C10)acyl, an(C1-C6)alcohol, a carboxylic group (—COOH), an(C6-C10)ester, an(C1-C6) amine, an amino group (—NH2), an amide (—CONH), an(C1-C6)imine, a nitro, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an(C1-C6)halogenoalkyl, a thiol (—SH), a(C6-C10)thioalkyl, a(C1-C6)sulfone, a(C1-C6)sulfide, and a combination thereof.

A first preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Diagram 6]

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the taxifolin O-α-glucoside, the eriodictyol O-α-glucoside, the dihydrorobinetin O-α-glucoside and the fustin O-α-glucoside.
A second preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the catechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, the galloカテchin O-α-glucoside, the epigalloカテchin O-α-glucoside, the gallocatechin gallate O-α-glucoside and the epigallocatechin gallate O-α-glucoside.

A third preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the rhamnetin O-α-glucoside, the fisetin O-α-glucoside, the robinetin O-α-glucoside, the gossypetin O-α-glucoside, the orientin O-α-glucoside, the homoorientin O-α-glucoside and the cirsilol O-α-glucoside.

A fourth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is —(CH₂)ₙ—COR or —(CH₂)ₙ—CONHR with n being an integer from 0 to 2, and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the homoprotocatechuic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the propyl gallate O-α-glucoside, the gallic acid O-α-glucoside, the hamamelitin O-α-glucoside and the protocatechuic acid O-α-glucoside.

In a fifth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is —(CR₁₂—CH)—COOR or —(CR₁₂—CH)—CONHR, R₁₂ being H or a C₁₋₃ linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the caffeic acid O-α-glucoside, the rosmarinic acid O-α-glucoside, the esculetin O-α-glucoside, the 4-methylesculetin O-α-glucoside, the nordalbergin (6,7-dihydroxyphe-nyloumarin) O-α-glucoside, the chlorogenic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the chiconic acid (diacetyl tartaric acid) O-α-glucoside, the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1→6))-4-(3-(3,4-dihydroxyphenyl)-2-propenoate) O-α-glucoside, beta-D-glucopyranoside O-α-glucoside and the verbascoside O-α-glucoside.

The phenolic compound O-α-glucoside is preferably selected from the group consisting of the pyrocatheol O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the 3-hydroxydaidzein O-α-glucoside, the oleanorin O-α-glucoside, and maritamine (3',4',6,7-tetrahydroxy-6-O-glucosylaurone) O-α-glucoside.
In a ninth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom. Preferably the phenolic compound O-α-glucoside is selected in the group consisting of the anthorarin O-α-glucoside and the sabololin (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydrodiosquinoline) O-α-glucoside.

In a preferred embodiment, the phenolic compound O-α-glucoside of the present invention has a α-glucosyl residue which is a glucose monomer, dimer, trimer or tetramer, preferably a monoglucoside.

Preferably, the phenolic compound O-α-glucosides of the present invention have a 20 folds higher solubility than the corresponding aglycone in the same physiological conditions.

The phenolic compound O-α-glucosides of the present invention can be cleaved by an enzyme to release the corresponding aglycone. Said enzyme is an O-α-glucosidase. Preferably, said enzyme is issued from human associated micro-organism, in particular human micro-organism associated to skin, mouth, intestinal tract, upper respiratory system or female genital tract, even more preferably skin associated micro-organisms.

The present invention further concerns phenolic compound O-α-glucosides of the present invention as medicament.

The present invention also concerns a pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside of the present invention.

The present invention also concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered topically, orally, rectally, nasally, or vaginally, wherein enzymes issued from micro-organisms associated to skin, mouth, intestinal tract, upper respiratory system or female genital tract release the corresponding aglycone.

The present invention also concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1**—Flavonoids: basic structure and numbering of carbon atoms.
**FIG. 2**—Proocatechic acid.
**FIG. 3**—Caffeic acid (3,4-dihydroxycinnamic acid).
**FIG. 4**—3,4-dihydroxymandelic acid.
**FIG. 5**—Esculetin (6,7-dihydroxycoumarin).
**FIG. 6**—Taxifolin.
**FIG. 7**—Glucoside of caffeic acid derivatives.
**FIG. 8**—Glucoside of 3,4 dihydroxybenzoic acid and other phenic acids.
**FIG. 9**—Glucoside of flavanol.
**FIG. 10**—Glucoside of flavonol, isoflavone, flavone and dihydroflavonol.
**FIG. 11**—Glucoside of neutral polyphenol.
**FIG. 12**—HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm). Incubation duration: 0 hours.
**FIG. 13**—HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm). Incubation duration: 22 hours.
**FIG. 14**—Mass spectrum of the substance eluted at around 8.13 minutes. Incubation duration: 22 hours.
**FIG. 15**—UV spectrum of the substance eluted at around 8.13 minutes. Incubation duration: 22 hours.
**FIG. 16**—Mass spectrum of the substance eluted at around 6.15 minutes. Incubation duration: 22 hours.
**FIG. 17**—UV spectrum of the substance eluted at around 6.15 minutes. Incubation duration: 22 hours.
**FIG. 18**—HPLC chromatogram of an aqueous solution of taxifolin and taxifolin glucoside (289 nm). (elution with an initial methanol concentration of 10%—method 1).
**FIG. 19**—HPLC chromatogram of a highly purified Taxifolin glucoside preparation (289 nm; method 2).
**FIG. 20**—Microbial growth in the presence or absence of Taxifolin glucoside (human skin micro-flora).
**FIG. 21**—Evolution of Taxifolin glucoside and Taxifolin concentrations in the presence of human skin micro-flora.

**DETAILED DESCRIPTION OF THE INVENTION**

Definitions

Phenolic compound or Phenolics: compound that possess an aromatic ring bearing one or more hydroxyl substituents.

Flavonoids: polyphenolic compounds possessing 15 carbon atoms, two benzene rings joined by a linear three carbon chain giving a system C6-C3-C6. The first benzene ring (ring A) forms with an oxygen atom and the three carbon atoms joining the two benzene rings a chromene skeleton (rings A and C). The chromene skeleton bears the second aromatic ring B in position 2, 3 or 4. In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring. Both the oxidation state of the heterocyclic C ring and the position of ring B are important in the classification of flavonoids.

Anthocyanins: ring C is a pyran which participates in a 3-hydroxychromene skeleton substituted in 2.

Catechin substances (flavanols): ring C is a hydroxylated tetrahydrophenyl which participates in a 3-hydroxy or a 3,4-dihydroxychromene skeleton substituted in 2 (catechin, epicatechin, galloceleatin and epigallocatechin forming the condensible tannins).

Flavones: ring C is a pyrone substituted in 2.

Flavonols: ring C is a pyrone hydroxylated in 3 and substituted in 2.

Flavanones: ring C is a dihydropyronone substituted in 2.

Dihydroflavonols: ring C is a dihydropyronone hydroxylated in 3 and substituted in 2.

Isoflavones: flavones with the substitution in 3 instead of 2.

Chalcones and dihydrochalcones: ring C is open and with a C2C3 double bond (chalcones) or not (dihydrochalcones).

Aurones: ring C is a five-membered ring.

Enzyme: protein molecule that catalyses chemical reactions on molecules (named substrates) to obtain other
molecules (named products). A recommended name, a systematic name which stresses the type of reaction and an Enzyme Commission (EC) code number are assigned to each enzyme. These code numbers, prefixed by EC, contain four elements separated by points. The first number shows to which of the six main divisions (classes) the enzyme belongs: oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6). The second number indicates the subclass, the third the sub-subclass and the fourth is the serial number of the enzyme in its sub-subclass.

0141 Bioavailability: the degree to which or rate at which a molecule or other substance is absorbed or becomes available at the site of physiological activity after administration or application.

0142 Glucosucrases: common name of glucosyltrans-
erases with the EC number 2.4.1.5 (see hereafter; KRALI S, VANG GEEL-SCUTTEN G H, DONDORFF M M G, KIR-

0143 Glycosyltransferase: enzyme that catalyzes the transfer of glycosyl group(s) from one compound (said donor) to another (said acceptor). Glycosyltransferase are classified as transferases, with the EC number EC 2.4. Trans-
ferases that transfer hexos (carbohydrate molecules that have six carbon atoms per molecule) are included in the sub-subclass EC 2.4.1. Transferases that transfer the glucose moiety of sucrose to an acceptor are EC 2.4.1.4 (sucrose: 1,4-α-D-glucan 4-α-D-glucosyltransferase; recommended name: amylosucrase). EC 2.4.1.5 (sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferase; recommended name: dextran-
sucrase). EC 2.4.1.7 (sorbose: orthophosphate α-D-glucosyltransferase; recommended name: sucrose phosphorylase).

0144 Glycone: chemical part of a glycosidic derivative which belongs to the carbohydrate family. If the glycone group is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronic acid.

0145 Glycosidic bond: chemical linkage between a gly-
cone and another glycone or a glycone. Depending on whether the glycosidic bond lies “below” or “above” the plane of the cyclic carbohydrate molecule when considering the HAWORTH projection, glycosides are classified as α-glycosides or β-glycosides.

0146 Aglycone: Chemical part of a glycosidic derivative which is not the glycone one.

0147 Where “comprising” is used, this can preferably be replaced by “consisting essentially of”, more preferably by “consisting of”.

0148 In the context of the present invention, the term “alkyl” more specifically means a group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl and the other iso-
meric forms thereof. (C1-C8)alkyl more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the other isomeric forms thereof. (C9-C18)alkyl more specifically means alkyl, ethyl, propyl, or iso-
propyl.

0149 The term “alkenyl” refers to an alkyl group defined hereinabove having at least one unsaturated ethylene bond and the term “alkynyl” refers to an alkyl group defined hereinabove having at least one unsaturated acetylene bond. (C2-
C6)alkenyl includes a ethenyl, a propenyl (1-propenyl or 2-propenyl).

0150 The “aryl” groups are mono-, bi- or tri-cyclic aro-
matic hydrocarbons having from 5 to 9 carbon atoms. Examples include a phenyl, in particular.

0151 “Heterocycle” groups are groups containing 1 to 3 rings comprising one or more heteroatoms, preferably 1 to 5 endocyclic heteroatoms. They may be mono-, bi- or tri-cyclic. They may be aromatic or not. Examples of aromatic hetero-
cycles include pyridine, pyridazine, pyrimidine, pyrazine, furan, thiophene, pyrrole, oxazole, thiazole, isothiazole, imi-
dazole, pyrazole, oxadiazole, triazole, thiadiazole and triazine groups. Examples of bicycles include in particular quinoline, isoquinoline and quinazoline groups (for two 6-membered rings) and indole, benzimidazole, benzoazol, benzothiazole and indazole (for a 6-membered ring and a 5-membered ring). Nonaromatic heterocycles comprise in particular piperazine, piperidine, etc.

0152 (C1-C8)alkoxy includes methoxy, ethoxy, propoxy-
xy, and isopropoxy.

0153 (C2-C8)acyl includes acetyl, propionyl, and isoprop-
ollyl.

0154 (C1-C8)alcohol includes methanol, ethanol, pro-
panol and isopropanol.

0155 (C2-C8)ester includes methylester and ethylester.

0156 (C1-C8)amine includes methylamine, ethylamine, and propylamine.

0157 (C1-C8)imine includes methylimine, ethylimine, and propylimine.

0158 The halogen can be Cl, Br, I, or F.

0159 (C1-C8)halogenoalkyl includes halogenomethyl, halogenoethyly, and halogenopropyl.

0160 (C1-C8)thioalkyl includes thiomethyl, thioethyl, and thiopropyl.

0161 (C1-C8)sulfone includes methylsulfone, ethylsul-
fone and propylsulfone.

0162 (C1-C8)sulfoxide includes methylsulfoxide, ethyl-
sulfoxide, propylsulfoxide and isopropylsulfoxide.

0163 “Heteroatom” denotes N, S, or O.

0164 This invention also relates to a process for preparing O-α-glucosides of phenolic compounds containing a cate-
chol structure and for instance selected among protocate-
chue acid and its esters derivatives, caffeic acid and its esters derivatives, especially rosmarinic acid, chlorogenic acid and caffeic acid phenethyl ester and hydrocaffeic acid or 3,4-dihydroxyhydrocinnamic acid, 3,4-dihydroxyphenylgly-
col, esculetin, taxifolin, fistin, eriodictyol, fisetin and rham-
netin. In particular, the phenolic compounds containing a catechol structure can be selected from the group consisting of the epicatechin gallate, the eriodictyol, the esculetin, the epicatechin, the fisetin, the fistin, the homoprotocatechue acid, the protocatechuic acid, the protocatechuic acid ethyl ester, the hydroxytyrosol, the maclurine, the nordih-
ydroguaiaretic acid, the oleuropein, the pyrocatechol, the rham-
netin, the rosmarinic acid, the taxifolin, the 3-hydroxychloro-
lupine, the 3,4-dihydroxybenzophonene, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the cate-
chin, the cistoiliol, the chlorogenic acid, the gossypetin, the orientin, the homoorientin, the 3,4-dihydroxybenzaldelyde, the buten, the 3,4-dihydroxyacetophenone, the marein, the
maritmein, the eriodictyolchalcone, the 4-methylesculetin, the
nordalbergin, the salsoinol, the choric acid, the echinacose,
the verbascoside, the anthrarinobin, the epigallocatech
echin, the dihydrorobinatin, the gallicatechin, the gallic acid,
the propyl gallate, the epigallocatechin gallate, the hamamelitin
and the robinein. The process for preparing O-α-
glucosides of phenolic compounds containing a catechol
structure can also be performed with cirsilol, 3',4',7-trihy-
droxyflavone and 3'-hydroxydaidzein (flavones and isofla
vone).

For this purpose, an enzymatic reaction is achieved
using sucrose, an abundant and rather cheap substance used in
the food and feed fields. This reaction consists in the transfer
of the glucose part of sucrose on an hydroxyl group of the
catechol ring by a glycosyltransferase (EC 2.4.1) or, once a
first glucosyl residue has been attached to an hydroxyl group
of the catechol ring, the transfer of the glucose part of sucrose
to an hydroxyl group of the fixed glucose, the position of this
hydroxyl group depending on the enzyme specificity. As each
phenolic compound cited above bears two hydroxyl groups
on said ring, two derivatives can be obtained by this enzy-
matic reaction. When a population of glycoside derivatives
results from the synthesis reaction (by population, it is under-
stood the compounds for which the catechol ring has one of its
hydroxyl group substituted or both of its hydroxyl group
substituted by one glucosyl residue or an oligosaccharide),
the entire population is said the product and corresponds to
the invention.

The present invention concerns a method for pro-
ducing a phenolic compound O-α-glucoside comprising
incubating sucrose and a glucansucrase from Leuconostoc
species, preferably from Leuconostoc mesenteroides NRRL
B-512F, in buffered water at pH convenient for the enzymatic
activity (well known by a skilled man) or in a buffered water
at pH convenient for the enzymatic activity-cosolvent mix-
ture with a phenolic compound having the following formula:

R1
\[\text{OH OH} \]
R2
\[\text{O R4} \]

R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH; and,

R7
\[\text{R8} \]

R7 is selected from the group consisting of

H, —OH or —OCOR and R8 is H or OH, with the proviso that

at least one among R7 and R8 represents OH;

R5
\[\text{R6} \]

R5 is OH or OCH3, R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H11O2, and R11 is H, OH or C6H11O2, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H11O2 then R11 is H;

R2
\[\text{R3} \]

—(CH2)n—COOR or —(CH2)n—CONHR, with n being an integer from 0 to 2;

R1
\[\text{R4} \]

—CR12—COOR or —CR12—CONHR, R12 being H or a C1—C4 linear, branched or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl;

R2
\[\text{R3} \]

—(CH3)n—OR or —(CH3)n—NHR with n being an integer from 0 to 2;

R4
\[\text{R5} \]

—(CH2)n—COR or —(CH2)n—COR with n being an integer from 0 to 2;

—H;
a C₁₋₃ hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (bi or tricyclic) together with the orthocarbon of R₁;

wherein R is H or a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C₁₋₃ hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkynyln, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C₁₋₃)aryl, an (C₆₋₃)heterocycle, an (C₅₋₃)alkoxy, an (C₃₋₃)alkyl, an (C₃₋₃)alcohol, a carboxylic group (—COOH), an (C₂₋₃)ester, an (C₃₋₃)amine, an amino group (—NH₂), an amide (—CONH₂), an (C₃₋₃)imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an (C₁₋₃)halogenoalkyl, a thiol (—SH), a (C₁₋₃)thioalkyl, a (C₃₋₃)sulfone, a (C₂₋₃)sulfoxide, and a combination thereof.

In a first embodiment, R₂ is H. In this embodiment, the phenolic compound can be for example the epigallocatechin gallate, the eriodictyol, the esculetin, the epicatechin, the fisetin, the fustin, the homoprotocatechuic acid, the protocatechuic acid, the protocatechuic acid ethyl ester, the hydroxytyrosol, the machurine, the nordihydroguaiaretic acid, the oleuropein, the pyrocatechol, the rhamnetin, the rosmarinic acid, the taxifolin, the 3-hydroxyflavone, the 3,4-dihydroxybenzophenone, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the catechin, the cirsiliol, the chlorogenic acid, the gossypetin, the orientin, the homoorientin, the 3,4-dihydroxybenzaldehyde, the butein, the 3,4-dihydroxycatechol, the marein, the maritimesin, the eriodictyol, the 4-methylesculetin, the nordalbergin, the salolsinol, the chicomeric acid, the echinacoside, the verbascoside and the anthrarobin.

In an alternative embodiment, R₂ is OH. In this embodiment, the phenolic compound can be for example the epigallocatechin, the dihydrodibobinin, the gallatechin, the gallic acid, the propyl gallate, the epigallocatechin gallate, the hamamelitannin and the robinetin.

In a particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein
R₂ is H or OH; and
R₁ is

wherein R₃ and R₄, independently, are H or OH, with the proviso that at least one among R₃ and R₄ represents OH.

In a preferred embodiment, R₃ and R₄ are OH. In another preferred embodiment, R₃ is H and R₄ is OH. In a further preferred embodiment, R₃ is OH and R₄ is H. In a particularly preferred embodiment, R₂ is H and R₃/R₄ are selected in the following combinations: OH/OH; H/OH; OH/H. In another preferred embodiment, R₂ is OH and R₃/R₄ are selected in the following combinations: OH/OH; H/OH; OH/H. Preferably, the phenolic compound is selected from the group consisting of the taxifolin, the eriodictyol, the dihydrodibobinin and the fustin.

In another particular embodiment of the method according to the present invention, the phenolic compound has the following formula:
wherein R2 is H or OH; and 

R1 is

wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is Hor OH, with the proviso that at least one among R7 and R8 represents OH. In a preferred embodiment, R8 is OH and R7 is OH or OCOR. In a more preferred embodiment, R7 and R8 are both OH. In another preferred embodiment, R7 is —OCOR and R8 is OH. In a particular preferred embodiment, R2 is H and R3/R4 are selected in the following combinations: H/OH, OH/H, OH/OH and OCOR/OH. In another particular preferred embodiment, R2 is OH and R3/R4 are selected in the following combinations: H/OH, OH/H, OH/OH and OCOR/OH. More preferably, R is

Preferably, the phenolic compound is selected from the group consisting of the catechin, the epicatechin, the catechin gallate, the epicatechin gallate, the epigallocatechin, the gallocatechin gallate and the epigallocatechin gallate.

In a further particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein R5 is OH or OCH3; R6 is H or OH; R9 is H or OH; R10 is H, OCH3 or C6H5O2, and R11 is H, OH or C6H5O2, with the proviso that R10 and R11 cannot be both H when R5 and R6 are both OH and that when R10 is C6H5O2, then R11 is H. In this embodiment, R6, R5 and R11 can be selected from the following combinations:

a) R6 is OH and R5 is OCH3 and R11 is OH;

b) R6 is OH and R5 is OH and R11 is OH;

c) R6 is OH and R5 is OH and R11 is C6H5O2; and,

d) R6 is H and R5 is OH and R11 is H; and

R9 is H or OH, and R10 is H or OCH3 or C6H5O2, with the proviso that when R10 is C6H5O2, then R11 is H.

In a preferred embodiment, R9 is OH, R10 is H and R11 is H, whereas R6 is OH and R5 is OCH3, or R6 is H and R5 is OH. Preferably, R2 is H. Alternatively, R2 is OH.

In another preferred embodiment, R6 is H and R10 is OCH3 or C6H5O2. In a particular aspect of this embodiment, R9 and R11 are H, R10 and R5 are OCH3, and R6 is OH.

In an additional preferred embodiment, R5 and R6 are both OH, R9 is H or OH, R10 is OH or C6H5O2, and R11 is H, OH or C6H5O2, with the proviso that when R10 is C6H5O2, then R11 is H. In another preferred embodiment, R6 and R5 are both OH, R9 is H or OH, R10 is H, and R11 is OH or C6H5O2.

In another preferred embodiment, R9 is H and R10 is H. In a further preferred embodiment, R9 is H, R10 and R5 are OCH3, and R6 is OH.

In a particular embodiment, R2, R5, R6, R9, R10 and R11 can be selected from the above mentioned combinations.

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<tr>
<th>R2</th>
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<th>R10</th>
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</table>
[0210] Preferably, the phenolic compound is selected from the group consisting of the rhamnetin, the fisetin, the robinetin, the gossypetin, the orientin, the homoorientin and the cirsiol.

[0211] In a further particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

\[
(\text{I}) \quad R_1 \quad R_2 \quad \text{OH} \quad \text{OH}
\]

wherein

[0212] wherein

[0213] \( R_2 \) is \( H \) or \( \text{OH} \); and \( R_1 \) is \(-(\text{CH}_2)_n-\text{COOR}\) or \(-(\text{CH}_2)_n-\text{CONHR}\) with \( n \) being an integer from 0 to 2. In a preferred embodiment, \( R_2 \) is \( H \). Alternatively, \( R_2 \) is \( \text{OH} \). Preferably, \( R \) is selected from the group consisting of \( H \), a \( \text{C}_1-\text{C}_4 \) alkyl, preferably methyl, ethyl or propyl, and

[0214] In a first more preferred embodiment, \( n \) is 0 and, \( R \) is preferably \( H \). In a second more preferred embodiment \( n \) is 1 and, \( R \) is preferably \( H \). In a third more preferred embodiment, \( n \) is 2 and \( R \) is preferably \( H \). In another preferred embodiment, \( n \) is 0 and \( R \) is a \( \text{C}_1-\text{C}_3 \) alkyl, preferably methyl, ethyl or propyl, or

[0215] In a preferred embodiment, \( R_1 \) is \(-(\text{CH}_2)_n-\text{COOR}\). In a preferred embodiment, \( R \) is \( H \).

[0216] Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid, the dihydroflavonic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitannin (2',5-di-O-galloyl-hamamelose) and the protocatechic acid.

[0217] In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

\[
(\text{I})
\]

wherein

[0218] wherein

[0219] \( R_2 \) is \( H \) or \( \text{OH} \); and \( R_1 \) is \(-(\text{CR}_1\text{R}_2=\text{CH})-\text{COOR}\) or \(-(\text{CR}_1\text{R}_2=\text{CH})-\text{CONHR}\), \( R_2 \) being \( H \) or a \( \text{C}_1-\text{C}_4 \) linear or cyclic alkyl or alkenny, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl. Preferably \( R_1 \) is \(-(\text{CH}=\text{CH})-\text{COOR}\) or \(-(\text{CH}=\text{CH})-\text{CONHR}\). In a preferred embodiment, \( R_2 \) is \( H \). Alternatively, \( R_2 \) is \( \text{OH} \). In a preferred embodiment, \( R_1 \) is \(-(\text{CH}=\text{CH})-\text{COOR}\). In a preferred embodiment, \( R \) is selected in the group consisting of \( H \);

[0220] \( \text{OH} \); and \( \text{CONHR} \);

[0221] \( \text{OH} \);
a bond attached to the phenyl group of formula (I) at the carbon in ortho of R1.

When R is a bond attached to the phenyl group of formula (I) at the carbon in ortho of R1, R12 can be in particular selected from the group consisting of H; methyl and phenyl. Then, the phenolic compound can have the following formula:

R12 being H or a C1-C6 linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl.

 Preferably, the phenolic compound is selected from the group consisting of the caffeic acid, the rosmarinic acid, the esculetin, the 4-methylesculetin, the nordalbergin (6,7-dihydroxyphenyleoumarin), the chlorogenic acid, the caffeic acid phenethyl ester, the chicoric acid (dicafeyle tartaric acid), the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1→3)—O-(beta-D-glucopyranosyl-(1→6))- 4-(3,4-dihydroxyphenyl)-2-propenoate), beta-D-glucopyranoside) and the verbascoside.

In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein

R2 is H or OH; and R1 is —(CH2)n—OR with n being an integer from 0 to 2. In a preferred embodiment n is 2. Preferably, the phenolic compound is the hydroxytyrosol.

In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein

R2 is H or OH; and R1 is —(CH2)n—COR or —(CH=CH)n—COR, with n being an integer from 0 to 2.

In a preferred embodiment, n is 0 or 1 and R is selected in the group consisting of

H; a C1-C3 alkyl, preferably methyl, ethyl or propyl, more preferably a methyl.
Preferably, \( n \) is 0. Alternatively, \( n \) is 1.

Preferably, the phenolic compound is selected from the group consisting of the maclurine, the 3,4-dihydroxybenzaldehyde, the 3,4-dihydroxybenzophenone, the butein (2',3, 4,4'-tetrahydroxychalcone), the 3,4-dihydroxyacetophenone, the marcin (2',3,3',4,4'-pentahydroxy-4'-glucosylchalcone), and the eriodictyol (2',4',6',3,4-pentahydroxychalcone).

In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

\[
\begin{align*}
R_1 & \quad \text{and} \\
R_2 & \quad \text{H or OH; and} \\
R_1 & \quad \text{is selected from the group consisting of}
\end{align*}
\]

Preferably, the phenolic compound is selected from the group consisting of the pyrocatechol, the nordihydroguaiaretic acid, the 3-hydroxydaidzein, the oleanone, and the martine (3',4',6,7-tetrahydroxy-6-O-glucosylaurone).

In this embodiment, \( R_1 \) of the phenolic compound is a \( C_1-C_{10} \) hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (bi or tricyclic) together with the ortho carbon of \( R_1 \). In particular, the phenolic compound can be selected from the group consisting of

Preferably, said fused ring can be optionally interrupted by at least one heteroatom and can be substituted by one or several substituents selected from the group consisting of: an \( (C_1-C_2) \) alkoxy, an \( (C_2-C_3) \) acyl, an \( (C_1-C_3) \) alcohol, a carboxylic group \((-\text{COOH})\), an \( (C_2-C_4) \) ester, an \( (C_1-C_2) \) amine, an amino group \(-\text{NH}_2\), an amide \(-\text{CONH}_2\), an \( (C_1-C_3) \) imine, a nitrile, an hydroxyl \(-\text{OH}\), an aldehyde group \(-\text{CHO}\), an halogen, an \( (C_1-C_2) \) halogenoalkyl, a thiol \(-\text{SH}\), a \( (C_1-C_2) \) thiolalkyl, a \( (C_1-C_3) \) sulfone, a \( (C_1-C_5) \) sulfone, and a combination thereof. In a particular preferred embodiment, the phenolic compound is
Nature and Source of the Enzyme

The enzymes that can be used for this condensation reaction are glycosyltransferases, more preferably hexosyltransferases (EC 2.4.1.1), and in a preferred manner glucosucrases (EC 2.4.1.5).

In a preferred embodiment, the enzyme used for the desired condensation of these phenolic compounds with glucose is a glucosucrase from a bacterial species, more precisely from a Leuconostoc species, and more preferably from Leuconostoc mesenteroides NRRL B-512F.

Alternative sources of enzyme may be the glucosucrase(s) from Leuconostoc mesenteroides NRRL B-742, Leuconostoc mesenteroides NRRL B-1299, Leuconostoc mesenteroides NRRL B-1355, or Leuconostoc mesenteroides NRRL B-23192.

Such enzymes can be obtained by a natural fermentation of the producing strains, followed by cell treatments and enzyme recovery and purification. Since glucosucrases are mainly extracellular large enzymes in solution in the culture broth or cells associated, the techniques that can be used for the recovery of the enzyme include but are not limited to centrifugation and tangential microfiltration, and, if it is a cell associated enzyme, the techniques aiming at cell disruption include but are not limited to French press homogenization, glass beads, sonication or any equivalent method. The techniques aiming at enzyme concentration include but are not limited to ultrafiltration with a molecular weight cut off ranging from 10 kDa to 300 kDa, and the techniques that can used for enzyme purification include but are not limited to phase partition with polyethylene glycol, gel permeation chromatography. An alternative solution consists in the recombinant expression of said enzymes in well known expression hosts such as E. coli, S. cerevisiae, Baculovirus, Y. lipolytica, Bacillus sp., Pseudomonas sp., H. polymorpha or mammalian cells (see as one reference “Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems” Wiley 2004—Gerde Gielissen Ed.), optionally followed by a purification step(s) using well known methods from man of the art.

The enzyme can also be obtained through well known from the man of the art random mutagenesis, directed mutagenesis or directed evolution methods (MIYAZAKI K, ARNOLD F H, (2004). In vitro DNA recombination. In Phage Display: A practical approach. Clarkson T and Lowman H, editors. New York: Oxford University Press Inc., 43-60). These technologies could enable to obtain enzymes with higher specific activity, lower products inhibition, dedicated region, chemio and stereo selectivity, better stability, or any combination thereof.

The process of the invention can thus be carried out with either whole cells, or with natural or recombinant crude or purified enzyme. The enzyme can be used under its “free” form, or as an immobilized catalyst. Such immobilisation procedures include but are not limited to gel encapsulation (calcium alginate,…), resin adsorption, glutaraldehyde reticulation, spray drying in the presence eventually of an adequate adjuvant to obtain an insoluble form of the enzyme, membrane reactors or any combination thereof, and are well known from the man of the art. The choice of one immobilisation approach relies on its economical cost and on the final yield of the process involving said immobilized enzyme.

The amount of enzymatic activity of an enzyme preparation can be estimated using the hydrolysis of sucrose and the measurement of the released reducing sugar (fructose) by means of colorimetric methods (such as the one involving 3,5-dinitro-salicylic acid; SUMNER J B, HOWELL R F (1935) A method for determination of invertase activity. J Biol Chem 108: 51-4). This enzymatic activity is expressed in units, wherein one unit (U) corresponds to the amount of enzyme that releases 1 umole of fructose per minute at 30°C, pH 5.2 (sucrose: 100 g/L; sodium acetate buffer: 50 mM; calcium chloride dihydrate: 10 mg/L).

Reaction Conditions

The reaction can be carried out in buffered water or in buffered water/cosolvent(s) mixture. Indeed, the inventors surprisingly observed that the enzyme is able to glycosylate in absence of cosolvents.

Preferably, the buffered water at a pH convenient for the enzymatic activity used either without cosolvent or in a mixture with a cosolvent consists of sodium or potassium acetate buffer at a concentration ranging from 20 to 500 mM in water but any other buffering substance without any negative effect on the enzymatic activity can be used. Preferably, the buffered water at a pH convenient for the enzymatic activity-cosolvent mixture consists in mixture of water, preferably a buffered water as previously described, and dimethyl sulfoxide (DMSO) with a ratio of less than 35% of DMSO (volume/volume), preferably between 15-25%, more preferably about 15%.

The reaction can be achieved in a water/cosolvent(s) mixture that enables both a proper activity of the enzyme and a good level of solubility of the phenolic compounds and of the glucose donor, i.e. sucrose. Such cosolvents can be the following water-miscible organic solvents dimethyl sulfoxide, dioxane, dimethyl formamide, ethanol, n-propanol, iso-propanol, ethylene glycol, glycerol, 1,2-propandiol, sulfolane, tetramethylurea, ethyl-lactate, diethyl ether of diethyleneglycol, dimethyl ether of triethyleneglycol used at different weight/volume ratio. In addition to these simple organic solvents, ionic liquids (imidazolium, pyridinium, phosphonium and ammonium salts) can also be envisaged. The cosolvents can also be the following water-immiscible organic solvents ethyl acetate, methyl ethyl ketone, methyl-2 butanole and a combination of water-miscible organic solvents with water-immiscible organic solvents.

In a preferred embodiment, the mixture is made of water and dimethyl sulfoxide (DMSO), with DMSO concentrations ranging from 5 to 70% (volume/volume). In a preferred embodiment, DMSO concentrations are between 5 and 50% (volume/volume). In a most preferred embodiment, DMSO concentration is between 10 to 35% (volume/volume). Indeed, the inventors surprisingly found that the reaction is highly more efficient when proceeded at a ratio of DMSO lower than 40%. The higher rate of product has been registered for a ratio of 15%. Therefore, a preferred ratio of
the method according to the present invention is comprised between 15-25%, preferably about 15% (±3%).

[0251] Each phenolic compound is incubated in this reaction mixture with sucrose and the enzyme in pH and temperature conditions that allow the enzyme to be active and to synthesize the maximum possible of desired glucoside. Preferably, the reaction medium contains, in addition, calcium cations in the form of calcium chloride (or in the form of any water soluble salt of calcium) to improve the stability of the enzyme. The condensation reaction can be performed at a pH ranging from 4 to 8, and preferably from 5 to 7 by introducing a low amount of acetate buffer in the reaction medium. The temperature of the synthesis medium is maintained at a value ranging from 10 to 40 degrees Celsius, and preferably approximately 25 to 33 degrees Celsius.

[0252] Typical reaction conditions with the glucosurase from Leuconostoc mesenteroides NRRL B-512F consists of a mixture of acetate buffer at 10 mM to 100 mM, DMSO at 10 to 35% (volume/volume), sucrose at 100 mM to 900 mM and phenolic compound at 2 to 200 mM, calcium salts at 0.5 mg to 1 g/l and the enzyme for a final concentration of 0.5 to 5 U/ml. This reaction is incubated at 30° C. for several hours (e.g., 10 to 48 hours), and the synthesis of the phenolic compound is determined, as well as the disappearance of said phenolic compound upon time is followed by HPLC analysis. A better characterization of the products can be achieved by high performance liquid chromatography coupled with a photodiode array detector coupled with a mass spectrometer to directly estimate the number of glucose moieties attached to the phenolic compound, and thus have a good analytical characterization of the synthesized derivatives.

[0253] In one embodiment of the present invention, such conditions allowing the analytical characterization of the synthesized derivatives can be as follows:

[0254] The synthesis media can be analyzed by high performance liquid chromatography coupled with a photodiode array detector (PDA Waters® 996) and a mass spectrometer (Micromass ZQ 2000, Waters®).

[0255] i) Operating conditions for chromatography:

[0256] Column: KROMASIL C18 5µ, 250 mm x 4.6 mm (reference: K2185; A.T. Chromato; 117 rue de Stalingrad; 78800 Fontenay)

[0257] Elution (method 1):

[0258] solvent A: deionized water containing 1% v/v acetic acid

[0259] solvent B: HPLC grade methanol containing 1% v/v acetic acid

[0260] 0 to 10 minutes: 90% to 80% A (linear); 10% to 20% B (linear); 1 ml/minute

[0261] 10 to 25 minutes: 80% to 50% A (linear); 20% to 50% B (linear); 1 ml/minute

[0262] 25 to 30 minutes: 50% A; 50% B; 1 ml/minute

[0263] 30 to 35 minutes: 50% to 90% A (linear); 50% to 10% B (linear); 1 ml/minute

[0264] 45 minutes: next injection

[0265] Column temperature: 30° C.

[0266] Injection volume: 10 µL

[0267] ii) Photodiode array detector

[0268] Start wavelength: 210 nm

[0269] End wavelength: 400 nm

[0270] Resolution: 1.2 nm

[0271] Sampling rate: 1 spectra/second

[0272] iii) LC mass spectrometer (single quadrupole)

[0273] Ionisation: electrospray in negative mode

[0274] Spray voltage: 3.0 kV

[0275] Source temperature: 150° C.

[0276] Cone tension: 20 or 40 V

[0277] Extractor: 3.0 V

[0278] Desolvation temperature: 300° C.

[0279] Cone gas flow: 30 L/hour

[0280] Desolvation gas flow: 600 L/hour

[0281] Full scan mass spectra: m/z from 100 to 2000

[0282] Purification

[0283] After synthesis, each phenolic compound O-α-glucosides can be either used directly or purified to reach a desired purity in terms of residual of non transformed phenolic compound, sugars, enzyme and cosolvents.

[0284] For example, the phenolic compound O-α-glucosides can be adsorbed on a synthetic macroporous adsorbent resin by taking advantage of the difference of absorbing ability of substances. Due to the presence of residual substances in the intestinal volume, the resin with the adsorbed phenolic substances is washed with water in order to completely flush out the enzyme, the sugars and the polysaccharide and the co-solvent. Then the resin can undergo an elution step with an appropriate solvent to recover the synthesized product. The appropriate solvent is pure methanol, ethanol, n-propanol, 2-propanol, acetone or a mixture of them or a mixture of them with water with no more than 20% volume/volume water. The solution containing the synthesized product(s) can be concentrated by evaporation under vacuum at moderate temperature (not higher than 50° C.) or with compatible membrane equipments for further purification, or directly used for further purification. Further purification steps such as liquid/liquid extraction, preparative HPLC, or other rounds of resin purification can be used to attain the required level of purity for the final application. Organic solvent that can be used for liquid-liquid extraction are ethyl acetate, butyl acetate, methyl ethyl ketone, depending on the solubility difference of the phenolic compound and phenolic compound glucoside.

[0285] Finally, a syrup containing the desired substance(s) can be obtained by removing the solvent (water or organic solvent) by evaporation under vacuum at moderate temperature (not higher than 50° C.) or with compatible membrane equipments and concentrating the resulting solution to give a prescribed concentration. This syrup can be dried (freeze drying, spray drying or any other way of drying that will preserve the integrity of the molecules) to obtain a powder.

[0286] The synthetic macroporous adsorbent resin can be used either in a tank (a sieve with a convenient mesh depending on the resin granulometry will be used to recover the resin) or located in a column fed with a pump. By synthetic macroporous adsorbent resin, it is understood non ionic and porous synthetic resins which have relatively large surface area such as those containing styrene-divinyl benzene copolymer, phenol-formaldehyde polymers, acrylic polymer and methacrylic polymer. Examples of such resins are Amberlite of the XAD type (Rohn and Haas Company, USA), Diaion of the HP family (Mitsubishi Chemical Industries, Japan).

[0287] The invention relates to O-α-glucosides of phenolic compounds containing a catechol structure and for instance selected among protocatechuic acid and its esters derivatives, caffeic acid and its esters derivatives, especially rosmarinic acid, chlorogenic acid and caffeic acid phenethyl ester and hydrocaffeic acid or 3,4-dihydroxyhydrocinnamic acid, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol, esculetin, taxifolin, fisetin, eriodictyol, fisetin and rhamnetin. In particular, the invention relates to O-α-glucosides of phenolic compounds containing a catechol structure and selected from the group consisting of the epicatechin gallate, the eriodictyol, the esculetin, the fisetin O-α-glucoside, the fisetin, the homoprotocatechuic acid, the protocatechuic acid, the
protocatechuic acid ethyl ester, the hydroxytyrosol, the maclurine, the nordihydroguaiaretic acid, the oleuropein, the pyrocatechol, the rhamnetin, the rosmarinic acid, the taxifolin, the 3-hydroxydaidzein, the 3,4-dihydroxybenzophenone, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the cirsiliol, the chlorogenic acid coside, the anthrarobin, the epigallocatechin, the dihydrorobinetin, the gallicatechin, the gallic acid, the propyl gallate, and the robinetin. These new phenolic compounds derivatives have a better bioavailability through an improved solubility in water and/or on in situ release of the aglycons during their usage through their hydrolysis by human natural microbiotes, and more specifically of human skin micro-organisms, or by a selected α-glucosidase such as the α-glucosidase produced by the yeast Saccharomyces cerevisiae.

In particular, the present invention concerns a phenolic compound O-α-glucoside having the following formula:

![Chemical structure](image)

wherein

- A and B, identical or different, are H or an α-α-glucosyl residue, with the proviso that at least one of A and B is an α-α-glucosyl residue;
- R2 is H or OH; and,
- R1 is selected from the group consisting of

![Chemical structures](image)

wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH;

![Chemical structures](image)

wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH, and at least one among R7 and R8 is OH;
[0301] a C₇-C₁₀ hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R₁, said ring being optionally interrupted by at least one heterotom;

[0302] wherein R is H or a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C₁-C₁₀ hydrocarbon group, optionally interrupted by at least one heterotom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkylenyl, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C₅-C₉)aryl, an (C₅-C₉)heterocycle, an (C₅-C₉)alkoxy, an (C₅-C₉)acyl, an (C₅-C₉)alkanol, a carboxylic group (—COOH), an (C₂-C₅)ester, an (C₅-C₉)amine, an amino group (—NH₃), an amide (—CONH₂), an (C₁-C₅)imine, a nitrile, an hydroxyl (—OH), a thiol (—SH), or a hydroxy group (—CH₂OH), an halogen, an (C₁-C₅)halogenoalkyl, a thiol (—SH), or an hydroxy group (—CH₂OH), an halogen, an (C₁-C₅)halogenoalkyl, a thiol (—SH), or an hydroxy group (—CH₂OH), an halogen, an (C₁-C₅)halogenoalkyl, or a combination thereof.

[0303] In a first embodiment, R₂ is H. In this embodiment, the phenolic compound O-α-glucoside can be for example the epicatechin gallate O-α-glucoside, the eriodictyol O-α-glucoside, the esculetin O-α-glucoside, the fisetin O-α-glucoside, the fisetin O-α-glucoside, the homoprotocatechuic acid O-α-glucoside, the protocatechuic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the hydroxytyrosol O-α-glucoside, the maclurine O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the oleanne O-α-glucoside, the pyrocatechol O-α-glucoside, the rhamnetin O-α-glucoside, the rosmarinic acid O-α-glucoside, the taxifolin O-α-glucoside, the 3-hydroxydaidzein O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the caffeic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the cirsiliol O-α-glucoside, the chlorogenic acid O-α-glucoside and the anthrobin O-α-glucoside.

[0304] In an alternative embodiment, R₂ is OH. In this embodiment, the phenolic compound O-α-glucoside can be for example the epicatechin gallate O-α-glucoside, the dihydroretin O-α-glucoside, the dihydrotechin O-α-glucoside, the gallic acid O-α-glucoside, the propyl gallate O-α-glucoside, and the robinetin O-α-glucoside.

[0305] In a particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

[0306] wherein

[0307] A and B, identical or different, are H or an ω-glucosyl residue, with the proviso that at least one of A and B is a ω-glucosyl residue;

[0308] R₂ is H or OH; and

[0309] R₁ is

[0310] wherein R₃ and R₄, independently, are H or OH, with the proviso that at least one among R₃ and R₄ represents OH.

[0311] In a particular embodiment, R₂ is H. In another embodiment, R₂ is OH.

[0312] In a preferred embodiment, R₃ and R₄ are OH. In another preferred embodiment, R₃ is H and R₄ is OH. In a further preferred embodiment, R₃ is OH and R₄ is H. In a particularly preferred embodiment, R₂ is H and R₃/R₄ are selected in the following combinations: OH/OH; H/OH; OH/H. In another preferred embodiment, R₂ is OH and R₃/R₄ are selected in the following combinations: OH/OH; H/OH; OH/H.

[0313] In particular, R₂ is H, R₃ is H and R₄ is OH (resulting in eriodictyol O-α-glucoside). Alternatively, R₂ is H, R₃ is OH and R₄ is H (resulting in fisetin O-α-glucoside). In a preferred embodiment, R₂ is H, and both R₃ and R₄ are OH (resulting in taxifolin O-α-glucoside).

[0314] Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the taxifolin O-α-glucoside, the eriodictyol O-α-glucoside, the dihydroretin O-α-glucoside, and the robinetin O-α-glucoside.

[0315] In another particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

[0316] wherein

[0317] A and B, identical or different, are H or a ω-glucosyl residue, with the proviso that at least one of A and B is a ω-glucosyl residue;

[0318] R₂ is H or OH; and

[0319] R₁ is

[0320] wherein R₇ is selected from the group consisting of H, —OCH₃ or —OCOR and R₈ is H or OH, with the proviso that, when R₂ is H, R₇ and R₈ are not both OH, and at least one among R₇ and R₈ represents OH.
In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

In a preferred embodiment, R2 is OH, R8 is OH and R7 is OH or OCOR. In a more preferred embodiment, R7 and R8 are both OH. In another preferred embodiment, R2 is H, R8 is OH and R7 is OCOR. In a further preferred embodiment, R2 is H or OH, R7 is —OCOR and R8 is OH. More preferably, R is OH.

Preferably, the phenolic compound O-α-glucose is selected from the group consisting of the epigallocatechin O-α-glucose, the gallocatechin O-α-glucose, and the epicatechin gallate O-α-glucose.

In a further particular embodiment of the present invention, the phenolic compound O-α-glucose has the following formula:

```
    O
   / \  
  /   \  
 /     \  
       O
```

wherein
A and B, identical or different, are H or a -α-glucosyl residue, with the proviso that at least one of A and B is an -α-glucosyl residue;

R2 is H or OH; and

R1 is

wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3, or C6H11O5, and R11 is H, OH or C6H11O5, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H11O5, then R11 is H. In particular, R6, R5 and R11 can be selected from the following combinations:

a) R6 is OH and R5 is OCH3, and R11 is H;
b) R6 is OH and R5 is OH and R11 is OH;
c) R6 is OH and R5 is OH and R11 is C6H11O5;
d) R6 is H and R5 is OH and R11 is H; and

e) R9 is H or OH, and R10 is H or OCH3, or C6H11O5,

with the proviso that when R10 is C6H11O5, R11 is H.

In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

In a preferred embodiment, R9 is OH, R10 is H and R11 is H, whereas R6 is OH and R5 is OCH3, or R6 is H and R5 is OH. Preferably, R2 is H. Alternatively, R2 is OH.

In another preferred embodiment, R9 is H and R10 is OCH3, or C6H11O5. In a particular aspect of this embodiment, R9 and R11 are H, R10 and R5 are OCH3, and R6 is OH.

In an additional preferred embodiment, R5 and R6 are both OH, R9 is H, R10 is OH, R11 is H, OH or C6H11O5, with the proviso that when R10 is C6H11O5 then R11 is H. In another preferred embodiment, R5 and R6 are both OH, R9 is H or OH, R10 is H, and R11 is OH or C6H11O5.

In another preferred embodiment, R9 is H and R10 is H. In a further preferred embodiment, R9 is H, R10 and R5 are OCH3, and R6 is OH.

In a particular embodiment, R2, R5, R6, R9, R10 and R11 can be selected from the above mentioned combinations.
In a particular embodiment, R2 is H and R1 is OH and R5 is OCH3 (resulting in rhamnetin O-α-glucoside), or R6 is H and R5 is OH (resulting in fisetin O-α-glucoside). Preferably, the phenolic compound O-α-glucose is selected from the group consisting of the rhamnetin O-α-glucose, the fisetin O-α-glucose, the robinetin O-α-glucose, the gossypetin O-α-glucose, the orientin O-α-glucose, the homoorientin O-α-glucose and the cirsiliol O-α-glucose.

In a further particular embodiment of the present invention, the phenolic compound O-α-glucose has the following formula:

![Chemical Structure]

In a preferred embodiment, R1 is —(CH2)n—COOR. In a preferred embodiment, R is H.

Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid, the dihydrocaffeic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitannin (2',5-di-O-galloyl-hamamelose) and the protocatechuic acid.

In a particular embodiment, R2 is H and R1 is —COOH (resulting in protocatechuic acid O-α-glucoside). In another particular embodiment, R2 is H and R1 is —(CH2)n—COOH (resulting in hydrocaffeic acid O-α-glucoside).

The present invention contemplates the ester thereof and the pharmaceutically acceptable salts thereof.

Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the propyl gallate O-α-glucoside, the gallic acid O-α-glucoside, the hamamelitannin (2',5-di-O-galloyl-hamamelose) O-α-glucoside and the protocatechuic acid O-α-glucoside.

In an additional particular embodiment of the present invention, the phenolic compound O-α-glucose has the following formula:

![Chemical Structure]

A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

R2 is H or OH; and

R1 is —(CH2)n—COOR or —(CH2)n—CONHRR with n being an integer from 0 to 2.

In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

Preferably, R is selected from the group consisting of H, a C1-C3 alkyl, preferably methyl, ethyl or propyl, and

![Chemical Structure]

In a first more preferred embodiment, n is 0 and R is preferably H. In a second more preferred embodiment n is 1 and R is preferably H. In a third more preferred embodiment, n is 2 and R is preferably H. In another preferred embodiment, n is 0 and R is a C1-C3 alkyl, preferably methyl, ethyl or propyl, or

![Chemical Structure]
and a bond attached to the phenyl group of formula (I) at the carbon in ortho of R1.

In a particular embodiment, R2 is H and R1 is —(CH=CH)—COOH (resulting in caffeic acid O-α-glucoside). The present invention contemplates the ester thereof and the pharmaceutically acceptable salts thereof. In particular, when R1 is —(CH=CH)—COOR, R is selected from 1,3,4,5-tetrahydroxy-5-carboxyhexanecarboxylic acid and being attached at position 3 (resulting in chlorogenic acid O-α-glucoside), (R)-1-carboxy-2-(3,4-dihydroxyphenyl)ethyl (resulting in rosmarinic acid O-α-glucoside), and phenethyl (resulting in caffeic acid phenethyl ester O-α-glucoside). In particular, when R1 is —(CR12=CH)—COOR, R is a bond attached to the phenyl group of formula (II) by the carbon in mets of OB giving the following formula:

![Chemical structure](image)

(i.e., when R12 is H, then esculetin O-α-glucoside, when R12 is methyl, then 4-methylesculetin O-α-glucoside, and when R12 is phenyl, then nardalbergin O-α-glucoside). In a particular embodiment, R12 is H.

Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the caffeic acid O-α-glucoside, the rosmarinic acid O-α-glucoside, the esculetin O-α-glucoside, the 4-methylesculetin O-α-glucoside, the nardalbergin, 6,7-dihydroxyphenylcoumarin, O-α-glucoside, the chlorogenic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the chloric acid (dicycloxy tartaric acid) O-α-glucoside, the echinacoside (2-(3,4-dihydroxyphenyl)ethyl -O-6-deoxy-alpha-L-mannopyranosyl-(1→3)-O-(beta-D-glucopyranosyl(1→6))-4-(3,4-dihydroxyphenyl)-2-propeniose) O-α-glucoside, beta-o-glucopyranoside O-α-glucoside and the verbascoside O-α-glucoside.

In an additional particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

![Chemical structure](image)

wherein

- A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;
- R2 is H or OH; and
- R1 is —(CH2)n—OR with n being an integer from 0 to 2.

In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

In a preferred embodiment n is 2. Preferably, the phenolic compound O-α-glucoside is the hydroxytyrosol O-α-glucoside.

In an additional particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

![Chemical structure](image)
wherein

A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

(II)

R2 is H or OH; and

R1 is \((\text{CH}_2)_n\text{-COR}\) or \((\text{CH}-\text{CH})_n\text{-COR}\) with n being an integer from 0 to 2.

In a particular embodiment, R2 is 0. Alternatively, R2 is OH.

In a preferred embodiment, n is 0 or 1 and R is selected in the group consisting of

H; a C₁-C₃ alkyl, preferably methyl, ethyl or propyl, more preferably a methyl;

Preferably, n is 0. Alternatively, n is 1.

Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the maclurine O-α-glucoside, the 3,4-dihydroxybenzaldehyde O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the butein (2',3',4',4'-tetrahydroxychalcone) O-α-glucoside, the 3,4-dihydroxyacetophenone O-α-glucoside, the marenin (2',3',3',4',4'-pentahydroxy-4'-glucosylichalcone) O-α-glucoside, and the eriodictyol chalcone (2',4',6',3,4-pentahydroxychalcone) O-α-glucoside.

In an additional particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

(II)
wherein A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

R2 is H or OH; and

R1 is a C7-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (b) or tricyclic together with the ortho carbon of R1. In particular, the phenolic compound O-α-glucoside can be selected from the group consisting of

\[
\begin{align*}
\text{AO} & \quad \text{BO} \\
\text{AO} & \quad \text{AO} \\
\text{BO} & \quad \text{AO} \\
\text{AO} & \quad \text{BO} \\
\end{align*}
\]

said fused ring can be optionally interrupted by at least one heteroatom and can be substituted by one or several substituents selected from the group consisting of: an (C1-C3) alkoxy, an (C2-C6)alkyl, an (C3-C6)alkenyl, a carboxylic group (—COOH), an (C2-C4)ester, an (C1-C2)amino, an amino group (—NH2), an amide (—CONH2), an (C1-C3) imine, a nitro, an hydroxyl (—OH), a halogen, an (C2-C3)halogenaalkyl, a thiol (—SH), a (C1-C3 thiicouacid), a (C1-C3)sulfonyl, a (C1-C5)sulfoxide, and a combination thereof. In a particular preferred embodiment, the phenolic compound O-α-glucoside is

\[
\begin{align*}
\text{AO} & \quad \text{BO} \\
\text{AO} & \quad \text{AO} \\
\end{align*}
\]

The O-α-glucosyl residue refers herein to a glucose monomer, dimer, trimer, tetramer, pentamer or more. Preferably, the O-α-glucosyl residue is a glucose monomer, dimer, or trimer, namely glucosyl, diglucosyl, or triglucosyl. Still preferably, the O-α-glucosyl residue is a glucose monomer. In a particular embodiment the O-α-glucosyl residue is attached to the phenolic compound by the carbon in position 1. In a preferred embodiment, OA is OH and OB is a O-α-glucosyl residue. In an other preferred embodiment, OB is OH and OA is a O-α-glucosyl residue.

In a particular embodiment, R can be a monon-carboxylic acid, in an other particular embodiment, R is a (C1-C6) alkyl or a (C1-C3)alkyl.

Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable base addition salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, perchloric, and the like. Representative examples of suitable organic acids include formic, acetic, trichloracetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, and in Handbook of Pharmaceutical Salts: Properties, Selection, and Use edited by P. Heinrich Stahl and Camille G. Wermuth 2002. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like. Examples of organic bases include lysine, arginine, guanidine, diethanolamine and the like.

In Situ Release of the Aglycones.

Surprisingly, the inventors found that the phenolic compound O-α-glucosides of the present invention can be cleaved by α-glucosidases leading to in situ releasing of the phenolic compounds.

All the phenolic compounds O-α-glucosides of the present invention have at least one O-α-glucoside bond. This bond can be specifically hydrolyzed by enzymes, such α-glucosidases (EC 3.2.1.20), to release the glucosyl residue and the aglycone part. When achieved in situ, this liberation has several advantages:

- it enables to release the poorly soluble aglycone (which may be more active than the glycoside derivative) after their administration/injection/application under a soluble glycoside form, and/or

- the in situ release can be time dependent if achieved by enzymes expressed by micro-organisms, the amount of releasing enzyme will be correlated to the number of micro-organisms: the more dense the bacterial population will be, the more aglycones release will occur), and/or

- the in situ release can be controlled by an in situ administration/injection/application of an α-glucosidase or of a micro-organism expressing such enzymatic activity.

These advantages are important in the formulation of phenolics in cosmetics or dermatocosmetics preparations. In a preferred embodiment of the present invention, said phenolic compound O-α-glucosides can be in situ activated by enzyme(s) expressed by human associated micro-organisms, and more preferentially by human skin associated micro-organisms. Known and non exhaustive examples of such
human commensal or non commensal micro-organisms include *Streptococcus* species, *Staphylococcus* species, *Enterococcus* species, *Escherichia coli*, *Bacilli*, *Corynebacterium* species, *Propionibacterium* species. When applied on skin, the phenolic compounds O-α-glucosides of the present invention are converted by skin associated micro-organisms into the aglycone part and the glucosyl residue. Such bacteria can be found in human beings in mouth, intestinal tract, genital tract and upper respiratory system.

[0405] In another preferred embodiment of the present invention, said phenolic compounds O-α-glucosides can be in situ activated by α-glucosidase (EC 3.2.1.20), such as the α-glucosidase from *Saccharomyces cerevisiae*.

[0406] So phenolic compounds O-α-glucosides of the present invention have a pro-drug status as the active part of the molecule (the aglycone) can be released in situ.

[0407] Therefore, the present invention concerns a pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside of the present invention or a pharmaceutically acceptable salt thereof. The present invention also concerns a phenolic compound O-α-glucosides of the present invention or a pharmaceutically acceptable salt thereof as a medicament. The medicament can be therapeutic or prophyllactic. Phenolic compound O-α-glucosides of the present invention have several activity among which antiviral, antibacterial, immune-stimulating, antiallergic, antihypertensive, anti-ischemic, antiarrhythmic, antithrombotic, hypocholesterolic, antilipoperoxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, antimutagenic, antineoplastic, anti-thrombotic, and vasodilatory actions.

[0408] In a particular embodiment, the composition can further comprise a O-α-glucosidase (EC 3.2.1.20) or a microorganism expressing O-α-glucosidase activity. Preferably, the O-α-glucosidase is from *Saccharomyces cerevisiae*. In particular, the O-α-glucosidase (EC 3.2.1.20) or a microorganism expressing O-α-glucosidase activity is present in the composition in an inactivated form and the O-α-glucosidase is activated just at the moment of administration. For instance, the composition can be formulated in dried form, the absence of water leading to the inactivation of O-α-glucosidase; after water addition, the enzyme will become active and will be then able to hydrolyze the glucosidic bond. The enzyme and the phenolic compound O-α-glucosides can be put in two different liquid preparations that will be mixed just at the moment of administration. If the enzyme and the phenolic compound O-α-glucosides are put into the same solution, it is possible to use an enzyme reversible inhibitor that will be diluted after administration, thus allowing the enzyme to recover its ability to hydrolyze the phenolic compound O-α-glucosides. Phenolic compound O-α-glucosides of the present invention and the O-α-glucosidase or a microorganism expressing O-α-glucosidase activity can also be physically separated (e.g., microcapsule).

[0409] The present invention concerns the use of a phenolic compound O-α-glucoside of the present invention or a pharmaceutically acceptable salt thereof for preparing a pharmaceutical or cosmetic composition for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder comprising administering a phenolic compound O-α-glucoside of the present invention or a pharmaceutically acceptable salt thereof. In addition, the method can further comprise the step of administering sequentially or simultaneously a O-α-glucosidase (EC 3.2.1.20) or a micro-organism expressing O-α-glucosidase activity. Preferably, the O-α-glucosidase (EC 3.2.1.20) or a micro-organism expressing O-α-glucosidase activity is administered by the same route.

[0410] In a particular embodiment, the present invention concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered topically (i.e., on skin), wherein enzymes issued from skin associated micro-organisms release the corresponding aglycone. In addition, the present invention concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered orally, wherein enzymes issued from mouth and intestinal tract associated micro-organisms release the corresponding aglycone. The present invention also concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered rectally, wherein enzymes issued from intestinal tract associated micro-organisms release the corresponding aglycone. The present invention further concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered nasally, wherein enzymes issued from upper respiratory system associated micro-organisms release the corresponding aglycone. The present invention further concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered vaginally, wherein enzymes issued from female genital tract associated micro-organisms release the corresponding aglycone.

[0411] The present invention also concerns a combination of a phenolic compound O-α-glucosides of the present invention or a pharmaceutically acceptable salt thereof with a O-α-glucosidase (EC 3.2.1.20) or a micro-organism expressing O-α-glucosidase activity for a simultaneous or sequential administration. When simultaneously administration is performed, the phenolic compound O-α-glucosides of the present invention or a pharmaceutically acceptable salt thereof and the O-α-glucosidase (EC 3.2.1.20) or a micro-organism expressing O-α-glucosidase activity can be administered in the same or different compositions.

[0412] Such a composition can comprise pharmaceutically acceptable carrier, stabilizers or excipients.

[0413] Use of Phenolic Compounds as Key Intermediates for the Development of Other Derivatives

[0414] Phenolic compound O-α-glucosides of the present invention can be directly used as active ingredients as cosmetics or as active substances alone or in combination with other products, including other active molecules with synergistic or complementary activities, or with stabilizers or excipients. These phenolic compounds derivatives can also be used as starting materials for additional chemical, physical or enzymatic modification(s) in order to produce second generation of derivatives. As the enzymatic reaction used in the present invention concerns specific hydroxyl positions on the catechol ring of the phenolic compound, the other hydroxyl groups can for example be used in a chemical reaction to create ester bonds, acyl bonds, sulphate or phosphate bonds.
Such modifications can improve already existing properties of the phenolic compounds O-α-glucosides of the present invention, or provide new properties for specific applications (higher therapeutic efficiency, lower cytotoxicity, higher stability after release of the glycone part by micro-organisms, . . . ).

[0415] Formulation of Said Derivatives for Cosmetic or Therapeutic Applications

[0416] The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrathecal, intradermic, intrathecal, intraskeletal and intracranial injection or infusion techniques. Preferably, the composition of the present invention are administered orally, by inhalation spray, topically, rectally, nasally, buccally, or vaginally. In a preferred embodiment, the pharmaceutical or cosmetic composition is administered topically.

[0417] New types of cosmetic products are constantly being developed, and new raw materials are adding to the cosmetic chemist’s selection of personal care ingredients. The phenolic compounds O-α-glucosides described in the present invention can easily be incorporated in a large panel of cosmetic products. Such preparations are well known from the man of the art: it can be creams, sticks, shampoo, shower gels, lotions, soaps, emulsions, gels. These formulations can include other ingredients such as but are not limited to: deionized water, magnesium aluminium silicate, xanthan gum, nylon-12, sodium PCA, propylene glycol, red iron oxides, talc, yellow iron oxides, black iron oxides, titanium dioxide, glycercyl stearate, stearic acid, DEA-cetyl phosphate, methylparaben, butylparaben, ethylparaben, propylparaben, isostearyl neopentanoate, isopropyl palmitate, ethylene/propylene/styrene, copolymers, butylene/ethylene/styrene copolymer, isopropyl palmitate, phenoxyethanol, tocopheryl acetate, glycercin, triethanolamine, stearic acid, propylene glycol stearate, mineral oil, butylene/ethylene/styrene copolymer, diazolidinyl urea, hydrogennated polyisobutene, octyl palmitate, tridecyl neopentanoate, isostearyl isostearate, isopropylparaben, isobutylparaben, octylhydroxyl neopentanoate, tocopheryl acetate, fragrance, octyl methoxycinnamate, benzophenone, octyl salicylate, isopropyl isostearate, propylene glycol isoceteth-3 acetate, or any combinations thereof.

[0418] For their use in therapeutic applications, phenolic compounds O-α-glucosides of the present invention can be incorporated in different galenic preparations such as pills, tablets, syrups, creams, lotions, gels using for example packing, standardisation, blending/homogenisation, sterile and nonsterile micronization, granulation/compacting, sieving or any combination thereof. Preparations of said phenolic compounds O-α-glucosides can include some excipients of the following non exhaustive list: talc, lactose, magnesium stearate, glycerol monostearate, colloidal silicon dioxide, precipitated silicon dioxide, crosslinked polyvinyl pyrrolidone, dibasic calcium phosphate dihydrate, microcrystalline cellulose, corn starch, povidone, sodium carboxy-methyl cellulose, poloxarbetal 80, lactic acid, carborner, cetyl alcohol, isopropyl myristate, isopropyl palmitate, glucose, dextrose, triethanolamine, glycercine, fructose, sucrose, polymers, nanostructures.

[0419] The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0420] Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[0421] The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0422] For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0423] For ophthalmic use, the compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic use, the compositions may be formulated in an ointment such as petrolatum.

[0424] The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0425] Sterile injectable forms of the compositions of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butandiol. Among the acceptable vehicles and solvents that may be employed are water, Ringer’s solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycer-
ides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutical-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Span and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

ADVANTAGES OF THE PRESENT INVENTION

[0426] The advantages of the method of the present invention over pre-existing methods appears clearly from the previous descriptions and embodiments. A non-exhaustive list of others advantages of the present invention are described below.

[0427] The present invention describes new original phenolic compounds O-α-glucosides of

[0428] protocatechuic acid and its esters derivatives,

[0429] caffeic acid and its esters derivatives, especially rosmarinic acid, chlorogenic acid and caffeic acid phenylethyl ester and hydrocaffeic acid or 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylethylglycol,

[0430] esculetin,

[0431] taxifolin,

[0432] fisetin,

[0433] eriodictyol,

[0434] and rhamnetin.

[0436] Preferably, the new original phenolic compounds O-α-glucosides of the present invention are selected in the group consisting of the epicatechin gallate O-α-glucoside, the eriodictyol O-α-glucoside, the esculetin O-α-glucoside, the fisetin O-α-glucoside, the fustin O-α-glucoside, the homoprotocatechuic acid O-α-glucoside, the protocatechuic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the hydroxytyrosol O-α-glucoside, the maclurin O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the oleuropein O-α-glucoside, the pyrochelol O-α-glucoside, the rhamnetin O-α-glucoside, the rosmanarinic acid O-α-glucoside, the taxifolin O-α-glucoside, the 3-hydroxyadaidzein O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the caffeic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the caffeic acid phenylesther O-α-glucoside, the cirsiliol O-α-glucoside, the chlorogenic acid O-α-glucoside, the anthrarin O-α-glucoside, the epigallocatechin O-α-glucoside, the dihydrodibeninetin O-α-glucoside, the gallatechelin O-α-glucoside, the gallic acid O-α-glucoside, the propyl gallate O-α-glucoside, and the robinetin O-α-glucoside.

[0437] These phenolic compound O-α-glucosides of high interest in the fields of cosmetic and therapy show improved water solubility. Indeed, an increase by at least 20, 30 or 50 folds of the solubility has been observed in comparison with the corresponding aglycone in the same physiological conditions.

[0438] These phenolic compound O-α-glucosides have an increased bioavailability. These phenolic compound O-α-glucosides can be “in situ activated” through their hydrolysis into the initial phenolic structure by human commensal microorganisms, giving them a “pro-drug” status of high interest for both cosmetic and therapy applications. They can also be activated with an α-glucosidase, such as the α-glucosidase produced by the yeast Saccharomyces cerevisiae.

[0439] These new phenolic compound O-α-glucosides are obtained through a proven, reliable, low cost, “green chemistry” enzymatic process that ensures high quality of these products (due to the specificity and selectivity of the enzyme used).

EXAMPLES

[0440] Any other embodiments and advantages of the present invention will appear from the following examples, that are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Example 1

Synthesis of Glucosylated Taxifolin; Solubility in Water of Highly Purified Glucosylated Taxifolin and Stability of the Glucosylated Derivative Molecule at Temperatures Ranging from 4°C to 45°C.

[0441] The conditions that were carried out for the synthesis of glucosylated taxifolin are as follows (amounts for 1 liter of reaction medium):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Origin</th>
<th>Amount</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution of Taxifolin at 90 g/L in pure DMSO</td>
<td>Taxifolin SIGMA T 4512</td>
<td>100 ml</td>
<td>Taxifolin 9 g/L</td>
</tr>
<tr>
<td>DMSO</td>
<td>Riedel de Haën 60153</td>
<td>250 ml</td>
<td>Total DMSO: 350 ml/L</td>
</tr>
<tr>
<td>Sodium acetate buffer 500 mM pH 5.2</td>
<td>Acetic acid: Prolabo 20104.298</td>
<td>40 ml</td>
<td>Sodium acetate: 20 mM</td>
</tr>
<tr>
<td></td>
<td>Sodium hydroxide: Riedel de Haën 6203</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose at 500 g/L (1.462 M)</td>
<td>Prolabo 27478.296</td>
<td>300 ml</td>
<td>150 g/L</td>
</tr>
<tr>
<td>Water</td>
<td>Deionized Qsp 1 L #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingredient</td>
<td>Origin</td>
<td>Amount</td>
<td>Concentration</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------------------------------------</td>
<td>----------</td>
<td>---------------</td>
</tr>
<tr>
<td>Calcium chloride, dihydrate</td>
<td>Merck 1,023,82,0500</td>
<td>10 mg</td>
<td>10 mg/L</td>
</tr>
<tr>
<td>(can also be introduced in the reaction medium in the form of a solution at 2 g/L; the dose is then 5 ml/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextranucrase preparation (18 U/ml)</td>
<td>Purified from L. mesenteroides NRRLB 512F culture broth</td>
<td>170 ml</td>
<td>3.1 U/ml</td>
</tr>
</tbody>
</table>

[0442] The reaction medium without the enzyme was first obtained by mixing the various solutions in the order reported in the table. The mixture was incubated at 30°C during a period of time sufficient to attain the desired temperature of 30°C (plus or minus 0.2°C). Then the reaction was started by introducing the enzyme preparation. The reaction medium may be moderately agitated.

[0443] The enzyme preparation has been obtained as follows: the culture broth of Leuconostoc mesenteroides NRRL B512-F containing an enzyme activity ranging from 4 to 6 U/ml is centrifuged in order to completely separate the microbial cells from the liquid containing the enzyme. The centrifugation supernatant was then concentrated 4 to 10 times by tangential ultrafiltration (molecular weight cut-off of 100 kDa). The retentate was then diluted 4 times with 20 mM acetate buffer pH 5.2 containing calcium chloride dihydrate at 10 mg/L and then concentrated 4 times in order to extensively remove the residual low molecular weight components of the cell culture medium containing the enzyme. The purified enzyme preparation has then been stored in a frozen form (–20°C) or freeze dried up to several months without loss of activity. As a general procedure, the activity of the enzyme preparation is adjusted by intensifying the concentration of the retentate in order that the volume of the enzyme preparation will not be higher than 20% of the final volume of the synthesis reaction medium.

[0444] The reaction medium was incubated at 30°C (plus or minus 0.2°C) during 22 hours. An aliquot of the reaction medium was taken off the reaction medium and diluted 50 times with a solution containing methanol and water in the proportions of 40/60. The methanolic solution was then analyzed by HPLC.

[0445] The analysis conditions were those previously described excepted that the profile of the methanol concentration was as follows (Method 2):

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Solvent A: deionized water containing 1% v/v acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 10</td>
<td>60% A; 40% B</td>
</tr>
<tr>
<td>10 to 12</td>
<td>60% to 20% A (linear); 40% to 80% B (linear)</td>
</tr>
<tr>
<td>12 to 14</td>
<td>20% A; 80% B</td>
</tr>
<tr>
<td>14 to 16</td>
<td>20% to 60% A (linear); 80% to 40% B (linear)</td>
</tr>
<tr>
<td>16 to 25</td>
<td>60% A; 40% B</td>
</tr>
<tr>
<td>25</td>
<td>next injection</td>
</tr>
</tbody>
</table>

[0454] FIG. 7 shows the HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm) at just the beginning of the incubation. The major peak at 8.15 minutes corresponds to Taxifolin.

[0455] FIG. 8 shows the HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm) after 22 hours of incubation. A peak with a retention time of 6.15 minutes is observed.

[0456] FIG. 9 shows the mass spectrum and FIG. 10 the UV spectrum of the peak eluted at around 8.15 minutes: the substance is Taxifolin (m/z [M–H]: 302.96 and m/z [M–H–H2O]: 284.96) which molecular weight is 304.

[0457] FIG. 11 shows the mass spectrum and FIG. 12 the UV spectrum of the peak eluted at around 6.15 minutes: the corresponding substance is Taxifolin glucoside (m/z [M–H]: 464.98) since its molecular weight is 466.

[0458] The substances eluted at 9.33 and 12.75 minutes are polyphenolic substances found in the Taxifolin preparation.

[0459] FIG. 13 shows a HPLC chromatogram of an aqueous solution containing Taxifolin and Taxifolin glucoside after carrying out purification to remove the enzyme, dextran, fructose and DMSO and a fraction of residual Taxifolin. The eluting conditions are those previously described in which the initial content of methanol is 10% (method 1). Taxifolin is eluted at 24.01 minutes and Taxifolin glucoside at 22.33 minutes.

[0460] Taxifolin glucoside has been purified extensively to reduce as much as possible the Taxifolin concentration. It was finally obtained a solution titrating more than 93 mM of Taxifolin glucoside with a Taxifolin residual concentration of less than 2 mM (FIG. 14; Taxifolin eluted at 8.95 minutes and Taxifolin glucoside eluted at 6.55 minutes).

[0461] Concentrations of Taxifolin glucoside were determined as follows: after having established the relationship between the molar concentration of Taxifolin and the peak areas with a precisely characterized Taxifolin preparation (SIGMA), concentrations of Taxifolin glucoside were determined by applying the relationship between area and concentration to Taxifolin glucoside, since Taxifolin and Taxifolin glucoside have the same UV spectra. Then, concentrations in g/L were obtained by multiplying the molar concentration by the value of the Taxifolin glucoside molecular weight (466). Whereas the Taxifolin solubility in water at 25°C is measured at 1.19 g/L (3.91 mM), the solubility of Taxifolin glucoside in water at 25°C is higher than 43.5 g/L (93.2 mM).
[0462] It is thus possible according to the described method to synthesize a new substance, Taxifolin glucoside with a molecular weight of 466 and a solubility in water at around 25° C. higher than 93 mM, corresponding to an increase in water solubility regarding the Taxifolin residue higher than 23. Taxifolin glucoside can be purified according to the techniques previously mentioned (resin adsorption, elution, concentration, liquid extraction, solvent removal and concentration and eventually drying).

[0463] The Taxifolin glucoside solution can be stored during a long period of time without loss of the glucosidic bond and with a quite satisfactory resistance to oxidation.

[0464] Accelerated shelf-life studies were performed using temperature chambers at 4° C., 22° C., 37° C. and 45° C. for 4 months. The Taxifolin glucoside content was frequently measured, and color and odor were roughly controlled. The Taxifolin glucoside content was determined by HPLC as previously described (500 fold dilution of an aliquot of the solution and analysis using method 2; detection: 210-400 nm).

[0465] The following table describes the observed quantity of Taxifolin glucoside versus the storage time at different storage temperatures.

| Measured quantity of taxifolin glucoside (in % of the initial quantity) |
|---|---|---|---|---|
| Days | Stored at +4° C. | Stored at +22° C. | Stored at +37° C. | Stored at +45° C. |
| 0  | 100  | 100  | 100  | 100  |
| 9  | 100  | 100  | 100  | 91  |
| 23 | 100  | 100  | 95  | 91  |
| 37 | 100  | 100  | 96  | 91  |
| 63 | 100  | 95  | 96  | 73  |
| 118| 100  | 95  | 91  | 73  |

[0466] No colour or odour changes have been observed whatever the temperature of storage.

[0467] Therefore, the glucoside bond between Taxifolin and the glucose moiety is stable in the above tested conditions. At 37° C. and 45° C., a slight degradation for the Taxifolin glucoside has been observed probably due to oxidation: indeed, no Taxifolin concentration increase that would indicate an hydrolysis of the glucoside bond was observed in the corresponding solutions. In the above mentioned conditions, half life of Taxifolin glucoside is estimated at 1.6 year at 37° C., and 0.67 year at 45° C.

[0468] This example demonstrates that Taxifolin glucoside has a high chemical stability even in harsh storage conditions.

Example 2

Influence of the DMSO Concentration on the Efficiency of the Synthesis of Taxifolin Glucoside

[0469] Taxifolin glucoside enzymatic synthesis was carried out as described in Example 1 with the following exceptions:

- enzyme concentration was 1 U/ml
- DMSO concentration was 35%, 25%, 15% or 5%.

[0470] After 22 hours of incubation, relative Taxifolin glucoside concentrations in the four reaction medium are reported in the following table.

<table>
<thead>
<tr>
<th>DMSO, %</th>
<th>35</th>
<th>25</th>
<th>15</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxifolin glucoside (relative concentration), %</td>
<td>100</td>
<td>133</td>
<td>161</td>
<td>17</td>
</tr>
</tbody>
</table>

[0473] The optimal DMSO concentration for the synthesis of Taxifolin glucoside appears to be at a value significantly lower than 30% and close to 15%.

Example 3

Activation of Taxifolin Glucoside by Human Skin Micro-Flora

[0474] Cutaneous flora was separately collected from 5 donors. The forearms and forehead of each donor were scraped with a cotton-wool swab saturated with NaCl solution (v=5 mL, 8 g/l). After each scraping, the swab was divided into the remaining NaCl solution and squeezed to deliver the sampled material. After two cycles of scraping/squeezing on both forearms and three on the forehead, the obtained trouble preparation was filtered (40 μm) to eliminate squama and finally centrifuged (4° C., 5000 g, 15 min). The microbial pellets were resuspended in a NaCl solution (v=1 mL, 8 g/l) and characterized by OD at 600 nm.

[0475] The five microbial samples were mixed to form the final microbial suspension used for the test. Microbial cells were cultivated using the Hickey-Tresner culture medium (yeast extract at 1.0 g/L, meat extract at 1.0 g/L, casein peptone at 2.0 g/L, starch at 10.0 g/L, cobalt chloride hexahydrate at 20 mg/L; pH=6). Microbial growth was carried out in 100 ml Erlemeyer flasks at 37° C. under continuous agitation (100 rpm). The sterile culture broth (20 ml) was inoculated with 0.1 mL of suspension. Microbial growth was controlled by measuring the OD at 600 nm.

[0476] Taxifolin glucoside was obtained as described in example 1 (highly purified preparation corresponding to the HPLC chromatogram reported in FIG. 14). Taxifolin glucoside was added or not at day 0 (V=0.5 mL of 0.20 μm sterilized solution). Control was made by growing the final microbial suspension without Taxifolin glucoside.

[0477] After centrifugation of an aliquot of cell culture media, the supernatant was diluted 4-fold with a solution containing methanol and water in the proportions of 40:60. Taxifolin glucoside and taxifolin concentrations in the supernatants were determined by HPLC (method 2).

[0478] FIG. 15 shows the apparent bacterial growth during a week in the Hickey-Tresner culture medium. From day 3 to day 7, the apparent biomass production is higher in presence of Taxifolin glucoside than in its absence. This might be explained by a higher concentration of carbon and energy source due to the liberation of the glucose from the taxifolin glucoside under the bacterial hydrolysis.
In FIG. 16, the hydrolysis of Taxifolin glucoside cannot be detected during the first three days. After three days of incubation, probably when the carbon and energy source becomes limiting, the concentration of Taxifolin glucoside diminishes in a significant manner and the aglycone flavonoid, Taxifolin, appears concomitantly. The nutritional stress undergone by the bacterial community originating from the human cutaneous flora might stimulate the liberation of the glucosyl residue through the action of the secreted enzymes.

This example demonstrates that human cutaneous flora recognizes and is able to hydrolyze the flavonoid glucosidic bond with a high output, offering a new pathway for the delivery of active ingredients.

Example 4
Activation of Taxifolin Glucoside by an α-Glucosidase Preparation

Taxifolin glucoside was incubated in the presence of an α-glucosidase enzyme in the following conditions:

- Taxifolin glucoside obtained as described in example 1 (highly purified preparation corresponding to the HPLC chromatogram reported in FIG. 14): 0.25 ml;
- α-glucosidase (from Saccharomyces cerevisiae; FLUKA 70797; lot 06413371; activity: 5.8 U/mg); 50.1 mg in 5 ml of potassium phosphate buffer 0.1 M, pH 7.3; no enzyme in the control reaction medium;
- Temperature: 30°C; Moderate agitation.

The reaction media were analysed by HPLC (method 2) after a 2-fold dilution of an aliquote with methanol.

After 18 hours of incubation, the Taxifolin glucoside molecule remained unchanged in the medium which did not contain the α-glucosidase enzyme whereas the Taxifolin glucoside molecule was totally converted into Taxifolin in the presence of the α-glucosidase enzyme.

These results show that an isolated enzyme specific for the hydrolysis of α-glucosidic bonds is able to hydrolyse the Taxifolin glucoside molecule: this indicates that the Taxifolin glucoside molecule contains Taxifolin and glucoside with glucose being linked to an hydroxyl group of Taxifolin through an α-glucosidic bond. For this reason, the synthesized new glucoside derivatives are claimed O-α-D-glucoside derivatives.

Example 5
Enzymatic Synthesis of O-α-D-Glycosides of Pyrocatechol, Protocatechuic Acid and Protocatechuic Acid Ethyl Ester

Reaction media were prepared as described in example 1, Taxifolin being replaced by Pyrocatechol (SIGMA, reference C 9510), or by Protocatechuic acid (ALDRICH, reference D10,980-0) or by Protocatechuic acid ethyl ester (ALDRICH, reference E 2, 485-9).

After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>m/z [M – H] weight</th>
<th>Identification (theoretical molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrocatechol</td>
<td>13.78</td>
<td>108.74</td>
<td>Pyrocatechol (110)</td>
</tr>
<tr>
<td>(chromatogram at 276 nm in FIG. 17)</td>
<td>16.80</td>
<td>271.03</td>
<td>Pyrocatechol monoglucoside (272)</td>
</tr>
<tr>
<td></td>
<td>14.88</td>
<td>433.05</td>
<td>Pyrocatechol diglucoside (434)</td>
</tr>
<tr>
<td></td>
<td>13.22</td>
<td>595.06</td>
<td>Pyrocatechol triglucoside (596)</td>
</tr>
<tr>
<td></td>
<td>11.87</td>
<td>919.35</td>
<td>Pyrocatechol pentaglucoside (920)</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>11.26</td>
<td>152.88</td>
<td>Protocatechuic acid (154)</td>
</tr>
<tr>
<td>(chromatogram at 294 nm in FIG. 18)</td>
<td>7.89</td>
<td>477.00</td>
<td>Protocatechuic acid diglucoside (478)</td>
</tr>
<tr>
<td></td>
<td>7.15</td>
<td>801.26</td>
<td>Protocatechuic acid tetraglucoside (802)</td>
</tr>
<tr>
<td>Protocatechuic acid ethyl ester</td>
<td>28.28</td>
<td>180.96</td>
<td>Protocatechuic acid ethyl ester (182)</td>
</tr>
<tr>
<td>(chromatogram at 295 nm in FIG. 19)</td>
<td>27.30</td>
<td>343.02</td>
<td>Protocatechuic acid ethyl ester monoglucoside (344)</td>
</tr>
<tr>
<td></td>
<td>24.99</td>
<td>505.05</td>
<td>Protocatechuic acid ethyl ester diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>20.54</td>
<td>829.30</td>
<td>Protocatechuic acid ethyl ester tetraglucoside (830)</td>
</tr>
</tbody>
</table>
It is thus possible according to the described method to synthesize the new glucosylated derivatives of Pyrocatechol, Protocatechuic acid and Protocatechuic acid ethyl ester; the resulting products are a family of substances containing at least monoglucosylated, diglucosylated, triglucosylated and tetraglucosylated derivatives.

Example 6
Enzymatic Synthesis of O-α-D-Glycosides of Caffeic Acid, 3,4-Dihydroxyhydrocinnamic Acid (Hydrocaffeic Acid) and Rosmarinic Acid

Reaction media were prepared as described in example 1, Taxifolin being replaced by Caffeic acid (SIGMA, reference C 0625), or by 3,4-Dihydroxyhydrocinnamic acid (ALDRICH, reference D10,980-0) or by Rosmarinic acid (FLUKA, reference 44699; the concentration of Rosmarinic acid in the reaction medium was 1 g/L).

After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>m/z [M – H]</th>
<th>Identification (theoretical molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (chromatogram at 322 nm in FIG. 20)</td>
<td>19.53</td>
<td>178.97</td>
<td>Caffeic acid (380)</td>
</tr>
<tr>
<td></td>
<td>15.46</td>
<td>341.09</td>
<td>Caffeic acid monoglucoside (342)</td>
</tr>
<tr>
<td></td>
<td>14.62</td>
<td>503.16</td>
<td>Caffeic acid diglucoside (504)</td>
</tr>
<tr>
<td>3,4-Dihydroxyhydrocinnamic acid (hydrocaffeic acid) (chromatogram at 278 nm in FIG. 21)</td>
<td>18.72</td>
<td>343.02</td>
<td>Hydrocaffeic acid monoglucoside (344)</td>
</tr>
<tr>
<td></td>
<td>17.93</td>
<td>505.05</td>
<td>Hydrocaffeic acid diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>17.80</td>
<td>180.96</td>
<td>Hydrocaffeic acid (182)</td>
</tr>
<tr>
<td></td>
<td>17.50</td>
<td>343.02</td>
<td>Hydrocaffeic acid monoglucoside (344)</td>
</tr>
<tr>
<td></td>
<td>17.06</td>
<td>657.21</td>
<td>Hydrocaffeic acid triglucoside (668)</td>
</tr>
<tr>
<td></td>
<td>16.23</td>
<td>829.25</td>
<td>Hydrocaffeic acid tetraglucoside (830)</td>
</tr>
<tr>
<td></td>
<td>16.01</td>
<td>505.05</td>
<td>Hydrocaffeic acid diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>15.70</td>
<td>992.36</td>
<td>Hydrocaffeic acid pentaglucoside (992)</td>
</tr>
<tr>
<td></td>
<td>14.70</td>
<td>657.21</td>
<td>Hydrocaffeic acid triglucoside (668)</td>
</tr>
<tr>
<td></td>
<td>13.92</td>
<td>829.39</td>
<td>Hydrocaffeic acid tetraglucoside (830)</td>
</tr>
<tr>
<td></td>
<td>13.22</td>
<td>991.50</td>
<td>Hydrocaffeic acid pentaglucoside (992)</td>
</tr>
<tr>
<td></td>
<td>12.61</td>
<td>1153.60</td>
<td>Hydrocaffeic acid hexaglucoside (1154)</td>
</tr>
<tr>
<td></td>
<td>12.08, 11.21</td>
<td>#</td>
<td>Polymerization degree higher than 6</td>
</tr>
<tr>
<td>Rosmarinic acid (chromatogram at 295 nm in FIG. 22)</td>
<td>28.36</td>
<td>350.09</td>
<td>Rosmarinic acid (360)</td>
</tr>
<tr>
<td></td>
<td>27.18</td>
<td>521.16</td>
<td>Rosmarinic acid monoglucoside (522)</td>
</tr>
</tbody>
</table>

It is thus possible according to the described method to synthesize the new glucosylated derivatives of Caffeic acid, Hydrocaffeic acid and Rosmarinic acid: the resulting products are a family of substances containing at least monoglucosylated, diglucosylated, triglucosylated and tetraglucosylated derivatives. As far as Hydrocaffeic acid is concerned, it clearly appears that both hydroxyl groups have been substituted: indeed, it can be seen at least two series of derivatives, both containing at least monoglucosylated (344), diglucosylated (506), triglucosylated (668), tetraglucosylated (830) and pentaglucosylated (992) derivatives. This shows that in some cases that can not be predicted by a skilled man, both hydroxylated groups can accept a glucose moiety.

Example 7
Enzymatic Synthesis of O-α-D-Glycosides of 3,4-Dihydroxymandelic Acid, Esculetin and Escculin

Reaction media were prepared as described in example 1, Taxifolin being replaced by 3,4-Dihydroxymandelic acid (ALDRICH, reference 151610), or by Esculetin (ALDRICH, reference 24, 657-3) or Esculin (SIGMA, reference E 8250).

After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>m/z [M – H]</th>
<th>Identification (theoretical molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Dihydroxymandelic acid (chromatogram at 322 nm in FIG. 23)</td>
<td>14.68</td>
<td>136.82</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>5.32</td>
<td>136.64</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>4.10</td>
<td>182.95</td>
<td>3,4-Dihydroxymandelic acid (184)</td>
</tr>
<tr>
<td></td>
<td>3.45</td>
<td>341.63</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
3,4-Dihydroxymandelic acid contains a pyrocatechol structure as Taxifolin, Pyrocatechol, Protocatechuic acid, Caffeic acid; nevertheless, no glucosylated derivative of 3,4-Dihydroxymandelic acid has been synthesized in the present conditions.

In an unexpected manner, the 6,7-dihydroxycoumarin skeleton is also a glucoside acceptor which leads to a series of glucosylated Esculetin. It has to be underlined that the synthesized Esculetin monoglucoside has a retention time of 15.65 minutes whereas the natural glucosylated Esculetin (Esculin or Esculetin 6-O-β-D-glucopyranoside) has a retention time of 13.69 minutes: this has to be attributed that the osidic bond in the case of the natural molecule is of the α-type whereas the osidic bond in esculin is of the type-β.

In an unexpected manner, Esculin is a glucoside acceptor, probably by its glucose moiety.

Example 8

Enzymatic Synthesis of O-α-D-Glycosides of Gallic Acid, Propyl Gallate and Epigallocatechin Gallate

Reaction media were prepared as described in example 1, Taxifolin being replaced by Gallic acid (FLUKA, reference 48630), or by Propyl Gallate (SIGMA, reference P3130) or by Epigallocatechin Gallate (SIGMA, reference 446999) and the DMSO concentration being reduced to 15% v/v.

After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min</th>
<th>Identification (theoretical molecular weight)</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>7.92</td>
<td>Gallic acid O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>6.75</td>
<td>Gallic acid O-α-glucoside</td>
<td></td>
</tr>
<tr>
<td>Propyl Gallate</td>
<td>27.22</td>
<td>Propyl Gallate O-α-glucoside</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.35</td>
<td>Propyl Gallate O-α-glucoside</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin Gallate</td>
<td>17.03</td>
<td>Epigallocatechin Gallate O-α-glucoside and Epigallocatechin Gallate di-O-α-glucoside</td>
<td>G1</td>
</tr>
</tbody>
</table>

Analysis Conditions:

G1: flow rate 1 ml/min; from 0 to 10 min: B increases linearly from 10 to 20%; from 10 to 25 min: B increases linearly from 20 to 50%; from 25 to 30 min: B is stable at 50%; from 30 to 35 min: B decreases linearly from 50 to 10%.

G6: flow rate 1 ml/min; from 0 to 20 min: B increases linearly from 2.5 to 25%; from 20 to 25 min: B is stable at 25%; from 25 to 28 min: B decreases linearly from 25 to 2.5% 

It is thus possible according to the described method to synthesize the new glucosylated derivatives of Gallic acid, Propyl Gallate and Epigallocatechin Gallate: the resulting products are a family of substances containing at least a monoglucosylated derivative.

Example 9

Enzymatic Synthesis of O-α-D-Glycosides of Caffeic Acid Phenethyl Ester, Chlorogenic Acid and 3,4-Dihydroxybenzophenone

Reaction media were prepared as described in example 1, Taxifolin being replaced by Caffeic acid Phenethyl ester (SIGMA, reference C8221), or by Chlorogenic acid (SIGMA, reference C3878) or by 3,4-dihydroxybenzophenone (ALDRICH, reference 579815) and the DMSO concentrations were 15% and 25% v/v. 

[0500] 3,4-Dihydroxymandelic acid contains a pyrocatechol structure as Taxifolin, Pyrocatechol, Protocatechuic acid, Caffeic acid; nevertheless, no glucosylated derivative of 3,4-Dihydroxymandelic acid has been synthesized in the present conditions.
After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min</th>
<th>Retention time, min</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (DMSO 15 and 25%)</td>
<td>20.15</td>
<td>17.42 and 16.88: majoritary products</td>
<td>G2</td>
</tr>
<tr>
<td>Phenethyl ester</td>
<td>Phenethyl ester</td>
<td>18.42, 15.65, 14.22 and 13.77 O-α-glucosides of Caffeic acid Phenethyl ester</td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>15.53</td>
<td>11.00 and 10.67 Chlorogenic acid mono-O-α-glucoside and Chlorogenic acid di-O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzophenone</td>
<td>32.35</td>
<td>27.98 and 27.68</td>
<td>G1</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzophenone C-glucoside and 3,4-Dihydroxybenzophenone O-α-glucoside and 3,4-Dihydroxybenzophenone di-O-α-glucoside</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis Conditions:

G1: see example 8

G2: flow rate 1 ml/min; from 0 to 20 min: B increases linearly from 40 to 80%; from 20 to 22 min: B is stable at 80%; from 22 to 27 min: B decreases linearly from 80 to 40%.

Example 10

Enzymatic Synthesis of O-α-D-Glycosides of Catechin, Eriodictyol, Fisetin, Oleuropein and Nordihydroguaiaretic Acid

Reaction media were prepared as described in example 1, Taxifolin being replaced by catechin (FLUKA, reference 22110), or by Eriodictyol (EXTRASYNTHESSE, reference 0056), or by Fisetin (SIGMA, reference F4043), or by Oleuropein (EXTRASYNTHESSE, reference 0204) or by Nordihydroguaiaretic acid (EXTRASYNTHESSE, reference 6135) and the DMSO concentrations were 15% and 25% v/v.

After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.
It is thus possible according to the described method to synthesize the new glucosylated derivatives of Catechin, Eriodictyol, Fisetin, Oleuropein and Nordihydroguaiaretic acid: the resulting products are a family of substances containing at least a monoglucosylated derivative.

Example 11

Enzymatic Synthesis of O-α-D-Glycosides of Catechin, 3,4-Dihydroxybenzoic Acid, Gallic Acid, Rosmarinic Acid, Caffeic Acid and Chlorogenic Acid in Strictly Aqueous Media

Reaction media were prepared as described in example 1, Taxifolin being replaced by Catechin (FLUKA, reference 22110) at a concentration of 7.5 g/L, or by 3,4-Dihydroxybenzoic acid (ALDRICH, reference D10, 980-0) at a concentration of 9.0 g/L, or by Gallic acid (FLUKA, reference 486SO) at a concentration of 9.0 g/L, or by Rosmarinic acid (FLUKA, reference 44699) at a concentration of 7.5 g/L, or by Caffeic acid (SIGMA, reference C0625) at a concentration of 9.0 g/L, or by Chlorogenic acid (SIGMA, reference C3878) at a concentration of 7.5 g/L. The DMSO was omitted whereas the sodium acetate buffer concentration was increased to 100 mM and the enzyme activity was reduced to 1.0 U/mL.

After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40:60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min</th>
<th>Retention time, min</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>13.57</td>
<td>12.57</td>
<td>G1</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>5.95</td>
<td>6.75</td>
<td>G1</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>18.62</td>
<td>14.27</td>
<td>G1</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>10.58</td>
<td>6.83</td>
<td>G1</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>27.65</td>
<td>10.95</td>
<td>G1</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>15.63</td>
<td>12.63</td>
<td>G1</td>
</tr>
</tbody>
</table>

Example 12

Attempt for the Enzymatic Synthesis of O-α-D-Glycosides of Ellagic Acid, Alizarin, Epinephrine, Rutin and Baicalein

Reaction media were prepared as described in example 1, Taxifolin being replaced by Ellagic acid (FLUKA, reference 45140), or by Rutin (SIGMA, reference RS143), or by Alizarin (EXTRASYNTHETE, reference 0411), or by Epinephrine (SIGMA, reference E4250), or by Baicalein (FLUKA, reference 11712). The DMSO concentration was 25% v/v.

After 6 hours and 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40:60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min</th>
<th>Retention time, min</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellagic acid</td>
<td>27.42</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G1</td>
</tr>
<tr>
<td>Rutin</td>
<td>26.33</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G1</td>
</tr>
<tr>
<td>Alizarin</td>
<td>19.17</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G2</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>5.96</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G6</td>
</tr>
<tr>
<td>Baicalein</td>
<td>11.60</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G4</td>
</tr>
</tbody>
</table>

Analysis Conditions:

G1: see example 8
G2: see example 9
G6: see example 8
0538 Though the tested substances contain a pyrocatechol structure, the substituents of the ring do not allow their recognition by the enzyme. In the case of rutin, the saccharide part of quercetin 3-O-rutinoside appears to be very important for the enzyme recognition since quercetin is glucosylated in the 3' and/or 4' position (BERTRAND et al.) whereas rutin is not.

1-25. (canceled)
26. A method for producing a phenolic compound O-α-glucoside comprising incubating sucrose and a glucoamylase from _Leuconostoc_ species with a phenolic compound having the following formula:

\[
\begin{align*}
R2 & \quad \text{H or OH; and} \\
R1 & \quad \text{is selected from the group consisting of:}
\end{align*}
\]

\[
\begin{align*}
R3 & \quad \text{H or OH; with the} \\
R4 & \quad \text{proviso that at least one among R3 and R4 represents} \\
R7 & \quad \text{OH;}
\end{align*}
\]

\[
\begin{align*}
R8 & \quad \text{R7 is selected from the group consisting of H,} \\
R9 & \quad \text{OH or —OCOR and R8 is H or OH, with the} \\
R10 & \quad \text{proviso that at least one among R7 and R8 represents} \\
R11 & \quad \text{OH;}
\end{align*}
\]

\[
\begin{align*}
R5 & \quad \text{OH or OCH}_3; \\
R6 & \quad \text{H or OH, R9 is H or OH,} \\
R10 & \quad \text{H, OCH}_3 \text{ or C}_6\text{H}_{11}\text{O}_3, \text{ and R11 is H, OH or} \\
R12 & \quad \text{C}_6\text{H}_{11}\text{O}_3, \text{ with the proviso that R10 and R11 cannot be} \\
R12 & \quad \text{both H when R5 and R6 are both OH and that when R10} \\
R12 & \quad \text{is C}_6\text{H}_{11}\text{O}_3 \text{ then R11 is H;}
\end{align*}
\]

\[
\begin{align*}
—(\text{CH}_2)_n—\text{CONHR, with n being an integer from 0 to 2;} \\
—(\text{CR12}—\text{CH})—\text{COOR} \quad \text{or} \quad —(\text{CR12}—\text{CH})—\text{CONHR,} \\
R12 & \quad \text{R12 being H or a C}_1—\text{C}_n \text{ linear or cyclic alkyl or alkenyl;}
\end{align*}
\]

\[
\begin{align*}
—(\text{CH}_2)_n—\text{OR} \quad \text{or} \quad —(\text{CH}_2)_n—\text{NHR with n being an integer from 0 to 2;}
\end{align*}
\]
—(CH₃)ₙ—COR or —(CH=CH)ₙ—COR with n being an integer from 0 to 2:

—H;

a C₁₋C₁₀ hydrocarbon group which forms with the represented ring of formula (I) a bicyclic or tricyclic fused ring together with the ortho carbon of R₁, wherein said ring can be interrupted by at least one heteroatom;

wherein R is H or a linear, branched, or cyclic, aromatic or non-aromatic, saturated or unsaturated, C₁₋C₁₀ hydrocarbon group, that may be interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkylnyl, which can be substituted by one or several substituents selected from the group consisting of an (C₁₋C₅)aryl, an (C₅₋C₂₀)heterocycle, an (C₁₋C₅)alkoxy, an (C₁₋C₅)acyl, an (C₁₋C₅) alcohol, a carboxylic group (—COOH), an (C₂₋C₅) ester, an (C₁₋C₅) amine, an amino group (—NH₂), an amide (—CONH₂), an (C₁₋C₅) imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an (C₁₋C₂) halogenoalkyl, a thiol (—SH), a (C₁₋C₅) thio-alkyl, a (C₁₋C₅) sulfone, a (C₁₋C₅) sulfoxide, and a combination thereof.

27. The method according to claim 26, wherein R₁ of the phenolic compound is

wherein R₇ is selected from the group consisting of H, —OH or —OCOR and R₈ is H or OH, with the proviso that at least one among R₇ and R₈ represents OH.

28. The method according to claim 27 wherein said phenolic compound is taxifolin, eriodictyol, ditydroorobinetin or fustin.

29. The method according to claim 26, wherein R₁ of the phenolic compound is

wherein R₅ is OH or OCH₃, R₆ is H or OH, R₉ is H or OH, R₁₀ is H, OCH₂ or C₅H₅O₃, and R₁₁ is H, OH or C₅H₅O₃, with the proviso that R₁₀ and R₁₁ cannot be both H when R₅ and R₆ are both OH and that when R₁₀ is C₅H₅O₃ then R₁₁ is H.

30. The method according to claim 29, wherein said phenolic compound is selected from catechin, epicatechin, catechin gallate, epicatechin gallate, gallolatechin, epiigallocatechin, gallolatechin gallate and epigallocatechin gallate.

31. The method according to claim 26, wherein R₁ of the phenolic compound is

wherein R₅ is OH or OCH₃, R₆ is H or OH, R₉ is H or OH, R₁₀ is H, OCH₂ or C₅H₅O₃, and R₁₁ is H, OH or C₅H₅O₃, with the proviso that R₁₀ and R₁₁ cannot be both H when R₅ and R₆ are both OH and that when R₁₀ is C₅H₅O₃ then R₁₁ is H.

32. The method according to claim 31, wherein the phenolic compound is selected from the group consisting of rhamnetin, fisetin, robinetin, gossypetin, orientin, homoorientin and cirsiliol.

33. The method according to claim 26, wherein R₁ of the phenolic compound is —(CH₂)ₙ—COOR or —(CH₂)ₙ—CONH₂ with n being an integer from 0 to 2.

34. The method according to claim 33, wherein the phenolic compound is selected from the group consisting of homoprotocatechuic acid, dihydrocaffeic acid, protocatechuic acid ethyl ester, propyl gallate, gallic acid, hamamelitannin (2',5'-O-galloyl-hamamelose) and protocatechuic acid.

35. The method according to claim 26, wherein R₁ of the phenolic compound is —(CR₁₂—CH)—COOR or
—(CR12—CH)—CONHR, R12 being H or a C1-C8 linear, branched or cyclic alkyl or alkenyl.

36. The method according to claim 35, wherein the phenolic compound is selected from the group consisting of caffeic acid, rosmarinic acid, esculetin, 4-methylesculetin, nardalbergin, chlorogenic acid, caffeic acid phenyl ester, chemic acid, echinacoside and verbascoside.

37. The method according to claim 26, wherein R1 of the phenolic compound is —(CH2)n—COR or —(CH=CH)n—COR with n being an integer from 0 to 2.

38. The method according to claim 37, wherein the phenolic compound is selected from the group consisting of maclurine, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzophenone, butein, 3,4-dihydroxyacetophenone, marcen, and eriodictyolalcohol one.

39. The method according to claim 26, wherein R1 of the phenolic compound is selected from the group consisting of:
   a) —(CH2)n—OR or —(CH2)n—NHR with n being an integer from 0 to 2;
   b) —H;

40. The method according to claim 26, wherein R1 of the phenolic compound is a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a bicyclic or tricyclic fused ring together with the ortho carbon of R1, wherein said ring may be interrupted by at least one heteroa tom.

41. The method according to claim 39, wherein the phenolic compound is selected from the group consisting of:

42. A phenolic compounds O-α-glucoside having the following formula:

wherein:
A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;
R2 is H or OH; and
R1 is selected from the group consisting of:

wherein R3 and R4, independently, are H or HO, with the proviso that at least one among R3 and R4 represents OH;
wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH, and at least one among R7 and R8 is OH;

wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3, or C6H5Ox, and R11 is H, OH or C6H5Ox, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H5Ox then R11 is H;

—(CH2)n—COOR or —(CH2)n—CONHR, with n being an integer from 0 to 2;

—(CR12—CH)—COOR or —(CR12—CH)—CONHR, R12 being H or a C1—C6 linear, branched or cyclic alkyl or alkenyl;

—(CH2)n—OR or —(CH2)n—NHR with n being an integer from 0 to 2;

—(CH2)n—COR or —(CH—CH)—COR with n being an integer from 0 to 2;

—H;

a C1—C10 hydrocarbon group which forms with the represented ring of formula (I) a bicyclic or tricyclic fused ring together with the ortho carbon of R1, and wherein said ring may be interrupted by at least one heteroatom; wherein R is H or a linear, branched, or cyclic, aromatic, non-aromatic, saturated or unsaturated C1—C10 hydrocarbon group, that may be interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkynyl, which can be substituted by one or several substituents selected from the group consisting of an (C3—C9)aryl, an (C4—C9)heterocycle, an (C1—C5)alkoxy, an (C2—C5)acyl, an (C1—C3) alcohol, a carboxylic group (—COOH), an (C2—C5)ester, an (C2—C5)amine, an amino group (—NH2), an amide (—CONH2), an (C1—C5)imine, a nitrite, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an (C1—C3)halogenoalkyl, a thiol (—SH), a (C1—C3)thioalkyl, a (C1—C5)sulfone, a (C1—C5)sulfoxide, and a combination thereof.

43. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is

44. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is

45. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is
46. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is \(-\left(CH_2\right)_n-\) COOR or \(-\left(CH_2\right)_n-\) CONHR with \(n\) being an integer from 0 to 2.

47. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is \(-\left(CR_12-CH\right)-\) COOR or \(-\left(CR_12-CH\right)-\) CONHR, R12 being H or a \(C_1-C_6\) linear or cyclic alkyl or alkenyl.

48. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is \(-\left(CH_2\right)_n-\) OR or \(-\left(CH_2\right)_n-\) NHR with \(n\) being an integer from 0 to 2.

49. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is \(-\left(CH_2\right)_n-\) COR or \(-\left(CH-CH\right)_n-\) COR with \(n\) being an integer from 0 to 2.

50. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is selected from the group consisting of:

51. The phenolic compound O-α-glucoside according to claim 42, wherein R1 is a \(C_1-C_{10}\) hydrocarbon group which forms with the represented ring of formula (I) a bicyclic or tricyclic fused ring together with the ortho carbon of R1, and said ring may be interrupted by at least one heteroatom.

52. The phenolic compound O-α-glucoside according to claim 42, wherein said O-α-glucosyl residue is a glucose monomer.

53. The phenolic compound O-α-glucoside according to claim 42, having a 20 fold higher solubility than the corresponding aglycone in the same physiological conditions.

54. The phenolic compound O-α-glucoside according to claim 42, wherein said phenolic compound O-α-glucoside can be cleaved by an enzyme to release the corresponding aglycone.

55. A pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside according to claim 42.

56. A method for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder in a subject, comprising administering to the subject an efficient amount of a phenolic compound O-α-glucoside according to claim 42.

* * * * *