Title: COMPOSITIONS AND PROCESSES FOR PREPARING THEM

Abstract: The present invention provides a topical or therapeutic composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof. The composition may for example be an anti-ageing composition or a composition for the prevention or reduction of ageing symptoms and the treatment or prophylaxis of a numbers of diseases and conditions.
COMPOSITIONS AND PROCESSES FOR PREPARING THEM

The invention relates to topical and therapeutic compositions comprising a flavan-3-ol compound or a derivative thereof and to the use of a flavan-3-ol compound or a derivative thereof in the preparation of pharmaceutical and cosmetic compositions.

Polyphenols have been referred to as nature's biological response modifiers because of strong experimental evidence demonstrating their ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. In addition, polyphenols act as powerful antioxidants, protecting against oxidative and free radical damage, and help to prevent various diseases associated with oxidative stress. To achieve these benefits, upon consumption, usually but not necessarily as a food or a food supplement, a polyphenol must be absorbed by the body and/or a non-naturally occurring derivative must be present in the bloodstream in intimate contact with sites where its activity is required.

However, it has been estimated that most people do not consume sufficient quantities to obtain the health benefits offered by polyphenols. For example, the average current US daily intake of the polyphenol epicatechin is 2 to 4 times lower than the projected dose required to achieve a CVD (cardiovascular disease) risk reduction (Gu et al. (2004) J Nutr 134(3): 613-7; Prior and Gu (2005) Phytochemistry 66(18): 2264-80). As a result, increasing intake through the diet has emerged as an important health goal.

Orally administered flavan-3-ols with a combined molecular weight of less than 600 Daltons are readily absorbed in the small intestine where they form non-naturally occurring or non-exogenous derivatives or
metabolites. These derivatives have been shown to circulate in the bloodstream of humans and non-human mammals alike.

Orally administered flavan-3-ols with a molecular weight greater than 600 Daltons are partially absorbed in small intestine in the range up to a molecular weight of 900 Daltons and essentially not absorbed in the small intestine for molecular weights greater than 900 Daltons. Flavan-3-ols which are known not to be absorbed in the small intestine can survive the digestive process to come into intimate contact with colon cancer cells.

Circulating flavan-3-ol derivatives demonstrate flow-mediated dilatation (FMD) or vasorelaxation in vivo in humans and are shown to exhibit relaxation of isolated pre-constricted aortic rings in vitro; (Schroeter, et al. 2006, PANS 103(4), 1024-1029). Furthermore, circulating flavan-3-ol derivatives in blood plasma have been shown to increase peripheral vascular relaxation and to improve skin tone and to reduce the depth and quantity of skin lines (Neukam K. et al. 2007 Eur. J. Nutr. ,46(l) 53-6).

There is an increasing trend among adult women and men, to maintain a youthful appearance. Currently, this trend is associated with seeking to eradicate or minimise the appearance of fine lines or wrinkles on the skin, which are reflected in particular by a loss of firmness, elasticity, tonicity and/or of suppleness of the skin. There is therefore a need for compositions designed to maintain the radiant and wrinkle-free nature of skin without resorting to surgical procedures.

Human skin is composed of three primary layers: the epidermis, which provides waterproofing and serves as a barrier to infection; the dermis, which serves as a location for the appendages of skin; and the hypodermis (subcutaneous adipose layer).
The main type of cells which make up the epidermis are keratinocytes, with melanocytes and Langerhans cells also present. The epidermis contains no blood vessels, and is nourished by diffusion from the dermis.

The dermis is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many mechanoreceptor/nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal to its own cells as well as the Stratum basale of the epidermis.

The dermis is structurally divided into two areas: a superficial area adjacent to the epidermis, called the papillary region, and a deep thicker area known as the reticular region.

The papillary region is composed of loose areolar connective tissue. It is named for its fingerlike projections called papillae, that extend toward the epidermis. The papillae provide the dermis with a "bumpy" surface that interdigitates with the epidermis, strengthening the connection between the two layers of skin.

The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenous, elastic, and reticular fibers that weave throughout it. These protein fibers give the dermis its properties of strength, extensibility, and elasticity. Also located within the reticular region are the roots of the hair, sebaceous glands, sweat glands, receptors, nails, and blood vessels.
The hypodermis is not part of the skin, and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the hypodermis contains 50% of body fat).

As skin ages, it becomes thinner and more easily damaged. Intensifying this effect is the decreasing ability of skin to heal itself as a person ages. Skin aging is caused by the fall in elasticity. Aging skin also receives less blood flow and lower gland activity.

Skin ageing is a natural physiological process; it is not considered to be a pathological condition or a therapeutic disorder. There is thus a great need for further cosmetic compositions intended to minimise the signs of ageing (e.g. enhance the mechanical properties of younger skin, such as its firmness, elasticity and suppleness).

Thus according to a first aspect of the invention there is provided a topical composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof. In one embodiment the topical composition comprises a non-naturally occurring flavan-3-ol compound.

Thus according to a second aspect of the invention there is provided an orally administered cosmetic composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof. In one embodiment the orally administered cosmetic composition comprises a non-naturally occurring flavan-3-ol compound.

According to a further aspect of the invention there is provided an anti-ageing composition comprising a flavan-3-ol compound or a non-naturally
occurring derivative thereof. In one embodiment the anti-ageing composition comprises a non-naturally occurring flavan-3-ol compound.

According to a yet further aspect of the invention there is provided the use of a flavan-3-ol compound or a non-naturally occurring derivative thereof in the prevention or reduction of ageing symptoms. In one embodiment a non-naturally occurring flavan-3-ol compound is used.

Thus according to a further aspect of the invention there is provided a pharmaceutical composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof. In one embodiment the pharmaceutical composition comprises a non-naturally occurring flavan-3-ol compound.

According to a yet further aspect of the invention there is provided the use of a flavan-3-ol compound or a non-naturally occurring derivative thereof for use in the treatment or prophylaxis of cardiovascular disease and cancer. In one embodiment a non-naturally occurring flavan-3-ol compound is used.

According to yet a further aspect of the invention there is provided the use of a flavan-3-ol compound or a non-naturally occurring derivative thereof for use as a vasodilator in the prophylaxis or treatment of metabolic syndrome. In one embodiment a non-naturally occurring flavan-3-ol compound is used.

According to a still further embodiment of the invention there is provided use of a flavan-3-ol compound or a non-naturally occurring derivative thereof in the manufacture of a medicament for treatment or prophylaxis of cardiovascular disease and cancer. In one embodiment a non-naturally occurring flavan-3-ol is used.
The plasma concentration of flavan-3-ols and derivatives with molecular weight less than 900 Daltons in human blood plasma has been shown to be very much less than the quantity orally administered. This is known as partial bioavailability, typically less than 10%, more typically less than 5% of the total ingested polyphenol. Even so it is shown that blood plasma concentrations of less than 100 nM of certain metabolites give rise to vasorelaxation in excess of 6% expressed in terms of the change (increase) in the diameter of the brachial artery or in terms of the reduction is arterial stiffness index (see WO 2008017845 A1).

The present invention is advantageous as it provides compositions consisting of non-exogenous and non-naturally occurring flavan-3-ol derivatives which require lower consumption levels to provide a pharmacologically active amount. Similarly, topically applied non-exogenous non-naturally occurring flavan-3-ol derivatives, for example with molecular weights less than 600 Daltons, can provide a cost effective and pharmacologically advantageous cosmetic administration routes.

References herein to "flavan-3-ol compound" include references to a compound of formula (I):

![Flavan-3-ol Compound](image)
Examples of flavan-3-ol compounds include catechin, gallocatechin, epicatechin and epigallocatechin. Catechin and epicatechin are epimers, with (-)-epicatechin and (+)-catechin being the most common optical isomers found in nature. Catechin was first isolated from the plant extract catechu, from which it derives its name. Epigallocatechin and gallocatechin contain an additional phenolic hydroxyl group when compared to epicatechin and catechin, respectively.

In one embodiment, the flavan-3-ol compound is epicatechin (e.g. (-)-epicatechin):

![Chemical structure of epicatechin]

or a non-naturally occurring derivative thereof.

In a further embodiment, the flavan-3-ol compound is an (-)-epicatechin alpha monomer. The term "alpha" (α) indicates that the substituent is oriented below the plane of the flavan ring.

In one embodiment the flavan-3-ol compound is a flavan-3-ol compound extract from an apple. In a further embodiment, the flavan-3-ol compound is obtained from an apple in accordance with the processes described in WO 2008/017842 and WO 2008/017845 (the extraction processes of which are herein incorporated by reference).

In yet another embodiment the flavan-3-ol compound is a flavan-3-ol compound produced by glucosylation of flavan-3-ols derived from an intermediate extract rich in fructose, pectins and starches in accordance
with WO 2008/017842 and WO 2008/017845 (the extraction processes of which are herein incorporated by reference).

In yet another embodiment the fructose rich extract is converted by the application of an enzyme such as a pectinase to degrade the pectin to low molecular weight polysaccharides, nominally at a concentration of 50 ml per 1000 litres of fructose rich extract, followed by the application of suitable amounts of glucoamylase, nominally 50 ml per 1000 litres of fructose rich extract, to reduce the starch to glucose such as to produce a glucose rich extract and the said extract contains high concentrations of (-)-epicatechin monomers.

In yet another embodiment the glucose rich extract can be processed in accordance with WO 2008/017842 and WO 2008/017845 (the extraction processes of which are herein incorporated by reference) into extracts.

In yet another embodiment the glucose rich extract can be partially evaporated to produce an intermediate which is a stable glucose rich syrup with a Brix° > 32 possessing a low viscosity such that self or spontaneous fermentation does not occur. Furthermore processing is facilitated by the above intermediate in particular the processing of the intermediate includes the addition of a suitable plant cell culture, (Shimouda et al. (2007) Chemistry Letter 36(10):1292-3) or a suitable enzyme such as glycosyltransferases to transfer the sugar moiety to the (-)-epicatechin monomer.

Without being bound by theory, the flavan-3-ol compound so produced is believed to exert its anti-ageing properties via activation of the SIRT1 enzyme. SIRT1 (also known as Sir2 1 or silent mating type information regulation 2 homolog S. cerevisiae) stands for sir2uin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae), referring to the fact...
that its sirtuin homolog (biological equivalent across species) in yeast (*S. cerevisiae*) is Sir2. SIRT1 is an enzyme which deacetylates proteins that contribute to cellular regulation (reaction to stressors, longevity). SIRT1 has also been shown to promote endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase (eNOS; Mattagajasingh *et al* (2007) PNAS 104(37), 14855-14860). This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, in yeast, the SIRT1 homologue sir2 has been linked to cellular senescence. Increasing the levels of SIRT1 homologues in fruit flies and roundworms have also been shown to extend lifespan (http://genomics.senescence.info/genes/entry.php?hugo=SIRT1).

Definitions

**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 (EC3), lyases (EC4), isomerases (EC5) and ligases (EC6). 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.

**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.

Glucansucrases: common name of glucosyltransferases with the EC number 2.4.1.5.

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. Transferases that transfer hexoses (carbohydrate molecules that have six carbon atoms per molecule) are included in the sub-subclass EC 2A.I. Transferases that transfer the glucose moiety of sucrose to an acceptor are EC 2A.I A (sucrose: 1,4-\(\alpha\)-D-glucan 4-\(\alpha\)-D-glucosyltransferase; recommended name: amylosucrase), EC 2.4.1.5 (sucrose: 1,6-\(\alpha\)-D-glucan 6-\(\alpha\)-D-glucosyltransferase; recommended name: dextranucrase), EC 2.4.1.7 (sucrose: orthophosphate (\(\alpha\)-D-glucosyltransferase; recommended name: sucrose phosphorylase).

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 glucuronide.

Glycosidic bond: chemical linkage between a glycone and an other glycone or a aglycone. 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 -glycosides or -glycosides.

Aglycone: Chemical part of a glycosidic derivative which is not the glycone one.
In the context of the present invention, the term "alkyl" refers to a linear or branched saturated hydrocarbon group and 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 isomeric forms thereof. (Cl-C6)alkyl more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the other isomeric forms thereof. (Cl-C3)alkyl more specifically means methyl, ethyl, propyl, or isopropyl.

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-C3)alkenyl includes an ethenyl, a propenyl (1-propenyl or 2-propenyl).

The term "aryl" groups are mono-, bi- or tri-cyclic aromatic hydrocarbons having from 5 to 9 carbon atoms. Examples include a phenyl, in particular.

"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 heterocycles include pyridine, pyridazine, pyrimidine, pyrazine, furan, thiophene, pyrrole, oxazole, thiazole, isothiazole, imidazole, 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, benzoazole, benzothiazole and indazole (for a 6-membered ring and a 5-
membered ring). Nonaromatic heterocycles comprise in particular piperazine, piperidine, etc.

(Cl-C3)alkoxy includes methoxy, ethoxy, propyloxy, and isopropyloxy.

(C2-C3)acyl includes acetyl, propylacyl and isopropylacyl.

(Cl-C3)alcohol includes methanol, ethanol, propanol and isopropanol.

(C2-C3)ester includes methylester and ethylester.

(Cl-C3)amine includes methylamine, ethylamine, and propylamine.

(Cl-C3)imine includes methylimine, ethylimine, and propylimine.

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

(Cl-C3)halogenoalkyl includes halogenomethyl, halogenoethyl, and halogenopropyl.

(Cl-C3)thioalkyl includes thiomethyl, thioethyl, and thiopropyl.

(Cl-C3)sulfone includes methylsulfone, ethylsulfone and propylsulfone.

(Cl-C3)sulfoxide includes methylsulfoxide, ethylsulfoxide, propylsulfoxide and isopropylsulfoxide.

"Heteroatom" denotes N, S, or O.

References herein to "derivative", unless otherwise indicated by the context, includes references to any natural or non-natural (e.g. synthetic) derivative or non exogenous derivatives and includes oxidation products,
esters, methylated derivatives and glucuronidated derivatives. Oxidation products may be prepared as disclosed in US 5,554,645 (the synthetic preparation of which is herein incorporated by reference). Esters, for example, esters with gallic acid, may be prepared using known esterification reactions and as described in US 6,420,572 (the synthetic preparation of which is herein incorporated by reference). Methylated derivatives, such as 3'0-methyl-, 4'0-methyl- and 3'O, 4'0-dimethyl-derivatives may be prepared as described in Cren-Olive et al (2002) J. Chem. Soc. Perkin Trans. 1, 821-830 and Donovan et al (1999) Journal of Chromatography B, 726, 277-283 (the synthetic preparations of which is herein incorporated by reference). Glucuronidated derivatives may be prepared as described in Yu et al (2000) Organic Letters 2(16), 2539-41 and Spencer et al (2001) Free Radical Biology and Medicine 31(9), 1139-46 (the synthetic preparations of which is herein incorporated by reference). Glucuronidation may take place at the 7, 5 and/or 3' position(s). Examples of glucuronidated products include 4'-O-methyl-epicatechin-O-β-D-glucuronide (e.g. 4'-O-methyl-epicatechin-7-O-β-D-glucuronide), 3'-O-methyl-epicatechin-O-β-D-glucuronide (e.g. 3'-O-methyl-epicatechin-5/7-O-β-D-glucuronidates), and epicatechin-O-β-D-glucuronide (e.g. epicatechin-7-O-β-D-glucuronide). In one embodiment, the flavan-3-ol derivative is 4'-O-methyl-epicatechin-7-O-β-D-glucuronide or epicatechin-7-O-β-D-glucuronide.

References herein to "anti-ageing" refer to the reduction of symptoms of ageing, for example, fine lines, wrinkles, a loss of firmness, elasticity, tonicity and/or of suppleness of the skin as well as the prevention of the onset of age related diseases such as the diseases of metabolic syndrome.

The phrase "non-naturally occurring" is intended to cover non-exogenous compounds or non-exogenous forms of compounds.
Where "comprising" is used, this can be replaced by "consisting essentially of" or by "consisting of".

In one embodiment a topical composition of the present invention may be in any form suitable for application to the body surface, and may comprise, for example, a cream, lotion, solution, gel, ointment, paste or the like, and/or may be prepared so as to contain liposomes, micelles, and/or microspheres. The composition may be directly applied to the body surface or may involve use of a drug delivery device. Thus, a formulation or drug reservoir may be aqueous, i.e., contain water, or may be non-aqueous and used in combination with an occlusive overlayer so that moisture evaporating from the body surface is maintained within the formulation or transdermal system during drug administration. In some cases, however, e.g., with an occlusive gel, a nonaqueous formulation may be used with or without an occlusive layer.

Suitable formulations for topical application include ointments, creams, gels, lotions, pastes, and the like. Ointments, as is well known in the art of pharmaceutical formulation, are semisolid preparations that are typically based on petrolatum or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g., emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, non-irritating and non-sensitizing. As explained in Remington: The Science and Practice of Pharmacy, 19th Ed. (Easton, Pa.: Mack Publishing Co., 1995), at pages 1399-1404, ointment bases may be grouped in four classes: oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and
semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (W/O) emulsions or oil-in-water (O/W) emulsions, and include, for example, cetyl alcohol, glycercyl monostearate, lanolin and stearic acid. Preferred water-soluble ointment bases are prepared from polyethylene glycols of varying molecular weight; again, see Remington: The Science and Practice of Pharmacy for further information.

Creams, are also well known in the art, are viscous liquids or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase, also called the "internal" phase, is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol. The aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation is generally a nonionic, anionic, cationic or amphoteric surfactant.

As will be appreciated by those working in the field of pharmaceutical formulation, gels are semisolid, suspension-type systems. Single-phase gels contain organic macromolecules distributed substantially uniformly throughout the carrier liquid, which is typically aqueous, but also, preferably, contain an alcohol and, optionally, an oil. Preferred "organic macromolecules," i.e., gelling agents, are crosslinked acrylic acid polymers such as the "carbomer" family of polymers, e.g., carboxypolyalkylenes that may be obtained commercially under the Carbopol® trademark. Also preferred are hydrophilic polymers such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers and polyvinylalcohol; cellullosic polymers such as hydroxypropyl cellulose,
hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and methyl cellulose; gums such as tragacanth and xanthan gum; sodium alginate; and gelatin. In order to prepare a uniform gel, dispersing agents such as alcohol or glycerin can be added, or the gelling agent can be dispersed by trituration, mechanical mixing or stirring, or combinations thereof.

Lotions, as is known in the art, are preparations to be applied to the skin surface without friction, and are typically liquid or semi-liquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and preferably, for the present purpose, comprise a liquid oily emulsion of the oil-in-water type. Lotions are preferred formulations herein for treating large body areas, because of the ease of applying a more fluid composition. It is generally necessary that the insoluble matter in a lotion be finely divided. Lotions will typically contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methylcellulose, sodium carboxymethyl-cellulose, or the like.

Pastes are semisolid dosage forms in which the active agent is suspended in a suitable base. Depending on the nature of the base, pastes are divided between fatty pastes or those made from a single-phase aqueous gels. The base in a fatty paste is generally petrolatum or hydrophilic petrolatum or the like. The pastes made from single-phase aqueous gels generally incorporate carboxymethylcellulose or the like as a base.

Formulations may also be prepared with liposomes, micelles, and microspheres. Liposomes are microscopic vesicles having a lipid wall comprising a lipid bilayer, and can be used as drug delivery systems herein as well. Liposome preparations for use in the instant invention
include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the tradename Lipofectin™ (GEBCO BRL, Grand Island, N.Y.). Similarly, anionic and neutral liposomes are readily available as well, e.g., from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphostatidyl ethanolamine (DOPE), among others. These materials can also be mixed with DOTMA in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

Micelles are known in the art as comprised of surfactant molecules arranged so that their polar headgroups form an outer spherical shell, while the hydrophobic, hydrocarbon chains are oriented towards the center of the sphere, forming a core. Micelles form in an aqueous solution containing surfactant at a high enough concentration so that micelles naturally result. Surfactants useful for forming micelles include, but are not limited to, potassium laurate, sodium octane sulfonate, sodium decane sulfonate, sodium dodecane sulfonate, sodium lauryl sulfate, docusate sodium, decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, tetradecyltrimethylammonium chloride, dodecylammonium chloride, polyoxyl 8 dodecyl ether, polyoxyl 12 dodecyl ether, nonoxynol 10 and nonoxynol 30. Micelle formulations can be used in conjunction with the present invention either by incorporation into the reservoir of a topical or transdermal delivery system, or into a formulation to be applied to the body surface.
Microspheres, similarly, may be incorporated into the present formulations and drug delivery systems. Like liposomes and micelles, microspheres essentially encapsulate a drug or drug-containing formulation. They are generally although not necessarily formed from lipids, preferably charged lipids such as phospholipids. Preparation of lipidic microspheres is well known in the art and described in the pertinent texts and literature.

Various additives, known to those skilled in the art, may be included in the compositions of the present invention. For example, solvents, including alcohol, may be used to facilitate solubilization of the active agent. Other optional additives include opacifiers, antioxidants, fragrance, colorant, gelling agents, thickening agents, stabilizers, and the like. Other agents may also be added, such as antimicrobial agents, to prevent spoilage upon storage, i.e., to inhibit growth of microbes such as yeasts and molds. Suitable antimicrobial agents are typically selected from the group consisting of the methyl and propyl esters of p-hydroxybenzoic acid (i.e., methyl and propyl paraben), sodium benzoate, sorbic acid, imidurea, and combinations thereof.

Specific compounds that may be used to enhance skin permeability include: the sulfoxides dimethylsulf oxide (DMSO) and decylmethylsulf oxide (ClO MSO); ethers such as diethylene glycol monoethyl ether (available commercially as Transcutol(TM)) and diethylene glycol monomethyl ether; surfactants such as sodium laurate, sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, Poloxamer(TM) (231, 182, 184), Tween(TM) (20, 40, 60, 80) and lecithin (U.S. Pat. No. 4,783,450); the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecyclazacycloheptan-2-one (available under the trademark Azone™ from Nelson Research & Development Co., Irvine,
Calif.; see U.S. Pat. Nos. 3,989,816, 4,316,893, 4,405,616 and 4,557,934; alcohols such as ethanol, propanol, octanol, benzyl alcohol, and the like; fatty acids such as lauric acid, oleic acid and valeric acid; fatty acid esters such as isopropyl myristate, isopropyl palmitate, methylpropionate, sorbitan sesquioleate, and ethyl oleate; polyols and esters thereof such as propylene glycol, ethylene glycol, glycerol, butanediol, polyethylene glycol, and polyethylene glycol monolaurate (PEGML; see, e.g., U.S. Pat. No. 4,568,343); amides and other nitrogenous compounds such as urea, dimethylacetamide (DMA), dimethylformamide (DMF), 2-pyrrolidone, l-methyl-2-pyrrolidone, ethanol amine, diethanol amine and trethanolamine; alkanones, and organic acids, particularly salicylic acid and salicylates, citric acid and succinic acid. Percutaneous Penetration Enhancers, eds. Smith et al. (CRC Press, 1995) provides an excellent overview of the field and further background information on a number of chemical and physical enhancers.

It will be appreciated that the term "treatment" and "treating" as used herein means the management and care of a patient for the purpose of combating a condition, such as a disease or a disorder. The term is intended to include the full spectrum of treatments for a given condition from which the patient is suffering, such as administration of the active compound to alleviate the symptoms or complications, to delay the progression of the disease, disorder or condition, to alleviate or relief the symptoms and complications, and/or to cure or eliminate the disease, disorder or condition as well as to prevent the condition, wherein prevention is to be understood as the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of the active compounds to prevent the onset of the symptoms or complications. The patient to be treated is preferably a mammal, in particular a human being, but it may also include animals, such as dogs, cats, cows, sheep, horses and pigs.
Combination Therapies

Many diseases are treated using more than one medicament in the treatment, either concomitantly administered or sequentially administered. It is therefore within the scope of the invention to use the compositions of the invention in therapeutic methods for the treatment of one of the above-mentioned diseases in combination with one another, or as an adjunct to, or in conjunction with, other established therapies normally used in the treatment said disease. In one embodiment, there is provided a pharmaceutical composition as hereinbefore defined in combination with one or more additional therapeutic agent.

By analogy, it is also within the scope of the invention to use the compositions of the invention in combination with other therapeutically active compounds normally used in the treatment of one of the above-mentioned diseases in the manufacture of a medicament for said disease.

The combination treatment may be carried out in any way as deemed necessary or convenient by the person skilled in the art and for the purpose of this specification, no limitations with regard to the order, amount, repetition or relative amount of the compounds to be used in combination is contemplated.

Compositions

In one aspect, the invention provides a dosage form, which comprises a composition of the invention.

The compositions of the invention may conveniently be administered alone or in combination with pharmaceutically acceptable carriers or excipients. The compositions of the invention may be formulated with pharmaceutically acceptable carriers or diluents as well as any other
known adjuvants and excipients in accordance with conventional
techniques such as those disclosed in Remington: The Science and

Suitable pharmaceutical carriers include inert solid diluents or fillers,
sterile aqueous solution and various organic solvents. Examples of solid
carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar,
pectin, acacia, magnesium stearate, stearic acid and lower alkyl ethers of
cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil,
phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water.

Similarly, the carrier or diluent may include any sustained release
material known in the art, such as glyceryl monostearate or glyceryl
distearate, alone or mixed with a wax. The pharmaceutical compositions
formed by combining the compositions of the invention and the
pharmaceutically acceptable carriers are then readily administered in a
variety of dosage forms suitable for the disclosed routes of
administration. The formulations may conveniently be presented in unit
dosage form by methods known in the art of pharmacy.

Thus, in a further aspect, there is provided a dosage form comprising one
or more flavan-3-ol or non-naturally occurring derivative thereof, or a
pharmaceutically acceptable salt, or prodrug thereof, and one or more
pharmaceutically acceptable carriers, excipients, or diluents for use in the
treatment of cardiovascular disease or metabolic syndrome.

Thus, in a further aspect, there is provided a dosage form comprising one
or more flavan-3-ol compound or non-naturally occurring derivative
thereof, or a pharmaceutically acceptable salt, or prodrug thereof, and
one or more pharmaceutically acceptable carriers, excipients, or diluents for use in the treatment of cancer.

The dosage forms may be formulated for administration by any suitable route such as the oral, rectal, nasal, pulmonary, topical (including buccal and sublingual), transdermal, intracisternal, intraperitoneal, vaginal and parenteral (including subcutaneous, intramuscular, intrathecal, intravenous and intradermal) route, the oral route being preferred. It will be appreciated that the preferred route will depend on the general condition and age of the subject to be treated, the nature of the condition to be treated and the active ingredient chosen.

For topical use, sprays, creams, ointments, jellies, gels, inhalants, dermal patches, implants, solutions of suspensions, etc., containing the compounds of the present invention are contemplated. For the purpose of this application, topical applications shall include mouthwashes and gargles.

Compositions for oral administration include solid dosage forms, such as hard or soft capsules, tablets, troches, dragees, pills, lozenges, powders, granules, and liquid dosage forms such as solutions, emulsions, aqueous or oily suspensions, syrups and elixirs, each containing a predetermined amount of the compositions of the invention, and which may include a suitable excipient. Compositions intended for oral use may be prepared according to any known method, and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations.

Aqueous suspensions may contain the compositions of the invention in admixture with excipients suitable for the manufacture of aqueous
suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide such as lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the compositions of the invention in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as a liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the compositions of the invention in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. They may also contain buffering agents such as citrate and
phosphate buffers, effervescent agents formed from carbonates, e.g. bicarbonates such as sodium or ammonium bicarbonate, and a solic acid, for example citric acid or an acid citrate salt. Additional excipients, for example, sweetening, flavouring, and colouring agents may also be present.

The pharmaceutical compositions of the present invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example a liquid paraffin, or a mixture thereof. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known methods using suitable dispersing or wetting agents and suspending agents described above. 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-butanediol. 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 conveniently employed as solvent or suspending
medium. For this purpose, any bland fixed oil may be employed using synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

In a further embodiment, the flavan-3-ol compound or non-naturally occurring derivative thereof can be dried, for example spray-dried or dried under vacuum at a temperature less than 30°C, e.g. less than 20°C and optionally freeze-dried, and formulated into a solid dosage form.

Tablets may contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch or alginic acid; binding agents, for example, starch, gelatine or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc.

The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period.

Formulations for oral use may also be presented as hard gelatine capsules where the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or a soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Dosage forms for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or
dispersions prior to use. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art. Depot injectable formulations are also contemplated as being within the scope of the present invention.

The compositions for rectal administration of the compounds may also be in the form of suppositories. These compositions can be prepared by mixing the compositions of the invention with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will thus melt in the rectum to release the compositions of the invention. Such materials include cocoa butter and polyethylene glycols, for example.

The compositions of the invention will generally be used in an amount effective to achieve the intended result, for example in an amount effective to treat or prevent the particular disease being treated. The compositions may be administered therapeutically to achieve therapeutic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated and/or eradication or amelioration of one or more of the systems associated with the underlying disorder. Therapeutic benefit also includes halting or slowing the progression of the disease, regardless of whether improvement is realised.

The exact dosage will depend upon the frequency and mode of administration, the sex, age, weight and general condition of the subject treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those
skilled in the art. Determination of the effective dosage is well within the capabilities of those skilled in the art.

When a composition of the invention or a pharmaceutically acceptable salt, solvate or prodrug thereof is used in combination with a second therapeutic agent active against the same disease state the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

This invention also relates to an enzymatic process for preparing O-β-D-glucosides of the flavan-3-ol compound (-)-epicatechin suspended in a stable glucose-rich intermediate extract described in WO 2008/017842 and WO 2008/017845 (the extraction processes of which are herein incorporated by reference).

For this purpose, an enzymatic reaction is achieved using glucose, an abundant substance existing as a primary extract described in WO 2008/017842 and WO 2008/017845 (the extraction processes of which are herein incorporated by reference).

This reaction consists in the transfer of the glucose onto a hydroxyl group of the catechol ring by a glycosyltransferase (EC 2.4.1). Two derivatives can be obtained by this enzymatic reaction, α and the β derivative. This invention relates to the β derivative only which is a non-naturally occurring and non-exogenously produced metabolite of flavan-3-ol monomers.

When a population of glycoside derivatives results from the synthesis reaction (by population, it is understood the compounds for which the catechol ring has one of its hydroxyl group substituted or both of its hydroxyl group substituted by one glucose residue or an oligosaccharide),
only the (-)-epicatechin-β-O-D-glucoside population is said to be the product and corresponds to the invention.

Therefore, the present invention concerns a method for producing a phenolic compound O-β-glucoside comprising incubating glucose-rich flavan-3-ol containing (-)-epicatechin monomers and a glycosyltransferase (EC 2.4.1.) suspended in a water-glucose mixture.

The enzymes that can be used for this condensation reaction are glycosyltransferases, more preferably hexosyltransferases (EC 2.4.1), and in a preferred manner glucansucrases (EC 2A.1.5).

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

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-dinitrosalycilic acid. This enzymatic activity is expressed in units, wherein one unit (U) corresponds to the amount of enzyme that releases 1 mole of fructose per minute at 30 deg. C, pH 5.2 (sucrose: 100 g/L; sodium acetate buffer: 50 mM; calcium chloride dihydrate: 10 mg/L).

The process reaction is such that it enables both a proper activity of the enzyme, and a good level of solubility of the phenolic compounds in the glucose-rich flavan-3-ol intermediate extract.
The glucose-rich flavan-3-ol intermediate is incubated with the enzyme at temperature conditions that allow the enzyme to be active and to synthesize the maximum possible of desired (-)-epicatechin-O-β-D-glucoside. The temperature of the synthesis medium is maintained at a value of less than 55 Degrees Celsius, preferably ranging from 10 to 40 degrees Celsius, and preferably approximately 25 to 33 degrees Celsius.

After synthesis, the non-naturally occurring flavan-3-ol derivative, namely (-)-epicatechin-7-O-β-D-glucoside can be either used directly or purified to reach a desired purity in terms of residual of non transformed phenolic compound, sugars, enzyme and/or cosolvents. The residual enzyme may be deactivated.

For example, the non-naturally occurring (-)-epicatechin-7-O-β-D-glucoside 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 interstitial volume, the resin with the adsorbed phenolic substances can be washed with deaerated and deionised water 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 (-)-epicatechin-7-O-β-D-glucoside product. The solution containing the non-naturally occurring derivative can be concentrated by evaporation under vacuum at moderate temperature (not higher than 55 deg. 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.

Example 1: Affect of processing enzymes on epicatechin extracted from freeze-dried apple powder
The enzymes under test are as follows.

1) Pectozyme
2) Pectozyme P
3) Diazyme X4

In the first instance the HPLC, UV signature of each of the above was tested to check for the presence of materials that may coincide with the retention time of epicatechin. Solutions comprising 10μL of enzyme and 1000μl of water were prepared and analysed using the standard HPLC method.

The chromatograms, which follow, show the UV response for each of the three enzymes. The retention time of epicatechin on this column was determined as 25.5 minutes. As can be seen from the chromatograms, there are no interferants present in any of the enzyme solutions.

Figure 1, Pectozyme
Affect of enzymes on epicatechin

Having established that the enzyme solutions themselves do not interfere with the analysis of epicatechin it was then necessary to determine if the enzymes acted directly on epicatechin by reacting each in sequence of use with a solution of 90% EPI in water.
HPLC samples, showing enzyme addition sequence.

Solution 1 (enzymes only)

enzepil = (-)-epicatechin monomer (1.77mg/ml)
enzepila = Pectozyme (incubate for 30 mins at 55°C)

enzepilb = Pectozyme PowerClear P + Diazyme X4 (incubate for 30 mins at 55°C)

Solution 2 (enzymes with ascorbic acid)

enzepi2 = (-)-epicatechin monomer (1.9mg/ml) + Ascorbic acid
enzepi2a = Pectozyme (incubate for 30 mins at 55°C)

enzepi2b = Pectozyme PowerClear P + Diazyme X4 (incubate for 30 mins at 55°C)

Solution 3 (thermal degradation test)

enzepi3 = (-)-epicatechin monomer (1.94mg/ml)
enzepi3 = repeat analysis after first incubation period
enzepi3 = repeat analysis after second incubation period

The results are as follows.

<table>
<thead>
<tr>
<th>EPC</th>
<th>EPC (mg/ml)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzepil1</td>
<td>16059.2</td>
<td>1.710</td>
</tr>
<tr>
<td>enzepil2</td>
<td>17117</td>
<td>1.823</td>
</tr>
<tr>
<td>enzepil3</td>
<td>17657.3</td>
<td>1.881</td>
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<tr>
<td>enzepila1</td>
<td>15717.3</td>
<td>1.674</td>
</tr>
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</table>
enzepl2a 17074.5 1.819 99.8
enzepl3a 17254.1 1.838 97.7
enzepl1b 15003.6 1.598 93.4
enzepl2b 16459.5 1.753 96.2
enzepl3b 16769.7 1.786 95.0

Figure 4. HPLC recovery data for epicatechin only test.

Figure 5. enzepil, Epicatechin (1.77mg/ml)

Figure 6. enzepila, Pectozyme (incubate for 30 mins at 55°C)
The EPC recovery data along with the above chromatography shows that although there is an ongoing decrease in the analysis figure for EPC, the results are comparable. This indicates that the enzymes have no direct affect on the level of (-)-epicatechin monomer in solution.

Example 2

Affect of enzymes on freeze-dried apple powder extract.

Three samples of apple powder were transferred to separate 50ml centrifuge tubes each was extracted with water at 95°C for 15 minutes then analysed. Samples 2 and 3 were then treated with Pectozyme then analysed. Sample three was then treated with and diazyme (see table below for sample sequence)

APP 1 Apple powder + 95°C water initial level
APP 1a Apple powder + 95°C water after first incubation time
APP 1b Apple powder + 95°C water after second incubation time
APP 2 Apple powder + 95°C water, after 45min incubation at 55°C
APP 2a Apple powder + 95°C water + Pektozyme, after 45min at 55°C
APP 2b Apple powder + 95°C water + Pektozyme, after 45min at 55°C

APP 3 Apple powder + 95°C water, after 90min incubation at 55°C
APP 3a Apple powder + 95°C water + Pektozyme, after 90min at 55°C
   Apple powder + 95°C water + Pektozyme + P
APP 3b + Diazyme X4, after 45min incubation at 55°C

Figure 8. Identification of HPLC samples, showing enzyme addition sequence.

Freeze dried apple powder no added Vit C
enzymes added at a level of 150μl
Ascorbic added to analysis vial as preservative at a 1.07mg

<table>
<thead>
<tr>
<th>Sample</th>
<th>wt (g)</th>
<th>Free vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.22</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>3.18</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>2.99</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>EPC Area</th>
<th>EPC (mg/ml)</th>
<th>EPC %</th>
<th>EPC mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP 1</td>
<td>2159.4</td>
<td>0.230</td>
<td>100.0</td>
<td>1428.6</td>
</tr>
<tr>
<td>APP 2</td>
<td>2155.1</td>
<td>0.230</td>
<td>100.0</td>
<td>1877.9</td>
</tr>
<tr>
<td>APP 3</td>
<td>2120.7</td>
<td>0.226</td>
<td>100.0</td>
<td>1965.5</td>
</tr>
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</table>
APP 1a  2273.1  0.242  105.3  1503.8
APP 2a  2349.0  0.250  109.0  2046.9
APP 3a  2320.8  0.247  109.4  2150.9
APP 1b  2236.9  0.238  103.6  1479.8
APP 2b  2317.5  0.247  107.5  2019.4
APP 3b  2299.6  0.245  108.4  2131.3

Figure 9. HPLC analysis data.

Figure 10. Chromatogram of apple extract prior to addition of Pectozyme Max Juice.
Figure 11. Chromatogram of apple extract after treatment with Pectozyme MaxJuice.

Figure 12. Chromatogram of apple extract after treatment with Pectozyme MaxJuice and subsequent additions of Pectozyme PowerClear P and Diazyme X4
Figure 13. Chromatography overlay of figures 10, 11 and 12.
Figure 14. Annotated chromatogram overlay of figures 10, 11 and 1.

When the mg/kg figures are examined for the sequence above it can be seen that there is a small fall off in the epicatechin amount. Examination of the spectra of the individual EPC peaks shows no change during the enzyme addition sequence. Also taking into account the information from tests using epicatechin alone, it can be concluded that the epicatechin extracted from freeze dried apple powder has not been affected by the addition of the enzymes.

**Difference Loss**

<table>
<thead>
<tr>
<th>mg/kg</th>
<th>mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP 1</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure 15. (-)-epicatechin monomer loss over time

This loss is most likely attributable to thermal degradation / oxidation of epicatechin due to time spent at elevated temperatures. The addition of a solution containing ascorbic acid to the apple powder prior to its initial extraction with water at 95°C would reduce this affect. However, the result of increasing the ambient levels of ascorbic acid in solution would be an overall reduction in solution pH. This lowering of the pH could affect the functionality of the enzymes.

In order to test the affect the above experiment was duplicated and ascorbic acid introduces as described, prior to the addition of the hot water.

Example 3

Affect of enzymes on freeze-dried apple powder extract with added ascorbic acid

APPV 1 Apple powder + 95°C water initial level
APPV Ia Apple powder + 95°C water after first incubation time
APPV Ib Apple powder + 95°C water after second incubation time

APPV 2 Apple powder + 95°C water, after 45min incubation at 55°C
APPV 2a Apple powder + 95°C water + Pektozyme, after 45min at 55°C
APPV 2b Apple powder + 95°C water + Pektozyme, after 45min at 55°C

APPV 3 Apple powder + 95°C water, after 90min incubation at 55°C
APPV 3a Apple powder + 95°C water + Pektozyme, after 90min at 55°C
APPV 3b Apple powder + 95°C water + Pektozyme + P + Diazyme X4, after 45min at 55°C

Figure 16. Identification of HPLC samples, showing enzyme addition sequence

Freeze dried apple powder added Vit C
enzymes added at a level of 150µl

<table>
<thead>
<tr>
<th>Sample</th>
<th>wt (g)</th>
<th>Free Vol</th>
</tr>
</thead>
<tbody>
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<td>3.43</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>3.36</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>3.48</td>
<td>27</td>
</tr>
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</table>

Ascorbic added, 32. ling per sample

<table>
<thead>
<tr>
<th></th>
<th>EPC Area (mg/ml)</th>
<th>EPC</th>
<th>EPC %</th>
<th>mg/kg</th>
<th>mg/kg</th>
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<tr>
<td>APPV 1</td>
<td>2204.8 0.235</td>
<td>100.0</td>
<td>1437.8 0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APPV 2</td>
<td>2289.3 0.244</td>
<td>100.0</td>
<td>1886.8 0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APPV 3</td>
<td>2325.6 0.248</td>
<td>100.0</td>
<td>1921.8 0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 17. HPLC analysis data
The addition of ascorbic acid has not affected the activity of the enzymes, and has preserved the extracted epicatechin from thermal degradation/oxidation.

Other affects of adding the enzymes have been noticed.
1). The ability to provide a clear extract when the samples were centrifuged, this leads to an increase in the level of recovery of (−)-epicatechin.
2). The removal of the pink colouration from the solution.
3). The level of chlorogenic acid is reduced, however a new peak (27.5 min) is seen which has the same UV signature as chlorogenic acid. See figure 14.

These affects are best shown in the following pictures, which were taken during the test run using ascorbic acid.
Figure 18. Test samples after initial water addition (no enzymes)

Figure 19. Test samples after addition of Pektozyme to 2 and 3
Figure 20. Test samples after addition of PowerClear P and Diazyme X4 to sample 3

The overall conclusion is that the addition of enzymes does not affect the epicatechin that is extracted into solution and that processing will be aided by the improved settling characteristics of the remaining solids in solution.
CLAIMS

1. A topical or therapeutic composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof.

2. An anti-ageing composition comprising a flavan-3-ol compound or a non-naturally occurring derivative thereof.

3. Use of a flavan-3-ol compound or a non-naturally occurring derivative thereof in the prevention or reduction of ageing symptoms.

4. Use of a flavan-3-ol compound or a non-naturally occurring derivative thereof in the reduction or prophylaxis of the symptoms of metabolic syndrome, including cardiovascular disease, peripheral vascular disease, diabetes, heart disease, vascular dementia and atherosclerosis.

5. The composition or use as defined in any preceding claim wherein the flavan-3-ol compound is a compound of formula (I):

\[
\begin{align*}
\text{HO} & \quad \text{O} & \quad \text{OH} \\
\text{HO} & \quad \text{O} & \quad \text{OH} \\
\text{6} & \quad \text{8} & \quad 2 \\
\text{3} & \quad 4 & \quad \text{OH} \\
\end{align*}
\]

6. The composition or use as defined in any of claims 1 to 3 wherein the flavan-3-ol compound is selected from catechin, gallocatechin, epicatechin and epigallocatechin.
7. The composition or use as defined in claim 5 wherein the flavan-3-
ol compound is an epicatechin monomer (e.g. (-)-epicatechin):

\[
\text{\includegraphics[width=0.2\textwidth]{image.png}}
\]

or a non-naturally occurring derivative thereof.

8. The composition or use as defined in claim 6 wherein the flavan-3-
ol compound is an (-)-epicatechin alpha monomer.

9. The composition or use as defined in any preceding claims wherein
the flavan-3-ol compound is a flavan-3-ol compound isolated from an
apple.

10. The composition or use as defined in any preceding claims wherein
the flavan-3-ol compound or a derivative thereof is selected from
oxidation products, esters, methylated derivatives and glucuronidated
derivatives.

11. The composition or use as defined in claim 10 wherein the flavan-
3-ol compound or a derivative thereof is a glucuronidated derivative.

12. The composition or use as defined in claim 10 or claim 11 wherein
the glucuronidated derivative is selected from 4'-O-methyl-epicatechin-O-
\( \beta \)-D-glucuronide (e.g. 4'-O-methyl-epicatechin-7-O- \( \beta \)-D-glucuronide), 3'-
O-methyl-epicatechin-O- \( \beta \)-D-glucuronide (e.g. 3'-O-methyl-epicatechin-
5/7-O- \( \beta \)-D-glucuronides), and epicatechin-O- \( \beta \)-D-glucuronide (e.g.
epicatechin-7-O- \( \beta \)-D-glucuronide).
13. The composition or use as defined in claim 11 wherein the glucuronidated derivative is selected from 4’-O-methyl-epicatechin-7-O-β-D-glucuronide or epicatechin-7-O-β-D-glucuronide.

14. The composition as defined in any of claims 1, 2 or 4 to 12 wherein the composition comprises a cream, lotion, solution, gel, ointment or paste.

15. The composition as defined in any claims 1, 2 or 4 to 12 wherein the composition comprises a pharmaceutical preparation.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/353 A61K31/7048 A61P39/00 A61P39/06

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

B. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search
19 August 2009

Date of mailing of the international search report
01/09/2009

Name and mailing address of the ISA/
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk
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Fax (+31-70) 340-3016

Authorized officer
Garabatos-Perera, J
<table>
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### DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>NATSUME MIDORI ET AL: &quot;Inhibitory effects of conjugated epicatechin metabolites on peroxynitrite-mediated nitrotyrosine formation&quot; JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, vol. 42, no. 1, January 2008 (2008-01), pages 50-53, XP002541444 ISSN: 0912-0009 abstract page 50, left-hand column, lines 1-11 page 50, right-hand column, lines 1-15 page 51, right-hand column, line 5 - page 52, left-hand column, line 18 page 51; figure 2</td>
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<td>STEFFEN ET AL: &quot;Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase&quot; ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 469, no. 2, 8 November 2007 (2007-11-08), pages 209-219, XP022399946 ISSN: 0003-9861 abstract page 209, left-hand column, lines 1-13 page 212, left-hand column, lines 30-40; table 1</td>
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This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a):

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically Claims Nos:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims it is covered by claims Nos.

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee
- [ ] The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the Invitation
- [ ] No protest accompanied the payment of additional search fees

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1 - 4, 9, 14 - 15 (partially); 5 - 8 (complete)
   Use of hydroxy-substituted flavan-3-ol s (e.g., catechin, gallocatechin, epicatechin and epigallocatechin) in the treatment / prevention / reduction of the symptoms of ageing and metabolic syndrome.

2. claims: 1 - 4, 9, 14 - 15 (partially); 10 - 13 (complete)
   Use of non-hydroxy-substituted flavan-3-ol s (e.g., esterified, methylated, glucuronated) in the treatment / prevention / reduction of the symptoms of ageing and metabolic syndrome.
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