



(51) International Patent Classification:

A61K 8/35 (2006.01) *A61K 8/97* (2006.01)
A61K 8/49 (2006.01) *A61Q 19/08* (2006.01)

(21) International Application Number:

PCT/EP2012/056979

(22) International Filing Date:

17 April 2012 (17.04.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

11167802.5 27 May 2011 (27.05.2011) EP

(71) **Applicant** (for AE, AG, AU, BB, BH, BW, BZ, CA, CY, EG, GB, GD, GH, GM, IE, IL, KE, KN, LC, LK, LS, MT, MW, MY, NA, NG, NZ, OM, PG, QA, RW, SC, SD, SG, SL, SZ, TT, TZ, UG, VC, ZA, ZM, ZW only): **UNILEVER PLC** [GB/GB]; Unilever House, 100 Victoria Embankment, London Greater London EC4Y 0DY (GB).

(71) **Applicant** (for all designated States except AE, AG, AU, BB, BH, BW, BZ, CA, CY, EG, GB, GD, GH, GM, IE, IL, IN, KE, KN, LC, LK, LS, MT, MW, MY, NA, NG, NZ, OM, PG, QA, RW, SC, SD, SG, SL, SZ, TT, TZ, UG, US, VC, ZA, ZM, ZW): **UNILEVER N.V.** [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).

(71) **Applicant** (for IN only): **HINDUSTAN UNILEVER LIMITED** [IN/IN]; Unilever House, B.D. Sawant Marg Chakala Andheri (East), Mumbai, Maharashtra 400 099 (IN).

(72) Inventors; and

(75) **Inventors/Applicants** (for US only): **FOWLER, Mark, Ian** [GB/GB]; Unilever R&D Colworth, Sharnbrook, Bed-

ford Bedfordshire MK44 1LQ (GB). **JENKINS, Gail** [GB/GB]; Unilever R&D Colworth, Sharnbrook, Bedford Bedfordshire MK44 1LQ (GB). **WAINWRIGHT, Linda, Jane** [GB/GB]; Unilever R&D Colworth, Sharnbrook, Bedford Bedfordshire MK44 1LQ (GB).

(74) **Agent**: **ACHAM, Nicholas, Clive**; Unilever PLC, Unilever Patent Group, Colworth House, Sharnbrook, Bedford Bedfordshire MK44 1LQ (GB).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: ANTI-AGEING COMPOSITION

(57) **Abstract**: This invention relates to the provision of a topical or oral anti-ageing skin composition. Skin ageing, such as the appearance of fine lines, wrinkles or sagging, is a major age-related consumer issue. There is a continuing need for effective products to prevent or reduce the on-set of skin ageing. Thus in one aspect of the invention, a topical or oral composition for delaying the visible signs of skin ageing is provided, the composition comprising curcumin and a composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution.



ANTI-AGEING COMPOSITION

This invention relates to the provision of a topical or oral anti-ageing skin composition.

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Skin ageing, such as the appearance of fine lines, wrinkles or sagging, is a major age-related consumer issue. There is a continuing need for effective products to prevent or reduce the on-set of skin ageing.

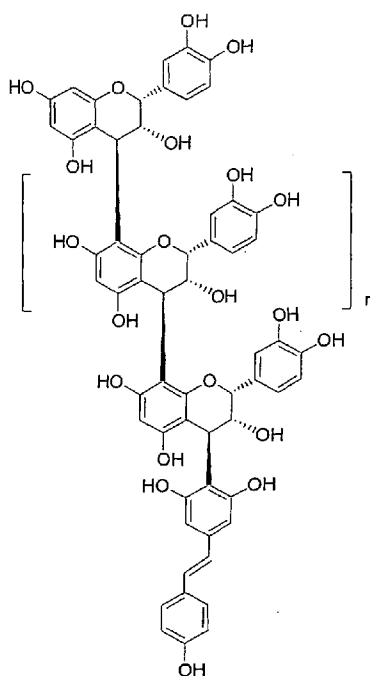
- 10 Gems et al (Mechanisms of Ageing and Development, 126, 381-387 (2005)) describes the green theory of ageing in which it is suggested that accumulation of lipophilic toxic by-products from stochastic errors of metabolism which are therefore not recognisable to the cell lead to molecular damage and ageing. Gems et al further describe how the smooth endoplasmic reticulum, a eukaryotic
- 15 organelle, acts as a cellular filter deploying phase 1 and phase 2 metabolism to mobilise and excrete the lipophilic toxins. Phase 1 metabolism results in addition of chemically reactive functional groups which allow further metabolism of otherwise unreactive lipophilic compounds. Phase 2 metabolism involves the addition of side groups which increase solubility aiding excretion. Cytochrome
- 20 P450 and short-chain dehydrogenases or reductases are agents of phase 1 metabolism in mammalian cells. Uridine 5'-diphospho-glucuronosyltransferases (also known as UDP-glucuronosyltransferases or UGT's), which are glycosyltransferases that catalyze addition of the glycosyl group from a uridine-5'-triphosphate (UTP)-sugar to a small hydrophobic molecule (known as the
- 25 glucuronidation reaction), are important agents of phase 2 metabolism.

- Mattson et al (Dose-Response, 5, 174-186 (2007)) disclose that curcumin is a potent inducer of numerous nuclear factor erythroid-derived 2 (Nrf2) transcription factor dependent phase 2 enzymes involved in xenobiotic detoxification. Lee et al
- 30 (Journal of Biochemistry and Molecular Biology, 37, 2, 139-143 (2004)) describe enzymes induced by curcumin include the antioxidant response element (ARE) targets: NQO1, γ -glutamylcysteine synthetase (GGCS), Heme Oxygenase-1

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(HO-1), glutathione transferases (GST), glucuronosyltransferases, and epoxide hydrolases.

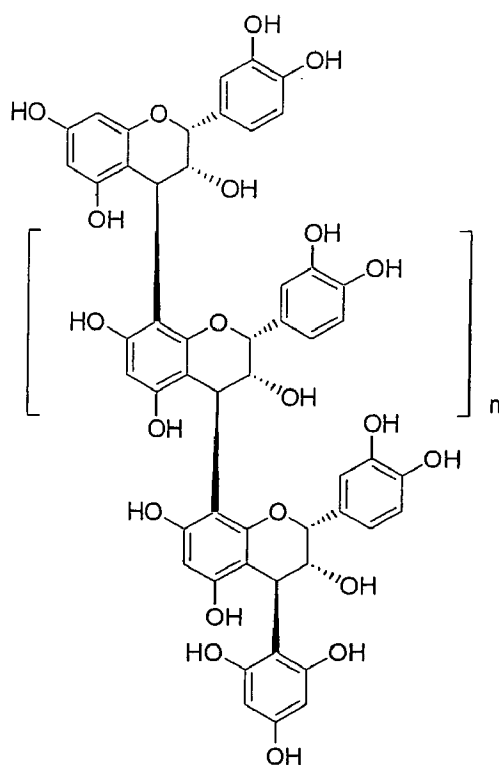
EP 1 852 430 A discloses a composition containing as its main component
5 proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating, for example at 90 to 100 degrees centigrade for 1 to 4 hours, plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol
10 ring structure or resorcinol ring structure in an acidic aqueous solution. The resulting mixture may then be filtered, concentrated, fractionated or dried using standard techniques known to the skilled person. A best mode for preparing the composition is disclosed in paragraphs [0015] to [0023] of EP 1 852 430 A and is incorporated herein by reference. Such a composition has been reduced in
15 molecular weight compared to the proanthocyanidin polymer feed stock to such a level that the oligomer can be more easily absorbed into a living body. The composition comprises compounds of structure (I):



Structure (I)

- 3 -

wherin n is 0 or an integer of 1 or 2, and/or structure (II):



Structure (II)

5

wherein n is 0 or an integer of 1 or 2. Oligomers of structure (I) and (II) are obtained by fractionating the aforementioned composition of EP 1 852 430 A using standard fractionation methods known to the skilled person in the art.

- 10 The plant material comprising proanthocyanidin polymer or extract thereof comprising proanthocyanidin polymer may be selected from the group consisting of grape, pine, *Chamaecyparis obtuse*, camphor tree, wax myrtle, cacao, date plum, banana, Chinese quince, apple, hawthorn, *Lychi chinensis*, *Myrica rubra* and *Cinnamomi cortex*. The substance having a phloroglucinol ring structure or
- 15 resorcinol ring structure may be selected from the group consisting of resveratrol, phloroglucinol, flavonoid and flavanoid. The substance having a phloroglucinol ring structure or resorcinol ring structure may also be selected from the group

consisting of green tea, fresh tea leaves, grape seed, grape seed coat, cube gambir, red algae and extracts thereof.

EP 1 852 430 A further discloses that such a composition exhibits significant anti-oxidative activity in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity assay and a Trolox Equivalent Antioxidant Capacity (TEAC) assay. It is also disclosed that application of such compositions leads to improved cell viability under ultra-violet radiation, significant anti-oxidant activity to blood serum and liver lipid peroxides (in mice) and reductions in blood serum levels of the liver enzymes glutamyl oxaloacetic transaminase (GOT) and glutamyl pyruvic transaminase (GPT). GOT and GPT are involved in transferring the amino group of amino acids from alpha-amino acids to alpha-keto acids. Transaminases are stored in the liver and are normally present only at low levels in blood serum. They are released into blood when the liver cells are damaged, thus detection of high levels of transaminases in blood serum is evidence of liver cell damage.

A composition according to EP 1 852 430 A is available commercially under the name Oligonol™ (Cognis) and comprises proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution., the plant material being an extract produced from a combination of fruit from Lychi chinensis and green leaf of Camellia sinensis.

The inventors have observed that a combination of a composition according to EP 1 852 430 A and curcumin synergistically boosts the performance of phase 2 detoxification enzymes.

30 **Summary of the invention**

Thus in a first aspect of the invention, a topical or oral composition for delaying the visible signs of skin ageing is provided, the composition comprising curcumin and

a composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising
5 proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution. The visible signs of skin ageing are selected from the group consisting of wrinkles, sagging, lines and dry skin.

- 10 The composition may comprise 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials
15 comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution.

The weight ratio of curcumin to composition containing as its main component
20 proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous
25 solution may be 50:1 to 1:50, preferably 20:1 to 1:20, most preferably 15:1 to 1:15.

The proanthocyanidin oligomer optionally has a degree of polymerisation of 2 to 4.

The plant material comprising proanthocyanidin polymer or aqueous extract
30 thereof comprising proanthocyanidin polymer may be selected from the group consisting of grape, pine, Chamaecyparis obtuse, camphor tree, wax myrtle,

cacao, date plum, banana, Chinese quince, apple, hawthorn, *Lychi chinensis*, *Myrica rubra* and *Cinnamomi cortex*.

5 The substance having a phloroglucinol ring structure or resorcinol ring structure may be selected from the group consisting of resveratrol, phloroglucinol, flavonoid and flavanoid.

10 The substance having a phloroglucinol ring structure or resorcinol ring structure may be selected from the group consisting of green tea, fresh tea leaves, grape seed, grape seed coat, cube gambir, red algae and extracts thereof.

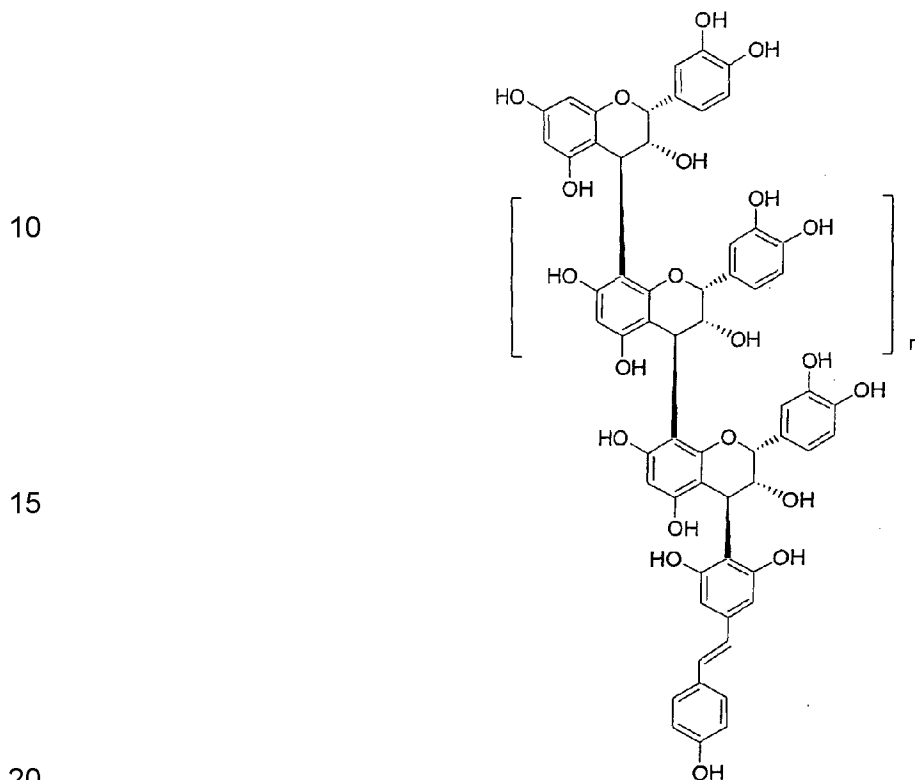
15 The composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution, is optionally obtainable by heating plant material being an extract produced from a combination of fruit from *Lychi chinensis* and green leaf of *Camellia sinensis* in an acidic aqueous solution.

25 The composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution, is optionally obtainable by heating plant material being an extract produced from a combination of fruit from *Lychi chinensis* and green leaf of *Camellia sinensis* in an acidic aqueous solution is Oligonol™.

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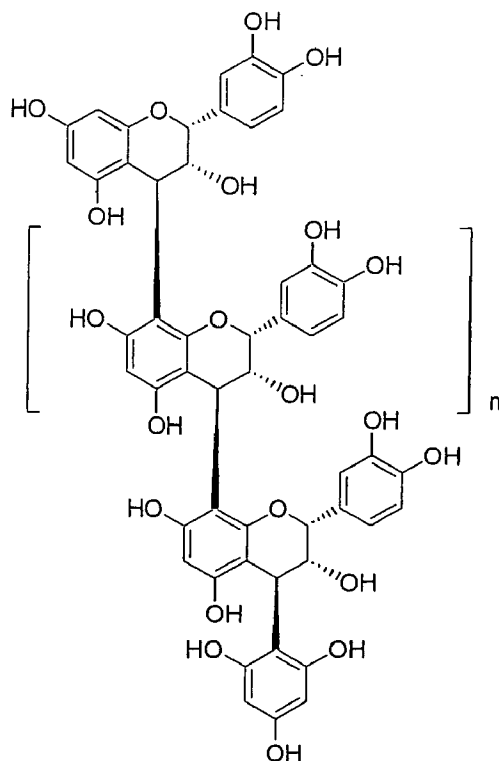
In a second aspect of the invention, a topical or oral composition for delaying the visible signs of skin ageing is provided, the composition comprising 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg a proanthocyanidin oligomer
 5 represented by structure (I):



wherein n is 0 or an integer of 1 or 2.

In a third aspect of the invention, a topical or oral composition for delaying the visible signs of skin ageing is provided, the composition comprising 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg a proanthocyanidin oligomer
 25 represented by structure (II):

- 8 -



wherein n is 0 or an integer of 1 or 2.

In a fourth aspect of the invention, a method for delaying the visible signs of skin ageing is provided, the method comprising the step of applying to the skin or imbibing the composition of any one of the preceding claims.

In a fifth aspect of the invention, use of the composition of any one of the first, second or third aspects of the invention is provided for delaying the visible signs of skin ageing.

In a sixth aspect of the invention, a composition according to any one of the first, second or third aspects of the invention is provided for use as a medicament.

In a seventh aspect of the invention, a composition according to any one of the first, second or third aspects of the invention is provided for use in delaying the visible signs of skin ageing.

In an eighth aspect of the invention, use of the composition of any one of the first, second or third aspects of the invention is provided for the manufacture of a medicament for delaying the visible signs of skin ageing.

5 **Summary of the figures**

The invention is illustrated with reference to

Figure 1 which shows in (a) procollagen 1 (PC-1) (ng per μ g protein) for control (untreated primary dermal fibroblasts), with 2.5 μ M 4-hydroxynonenal (a known toxic lipophilic metabolite), and with 2.5 μ M 4-hydroxynonenal and 10 μ M
10 curcumin (a known phase 2 detox booster) and in; (b) fibroblast collagenase, also known as matrix metalloproteinase 1 (MMP-1), (pg/ml) for control (untreated primary dermal fibroblasts), with 2.5 μ M 4-hydroxynonenal (a known toxic lipophilic metabolite), and with 2.5 μ M 4-hydroxynonenal and 10 μ M curcumin;

15 Figure 2 shows heme oxygenase 1 (HO-1) (pg per μ g protein) for control (untreated primary dermal fibroblasts), 2 μ M curcumin, 10 μ g/ml Oligonol™, and a combination of 2 μ M curcumin and 10 μ g/ml Oligonol™ (p=0.003); and

Figure 3 shows heme oxygenase 1 (HO-1) (pg per μ g protein) for control
20 (untreated primary dermal fibroblasts), 10 μ M ferulic acid, 10 μ g/ml Oligonol™, and a combination of 10 μ M ferulic acid and 10 μ g/ml Oligonol™.

Detailed description of the invention

Any commercially acceptable and conventional vehicles may be used in the
25 topical compositions of the invention, acting as diluents, dispersants and/or carriers for the aforementioned agents that synergistically boost the performance of phase 2 detoxification enzymes described herein and for any other optional but often preferred ingredients. Therefore a cosmetically acceptable vehicle suitable for use in this invention may be aqueous-based, anhydrous or an emulsion, a
30 water-in-oil or oil-in-water emulsion being generally preferred. If the use of water is desired, water typically makes up the balance of the composition, and preferably makes up from about 5 to about 99%, and most preferably from about

40 to about 80% by weight of the topical composition, including all ranges subsumed therein.

In addition to water, organic solvents may be optionally included to act as carriers or to assist carriers within the compositions of the present invention. Illustrative and non-limiting examples of the types of organic solvents suitable for use in the present invention include alkanols like ethyl and isopropyl alcohol, mixtures thereof or the like.

Other optional additives suitable for use include ester oils like isopropyl myristate, cetyl myristate, 2-octyldodecyl myristate, avocado oil, almond oil, olive oil, neopentylglycol dicaprate, mixtures thereof or the like. Typically, such ester oils assist in emulsifying the composition of this invention, and an effective amount is often used to yield a stable, and most preferably, water-in-oil emulsion.

Emollients may also be used, if desired, as carriers within the composition of the present invention. Alcohols like 1-hexadecanol (i.e. cetyl alcohol) are often desired as are the emollients generally classified as silicone oils and synthetic esters. Silicone oils suitable for use include cyclic or linear polydimethylsiloxanes containing from 3 to 9, preferably from 4 to 5, silicon atoms. Non-volatile silicone oils useful as an emollient material in the inventive composition described herein include polyalkyl siloxanes, polyalkylaryl siloxanes and polyether siloxane copolymers. The essentially non-volatile polyalkyl siloxanes useful herein include, for example, polydimethylsiloxanes.

Ester emollients that may optionally be used are:

- (1) Alkenyl or alkyl esters of fatty acids having 10 to 20 carbon atoms. Examples thereof include isoarachidyl neopentanoate, isononyl isonanonoate, oleyl myristate, oleyl stearate and oleyl oleate.
- (2) Ether-esters such as fatty acid esters of ethoxylated fatty alcohols.

- (3) Polyhydric alcohol esters such as ethylene glycol mono- and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, polyethylene glycol (200-6000) mono- and di-fatty acid esters, propylene glycol mono- and di-fatty acid esters, polypropylene glycol 2000 monooleate, polypropylene glycol 2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol poly-fatty esters, ethoxylated glyceryl mono-stearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol distearate, polyoxyethylene polyol fatty acid ester, sorbitan fatty acid esters, and polyoxyethylene sorbitan fatty acid esters are satisfactory.
- (4) Wax esters such as beeswax, spermaceti, stearyl stearate and arachidyl behenate.
- (5) Sterols esters, of which cholesterol fatty acid esters are examples.

- Emollients, when used, typically make up from about 0.1 to about 50% by weight of the composition.

Fatty acids having from 10 to 30 carbon atoms may also be included as acceptable carriers within the composition of the present invention. Illustrative examples of such fatty acids include pelargonic, lauric, myristic, palmitic, stearic, isostearic, oleic, linoleic, arachidic, behenic or erucic acid, and mixtures thereof. Compounds that are believed to enhance skin penetration, like dimethyl sulfoxide, may also be used as an optional carrier.

Humectants of the polyhydric alcohol type may also be employed in the compositions of this invention. The humectant often aids in increasing the effectiveness of the emollient, reduces scaling, stimulates removal of built-up scale and improves skin feel. Typical polyhydric alcohols include glycerol, polyalkylene glycols and more preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, hexylene glycol, 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycerol, propoxylated glycerol

and mixtures thereof. For best results the humectant is preferably propylene glycol or sodium hyaluronate. The amount of humectant may range anywhere from 0.2 to 25%, and preferably, from about 0.5 to about 15% by weight of the composition, based on total weight of the composition and including all ranges
5 subsumed therein.

Thickeners may also be utilized as part of the acceptable carrier in the compositions of the present invention. Typical thickeners include cross-linked acrylates (e.g. Carbopol 982), hydrophobically-modified acrylates (e.g. Carbopol
10 1382), cellulosic derivatives and natural gums. Among useful cellulosic derivatives are sodium carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, ethyl cellulose and hydroxymethyl cellulose. Natural gums suitable for the present invention include guar, xanthan, sclerotium, carrageenan, pectin and combinations of these gums. Amounts of the
15 thickener may range from 0.0001 to 5%, usually from 0.001 to 1%, optimally from 0.01 to 0.5% by weight of the composition and including all ranges subsumed therein.

Collectively the water, solvents, silicones, esters, fatty acids, humectants and/or
20 thickeners will constitute the acceptable carrier in amounts from 1 to 99.9%, preferably from 80 to 99% by weight of the composition and including all ranges subsumed therein.

Surfactants may also be present in compositions of the present invention. Total
25 concentration of the surfactant will range from about 0.001 to about 40%, and preferably from about 0.001 to about 20%, optimally from about 0.01 to about 5% by weight of the composition and including all ranges subsumed therein. The surfactant may be selected from the group consisting of anionic, nonionic, cationic and amphoteric actives. Particularly preferred nonionic surfactants are those with
30 a C10-C20 fatty alcohol or acid hydrophobe condensed with from 2 to 100 moles of ethylene oxide or propylene oxide per mole of hydrophobe; mono- and di- fatty acid esters of ethylene glycol; fatty acid monoglyceride; sorbitan, mono- and di-

C8-C20 fatty acids; block copolymers (ethylene oxide/propylene oxide); and polyoxyethylene sorbitan as well as combinations thereof. Alkyl polyglycosides and saccharide fatty amides (e.g. methyl gluconamides) are also suitable nonionic surfactants.

5

Preferred anionic surfactants include soap, alkyl ether sulfate and sulfonates, alkyl sulfates and sulfonates, alkylbenzene sulfonates, alkyl and dialkyl sulfosuccinates, C8-C20 acyl isethionates, acyl glutamates, C8-C20 alkyl ether phosphates and combinations thereof.

10

Fragrances may be used in the composition of this invention. Illustrative non-limiting examples of the types of fragrance that may be used include those comprising terpenes and terpene derivatives like those described in Bauer, K., et al., Common Fragrance and Flavor Materials, VCH Publishers (1990). Illustrative yet non-limiting examples of the types of fragrances that may be used in this invention include myrcene, dihydromyrenol, citral, tagetone, cis-geranic acid, citronellic acid, mixtures thereof or the like. Preferably, the amount of fragrance employed in the composition of this invention is in the range from about 0.000001 % to about 10 %, more preferably about 0.00001 % to about 5 %, most preferably about 0.0001 % to about 2 % by weight of the compound and including all ranges subsumed therein.

15

20

Various types of optional additional active ingredients may be used in the compositions of the present invention. Actives are defined as skin benefit agents other than emollients and other than ingredients that merely improve the physical characteristics of the composition. Although not limited to this category, general examples include talcs and silicas, as well as alpha-hydroxy acids, beta-hydroxy acids, zinc salts, and sunscreens.

25

Beta-hydroxy acids include salicylic acid, for example. Zinc pyrithione is an example of the zinc salts useful in the composition of the present invention.

30

Sunscreens include those materials commonly employed to block ultra-violet radiation. Illustrative compounds are the derivatives of para-aminobenzoic acid (PABA), cinnamate and salicylate. For example, avobenzophenone (Parsol 1789®) octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also
5 known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trade marks, Parsol MCX™ and Benzophenone-3™, respectively. The exact amount of sunscreen employed in the compositions can vary depending upon the degree of protection desired from the sun's ultra-violet radiation. Additives that reflect or scatter the
10 suns rays may also be employed. These additives include oxides like zinc oxide and titanium dioxide.

Many compositions, especially those containing water, should be protected against the growth of potentially harmful microorganisms. Anti-microbial
15 compounds, such as triclosan, and preservatives are, therefore, typically necessary. Suitable preservatives include alkyl esters of p-hydroxybenzoic acid, hydantoin derivatives, propionate salts, and a variety of quaternary ammonium compounds. Particularly preferred preservatives of this invention are methyl paraben, propyl paraben, phenoxyethanol and benzyl alcohol. Preservatives will
20 usually be employed in amounts ranging from about 0.1% to 2% by weight of the composition.

Still other optional ingredients that may be used with the composition of this invention include dioic acids (e.g. malonic acid and sebacic acid), antioxidants like
25 vitamin E, retinoids, including retinoic acid, retinal, retinol and retinyl esters, conjugated linoleic acid, petroselinic acid and mixtures thereof, as well as any other conventional ingredients well known for wrinkle-reducing, anti-acne effects and reducing the impact of sebum.

30 When making a topical composition of the present invention, the desired ingredients are mixed in no particular order and usually at temperatures from about 70 to about 80°C and under atmospheric pressure. The packaging for the

topical composition of the invention can be a patch, bottle, tube, roll-ball applicator, propellant driven aerosol device, squeeze container or lidded jar.

Oral compositions of the invention may be in the form of capsules, pills, tablets, granules, solutions, suspensions or emulsions. The amount of curcumin or composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution in a capsule, pill or tablet will not be in excess of the recommended daily limit for an adult person. Preparation of compositions of the invention in the aforementioned oral formats is well known to the person skilled in the art.

Example 1

Primary dermal fibroblasts were plated out at approximately 20,000 cells/cm² in Dulbecco's Modified Eagle Serum (DMEM) with 10% foetal calf serum (FCS) and incubated at 37°C until the cells attached and approached near confluence (routinely ~2 days). 2.5 µM 4-hydroxynonenal (a known toxic lipophilic metabolite) in ethanol was added for 24 hours with and without 10 µM curcumin (a known phase II detox booster) and cells were harvested by adding 1 ml of trypsin/EDTA solution (Invitrogen) and incubated at 37°C until detached. The cells were then centrifuged immediately at 13000 rpm for 10 minutes and the supernatant discarded. The resulting cell pellet was washed with 500 µl of Dulbecco's phosphated buffer solution (PBS) and centrifuged as before. The supernatant was discarded as before and the cell pellet stored at -20°C prior to cell lysis.

All cell pellets were lysed on ice for 30 minutes in 1 ml cell lysis buffer per 2.5 x 10⁶ cells. The lysis buffer contained 1% nonyl phenoxypolyethoxylethanol (Tergitol-type NP-40), 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 6 mM sodium chloride and 0.05M tris(hydroxymethyl)aminomethane at pH

7.6. Protease inhibitor cocktail (1000X; Sigma P8340) was added prior to use at a level of 10 µl/ml of lysis buffer. The partially lysed cell pellets were completely homogenised with a pellet pestle and unwanted cell debris removed by centrifugation for 20 minutes at 20,000g at 4°C. The clarified cell lysate was
5 frozen at -80°C until needed.

Cell lysate was examined for cytotoxicity using the Promega CytoTox 96 non-radioactive cytotoxicity assay. This assay quantitatively measures lactate dehydrogenase (LDH) released upon cell lysis and is a good indication of cell
10 viability. 50 µl of cell lysate or control medium was added to duplicate wells of a 96 well plate. 50 µl of CytoTox reagent was added and mixed well. The plate was incubated in the dark at room temperature for 30 minutes after which 50 µl of stop solution was added to each well and the absorbance of the plate read at 492 nm. Any test samples giving an absorbance value of more than double that of the
15 control medium was considered to be cytotoxic. No results have been included from samples that showed any signs of cytotoxicity.

The total protein concentration of each cell lysate was measured using the Pierce BCA protein assay kit so that the response to effect of the test substances could
20 be normalised to ng protein. A set of eight standard solutions ranging from 0 to 1200 µg/ml protein was prepared from the supplied 2 mg/ml bovine serum albumin (BSA) stock solution. 10 µl of standard or cell lysate was added to duplicate wells of a flat-bottomed 96-well plate. The reagent solution was prepared according to the kit instructions from 50 parts reagent A and 1 part
25 reagent B. 200 µl of the final reagent was added to each well. The plate was mixed, covered and incubated at 37°C for 30 minutes and absorbance read at 562 nm. A protein standard curve was constructed and used to determine the protein concentration of each cell lysate.

30 Collagen 1 is synthesised as the precursor molecule procollagen 1 and therefore the amount of free propeptide reflects stoichiometrically the amount of collagen 1

synthesised. The Procollagen Type I C-peptide Enzyme Immunoassay kit (Takara Bio Incorporated) allows for the quantitative determination of procollagen 1.

Eight procollagen 1 standards were prepared in sample diluent at concentrations ranging from 0 to 640 ng/ml. 100 µl of antibody-peroxidase conjugate solution and 20 µl of cell lysate (1 µg protein) or standard was added to duplicate wells. The plate was sealed and incubated at 37°C for 3 hours before being washed four times with 400 µl of phosphated buffer solution (PBS). Each well then received 100 µl of substrate solution and the plate was incubated at room temperature on the benchtop for 15 minutes. After this period, 100 µl stop solution was added to each well and absorbance measured at 450nm with a plate reader.

A standard curve was plotted of mean absorbance versus procollagen 1 concentration and the line of best fit calculated by regression analysis. The unknown concentration of procollagen 1 in all the samples was estimated from this.

The active fibroblast collagenase, also known as matrix metalloprotease 1 (MMP-1) concentration of the cell lysate was assayed using the Fluorokine E Human Active MMP-1 ELISA assay (R&D Systems DY206) according to the manufacturer's instructions. The assay is a fluorometric assay designed to quantitatively measure enzyme activity

Eight MMP-1 standards were prepared in reagent diluent at concentrations ranging from 0 to 25 ng/ml. A monoclonal antibody specific for MMP-1 had been pre-coated onto a black microplate. Samples of cell lysate were diluted appropriately in calibrator diluent. 100 µl of assay diluent was added to each test well followed by the addition of 150 µl of standard or sample to duplicate wells. The plate was sealed and incubated on a shaker at room temperature for 3 hours before being washed 4 times with 400 µl of wash buffer. 200 µl of diluted amino phenyl mercuric acetate (APMA), an activation agent, was added to each well and the plate was sealed and incubated at 37°C in a humidified environment in the

dark for 2 hours before being washed as before. 200 µl of substrate was added to each well and the plate incubated at 37°C in a humidified environment in the dark for 17-20 hours. The relative fluorescence units (RFU) of each well was determined using a fluorescence plate reader set with the following parameters:

5 excitation wavelength set to 320 nm and emission wavelength set to 405 nm; endpoint mode; 1 x 20 milliseconds integration time; plate speed = 6.

A standard curve was plotted of mean RFU versus MMP-1 concentration and the line of best fit calculated by regression analysis. The unknown concentration of

10 MMP-1 in all the samples was then estimated from this.

Figure 1 shows in (a) procollagen 1 (ng/µg protein) for control (untreated primary dermal fibroblasts), with 2.5 µM 4-hydroxynonenal (a known toxic lipophilic metabolite), and with 2.5 µM 4-hydroxynonenal and 10 µM curcumin and in; (b)

15 fibroblast collagenase (pg/ml for control (untreated primary dermal fibroblasts), with 2.5 µM 4-hydroxynonenal (a known toxic lipophilic metabolite), and with 2.5 µM 4-hydroxynonenal and 10 µM curcumin (a phase 2 detox booster). Thus when primary dermal fibroblasts were treated with 4-hydroxynonenal, a known toxic lipophilic metabolite, a significant reduction in collagen and a significant increase

20 in fibroblast collagenase were observed. Such changes are typically associated with the on-set of wrinkles, sagging and lines in skin. When the primary dermal fibroblasts were subsequently treated with curcumin, significant protection from loss of collagen and from an increase in fibroblast collagenase was observed. Curcumin is known to enhance the effect of phase 2 metabolism and thus it can

25 be seen that this effect leads directly to protection from loss of collagen or from an increase in fibroblast collagenase resulting from the effects of build-up of toxic lipophilic metabolites and hence indirectly preventing or reducing the on-set of skin ageing.

Example 2

Primary dermal fibroblasts were treated with 5 μ M 4-hydroxynonenal and aqueous solutions of the test substances for 24 hours as described in example 1 and then the level of heme oxygenase 1, a phase II metabolism enzyme, measured.

5

The preparation of the lysate and cytotoxicity and protein tests were as described for example 1. Primary dermal fibroblast cell lysate was diluted in sample diluent, and 100 μ l transferred to a pre-coated anti-human heme oxygenase 1 immunoassay plate (Assay Designs). A 7-point recombinant heme oxygenase 1 standard curve, ranging from 25 to 0.39 ng/ml, was also prepared in sample diluent. The immunoassay plate was incubated at room temperature for 30 minutes, washed six times with wash buffer, and incubated for a further 60 minutes at room temperature with 100 μ l/well of anti-heme oxygenase 1 antibody solution. The plate was washed as above and incubated for 30 minutes at room temperature with 100 μ l/well of horseradish peroxidase conjugate solution. Again the plate was washed as above and 100 μ l/well of tetramethylbenzidine substrate added to each well and stored for 15 minutes at room temperature in the dark. Following the addition of 100 μ l/well of stop solution, the absorbance was read at 450 nm and the unknown biopsy levels of heme oxygenase 1 were extrapolated from the standard curve.

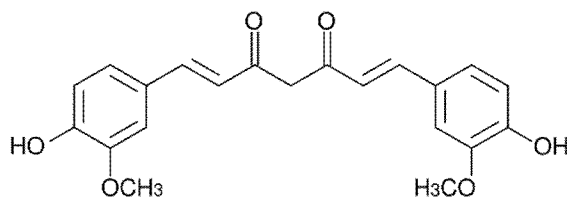
Figure 2 shows heme oxygenase 1 (HO-1) (pg per μ g protein) for a control (untreated primary dermal fibroblasts), 2 μ M curcumin, 10 μ g/ml Oligonol™, and a combination of 2 μ M curcumin and 10 μ g/ml Oligonol™. Both curcumin and Oligonol™ appear to increase the level of heme oxygenase 1 and thus boost phase 2 metabolism. However a combination of 10 μ M curcumin and 10 μ g/ml Oligonol™ appears to significantly ($p=0.003$) and synergistically increase heme oxygenase 1 levels and hence will indirectly prevent or reduce the on-set of skin ageing.

As already mentioned, Nrf2 dependent HO-1 is a phase 2 detoxification enzyme which represents a prime cellular defence mechanism. It may be activated using a

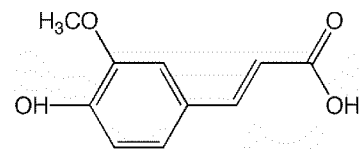
number of different routes (via induction of Nrf2 gene expression and nuclear translocation). Nrf2 transcription factors up-regulates the messenger ribonucleic acid (mRNA), protein and activity for HO-1 and it is believed that both Oligonol™ and curcumin target and activate different parts of the Nrf2 pathway. The results now show that this up-regulation occurs in a synergistic manner, for example, through conformational changes or increases in phosphorylation which may result in increases in nuclear translocation of Nrf2 transcription factors.

Example 3

Example 2 was repeated substituting ferulic acid for curcumin. As can be seen below, ferulic acid is the monomer of curcumin and could be assumed to behave similarly to curcumin in combination with Oligonol™.



Curcumin



Ferulic acid

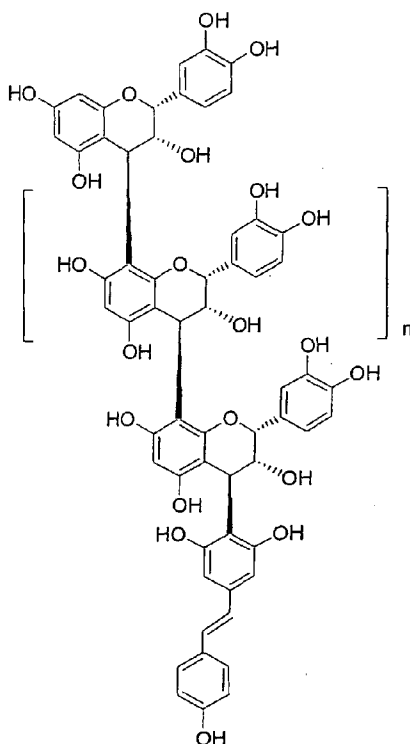
Figure 3 shows heme oxygenase 1 (HO-1) (pg per µg protein) for a control (untreated primary dermal fibroblasts), 10 µM ferulic acid, 10 µg/ml Oligonol™, and a combination of 10 µM ferulic acid and 10 µg/ml Oligonol™. In contrast to curcumin, ferulic acid does not appear to increase the level of heme oxygenase 1 and hence boost phase 2 metabolism, nor does a combination of 10 µM ferulic acid and 10 µg/ml Oligonol™ appear to significantly and synergistically increase heme oxygenase 1 levels.

Claims

1. A topical or oral composition for delaying the visible signs of skin ageing, the composition comprising curcumin and a composition containing as its
5 main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring
10 structure in an acidic aqueous solution.
2. A composition according to claim 1 comprising 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg composition containing as its main
15 component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring
20 structure in an acidic aqueous solution.
3. A composition according to claim 1 or claim 2 wherein the weight ratio of curcumin to composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol
25 ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution is 50:1 to 1:50, preferably 20:1 to 1:20, most
30 preferably 15:1 to 1:15.

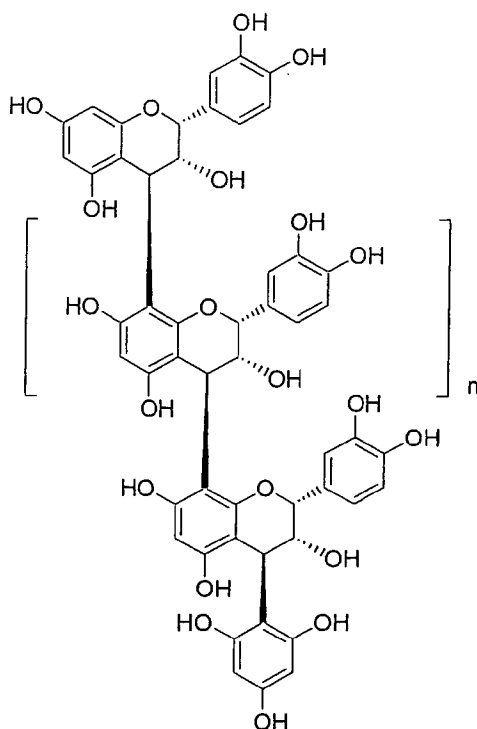
4. A composition according to any one of the preceding claims wherein the proanthocyanidin oligomer has a degree of polymerisation of 2 to 4.
5. A composition according to any one of the preceding claims wherein the
5 plant material comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer may be selected from the group consisting of grape, pine, Chamaecyparis obtuse, camphor tree, wax myrtle, cacao, date plum, banana, Chinese quince, apple, hawthorn, Lychi chinensis, Myrica rubra and Cinnamomi cortex.
10
6. A composition according to any one of the preceding claims wherein the substance having a phloroglucinol ring structure or resorcinol ring structure may be selected from the group consisting of resveratrol, phloroglucinol, flavonoid and flavanoid.
15
7. A composition according to any one of claims 1 to 5 wherein the substance having a phloroglucinol ring structure or resorcinol ring structure may be selected from the group consisting of green tea, fresh tea leaves, grape seed, grape seed coat, cube gambir, red algae and extracts thereof.
20
8. A composition according to any one of claims 1 to 5 and 7 wherein the composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant
25 materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution, is obtainable by heating plant material being an extract produced from a combination of fruit from Lychi chinensis and green leaf of
30 Camellia sinensis in an acidic aqueous solution.

9. A composition according to claim 8 wherein the composition containing as its main component proanthocyanidin oligomer to which a substance having a phloroglucinol ring structure or resorcinol ring structure has been bonded, which is obtainable by heating plant materials comprising proanthocyanidin polymer or aqueous extract thereof comprising proanthocyanidin polymer, with a substance having a phloroglucinol ring structure or resorcinol ring structure in an acidic aqueous solution, is obtainable by heating plant material being an extract produced from a combination of fruit from *Lychi chinensis* and green leaf of *Camellia sinensis* in an acidic aqueous solution is Oligonol™.
10. A topical or oral composition for delaying the visible signs of skin ageing or reducing the onset of atherosclerosis, the composition comprising 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg a proanthocyanidin oligomer represented by structure (I):



wherein n is 0 or an integer of 1 or 2.

11. A topical or oral composition for delaying the visible signs of skin ageing, the composition comprising 10-5000, preferably 50-2500, most preferably 100-300 mg curcumin and 10-5000, preferably 50-2500, most preferably 100-300 mg a proanthocyanidin oligomer represented by structure (II):



5

wherein n is 0 or an integer of 1 or 2.

12. A composition according to any one of the preceding claims wherein the visible signs of skin ageing are selected from the group consisting of wrinkles, sagging, lines and dry skin.
13. A method for delaying the visible signs of skin ageing comprising the step of applying to the skin or imbibing the composition of any one of the preceding claims.
14. Use of the composition of any one of claims 1 to 11 for delaying the visible signs of skin ageing.

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- 25 -

15. A composition according to any one of claims 1 to 11 for use as a medicament.
16. A composition according to any one of claims 1 to 11 for use in delaying
5 the visible signs of skin ageing.
17. Use of the composition of any one of claims 1 to 11 for the manufacture of a medicament for delaying the visible signs of skin ageing.

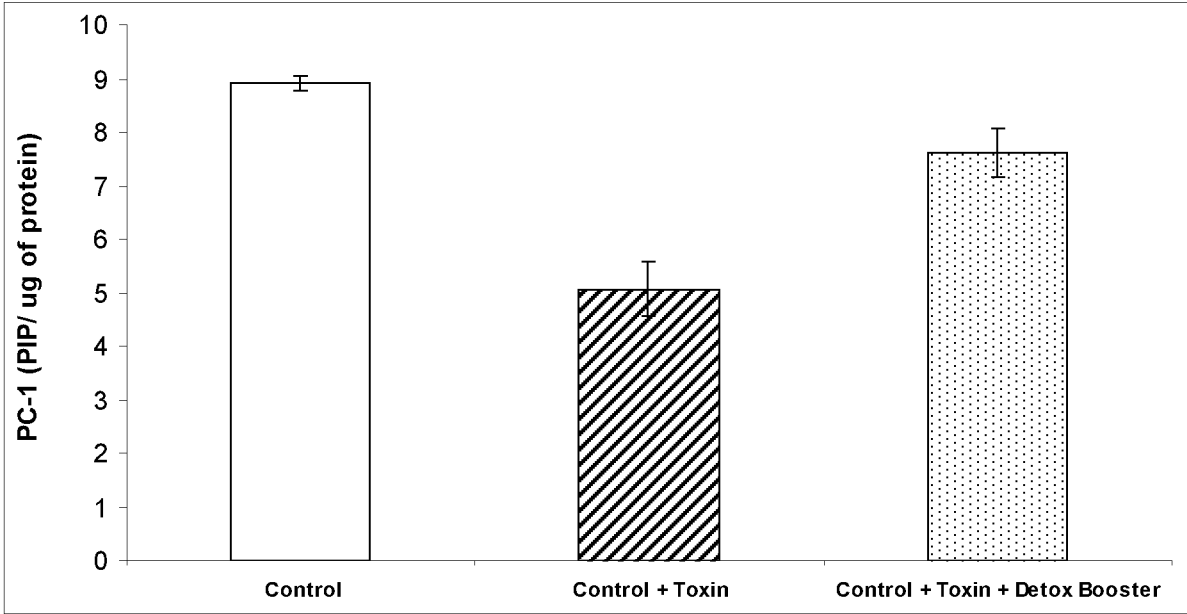


Figure 1a

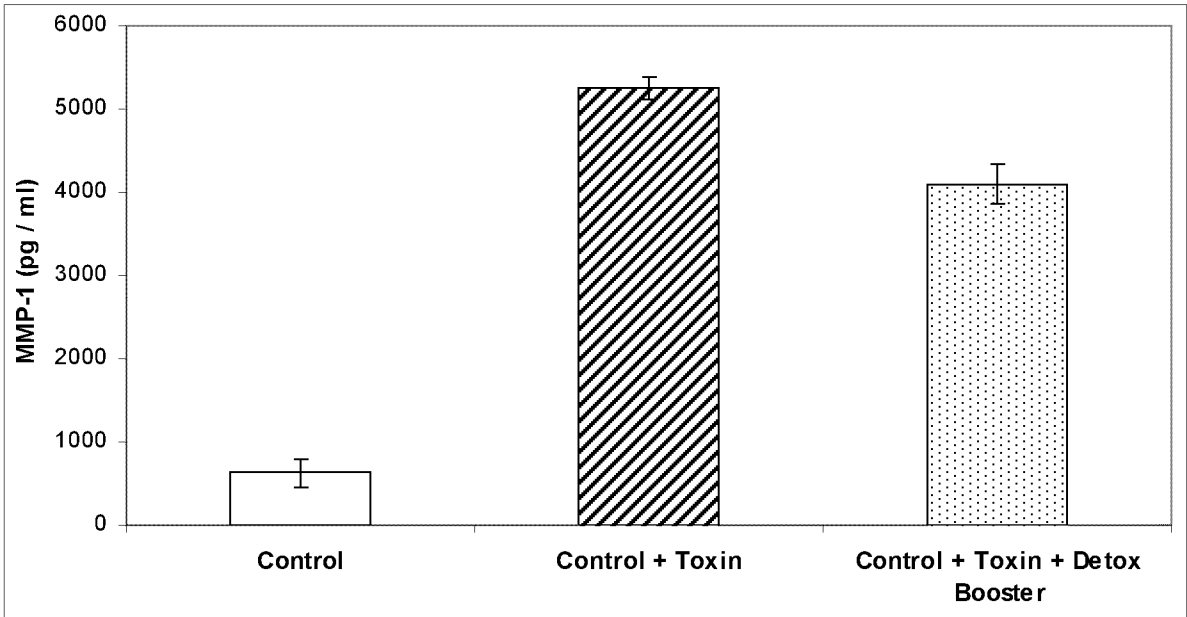


Figure 1b

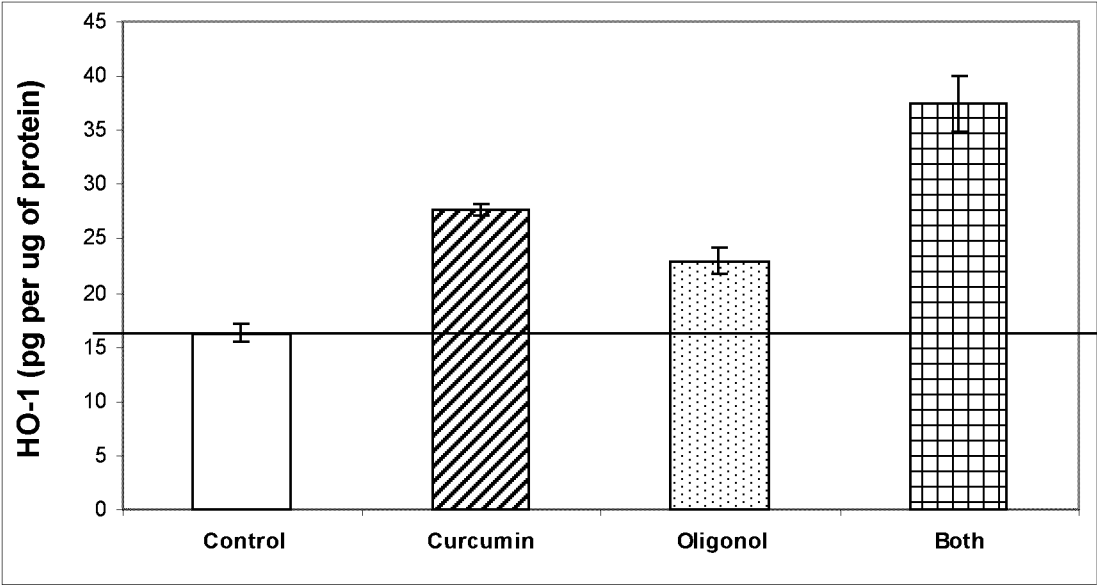


Figure 2

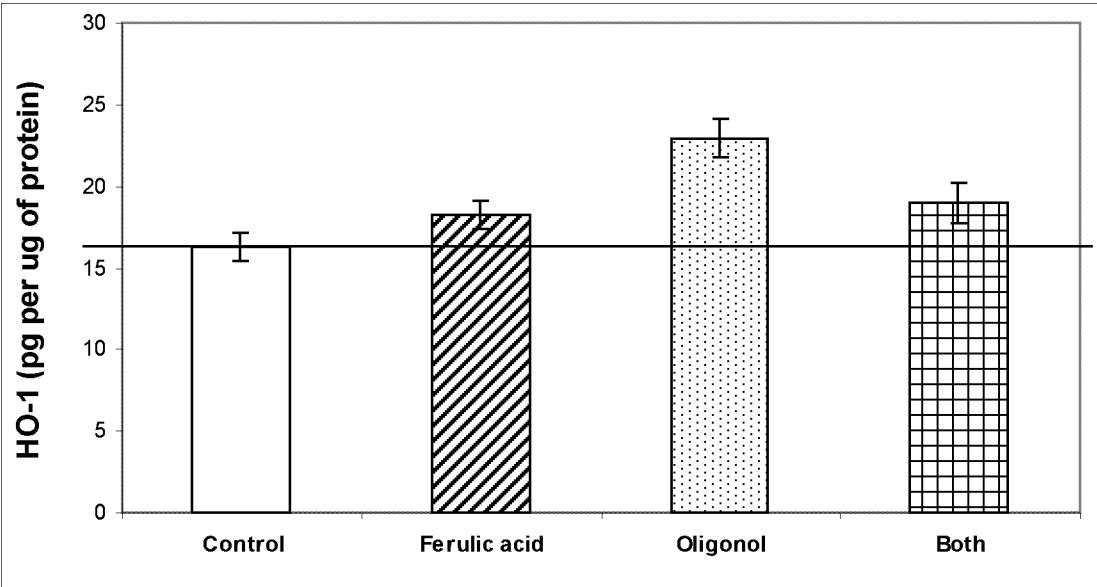


Figure 3