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(72) Inventeurs/Inventors:
PELLE, EDWARD, US;
PERNODET, NADINE A., US;
YAROSH, DANIEL B., US
(73) Propriétaire/Owner:
ELC MANAGEMENT LLC, US
(74) Agent: OSLER, HOSKIN & HARCOURT LLP

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CELLULES DE LA PEAU
(54) Title: METHOD AND COMPOSITIONS FOR INCREASING *per1* GENE EXPRESSION IN SKIN CELLS

(57) **Abrégé/Abstract:**

A method for increasing and/or synchronizing *per1* gene expression in skin cells having decreased, irregular, or asynchronous *per1* gene expression comprising treating the skin cells with an effective amount of cichoric acid, and the resulting compositions.



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(71) Applicant (*for all designated States except US*): **ELC MANAGEMENT LLC** [US/US]; 767 Fifth Avenue, New York, New York 10153 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **PELLE, Edward (Dr.)** [US/US]; 140 Meyer Avenue, Valley Stream, New York 11580 (US). **PERNODET, Nadine A.** [FR/US]; 3 Berry Court, Huntington Station, New York 11746 (US). **YAROSH, Daniel B.** [US/US]; 2915 Shore Drive, Merrick, New York 11566 (US).

(74) Agents: **BLACKBURN, Julie M.** et al.; 155 Pinelawn Road, Suite 345 South, Melville, New York 11747 (US).

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(54) Title: METHOD AND COMPOSITIONS FOR TREATING SKIN

(57) Abstract: A method for increasing and/or synchronizing per1 gene expression in skin cells having decreased, irregular, or asynchronous per1 gene expression comprising treating the skin cells with an effective amount of cichoric acid, and the resulting compositions.

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METHODS AND COMPOSITIONS FOR INCREASING
per1 GENE EXPRESSION IN SKIN CELLS

Technical Field

5 The invention is in the field of methods for treating skin cells to increase per1 gene expression in order to ameliorate the adverse effects of natural aging of skin, UV radiation, environmental toxins, environmental pollution, and the like, and the compositions used in the method.

Background of the Invention

10 It is well known that environmental aggressors such as UV radiation, environmental pollution, environmental toxins, physiological stress, and the natural process of aging can be very detrimental to skin. The skin on the face is made up of keratinocytes, fibroblasts, melanocytes, T-cells and so on. Environmental aggressors cause damage to DNA of skin cells
15 and affects the cellular circadian rhythm in general. The body's natural circadian rhythms are synchronized such that during exposure to environmental aggressors -- usually during daylight hours - certain genes in the cells are activated to produce proteins that protect the cells against damage.

 Genes associated with natural bodily circadian rhythms have been identified and
20 include the clock (Circadian Locomotor Output Cycles Kaput) gene and the per1 (Period Homolog 1) gene, both of which encode proteins (CLK and PER1) that regulate circadian rhythms. Clock and per1 genes are also present in skin cells. The induction of per1 gene expression initiates a program of cellular activity that is associated with biological processes that take place at night (e.g. repair). It is known that skin cells exposed to environmental
25 aggressors will often exhibit decreased, irregular, or asynchronous clock or per1 gene expression. This in turn causes disruption of normal circadian rhythm in the exposed skin cells. Over a prolonged period of time disruption of normal cellular circadian rhythm and

synchronicity can accelerate the natural aging process of skin which leads to wrinkles, fine lines, skin laxity, uneven pigmentation, age spots, mottling, and the like.

For this reason it is very advantageous to maintain natural cellular circadian rhythm to the greatest extent possible. To date, the only known mechanisms for synchronizing skin cells
5 are to (1) starve the cells for an extended period of time by removing nutrients and energy sources, and then suddenly resupply the nutrients; or (2) treating the skin cells with a peptide having the C.T.F.A. name Tripeptide-32. The practical difficulties in starving skin cells not in culture are obvious. In addition, the ingredient known to have this activity is expensive.

It has been discovered that cichoric acid is an effective stimulator of per1 gene
10 expression in skin cells. The ability to increase per1 gene expression in skin cells means that skin cells with irregular or decreased synchronicity will become synchronized (e.g. all cells exhibit same level of per1 gene expression at the same time). When skin cells are synchronized, treatment of the skin cells with active ingredients that repair cellular damage are the most effective. It is most beneficial to treat skin cells to increase per1 gene expression and
15 thereby cause the treated cells to be synchronized so that the active ingredients in skin treatment products may be optimally beneficial to the skin cells. In one most preferred embodiment of the invention the skin cells are treated with a cichoric acid-containing composition to increase per1 gene expression and synchronicity at night prior to retiring.

20 Summary of the Invention

The invention is directed to a method for increasing and/or synchronizing per1 gene expression in skin cells having decreased, irregular, or asynchronous per1 gene expression comprising treating the skin cells with an effective amount of cichoric acid.

25 The invention is also directed to a method for increasing and/or synchronizing per1 gene expression in skin cells having decreased, irregular, or asynchronous per1 gene

expression due to exposure to UV radiation comprising treating the skin cells with cichoric acid in an amount sufficient to increase the per1 gene expression of the treated cells.

The invention is also directed to a method for increasing and/or synchronizing per1 gene expression in skin cells having decreased, irregular, or asynchronous per1 gene expression due to the natural aging process comprising treating the skin cells with cichoric acid in an amount sufficient to increase the per1 gene expression of the treated cells.

The invention is also directed to a method for treating skin cells with a regimen comprising:

- (a) During the day treating the skin cells with a composition containing chemical or physical UV sunscreens;
- (b) At night, treating skin cells that may have decreased, irregular, or asynchronous per1 gene expression due to UV exposure during day hours, with cichoric acid in an amount sufficient to increase and/or synchronize the per1 gene expression of the treated cells.

The invention is also directed to a composition comprising cichoric acid and at least one DNA repair enzyme.

Brief Description of the Figures

Figure 1 depicts the per1 gene expression of *Ecchinacea purpurea* extract.

Figure 2 depicts the per1 gene expression of cichoric acid.

Detailed Description

I. Definitions

All percentages mentioned herein are percentages by weight unless otherwise indicated.

“Environmental pollution” means contaminants typically found in the environment such as smog, cigarette smoke, dust, pollen, motor vehicle exhaust, and the like.

“Environmental toxins” means ingredients found in products that consumers may use in day to day life such as cleaning products containing solvents, chemicals found in
5 contaminated ground water, plastic by products, and the like.

The term “DNA repair enzyme” means an enzyme that is operable to repair DNA base mutagenic damage. Such enzymes are often categorized by the type of DNA damage they repair, for example base excision repair (BER) enzymes, nucleotide excision repair (NER) enzymes; mismatch repair (MMR) enzymes; DNA helicases; DNA polymerases, and so on.
10 For example, DNA lesions such as 8-oxo-7,8-dihydro-2'-deoxyguanosine may be repaired by OGG1 (8-oxoGuanine glycosylase); T-T dimers which may be repaired by NER (nucleotide excision repair) or CPD Photolyase); 6-4 photoproducts (which may be repaired by NER or 6-4 Photolyase); and 06-methyl guanine (which may be repaired by 06-alkyl guanine DNA transferase (AGT)).

15 “Natural aging process” means the natural process of skin aging which includes formation of wrinkles, fine lines, skin laxity, uneven pigmentation, skin mottling, yellowness, and so on.

“per1 gene expression” means the expression of per1 genes in skin cells which may be measured by, among other things, synthesis of proteins transcribed by that gene.

20 “Physiological stress” means stress conditions to which skin cells may be exposed such as wind burn, itching, chafing, extreme heat or cold.

“skin cells” when used herein means cells that make up skin including but not limited to keratinocytes, melanocytes, fibroblasts, T-cells, and so on.

The term “synchronizing per1 gene expression” means that the per1 gene expression of
25 the treated skin cells is synchronized.

“UV radiation” means ultraviolet radiation in the UVA and UVB wavelength ranges.

II. The Method of the Invention

In the method of the invention cichoric acid is applied to skin cells, preferably those found on the face or body skin in an amount sufficient to increase and/or synchronize the per1 gene expression in the treated cells. Most preferably the cichoric acid is incorporated into a cosmetic composition and that composition is used to treat skin cells, preferably keratinocytes. In such a case, suggested ranges of cichoric acid are from about 0.000001 to about 40%, preferably from about 0.000005 to 35%, more preferably from about 0.00001 to 25%.

Cichoric acid may be synthetic or naturally derived. Synthetic cichoric acid may be purchased from a number of commercial manufacturers including Sigma Aldrich. Cichoric acid may also be extracted from botanical sources that are known to contain cichoric acid such as *Echinacea*, *Cichorium*, *Taraxacum*, *Ocimum*, *Melissa*, or from algae or sea grasses. More specifically, botanical extracts such as *Echinacea purpurea*, *Cichorium intybus*, *Taraxacum officinale*, *Ocimum basilicum*, or *Melissa officinalis*. The term “cichoric acid” when used herein also includes any isomers thereof that are operable to increase per1 gene expression in skin cells.

Most preferred is a botanical extract from *Echinacea purpurea* sold by Symrise under the brand name Symfinity™ 1298 which is an extract of *Echinacea purpurea* which is standardized during the extraction process to contain about 3% by weight of the total extract composition of cichoric acid. *Echinacea* extracts from different sources will vary in cichoric acid content, and as such will yield variable results in induction of per1 gene expression. For example, we have observed that another component commonly found in extracts of *Echinacea*, specifically caftaric acid, does not increase per1 gene expression in skin cells. Moreover, each species of *Echinacea* will differ in content of phenolic and cichoric acids. Ethanol extract of the roots of *Echinacea purpurea* will provide more cichoric acid than ethanol extracts of *Echinacea angustifolia* or *Echinacea pallida*. The content of active

ingredients in any extract is also very dependent on the method of extraction. For example, it is known that in many cases enzymatic browning during the extraction process will reduce the phenolic acid content of the resulting extract.

In the method of the invention the cichoric acid containing composition may be applied
5 to the skin in the form of a skin cream, lotion, foundation makeup, lipstick, concealer, blush, serum, eye shadow, cleanser or toner. The composition may be applied to the skin one or more times per day and in any regimen. The cichoric acid may be found in skin cream or lotion or in one or more of a cleanser, toner, or skin treatment product. Preferably the cichoric acid-containing composition is applied to the skin at night, prior to retiring, to maximize the
10 skin's natural repair process.

The composition into which the cichoric acid is formulated may contain other ingredients including but not limited to those set forth herein.

III. DNA Repair Enzymes

15 The composition used in the method of the invention also contains at least one DNA repair enzyme. Suggested ranges are from about 0.00001 to about 35%, preferably from about 0.00005 to about 30%, more preferably from about 0.0001 to about 25% of one or more DNA repair enzymes.

20 DNA repair enzymes as disclosed in U.S. Patent Nos. 5,077,211; 5,190,762; 5,272,079; and 5,296,231 are suitable for use in the compositions and method of the invention. One example of such a DNA repair enzyme may be purchased from AGI/Dermatics under the trade name Roxisomes®, and has the INCI name *Arabidopsis Thaliana* extract. It may be present alone or in admixture with lecithin and water. This DNA repair enzyme is known to be
25 effective in repairing 8-oxo-Guanine base mutation damage.

Another type of DNA repair enzyme that may be used is one that is known to be effective in repairing 06-methyl guanine base mutation damage. It is sold by AGI/Dermatics under the tradename Adasomes®, and has the INCI name Lactobacillus ferment, which may be added to the composition of the invention by itself or in admixture with lecithin and water.

5 Another type of DNA repair enzyme that may be used is one that is known to be effective in repairing T-T dimers. The enzymes are present in mixtures of biological or botanical materials. Examples of such ingredients are sold by AGI/Dermatics under the tradenames Ultrasomes® or Photosomes®. Ultrasomes® comprises a mixture of *Micrococcus* lysate (an end product of the controlled lysis of various species of micrococcus), lecithin, and
10 water. Photosomes® comprises a mixture of plankton extract (which is the extract of marine biomass which includes one or more of the following organisms: thalassoplankton, green micro-algae, diatoms, greenish-blue and nitrogen-fixing seaweed), water, and lecithin.

Another type of DNA repair enzyme may be a component of various inactivated bacterial lysates such as *Bifida* lysate or *Bifida* ferment lysate, the latter a lysate from *Bifido*
15 bacteria which contains the metabolic products and cytoplasmic fractions when *Bifido* bacteria are cultured, inactivated and then disintegrated. This material has the INCI name *Bifida* Ferment Lysate.

Other suitable DNA repair enzymes include T4 Endonuclease V, which may be produced by the denV gene of the bacteriophage T4. Also suitable are base glycosylases such
20 as uracil- and hypoxanthine-DNA glycosylases; apyrimidinic/apurinic endonucleases; DNA exonucleases, damaged-bases glycosylases (e.g., 3-methyladenine-DNA glycosylase); correndonucleases either alone or in complexes (e.g., *E. coli* uvrA/uvrB/uvrC endonuclease complex); APEX nuclease, which is a multi-functional DNA repair enzyme often referred to as “APE”; dihydrofolate reductase; terminal transferase; topoisomerase.

25 Other types of suitable DNA repair enzymes may be categorized by the type of repair facilitated and include BER (base excision repair) or BER factor enzymes such as uracil-DNA

glycosylase (UNG); single strand selective monofunctional uracil DNA glycosylase (SMUG1); 3,N(4)-ethenocytosine glycosylase (MBD4); thymine DNA-glycosylase (TDG); A/G-specific adenine DNA glycosylase (MUTYH); 8-oxoguanine DNA glycosylase (OGG1); endonuclease III-like (NTHL1); 3-methyladenine DNA glycosidase (MPG); DNA

5 glycosylase/AP lyase (NEIL1 or 2); AP endonuclease (APEX 1 and 2), DNA ligase (LIG3), ligase accessory factor (XRCC1); DNA 5'-kinase/3'-phosphatase (PNKP); ADP-ribosyltransferase (PARP1 or 2).

Another category of DNA repair enzymes includes those that are believed to directly reverse methylpurine damage such as 1-meA dioxygenase (ALKBH2 or ALKBH3).

10 Yet another category of enzymes operable to repair DNA/protein crosslinks includes Tyr-DNA phosphodiesterase (TDP1).

Also suitable are MMR (mismatch excision repair) DNA repair enzymes such as MutS protein homolog (MSH2); mismatch repair protein (MSH3); mutS homolog 4 (MSH4); MutS homolog 5 (MSH5); or G/T mismatch-binding protein (MSH6); DNA mismatch repair protein

15 (PMS1, PMS2, MLH1, MLH3); Postmeiotic segregation increased 2-like protein (PMS2L3); or postmeiotic segregation increased 2-like 4 pseudogene (PMS2L4).

Also suitable are DNA repair enzymes known as nucleotide excision repair (NER) enzymes and include those such as Xeroderma pigmentosum group C-complementing protein (XPC); RAD23 (*S. cerevisiae*) homolog (RAD23B); caltractin isoform (CETN2); RFA

20 Protein 1, 2, of 3 (RPA1, 2, or 3); 3' to 5' DNA helicase (ERCC3); 5' to 3' DNA helicase (ERCC2); basic transcription factor (GTF2H1, GTF2H2, GTF2H3, GTF2H4, GTF2H5); CDK activating kinase (CDK7, CCNH); cyclin G1-interacting protein (MNAT1); DNA excision repair protein ERCC-51; excision repair cross-complementing 1 (ERCC1); DNA ligase 1 (LIG1); ATP-dependent helicase (ERCC6); and the like.

25 Also suitable may be DNA repair enzymes in the category that facilitate homologous recombination and include, but are not limited to DNA repair protein RAD51 homolog

(RAD51, RAD51L1, RAD51B etc.); DNA repair protein XRCC2; DNA repair protein XRCC3; DNA repair protein RAD52; ATPase (RAD50); 3' exonuclease (MRE11A); and so on.

DNA repair enzymes that are DNA polymerases are also suitable and include DNA
5 polymerase beta subunit (POLB); DNA polymerase gamma (POLG); DNA polymerase subunit delta (POLD1); DNA polymerase II subunit A (POLE); DNA polymerase delta auxiliary protein (PCNA); DNA polymerase zeta (POLZ); MAD2 homolog ((REV7); DNA polymerase eta (POLH); DNA polymerase kappa (POLK); and the like.

Various types of DNA repair enzymes that are often referred to as “editing and
10 processing nucleases” include 3'-nuclease; 3'-exonuclease; 5'-exonuclease; endonuclease; and the like.

Other examples of DNA repair enzymes include DNA helicases including such as ATP DNA helicase and so on.

The DNA repair enzymes may be present as components of botanical extracts,
15 bacterial or yeast lysates, biological materials, and the like. For example, botanical extracts may contain DNA repair enzymes.

The compositions of the invention may contain one or more DNA repair enzymes. Preferably, the composition contains other ingredients that will provide a cosmetically or pharmaceutically acceptable product.

20

IV. Other Ingredients

The composition used in the method of the invention may be in the form of an emulsion, aqueous solution or dispersion, gel, or anhydrous composition. If in the form of an
25 emulsion, it may be a water-in-oil or oil-in-water emulsion. If in the form of an emulsion, the composition may contain from about 1-99%, preferably from about 5-90%, more preferably from about 10-85% water and from about 1-99%, preferably from about 5-90%, more

preferably from about 5-75% of oil. If in the form of an aqueous suspension or dispersion, the composition may generally contain from about 1-99.9%, preferably from about 5-95%, more preferably from about 10-90% water, with the remaining ingredients being the active ingredients or other formula ingredients.

5

A. Humectants

The composition used in the method of the invention may contain one or more humectants. If present, they may range from about 0.1 to 75%, preferably from about 0.5 to 70%, more preferably from about 0.5 to 40%. Examples of suitable humectants include
10 glycols, sugars, and the like. Suitable glycols are in monomeric or polymeric form and include polyethylene and polypropylene glycols such as PEG 4-10, which are polyethylene glycols having from 4 to 10 repeating ethylene oxide units; as well as C₁₋₆ alkylene glycols such as propylene glycol, butylene glycol, pentylene glycol, and the like. Suitable sugars, some of which are also polyhydric alcohols, are also suitable humectants. Examples of such sugars
15 include glucose, fructose, honey, hydrogenated honey, inositol, maltose, mannitol, maltitol, sorbitol, sucrose, xylitol, xylose, and so on. Also suitable is urea. Preferably, the humectants used in the composition of the invention are C₁₋₆, preferably C₂₋₄ alkylene glycols, most particularly butylene glycol.

20

B. Surfactants

It may be desirable for the composition used in the method of the invention to contain one more surfactants, especially if in the emulsion form. However, such surfactants may be used if the compositions are solutions, suspensions, or anhydrous also, and will assist in dispersing ingredients that have polarity, for example pigments. Such surfactants may be
25 silicone or organic based. The surfactants will also aid in the formation of stable emulsions of either the water-in-oil or oil-in-water form. If present, the surfactant may range from about

0.001 to 30%, preferably from about 0.005 to 25%, more preferably from about 0.1 to 20% by weight of the total composition.

1. Organic Nonionic Surfactants

The composition used in the method of the invention may comprise one or more
5 nonionic organic surfactants. Suitable nonionic surfactants include alkoxylated alcohols or ethers, formed by the reaction of an alcohol with an alkylene oxide, usually ethylene or propylene oxide. Suitable alcohols include mono-, di-, or polyhydric short chain (C1-6) alcohols; aromatic or aliphatic saturated or unsaturated fatty (C12-40) alcohols, of cholesterol; and so on.
10 Cholesterol is suitable, or an aromatic or aliphatic saturated or unsaturated fatty alcohol which may have from 6 to 40, preferably from about 10 to 30, more preferably from about 12 to 22 carbon atoms. Examples include oleyl alcohol, cetearyl alcohol, cetyl alcohol, stearyl alcohol, isostearyl alcohol, behenyl alcohol, and the like. Examples of such ingredients include Oleth 2-100; Steareth 2-100; Beheneth 5-30; Cetareth 2-100; Ceteth 2-100; Choleth
15 2-100 wherein the number range means the number of repeating ethylene oxide units, e.g. Ceteth 2-100 means Ceteth where the number of repeating ethylene oxide units ranges from 2 to 100. Derivatives of alkoxylated alcohols are also suitable, such as phosphoric acid esters thereof.

Some preferred organic nonionic surfactants include Oleth-3, Oleth-5, Oleth-3
20 phosphate, Choleth-24; Ceteth-24; and so on.

Also suitable are alkoxylated alcohols formed with mono-, di-, or polyhydric short chain alcohols, for example those having from about 1 to 6 carbon atoms. Examples include glucose, glycerin, or alkylated derivatives thereof. Examples include glycereth 2-100; gluceth
25 2-100; methyl gluceth 2-100 and so on. More preferred are methyl gluceth-20; glycereth-26 and the like.

Other types of alkoxyated alcohols are suitable surfactants, including ethylene oxide polymers having varying numbers of repeating EO groups, generally referred to as PEG 12 to 200. More preferred are PEG-75, which is may be purchased from Dow Chemical under the trade name Carbowax PEG-3350.

- 5 Other suitable nonionic surfactants include alkoxyated sorbitan and alkoxyated sorbitan derivatives. For example, alkoxylation, in particular ethoxylation of sorbitan provides polyalkoxyated sorbitan derivatives. Esterification of polyalkoxyated sorbitan provides sorbitan esters such as the polysorbates. For example, the polyalkoxyated sorbitan can be esterified with C6-30, preferably C12-22 fatty acids. Examples of such ingredients include
- 10 Polysorbates 20-85, sorbitan oleate, sorbitan sesquioleate, sorbitan palmitate, sorbitan sesquiisostearate, sorbitan stearate, and so on.

2. Silicone or Silane Surfactants

- Also suitable are various types of silicone or silane-based surfactants. Examples include organosiloxanes substituted with ethylene oxide or propylene oxide groups such as
- 15 PEG dimethicones which are dimethicones substituted with polyethylene glycols including those having the INCI names PEG-1 dimethicone; PEG-4 dimethicone; PEG-8 dimethicone; PEG-12 dimethicone; PEG-20 dimethicone; and so on.

- Also suitable are silanes substituted with ethoxy groups or propoxy groups or both, such as various types of PEG methyl ether silanes such as bis-PEG-18 methyl ether dimethyl
- 20 silane; and so on.

Further examples of silicone based surfactants include those having the generic names dimethicone copolyol; cetyl dimethicone copolyol; and so on.

C. Botanical Extracts

- 25 It may be desirable to incorporate one more additional botanical extracts (other than those that contain cichoric acid as a component) into the composition. If present suggested

ranges are from about 0.0001 to 20%, preferably from about 0.0005 to 15%, more preferably from about 0.001 to 10%. Suitable botanical extracts include extracts from plants (herbs, roots, flowers, fruits, seeds) such as flowers, fruits, vegetables, and so on, including yeast ferment extract, *Padina pavonica* extract, *Thermus thermophilis* ferment extract, *Camelina* 5 *sativa* seed oil, *Boswellia serrata* extract, olive extract, *Acacia dealbata* extract, *Acer saccharinum* (sugar maple), *Acidopholus*, *Acorus*, *Aesculus*, *Agaricus*, *Agave*, *Agrimonia*, algae, aloe, citrus, *Brassica*, cinnamon, orange, apple, blueberry, cranberry, peach, pear, lemon, lime, pea, seaweed, caffeine, green tea, chamomile, willowbark, mulberry, poppy, and those set forth on pages 1646 through 1660 of the CTFA Cosmetic Ingredient Handbook, 10 Eighth Edition, Volume 2. Further specific examples include, but are not limited to, *Glycyrrhiza glabra*, *Salix nigra*, *Macrocystis pyrifera*, *Pyrus malus*, *Saxifraga sarmentosa*, *Vitis vinifera*, *Morus nigra*, *Scutellaria baicalensis*, *Anthemis nobilis*, *Salvia sclarea*, *Rosmarinus officianalis*, *Citrus limonum*, *Panax ginseng*, *Siegesbeckia orientalis*, *Fructus mume*, *Ascophyllum nodosum*, *Glycine soja* extract, *Beta vulgaris*, *Haberlea rhodopensis*, 15 *Polygonum cuspidatum*, *Citrus aurantium dulcis*, *Vitis vinifera*, *Selaginella tamariscina*, *Humulus lupulus*, *Citrus reticulata* Peel, *Punica granatum*, *Asparagopsis*, *Curcuma longa*, *Menyanthes trifoliata*, *Helianthus annuus*, *Hordeum vulgare*, *Cucumis sativus*, *Evernia prunastri*, *Evernia furfuracea*, *Kola acuminata*, and mixtures thereof.

20 D. Biological Materials

Also suitable are various types of biological materials such as those derived from cells, fermented materials, and so on. If present such materials may range from about 0.001 to 30%, preferably from about 0.005 to 25%, more preferably from about 0.01 to 20%. Examples include fragments of cellular RNA or DNA, or probiotic microorganisms. Particularly 25 preferred are RNA fragments.

E. Oils

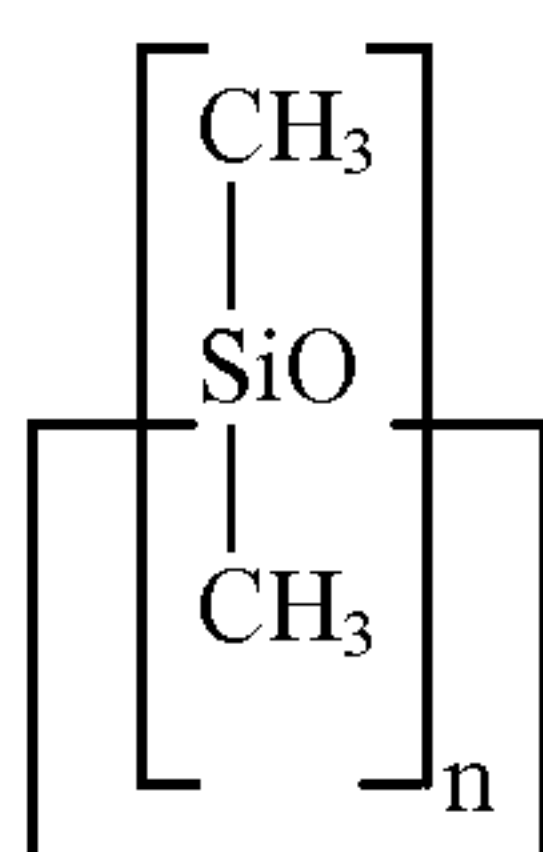
In the event the compositions used in the method of the invention are in emulsion form, the composition will comprise an oil phase. Oily ingredients are desirable for the skin moisturizing and protective properties. Suitable oils include silicones, esters, vegetable oils, synthetic oils, including but not limited to those set forth herein. The oils may be volatile or nonvolatile, and are preferably in the form of a pourable liquid at room temperature. The term "volatile" means that the oil has a measurable vapor pressure, or a vapor pressure of at least about 2 mm. of mercury at 20° C. The term "nonvolatile" means that the oil has a vapor pressure of less than about 2 mm. of mercury at 20° C.

1. Volatile Oils

Suitable volatile oils generally have a viscosity ranging from about 0.5 to 5 centistokes at 25° C. and include linear silicones, cyclic silicones, paraffinic hydrocarbons, or mixtures thereof.

(a). Volatile Silicones

Cyclic silicones are one type of volatile silicone that may be used in the composition. Such silicones have the general formula:



where n=3-6, preferably 4, 5, or 6.

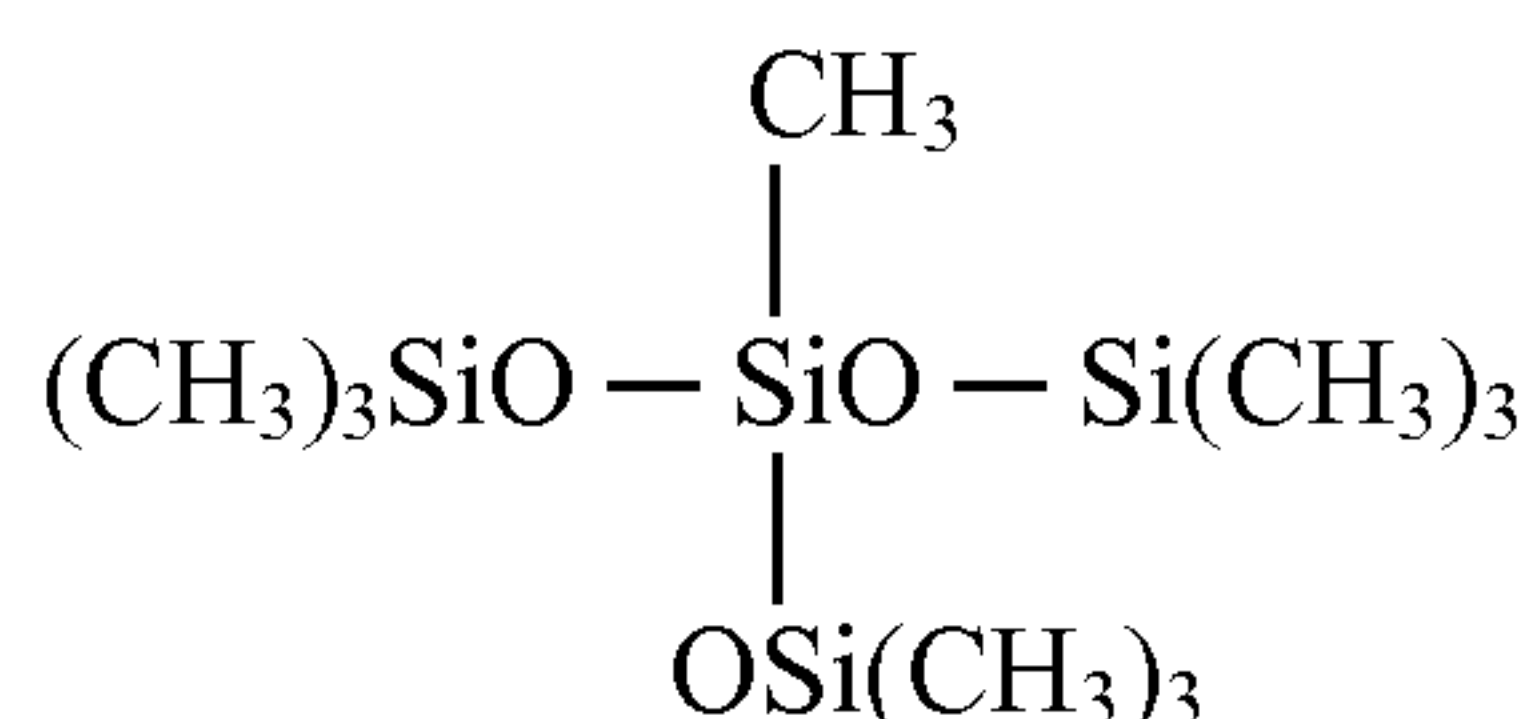
Also suitable are linear volatile silicones, for example, those having the general formula:



where $n=0, 1, 2, 3, 4$, or 5 , preferably $0, 1, 2, 3$, or 4 .

Cyclic and linear volatile silicones are available from various commercial sources including Dow Corning Corporation and General Electric. The Dow Corning linear volatile silicones are sold under the tradenames Dow Corning 244, 245, 344, and 200 fluids. These fluids include hexamethyldisiloxane (viscosity 0.65 centistokes (abbreviated cst)), octamethyltrisiloxane (1.0 cst), decamethyltetrasiloxane (1.5 cst), dodecamethylpentasiloxane (2 cst) and mixtures thereof, with all viscosity measurements being at 25°C .

Suitable branched volatile silicones include alkyl trimethicones such as methyl trimethicone, a branched volatile silicone having the general formula:



Methyl trimethicone may be purchased from Shin-Etsu Silicones under the tradename TMF-1.5, having a viscosity of 1.5 centistokes at 25°C .

(b). Volatile Paraffinic Hydrocarbons

Also suitable as the volatile oils are various straight or branched chain paraffinic hydrocarbons having 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms, more preferably 8 to 16 carbon atoms. Suitable hydrocarbons include pentane, hexane, heptane, decane, dodecane, tetradecane, tridecane, and C_{8-20} isoparaffins as disclosed in U.S. Pat. Nos. 3,439,088 and 3,818,105, both of which are hereby incorporated by reference. Preferred volatile paraffinic hydrocarbons have a molecular weight of 70-225, preferably 160 to 190 and a boiling point range of 30 to 320, preferably 60 to 260 $^{\circ}\text{C}$., and a viscosity of less than about 10 cst. at 25°C . Such paraffinic hydrocarbons are available from EXXON under the ISOPARS trademark, and from the Permethyl Corporation. Suitable C_{12} isoparaffins are manufactured by Permethyl Corporation under the tradename Permethyl 99A. Various C_{16}

isoparaffins commercially available, such as isohexadecane (having the tradename Permethyl R), are also suitable.

2. Non-Volatile Oils

A variety of nonvolatile oils are also suitable for use in the compositions of the invention. The nonvolatile oils generally have a viscosity of greater than about 5 to 10 centistokes at 25° C., and may range in viscosity up to about 1,000,000 centipoise at 25° C. Examples of nonvolatile oils include, but are not limited to:

(a). Esters

Suitable esters are mono-, di-, and triesters. The composition may comprise one or more esters selected from the group, or mixtures thereof.

(i) Monoesters

Monoesters are defined as esters formed by the reaction of a monocarboxylic acid having the formula R-COOH, wherein R is a straight or branched chain saturated or unsaturated alkyl having 2 to 45 carbon atoms, or phenyl; and an alcohol having the formula R-OH wherein R is a straight or branched chain saturated or unsaturated alkyl having 2-30 carbon atoms, or phenyl. Both the alcohol and the acid may be substituted with one or more hydroxyl groups. Either one or both of the acid or alcohol may be a "fatty" acid or alcohol, and may have from about 6 to 30 carbon atoms, more preferably 12, 14, 16, 18, or 22 carbon atoms in straight or branched chain, saturated or unsaturated form. Examples of monoester oils that may be used in the compositions of the invention include hexyl laurate, butyl isostearate, hexadecyl isostearate, cetyl palmitate, isostearyl neopentanoate, stearyl heptanoate, isostearyl isononanoate, stearyl lactate, stearyl octanoate, stearyl stearate, isononyl isononanoate, and so on.

(ii). Diesters

Suitable diesters are the reaction product of a dicarboxylic acid and an aliphatic or aromatic alcohol or an aliphatic or aromatic alcohol having at least two substituted hydroxyl

groups and a monocarboxylic acid. The dicarboxylic acid may contain from 2 to 30 carbon atoms, and may be in the straight or branched chain, saturated or unsaturated form. The dicarboxylic acid may be substituted with one or more hydroxyl groups. The aliphatic or aromatic alcohol may also contain 2 to 30 carbon atoms, and may be in the straight or branched chain, saturated, or unsaturated form. Preferably, one or more of the acid or alcohol is a fatty acid or alcohol, i.e. contains 12-22 carbon atoms. The dicarboxylic acid may also be an alpha hydroxy acid. The ester may be in the dimer or trimer form. Examples of diester oils that may be used in the compositions of the invention include diisostearyl malate, neopentyl glycol dioctanoate, dibutyl sebacate, dicetearyl dimer dilinoleate, dicetyl adipate, diisocetyl adipate, diisononyl adipate, diisostearyl dimer dilinoleate, diisostearyl fumarate, diisostearyl malate, dioctyl malate, and so on.

(iii). Triesters

Suitable triesters comprise the reaction product of a tricarboxylic acid and an aliphatic or aromatic alcohol or alternatively the reaction product of an aliphatic or aromatic alcohol having three or more substituted hydroxyl groups with a monocarboxylic acid. As with the mono- and diesters mentioned above, the acid and alcohol contain 2 to 30 carbon atoms, and may be saturated or unsaturated, straight or branched chain, and may be substituted with one or more hydroxyl groups. Preferably, one or more of the acid or alcohol is a fatty acid or alcohol containing 12 to 22 carbon atoms. Examples of triesters include esters of arachidonic, citric, or behenic acids, such as triarachidin, tributyl citrate, triisostearyl citrate, tri C₁₂₋₁₃ alkyl citrate, tricaprylin, tricaprylyl citrate, tridecyl behenate, trioctyldodecyl citrate, tridecyl behenate; or tridecyl cocoate, tridecyl isononanoate, and so on.

Esters suitable for use in the composition are further described in the C.T.F.A. Cosmetic Ingredient Dictionary and Handbook, Eleventh Edition, 2006, under the classification of "Esters".

(b). Hydrocarbon Oils

It may be desirable to incorporate one or more nonvolatile hydrocarbon oils into the composition used in the method of the invention. Suitable nonvolatile hydrocarbon oils include paraffinic hydrocarbons and olefins, preferably those having greater than about 20 carbon atoms. Examples of such hydrocarbon oils include C₂₄₋₂₈ olefins, C₃₀₋₄₅ olefins, C₂₀₋₄₀ isoparaffins, hydrogenated polyisobutene, polyisobutene, polydecene, hydrogenated polydecene, mineral oil, pentahydrosqualene, squalene, squalane, and mixtures thereof. In one preferred embodiment such hydrocarbons have a molecular weight ranging from about 300 to 1000 Daltons.

(c). Glycerol Esters of Fatty Acids

Synthetic or naturally occurring glycerol esters of fatty acids, or triglycerides, are also suitable for use in the compositions. Both vegetable and animal sources may be used.

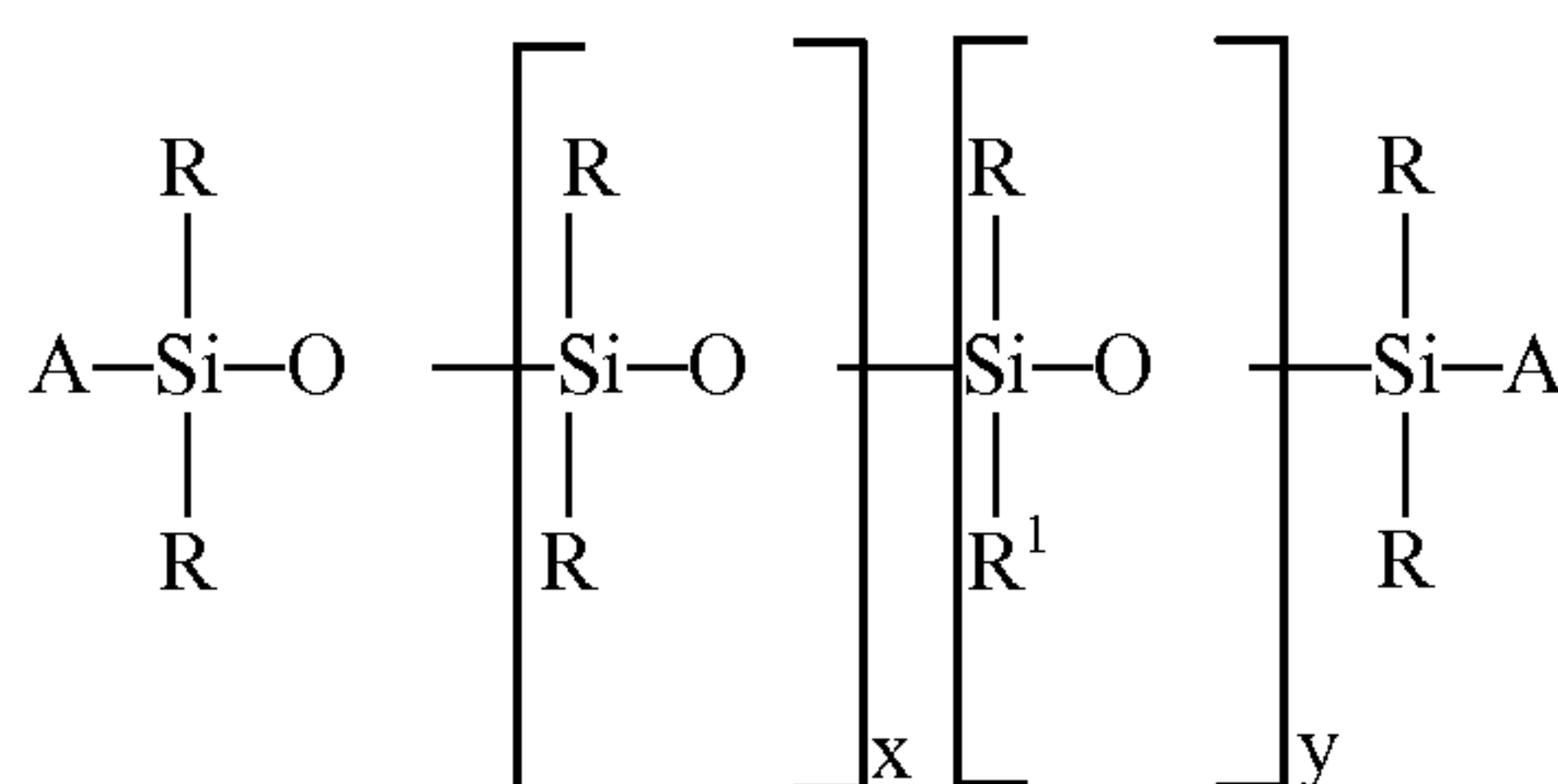
Examples of such oils include castor oil, lanolin oil, C₁₀₋₁₈ triglycerides, caprylic/capric/triglycerides, sweet almond oil, apricot kernel oil, sesame oil, camelina sativa oil, tamanu seed oil, coconut oil, corn oil, cottonseed oil, linseed oil, ink oil, olive oil, palm oil, illipe butter, rapeseed oil, soybean oil, grapeseed oil, sunflower seed oil, walnut oil, and the like.

Also suitable are synthetic or semi-synthetic glycerol esters, such as fatty acid mono-, di-, and triglycerides which are natural fats or oils that have been modified, for example, mono-, di- or triesters of polyols such as glycerin. In an example, a fatty (C₁₂₋₂₂) carboxylic acid is reacted with one or more repeating glycerol groups. glycerol stearate, diglycerol diisostearate, polyglycerol-3 isostearate, polyglycerol-4 isostearate, polyglycerol-6 ricinoleate, glycerol dioleate, glycerol diisostearate, glycerol tetraisostearate, glycerol trioctanoate, diglycerol distearate, glycerol linoleate, glycerol myristate, glycerol isostearate, PEG castor oils, PEG glycerol oleates, PEG glycerol stearates, PEG glycerol tallowates, and so on.

(d). Nonvolatile Silicones

Nonvolatile silicone oils, both water soluble and water insoluble, are also suitable for use in the composition. Such silicones preferably have a viscosity ranging from about greater than 5 to 800,000 cst, preferably 20 to 200,000 cst at 25° C. Suitable water insoluble silicones include amine functional silicones such as amodimethicone.

For example, such nonvolatile silicones may have the following general formula:



wherein R and R' are each independently C₁₋₃₀ straight or branched chain, saturated or unsaturated alkyl, phenyl or aryl, trialkylsiloxy, and x and y are each independently 1-1,000,000; with the proviso that there is at least one of either x or y, and A is alkyl siloxy endcap unit. Preferred is where A is a methyl siloxy endcap unit; in particular trimethylsiloxy, and R and R' are each independently a C₁₋₃₀ straight or branched chain alkyl, phenyl, or trimethylsiloxy, more preferably a C₁₋₂₂ alkyl, phenyl, or trimethylsiloxy, most preferably methyl, phenyl, or trimethylsiloxy, and resulting silicone is dimethicone, phenyl dimethicone, diphenyl dimethicone, phenyl trimethicone, or trimethylsiloxyphenyl dimethicone. Other examples include alkyl dimethicones such as cetyl dimethicone, and the like wherein at least one R is a fatty alkyl (C₁₂, C₁₄, C₁₆, C₁₈, C₂₀, or C₂₂), and the other R is methyl, and A is a trimethylsiloxy endcap unit, provided such alkyl dimethicone is a pourable liquid at room temperature. Phenyl trimethicone can be purchased from Dow Corning Corporation under the tradename 556 Fluid. Trimethylsiloxyphenyl dimethicone can be purchased from Wacker-Chemie under the tradename PDM-1000. Cetyl dimethicone, also

referred to as a liquid silicone wax, may be purchased from Dow Corning as Fluid 2502, or from DeGussa Care & Surface Specialties under the trade names Abil Wax 9801, or 9814.

F. Vitamins and Antioxidants

5 It may be desirable to incorporate one or more vitamins or antioxidants in the composition used in the method of the invention. If present, suggested ranges are from about 0.001 to 20%, preferably from about 0.005 to 15%, more preferably from about 0.010 to 10%. Preferably such vitamins, vitamin derivatives and/or antioxidants are operable to scavenge free radicals in the form of singlet oxygen. Such vitamins may include tocopherol or its
10 derivatives such as tocopherol acetate, tocopherol ferulate; ascorbic acid or its derivatives such as ascorbyl palmitate, magnesium ascorbyl phosphate; Vitamin A or its derivatives such as retinyl palmitate; or vitamins D, K, B, or derivatives thereof.

G. Preferred Compositions

15 Preferred compositions used in the method of the invention are in the aqueous solution or emulsion form and contain cichoric acid and at least one DNA repair enzyme.

More preferred is where the composition used in the method of the invention comprises at least one nonionic organic surfactant which is an alkoxylated alcohol and the at least one oil is an organic ester or hydrocarbon.

20 The invention will be further described in connection with the following examples which are set forth for the purposes of illustration only.

EXAMPLE 1

25 A skin treatment composition is prepared as follows:

Ingredient	w/w%
Oleth-3 phosphate	0.45
Oleth-3	0.35
Oleth-5	0.24

Butylene glycol	0.20
Squalane	0.50
BHT	0.10
Ethylhexyl methoxycinnamate	0.10
Choleth-24/ceteth-24	0.10
Triethanolamine	0.11
Retinyl palmitate/zea mays (corn) oil/BHT/BHA	0.10
Butylene glycol	1.1
Chamomile	0.03
Bisabolol	0.10
Water	QS
Methyl paraben	0.46
PEG-75	4.00
Bis-PEG-18 methyl ether dimethyl silane	2.00
Glycereth-26	1.00
Methyl gluceth-20	4.00
Trisodium EDTA	0.10
Pantethine	0.14
Caffeine	0.05
Xanthan gum	0.075
Carbomer	0.26
Triethanolamine	0.50
Phenoxyethanol	0.70
Benzyl alcohol	0.10
Bifida ferment lysate	9.40
Water/bifida ferment lysate/hydrogenated lecithin	3.00
Butylene glycol/water/ <i>Cola Acuminata</i> extract	3.00
Sodium ribonucleic acid	0.01
Cichoric acid	0.20
Lactobacillus ferment/lecithin/water	0.05
Water/ <i>Arabidopsis Thaliana</i> extract/lecithin	0.05
Phenoxyethanol	0.02
Sodium hyaluronate	0.01
FD&C Red No. 4 (1% aqueous solution with butylene glycol)	0.04
FD&C Yellow No. 5 (1% aqueous solution with butylene glycol)	0.09
D&C Green No. 5 (0.1% solution with butylene glycol)	0.001

The composition is prepared by combining the ingredients and mixing well to form a liquid. The composition is stored in glass bottles.

EXAMPLE 2

Cichoric acid and *Ecchinacea purpurea* extract (Symfinity 1298, Symrise) were tested to determine per1 gene expression.

- 5 Normal human epidermal keratinocytes (NHEK) were diluted in EpiLife Media MEP1500CA with supplement S001-5 added (Gibco, Cascade Biologics, Invitrogen) to form a concentration of 3×10^4 and plated onto a black walled 96 well microtiter plate by adding 100 μ l in each well. The plate was incubated for 3 hours at 37° C. in 5% CO₂. The media was removed and remaining adhered cells rinsed with EpiLife Media MEP1500CA, supplement
- 10 free. A plasmid solution was prepared by diluting Tris-EDTA pH 8.0 buffer with plasmid pGL4.11 (DNA 2.0, Carlsbad, CA) to form a 1 mg/ml solution. This plasmid contained a per1 gene sequence and a luciferase reporter gene sequence. A transfection mixture was prepared by diluting EpiLife Media MEP1500CA, supplement free, to form a solution with 0.31 μ g/ml of plasmid (obtained by adding appropriate amount of plasmid solution prepared above),
- 15 1.28% Plus™ Reagent (Catalog No. 11514-015, Invitrogen, Carlsbad, CA), and 3.22% Lipofectamine™ Transfection Reagent (Catalog No. 18324-012, Invitrogen, Carlsbad, CA). Transfection mixture, 40 μ l, was added to each well and the plate incubated for 4 hours at 37° C. in 5% CO₂. Then 80 μ l of EpiLife Media MEP1500CA with supplement S001-5 was added to the well containing the control (media alone), the transfection mixture control
- 20 (transfection mixture without any active ingredient). Test wells and transfection control wells. To the test wells 80 μ l of test material solution (test material diluted in EpiLife Media MEP1500CA with supplement S001-5) was added. The wells were incubated for 16 hours at 37° C. in 5% CO₂. Immediately prior to reading, Bright-Glo™ (Pro-Mega, Madison WI) was added to each well plate in the same volume as is currently in the well (e.g. a 1:1 dilution).
- 25 Luminescence was measured in an LMax™ Microplate Luminometer (Molecular Devices,

Sunnyvale, CA). The results are set forth in figures 1 and 2. These results demonstrate that both *Ecchinacea purpurea* extract and cichoric acid stimulate per1 gene expression in keratinocytes.

While the invention has been described in connection with the preferred embodiment,
5 it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. Use of a composition containing an effective amount of cichoric acid in the form of *Echinacea* extract in combination with inactivated bacterial lysates of *Bifido* bacteria for increasing and/or synchronizing per1 gene expression in skin cells having decreased, irregular, or asynchronous per1 gene expression for topical application at least once per day in a subject in need thereof.
2. The use of claim 1 wherein the skin cells have decreased, irregular, or asynchronous per1 gene expression due to the natural aging process, or due to exposure to one or more of UV radiation, environmental pollution, environmental toxins, or physiological stress.
3. The use of claim 1 wherein the skin cells are keratinocytes and the *Echinacea* extract is from *Echinacea purpurea*.
4. The use of claim 1 wherein the cichoric acid is contained in a composition in the form of a skin cream, lotion, foundation makeup, lipstick, concealer, blush, serum, eye shadow, cleanser or toner.
5. The use of claim 4 wherein the composition is for application to the skin in the evening prior to retiring.
6. Use of a composition containing an extract from *Echinacea purpurea* containing cichoric acid in combination with inactivated bacterial lysates of *Bifido* bacteria for topical application to skin cells in a subject in need thereof for increasing and/or synchronizing per1 gene expression in the skin cells of the subject having decreased, irregular, or asynchronous per1 gene expression.
7. Use of a composition containing an extract from *Echinacea purpurea* containing cichoric acid and inactivated bacterial lysates of *Bifido* bacteria in an amount sufficient to increase and/or synchronize the per1 gene expression for treating skin cells:
 - (a) During the day in a composition containing chemical or physical UV sunscreens; and/or

- (b) At night, in skin cells that may have decreased, irregular, or asynchronous *per1* gene expression due to UV exposure during the day.

8. A composition comprising cichoric acid in the form of *Echinacea purpurea* in combination with inactivated bacteria lysates of *Bifido* bacteria.

9. The composition of claim 8 wherein the inactivated bacterial lysates from *Bifido* bacteria are *Bifido* lysate, *Bifido* ferment lysate or mixtures thereof.

10. The use of claim 6 wherein the inactivated bacterial lysates of *Bifido* bacterium is *Bifido* ferment lysate.

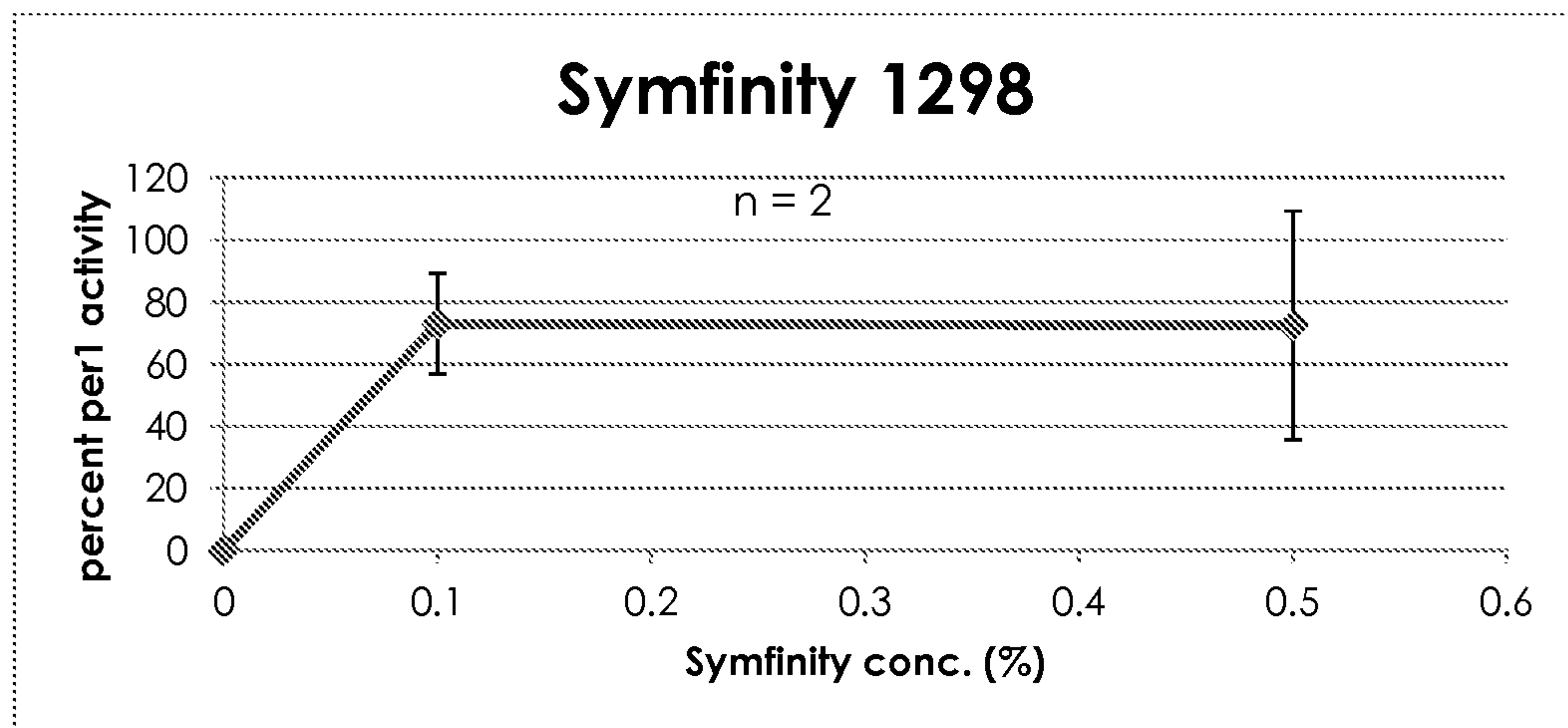


Figure 1

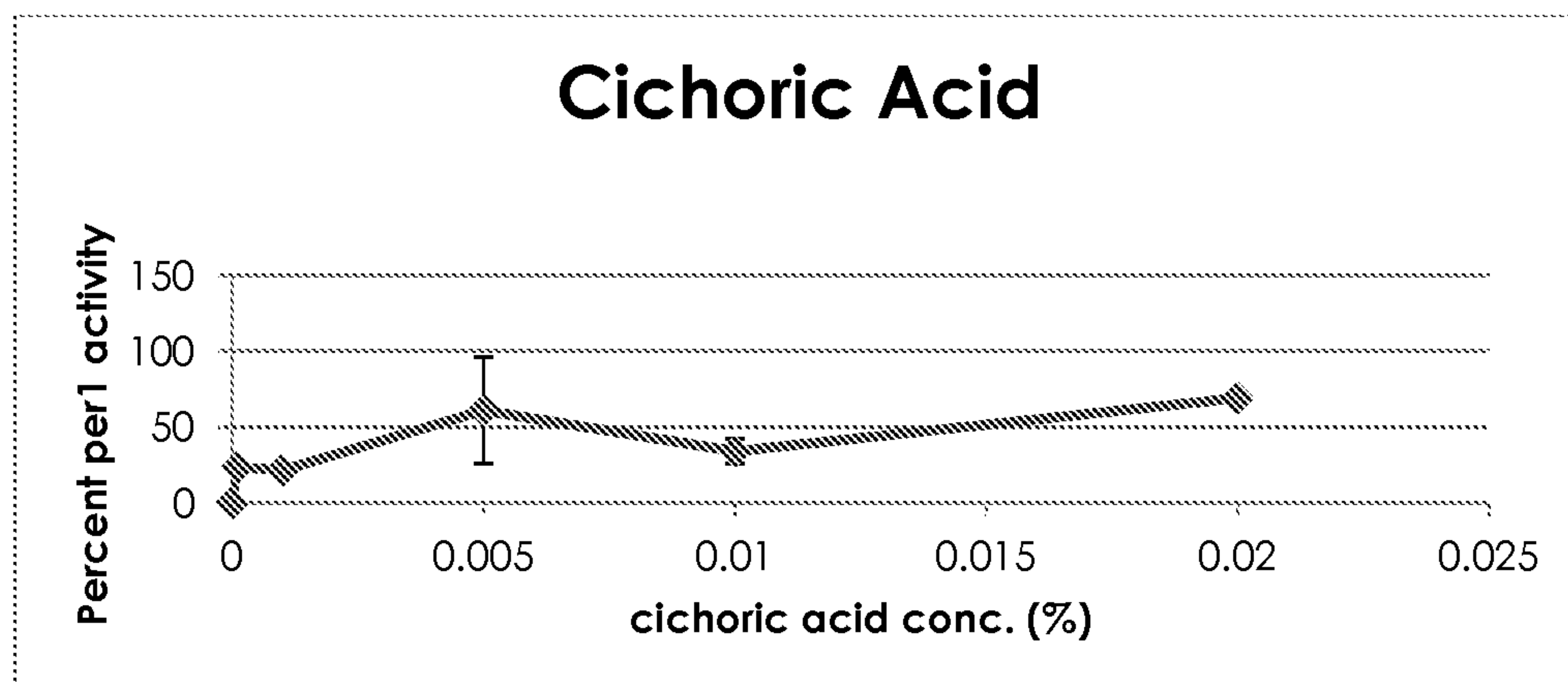


Figure 2