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**Topical compositions comprising Pichia anomala and retinol**

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ABSTRACT

The present invention provides a topical composition comprising an extract of *Pichia anomala* and retinol.

## TOPICAL COMPOSITIONS COMPRISING *PICHIA ANOMALA* AND RETINOL

### FIELD OF THE INVENTION

The present invention provides a method of treating skin by topically applying to skin a combination of an extract of *Pichia anomala* and retinol. Additionally, a topical composition comprising a combination of an extract of *Pichia anomala* and retinol is provided.

### BACKGROUND OF THE INVENTION

Hyaluronic acid is found in skin at the periphery of collagen and elastin fibers and where these fibers intersect. Hyaluronic acid is localized not only in the dermis but also in the epidermal intercellular spaces, especially the middle spinous layer, but not in the stratum corneum (SC) or stratum granulosum. In aged skin, the level of hyaluronic acid decreases and it disassociates from collagen and elastin. Skin containing reduced levels of hyaluronic acid also demonstrates reduced water binding, which may be involved in the changes noted in aged skin, including wrinkling, altered elasticity, reduced turgidity and diminished capacity to support the microvasculature of the skin. As one of the primary GAGs, hyaluronic acid can bind 1000 times its weight in water, and may help the skin retain and maintain water. It is found in all connective tissue and is produced mainly by fibroblasts and keratinocytes in the skin.

Different methods have been proposed for combating wrinkles and fine lines, including injection of hyaluronic acid. Injection of exogenous hyaluronic acid is used as a temporary dermal filling agent in soft tissue augmentation procedures. However, injected hyaluronic acid has a limited lifetime. On the other hand, penetration of exogenous hyaluronic acid into the skin has proved difficult to accomplish by topical application.

*Pichia* is a genus of yeasts in the family Saccharomycetaceae. More than 100 species of this genus are known. The most well-known species include *Pichia anomala*, *Pichia guilliermondii*, *Pichia norvegensis*, and *Pichia ohmeri*.

*Pichia anomala* (formerly named *Hansenula anomala*) can be found in raw milk and cheese. The extracts of yeasts of the genus *Pichia* are rich in mannans, polysaccharides

composed of mannose monomers. *Pichia anomala* and mannans are known to be used in the treatment of aging skin. See, for example, FR 2938768, FR 2906719, FR 2897266 and FR 2976490.

PRO-LIPISKIN® is a commercially available cosmetic ingredient containing extract of *Pichia anomala*. It is produced by a *Pichia* strain isolated from sugar cane. It is available from Silab-France.

US 2017/0172913A1 relates to topical compositions comprising combinations of *Pichia anomala* extract and chicory root extract that provide increased production of hyaluronic acid, along with methods of treating signs of skin aging and improving skin barrier protection and skin moisturization.

Retinoids including retinol are well known anti-aging actives. They are used in a variety of cosmetic products for topical application. Retinol is used for example in NEUTROGENA® Rapid Wrinkle Repair® commercially available from Johnson & Johnson Consumer Inc. NEUTROGENA® Rapid Wrinkle Repair® is used to fade the look of wrinkles in skin, smooth fine lines, improve skin texture, and brighten skin tone. Retinol in particular has proven to be a highly efficacious and cost effective cosmetic ingredient.

*Pichia anomala* extract and retinol work through different biological mechanisms and result in improvement of different clinical benefits. Retinol is known as the gold standard for anti-aging benefits, and is known to work through a biological pathway including the markers CRABP2 & HBEGF. *Pichia anomala* extract works through a hydration pathway by increasing the activity of Hyaluronic Acid Synthase 2 enzyme that translates to the clinical benefits including hydration, improved skin barrier, firming, etc.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Although the art provides topical uses for extracts of *Pichia anomala* and retinol separately, applicants have now discovered that topical application of a combination of these two ingredients beneficially boosts the activity of retinol and the activity of *Pichia anomala* extract. This unexpectedly provides significant benefits for skin, including improving, reducing, inhibiting, or delaying the appearance of at least one sign of aging in

skin, and enhancing skin barrier protection and skin moisturization. Accordingly, new methods of treating signs of skin aging, for example, are now available.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

#### SUMMARY OF THE INVENTION

The present invention relates to a topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.

The invention also relates to a method of treating a sign of skin aging, comprising topically applying to skin in need of treatment for skin aging a topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.

The invention further provides a method of improving skin barrier function and moisturization, comprising topically applying to skin in need of improving skin barrier function and moisturization a topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.

The invention also provides a method of increasing the amount of hyaluronic acid produced by skin when contacted with an extract of *Pichia anomala*, comprising contacting the skin with the extract in combination with retinol; wherein the skin is contacted with a weight ratio of the extract of *Pichia anomala* to retinol of about 1.3.

The invention further provides a use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for treating a sign of skin aging; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.

The invention also provides a use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for improving skin barrier function and moisturization; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.

The invention further provides a use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for increasing the amount of hyaluronic acid produced by skin; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.

5 DETAILED DESCRIPTION

The topical composition of the present invention improves the production of hyaluronic acid in the skin by synergistic action of *Pichia anomala* extract and retinol.

0 It is believed that one skilled in the art can, based upon the description herein, utilize the present invention to its fullest extent. The following specific embodiments are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference. Unless otherwise indicated, percentages used to express amounts of ingredients are percentage by weight (i.e., % (W/W)). Similarly, weight ratios used to express relative proportions of ingredients are also determined using

percentage by weight (i.e., weight ratios are calculated by dividing the percentage by weight of one ingredient by another). Unless stated otherwise, all ranges are inclusive of the endpoints, e.g., “from 4 to 9” includes the endpoints 4 and 9.

5 As used herein, a “product” is optionally in finished packaged form. In one embodiment, the package is a container such as a plastic, metal or glass tube or jar containing the composition. The product may further contain additional packaging such as a plastic or cardboard box for storing such container. In one embodiment, the product comprises a composition of the invention and contains instructions directing the user to apply the composition to the skin or hair.

0 As used herein, “topically applying” means directly laying on or spreading on outer skin, the scalp, or hair, e.g., by use of the hands or an applicator such as a wipe, roller, or spray.

5 As used herein, “cosmetic” refers to a beautifying substance or preparation which preserves, restores, bestows, simulates, or enhances the appearance of bodily beauty or appears to enhance the beauty or youthfulness, specifically as it relates to the appearance of tissue or skin.

As used herein, “cosmetically acceptable” means that the ingredients the term describes are suitable for use in contact with tissues (e.g., the skin or hair) without undue toxicity, incompatibility, instability, irritation, allergic response, or the like.

20 In certain embodiments, the compositions of the present invention are suitable for treating signs of skin aging. As used herein, “signs of skin aging” includes the presence of lines and wrinkles, loss of elasticity, uneven skin, and blotchiness. In a particularly preferred embodiment, the sign of aging is the presence of lines and wrinkles and/or loss of elasticity.

25 As used herein, “treating signs of skin aging” refers to mitigating, reducing, preventing, improving, or eliminating the presence or signs of skin aging described above.

As used herein, “wrinkle” includes fine lines, fine wrinkles, or coarse wrinkles. Examples of wrinkles include, but are not limited to, fine lines around the eyes (e.g., “crow's feet”), forehead and cheek wrinkles, frown-lines, and laugh-lines around the mouth.

5 As used herein, “loss of elasticity” includes loss of elasticity or structural integrity of the skin or tissue, including but not limited to sagging, lax and loose tissue. The loss of elasticity or tissue structure integrity may be a result of a number of factors, including but not limited to disease, aging, hormonal changes, mechanical trauma, environmental damage, or the result of an application of products, such as a cosmetics or pharmaceuticals, to the tissue.

As used herein, “uneven skin” means a condition of the skin associated with diffuse or mottled pigmentation, which may be classified as hyperpigmentation, such as post-inflammatory hyperpigmentation.

0 As used herein, “blotchiness” means a condition of the skin associated with redness or erythema.

As used herein, “improving the firmness of skin” means the enhancing of the firmness or elasticity of the skin, preventing the loss of firmness or elasticity of skin, or preventing or treating sagging, lax and loose skin. The firmness or elasticity of the skin can be measured by use of a cutometer. See *Handbook Of Non-Invasive Methods And The Skin*, eds. J. Serup, G. Jemec & G. Grove, Chapter 66.1 (2006). The loss of skin elasticity or firmness may be a result of a number of factors, including but not limited to aging, environmental damage, or the result of an application of a cosmetic to the skin.

10 As used herein, “improving the texture of skin” means the smoothing of the surface of the skin to remove either bumps or crevasses on the skin surface.

As used herein, "improving the appearance of wrinkles in skin" means preventing, retarding, arresting, or reversing the process of wrinkle and fine line formation in skin.

25 As used herein, the term “safe and effective amount” means an amount sufficient to induce the desired effect, but low enough to avoid serious side effects. The safe and effective amount of the compound, extract, or composition will vary with, e.g., the age, health and environmental exposure of the end user, the duration and nature of the treatment, the specific extract, ingredient, or composition employed, the particular carrier utilized, and like factors.

As used herein, “skin in need of improving skin barrier function and moisturization” means skin that is, but not limited to, lacking in moisture, lacking in sebum, cracked, dry, itchy, scaly, xerodermic, dehydrated, lacks suppleness, lacks radiance, dull, or lacks lipids.

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

As described herein, applicants have discovered that topical application of a combination of an extract of *Pichia anomala* and retinol provides unexpectedly good skin barrier function, skin moisturization, and skin anti-aging benefits.

Applicants have also discovered in particular that topical application of a composition containing a combination of an extract of *Pichia anomala* and retinol enhances the endogenous hyaluronic acid (“HA”) levels in skin, providing improvements in hydration and the appearance of at least one sign of skin aging. Topical use of such a composition can increase the levels of hyaluronic acid to a direction found in younger skin thereby providing the structural support to skin to reduce the appearance of signs of aging in skin.

### ***Pichia anomala***

The topical composition comprises one or more extracts of *Pichia anomala*. In particular, such extracts may be extracts produced using one of the various strains of *Pichia anomala* isolated from the fruit or other aerial parts of a plant. Any cosmetically acceptable extract of *Pichia anomala* may be used.

One example of a suitable extract of *Pichia anomala* is PRO-LIPISKIN, commercially available from Silab-France. It is produced from a strain of *Pichia anomala* present on sugar cane.

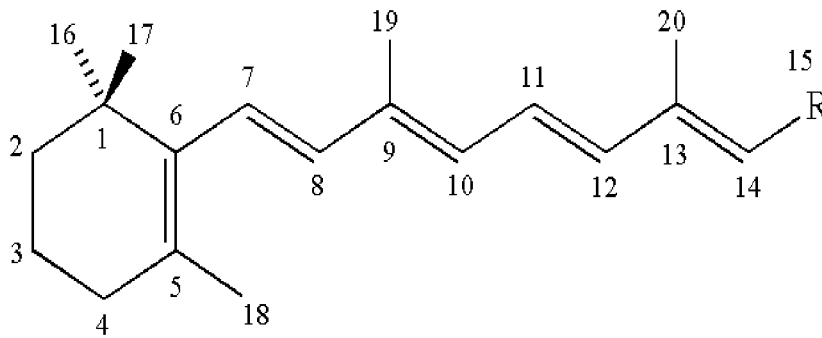
Another example of a suitable extract of *Pichia anomala* is produced from a strain of *Pichia anomala* present on fruit or leaves of Kiwi plant.

The extract of *Pichia anomala* may be provided as a solution containing dry matter (the extract) in the range of about 20 wt%, more specifically 2 to 10 wt%, most specifically 3 to 7 wt%.

Solvents for such solutions include water, alcohols, glycols and the like. In one embodiment, the solvent is at least about 90 wt% water, or at least about 95 wt% water.

### Retinol

The topical composition also contains retinol. Retinol has the formula:



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wherein R is CH<sub>2</sub>OH.

### Amounts

Any suitable amounts of *Pichia anomala* extract and retinol may be used in the compositions of the present invention. Preferably, the compositions comprise safe and effective amounts of both ingredients. In particular, the amounts of *Pichia anomala* extract and retinol used are cosmetically acceptable and are selected to achieve the desired treatment of skin for a particular condition, such as signs of aging, decreased barrier function, or decreased moisturization.

In certain preferred embodiments, the compositions comprise from about 0.01 to about 1 % by weight of *Pichia anomala* extract, more preferably about 0.065 to about 0.26 % by weight of *Pichia anomala* extract. In one embodiment, the composition comprises about 0.13 % by weight of *Pichia anomala* extract.

In certain preferred embodiments, the compositions comprise from about 0.01 to about 10 % by weight, more preferably about 0.05 to about 1 % by weight, of retinol. In one embodiment, the composition comprises about 0.1 % by weight of retinol.

In certain embodiments, the weight ratio of *Pichia anomala* extract to retinol in the compositions is from about 0.5 to about 2. In one embodiment, the weight ratio of *Pichia anomala* extract to retinol in the compositions is about 1.3.

### Topical Compositions

The compositions of the present invention are applied topically to human skin or hair. Accordingly, the composition may further include a cosmetically acceptable topical carrier that may be from about 50% to about 99.99%, by weight, of the composition (e.g., from about 80% to about 99%, by weight, of the composition). In a preferred embodiment of the invention, the cosmetically acceptable topical carrier includes water.

The compositions may be made into a wide variety of product types that include but are not limited to lotions, creams, gels, sticks, sprays, ointments, cleansing liquid washes and solid bars, shampoos and hair conditioners, hair fixers, pastes, foams, powders, mousses, shaving creams, wipes, patches, hydrogels, film-forming products, facial masks and skin masks, films and make-up such as foundations, and mascaras. These product types may contain several types of cosmetically acceptable topical carriers including, but not limited to solutions, suspensions, emulsions such as microemulsions and nanoemulsions, gels, solids and liposomes. The following are non-limiting examples of such carriers. Other carriers can be formulated by those of ordinary skill in the art.

The compositions useful in the present invention can be formulated as solutions. Solutions typically include an aqueous or organic solvent (e.g., from about 50% to about 99.99% or from about 90% to about 99% of a cosmetically acceptable aqueous or organic solvent). Examples of suitable organic solvents include propylene glycol, polyethylene glycol, polypropylene glycol, glycerol, 1,2,4-butanetriol, sorbitol esters, 1,2,6-hexanetriol, ethanol, and mixtures thereof.

Compositions useful in the subject invention may be formulated as a solution comprising an emollient. Such compositions preferably contain from about 2% to about 50% of an emollient(s). As used herein, "emollients" refer to materials used for the prevention or relief of dryness, such as by preventing the transepidermal loss of water from the skin. Examples of emollients include those known in the art. Examples of particularly suitable emollients include vegetable oils, mineral oils, fatty esters, and the like.

A lotion can be made from such a solution. Lotions typically contain from about 1% to about 20% (e.g., from about 5% to about 10%) of an emollient(s) and from about 50% to about 90% (e.g., from about 60% to about 80%) of water.

Another type of product that may be formulated from a solution is a cream. A cream typically contains from about 5% to about 50% (e.g., from about 10% to about 20%) of an emollient(s) and from about 45% to about 85% (e.g., from about 50% to about 75%) of water.

The composition of the present invention may include water or alternatively be anhydrous or be an ointment that includes no water but organic and/or silicone solvents, oils, lipids and waxes. An ointment may contain a simple base of animal or vegetable oils or semi-solid hydrocarbons. An ointment may contain from about 2% to about 10% of an emollient(s) plus from about 0.1% to about 2% of a thickening agent(s).

The composition may be formulated as an emulsion. If the topical carrier is an emulsion, from about 1% to about 10% (e.g., from about 2% to about 5%) of the topical carrier contains an emulsifier(s). Emulsifiers may be nonionic, anionic or cationic. Examples of emulsifiers are well known in the art.

Lotions and creams can be formulated as emulsions. Typically, such lotions contain from 0.5% to about 5% of an emulsifier(s). Such creams typically contain from about 1% to about 20% (e.g., from about 5% to about 10%) of an emollient(s); from about 20% to about 80% (e.g., from 30% to about 70%) of water; and from about 1% to about 10% (e.g., from about 2% to about 5%) of an emulsifier(s).

Single emulsion skin care preparations, such as lotions and creams, of the oil-in-water type and water-in-oil type are well-known in the cosmetic art and are useful in the subject invention. Multiphase emulsion compositions, such as the water-in-oil-in-water type or the oil-in-water-in-oil type, are also useful in the subject invention. In general, such single or multiphase emulsions contain water, emollients, and emulsifiers as essential ingredients.

The compositions of this invention can also be formulated as a gel (e.g., an aqueous, alcohol, alcohol/water, or oil gel using a suitable gelling agent(s)). Suitable gelling agents for aqueous and/or alcoholic gels include, but are not limited to, natural gums, acrylic acid and acrylate polymers and copolymers, and cellulose derivatives (e.g., hydroxymethyl cellulose and hydroxypropyl cellulose). Suitable gelling agents for oils (such as mineral oil) include, but are

not limited to, hydrogenated butylene/ethylene/styrene copolymer and hydrogenated ethylene/propylene/styrene copolymer. Such gels typically contain between about 0.1% and 5%, by weight, of such gelling agents.

5 In one embodiment, the composition is a gel cream. The gel cream aesthetic is characterized with a watery break, semi-translucent aspect and light after-feel. As used herein, the term "gel cream" means a formulation with low levels of oil droplets suspended in aqueous gel matrix.

0 The compositions of the present invention can also be formulated into a solid formulation (e.g., a wax-based stick, soap bar composition, powder, or a wipe containing powder).

The compositions may contain, in addition to the components above, a wide variety of additional oil-soluble materials and/or water-soluble materials conventionally used in compositions for use on skin and hair, at their art-established levels.

#### **Additional Cosmetically Active Agents**

5 The compositions of the present invention may further comprise any of a variety of additional cosmetically active agents. Examples of suitable additional active agents include: skin lightening agents, darkening agents, additional anti-aging agents, tropoelastin promoters, collagen promoters, anti-acne agents, shine control agents, anti-microbial agents such as anti-yeast agents, anti-fungal, and anti-bacterial agents, anti-inflammatory agents, anti-parasite agents, external analgesics, sunscreens, photoprotectors, antioxidants, keratolytic agents, detergents/surfactants, moisturizers, nutrients, vitamins, energy enhancers, anti-perspiration agents, astringents, deodorants, hair removers, hair growth enhancing agents, hair growth delaying agents, firming agents, hydration boosters, efficacy boosters, anti-callous agents, agents for skin conditioning, anti-cellulite agents, odor-control agents such as odor masking or pH-changing agents, and the like.

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Examples of various suitable additional cosmetically acceptable actives include hydroxy acids; benzoyl peroxide; D-panthenol; UV filters such as but not limited to avobenzene (PARSOL 1789), bisdisulizole disodium (NEO HELIOPAN AP), diethylamino hydroxybenzoyl hexyl benzoate (UVINUL A Plus), ecamsule (MEXORYL SX), methyl anthranilate, 4-aminobenzoic acid (PABA), cinoxate, ethylhexyl triazone (UVINUL T 150),

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homosalate, 4-methylbenzylidene camphor (PARSOL 5000), octyl methoxycinnamate (Octinoxate), octyl salicylate (Octisalate), padimate O (ESCALOL 507), phenylbenzimidazole sulfonic acid (ENSULIZOLE), polysilicone-15 (PARSOL SLX), trolamine salicylate, Bemotrizinol (TINOSORB S), benzophenones 1–12, dioxybenzone, drometrizole trisiloxane (MEXORYL XL), iscotrizinol (UVASORB HEB), octocrylene, oxybenzone (EUSOLEX 4360), sulisobenzone, bisoctrizole (TINOSORB M), titanium dioxide, zinc oxide; carotenoids; free radical scavengers; spin traps; other retinoids and retinoid precursors such as retinoic acid and retinyl palmitate; ceramides; polyunsaturated fatty acids; essential fatty acids; enzymes; enzyme inhibitors; minerals; hormones such as estrogens; steroids such as hydrocortisone; 2-dimethylaminoethanol; copper salts such as copper chloride; peptides containing copper, coenzyme Q10; amino acids such as proline; vitamins; lactobionic acid; acetyl-coenzyme A; niacin; riboflavin; thiamin; ribose; electron transporters such as NADH and FADH<sub>2</sub>; and other botanical extracts such as oat, aloe vera, Feverfew, Soy, Shiitake mushroom extracts, and derivatives and mixtures thereof.

In certain preferred embodiments, the compositions comprise a combination of *Pichia anomala* extract, retinol, and at least one additional skin moisturizing active agent.

In certain preferred embodiments, the compositions comprise the combination of *Pichia anomala* extract, retinol, and at least one additional agent for improving the appearance of at least one sign of aging in skin. Examples of suitable additional agents improving the appearance of at least one sign of aging in skin include, but are not limited to, tropoelastin promoters, collagen promoters, retinoids, hyaluronic acid including cross-linked hyaluronic acid, chondroitin sulfate including cross-linked chondroitin sulfate, dimethylaminoethanol, N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine, alpha hydroxy acids, polyhydroxyacids, sugar amines, and combinations of two or more thereof.

“Tropoelastin promoters,” as used herein, refers to a class of compounds that possess the biological activity of enhancing the production of tropoelastin. Tropoelastin promoters, according to the present invention, include all natural or synthetic compounds capable of enhancing the production of tropoelastin in the human body.

Examples of suitable tropoelastin promoters include, but are not limited to, blackberry extracts, cotinus extracts, feverfew extracts, and bimetal complexes having

5 copper and/or zinc constituents. The bimetal complex having copper and/or zinc constituents may be, for example, copper-zinc citrate, copper-zinc oxalate, copper-zinc tartarate, copper-zinc malate, copper-zinc succinate, copper-zinc malonate, copper-zinc maleate, copper-zinc aspartate, copper-zinc glutamate, copper-zinc glutarate, copper-zinc fumarate, copper-zinc glucarate, copper-zinc polyacrylic acid, copper-zinc adipate, copper-zinc pimelate, copper-zinc suberate, copper-zinc azealate, copper-zinc sebacate, copper-zinc dodecanoate, or combinations thereof. In a preferred embodiment, the tropoelastin promoter is selected from blackberry extracts, cotinus extracts, feverfew extracts, and combinations thereof. In a particularly preferred embodiment, the tropoelastin promoter is selected from blackberry extracts, feverfew extracts, and combinations thereof.

0 By "blackberry extract," it is meant a blend of compounds isolated from the plant of the genus *Rubus*, and preferably *Rubus fruticosus*. In one embodiment, the compounds are isolated from the flowers of the plant. In a further embodiment, the compounds are isolated from dried flowers of the plant. Such compounds may be isolated from one or more part of the plant (*e.g.*, the whole plant, flower, seed, root, rhizome, stem, fruit and/or leaf of the plant). In a preferred embodiment, the blackberry extract is a blackberry leaf extract. One particularly suitable blackberry extract is produced by extracting the leaves of *Rubus fruticosus* with a mixture of water and ethanol compounded to an activity of about 5% to about 10%, with a maltodextrin matrix, commercially available from Symrise Inc. of Teterboro, NJ, and is sold under the name SYMMATRIX.

5 Compositions of the present invention may include a cosmetically effective amount of one or more tropoelastin promoters such as those described above. The compositions preferably include, on an active basis, from about 0.1% to about 10% of the tropoelastin promoters, more preferably from about 0.5% to about 5% of tropoelastin promoters, and most preferably from about 0.5% to about 2% of the tropoelastin promoters.

25 "Collagen promoter," as used herein, refers to compounds that possess the biological activity of enhancing the production of collagen. "Non-retinoid collagen promoters" according to the present invention include all natural or synthetic compounds that are not retinoids, or derived from retinoids, and are capable of enhancing the production of collagen in the human body.

Examples of suitable collagen promoters include, but are not limited to the following: Retinoids, extracts of feverfew (*Tanacetum parthenium*), extracts of *Centella asiatica*, and extracts of *Siegesbeckia orientalis*; extracts of soy; collagen-promoting peptides; ursolic acid; and asiaticoside.

5 *Centella asiatica*, also known as *Violette marronne* on Reunion Island, Gotu Kola or Indian pennywort in India, *Centella repanda* in North America, and Talapetraka in Madagascar, is a polymorphous herb and belongs to the family of *Umbelliferae (Apiaceae)*, particularly to the *Hydrocotyle* subfamily. It grows wild throughout the tropics and prefers moist and shady regions at an altitude of about 600 to 1200 meters above sea level.

0 *Centella asiatica* has three varieties: *Typica*, *Abyssinica*, and *Floridana*. The herb is known and used for its healing, sedative, analgesic, antidepressant, antiviral and antimicrobial properties. The biological activity of the herb appears to be due to the presence of triterpene molecules in the herb. A suitable extract of *Centella asiatica* is available as TECA from Bayer Consumer HealthCare of Basel, Switzerland.

5 By "extracts of *Siegesbeckia orientalis*," is meant any of various extracts of the plant *Siegesbeckia orientalis*, including Darutoside available from Sederma (Croda International Group of Edison, NJ).

Suitable collagen-promoting peptides include the following matrikine peptides, (*i.e.*, a peptide derived from the degradation of extracellular matrix proteins - collagen, elastin, or  
10 proteoglycan) including palmitoyl pentapeptides, such as MATRIXYL from Sederma (Croda International Group of Edison, NJ); GHK copper peptide available as PROCYTE from Photomedex of Montgomeryville, PA; Palmitoyl GHK peptide available as Biopopeptide CL from Sederma (Croda International Group of Edison, NJ); Biomimetic tetrapeptides, such as those available as Chronoline Tri Peptide from Unipex of Québec, Canada ; and Palmitoyl tri-  
25 peptide, available as Syn-Coll from DSM of Basel, Switzerland.

Ursolic acid is also known as pentacyclic triterpene acid, Prunol, Malol, Urson, beta-ursolic acid and 3-Beta-Hydroxy-Urs-12-En-28-Oic Acid. It is commercially available for example from Sigma-Aldrich of St. Louis, MO.

Asiaticoside, also known chemically as: [6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-  
30 (3,4,5-trihydroxy-6-methyloxan-2-yl)oxyoxan-2-yl]oxymethyl]-3,4,5-trihydroxyoxan-2-yl]

10,11-dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-  
2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylate) is  
commercially available for example from Bayer Santé Familiale Division Serdex, 69,  
Boulevard Victor Hugo 93400 SAINT-OUEN France.

5 Compositions of the present invention may include a cosmetically effective amount  
of one or more collagen promoters. The compositions preferably include, on an active  
basis, from about 0.1% to about 10% of the collagen promoters, more preferably from  
about 0.5% to about 5% of collagen promoters, and most preferably from about 0.5% to  
about 2% of the collagen promoters.

0 The compositions of the present invention may comprise additionally at least one  
skin lightening active agent. Examples of suitable skin lightening active agents include, but  
are not limited to, tyrosinase inhibitors, melanin-degradation agents, melanosome transfer  
inhibiting agents including PAR-2 antagonists, exfoliants, sunscreens, retinoids, antioxidants,  
Tranexamic acid, tranexamic acid cetyl ester hydrochloride, skin bleaching agents, linoleic  
5 acid, adenosine monophosphate disodium salt, Chamomilla extract, allantoin, opacifiers,  
talcs and silicas, zinc salts, and the like, and other agents as described in Solano *et al.*  
*Pigment Cell Res.* 19 (550-571) and Ando *et al.* *Int J Mol Sci* 11 (2566-2575).

Examples of suitable tyrosinase inhibitors include but, are not limited to, Vitamin C  
and its derivatives, Vitamin E and its derivatives, Kojic Acid, Arbutin, resorcinols,  
10 hydroquinone, Flavones e.g. Licorice flavanoids, Licorice root extract, Mulberry root extract,  
Dioscorea Coposita root extract, Saxifraga extract and the like, Ellagic acid, Salicylates and  
derivatives, Glucosamine and derivatives, Fullerene, Hinokitiol, Dioic acid, Acetyl  
glucosamine, 5,5'-dipropyl-biphenyl-2,2'-diol (Magnolignan), 4-(4-hydroxyphenyl)-2-butanol  
(4-HPB), combinations of two or more thereof, and the like. Examples of vitamin C  
25 derivatives include, but are not limited to, ascorbic acid and salts, Ascorbic Acid-2-Glucoside,  
sodium ascorbyl phosphate, magnesium ascorbyl phosphate, and natural extract enriched in  
vitamin C. Examples of vitamin E derivatives include, but are not limited to, alpha-  
tocopherol, beta, tocopherol, gamma-tocopherol, delta-tocopherol, alpha-tocotrienol, beta-  
tocotrienol, gamma-tocotrienol, delta-tocotrienol and mixtures thereof, tocopherol acetate,  
30 tocopherol phosphate and natural extracts enriched in vitamin E derivatives. Examples of  
resorcinol derivatives include, but are not limited to, resorcinol, 4-substituted resorcinols

like 4-alkylresorcinols such as 4-butyresorcinol (rucinol), 4-hexylresorcinol (Synovea HR, Sytheon), phenylethyl resorcinol (Symwhite, Symrise), 1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)-Propane (nivotol, Unigen) and the like and natural extracts enriched in resorcinols. Examples of salicylates include, but are not limited to, 4-methoxy potassium salicylate, salicylic acid, acetylsalicylic acid, 4-methoxysalicylic acid and their salts. In certain preferred embodiments, the tyrosinase inhibitors include a 4-substituted resorcinol, a vitamin C derivative, or a vitamin E derivative. In more preferred embodiments, the tyrosinase inhibitor comprises Phenylethyl resorcinol, 4-hexyl resorcinol, or ascorbyl-2-glucoside.

Examples of suitable melanin-degradation agents include, but are not limited to, peroxides and enzymes such as peroxidases and ligninases. In certain preferred embodiments, the melanin-inhibiting agents include a peroxide or a ligninase.

Examples of suitable melanosome transfer inhibiting agents including PAR-2 antagonists such as soy trypsin inhibitor or Bowman-Birk Inhibitor, Vitamin B3 and derivatives such as Niacinamide, Essential soy, Whole Soy, Soy extract. In certain preferred embodiments, the melanosome transfer inhibiting agents includes a soy extract or niacinamide.

Examples of exfoliants include, but are not limited to, alpha-hydroxy acids such as lactic acid, glycolic acid, malic acid, tartaric acid, citric acid, or any combination of any of the foregoing, beta-hydroxy acids such as salicylic acid, polyhydroxy acids such as lactobionic acid and gluconic acid, and mechanical exfoliation such as microdermabrasion. In certain preferred embodiments, the exfoliants include glycolic acid or salicylic acid.

Examples of retinoids other than retinol include, but are not limited to, retinal (Vitamin A aldehyde), retinyl acetate, retinyl propionate, retinyl linoleate, retinoic acid, retinyl palmitate, isotretinoin, tazarotene, bexarotene, adapalene, combinations of two or more thereof and the like. In certain preferred embodiments, the retinoid is selected from the group consisting of retinal, retinyl acetate, retinyl propionate, retinyl linoleate, and combinations of two or more thereof.

Examples of antioxidants include, but are not limited to, water-soluble antioxidants such as sulfhydryl compounds and their derivatives (e.g., sodium metabisulfite and N-acetyl-

cysteine, glutathione), lipoic acid and dihydrolipoic acid, stilbenoids such as resveratrol and derivatives, lactoferrin, iron and copper chelators and ascorbic acid and ascorbic acid derivatives (*e.g.*, ascorbyl-2-glucoside, ascorbyl palmitate and ascorbyl polypeptide). Oil-soluble antioxidants suitable for use in the compositions of this invention include, but are not limited to, butylated hydroxytoluene, retinoids, tocopherols (*e.g.*, tocopherol acetate), tocotrienols, and ubiquinones. Natural extracts containing antioxidants suitable for use in the compositions of this invention, include, but not limited to, extracts containing flavonoids and isoflavonoids and their derivatives (*e.g.*, genistein and diadzein), extracts containing resveratrol and the like. Examples of such natural extracts include grape seed, green tea, black tea, white tea, pine bark, feverfew, parthenolide-free feverfew, oat extracts, blackberry extract, cotinus extract, soy extract, pomelo extract, wheat germ extract, Hesperedin, Grape extract, Portulaca extract, Licochalcone, chalcone, 2,2'-dihydroxy chalcone, Primula extract, propolis, and the like.

The additional cosmetically active agent may be present in a composition in any suitable amount, for example, in an amount of from about 0.0001% to about 20% by weight of the composition, *e.g.*, about 0.001% to about 10% such as about 0.01% to about 5%. In certain preferred embodiments, in an amount of 0.1% to 5% and in other preferred embodiments from 1% to 2%.

Compositions of the present invention may include a cosmetically effective amount of one or more anti-inflammatory compounds.

Examples of suitable anti-inflammatory agents include substituted resorcinols, (E)-3-(4-methylphenylsulfonyl)-2-propenenitrile (such as "Bay 11-7082," commercially available from Sigma-Aldrich of St. Louis, Missouri), tetrahydrocurcuminoids (such as Tetrahydrocurcuminoid CG, available from Sabinsa Corporation of Piscataway, NJ), extracts and materials derived from the following: *Phellodendron amurense* Cortex Extract (PCE), Non-Denatured Soy (*Glycine max*), Feverfew (*Tanacetum parthenium*), Ginger (*Zingiber officinale*), Ginko (*Ginkgo biloba*), Madecassoside (*Centella asiatica* extract ingredient), Cotinus (*Cotinus coggryria*), Butterbur Extract (*Petasites hybridus*), Goji Berry (*Lycium barbarum*), Milk Thistle Extract (*Silybum marianum*), Honeysuckle (*Lonicera japonica*), Basalm of Peru (*Myroxylon pereirae*), Sage (*Salvia officinalis*), Cranberry Extract (*Vaccinium oxycoccos*), Amaranth Oil (*Amaranthus cruentus*), Pomegranate (*Punica granatum*), Yerbe

Mate (*Ilex paraguariensis* Leaf Extract), White Lily Flower Extract (*Lilium candidum*), Olive Leaf Extract (*Olea europaea*), Phloretin (apple extract), Oat Flour (*Avena sativa*), Lifenol (Hops: *Humulus lupulus*) Extract, Bugrane P (*Ononis spinosa*), Licochalcone (Licorice: *Glycyrrhiza inflata* extract ingredient), Symrelief (Bisabolol and Ginger extract), combinations of two or more thereof, and the like.

In one embodiment, the anti-inflammatory agent is a resorcinol. Particularly suitable substituted resorcinols include 4-hexyl resorcinol and 4-octylresorcinol, particularly 4-hexyl resorcinol. 4-Hexyl resorcinol is commercially available as SYNOVEA HR from Sytheon of Lincoln Park, NJ. 4-Octylresorcinol is commercially available from City Chemical LLC of West Haven, Connecticut.

By "extracts of feverfew," it is meant extracts of the plant "*Tanacetum parthenium*," such as may be produced according to the details set forth in US Patent Application Publication No. 2007/0196523, entitled "PARTHENOLIDE FREE BIOACTIVE INGREDIENTS FROM FEVERFEW (TANACETUM PARTHENIUM) AND PROCESSES FOR THEIR PRODUCTION."

One particularly suitable feverfew extract is commercially available as about 20% active feverfew, from Integrated Botanical Technologies of Ossining, NY.

In the skin care composition of the invention, the ratio of the amounts of the combined *Pichia anomala* extract and retinol to the anti-inflammatory compound may be varied. For example, the extract and the anti-inflammatory compound may be present in a weight ratio (which is determined by dividing the amount by weight of the dry extract by the amount by weight of the anti-inflammatory compound) of about 0.001 to about 100, preferably about 0.01 to about 10, more preferably about 0.25 to about 2.

A variety of other materials may also be present in the compositions of the present invention. In certain preferred embodiments, the composition comprises one or more topical ingredients selected from the group consisting of: surfactants, chelating agents, emollients, humectants, conditioners, preservatives, opacifiers, fragrances and the like.

What is meant by an emollient is a compound that helps to maintain the soft, smooth, and pliable appearance of the skin (*e.g.*, by remaining on the skin surface or in the stratum corneum to act as a lubricant). Examples of suitable emollients include those found in Chapter 35, pages 399-415 (Skin Feel Agents, by G Zocchi) in *Handbook of Cosmetic*

*Science and Technology* (edited by A. Barel, M. Paye and H. Maibach, published in 2001 by Marcel Dekker, Inc., New York, NY), and include, but are not limited to, petrolatum, hexyldecyl stearate and plant, nut, and vegetable oils such as macadamia nut oil, rice bran oil, grape seed oil, palm oil, prim rose oil, hydrogenates peanut oil, and avocado oil.

5 What is meant by a humectant is a compound intended to increase the water content of the top layers of skin (*e.g.*, hygroscopic compounds). Examples of suitable humectants include those found Chapter 35, pages 399-415 (Skin Feel Agents, by G Zocchi) in Handbook of Cosmetic Science and Technology (edited by A. Barel, M. Paye and H. Maibach, Published in 2001 by Marcel Dekker, Inc New York, NY) and include, but are not  
0 limited to, glycerin, sorbitol or trehalose (*e.g.*,  $\alpha,\alpha$ -trehalose,  $\beta,\beta$ -trehalose,  $\alpha,\beta$ -trehalose) or a salt or ester thereof (*e.g.*, trehalose 6-phosphate).

In one embodiment, the composition contains glycerin. For example, the composition contains at least 10 wt % glycerin. The composition may contain at least 12 wt % glycerin.

5 In another embodiment, the composition comprises 0.5 to 25 weight percent glycerin. In one embodiment, the composition comprises 1 to 6 weight percent glycerin.

In another embodiment, the composition has a pH of 6.5 or less. For example, the composition may have a pH of 5.5 or less.

20 In a particular embodiment, the composition contains at least 10 wt% glycerin and has a pH or 6.5 or less.

In another embodiment, the composition comprises 0.1 to 5 weight percent of cetearyl olivate. In one embodiment, the composition comprises 0.1 to 2 weight percent of cetearyl olivate.

25 In a further embodiment, the composition also comprises 0.1 to 5 weight percent of sorbitan olivate. In one embodiment, the composition comprises 0.1 to 2 weight percent of sorbitan olivate.

A convenient source of cetearyl olivate and sorbitan olivate is Olivem 1000, commercially available from Hallstar Itali.

5 What is meant by a surfactant is a surface-active agent intended to cleanse or emulsify. Examples of suitable surfactants include those found in Chapter 37, pages 431-450 (Classification of surfactants, by L. Oldenhove de Guertechin) in *Handbook of Cosmetic Science and Technology* (edited by A. Barel, M. Paye and H. Maibach, Published in 2001 by Marcel Dekker, Inc., New York, NY) and include, but are not limited to anionic surfactants such as sulfates, cationic surfactants such as betaines, amphoteric surfactants such as sodium coco glycinate, noionic surfactants such as alkyl polyglucosides.

0 Examples of suitable chelating agents include those which are capable of protecting and preserving the compositions of this invention. Preferably, the chelating agent is ethylenediamine tetracetic acid ("EDTA"), and more preferably is tetrasodium EDTA, available commercially from Dow Chemical Company of Midland, Michigan under the trade name VERSENE 100XL.

5 Suitable preservatives include, for example, parabens, quaternary ammonium species, phenoxyethanol, benzoates, DMDM hydantoin, organic acids and are present in the composition in an amount, based upon the total weight of the composition, from about 0 to about 1 percent or from about 0.05 percent to about 0.5 percent.

10 Any of a variety of conditioners which impart additional attributes, such as gloss to the hair, are suitable for use in this invention. Examples include, but are not limited to, volatile silicone conditioning agent having an atmospheric pressure boiling point less than about 220°C. Examples of suitable volatile silicones nonexclusively include polydimethylsiloxane, polydimethylcyclosiloxane, hexamethyldisiloxane, cyclomethicone fluids such as polydimethylcyclosiloxane available commercially from Dow Corning Corporation of Midland, Michigan under the tradename, "DC-345" and mixtures thereof, and preferably include cyclomethicone fluids. Other suitable conditioners include cationic polymers, including polyquarterniums, cationic guar, and the like.

25 Any of a variety of commercially available pearlescent or opacifying agents are suitable for use in the composition. Examples of suitable pearlescent or opacifying agents include, but are not limited to, mono or diesters of (a) fatty acids having from about 16 to about 22 carbon atoms and (b) either ethylene or propylene glycol; mono or diesters of (a) fatty acids having from about 16 to about 22 carbon atoms (b) a polyalkylene glycol of the

formula: HO-(JO)<sub>a</sub>-H, wherein J is an alkylene group having from about 2 to about 3 carbon atoms; and a is 2 or 3; fatty alcohols containing from about 16 to about 22 carbon atoms; fatty esters of the formula: KCOOCH<sub>2</sub>L, wherein K and L independently contain from about 15 to about 21 carbon atoms; inorganic solids insoluble in the shampoo composition, and mixtures thereof.

Any fragrance compositions suitable for use on skin may be used in accord with the present invention.

In certain preferred embodiments, the present invention is in the form of a substrate comprising a composition of the present invention. Any suitable substrate may be used. Examples of suitable substrates and substrate materials are disclosed, for example, in U.S. Published Application Nos. 2005/0226834 and 2009/0241242 which are incorporated herein by reference in their entirety.

In certain preferred embodiments, the substrate is a wipe, glove, or a facial mask. Preferably, such embodiments comprise a water-insoluble substrate as such is defined in the cited references above. For certain embodiments, the water-insoluble substrate may have a size and shape such that it covers the face of a human user to facilitate placing the water-insoluble substrate about the face of the user as a mask substrate. For example, the water-insoluble mask substrate may have openings for a mouth, nose, and/or eyes of the user. Alternatively, the water-insoluble substrate may have no such openings. Such a configuration without openings may be useful for embodiments of the invention in which the water-insoluble substrate is intended to be draped over a non-facial expanse of skin or if the water-insoluble substrate is intended to be used as wipe. The water-insoluble substrate may have various shapes, such as an angular shape (*e.g.*, rectangular) or an arcuate shape such as circular or oval. For certain embodiments, the substrate is a glove such as described in U.S. Published Application No 2006/0141014 which is incorporated herein in its entirety. In one embodiment of the invention, the product includes a plurality of water-insoluble substrates of different shapes.

The present invention further comprises a method of improving the barrier function and/or moisturization of skin by applying to skin in need of improving skin barrier function and/or moisturization a combination of *Pichia anomala* extract and retinol. The method

comprises for example topically applying a composition of the present invention comprising combined *Pichia anomala* extract and retinol to skin in need of improving skin barrier function and moisturization. Such topical application may be to any skin in need of treatment on the body, for example skin of the face, lips, neck, chest, back, arms, axilla, hands, feet and/or legs. The combined *Pichia anomala* extract and retinol are preferably applied in an effective amount that results in the desired improvement of skin barrier function being achieved.

The present invention further comprises a method of improving the appearance of at least one sign of skin aging by applying to skin in need of improving the appearance of at least one sign of skin aging a combination of *Pichia anomala* extract and retinol. The method comprises for example topically applying a composition of the present invention comprising *Pichia anomala* extract and retinol to skin in need of treatment of at least one sign of skin aging. Such topical application may be to any skin in need of treatment on the body, for example skin of the face, lips, neck, chest, back, arms, axilla, hands, feet and/or legs. The combined *Pichia anomala* extract and retinol are preferably applied in an effective amount that results in the desired improvement in the appearance of at least one sign of skin aging being achieved.

Any suitable method of applying the composition to the skin in need may be used. For example, the composition may be applied directly from a package to the skin in need, by hand to the skin in need, or may be transferred from a substrate such as a wipe or mask, or a combination of two or more thereof. In other embodiments, the composition may be applied via a dropper, tube, roller, spray, and patch or added to a bath or otherwise to water to be applied to the skin, and the like. The composition may be applied in a variety of manners /forms, including, without limitation, as a leave-on cream, mask, and / or serum.

In certain embodiments, the methods of the present invention comprise applying at least two different compositions or products comprising *Pichia anomala* extract and retinol to the skin. For example, the methods may comprise applying a first composition comprising an extract of *Pichia anomala*, followed by applying a second composition comprising retinol that is different from the first composition, to the skin in need of treatment.

In certain preferred embodiments, the first and second composition may be independently selected from the group consisting of lotions, cleansers, masks, wipes, creams, serums, gels, and the like. In certain preferred embodiments, at least one of the first and second compositions is a cleanser, lotion, cream, essence, or serum and the other is a facial mask or wipe. In certain other preferred embodiments, at least one of the first and second compositions is a cleanser and the other is a lotion or cream.

The composition and formulations and products containing such compositions of the present invention may be prepared using methodology that is well known by an artisan of ordinary skill. These compositions may be useful in treating skins of aging such as wrinkles, loss of elasticity, uneven skin including reducing blotchiness. The composition may be used on a routine basis and is substantially free of skin irritants.

The following non-limiting examples further illustrate the present invention.

#### Example 1

Hyaluronic acid (HA) production by human skin explants was determined after treating them with test Compositions 1-4 containing different amounts of *Pichia anomala* extract as follows.

First, a gel was made containing the following ingredients:

INCI	Weight %
Phenoxyethanol; Methylparaben; Ethylparaben; Propylparaben	0.7
Carbomer	1
20% Sodium Hydroxide Solution	QS (pH = 6)
Water	98.3

Composition 1-4 were made by combining 2 wt% of the gel with different amounts of a 5% aqueous solution of *Pichia anomala* extract, along with additional ingredients. The *Pichia anomala* had been grown on kiwi plant. The 5% aqueous solution was treated as a 100% stock solution.

Compositions 1-4 contained the ingredients shown in Table 1.

20192222871 28 Aug 2019

TABLE 1

INCI	Composition 1 (weight %)	Composition 2 (weight %)	Composition 3 (weight %)	Composition 4 (weight %)
Gel	2	2	2	2
Cetearyl Alcohol; Cetearyl Glucoside	7	7	7	7
Isononyl Isononanoate	8	8	8	8
Phenoxyethanol; Methylparaben; Ethylparaben; Propylparaben	0.7	0.7	0.7	0.7
5% Aq. Solution of <i>Pichia anomala</i> extract	2.5 (0.065 wt% <i>Pichia anomala</i> extract)	5 (0.13 wt% <i>Pichia anomala</i> extract)	7.5 (0.195 wt% <i>Pichia anomala</i> extract)	10 (0.26 wt% <i>Pichia anomala</i> extract)
Water	79.8	77.3	74.8	72.3

HA production by Compositions 1-4 was determined using immunohistology on normal explants of human skin from three donors (29, 30, 55 years old). Eight mm diameter punches were cut from the explants and deposited on pieces of sterile gauze and placed, one explant per well, in six well plates with 3mL of culture media. The culture media was sold under the tradename GIBCO DMEM/F-12 (ThermoFisher Scientific, Waltham, MA, catalog #11514436) with 1% GIBCO Penicillin-Streptomycin (ThermoFisher Scientific, catalog #11528876) and 0.1% amphotericin B sold under the tradename FUNGIZONE (ThermoFisher Scientific, catalog #11510496).

For each test composition, 5  $\mu$ l of test composition was applied to an explant once a day for 5 days. An untreated explant sample was used as the control. On day 7 the explants were recovered, wiped with a sterile gauze, then cut in half vertically and fixed in 4% paraformaldehyde (V/V). On day 8 the explants were dehydrated and embedded in paraffin. Each test composition was tested in triplicate.

The paraffinized slides were stripped with xylene and epitope retrieval was carried out with PT link (Agilent, Santa Clara, CA) and target retrieval solution sold under the tradename ENVISION Flex, High pH (Dako, DM828, Agilent, Santa Clara, CA). Slides were then rinsed with wash buffer sold under the tradename ENVISION (Dako, DM831, Agilent, Santa Clara, CA) one time for 10 mins. Permeabilization and saturation were done with PBS 0.3% Triton/5% goat serum (Dako, Santa Clara, CA, catalog #CP3418/X090710-8) for 30 mins, followed by labeling

with Hyaluronic Acid Binding Protein (“HAPB” from Calbiochem, catalog #385911, Millipore Sigma, St. Louis, NJ) overnight at 4°C. The next day the slides were rinsed with PBS three times for 5 minutes each. Antibody was revealed with biotin-binding protein covalently attached to a fluorescent label sold under the tradename ALEXA FLUOR 488 streptavidin (Invitrogen™, catalog #S11223) and staining of nuclei was done with DAPI solution (Dako, Santa Clara, CA) at 5µg/ml for 30 min at ambient temperature. Slides were then rinsed with PBS and mounted with Fluoprep mounting medium (bioMerieux UK Ltd.,UK catalog #75521).

Pictures of the skin sections were taken with an Olympus IX70 Fluorescence microscope (Olympus Corporation, Japan) coupled to an image analysis system (NIS-Elements AR software, Nikon Instruments, Melville, NY). Quantitative analysis of images was conducted with ImageJ software.

The results are shown in Table 2. The results are expressed as average fluorescence intensity of the dermis (in Arbitrary Units (AU)). Fluorescence intensity (green) is proportional to the synthesis of HAPB.

**TABLE 2**

	<b>Amount of 5% Solution of <i>Pichia anomala</i> extract</b>	<b>Weight percent of <i>Pichia anomala</i> extract</b>	<b>Delta of HAPB as compared to untreated control</b>
Composition 1	2.5%	0.065%	10.36583333
Composition 2	5%	0.13%	17.73
Composition 3	7.5%	0.195%	15.23666667
Composition 4	10%	0.26%	15.4125

These results suggest that maximum HA production by skin is obtained using about 0.13% weight percent of the *Pichia anomala* extract. Above this amount, production of HA leveled off.

### Example 2

Hyaluronic acid (HA) production by human skin explants was determined after treating them with four test compositions: Composition 2 described in Example 1 (containing *Pichia anomala* extract), Composition 5 (containing retinol), and Compositions 6 and 7 (containing both) according to the invention.

Composition 5 contained 0.1 wt % retinol and the other ingredients shown in Table 3.

**TABLE 3:** Composition 5

INCI	Weight %
Water	62.18
Sodium Acryloyldimethyltaurate/VP Crosspolymer	0.5
Disodium EDTA	0.1
Glycerin	5
Butylene Glycol	2
Sodium Hyaluronate	0.1
Caprylyl Glycol	0.5
Stearyl Alcohol; Ceteareth-20	3
Cetearyl Alcohol; Ceteareth-20	3
Isohexadecane	1.5
PPG-15 Stearyl Ether	4.5
Pentaerythrityl Tetraethylhexanoate	7
BHT	0.1
Polyacrylamide; Laureth-7; C13-14 Isoparaffin	1
Dimethicone; Dimethicone Crosspolymer	5
Dimethicone; Trisiloxane; Dimethicone; Dimethicone	3
Ascorbic Acid	0.05
Phenoxyethanol	0.5
Chlorphenesin	0.2
Polyethylene; PTFE	0.5
Polysorbate 20; Retinol	0.23
Sodium Hydroxide	0.04

Composition 5 was prepared as follows. Removed Polysorbate 20; Retinol from cold  
 5 storage. Placed in 50°C oven until melted. Ascorbic Acid Premix: Mixed Ascorbic Acid with 2%  
 of water and adjusted to pH ~5. Set aside for post add under 40°C. Main Phase: Added water  
 (-2% for Ascorbic Acid Premix) and began mixing until uniform. Then added Disodium EDTA.  
 Added Sodium Acryloyldimethyltaurate/VP Crosspolymer under high mixing. Made sure  
 homogeneous/fully hydrated before proceeding. Began heating to target 73°C. Glycerin and  
 10 Butylene Glycol were added. Above 65°C added Caprylyl Glycol, Chlorphenesin,  
 Phenoxyethanol, and Sodium Hyaluronate. Oil Phase: Added Isohexadecane, PPG 15 Stearyl  
 Ether, Pentaerythrityl Tetraethylhexanoate, Dimethicone; Dimethicone Crosspolymer,

Dimethicone; Trisiloxane; Dimethicone; Dimethicone Stearyl Alcohol; Cetareth-20, and Cetearyl Alcohol; Cetareth-20. Heated to Target 73°C while mixing. Added BHT 5 minutes before emulsification. Emulsification: When Main Phase and Oil Phase were ~ 73°C, slowly added Oil Phase to Water Phase. Mixed 10-15 min then added Polyacrylamide; Laureth-7; C13-14 Isoparaffin. Homogenized (Silverson, 4000 RPM) for 1 min. Started cooling under prop mixing. Post Adds: At or below 40°C added Polyethylene; PTFE, and Premix 1 (Ascorbic Acid). Measured pH, and adjusted to target pH of 6.5 - 6.8. Under Yellow Light and Argon Gas, added Retinol 50°C and mixed thoroughly for 10-15 min. Allowed to cool to 30-35°C.

Compositions 6 and 7 were prepared in the same way as Composition 5, except that 2.5 wt% or 5 wt%, respectively, of the 5% solution of *Pichia anomala* extract described in Example 1 was added after the ascorbic acid premix, and the amount of water adjusted QS.

HA production was determined using immunohistology as follows. Abdominal skin samples were obtained from human adults undergoing abdominoplasty surgery. Informed consent was obtained from each patient, and all experimental steps were approved by an institutional review board (IRB). Subcutaneous fat was carefully removed and skin biopsies of 0.93 cm<sup>2</sup> were prepared under sterile conditions and acclimated in culture medium DMEM/F12 (1:1) (Thermofisher Scientific, Bridgewater, NJ, catalog #11320082), with 2% heat-inactivated fetal bovine serum (Thermofisher Scientific, Bridgewater, NJ catalog #A3840202), 10 µg/mL insulin (Sigma Aldrich, Allentown, PA, catalog #I0516-5ML), 10 ng/mL hydrocortisone (Sigma Aldrich, Allentown, PA, catalog #H 0135), 10 ng/mL EGF(Sigma Aldrich, Allentown, PA, catalog #11376454001), 1x ABAM (Thermofisher Scientific, Bridgewater, NJ, catalog #15240062), under a 5% CO<sub>2</sub> humidified atmosphere overnight.

The explants were treated topically with 4 µl of test composition daily for 5 days. An untreated explant sample was used as the control. On day 6 the tissues were collected. The collected tissues were fixed in buffered formalin for 24 hrs. After fixation the samples were dehydrated and impregnated in paraffin using a Leica TP 1020 dehydration automat (Leica Biosystems, Buffalo Grove, IL). The samples were embedded using a Leica EG 1160 embedding station (Leica Biosystems). 5 µm thick sections were realized using a Leica RM 2125 Minot-type microtome (Leica Biosystems), and the sections were mounted on histological glass slides sold under the tradename SUPERFROST. The microscopic observations were realized using a Leica DMLB (Leica Biosystems) or Olympus BX43 microscope (Olympus Corporation, Japan). Pictures

were digitized with a numeric DP72 Olympus camera with CellD storing software (Olympus Corporation, Japan). The staining of hyaluronic acid was performed on paraffinized sections using a biotinylated HABP (AMS Biotechnology Limited, Abingdon, UK, product code AMS.HKD.BC41) diluted 1:100 in PBS-BSA 0.3% during 1h at room temperature and enhanced with a streptavidin/biotin system and revealed using a peroxidase substrate sold under the trademark Vector VIP (Vector Laboratories, Burlingame CA, catalog #SK-4600). The staining was assessed by microscopical observation.

The results are shown in Table 4.

**TABLE 4**

Composition	Increase in HABP	% increase over untreated
Untreated	31.425	
Composition 5 (0.1 wt% Retinol)	44.775	42.48
Composition 2 (0.13 wt% <i>Pichia anomala</i> extract)	44.28	40.90
Composition 6 (0.1% Retinol + 0.065 wt% <i>Pichia anomala</i> extract)	47.075	49.80
Composition 7 (0.1% Retinol + 0.13 wt% <i>Pichia anomala</i> extract)	54.175	72.39

Treatment of the tissue explants with Compositions 6 and 7 containing combinations of *Pichia anomala* extract and retinol according to the invention provided increased HA production compared with either Composition 5 containing only retinol or Composition 2 containing only *Pichia anomala* extract.

Moreover, Composition 7 containing 0.1% Retinol and 0.13 wt% *Pichia anomala* extract provided a percent increase in HAPB over untreated of 72.39, whereas Composition 2 containing 0.13 wt% *Pichia anomala* extract provided only a percent increase over untreated of 40.90 and Composition 5 containing 0.1 wt% retinol provided only a percent increase over untreated of 42.48.

**Example 3**

Gene expression for Heparin Binding Epidermal Growth Factor (HBEGF), Hyaluronic Acid Synthase 2 (HAS 2), and CD44 were measured for Composition 5 (0.1 wt % retinol), the 5% solution of extract of *Pichia anomala* described in Example 1, and combined application of Composition 5 and the 5% solution of extract of *Pichia anomala*.

First, abdominal skin samples were obtained from human adults undergoing abdominoplasty surgery. Informed consent was obtained from each patient, and all experimental steps were approved by an institutional review board (IRB). Subcutaneous fat was carefully removed and skin biopsies of 0.93 cm<sup>2</sup> were prepared under sterile conditions and acclimated in keratinocyte growth media sold under the tradename KGM-GOLD BULLETKIT (Lonza, Walkersville, MD), with 10% antibiotics under a 5% CO<sub>2</sub> humidified atmosphere overnight.

The skin explants were treated topically with 4 μL of the various formulations and left to incubate for 48 hours. An untreated explant sample was used as the control. In the case of combined application of Composition 5 and the 5% solution of *Pichia anomala* extract, 4 μL of each were applied. At the end of the 48h, epidermis and dermis were separated by heating. Each of them was lysed in 400μl of lysis buffer, consisting of 100 parts RLT buffer (RNeasy Mini kit, Qiagen, Valencia, CA), to one part 2-mercaptoethanol inside a reinforced tube with screw cap and o-ring closure, and ceramic beads in the tube for tissue grinding (sold under the tradename PRECELLYS CKMix50-R, Bertin Corp, Rockville, MD). The tubes were shaken for 40 sec at 6300 rpm. RNA was extracted from the solutions using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions and RNA was eluted in 25 μL RNase-free water. Reverse transcription (RT) was performed using the Applied Biosystems High Capacity Reverse Transcription Kit (ThermoFisher Scientific, Bridgewater, NJ).

Gene expression assays were performed by real time quantitative polymerase chain reaction (PCR) based on the SyBR green method. qPCR was run on a real time PCR system sold under the tradename CFX96 TOUCH (Bio-Rad Laboratories, Inc., Hercules, CA). The expression of these genes was normalized against the expression of the human RNA 18S

housekeeping gene. The fold changes were calculated in comparison to the untreated controls and ANOVA statistical test was performed.

The results are shown in Tables 5, 6 and 7.

**TABLE 5**

HBEGF expression					
Formulations	Observed	Ttest	Observed Normalized to untreated	Expected additive	% synergy
Untreated	100				
Composition 5 (0.1% Retinol)	391	0.011330802	291		
5% solution of <i>Pichia anomala</i> extract	77	0.308217589	0		
Composition 5 + 5% solution of <i>Pichia anomala</i> extract	594	0.001658771	494	291	69.76

5

Application of a combination of retinol and *Pichia anomala* extract resulted in an unexpected boost in HBEGF expression (494) compared with the HBEGF expression resulting from retinol alone (291). This was almost a 70% increase.

0

**TABLE 6**

HAS 2 expression					
Formulations	Observed	Ttest	Observed Normalized to untreated	Expected additive	% synergy
Untreated	100				
Composition 5 (0.1% Retinol)	122	0.406565333	22		
5% solution of <i>Pichia anomala</i> extract	93	0.704495851	0		
0.1% Retinol + 5% Extract of <i>Pichia anomala</i> solution	137	0.244891449	37	22	68.18

Application of a combination of retinol and *Pichia anomala* extract resulted in an unexpected increase -- almost 70% -- in the stimulation of HAS 2 expression (37) compared with the expected additive result of retinol alone plus *Pichia anomala* extract alone (22).

TABLE 7

CD44 expression					
Formulations	Observed	Ttest	Observed Normalized to untreated	Expected additive	% of synergy
Untreated	100				
Composition 5	120	0.407059542	20		
5% solution of <i>Pichia anomala</i> extract	90	0.397819867	0		
NTG RWR + Extract of <i>Pichia anomala</i> 5%	165	0.11812913	65	20	225

Application of a combination of retinol and *Pichia anomala* extract resulted in an unexpected stimulation of CD44 expression (65) compared with the expected additive result of retinol alone plus *Pichia anomala* extract alone (20). This was a 225% increase.

We claim:

1. A topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.
2. The topical composition of claim 1, wherein the extract of *Pichia anomala* is prepared from a strain of *Pichia anomala* present on kiwi fruit or leaves.
3. The topical composition of claim 1, wherein the extract of *Pichia anomala* is prepared from a strain of *Pichia anomala* present on sugar cane.
4. The topical composition of claim 1 further comprising at least 10 wt % glycerin.
5. The topical composition of claim 1 having a pH of 6.5 or less.
6. A method of treating a sign of skin aging, comprising topically applying to skin in need of treatment for skin aging a topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.
7. A method of improving skin barrier function and moisturization, comprising topically applying to skin in need of improving skin barrier function and moisturization a topical composition comprising an extract of *Pichia anomala* and retinol; wherein the weight ratio of the extract of *Pichia anomala* to retinol in the topical composition is about 1.3.
8. A method of increasing the amount of hyaluronic acid produced by skin when contacted with an extract of *Pichia anomala*, comprising contacting the skin with the extract in combination with retinol; wherein the skin is contacted with a weight ratio of the extract of *Pichia anomala* to retinol of about 1.3.
9. The use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for treating a sign of skin aging; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.

10. The use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for improving skin barrier function and moisturization; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.
11. The use of an extract of *Pichia anomala* and retinol for the manufacture of a medicament for increasing the amount of hyaluronic acid produced by skin; wherein the weight ratio of the extract of *Pichia anomala* to retinol is about 1.3.