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(54) **COSMETIC COMPOSITIONS AND METHODS FOR THEIR USE IN FIRING SKIN**

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(57) **ABSTRACT**

A method of reducing the appearance of loose, sagging, or flaccid skin is disclosed. The method can include topically applying to skin in need thereof a composition that includes 0.0001 wt. % to 10 wt. % *Argania spinosa* kernel extract, 0.0001 wt. % to 10 wt. % dill extract, 0.0001 wt. % to 10 wt. % *Myrciaria dubia* fruit extract, and 0.0001 wt. % to 10 wt. % *Croton lechleri* extract or *Morus alba* fruit extract. Topical application of the composition can increase elastin expression, increase elastin secretion, or increase elastin fiber formation in the skin in need thereof, and the appearance of loose, sagging, or flaccid skin can be reduced.

## COSMETIC COMPOSITIONS AND METHODS FOR THEIR USE IN FIRMING SKIN

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 16/007,143 filed Jun. 13, 2018, which claims priority to U.S. Provisional Application No. 62/518,847 filed Jun. 13, 2017. The contents of the referenced applications are incorporated into the present application by reference.

### BACKGROUND OF THE INVENTION

#### A. Field of the Invention

[0002] The present invention relates generally to cosmetic compositions that can be used to improve the skin's visual appearance by firming or tightening the skin. The composition can include a combination of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract to achieve these effects. The combination is chemically compatible and can be incorporated into a wide-range of product formulations (e.g., masks, serums, creams, cleansers, toners, gels, emulsions, gel emulsions, gel serums, etc.).

#### B. Description of Related Art

[0003] Ageing, chronic exposure to adverse environmental factors, malnutrition, fatigue, etc., can change the visual appearance, physical properties, or physiological functions of skin in ways that are considered visually undesirable. Some of the more notable and obvious changes include increased sagging, loss of firmness, and loss of elasticity. This can result in an "aged" appearance where the person looks older than their age. Further, loose skin can also result in the development of fine lines and wrinkles as well as coarse surface texture. Less obvious but measurable changes which occur as skin ages or endures chronic environmental insult include a general reduction in cellular and tissue vitality, reduction in cell replication rates, reduced cutaneous blood flow, reduced moisture content, accumulated errors in structure and function, alterations in the normal regulation of common biochemical pathways, and a reduction in the skin's ability to remodel and repair itself. Many of the alterations in appearance and function of the skin are caused by changes in the outer epidermal layer of the skin, while others are caused by changes in the lower dermis.

[0004] One proposed solution to treating loose or sagging skin is cosmetic/plastic surgery. This can involve making incisions in facial or neck skin, pulling the skin tight, and then suturing the incisions. Some drawbacks with such surgery are risk of infection and the expenses associated with the surgery. Further, the skin can have an unnatural waxy or plastic visual appearance that may not be desirable.

[0005] Another solution involves the use of lasers, which can be less invasive when compared to the aforementioned cosmetic/plastic surgery option. This is oftentimes referred to as laser skin tightening, which uses an infrared light source to tighten skin by heating the collagen under the skin's surface. As the collagen is heated, it can cause the skin to contract or tighten. A benefit of this process is that you can see an immediate result. A downside is that the skin tightening effect dissipates over time, which can require multiple

treatments per month. Further, laser treatments are usually performed by a professional, which can require office visits. [0006] There have also been numerous attempts to tighten loose skin through the use of topical skin compositions. Such compositions typically include active ingredients that claim to help reduce the appearance of sagging or loose skin. By way of example, hyaluronic acid or salts thereof (e.g., sodium hyaluronate) are ingredients that are designed to attract and retain water at the skin. In particular, water can be used to fill up spaces between the connective fibers collagen and elastin in the dermis layer of skin, thereby making the skin appear to have a more tightened or less-loose appearance. One of the downsides of hyaluronic acid is that it may not have a lasting effect, thereby requiring large amounts and multiple applications, which can result in increased expenses and complications associated with its use. There have also been attempts to use plant extracts and chemical compounds to tighten skin. By way of example, US Publication 2008/0292651 claims that a combination of *Polygonum fagopyrum* seed extract, *Chlorella vulgaris* extract, palmitoyl wheat protein hydrolysate, algae extract, and a tripeptide (e.g., glutathione (2-amino-5-{[2-[(carboxymethyl)amino]-1-(mercaptomethyl)-2-oxoethyl]amino}-5-oxopentanoic acid or  $\gamma$ -glutamylcysteinylglycine) can be used to improve skin firmness, lift the skin, and prevent skin sagging. The use of compounds such as chemically modified peptides can be expensive and can result in skin irritation.

[0007] While there have been countless efforts to treat loose or sagging skin, these efforts oftentimes require surgery, special tools, or use ingredients that can be costly, ineffective over longer periods of time, and/or cause skin irritation. The current slate of options are failing to meet the needs of people having loose or sagging skin.

### SUMMARY OF THE INVENTION

[0008] The inventors have identified a solution to the problems associated with loose/sagging/facced skin. The solution is premised on a combination of plant-based ingredients that includes *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract. This combination can be used to create topical skin compositions that reduce the appearance of loose, sagging, and flaccid skin, such as under the neck, and improve facial contouring. Without wishing to be bound by theory, the efficacy of this combination can be linked to its ability to modify specific biochemical pathways in skin that can result in improved skin firmness, skin elasticity, and/or facial contouring. In particular, and as illustrated in a non-limiting manner in the Examples section, this combination of plant-based ingredients can be used to increase the expression of elastin, collagen, laminin, and/or fibronectin in skin cells, which are proteins associated with the structure of skin. By increasing the amount of these proteins in the skin, it is believed that the skin will have a more firm, taught, supple, and/or elastic appearance. Even further, it has been discovered that the particular combination of *Morus alba* fruit extract and *Croton lechleri* extract can increase the production of fibulin-5 in skin. Fibulin-5 is an elastin-binding protein that can have the ability to provide mechanical elasticity to skin tissue, which further helps with improving skin tightness and/or elasticity. Further, the combination of plant-based ingredients are compatible with one another and can there-

fore exist in a single topical skin care composition, which can be beneficial in that only one composition needs to be applied to skin rather than multiple compositions that often-times come in the form of a kit or regimen. While kits and regimens are contemplated in the context of the present invention, they are not required to obtain the beneficial effects offered by the compositions of the present invention.

**[0009]** In some aspects, there is disclosed a topical composition. In some aspects, the topical composition includes any one of, any combination of, or all of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract, and *Morus alba* fruit extract.

**[0010]** In some instances, the topical composition includes an effective amount of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract, and/or *Morus alba* fruit extract to reduce the appearance of loose, sagging, and flaccid skin, and improve facial contouring. In some instances, the topical composition includes an effective amount of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract to increase elasticity of skin. In some instances, the topical composition increases elastin synthesis. In some instances, the topical composition increases collagen expression. In some instances, the topical composition increases production of laminin. In some instances, the topical composition increases production of fibronectin. In some instances, the topical composition increases expression of proteins that are involved in elastin organization in fibroblasts (e.g., fibulin-5). In some instances, the *Argania spinosa* kernel extract and/or dill extract is a water extract. In some instances, the *Croton lechleri* extract and/or *Morus alba* fruit extract is a water and glycerol extract. In some instances, the *Myrciaria dubia* fruit extract is a dry fruit powder.

**[0011]** The topical compositions disclosed herein may further comprise one or more ingredients described herein. For example, the composition may comprise one or more additional ingredients selected from one or more conditioning agents, moisturizing agents, pH adjusters, structuring agents, inorganic salts, and preservatives. In some instances, the topical composition further includes water. The amounts of the ingredients within the composition can vary (e.g., amounts can be as low as 0.000001% to as high as 98% w/w or any range therein). In some instances, the topical composition is an emulsion, serum, gel, gel emulsion, or gel serum. In some instances, the topical composition can further contain one or more, or all of, water, disodium EDTA, HDI/trimethylol hexyllactone crosspolymer, nylon-12, acrylates/C10-30 alkyl crosspolymer, glyceryl stearate, PEG-100 stearate, ethylhexyl palmitate, pentylene glycol, dimethyl isosorbide, triethanolamine, iodopropynyl butylcarbamate, dimethicone, dimethicone crosspolymer, phenoxyethanol, decylene glycol, 1,2-hexanediol, glycerin, and sodium polyacrylate. In some instances, the topical composition contains 40 to 80% w/w of water, 0.001 to 1% w/w of disodium EDTA, 0.1 to 5% w/w of HDI/trimethylol hexyllactone crosspolymer, 0.01 to 3% w/w of nylon-12, 0.001 to 1% w/w of acrylates/C10-30 alkyl crosspolymer, 1 to 10% w/w of a combination of glyceryl stearate and PEG-100 stearate, 1 to 15% w/w of ethylhexyl palmitate, 1 to 5% w/w of pentylene glycol, 0.01 to 3% w/w of dimethyl isosorbide, 0.01 to 3% w/w of triethanolamine, 0.001 to 1% w/w of iodopropynyl butylcarbamate, 5 to 20% w/w of a combination of dimethicone and dimethicone crosspolymer, 0.01 to

3% w/w of a combination of phenoxyethanol, decylene glycol, and 1,2-hexanediol, and 5 to 20% w/w of a combination of glycerin, water, and sodium polyacrylate.

**[0012]** Methods of use for the compositions disclosed herein are also disclosed. In some aspects, a method is disclosed of improving a condition or appearance of skin, comprising applying any one of the compositions disclosed herein to skin in need thereof. In one aspect, any one of the compositions disclosed herein are applied to skin and the composition is left on the skin, or alternatively removed from the skin after a period of time. In another aspect, the compositions disclosed herein are used to treat and/or reduce the appearance of loose, sagging, and flaccid skin. In another aspect, the compositions disclosed here are used to improve facial contouring. In another aspect, a method for increasing skin elasticity is disclosed herein. In another aspect, a method for increasing elastin synthesis is disclosed herein. In another aspect, a method for increasing expression of proteins that are involved in elastin organization is disclosed herein. In another aspect, a method for increasing the production of fibulin-5 is disclosed herein. In another aspect, a method for increasing the production of fibronectin is disclosed herein. In another aspect, a method for increasing the production of laminin is disclosed herein. In another aspect, a method for increasing the expression of collagen is disclosed herein. In some aspects, the methods include applying any one of the topical compositions described herein to skin. In some aspects, the methods include applying the composition to skin of the neck. In some aspects, the methods include applying the composition to skin of the face. In some aspects, the methods include applying the composition to skin of a jawl.

**[0013]** In particular aspects, the compositions of the present invention are formulated as a topical skin composition. The composition can have a dermatologically acceptable vehicle or carrier for the compounds and extracts. The composition can further include a moisturizing agent or a humectant, a surfactant, a silicone containing compounds, a UV agent, an oil, and/or other ingredients identified in this specification or those known in the art. The composition can be a mask, lotion, cream, gel, serum, emulsion (e.g., oil-in-water, water-in-oil, silicone-in-water, water-in-silicone, water-in-oil-in-water, oil-in-water-in-oil, oil-in-water-in-silicone, etc.), solutions (e.g., aqueous or hydro-alcoholic solutions), anhydrous bases (e.g., lipstick or a powder), ointments, milk, paste, aerosol, solid forms, eye jellies, gel serums, gel emulsions, etc. The composition can be formulated for topical skin application at least 1, 2, 3, 4, 5, 6, 7, or more times a day during use. In other aspects of the present invention, compositions can be storage stable or color stable, or both. It is also contemplated that the viscosity of the composition can be selected to achieve a desired result, e.g., depending on the type of composition desired, the viscosity of such composition can be from about 1 cps to well over 1 million cps or any range or integer derivable therein (e.g., 2 cps, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, 20000, 30000, 40000, 50000, 60000, 70000, 80000, 90000, 100000, 200000, 300000, 400000, 500000, 600000, 700000, 800000, 900000, 1000000, 2000000, 3000000, 4000000, 5000000, 10000000, cps, etc., as measured on a Brookfield Viscometer using a TC spindle at 2.5 rpm at 25° C.).

**[0014]** The compositions in non-limiting aspects can have a pH of about 6 to about 9. In other aspects, the pH can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14. The compositions can include a triglyceride. Non-limiting examples include small, medium, and large chain triglycerides. In certain aspects, the triglyceride is a medium chain triglyceride (e.g., caprylic capric triglyceride). The compositions can also include preservatives. Non-limiting examples of preservatives include methylparaben, propylparaben, or a mixture of methylparaben and propylparaben. In some embodiments, the composition is paraben-free.

**[0015]** Compositions of the present invention can have UVA and UVB absorption properties. The compositions can have an sun protection factor (SPF) of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more, or any integer or derivative therein. The compositions can be sunscreen lotions, sprays, or creams.

**[0016]** The compositions of the present invention can also include any one of, any combination of, or all of the following additional ingredients: water, a chelating agent, a moisturizing agent, a preservative, a thickening agent, a silicone containing compound, an essential oil, a structuring agent, a vitamin, a pharmaceutical ingredient, or an antioxidant, or any combination of such ingredients or mixtures of such ingredients. In certain aspects, the composition can include at least two, three, four, five, six, seven, eight, nine, ten, or all of these additional ingredients identified in the previous sentence. Non-limiting examples of these additional ingredients are identified throughout this specification and are incorporated into this section by reference. The amounts of such ingredients can range from 0.0001% to 99.9% by weight or volume of the composition, or any integer or range in between as disclosed in other sections of this specification, which are incorporated into this paragraph by reference.

**[0017]** Kits that include the compositions of the present invention are also contemplated. In certain embodiments, the composition is comprised in a container. The container can be a bottle, dispenser, or package. The container can dispense a pre-determined amount of the composition. In certain aspects, the compositions is dispensed in a spray, mist, dollop, or liquid. The container can include indicia on its surface. The indicia can be a word, an abbreviation, a picture, or a symbol.

**[0018]** It is also contemplated that the compositions disclosed throughout this specification can be used as a leave-on or rinse-off composition. By way of example, a leave-on composition can be one that is topically applied to skin and remains on the skin for a period of time (e.g., at least 5, 6, 7, 8, 9, 10, 20, or 30 minutes, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 hours, or overnight or throughout the day). Alternatively, a rinse-off composition can be a product that is intended to be applied to the skin and then removed or rinsed from the skin (e.g., with water) within a period of time such as less than 5, 4, 3, 2, or 1 minute. An example of a rinse off composition can be a skin cleanser, shampoo, conditioner, or soap. An example of a leave-on composition can be a skin moisturizer, sunscreen, mask, overnight cream, or a day cream.

**[0019]** It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa.

Furthermore, compositions of the invention can be used to achieve methods of the invention.

**[0020]** In one embodiment, compositions of the present invention can be pharmaceutically or cosmetically elegant or can have pleasant tactile properties. "Pharmaceutically elegant," "cosmetically elegant," and/or "pleasant tactile properties" describes a composition that has particular tactile properties which feel pleasant on the skin (e.g., compositions that are not too watery or greasy, compositions that have a silky texture, compositions that are non-tacky or sticky, etc.). Pharmaceutically or cosmetically elegant can also relate to the creaminess or lubricity properties of the composition or to the moisture retaining properties of the composition.

**[0021]** Also contemplated is a product comprising a composition of the present invention. In non-limiting aspects, the product can be a cosmetic product. The cosmetic product can be those described in other sections of this specification or those known to a person of skill in the art. Non-limiting examples of products include a moisturizer, a cream, a lotion, a skin softener, a gel, a wash, a foundation, a night cream, a lipstick, a cleanser, a toner, a sunscreen, a mask, an anti-aging product, a deodorant, an antiperspirant, a perfume, a cologne, etc.

**[0022]** Also disclosed are the following Embodiments 1 to 24 of the present invention. Embodiment 1 is a method of treating skin, the method comprising topically applying to the skin an effective amount of a topical composition comprising *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract, wherein the skin is treated. Embodiment 2 is the method of Embodiment 1, wherein the skin is treated to improve the appearance of loose, sagging, and/or flaccid skin and/or to improve facial contouring, and wherein the appearance of loose, sagging, and/or flaccid skin is improved and/or facial contouring is improved. Embodiment 3 is the method of any of Embodiments 1 and 2, wherein the skin is treated to increase elasticity and wherein skin elasticity is increased. Embodiment 4 is the method of any of Embodiments 1 to 3, wherein the skin is treated to increase the expression of elastin, and wherein expression of elastin is increased. Embodiment 5 is the method of any of Embodiments 1 to 4, wherein the skin is treated to increase the expression of collagen, and wherein the expression of collagen is increased. Embodiment 6 is the method of any of Embodiments 1 to 5, wherein the skin is treated to increase the production of laminin and/or fibronectin, and wherein the production of laminin and/or fibronectin is increased. Embodiment 7 is the method of any of Embodiments 1 to 6, wherein the skin is treated to increase the production of fibulin-5, and wherein the production of fibulin-5 is increased. Embodiment 8 is the method of any of Embodiments 1 to 7, wherein the topical composition further comprises water. Embodiment 9 is the method of any of Embodiments 1 to 8, wherein the *Argania spinosa* kernel extract is a water extract, dill extract is a water extract, *Croton lechleri* extract is a water and glycerol extract, the *Morus alba* fruit extract is a water and glycerol extract, and/or the *Myrciaria dubia* fruit extract is a dried fruit powder. Embodiment 10 is the method of any of Embodiments 1 to 9, wherein the topical composition is an emulsion, serum, gel, gel emulsion, or gel serum. Embodiment 11 is the method of any of Embodiments 1 to 10, wherein the topical composition is an oil in water emulsion or a water in

oil emulsion. Embodiment 12 is the method of any of Embodiments 1 to 11, wherein the composition is applied to skin of a jowl. Embodiment 13 is a topical composition comprising *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract. Embodiment 14 is the topical composition of Embodiment 13, wherein the topical composition comprises an effective amount of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract to improve the appearance of loose, sagging, and/or flaccid skin and/or to improve facial contouring. Embodiment 15 is the topical composition of any of Embodiments 13 and 14, wherein the topical composition comprises an effective amount of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract to increase skin elasticity. Embodiment 16 is the topical composition of any of Embodiments 13 to 15, wherein the topical composition comprises an effective amount of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract to increase elastin synthesis, increase collagen expression, increase laminin production, increase fibronectin production, and/or increase fibulin-5 production. Embodiment 17 is the topical composition of any of Embodiments 13 to 16, wherein the *Argania spinosa* kernel extract is a water extract, dill extract is a water extract, *Croton lechleri* extract is a water and glycerol extract, the *Morus alba* fruit extract is a water and glycerol extract, and/or the *Myrciaria dubia* fruit extract is a dried fruit powder. Embodiment 18 is the topical composition of any of Embodiments 13 to 17, further comprising water. Embodiment 19 is the topical composition of any of Embodiments 13 to 18, wherein the topical composition is an emulsion, serum, gel, gel emulsion, or gel serum. Embodiment 20 is the topical composition of any of Embodiments 13 to 19, wherein the topical composition is an oil in water emulsion or water in oil emulsion. Embodiment 21 is the topical composition of any of Embodiments 13 to 20, wherein the topical composition comprises water, disodium EDTA, HDI/trimethylol hexyllactone crosspolymer, nylon-12, acrylates/C10-30 alkyl crosspolymer, glyceryl stearate, PEG-100 stearate, ethylhexyl palmitate, pentylene glycol, dimethyl isosorbide, triethanolamine, iodopropynyl butylcarbamate, dimethicone, dimethicone crosspolymer, phenoxyethanol, decylene glycol, 1,2-hexanediol, glycerin, and sodium polyacrylate. Embodiment 22 is the topical composition of Embodiment 21, comprising 40 to 80% w/w of water, 0.001 to 1% w/w of disodium EDTA, 0.1 to 5% w/w of HDI/trimethylol hexyllactone crosspolymer, 0.01 to 3% w/w of nylon-12, 0.001 to 1% w/w of acrylates/C10-30 alkyl crosspolymer, 1 to 10% w/w of a combination of glyceryl stearate and PEG-100 stearate, 1 to 15% w/w of ethylhexyl palmitate, 1 to 5% w/w of pentylene glycol, 0.01 to 3% w/w of dimethyl isosorbide, 0.01 to 3% w/w of triethanolamine, 0.001 to 1% w/w of iodopropynyl butylcarbamate, 5 to 20% w/w of a combination of dimethicone and dimethicone crosspolymer, 0.01 to 3% w/w of a combination of phenoxyethanol, decylene glycol, and 1,2-hexanediol, and 5 to 20% w/w of a combination of glycerin, water, and sodium polyacrylate. Embodiment 23, is the method of any of Embodiments 1 to 12, wherein the topical composition comprises water, disodium EDTA, HDI/trimethylol hexyllactone crosspolymer, nylon-12, acrylates/

C10-30 alkyl crosspolymer, glyceryl stearate, PEG-100 stearate, ethylhexyl palmitate, pentylene glycol, dimethyl isosorbide, triethanolamine, iodopropynyl butylcarbamate, dimethicone, dimethicone crosspolymer, phenoxyethanol, decylene glycol, 1,2-hexanediol, glycerin, and sodium polyacrylate. Embodiment 24 is the method of Embodiment 23, wherein the topical composition comprises 40 to 80% w/w of water, 0.001 to 1% w/w of disodium EDTA, 0.1 to 5% w/w of HDI/trimethylol hexyllactone crosspolymer, 0.01 to 3% w/w of nylon-12, 0.001 to 1% w/w of acrylates/C10-30 alkyl crosspolymer, 1 to 10% w/w of a combination of glyceryl stearate and PEG-100 stearate, 1 to 15% w/w of ethylhexyl palmitate, 1 to 5% w/w of pentylene glycol, 0.01 to 3% w/w of dimethyl isosorbide, 0.01 to 3% w/w of triethanolamine, 0.001 to 1% w/w of iodopropynyl butylcarbamate, 5 to 20% w/w of a combination of dimethicone and dimethicone crosspolymer, 0.01 to 3% w/w of a combination of phenoxyethanol, decylene glycol, and 1,2-hexanediol, and 5 to 20% w/w of a combination of glycerin, water, and sodium polyacrylate

**[0023]** “Topical application” means to apply or spread a composition onto the surface of lips or keratinous tissue. “Topical skin composition” includes compositions suitable for topical application on skin and/or keratinous tissue. Such compositions are typically dermatologically-acceptable in that they do not have undue toxicity, incompatibility, instability, allergic response, and the like, when applied to skin and/or keratinous tissue. Topical skin care compositions of the present invention can have a selected viscosity to avoid significant dripping or pooling after application to skin and/or keratinous tissue.

**[0024]** “Keratinous tissue” includes keratin-containing layers disposed as the outermost protective covering of mammals and includes, but is not limited to, lips, skin, hair, and nails.

**[0025]** The term “about” or “approximately” are defined as being close to as understood by one of ordinary skill in the art. In one non-limiting embodiment the terms are defined to be within 10%, preferably within 5%, more preferably within 1%, and most preferably within 0.5%.

**[0026]** The term “substantially” and its variations are refers to ranges within 10%, within 5%, within 1%, or within 0.5%.

**[0027]** The terms “inhibiting” or “reducing” or “preventing” or “avoiding” or any variation of these terms includes any measurable decrease or complete inhibition to achieve a desired result. The terms “promote” or “increase” or any variation of these terms includes any measurable increase or production of a protein or molecule (e.g., matrix proteins such as fibronectin, laminin, collagen, or elastin or molecules such as hyaluronic acid) to achieve a desired result.

**[0028]** The term “effective,” as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result.

**[0029]** The use of the word “a” or “an” when used in conjunction with the terms “comprising,” “including,” “having,” or “containing,” or any variations of these terms, in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

**[0030]** The terms “wt. %,” “vol. %,” or “mol. %” refers to a weight, volume, or molar percentage of a component, respectively, based on the total weight, the total volume of material, or total moles, that includes the component. In a

non-limiting example, 10 grams of component in 100 grams of the material is 10 wt. % of component.

**[0031]** As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

**[0032]** The compositions and methods for their use can “comprise,” “consist essentially of,” or “consist of” any of the ingredients or steps disclosed throughout the specification. With respect to the phrase “consisting essentially of,” a basic and novel property of the compositions and methods of the present invention is the ability to reduce the appearance of loose skin under the neck and/or improve facial contouring.

**[0033]** Other objects, features, and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the examples, while indicating specific embodiments of the invention, are given by way of illustration only. Additionally, it is contemplated that changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this

## DETAILED DESCRIPTION

### Description of Illustrative Embodiments

**[0034]** As noted above, several of the unique aspects of the present invention are to combine in a topical cosmetic composition *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract. This allows for the benefits of reducing the appearance of loose, sagging, and flaccid skin, such as under the neck, and improving facial contouring.

**[0035]** These and other non-limiting aspects of the present invention are described in the following sections.

### A. Active Ingredients

**[0036]** The present invention is premised on a determination that a combination of active ingredients—*Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract and/or *Morus alba* fruit extract—can be used to reduce the appearance of loose, sagging, and flaccid skin, and improving facial contouring.

**[0037]** This combination of ingredients can be used in different products to treat various skin conditions. By way of non-limiting examples, the combination of ingredients can be formulated in an emulsion (e.g. oil in water, water in oil), a gel, a serum, a gel emulsion, a gel serum, a lotion, a mask, or a body butter.

**[0038]** *Argania spinosa* kernel extract is an extraction from the kernel of the Argan tree, which is native to the Mediterranean region. In some instances, *Argania spinosa* kernel extract is commercially available. In some instances, *Argania spinosa* kernel extract can be supplied by BASF under the tradename Argatensyl. In some instances, the extract can be an aqueous extract or an alcohol extract or a combination thereof, wherein the extractant is water, an

alcohol (e.g., methanol, ethanol, propanol, butanol, a polyhydric alcohol such as glycerol, etc.) or a mixture of water and alcohol. In some instances, the extract is a water extract. The extract can be in liquid form or can be dried to be in powdered form by removing the extracting liquid (e.g., water or alcohol or both).

**[0039]** Dill extract is an extract from the annual herb, *Peucedanum graveolens*. In some instances, dill extract is commercially available. In some instances, dill extract can be supplied by BASF under the tradename LYS’LASTINE™. In some instances, the extract can be an aqueous extract or an alcohol extract or a combination thereof, wherein the extractant is water, an alcohol (e.g., methanol, ethanol, propanol, butanol, a polyhydric alcohol such as glycerol, etc.) or a mixture of water and alcohol. In some instances, the extract is a water extract. The extract can be in liquid form or can be dried to be in powdered form by removing the extracting liquid (e.g., water or alcohol or both).

**[0040]** *Myrciaria dubia* fruit extract is an extract from a small bushy river side tree commonly known as camu camu. Camu camu is endogenous to the Amazon Rainforest vegetation in Peru and Brazil and bears a red/purple cherry-like fruit rich in vitamin C. In some instances, *Myrciaria dubia* fruit extract is commercially available. In some instances, *Myrciaria dubia* fruit extract can be supplied by AMAX under the tradename Camu Camu. In some instances, the extract can be pulp, an aqueous extract, or an alcohol extract or a combination thereof, wherein the extractant is water, an alcohol (e.g., methanol, ethanol, propanol, butanol, a polyhydric alcohol such as glycerol, etc.) or a mixture of water and alcohol. The extract can be in liquid form or can be dried to be in powdered form by removing the extracting liquid (e.g., water or alcohol or both). In one instance, the extract is a dried fruit powder where the fruit is obtained and dried without the use of any extracting solvent. In some instances, the *Myrciaria dubia* fruit extract does not contain an extract of *Myrciaria dubia* seed.

**[0041]** *Croton lechleri* extract is an extract from a tree native to the foot of the Peruvian Andes. The *Croton lechleri* tree is also known as dragon’s blood because of its thick red latex. In some instances, *Croton lechleri* extract is commercially available. In some instances, *Croton lechleri* extract can be supplied by Naturex under the tradename Dragon’s Blood. In some instances the *Croton lechleri* extract is an extract of *Croton lechleri* resin. In some instances, the extract can be an aqueous extract or an alcohol extract or a combination thereof, wherein the extractant is water, an alcohol (e.g., methanol, ethanol, propanol, butanol, a polyhydric alcohol such as glycerol, etc.) or a mixture of water and alcohol. The extract can be in liquid form or can be dried to be in powdered form by removing the extracting liquid (e.g., water or alcohol or both). In some instances, the extract is a water+glycerol extract.

**[0042]** *Morus alba* fruit extract is an extract of white mulberry fruit, a tree native to northern China. In some instances, *Morus alba* fruit extract is commercially available. In some instances, *Morus alba* fruit extract can be supplied by Rahn AG under the tradename DERMOFEEL® Enlight. In some instances, the extract can be an aqueous extract or an alcohol extract or a combination thereof, wherein the extractant is water, an alcohol (e.g., methanol, ethanol, propanol, butanol, a polyhydric alcohol such as glycerol, etc.) or a mixture of water and alcohol. The extract

can be in liquid form or can be dried to be in powdered form by removing the extracting liquid (e.g., water or alcohol or both). In some instances, the extract is a water+glycerol extract.

**[0043]** It is also contemplated that any combination of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract and/or *Morus alba* fruit extract can be used. For example, at least the following combinations are contemplated to be useful in the context of the present invention such as reducing the appearance of loose, sagging, and flaccid skin, and/or improving facial contouring: (1) *Argania spinosa* kernel extract and dill extract; (2) *Argania spinosa* kernel extract and *Myrciaria dubia* fruit extract; (3) *Argania spinosa* kernel extract and *Croton lechleri* extract; (4) *Argania spinosa* kernel extract and *Morus alba* fruit extract; (5) *Argania spinosa* kernel extract, dill extract and *Myrciaria dubia* fruit extract; (6) *Argania spinosa* kernel extract, dill extract and *Croton lechleri* extract; (7) *Argania spinosa* kernel extract, dill extract, and *Morus alba* fruit extract; (8) *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract and *Croton lechleri* extract; (9) *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Morus alba* fruit extract; (10) dill extract and *Myrciaria dubia* fruit extract; (11) dill extract and *Croton lechleri* extract; (12) dill extract and *Morus alba* fruit extract; (13) dill extract, *Myrciaria dubia* fruit extract and *Croton lechleri* extract; (14) dill extract, *Myrciaria dubia* fruit extract and *Morus alba* fruit extract; (15) dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract and *Morus alba* fruit extract; (16) *Myrciaria dubia* fruit extract and *Croton lechleri* extract; (17) *Myrciaria dubia* fruit extract and *Morus alba* fruit extract; (18) *Myrciaria dubia* fruit extract, *Croton lechleri* extract and *Morus alba* fruit extract; and (19) *Croton lechleri* extract and *Morus alba* fruit extract.

**[0044]** The extracts described herein can be extracts made through extraction methods known in the art and combinations thereof. Non-limiting examples of extraction methods include the use of liquid-liquid extraction, solid phase extraction, aqueous extraction, ethyl acetate, alcohol (e.g., monohydric and polyhydric alcohols), acetone, oil, supercritical carbon dioxide, heat, pressure, pressure drop extraction, ultrasonic extraction, etc. Extracts can be a liquid, solid, dried liquid, re-suspended solid, etc.

#### B. Amounts of Ingredients

**[0045]** It is contemplated that the compositions of the present invention can include any amount of the ingredients discussed in this specification. The compositions can also include any number of combinations of additional ingredients described throughout this specification (e.g., pigments, or additional cosmetic or pharmaceutical ingredients). The concentrations of the any ingredient within the compositions can vary. In non-limiting embodiments, for example, the compositions can comprise, consisting essentially of, or consist of, in their final form, for example, at least about 0.0001%, 0.0002%, 0.0003%, 0.0004%, 0.0005%, 0.0006%, 0.0007%, 0.0008%, 0.0009%, 0.0010%, 0.0011%, 0.0012%, 0.0013%, 0.0014%, 0.0015%, 0.0016%, 0.0017%, 0.0018%, 0.0019%, 0.0020%, 0.0021%, 0.0022%, 0.0023%, 0.0024%, 0.0025%, 0.0026%, 0.0027%, 0.0028%, 0.0029%, 0.0030%, 0.0031%, 0.0032%, 0.0033%, 0.0034%, 0.0035%, 0.0036%, 0.0037%, 0.0038%, 0.0039%, 0.0040%, 0.0041%, 0.0042%, 0.0043%, 0.0044%, 0.0045%, 0.0046%, 0.0047%, 0.0048%,

0.0049%, 0.0050%, 0.0051%, 0.0052%, 0.0053%, 0.0054%, 0.0055%, 0.0056%, 0.0057%, 0.0058%, 0.0059%, 0.0060%, 0.0061%, 0.0062%, 0.0063%, 0.0064%, 0.0065%, 0.0066%, 0.0067%, 0.0068%, 0.0069%, 0.0070%, 0.0071%, 0.0072%, 0.0073%, 0.0074%, 0.0075%, 0.0076%, 0.0077%, 0.0078%, 0.0079%, 0.0080%, 0.0081%, 0.0082%, 0.0083%, 0.0084%, 0.0085%, 0.0086%, 0.0087%, 0.0088%, 0.0089%, 0.0090%, 0.0091%, 0.0092%, 0.0093%, 0.0094%, 0.0095%, 0.0096%, 0.0097%, 0.0098%, 0.0099%, 0.0100%, 0.0200%, 0.0250%, 0.0275%, 0.0300%, 0.0325%, 0.0350%, 0.0375%, 0.0400%, 0.0425%, 0.0450%, 0.0475%, 0.0500%, 0.0525%, 0.0550%, 0.0575%, 0.0600%, 0.0625%, 0.0650%, 0.0675%, 0.0700%, 0.0725%, 0.0750%, 0.0775%, 0.0800%, 0.0825%, 0.0850%, 0.0875%, 0.0900%, 0.0925%, 0.0950%, 0.0975%, 0.1000%, 0.1250%, 0.1500%, 0.1750%, 0.2000%, 0.2250%, 0.2500%, 0.2750%, 0.3000%, 0.3250%, 0.3500%, 0.3750%, 0.4000%, 0.4250%, 0.4500%, 0.4750%, 0.5000%, 0.5250%, 0.5500%, 0.5750%, 0.6000%, 0.6250%, 0.6500%, 0.6750%, 0.7000%, 0.7250%, 0.7500%, 0.7750%, 0.8000%, 0.8250%, 0.8500%, 0.8750%, 0.9000%, 0.9250%, 0.9500%, 0.9750%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3.0%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, 4.0%, 4.1%, 4.2%, 4.3%, 4.4%, 4.5%, 4.6%, 4.7%, 4.8%, 4.9%, 5.0%, 5.1%, 5.2%, 5.3%, 5.4%, 5.5%, 5.6%, 5.7%, 5.8%, 5.9%, 6.0%, 6.1%, 6.2%, 6.3%, 6.4%, 6.5%, 6.6%, 6.7%, 6.8%, 6.9%, 7.0%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, 8.0%, 8.1%, 8.2%, 8.3%, 8.4%, 8.5%, 8.6%, 8.7%, 8.8%, 8.9%, 9.0%, 9.1%, 9.2%, 9.3%, 9.4%, 9.5%, 9.6%, 9.7%, 9.8%, 9.9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% or any range derivable therein, of at least one of the ingredients that are mentioned throughout the specification and claims. In non-limiting aspects, the percentage can be calculated by weight or volume of the total composition. A person of ordinary skill in the art would understand that the concentrations can vary depending on the addition, substitution, and/or subtraction of ingredients in a given composition.

#### C. Vehicles

**[0046]** The compositions of the present invention can include or be incorporated into all types of vehicles and carriers. The vehicle or carrier can be a pharmaceutically or dermatologically acceptable vehicle or carrier. Non-limiting examples of vehicles or carriers include water, glycerin, alcohol, oil, a silicon containing compound, a silicone compound, and wax. Variations and other appropriate vehicles will be apparent to the skilled artisan and are appropriate for use in the present invention. In certain aspects, the concentrations and combinations of the compounds, ingredients, and agents can be selected in such a way that the combinations are chemically compatible and do not form complexes which precipitate from the finished product.

#### D. Structure

**[0047]** The compositions of the present invention can be structured or formulated into a variety of different forms. Non-limiting examples include emulsions (e.g., water-in-oil, water-in-oil-in-water, oil-in-water, silicone-in-water, water-

in-silicone, oil-in-water-in-oil, oil-in-water-in-silicone emulsions), creams, lotions, solutions (both aqueous and hydro-alcoholic), anhydrous bases (such as lipsticks and powders), gels, masks, peels, and ointments. Variations and other structures will be apparent to the skilled artisan and are appropriate for use in the present invention.

#### E. Additional Ingredients

**[0048]** In addition to the combination of ingredients disclosed by the inventors, the compositions can also include additional ingredients such as cosmetic ingredients and pharmaceutical active ingredients. Non-limiting examples of these additional ingredients are described in the following subsections.

##### 1. Cosmetic Ingredients

**[0049]** The CTFA International Cosmetic Ingredient Dictionary and Handbook (2004 and 2008) describes a wide variety of non-limiting cosmetic ingredients that can be used in the context of the present invention. Examples of these ingredient classes include: fragrance agents (artificial and natural; e.g., gluconic acid, phenoxyethanol, and triethanolamine), dyes and color ingredients (e.g., Blue 1, Blue 1 Lake, Red 40, titanium dioxide, D&C blue no. 4, D&C green no. 5, D&C orange no. 4, D&C red no. 17, D&C red no. 33, D&C violet no. 2, D&C yellow no. 10, and D&C yellow no. 11), flavoring agents/aroma agents (e.g., *Stevia rebaudiana* (sweetleaf) extract, and menthol), adsorbents, lubricants, solvents, moisturizers (including, e.g., emollients, humectants, film formers, occlusive agents, and agents that affect the natural moisturization mechanisms of the skin), water-repellants, UV absorbers (physical and chemical absorbers such as para-aminobenzoic acid ("PABA") and corresponding PABA derivatives, titanium dioxide, zinc oxide, etc.), essential oils, vitamins (e.g., A, B, C, D, E, and K), trace metals (e.g., zinc, calcium and selenium), anti-irritants (e.g., steroids and non-steroidal anti-inflammatories), botanical extracts (e.g., Aloe vera, chamomile, cucumber extract, Ginkgo biloba, ginseng, and rosemary), anti-microbial agents, antioxidants (e.g., BHT and tocopherol), chelating agents (e.g., disodium EDTA and tetrasodium EDTA), preservatives (e.g., methylparaben and propylparaben), pH adjusters (e.g., sodium hydroxide and citric acid), absorbents (e.g., aluminum starch octenylsuccinate, kaolin, corn starch, oat starch, cyclodextrin, talc, and zeolite), skin bleaching and lightening agents (e.g., hydroquinone and niacinamide lactate), humectants (e.g., sorbitol, urea, methyl gluceth-20, saccharide isomerate, and mannitol), exfoliants, waterproofing agents (e.g., magnesium/aluminum hydroxide stearate), skin conditioning agents (e.g., aloe extracts, allantoin, bisabolol, ceramides, dimethicone, hyaluronic acid, biosaccharide gum-1, ethylhexylglycerin, pentylene glycol, hydrogenated polydecene, octyldodecyl oleate, and dipotassium glycyrrhizate). Non-limiting examples of some of these ingredients are provided in the following subsections.

##### a. UV Absorption and/or Reflecting Agents

**[0050]** UV absorption and/or reflecting agents that can be used in combination with the compositions of the present invention include chemical and physical sunblocks. Non-limiting examples of chemical sunblocks that can be used include para-aminobenzoic acid (PABA), PABA esters (glyceryl PABA, amyldimethyl PABA and octyldimethyl

PABA), butyl PABA, ethyl PABA, ethyl dihydroxypropyl PABA, benzophenones (oxybenzone, sulisobenzone, benzophenone, and benzophenone-1 through 12), cinnamates (octyl methoxycinnamate (octinoxate), isoamyl p-methoxycinnamate, octylmethoxy cinnamate, cinoxate, diisopropyl methyl cinnamate, DEA-methoxycinnamate, ethyl diisopropylcinnamate, glyceryl octanoate dimethoxycinnamate and ethyl methoxycinnamate), cinnamate esters, salicylates (homomethyl salicylate, benzyl salicylate, glycol salicylate, isopropylbenzyl salicylate, etc.), anthranilates, ethyl urocanate, homosalate, octisalate, dibenzoylmethane derivatives (e.g., avobenzene), octocrylene, octyl triazone, digalloyl trioleate, glyceryl aminobenzoate, lawsone with dihydroxyacetone, ethylhexyl triazone, dioctyl butamido triazone, benzylidene malonate polysiloxane, terephthalylidene dicamphor sulfonic acid, disodium phenyl dibenzimidazole tetrasulfonate, diethylamino hydroxybenzoyl hexyl benzoate, bis diethylamino hydroxybenzoyl benzoate, bis benzoxazolylphenyl ethylhexylimino triazine, drometizole trisiloxane, methylene bis-benzotriazolyl tetramethylbutylphenol, and bis-ethylhexyloxyphenol methoxyphenyltriazine, 4-methylbenzylidene camphor, and isopentyl 4-methoxycinnamate. Non-limiting examples of physical sunblocks include, kaolin, talc, petrolatum and metal oxides (e.g., titanium dioxide and zinc oxide).

##### b. Moisturizing Agents

**[0051]** Non-limiting examples of moisturizing agents that can be used with the compositions of the present invention include amino acids, chondroitin sulfate, diglycerin, erythritol, fructose, glucose, glycerin, glycerol polymers, glycol, 1,2,6-hexanetriol, honey, hyaluronic acid, hydrogenated honey, hydrogenated starch hydrolysate, inositol, lactitol, maltitol, maltose, mannitol, natural moisturizing factor, PEG-15 butanediol, polyglyceryl sorbitol, salts of pyrrolidone carboxylic acid, potassium PCA, propylene glycol, saccharide isomerate, sodium glucuronate, sodium PCA, sorbitol, sucrose, trehalose, urea, and xylitol.

**[0052]** Other examples include acetylated lanolin, acetylated lanolin alcohol, alanine, algae extract, *Aloe barbadensis*, *Aloe barbadensis* extract, *Aloe barbadensis* gel, *Althea officinalis* extract, apricot (*Prunus armeniaca*) kernel oil, arginine, arginine aspartate, *Arnica montana* extract, aspartic acid, avocado (*Persea gratissima*) oil, barrier sphingolipids, butyl alcohol, beeswax, behenyl alcohol, beta-sitosterol, birch (*Betula alba*) bark extract, borage (*Borago officinalis*) extract, butcherbroom (*Ruscus aculeatus*) extract, butylene glycol, *Calendula officinalis* extract, *Calendula officinalis* oil, candelilla (*Euphorbia cerifera*) wax, canola oil, caprylic/capric triglyceride, cardamom (*Elettaria cardamomum*) oil, carnauba (*Copernicia cerifera*) wax, carrot (*Daucus carota sativa*) oil, castor (*Ricinus communis*) oil, ceramides, ceresin, cetareth-5, cetareth-12, cetareth-20, cetearyl octanoate, ceteth-20, ceteth-24, cetyl acetate, cetyl octanoate, cetyl palmitate, chamomile (*Anthemis nobilis*) oil, cholesterol, cholesterol esters, cholesteryl hydroxystearate, citric acid, clary (*Salvia sclarea*) oil, cocoa (*Theobroma cacao*) butter, coco-caprylate/caprate, coconut (*Cocos nucifera*) oil, collagen, collagen amino acids, corn (*Zea mays*) oil, fatty acids, decyl oleate, dimethicone copolyol, dimethiconol, dioctyl adipate, dioctyl succinate, dipentaerythryl hexacaprylate/hexacaprate, DNA, erythritol, ethoxydiglycol, ethyl linoleate, *Eucalyptus globulus* oil, evening primrose (*Oenothera biennis*) oil, fatty acids, *Gera-*



*nium maculatum* oil, glucosamine, glucose glutamate, glutamic acid, glycereth-26, glycerin, glycerol, glyceryl distearate, glyceryl hydroxystearate, glyceryl laurate, glyceryl linoleate, glyceryl myristate, glyceryl oleate, glyceryl stearate, glyceryl stearate SE, glycine, glycol stearate, glycol stearate SE, glycosaminoglycans, grape (*Vitis vinifera*) seed oil, hazel (*Corylus americana*) nut oil, hazel (*Corylus avellana*) nut oil, hexylene glycol, hyaluronic acid, hybrid safflower (*Carthamus tinctorius*) oil, hydrogenated castor oil, hydrogenated coco-glycerides, hydrogenated coconut oil, hydrogenated lanolin, hydrogenated lecithin, hydrogenated palm glyceride, hydrogenated palm kernel oil, hydrogenated soybean oil, hydrogenated tallow glyceride, hydrogenated vegetable oil, hydrolyzed collagen, hydrolyzed elastin, hydrolyzed glycosaminoglycans, hydrolyzed keratin, hydrolyzed soy protein, hydroxylated lanolin, hydroxyproline, isocetyl stearate, isocetyl stearyl stearate, isodecyl oleate, isopropyl isostearate, isopropyl lanolate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, isostearamide DEA, isostearic acid, isostearyl lactate, isostearyl neopentanoate, jasmine (*Jasminum officinale*) oil, jojoba (*Buxus chinensis*) oil, kelp, kukui (*Aleurites moluccana*) nut oil, lactamide MEA, laneth-16, laneth-10 acetate, lanolin, lanolin acid, lanolin alcohol, lanolin oil, lanolin wax, lavender (*Lavandula angustifolia*) oil, lecithin, lemon (*Citrus medica limonum*) oil, linoleic acid, linolenic acid, *Macadamia ternifolia* nut oil, maltitol, matricaria (*Chamomilla recutita*) oil, methyl glucose sesquistearate, methylsilanol PCA, mineral oil, mink oil, mortierella oil, myristyl lactate, myristyl myristate, myristyl propionate, neopentyl glycol dicaprylate/dicaprate, octyldodecanol, octyldodecyl myristate, octyldodecyl stearyl stearate, octyl hydroxystearate, octyl palmitate, octyl salicylate, octyl stearate, oleic acid, olive (*Olea europaea*) oil, orange (*Citrus aurantium dulcis*) oil, palm (*Elaeis guineensis*) oil, palmitic acid, pantethine, panthenol, panthenyl ethyl ether, paraffin, PCA, peach (*Prunus persica*) kernel oil, peanut (*Arachis hypogaea*) oil, PEG-8 C12-18 ester, PEG-15 cocamine, PEG-150 distearate, PEG-60 glyceryl isostearate, PEG-5 glyceryl stearate, PEG-30 glyceryl stearate, PEG-7 hydrogenated castor oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-20 methyl glucose sesquistearate, PEG-40 sorbitan peroleate, PEG-5 soy sterol, PEG-10 soy sterol, PEG-2 stearate, PEG-8 stearate, PEG-20 stearate, PEG-32 stearate, PEG-40 stearate, PEG-50 stearate, PEG-100 stearate, PEG-150 stearate, pentadecalactone, peppermint (*Mentha piperita*) oil, petrolatum, phospholipids, plankton extract, polyamino sugar condensate, polyglyceryl-3 diisostearate, polyquaternium-24, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polysorbate 85, potassium myristate, potassium palmitate, propylene glycol, propylene glycol dicaprylate/dicaprate, propylene glycol dioctanoate, propylene glycol dipelargonate, propylene glycol laurate, propylene glycol stearate, propylene glycol stearate SE, PVP, pyridoxine dipalmitate, retinol, retinyl palmitate, rice (*Oryza sativa*) bran oil, RNA, rosemary (*Rosmarinus officinalis*) oil, rose oil, safflower (*Carthamus tinctorius*) oil, sage (*Salvia officinalis*) oil, sandalwood (*Santalum album*) oil, serine, serum protein, sesame (*Sesamum indicum*) oil, shea butter (*Butyrospermum parkii*), silk powder, sodium chondroitin sulfate, sodium hyaluronate, sodium lactate, sodium palmitate, sodium PCA, sodium polyglutamate, soluble collagen, sorbitan laurate, sorbitan oleate, sorbitan palmitate, sorbitan sesqui-

oleate, sorbitan stearate, sorbitol, soybean (*Glycine soja*) oil, sphingolipids, squalane, squalene, stearamide MEA-stearate, stearic acid, stearoxy dimethicone, stearoxytrimethylsilane, stearyl alcohol, stearyl glyceryl ether, stearyl heptanoate, stearyl stearate, sunflower (*Helianthus annuus*) seed oil, sweet almond (*Prunus amygdalus dulcis*) oil, synthetic beeswax, tocopherol, tocopheryl acetate, tocopheryl linoleate, tribehnenin, tridecyl neopentanoate, tridecyl stearate, triethanolamine, tristearin, urea, vegetable oil, water, waxes, wheat (*Triticum vulgare*) germ oil, and ylang ylang (*Cananga odorata*) oil.

#### c. Antioxidants

[0053] Non-limiting examples of antioxidants that can be used with the compositions of the present invention include acetyl cysteine, ascorbic acid polypeptide, ascorbyl dipalmitate, ascorbyl methylsilanol pectinate, ascorbyl palmitate, ascorbyl stearate, BHA, BHT, t-butyl hydroquinone, cysteine, cysteine HCl, diamylhydroquinone, di-t-butylhydroquinone, dicetyl thiodipropionate, dioleoyl tocopheryl methylsilanol, disodium ascorbyl sulfate, distearyl thiodipropionate, ditridecyl thiodipropionate, dodecyl gallate, erythorbic acid, esters of ascorbic acid, ethyl ferulate, ferulic acid, gallic acid esters, hydroquinone, isoctyl thioglycolate, kojic acid, magnesium ascorbate, magnesium ascorbyl phosphate, methylsilanol ascorbate, natural botanical anti-oxidants such as green tea or grape seed extracts, nordihydroguaiaretic acid, octyl gallate, phenylthioglycolic acid, potassium ascorbyl tocopheryl phosphate, potassium sulfite, propyl gallate, quinones, rosmarinic acid, sodium ascorbate, sodium bisulfite, sodium erythorbate, sodium metabisulfite, sodium sulfite, superoxide dismutase, sodium thioglycolate, sorbityl furfural, thiodiglycol, thiodiglycolamide, thiodiglycolic acid, thioglycolic acid, thiolactic acid, thiosalicylic acid, tocophereth-5, tocophereth-10, tocophereth-12, tocophereth-18, tocophereth-50, tocopherol, tocophersolan, tocopheryl acetate, tocopheryl linoleate, tocopheryl nicotinate, tocopheryl succinate, and tris(nonylphenyl)phosphite.

#### d. Structuring Agents

[0054] In other non-limiting aspects, the compositions of the present invention can include a structuring agent. Structuring agent, in certain aspects, assist in providing rheological characteristics to the composition to contribute to the composition's stability. In other aspects, structuring agents can also function as an emulsifier or surfactant. Non-limiting examples of structuring agents include stearic acid, palmitic acid, stearyl alcohol, cetyl alcohol, behenyl alcohol, stearic acid, palmitic acid, the polyethylene glycol ether of stearyl alcohol having an average of about 1 to about 21 ethylene oxide units, the polyethylene glycol ether of cetyl alcohol having an average of about 1 to about 5 ethylene oxide units, and mixtures thereof.

#### e. Emulsifiers

[0055] In certain aspects of the present invention, the compositions do not include an emulsifier. In other aspects, however, the compositions can include one or more emulsifiers. Emulsifiers can reduce the interfacial tension between phases and improve the formulation and stability of an emulsion. The emulsifiers can be nonionic, cationic, anionic, and zwitterionic emulsifiers (See McCutcheon's (1986); U.S. Pat. Nos. 5,011,681; 4,421,769; 3,755,560).

Non-limiting examples include esters of glycerin, esters of propylene glycol, fatty acid esters of polyethylene glycol, fatty acid esters of polypropylene glycol, esters of sorbitol, esters of sorbitan anhydrides, carboxylic acid copolymers, esters and ethers of glucose, ethoxylated ethers, ethoxylated alcohols, alkyl phosphates, polyoxyethylene fatty ether phosphates, fatty acid amides, acyl lactylates, soaps, TEA stearate, DEA oleth-3 phosphate, polyethylene glycol 20 sorbitan monolaurate (polysorbate 20), polyethylene glycol 5 soya sterol, steareth-2, steareth-20, steareth-21, cetareth-20, cetearyl glucoside, cetearyl alcohol, C12-13 parath-3, PPG-2 methyl glucose ether distearate, PPG-5-ceteth-20, bis-PEG/PPG-20/20 dimethicone, ceteth-10, polysorbate 80, cetyl phosphate, potassium cetyl phosphate, diethanolamine cetyl phosphate, polysorbate 60, glyceryl stearate, PEG-100 stearate, arachidyl alcohol, arachidyl glucoside, and mixtures thereof.

#### f. Silicone Containing Compounds

**[0056]** In non-limiting aspects, silicone containing compounds include any member of a family of polymeric products whose molecular backbone is made up of alternating silicon and oxygen atoms with side groups attached to the silicon atoms. By varying the —Si—O— chain lengths, side groups, and crosslinking, silicones can be synthesized into a wide variety of materials. They can vary in consistency from liquid to gel to solids.

**[0057]** The silicone containing compounds that can be used in the context of the present invention include those described in this specification or those known to a person of ordinary skill in the art. Non-limiting examples include silicone oils (e.g., volatile and non-volatile oils), gels, and solids. In certain aspects, the silicon containing compounds includes a silicone oils such as a polyorganosiloxane. Non-limiting examples of polyorganosiloxanes include dimethicone, cyclomethicone, polysilicone-11, phenyl trimethicone, trimethylsilylamodimethicone, stearyoxytrimethylsilane, or mixtures of these and other organosiloxane materials in any given ratio in order to achieve the desired consistency and application characteristics depending upon the intended application (e.g., to a particular area such as the skin, hair, or eyes). A “volatile silicone oil” includes a silicone oil have a low heat of vaporization, i.e. normally less than about 50 cal per gram of silicone oil. Non-limiting examples of volatile silicone oils include: cyclomethicones such as Dow Corning 344 Fluid, Dow Corning 345 Fluid, Dow Corning 244 Fluid, and Dow Corning 245 Fluid, Volatile Silicon 7207 (Union Carbide Corp., Danbury, Conn.); low viscosity dimethicones, i.e. dimethicones having a viscosity of about 50 cst or less (e.g., dimethicones such as Dow Corning 200-0.5 cst Fluid). The Dow Corning Fluids are available from Dow Corning Corporation, Midland, Mich. Cyclomethicone and dimethicone are described in the Third Edition of the CTFA Cosmetic Ingredient Dictionary (incorporated by reference) as cyclic dimethyl polysiloxane compounds and a mixture of fully methylated linear siloxane polymers end-blocked with trimethylsiloxy units, respectively. Other non-limiting volatile silicone oils that can be used in the context of the present invention include those available from General Electric Co., Silicone Products Div., Waterford, N.Y. and SWS Silicones Div. of Stauffer Chemical Co., Adrian, Mich.

#### g. Exfoliating Agent

**[0058]** Exfoliating agents include ingredients that remove dead skin cells on the skin’s outer surface. These agents may act through mechanical, chemical, and/or other means. Non-limiting examples of mechanical exfoliating agents include abrasives such as pumice, silica, cloth, paper, shells, beads, solid crystals, solid polymers, etc. Non-limiting examples of chemical exfoliating agents include acids and enzyme exfoliants. Acids that can be used as exfoliating agents include, but are not limited to, glycolic acid, lactic acid, citric acid, alpha hydroxy acids, beta hydroxy acids, etc. Other exfoliating agents known to those of skill in the art are also contemplated as being useful within the context of the present invention.

#### h. Essential Oils

**[0059]** Essential oils include oils derived from herbs, flowers, trees, and other plants. Such oils are typically present as tiny droplets between the plant’s cells, and can be extracted by several method known to those of skill in the art (e.g., steam distilled, enfleurage (i.e., extraction by using fat), maceration, solvent extraction, or mechanical pressing). When these types of oils are exposed to air they tend to evaporate (i.e., a volatile oil). As a result, many essential oils are colorless, but with age they can oxidize and become darker. Essential oils are insoluble in water and are soluble in alcohol, ether, fixed oils (vegetal), and other organic solvents. Typical physical characteristics found in essential oils include boiling points that vary from about 160° to 240° C. and densities ranging from about 0.759 to about 1.096.

**[0060]** Essential oils typically are named by the plant from which the oil is found. For example, rose oil or peppermint oil are derived from rose or peppermint plants, respectively. Non-limiting examples of essential oils that can be used in the context of the present invention include sesame oil, macadamia nut oil, tea tree oil, evening primrose oil, Spanish sage oil, Spanish rosemary oil, coriander oil, thyme oil, pimento berries oil, rose oil, anise oil, balsam oil, bergamot oil, rosewood oil, cedar oil, chamomile oil, sage oil, clary sage oil, clove oil, cypress oil, eucalyptus oil, fennel oil, sea fennel oil, frankincense oil, geranium oil, ginger oil, grapefruit oil, jasmine oil, juniper oil, lavender oil, lemon oil, lemongrass oil, lime oil, mandarin oil, marjoram oil, myrrh oil, neroli oil, orange oil, patchouli oil, pepper oil, black pepper oil, petitgrain oil, pine oil, rose otto oil, rosemary oil, sandalwood oil, spearmint oil, spikenard oil, vetiver oil, wintergreen oil, or ylang ylang. Other essential oils known to those of skill in the art are also contemplated as being useful within the context of the present invention.

#### i. Thickening Agents

**[0061]** Thickening agents, including thickener or gelling agents, include substances which that can increase the viscosity of a composition. Thickeners includes those that can increase the viscosity of a composition without substantially modifying the efficacy of the active ingredient within the composition. Thickeners can also increase the stability of the compositions of the present invention. In certain aspects of the present invention, thickeners include hydrogenated polyisobutene, trihydroxystearin, ammonium acryloyldimethyltaurate/vp copolymer, or a mixture of them.

**[0062]** Non-limiting examples of additional thickening agents that can be used in the context of the present

invention include carboxylic acid polymers, crosslinked polyacrylate polymers, polyacrylamide polymers, polysaccharides, and gums. Examples of carboxylic acid polymers include crosslinked compounds containing one or more monomers derived from acrylic acid, substituted acrylic acids, and salts and esters of these acrylic acids and the substituted acrylic acids, wherein the crosslinking agent contains two or more carbon-carbon double bonds and is derived from a polyhydric alcohol (see U.S. Pat. Nos. 5,087,445; 4,509,949; 2,798,053; CTFA International Cosmetic Ingredient Dictionary, Fourth edition, 1991, pp. 12 and 80). Examples of commercially available carboxylic acid polymers include carbomers, which are homopolymers of acrylic acid crosslinked with allyl ethers of sucrose or pentaerythritol (e.g., Carbopol™ 900 series from B. F. Goodrich).

**[0063]** Non-limiting examples of crosslinked polyacrylate polymers include cationic and nonionic polymers. Examples are described in U.S. Pat. Nos. 5,100,660; 4,849,484; 4,835,206; 4,628,078; 4,599,379).

**[0064]** Non-limiting examples of polyacrylamide polymers (including nonionic polyacrylamide polymers including substituted branched or unbranched polymers) include polyacrylamide, isoparaffin and laureth-7, multi-block copolymers of acrylamides and substituted acrylamides with acrylic acids and substituted acrylic acids.

**[0065]** Non-limiting examples of polysaccharides include cellulose, carboxymethyl hydroxyethylcellulose, cellulose acetate propionate carboxylate, hydroxyethylcellulose, hydroxyethyl ethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, methyl hydroxyethylcellulose, microcrystalline cellulose, sodium cellulose sulfate, and mixtures thereof. Another example is an alkyl substituted cellulose where the hydroxy groups of the cellulose polymer is hydroxyalkylated (preferably hydroxy ethylated or hydroxypropylated) to form a hydroxyalkylated cellulose which is then further modified with a C10-C30 straight chain or branched chain alkyl group through an ether linkage. Typically these polymers are ethers of C10-C30 straight or branched chain alcohols with hydroxyalkylcelluloses. Other useful polysaccharides include scleroglucans comprising a linear chain of (1-3) linked glucose units with a (1-6) linked glucose every three unit.

**[0066]** Non-limiting examples of gums that can be used with the present invention include acacia, agar, algin, alginic acid, ammonium alginate, amylopectin, calcium alginate, calcium carrageenan, carnitine, carrageenan, dextrin, gelatin, gellan gum, guar gum, guar hydroxypropyltrimonium chloride, hectorite, hyaluronic acid, hydrated silica, hydroxypropyl chitosan, hydroxypropyl guar, karaya gum, kelp, locust bean gum, natto gum, potassium alginate, potassium carrageenan, propylene glycol alginate, sclerotium gum, sodium carboxymethyl dextran, sodium carrageenan, tragacanth gum, xanthan gum, and mixtures thereof.

#### j. Preservatives

**[0067]** Non-limiting examples of preservatives that can be used in the context of the present invention include quaternary ammonium preservatives such as polyquaternium-1 and benzalkonium halides (e.g., benzalkonium chloride ("BAC") and benzalkonium bromide), parabens (e.g., methylparabens and propylparabens), phenoxyethanol, benzyl alcohol, chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

## 2. Pharmaceutical Ingredients

**[0068]** Pharmaceutical active agents are also contemplated as being useful with the compositions of the present invention. Non-limiting examples of pharmaceutical active agents include anti-acne agents, agents used to treat rosacea, analgesics, anesthetics, anorectals, antihistamines, anti-inflammatory agents including non-steroidal anti-inflammatory drugs, antibiotics, antifungals, antivirals, antimicrobials, anti-cancer actives, scabicides, pediculicides, antineoplastics, antiperspirants, antipruritics, antipsoriatic agents, anti-seborrheic agents, biologically active proteins and peptides, burn treatment agents, cauterizing agents, depigmenting agents, depilatories, diaper rash treatment agents, enzymes, hair growth stimulants, hair growth retardants including DFMO and its salts and analogs, hemostatics, keratolytics, canker sore treatment agents, cold sore treatment agents, dental and periodontal treatment agents, photosensitizing actives, skin protectant/barrier agents, steroids including hormones and corticosteroids, sunburn treatment agents, sunscreens, transdermal actives, nasal actives, vaginal actives, wart treatment agents, wound treatment agents, wound healing agents, etc.

#### F. Kits

**[0069]** Kits are also contemplated as being used in certain aspects of the present invention. For instance, compositions of the present invention can be included in a kit. A kit can include a container. Containers can include a bottle, a metal tube, a laminate tube, a plastic tube, a dispenser, a pressurized container, a barrier container, a package, a compartment, a lipstick container, a compact container, cosmetic pans that can hold cosmetic compositions, or other types of containers such as injection or blow-molded plastic containers into which the dispersions or compositions or desired bottles, dispensers, or packages are retained. The kit and/or container can include indicia on its surface. The indicia, for example, can be a word, a phrase, an abbreviation, a picture, or a symbol.

**[0070]** The containers can dispense a pre-determined amount of the composition. In other embodiments, the container can be squeezed (e.g., metal, laminate, or plastic tube) to dispense a desired amount of the composition. The composition can be dispensed as a spray, an aerosol, a liquid, a fluid, or a semi-solid. The containers can have spray, pump, or squeeze mechanisms. A kit can also include instructions for employing the kit components as well the use of any other compositions included in the container. Instructions can include an explanation of how to apply, use, and maintain the compositions.

## EXAMPLES

**[0071]** The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

**[0072]** All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit, and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### A. Example 1

**[0073]** Combinations of active ingredients disclosed herein can be included in a wide-range of topical product formulations for skin and/or hair. Non-limiting examples of combinations of active ingredients include those found in Table 1 and Table 2.

TABLE 1

Ingredient
<i>Croton Lechleri</i> extract (Dragon's Blood by Naturex)
<i>Argania Spinosa</i> Kernel Extract (Argatensyl by BASF)
Dill ( <i>Peucedanum graveolens</i> ) Extract (LYS'LASTINE™ by BASF)
<i>Myrciaria dubia</i> fruit extract (Camu Camu by AMAX)

TABLE 2

Ingredient
<i>Morus Alba</i> Fruit Extract
<i>Argania Spinosa</i> Kernel Extract (Argatensyl by BASF)
Dill ( <i>Peucedanum graveolens</i> ) Extract (LYS'LASTINE™ by BASF)
<i>Myrciaria dubia</i> fruit extract (Camu Camu by AMAX)

#### B. Example 2

**[0074]** Tables 3 and 4 describe generic formulations or skin testing formulations in which an active ingredient can be incorporated into. These formulations can also be used to determine the types of skin benefits that can be attributed to the active ingredient. These formulations are prepared in such a manner that any resulting skin benefit from topical application of the formula to skin can be directly attributed to the active ingredient being tested. In the context of aspects of the present invention, the active ingredient that can be tested can be *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract, *Morus alba* fruit extract, or any combination thereof, or all of such active ingredients, or at least 1, 2, 3, 4, and/or 5 of such active ingredients. It should be recognized that other standard testing vehicles can also be used to determine the skin benefit properties of active ingredient and that the following formulations are non-limiting testing vehicles.

TABLE 3\*

Ingredient	% Concentration (by weight)
<b>Phase A</b>	
Water	84.80
Xanthan gum	0.1
M-paraben	0.15
P-paraben	0.1
Citric acid	0.1
<b>Phase B</b>	
Cetyl alcohol	4.0
Glyceryl stearate + PEG 100	4.0
Octyl palmitate	4.0
Dimethicone	1.0
Tocopheryl acetate	0.2
<b>Phase C</b>	
Active Ingredient**	2.0
<b>TOTAL</b>	<b>100</b>

\*Procedure for making composition: Sprinkle Xanthan gum in water and mix for 10 min. Subsequently, add all ingredients in phase A and heat to 70-75° C. Add all items in phase B to separate beaker and heat to 70-75° C. Mix phases A and B at 70-75° C. Continue mixing and allow composition to cool to 30° C. Subsequently, add phase C ingredient while mixing.

\*\*The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

TABLE 4\*

Ingredient	% Concentration (by weight)
<b>Phase A</b>	
Water	78.6
M-paraben	0.2
P-paraben	0.1
Na <sub>2</sub> EDTA	0.1
Shea butter	4.5
Petrolatum	4.5
Glycerin	4.0
Propylene Glycol	2.0
Finsolve TN	2.0
<b>Phase B</b>	
Sepigel 305	2.0
<b>Phase C</b>	
Active Ingredient **	2.0
<b>TOTAL</b>	<b>100</b>

\*Add ingredients in phase A to beaker and heat to 70-75° C. while mixing. Subsequently, add the phase B ingredient with phase A and cool to 30° C. with mixing. Subsequently, add phase C ingredient while mixing.

\*\* The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

#### C. Example 3

**[0075]** The formulations represented in Table 5-11 are non-limiting examples of the types of formulations that can be prepared in the context of the present invention. Any standard method can be used to prepare such formulations. For instance, simple mixing of the ingredients in a beaker can be used. One should mix such ingredients and add heat as necessary to obtain a homogenous composition. The active ingredients that can be used in the formulations can include *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract, *Morus*

*alba* fruit extract, or any combination thereof, or all of such active ingredients, or at least 1, 2, 3, 4, and/or 5 of such active ingredients.

[0076] Table 5 includes a non-limiting example of a composition of the present invention. The composition can be formulated into an emulsion (e.g., oil in water (o/w), water in oil (w/o), oil in water in oil (o/w/o), water in oil in water (w/o/w), etc.) and the additional ingredients identified throughout the specification can be included into the Table 5 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 5 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.).

TABLE 5

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient *	0.1% to 10%
Glycerin	3 to 40%
Butylene glycol	0.0001 to 10%
Propylene glycol	0.0001 to 10%
Phenoxyethanol	0.0001 to 10%
Disodium EDTA	0.0001 to 10%
Steareth-20	0.0001 to 10%
Chlorhexidine Diglunonate	0.0001 to 10%
Potassium Sorbate	0.0001 to 10%
Preservative**	0.0001 to 2%
TOTAL	100

\* The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

\*\*Any preservative can be used identified in the specification or those known in the art.

[0077] Table 6 includes a non-limiting example of a composition of the present invention. The composition can be formulated into an emulsion (e.g., o/w, w/o, o/w/o, w/o/w, etc.) and the additional ingredients identified throughout the specification can be included into the Table 6 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 6 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.).

TABLE 6

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient*	0.1% to 10%
Dimethicone	0.0001 to 10%
Triethanolamine	0.0001 to 10%
Phenonip	0.0001 to 10%
Betaine	0.0001 to 10%
Disodium EDTA	0.0001 to 10%
Tocopheryl acetate	0.0001 to 10%
Prodex 400	0.0001 to 10%
Preservative**	0.0001 to 2%
TOTAL	100

\* The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

\*\*Any preservative can be used identified in the specification or those known in the art.

[0078] Table 7 includes a non-limiting example of a composition of the present invention. The composition can

be formulated into an emulsion (e.g., o/w, w/o, o/w/o, w/o/w, etc.) and the additional ingredients identified throughout the specification can be included into the Table 7 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 7 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.). In particular embodiments, the Table 7 composition can be a moisturizer.

TABLE 7

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient *	0.1% to 10%
Glycerin	0.0001 to 10%
Pentylene Glycol	0.0001 to 10%
Capryl Glycol	0.0001 to 10%
Disodium EDTA	0.0001 to 10%
Capric/Caprylic Triglyceride	0.0001 to 10%
Lipex 205 (Shea Butter)	0.0001 to 10%
Squalane	0.0001 to 10%
Cetyl Alcohol	0.0001 to 10%
Dimethicone	0.0001 to 10%
Ceramide II	0.0001 to 10%
Stearic Acid	0.0001 to 10%
Super Sterol Ester	0.0001 to 10%
Arlacel 165	0.0001 to 10%
Simulgel 600	0.0001 to 10%
TOTAL	100

\*The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

[0079] Table 8 includes a non-limiting example of a composition of the present invention. The composition can be formulated into an emulsion (e.g., o/w, w/o, o/w/o, w/o/w, etc.) and the additional ingredients identified throughout the specification can be included into the Table 8 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 8 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.). In particular embodiments, the Table 8 composition can be a moisturizer.

TABLE 8

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient *	0.1% to 10%
Glycerin	0.0001 to 10%
Pentylene Glycol	0.0001 to 10%
Capryl Glycol	0.0001 to 10%
Disodium EDTA	0.0001 to 10%
Petrolatum	0.0001 to 10%
Squalane	0.0001 to 10%
Cetyl Alcohol	0.0001 to 10%
Arlacel 165	0.0001 to 10%
Dimethicone	0.0001 to 10%
Simulgel 600	0.0001 to 10%
TOTAL	100

\* The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

[0080] Table 9 includes a non-limiting example of a composition of the present invention. The composition can be formulated into an emulsion (e.g., o/w, w/o, o/w/o, w/o/w,

etc.) and the additional ingredients identified throughout the specification can be included into the Table 9 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 9 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.). In particular embodiments, the Table 9 composition can be a sunscreen lotion.

TABLE 9

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient *	0.1% to 10%
Xanthan Gum	0.0001 to 10%
Disodium EDTA	0.0001 to 10%
Pentylene Glycol	0.0001 to 10%
Capryl Glycol	0.0001 to 10%
Pemulen TR-1	0.0001 to 10%
Triethanolamine	0.0001 to 10%
PVP/Hexadecene Copolymer	0.0001 to 10%
Finsolv TN	10 to 30%
Sorbitan Isostearate	0.0001 to 10%
Sunscreen Ingredient**	2 to 25%
TOTAL	100

\* The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

\*\*Sunscreen ingredient can be any sunscreen ingredient, or combination of such ingredients, identified in the specification (e.g. UV absorbing and/or reflecting agents) or known to those of ordinary skill in the art. In one embodiment, the sunscreen ingredient is a combination of zinc oxide and titanium dioxide.

[0081] Table 10 includes a non-limiting example of a composition of the present invention. The additional ingredients identified throughout the specification can be included into the Table 10 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 10 can vary depending on a desired formulation (e.g., cream, lotion, moisturizer cleanser, etc.). In particular embodiments, the Table 10 composition can be a cleanser.

TABLE 10

Ingredient	% Concentration (by weight)
Water	q.s.
Active Ingredient*	0.1% to 10%
Disodium EDTA	0.0001 to 10%
Citric Acid	0.0001 to 10%
Pentylene Glycol	0.0001 to 10%
Capryl Glycol	0.0001 to 10%
sodium methyl cocoyl taurate	10 to 30%
sodium cocoamphodiacetate	1 to 10%
TOTAL	100

\*The active ingredients identified throughout this specification can be incorporated into composition as the active ingredient. The active ingredients can be individually used or combined in this composition. The concentration ranges of the active ingredients (or combination of active ingredients) can be modified as desired or needed by increasing or decreasing the amount of water.

[0082] Table 11 includes a non-limiting example of a composition of the present invention. The composition can be formulated into an emulsion (e.g., o/w, w/o, o/w/o, w/o/w, etc.) and the additional ingredients identified throughout the specification can be included into the Table 11 composition (e.g., by adjusting the water content of composition). Further, the concentration ranges of the ingredients identified in Table 11 can vary depending on a desired formulation (e.g.,

cream, lotion, moisturizer cleanser, etc.). In particular embodiments, the Table 11 composition can be a cream.

TABLE 11<sup>^</sup>

Ingredient	% Concentration (by weight)
Water	51
Disodium EDTA	0.1
HDI/Trimethylol Hexyllactone	2.0
Crosspolymer	
Nylon-12	0.5
Acrylates/C10-30 Alkyl Crosspolymer	0.2
Dow Corning 9041**	10
Glyceryl Stearate & PEG-100 Stearate	4.5
Ethylhexyl Palmitate	8.0
Pentylene Glycol	3.0
Dimethyl Isosorbide	1.0
Argatensyl LS 9735**	2.0
<i>Peucedanum graveolens</i> (dill) extract	1.0
<i>Myrciaria dubia</i> fruit extract	1.0
<i>Croton lechleri</i> resin extract	2.0
Triethanolamine	0.3
SYMOCIDE ® PS**	1.1
Iodopropynyl butylcarbamate	0.2
Inagel V.V.OP**	13
Excipients	q.s.
TOTAL	100

<sup>^</sup>Formulation can be prepared by mixing the ingredients in a beaker under heat 70-75° C. until homogenous. Subsequently, the formulation can be cooled to standing room temperature (20-25° C.). Further, and if desired, additional ingredients can be added, for example, to modify the rheological properties of the composition or ingredients that provide benefits to skin.

\*Excipients can be added, for example, to modify the rheological properties of the composition. Alternatively, the amount of water can be varied so long as the amount of water in the composition is at least 30% w/w, and preferably between 50 to 80% w/w.

\*\*Dow Corning 9041 comprises dimethicone and dimethicone crosspolymer; Argatensyl LS 9735 comprises *Argania spinosa* kernel extract; SYMOCIDE ® PS comprises phenoxyethanol, decylene glycol, and 1,2-hexanediol; Inagel V.V.OP comprises glycerin, water, and sodium polyacrylate.

#### D. Example 4 (Clinical Results)

[0083] In a twelve week, controlled clinical study, it has been determined that the combination of *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract can increase elasticity of skin. See Table 12.

[0084] Briefly, twenty-one women subjects with mild to moderate neck sagging were instructed to apply a cream containing *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, and *Croton lechleri* extract twice daily to the jawline, morning and evening after cleansing. The subjects used the product for twelve consecutive weeks. Elasticity measurements were taken before the first application of the composition (baseline) and then at three weeks, six weeks, and twelve weeks of consecutive use.

[0085] The elasticity measurements were taken using a DERMALAB® Suction Cup along the jawline between the chin and neck. Appropriate statistical analysis were performed comparing the baseline to the measurements at three, six, and twelve weeks. For all analyses, p<0.05 was taken as the level of significance. Reduction in reaction time indicates an increase in elasticity of the skin.

TABLE 12

Descriptive	Reaction Time (ms)			
	Baseline	Week 3	Week 6	Week 12
Mean	261.5	246.1	213.9	215.8
Percent Change		-5.91%	-18.19%	-17.49%
P-Value		P > 0.05	P < 0.01	P < 0.01

## E. Example 5 (In Vitro Assays)

**[0086]** It has been determined that *Argania spinosa* kernel extract, dill extract, *Myrciaria dubia* fruit extract, *Croton lechleri* extract, and/or *Morus alba* fruit extract can increase elastin synthesis, increase collagen expression, increase production of laminin, increase production of fibronectin, and/or increase the expression of proteins that are involved in elastin organization in fibroblasts (e.g., Fibulin-5). A summary of the results are found in Table 13 and the methods used to determine the properties of the ingredients are provided below. Taken together, a combination of these ingredients is expected to also have the same activities as the individual ingredients therein. These activities can reduce the appearance of loose, sagging, and flaccid skin, improve facial contouring (e.g., change the appearance of the shape of the face by tightening the skin), and increase elasticity of skin.

TABLE 13

Assay	Ingredient	Activity
Elastin Expression	<i>Argania spinosa</i> kernel extract	+200%
Collagen Expression	<i>Croton lechleri</i> extract	+81%
	<i>Myrciaria dubia</i> fruit extract	+250%
	<i>Croton lechleri</i> extract	+281%
	<i>Morus alba</i> fruit extract (0.1% extract)	+27%
Laminin Production	<i>Myrciaria dubia</i> fruit extract	+240%
	<i>Argania spinosa</i> kernel extract	+78%
	<i>Morus alba</i> fruit extract (0.1% extract)	+14.9%
Fibronectin Production	<i>Argania spinosa</i> kernel extract	+33%
	<i>Morus alba</i> fruit extract (0.1% extract)	+12.9%
Fibulin-5 Production	<i>Morus alba</i> fruit extract	Significant Increase
	<i>Croton lechleri</i> extract	Significant Increase

**[0087]** Elastin Stimulation Assay: Elastin is a connective tissue protein that helps skin resume shape after stretching or contracting. Elastin is also an important load-bearing protein used in places where mechanical energy is required to be stored. Elastin is made by linking many soluble tropoelastin protein molecules, in a reaction catalyzed by lysyl oxidase. Elastin secretion and elastin fibers were monitored in cultured human fibroblasts by staining of cultured human fibroblasts using immunofluorescent antibodies directed against elastin by a direct ELISA sandwich method. A Meso Scale Discovery system SECTOR 2400 Imaging system was used to analyze the results. Changes in elastin secretion and elastin fibers caused by *Argania spinosa* kernel extract and *Croton lechleri* extract were determined by incubating cultured human fibroblasts with the active ingredient for a period of time before probing the cells or a lysate thereof with antibodies directed against elastin. It was shown that *Argania spinosa* kernel extract and *Croton lechleri* extract increased elastin synthesis by 200% and 81%, respectively.

**[0088]** Collagen Stimulation Assay: Collagen is an extracellular matrix protein critical for skin structure. Increased synthesis of collagen helps improve skin firmness and elasticity. This bioassay was used to examine the effect of *Myrciaria dubia* fruit extract, *Croton lechleri* extract, and *Morus alba* fruit extract (*Morus alba* fruit extract at 0.1% extract concentration) on the production of type I procollagen peptide (a precursor to collagen) by human epidermal fibroblasts. The endpoint of this assay was a spectrophotometric measurement that reflects the presence of procollagen peptide and cellular viability. The assay employed the quantitative sandwich enzyme immunoassay technique whereby a monoclonal antibody specific for procollagen peptide has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any procollagen peptide present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for the procollagen peptide was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color was developed in proportion to the amount of procollagen peptide bound in the initial step. Color development was stopped and the intensity of the color at 450 nm was measured using a microplate reader.

**[0089]** For generation of samples and controls, subconfluent normal human adult epidermal fibroblasts (Cascade Biologics) were cultivated in standard DMEM growth medium with 10% fetal bovine serum (Mediatech) at 37° C. in 10% CO<sub>2</sub>. The cells were treated with each of the tested ingredients and controls for 3 days. Following incubation, cell culture medium was collected and the amount of procollagen peptide secretion was quantified using the sandwich enzyme linked immuno-sorbant assay (ELISA) from Takara (#MK101) as explained above. It was shown that *Myrciaria dubia* fruit extract, *Croton lechleri* extract, and *Morus alba* fruit extract increased collagen production by 250%, 81%, and 27%, respectively.

**[0090]** Laminin and Fibronectin Stimulation Assay: Laminin and fibronectin are major proteins in the dermal-epidermal junction (DEJ) (also referred to as the basement membrane). The DEJ is located between the dermis and the epidermis interlocks forming fingerlike projections called rete ridges. The cells of the epidermis receive their nutrients from the blood vessels in the dermis. The rete ridges increase the surface area of the epidermis that is exposed to these blood vessels and the needed nutrients. The DEJ provides adhesion of the two tissue compartments and governs the structural integrity of the skin. Laminin and fibronectin are two structural glycoproteins located in the DEJ. Considered the glue that holds the cells together, laminin and fibronectin are secreted by dermal fibroblasts to help facilitate intra- and inter-cellular adhesion of the epidermal cells to the DEJ. Laminin and fibronectin secretion was monitored by quantifying laminin and fibronectin in cell supernatants of cultured human fibroblasts treated for 3 days with culture medium with or without 1.0% final concentration of the test ingredient(s), except that *Morus alba* fruit extract was tested at 0.1% final concentration. Following incubation, laminin and fibronectin content was measured using immunofluorescent antibodies directed against each protein in an enzyme linked immuno-sorbant assay (ELISA). Measurements were normalized for cellular metabolic activity, as determined by bioconversion of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tet-

razolium (MTS). For laminin production, *Myrciaria dubia* fruit extract, *Argania spinosa* kernel extract, and *Morus alba* fruit extract were shown to increase laminin production by 240%, 78%, and 14.9%, respectively. For fibronectin, *Argania spinosa* kernel extract and *Morus alba* fruit extract were shown to increase fibronectin production by 33% and 12.9%, respectively.

**[0091]** Fibulin-5 Expression Assay: Fibulin-5 is a secreted extracellular-matrix protein that regulates elastic fiber assembly. Fibulin-5 expression levels were determined by immunostaining of Fibulin 5 with a monoclonal anti-fibulin 5 antibody, clone 393904 (R&D Systems, Ref.MAB3095) of paraffinized sections of human ex-vivo skin explants. The antibody was diluted at 1:50 in PBS-BSA 0.3% and incubated with the paraffinized sections overnight at room temperature. The staining was enhanced with a biotin/streptavidin enhancement system (VECTASTAIN® R.T.U. Universal, VECTOR®) and revealed by VIP (VECTOR®, Ref. SK-4600). The immunostaining was assessed by observation using a microscope. *Croton lechleri* extract and *Morus alba* fruit extract significantly increased the expression of fibulin-5.

#### F. Example 6 (Additional Assays)

**[0092]** Assays that can be used to determine the efficacy of any one of the ingredients or any combination of ingredients or compositions having said combination of ingredients disclosed throughout the specification and claims can be determined by methods known to those of ordinary skill in the art. The following are non-limiting assays that can be used in the context of the present invention. It should be recognized that other testing procedures can be used, including, for example, objective and subjective procedures.

**[0093]** Appearance of Loose, Sagging, and Flaccid Skin: Skin looseness, sagging, and flaccidity can be evaluated with clinical grading techniques. Evaluations can be made independently by two clinicians and averaged. Changes in skin looseness, sagging, and/or flaccidity caused by any one of, all of, or any combination of the active ingredients disclosed herein can be determined by measuring the skin looseness, sagging, and flaccidity at a baseline time, then applying the active ingredient(s) to skin once, twice, three times, or more over a period of time, such as 1 minute, 10 minute, 1 hour, 6 hours, 12 hours, 1 day, 2 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, or more, or any time therein, before measuring the skin looseness, sagging, and flaccidity again. Negative controls can be used to subtract out any effects of the carrier of the active ingredient(s).

**[0094]** Surface Contour of the Skin Assay with a Profilometer/Stylus Method: The surface contour of the skin can be measured by using the profilometer/Stylus method. This includes either shining a light or dragging a stylus across the replica surface. The vertical displacement of the stylus can be fed into a computer via a distance transducer, and after scanning a fixed length of replica a cross-sectional analysis of skin profile can be generated as a two-dimensional curve. This scan can be repeated any number of times along a fix axis to generate a simulated 3-D picture of the skin. Ten random sections of the replicas using the stylus technique can be obtained and combined to generate average values. The values of interest include Ra which is the arithmetic mean of all roughness (height) values computed by integrating the profile height relative to the mean profile height. Rt which is the maximum vertical distance between the highest

peak and lowest trough, and Rz which is the mean peak amplitude minus the mean peak height. Values are given as a calibrated value in mm. Equipment should be standardized prior to each use by scanning metal standards of known values. Ra Value can be computed by the following equation:  $R_a = \text{Standardize roughness}$ ;  $l_m = \text{the traverse (scan) length}$ ; and  $y = \text{the absolute value of the location of the profile relative to the mean profile height (x-axis)}$ .

**[0095]** Changes in facial contouring caused by any one of, all of, or any combination of the active ingredients disclosed herein can be determined by measuring the contour at a baseline time, then applying the active ingredient(s) to skin once, twice, three times, or more over a period of time, such as 1 minute, 10 minute, 1 hour, 6 hours, 12 hours, 1 day, 2 days, 1 week, 2 weeks, 4 weeks, 6 weeks, 12 weeks, or more, or any time therein, before measuring the skin contour again. Negative controls can be used to subtract out any effects of the carrier of the active ingredient(s).

**[0096]** B16 Pigmentation Assay: Melanogenesis is the process by which melanocytes produce melanin, a naturally produced pigment that imparts color to skin, hair, and eyes. Inhibiting melanogenesis is beneficial to prevent skin darkening and lighten dark spots associated with aging. This bioassay utilizes B16-F1 melanocytes (ATCC), an immortalized mouse melanoma cell line, to analyze the effect of compounds on melanogenesis. The endpoint of this assay is a spectrophotometric measurement of melanin production and cellular viability. B16-F1 melanocytes, can be cultivated in standard DMEM growth medium with 10% fetal bovine serum (Mediatech) at 37° C. in 10% CO<sub>2</sub> and then treated with any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification for 6 days. Following incubation, melanin secretion is measured by absorbance at 405 nm and cellular viability is quantified.

**[0097]** Fibrillin-1 Expression Assay: Fibrillin-1 is an extracellular matrix protein that forms a scaffold for the deposition of elastin and provides structural support in elastic and nonelastic connective tissue. Fibrillin-1 is secreted by fibroblasts. Fibrillin-1 expression levels can be determined by a quantitative sandwich enzyme immunoassay technique using a microtiter plate pre-coated with an anti-fibrillin-1 antibody. Samples containing, cells, lysed cells, and/or culture medium of cells from cells cultured in the presence of negative controls or any one of, all of, or any combination of the active ingredients disclosed herein can be tested for the presence and concentration of fibrillin-1 under each test condition. Samples can be added to the microtiter wells to allow the fibrillin-1 to bind. The unbound contents of the well can then be washed out and the concentration of the fibrillin-1 bound in the well determined by a substrate solution that develops color in proportion to the amount of fibrillin-1 bound in the initial step. Color can be determined using a microplate reader for detection.

**[0098]** Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) Assay: The prototype ligand of the TNF superfamily, TNF- $\alpha$ , is a pleiotropic cytokine that plays a central role in inflammation. Increase in its expression is associated with an up regulation in pro-inflammatory activity. This bioassay can be used to analyze the effect of any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification on the production of TNF- $\alpha$  by human epidermal keratinocytes. The endpoint of this assay can be a spectrophotometric



measurement that reflects the presence of TNF- $\alpha$  and cellular viability. The assay employs the quantitative sandwich enzyme immunoassay technique whereby a monoclonal antibody specific for TNF- $\alpha$  has been pre-coated onto a microplate. Standards and samples can be pipetted into the wells and any TNF- $\alpha$  present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TNF- $\alpha$  can be added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution can be added to the wells and color develops in proportion to the amount of TNF- $\alpha$  bound in the initial step using a microplate reader for detection at 450 nm. The color development can be stopped and the intensity of the color can be measured. Subconfluent normal human adult keratinocytes (Cascade Biologics) cultivated in EpiLife standard growth medium (Cascade Biologics) at 37° C. in 5% CO<sub>2</sub>, can be treated with phorbol 12-myristate 13-acetate (PMA, 10 ng/ml, Sigma Chemical, #P1585-1MG) and any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification for 6 hours. PMA has been shown to cause a dramatic increase in TNF- $\alpha$  secretion which peaks at 6 hours after treatment. Following incubation, cell culture medium can be collected and the amount of TNF- $\alpha$  secretion quantified using a sandwich enzyme linked immuno-sorbant assay (ELISA) from R&D Systems (#DTA00C).

**[0099]** Antioxidant (AO) Assay: An in vitro bioassay that measures the total anti-oxidant capacity of any one of the ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification. The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2'-azino-di-[3-ethylbenzothiazoline sulphoxide]) to ABTS®+by metmyoglobin. The antioxidant system of living organisms includes enzymes such as superoxide dismutase, catalase, and glutathione peroxidase; macromolecules such as albumin, ceruloplasmin, and ferritin; and an array of small molecules, including ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, reduced glutathione, uric acid, and bilirubin. The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the extracellular fluid. Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals, than any single compound alone. Thus, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids. The capacity of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and is quantified as molar Trolox equivalents. Anti-Oxidant capacity kit #709001 from Cayman Chemical (Ann Arbor, Mich. USA) can be used as an in vitro bioassay to measure the total anti-oxidant capacity of each of any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification. The protocol can be followed according to manufacturer recommendations.

**[0100]** ORAC Assay: Oxygen Radical Absorption (or Absorbance) Capacity (ORAC) of any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification can also be assayed by measuring the antioxidant activity of

such ingredients or compositions. Antioxidant activity indicates a capability to reduce oxidizing agents (oxidants). This assay quantifies the degree and length of time it takes to inhibit the action of an oxidizing agent, such as oxygen radicals, that are known to cause damage to cells (e.g., skin cells). The ORAC value of any one of the active ingredients, combination of ingredients, or compositions having said combinations disclosed in the specification can be determined by methods known to those of ordinary skill in the art (see U.S. Publication Nos. 2004/0109905 and 2005/0163880; and commercially available kits such as Zen-Bio ORAC Anti-oxidant Assay kit (#AOX-2)). The Zen-Bio ORAC Anti-oxidant Assay kit measures the loss of fluorescein fluorescence over time due to the peroxy-radical formation by the breakdown of AAPH (2,2'-azobis-2-methylpropanimidamide, dihydrochloride). Trolox, a water soluble vitamin E analog, serves as positive control inhibition fluorescein decay in a dose dependent manner.

**[0101]** Mushroom tyrosinase activity assay: In mammalian cells, tyrosinase catalyzes two steps in the multi-step biosynthesis of melanin pigments from tyrosine (and from the polymerization of dopachrome). Tyrosinase is localized in melanocytes and produces melanin (aromatic quinone compounds) that imparts color to skin, hair, and eyes. Purified mushroom tyrosinase (Sigma) can be incubated with its substrate L-Dopa (Fisher) in the presence or absence of each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification. Pigment formation can be evaluated by colorimetric plate reading at 490 nm. The percent inhibition of mushroom tyrosinase activity can be calculated compared to non-treated controls to determine the ability of test ingredients or combinations thereof to inhibit the activity of purified enzyme. Test extract inhibition was compared with that of kojic acid (Sigma).

**[0102]** Matrix Metalloproteinase 3 and 9 Enzyme Activity (MMP3; MMP9) Assay: An in vitro matrix metalloprotease (MMP) inhibition assay. MMPs are extracellular proteases that play a role in many normal and disease states by virtue of their broad substrate specificity. MMP3 substrates include collagens, fibronectins, and laminin; while MMP9 substrates include collagen VII, fibronectins and laminin. Using Colorimetric Drug Discovery kits from BioMol International for MMP3 (AK-400) and MMP-9 (AK-410), this assay is designed to measure protease activity of MMPs using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC2H5)5,6. The MMP cleavage site peptide bond is replaced by a thioester bond in the thiopeptide. Hydrolysis of this bond by an MMP produces a sulfhydryl group, which reacts with DTNB [5,5'-dithiobis(2-nitrobenzoic acid), Ellman's reagent] to form 2-nitro-5-thiobenzoic acid, which can be detected by its absorbance at 412 nm ( $\epsilon=13,600$  M<sup>-1</sup> cm<sup>-1</sup> at pH 6.0 and above 7). The active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be assayed.

**[0103]** Matrix Metalloproteinase 1 Enzyme Activity (MMP1) Assay: An in vitro matrix metalloprotease (MMP) inhibition assay. MMPs are extracellular proteases that play a role in many normal and disease states by virtue of their broad substrate specificity. MMP1 substrates include collagen IV. The Molecular Probes Enz/Chek Gelatinase/ Collagenase Assay kit (#E12055) utilizes a fluorogenic gelatin substrate to detect MMP1 protease activity. Upon prote-

olytic cleavage, bright green fluorescence is revealed and may be monitored using a fluorescent microplate reader to measure enzymatic activity.

**[0104]** The EnzChek Gelatinase/Collagenase Assay kit (#E12055) from Invitrogen is designed as an in vitro assay to measure MMP1 enzymatic activity. The active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be assayed. The assay relies upon the ability of purified MMP1 enzyme to degrade a fluorogenic gelatin substrate. Once the substrate is specifically cleaved by MMP1 bright green fluorescence is revealed and may be monitored using a fluorescent microplate reader. Test materials are incubated in the presence or absence of the purified enzyme and substrate to determine their protease inhibitor capacity.

**[0105]** Cyclooxygenase (COX) Assay: An in vitro cyclooxygenase-1 and -2 (COX-1, -2) inhibition assay. COX is a bifunctional enzyme exhibiting both cyclooxygenase and peroxidase activities. The cyclooxygenase activity converts arachidonic acid to a hydroperoxy endoperoxide (Prostaglandin G<sub>2</sub>; PGG<sub>2</sub>) and the peroxidase component reduces the endoperoxide (Prostaglandin H<sub>2</sub>; PGH<sub>2</sub>) to the corresponding alcohol, the precursor of prostaglandins, thromboxanes, and prostacyclins. This COX Inhibitor screening assay measures the peroxidase component of cyclooxygenases. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD). This inhibitor screening assay includes both COX-1 and COX-2 enzymes in order to screen isozyme-specific inhibitors. The Colorimetric COX (ovine) Inhibitor screening assay (#760111, Cayman Chemical) can be used to analyze the effects of each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification on the activity of purified cyclooxygenase enzyme (COX-1 or COX-2). According to manufacturer instructions, purified enzyme, heme and test extracts can be mixed in assay buffer and incubated with shaking for 15 min at room temperature. Following incubation, arachidonic acid and colorimetric substrate can be added to initiate the reaction. Color progression can be evaluated by colorimetric plate reading at 590 nm. The percent inhibition of COX-1 or COX-2 activity can be calculated compared to non-treated controls to determine the ability of test extracts to inhibit the activity of purified enzyme.

**[0106]** Lipoxygenase (LO) Assay: An in vitro lipoxygenase (LO) inhibition assay. LOs are non-heme iron-containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids. Linoleate and arachidonate are the main substrates for LOs in plants and animals. Arachadonic acid may then be converted to hydroxyeicosotrienoic (HETE) acid derivatives, that are subsequently converted to leukotrienes, potent inflammatory mediators. This assay provides an accurate and convenient method for screening lipoxygenase inhibitors by measuring the hydroperoxides generated from the incubation of a lipoxygenase (5-, 12-, or 15-LO) with arachidonic acid. The Colorimetric LO Inhibitor screening kit (#760700, Cayman Chemical) can be used to determine the ability of each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification to inhibit enzyme activity. Purified 15-lipoxygenase and test ingredients can be mixed in assay buffer and

incubated with shaking for 10 min at room temperature. Following incubation, arachidonic acid can be added to initiate the reaction and the mixtures can be incubated for an additional 10 min at room temperature. Colorimetric substrate can be added to terminate catalysis and color progression can be evaluated by fluorescence plate reading at 490 nm. The percent inhibition of lipoxygenase activity can be calculated compared to non-treated controls to determine the ability of each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification to inhibit the activity of purified enzyme.

**[0107]** Elastase Assay: EnzChek® Elastase Assay (Kit #E-12056) from Molecular Probes (Eugene, Oreg. USA) can be used as an in vitro enzyme inhibition assay for measuring inhibition of elastase activity for each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification. The EnzChek kit contains soluble bovine neck ligament elastin that can be labeled with dye such that the conjugate's fluorescence can be quenched. The non-fluorescent substrate can be digested by elastase or other proteases to yield highly fluorescent fragments. The resulting increase in fluorescence can be monitored with a fluorescence microplate reader. Digestion products from the elastin substrate have absorption maxima at ~505 nm and fluorescence emission maxima at ~515 nm. The peptide, N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone, can be used as a selective, collective inhibitor of elastase when utilizing the EnzChek Elastase Assay Kit for screening for elastase inhibitors.

**[0108]** Oil Control Assay: An assay to measure reduction of sebum secretion from sebaceous glands and/or reduction of sebum production from sebaceous glands can be assayed by using standard techniques known to those having ordinary skill in the art. In one instance, the forehead can be used. Each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be applied to one portion of the forehead once or twice daily for a set period of days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days), while another portion of the forehead is not treated with the composition. After the set period of days expires, then sebum secretion can be assayed by application of fine blotting paper to the treated and untreated forehead skin. This is done by first removing any sebum from the treated and untreated areas with moist and dry cloths. Blotting paper can then be applied to the treated and untreated areas of the forehead, and an elastic band can be placed around the forehead to gently press the blotting paper onto the skin. After 2 hours the blotting papers can be removed, allowed to dry and then transilluminated. Darker blotting paper correlates with more sebum secretion (or lighter blotting paper correlates with reduced sebum secretion).

**[0109]** Erythema Assay: An assay to measure the reduction of skin redness can be evaluated using a Minolta Chromometer. Skin erythema may be induced by applying a 0.2% solution of sodium dodecyl sulfate on the forearm of a subject. The area is protected by an occlusive patch for 24 hrs. After 24 hrs, the patch is removed and the irritation-induced redness can be assessed using the a\* values of the Minolta Chroma Meter. The a\* value measures changes in skin color in the red region. Immediately after reading, the

area is treated with the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification. Repeat measurements can be taken at regular intervals to determine the formula's ability to reduce redness and irritation.

**[0110]** Skin Moisture/Hydration Assay: Skin moisture/hydration benefits can be measured by using impedance measurements with the Nova Dermal Phase Meter. The impedance meter measures changes in skin moisture content. The outer layer of the skin has distinct electrical properties. When skin is dry it conducts electricity very poorly. As it becomes more hydrated increasing conductivity results. Consequently, changes in skin impedance (related to conductivity) can be used to assess changes in skin hydration. The unit can be calibrated according to instrument instructions for each testing day. A notation of temperature and relative humidity can also be made. Subjects can be evaluated as follows: prior to measurement they can equilibrate in a room with defined humidity (e.g., 30-50%) and temperature (e.g., 68-72° C.). Three separate impedance readings can be taken on each side of the face, recorded, and averaged. The T5 setting can be used on the impedance meter which averages the impedance values of every five seconds application to the face. Changes can be reported with statistical variance and significance. Each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be assayed according to this process.

**[0111]** Skin Clarity and Reduction in Freckles and Age Spots Assay: Skin clarity and the reduction in freckles and age spots can be evaluated using a Minolta Chromometer. Changes in skin color can be assessed to determine irritation potential due to product treatment using the a\* values of the Minolta Chroma Meter. The a\* value measures changes in skin color in the red region. This is used to determine whether each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification is inducing irritation. The measurements can be made on each side of the face and averaged, as left and right facial values. Skin clarity can also be measured using the Minolta Meter. The measurement is a combination of the a\*, b, and L values of the Minolta Meter and is related to skin brightness, and correlates well with skin smoothness and hydration. Skin reading is taken as above. In one non-limiting aspect, skin clarity can be described as L/C where C is chroma and is defined as  $(a^2+b^2)^{1/2}$ .

**[0112]** Skin Dryness, Surface Fine Lines, Skin Smoothness, and Skin Tone Assay: Skin dryness, surface fine lines, skin smoothness, and skin tone can be evaluated with clinical grading techniques. For example, clinical grading of skin dryness can be determined by a five point standard Kligman Scale: (0) skin is soft and moist; (1) skin appears normal with no visible dryness; (2) skin feels slightly dry to the touch with no visible flaking; (3) skin feels dry, tough, and has a whitish appearance with some scaling; and (4) skin feels very dry, rough, and has a whitish appearance with scaling. Evaluations can be made independently by two clinicians and averaged.

**[0113]** Clinical Grading of Skin Tone Assay: Clinical grading of skin tone can be performed via a ten point analog numerical scale: (10) even skin of uniform, pinkish brown color. No dark, erythremic, or scaly patches upon examination with a hand held magnifying lens. Microtexture of the

skin very uniform upon touch; (7) even skin tone observed without magnification. No scaly areas, but slight discolorations either due to pigmentation or erythema. No discolorations more than 1 cm in diameter; (4) both skin discoloration and uneven texture easily noticeable. Slight scaliness. Skin rough to the touch in some areas; and (1) uneven skin coloration and texture. Numerous areas of scaliness and discoloration, either hypopigmented, erythremic or dark spots. Large areas of uneven color more than 1 cm in diameter. Evaluations were made independently by two clinicians and averaged.

**[0114]** Clinical Grading of Skin Smoothness Assay: Clinical grading of skin smoothness can be analyzed via a ten point analog numerical scale: (10) smooth, skin is moist and glistening, no resistance upon dragging finger across surface; (7) somewhat smooth, slight resistance; (4) rough, visibly altered, friction upon rubbing; and (1) rough, flaky, uneven surface. Evaluations were made independently by two clinicians and averaged.

**[0115]** Skin Smoothness and Wrinkle Reduction Assay With Methods Disclosed in Packman et al. (1978): Skin smoothness and wrinkle reduction can also be assessed visually by using the methods disclosed in Packman et al. (1978). For example, at each subject visit, the depth, shallowness and the total number of superficial facial lines (SFLs) of each subject can be carefully scored and recorded. A numerical score was obtained by multiplying a number factor times a depth/width/length factor. Scores are obtained for the eye area and mouth area (left and right sides) and added together as the total wrinkle score.

**[0116]** Skin Firmness Assay with a Hargens Ballistometer: Skin firmness can be measured using a Hargens ballistometer, a device that evaluates the elasticity and firmness of the skin by dropping a small body onto the skin and recording its first two rebound peaks. The ballistometry is a small lightweight probe with a relatively blunt tip (4 square mm-contact area) was used. The probe penetrates slightly into the skin and results in measurements that are dependent upon the properties of the outer layers of the skin, including the stratum corneum and outer epidermis and some of the dermal layers.

**[0117]** Skin Softness/Suppleness Assay with a Gas Bearing Electrodynamometer: Skin softness/suppleness can be evaluated using the Gas Bearing Electrodynamometer, an instrument that measures the stress/strain properties of the skin. The viscoelastic properties of skin correlate with skin moisturization. Measurements can be obtained on the predetermined site on the cheek area by attaching the probe to the skin surface with double-stick tape. A force of approximately 3.5 gm can be applied parallel to the skin surface and the skin displacement is accurately measured. Skin suppleness can then be calculated and is expressed as DSR (Dynamic Spring Rate in gm/mm).

**[0118]** Appearance of Lines and Wrinkles Assay with Replicas: The appearance of lines and wrinkles on the skin can be evaluated using replicas, which is the impression of the skin's surface. Silicone rubber like material can be used. The replica can be analyzed by image analysis. Changes in the visibility of lines and wrinkles can be objectively quantified via the taking of silicon replicas from the subjects' face and analyzing the replicas image using a computer image analysis system. Replicas can be taken from the eye area and the neck area, and photographed with a digital camera using a low angle incidence lighting. The digital images can be

analyzed with an image processing program and are of the replicas covered by wrinkles or fine lines was determined.

**[0119]** MELANODERM™ Assay: In other non-limiting aspects, the efficacy of each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be evaluated by using a skin analog, such as, for example, MELANODERM™. Melanocytes, one of the cells in the skin analog, stain positively when exposed to L-dihydroxyphenyl alanine (L-DOPA), a precursor of melanin. The skin analog, MELANODERM™, can be treated with a variety of bases containing each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification or with the base alone as a control. Alternatively, an untreated sample of the skin analog can be used as a control.

**[0120]** Production of Filaggrin: Changes in the production of filaggrin in keratinocytes due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. Filaggrin is the precursor to Natural Moisturizing Factor (NMF) in the skin. Increased NMF increases the moisture content of the skin. Filaggrin production in treated and non-treated keratinocytes can be determined using a bioassay that analyzes filaggrin concentration in keratinocyte cell lysates. A non-limiting example of a bioassay that can be used to quantify filaggrin production is the PROTEINSIMPLE® Simon™ western blotting protocol. For each sample, normal human epidermal keratinocytes (NHEK) are grown in EPI-200—Mattek Epilife® growth media with calcium from Life Technologies (M-EP-500-CA). NHEK are incubated in growth medium overnight at 37° C. in 5% CO<sub>2</sub> prior to treatment. NHEK are then incubated in growth medium with 1% test compound/extract or no compound/extract (negative control) for 24 to 36 hours. The NHEK can then be washed, collected, and stored on ice or colder until lysed on ice using a lysis buffer and sonication. The protein concentrations of the samples can be determined and used to normalize the samples. The lysates can be stored at -80° C. until use in the quantification assay.

**[0121]** The PROTEINSIMPLE® Simon™ western blotting bioassay assay employs a quantitative western blotting immunoassay technique using an antibody specific for filaggrin to quantitatively detect filaggrin in the test samples. Cell samples are lysed and normalized for protein concentration. Normalized samples and molecular weight standards can then be loaded and ran on a denatured protein separation gel using capillary electrophoresis. The proteins in the gel are immobilized and immunoprobed using a primary antibody specific for filaggrin. The immobilized proteins can then be immunoprobed with an enzyme-linked detection antibody that binds the primary antibody. A chemiluminescent substrate solution can then be added to the immobilized proteins to allow chemiluminescent development in proportion to the amount of filaggrin bound in the immobilization. The chemiluminescent development is stopped at a specific time and the intensity of the chemiluminescent signal can be measured and compared to positive and negative controls.

**[0122]** Production of Occludin: Changes in the production of occludin in keratinocytes due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. Occludin is a protein critical to the formulation of tight junctions and the skin's moisture

barrier function. A non-limiting example of how occludin production in treated and non-treated keratinocytes can be determined is by the use of a bioassay that analyzes occludin concentration in keratinocyte cell lysates. The bioassay can be performed using PROTEINSIMPLE® Simon™ western blotting protocol. For the samples, adult human epidermal keratinocytes (HEKa) from Life Technologies (C-005-5C) can be grown at 37° C. and 5% CO<sub>2</sub> for 24 hours in Epilife growth media with calcium from Life Technologies (M-EP-500-CA) supplemented with Keratinocyte Growth Supplement (HKGS) from Life Technologies (S-101-5). HEKa are then incubated in growth medium with test compound/extract, no compound/extract for negative control, or with 1 mM CaCl<sub>2</sub> for positive control for 24 to 48 hours. The HEKa are then washed, collected, and stored on ice or colder until lysed on ice using a lysis buffer and sonication. The protein concentrations of the samples can be determined and used to normalize the samples. The lysates are stored at -80° C. until use in the bioassay.

**[0123]** The PROTEINSIMPLE® Simon™ western blotting bioassay assay employs a quantitative western blotting immunoassay technique using an antibody specific for occludin to quantitatively detect occludin in the test samples. Cell samples are lysed and normalized for protein concentration. Normalized samples and molecular weight standards are then loaded and ran on a denatured protein separation gel using capillary electrophoresis. The proteins in the gel are then immobilized and immunoprobed using a primary antibody specific for occludin. The immobilized proteins are immunoprobed with an enzyme-linked detection antibody that binds the primary antibody. A chemiluminescent substrate solution is then added to the immobilized proteins to allow chemiluminescent development in proportion to the amount of occludin bound in the immobilization. The chemiluminescent development can be stopped at a specific time and the intensity of the chemiluminescent signal can be measured and compared to positive and negative controls.

**[0124]** Keratinocyte Monolayer Permeability: Changes in the permeability of a keratinocyte monolayer due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. Keratinocyte monolayer permeability is a measure of skin barrier integrity. Keratinocyte monolayer permeability in treated and non-treated keratinocytes can be determined using, as a non-limiting example, the In Vitro Vascular Permeability assay by Millipore (ECM642). This assay analyzes endothelial cell adsorption, transport, and permeability. Briefly, adult human epidermal keratinocytes from Life Technologies (C-005-5C) can be seeded onto a porous collagen-coated membrane within a collection well. The keratinocytes are then incubated for 24 hours at 37° C. and 5% CO<sub>2</sub> in Epilife growth media with calcium from Life Technologies (M-EP-500-CA) supplemented with Keratinocyte Growth Supplement (HKGS) from Life Technologies (S-101-5). This incubation time allows the cells to form a monolayer and occlude the membrane pores. The media is then replaced with fresh media with (test sample) or without (non-treated control) test compounds/extracts and the keratinocytes are incubated for an additional 48 hours at 37° C. and 5% CO<sub>2</sub>. To determine permeability of the keratinocyte monolayer after incubation with/without the test compound/extract, the media is replaced with fresh media containing a high

molecular weight Fluorescein isothiocyanate (FITC)-Dextran and the keratinocytes are incubated for 4 hours at 37° C. and 5% CO<sub>2</sub>. During the 4 hours incubation, FITC can pass through the keratinocytes monolayer and porous membrane into the collection well at a rate proportional to the monolayer's permeability. After the 4 hour incubation, cell viability and the content of FITC in the collection wells can be determined. For the FITC content, the media in the collection well is collected and fluorescence of the media determined at 480 nm (Em) when excited at 520 nm. Percent permeability and percent change in comparison to the non-treated controls can be determined by the following equations: Percent Permeability= $(\text{Mean Ex/Em of test sample} / \text{Mean Ex/Em untreated control}) * 100$ ; Percent Change= $\text{Percent Permeability of test sample} - \text{Percent Permeability of untreated control}$ .

**[0125]** Production of Hyaluronic Acid: Changes in the production of hyaluronic acid in human dermal fibroblasts due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. HA is a polysaccharide involved in stabilization of the structure of the matrix and is involved in providing turgor pressure to tissue and cells. As one non-limiting example, HA production in treated and non-treated adult human dermal fibroblasts (HDFa) cells can be determined using the Hyaluronan DuoSet ELISA kit from R&D Systems (DY3614). In this assay, for production of samples, subconfluent HDFa cells from Cascade Biologics (C-13-5C) are incubated at 37° C. and 10% CO<sub>2</sub> in starvation medium (0.15% fetal bovine serum and 1% Penicillin Streptomycin solution in Dulbecco's Modified Eagle Medium) for 72 hours prior to treatment. The cells are then incubated with fresh starvation medium with either test compound, positive control (phorbol 12-myristate 13-acetate from Sigma-Aldrich (P1585) and platelet derived growth factor from Sigma-Aldrich (P3201)), or no additive for 24 hours. Media is then collected and frozen at -80° C. until use in the ELISA assay.

**[0126]** Briefly, the ELISA assay employs a quantitative sandwich enzyme immunoassay technique whereby a capture antibody specific for HA can be pre-coated onto a microplate. Standards and media from treated and untreated cells are pipetted into the microplate wells to enable any HA present to be bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked detection antibody specific for HA is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells to allow color development in proportion to the amount of HA bound in the initial step. The color development is stopped at a specific time and the intensity of the color at 450 nm can be measured using a microplate reader.

**[0127]** Inhibition of Hyaluronidase Activity: Changes in the activity of hyaluronidase due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. Hyaluronidase is an enzyme that degrades HA. HA is a polysaccharide involved in stabilization of the structure of the matrix and is involved in providing turgor pressure to tissue and cells. As one non-limiting example, hyaluronidase activity can be determined using an in vitro protocol modified from Sigma-Aldrich protocol #EC 3.2.1.35. Briefly, hyaluronidase type 1-S from

Sigma-Aldrich (H3506) is added to microplate reaction wells containing test compound or controls. Tannic acid can be used as a positive control inhibitor, no test compound can be added for the control enzyme, and wells with test compound or positive control but without hyaluronidase can be used as a background negative control. The wells are incubated at 37° C. for 10 minutes before addition of substrate (HA). Substrate is added and the reactions incubated at 37° C. for 45 minutes. A portion of each reaction solution is then transferred to and gently mixed in a solution of sodium acetate and acetic acid pH 3.75 to stop that portion of the reaction (stopped wells). The stopped wells and the reaction wells should both contain the same volume of solution after addition of the portion of the reaction solution to the stopped wells. Both the reaction wells and the stopped wells are incubated for 10 minutes at room temperature. Absorbance at 600 nm is then measured for both the reaction wells and the stopped wells. Inhibition can be calculated using the following formulas: Inhibitor (or control) activity= $(\text{Inhibitor stopped wells absorbance at 600 nm} - \text{inhibitor reaction wells absorbance at 600 nm}) / \text{Initial activity} = \text{control enzyme absorbance at 600 nm}$ ; Percent Inhibition= $[(\text{Initial activity} / \text{Inhibitor Activity}) * 100] - 100$ .

**[0128]** Peroxisome Proliferator-Activated Receptor Gamma (PPAR- $\gamma$ ) Activity: Changes in the activity of PPAR- $\gamma$  due to each of the active ingredients, any one of the combination of ingredients, or compositions having said combinations disclosed in the specification can be measured. PPAR- $\gamma$  is a receptor critical for the production of sebum. As one non-limiting example, the activity of PPAR- $\gamma$  can be determined using a bioassay that analyzes the ability of a test compound or composition to inhibit binding of a ligand. Briefly, fluorescent small-molecule pan-PPAR ligand, FLUORMONE™ Pan-PPAR Green, available from Life Technologies (PV4894), can be used to determine if test compounds or compositions are able to inhibit binding of the ligand to PPAR- $\gamma$ . The samples wells include PPAR- $\gamma$  and fluorescent ligand and either: test compound or composition (test); a reference inhibitor, rosiglitazone (positive control); or no test compound (negative control). The wells are incubated for a set period of time to allow the ligand opportunity to bind the PPAR- $\gamma$ . The fluorescence polarization of each sample well can then be measured and compared to the negative control well to determine the percentage of inhibition by the test compound or composition.

**[0129]** Cytokine array: Human epidermal keratinocytes are cultured to 70-80% confluency. The media in the plate is aspirated and 0.025% trypsin/EDTA is added. When the cells became rounded, the culture dish is gently tapped to release the cells. The trypsin/EDTA containing cells are removed from the culture dish and neutralized. Cells are centrifuged for 5 min. at 180xg to form a pellet of cells. The supernatant is aspirated. The resulting pellet is resuspended in EpiLife media (Cascade Biologics). The cells are seeded in 6-well plates at approximately 10-20% confluency. After the cells became approximately 80% confluent, the media is aspirated and 1.0 ml of EpiLife™, along with phorbol 13-Myristate 12-acetate ("PMA") (a known inducer of inflammation) and the test composition dilutions are added to two replicate wells (i.e., 1.0% (100  $\mu$ l of 100X stock) and 0.1% (10  $\mu$ l of 100X stock) test compositions are diluted into a final volume of 1 ml EpiLife Growth Medium). The media is gently swirled to ensure adequate mixing. In addition, 1.0 ml of EpiLife™ is added to the control wells, with and

without additional PMA. The plates are then incubated at  $37\pm 1^\circ\text{C}$ . and  $5.0\pm 1\%$   $\text{CO}_2$  for approximately 5 hours after dosing. Following this 5-hour incubation, all media is collected in conical tubes and frozen at  $-70^\circ\text{C}$ .

**[0130]** For analysis, a 16-pad hybridization chamber is attached to 16-pad FAST slides arrayed in triplicate with 16 anti-cytokine antibodies plus experimental controls (Whatman BioSciences), and the slides are placed into a FASTFrame (4 slides per frame) for processing. Arrays are blocked for 15 min. at room temp. using 70 ml S&S Protein Array Blocking buffer (Whatman Schleicher and Scheull). Blocking buffer is removed and 70 ml of each supernatant sample is added to each array. Arrays are incubated for 3 hours at room temp. with gentle agitation. Arrays are washed 3 times with TBS-T. Arrays are treated with 70 ml of an antibody cocktail, containing one biotinylated antibody corresponding to each of the arrayed capture antibodies. Arrays are incubated for 1 hour at room temp. with gentle agitation. Arrays are washed 3 times with TBS-T. Arrays are incubated with 70 ml of a solution containing streptavidin-Cy5 conjugate for 1 hour at room temp. with gentle agitation. Arrays are washed 3 times with TBS-T, quickly rinsed in de-ionized water, and dried.

**[0131]** Slides can be imaged in a Perkin-Elmer ScanArray 4000 confocal fluorescent imaging system. Array images can be saved and analyzed using Imaging Research Array-Vision software. Briefly, spot intensities are determined by subtracting background signal. Spot replicates from each sample condition can be averaged and then compared to the appropriate controls.

**[0132]** Endothelial Tube Formation: Endothelial tube formation is involved in angiogenesis and micro-vessel capillary formation. Capillary formation and angiogenesis may contribute to redness and rosacea of the skin. The ability for endothelial cells to form tubes in the presence or absence of test extracts and compounds may be determined using a capillary tubule disruption assay with pre-formed primary human umbilical vein endothelial cells (HUVEC) in a cell culture system.

**[0133]** Briefly, HUVECs are cultured in vitro on Extracellular Matrix, which stimulates the attachment and tubular morphogenesis of endothelial cells to form capillary-like lumen structures. These in vitro formed capillary tubules are similar to human blood vessel capillaries in many aspects. The capillary tube assay is based on this phenomenon and is used for evaluation of potential vasculature targeting agents.

**[0134]** HUVEC cultures are grown in a  $5\%$   $\text{CO}_2$   $37^\circ\text{C}$ . cell incubator. The full growth medium for HUVECs is Endothelial Cell Basal Medium (EBM) supplemented with  $2\%$  fetal bovine serum (FBS),  $12\ \mu\text{g/ml}$  bovine brain extract,  $1\ \mu\text{g/ml}$  hydrocortisone, and  $1\ \mu\text{g/ml}$  GA-1000 (gentamicin-amphotericin). HUVEC cultures between passage 3 and 8 may be used for all assay experiments.

**[0135]** HUVECs are pre-labeled with fluorescent agent Calcein AM and seeded in Extracellular Matrix coated 96-well culture plate with their full growth medium. After about four hours of the morphogenesis process, the endothelial capillary tubes should be formed. Then, test agent in designed doses in  $50\ \mu\text{l}$  volume is applied into the formed capillary tube cultures as treatment conditions. The no-treatment controls can be added with vehicle of test agents. Sutent, a FDA approved anti-angiogenic drug one concentration can be included as assay performance control. After about six hours of treatment, the endothelial tubule mor-

phology in each well is examined by microscopy, imaged, and the capillary disrupting activities under treatment conditions can be quantitatively analyzed. Each test conditions can be conducted in duplicate wells, including controls.

**[0136]** All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### References

**[0137]** The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

**[0138]** 1. Cosmetic Ingredient Dictionary, Third Edition, CTFA, 1982

**[0139]** 2. International Cosmetic Ingredient Dictionary, Fourth edition, CTFA, 1991

**[0140]** 3. International Cosmetic Ingredient Dictionary and Handbook, Tenth Edition, CTFA, 2004

**[0141]** 4. International Cosmetic Ingredient Dictionary and Handbook, Twelfth Edition, CTFA, 2008

1. A method of reducing the appearance of loose, sagging, or flaccid skin, the method comprising topically applying to skin in need thereof a composition comprising  $0.0001\ \text{wt.}\%$  to  $10\ \text{wt.}\%$  *Argania spinosa* kernel extract,  $0.0001\ \text{wt.}\%$  to  $10\ \text{wt.}\%$  dill extract,  $0.0001\ \text{wt.}\%$  to  $10\ \text{wt.}\%$  *Myrciaria dubia* fruit extract, and  $0.0001\ \text{wt.}\%$  to  $10\ \text{wt.}\%$  *Croton lechleri* extract or *Morus alba* fruit extract, wherein topical application of the composition increases elastin expression, increases elastin secretion, or increases elastin fiber formation in the skin in need thereof, and wherein the appearance of loose, sagging, or flaccid skin is reduced.

2. The method of claim 1, wherein topical application of the composition increases skin elasticity in the skin in need thereof.

3. The method of claim 1, wherein topical application of the composition increases elastin expression in the skin in need thereof.

4. The method of claim 1, wherein topical application of the composition increases elastin expression by at least  $75\%$  in the skin in need thereof as compared to skin which has not been treated with the composition.

5. The method of claim 1, wherein topical application of the composition increases collagen expression in the skin in need thereof.

6. The method of claim 1, wherein topical application of the composition increases laminin or fibronectin production in the skin in need thereof.

7. The method of claim 1, wherein topical application of the composition increases fibulin-5 production in the skin in need thereof.

8. The method of claim 1, wherein the skin in need thereof is skin of a jawl.

9. The method of claim 1, wherein the skin in need thereof is neck skin.

10. The method of claim 1, wherein the skin in need thereof is facial skin.

11. The method of claim 1, wherein the composition is an emulsion.

12. The method of claim 11, wherein the emulsion is an oil-in-water emulsion.

13. The method of claim 1, wherein the composition is a gel.

14. The method of claim 1, wherein the composition is an aqueous solution.

15. The method of claim 1, wherein the composition is a hydro-alcoholic solution.

16. The method of claim 1, wherein each extract is an aqueous extract, an alcoholic extract, or an aqueous-alcoholic extract.

17. The method of claim 1, wherein the composition is applied daily.

18. The method of claim 1, wherein the composition comprises 0.0001 wt. % to 10 wt. % *Croton lechleri* extract.

19. The method of claim 1, wherein the composition comprises 0.0001 wt. % to 10 wt. % *Morus alba* fruit extract.

20. The method of claim 1, wherein the composition comprises 0.1 wt. % to 5 wt. % *Argania spinosa* kernel extract, 0.1 wt. % to 5 wt. % dill extract, 0.1 wt. % to 5 wt. % *Myrciaria dubia* fruit extract, and 0.1 wt. % to 5 wt. % *Croton lechleri* extract or *Morus alba* fruit extract.

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