Abstract:

A dermatocosmetic composition having a dermatologically-acceptable carrier, a therapeutically-effective amount of a proteinaceous extract of M. miehei disposed in the dermatologically-acceptable carrier and a therapeutically-effective amount of a polysaccharides extract derived from a M. rouxii or M. miehei disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition.
COMPOSITIONS CONTAINING EXTRACTS OF MUCOR MIEHEI AND MUCOR ROUXII

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] None.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates in general to the field of topical compositions containing extracts of Mucor miehei (M. Miehei) and Mucor rouxii (M. Rouxii) for use in treating dermatologic conditions, specifically for promoting non-irritating exfoliation and reducing the appearance of signs of skin aging.

STATEMENT OF FEDERALLY FUNDED RESEARCH

[0003] None.

INCORPORATION-BY-REFERENCE OF MATERIALS FILED ON COMPACT DISC

[0004] None.

BACKGROUND OF THE INVENTION

[0005] Without limiting the scope of the invention, its background is described in connection with treating dermatologic conditions with a composition containing extracts of M. Miehei and M. rouxii or M. miehei for promoting non-irritating exfoliation and reducing the appearance of signs of skin aging.

[0006] For example, U.S. Patent No. 6,656,701 discloses compositions having one or more of an acid protease and an acidic buffer, the acidic buffer comprising an acid and a pharmaceutically or cosmetically acceptable carrier, vehicle or excipient, useful for treating or preventing abnormal biological conditions, diseases or disorders, and/or for improving the texture or appearance of the skin, and/or for enhancing epidermal exfoliation and/or for enhancing epidermal cell renewal and to methods for the use of the compositions. The acid protease comprises one or more proteolytic enzymes which exhibit proteolytic activity at pH values below that of the surface of the skin, i.e.,
approximately pH 5.5. The acidic buffer comprises at least one acidic buffering component that can reversibly disassociate hydrogen ions and has buffering capacity at pH values below that of the surface of the skin, i.e., approximately pH 5.5 or mixtures thereof with a pharmaceutically or cosmetically acceptable carrier, vehicle or excipient. The buffer is capable of reducing the pH of the surface of the skin to less than pH 5.5 and is susceptible to neutralization by normal epidermal processes. Such types of abnormal biological conditions, diseases or disorders include skin atrophy, i.e., the thinning and/or general degradation of the dermis often characterized by a decrease in collagen and/or elastin as well as decreased number, size and doubling potential of fibroblast cells, and other maladies including, but are not limited to, dry skin, severe dry skin, dandruff, acne, keratoses, psoriasis, eczema, skin flakiness, pruritus, age spots, lentigines, melasmas, wrinkles, warts, blemished skin, hyperpigmented skin, hyperkeratotic skin, inflammatory dermatoses, age-related skin changes and skin in need of cleansers.

[0007] For example, U.S. Patent No. 6,846,812 discloses 7-Oxo-DHEA derivatives, variations of which are themselves novel compounds for cosmetically/therapeutically treating adverse conditions/afflictions of a keratinous substrate/material, notably of human skin, hair, eyelashes and nails, to improve the appearance thereof, in particular to prevent or treat signs of aging of the skin and/or a dull complexion and/or skin or hair pigmentation disorders and/or dryness of the skin and/or hyperseborrhoea and/or hyperseborrhoea-related imperfections and/or sensitive skin and/or dandruff and/or natural hair loss and/or baldness.

[0008] For example, U.S. Patent No. 6,569,437 discloses novel compositions having one or more of an acid protease and an acidic buffer, the acidic buffer comprising an acid and a pharmaceutically or cosmetically 5 acceptable carrier, vehicle or excipient, useful for treating or preventing abnormal biological conditions, diseases or disorders, and/or for improving the texture or appearance of the skin, and/or for enhancing epidermal exfoliation and/or for enhancing epidermal cell renewal and to methods for the use of the compositions. The acid protease comprises one or more proteolytic enzymes that exhibit proteolytic activity at pH values below that of the surface of the skin, i.e., approximately pH 5.5. The acidic buffer comprises inorganic and/or organic acids or mixtures thereof with a pharmaceutically or cosmetically acceptable carrier, vehicle or excipient. The
buffer is capable of reducing the pH of the surface of the skin to less than pH 5.5 and is susceptible to neutralization by normal epidermal processes.

SUMMARY OF THE INVENTION

The present invention provides a dermatocosmetic composition for reducing the signs of aging having a dermatologically-acceptable carrier; a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable carrier; and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition. In one embodiment, the isolated and purified proteinaceous extract is a protease from *M. miehei* and the isolated and purified amino sugar extract is derived from a cell wall from *M. rouxii* or *M. miehei*.

The present invention provides a dermatocosmetic composition having a therapeutically-effective amount of a protease and a therapeutically-effective amount of an amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition. For example, the dermatocosmetic composition may be a therapeutically-effective amount of a protease extract of a *M. miehei* and a therapeutically-effective amount of an amino sugar extract derived from a *M. rouxii* or *M. miehei* disposed in the dermatologically-acceptable carrier for exfoliation. The dermatocosmetic composition may further include one or more additives for even greater effects through the addition of retinoids, peptides, and other exfoliants. The polysaccharides extract may be derived from a cell wall components of a *Mucor rouxii* or *Mucor miehei*. Although *Mucor* is provided as an example others may be used including fungi, yeast, and chitin.

The present invention provides a method for increasing skin moisture content by administering to a person in need of a composition of a therapeutically-effective amount of a protease extract of a *M. miehei* and a therapeutically-effective amount of an amino sugar extract derived from a *M. rouxii* or *M. miehei* disposed in the dermatologically-acceptable carrier.

The present invention provides a method for exfoliating a skin surface by administering to a person in need thereof an exfoliating composition comprising a dermatologically-acceptable exfoliating carrier, a therapeutically-effective amount of an
isolated and purified proteinaceous extract disposed in the dermatologically-acceptable exfoliating carrier and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable exfoliating carrier to form an exfoliating composition.

[0013] The present invention provides a dermatocosmetic composition for reducing the signs of aging having a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in a dermatologically-acceptable carrier to form a dermatocosmetic composition.

[0014] The present invention provides a composition for increasing the protease activity having a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in a dermatologically-acceptable carrier to form a dermatocosmetic composition.

[0015] The present invention provides a method for increasing skin firmness comprising administering to a person in need of a composition of a therapeutically-effective amount of a protease extract of a M. miehei and a therapeutically-effective amount of a amino sugar extract derived from a M. rouxii or M. miehei disposed in the dermatologically-acceptable carrier.

[0016] The present invention provides a composition for increasing the protease activity having a dermatologically-acceptable carrier; a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable carrier; and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition.

[0017] The present invention provides a method for increasing the protease activity on the skin by administering to a skin surface an activating composition having a dermatologically-acceptable exfoliating carrier, a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier, wherein the activating composition increases the protease activity on the surface of the skin.

[0018] The present invention provides an exfoliating composition having a dermatologically-acceptable exfoliating carrier; a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable ...
exfoliating carrier; and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable exfoliating carrier to form an exfoliating composition.

DETAILED DESCRIPTION OF THE INVENTION

[0019] While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

[0020] To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

[0021] The present invention provides a topical composition having a peptide extract and a polysaccharide extract. The peptide extract may be an enzymatic composition, while the polysaccharides extract is an amino sugar.

[0022] The present invention relates to a method of exfoliating and moisturizing the skin by topically applying a cosmetic or pharmaceutical composition having a peptide extract and an amino sugar. The present invention provides the use of any protein, peptide or enzyme and any amino sugar. In one embodiment, the protein, peptide or enzyme is from an animal, plant, bacteria or fungi and the amino sugar is from a similar or different animal, plant, bacteria or fungi. For example, the proteinaceous extract may be from bacteria and the amino sugar may be chemically synthesized; the proteinaceous extract may be from fungi and the amino sugar may be from a plant; or the proteinaceous extract may be from a fungi and the amino sugar may be from a fungi. Another embodiment includes an amino sugar composition that interacts or activates endogenous proteins to have a synergistic effect.
In other embodiments, the dermatocosmetic composition includes a carrier; a protein disposed in the carrier; and an amino sugar disposed in the carrier and there is no need for isolation and purification.

In general, an amino sugar contains an amine group in place of a hydroxyl group. Derivatives of amine containing sugars, such as glucosamine, N-acetylglicosamine and sialic acid, while not formally containing an amine, are also considered amino sugars. The amino sugar may be in the form of a mono-, di-, or polysaccharide with the amino substituted at any position and in any number. For example, a non limiting list of amino sugars includes galactosamine, mannosamine, glucosamine, allosamine, altrosamine, ribosamine, arabinosamine, gulosamine, idosamine, fucosamine, talosamine, xylosamine, lyxosamine, sorbosamine, tagatosamine, psicosamine, fructosamine, D-mannosamine, D-glucosamine, D-allosamine, D-altrosamine, D-ribosamine, D-arabinosamine, L-gulosamine, L-idosamine, L-galactosamine, L-fucosamine, L-talosamine, L-xylosamine and L-lyxosamine. Amino aldoses, such as mannosamine, glucosamine, allosamine, altrosamine, ribosamine, arabinosamine, gulosamine, idosamine, galactosamine, talosamine, xylosamine, lyxosamine. In particular D-mannosamine, D-glucosamine, D-allosamine, D-altrosamine, D-ribosamine, D-arabinosamine, L-gulosamine, L-idosamine, L-galactosamine, L-talosamine, L-xylosamine, L-lyxosamine. Amino ketoses, such as sorbosamine, tagatosamine, psicosamine, fructosamine. In particular D-sorbosamine, D-tagatosamine, L-psicosamine, L-fructosamine. Amino deoxyaldoses, such as derivatives of fucose, for example fucosamine and fucosylamine, preferably L-fucosamine. Examples of amino sugars include mannosamine, glucosamine, galactosamine, fructosamine. In particular D-mannosamine, D-glucosamine, L-galactosamine, L-fucosamine. Examples of disaccharides, oligosaccharides, and polysaccharides having an amino group in one part of the molecule may be used. For example the amino sugar may be any disaccharide derivative having an amino group, wherein at least one of the monosaccharide subunits of the disaccharide is selected from the group consisting of mannose and glucose. Examples of disaccharides include saccharose derivatives, such as saccharosamine, and maltose derivatives, such as maltosamine, comprising an amino group, as well as agarosamine, cellullosamine, and amylosamine. Another disaccharide derivative is saccharosamine. Furthermore the amino sugar may for example be a
multimer of glucosamine, such as a glucosamine dimer, a glucosamine oligomer or a glucosamine polymer. See also, U.S. Patent No. 7,049,414 disclosing the isolation of lectins and is incorporated herein by reference in its entirety. U.S. Patent No. 6,413,525 disclosing methods of exfoliation using n-acetyl glucosamine and Compositions for topical application containing N-acetyl-D-glucosamine have been disclosed for example, in JP 59013708, WO 98/152576, and U.S. Patent No. 5,866,142, each incorporated herein by reference. To soften and moisturize the skin, a cosmetic containing an N-acetyl amino sugar is disclosed in JP 59013708. A composition for alleviating itching and pain containing N-acetyl-D-glucosamine is disclosed in WO 98/52576. In U.S. Pat. No. 5,866,142, a composition for exfoliating the skin has been disclosed, which includes N-acetyl-D-glucosamine. U.S. Patent Nos. 5,976,556; 6,656,701; 7,524,504; and 7,776,504 are incorporated herein by reference in their entirety.

[0025] The topical composition of the present invention includes a combination of an extract of M. miehei and an extract of M. rouxii or M. miehei in a carrier. Specifically, a composition having a therapeutically-effective amount of a protease and a therapeutically-effective amount of an amino sugar extract disposed in the dermatologically-acceptable carrier. More specifically, the present invention includes a combination of an extract of peptides derived from M. miehei and an extract of polysaccharides derived from cell wall components from M. rouxii or M. miehei in a dermatologically-acceptable carrier. In some embodiments the amino sugar are used without the peptide to provide a composition having an amino sugar and is substantially devoid of acid protease activity.

[0026] As used herein the term "amino sugar" means that a nitrogen is substituted as a functional group on at least one sugar.

[0027] As used herein the term "exfoliation" denotes is a technique whereby dead skin cells are removed or sloughed from the skin surface to promote a healthier and more youthful appearance to the skin.

[0028] As used herein the term One HUT unit of proteolytic activity is defined as that amount of enzyme that produces, in one minute under the specified conditions of the assay, a hydrolysate whose absorbance at 280 nm is the same as that of a solution containing 1.10 µg per ml of tyrosine in 0.006 N hydrochloric acid. HUT units per gram are determined by the following formula: HUT/g = (absorbance at 280 nm x V)/(0.0084...
x T x W), where V is the final volume of the test solution, T is the reaction time in minutes, and W is the dry weight of the original enzyme sample used in the assay. Protein concentration is determined by a method known in the art, such as, for example, the Bradford Assay which is described in Ausubel et al, (Eds.), Current Protocols in Molecular Biology (John Wiley & Sons, Inc., New York, 1994).

[0029] As used herein the term "dermatologically-acceptable carrier" denotes one that is suitable for topical application to the keratinous tissue and is compatible with the dermatocosmetic active ingredients described below. The carrier can be in a wide variety of forms, including, but not limited to, oil-in-water emulsions, water-in-oil emulsions, water-in-silicone emulsions, silicone-in-water emulsions, water-in-oil-in-water, and oil-in-water-in-oil emulsions, and oil-in-water-in-silicone emulsions. Suitable surfactants include anionic, cationic, amphoteric, zwitterionic and non-ionic, including those listed in U.S. Patent No. 6,197,319. The International Cosmetic Ingredient Dictionary and Handbook (10th Edition, 2004), published by the Cosmetic, Toiletries & Fragrance Association, describes a wide variety of non-limiting cosmetic and dermatopharmaceutical ingredients commonly used in the skin care industry, which are suitable for use in combination with the M. miehei and M. rouxii extracts of the present invention. Examples of these ingredients include: antioxidants, anti-inflammatory agents, anti-acne agents, antimicrobial agents, astringents, humectants, moisturizers, pH adjusters, skin bleaching/lightening agents, skin soothing/healing agents and agents that help decrease the appearance of signs of aging.

[0030] As used herein the term "anti-acne ingredients" suitable for use in compositions of the present invention include: resorcinol, sulfur, salicylic acid, benzoyl peroxide, erythromycin, and zinc. Further examples of suitable anti-acne actives are described in U.S. Patent No. 5,607,980.

[0031] As used herein the term "skin bleaching" and "lightening agents" which may be topically delivered in the present invention include: hydroquinone, kojic acid, glabradin, ascorbic acid, magnesium ascorbyl phosphate and ascorbyl glucosamine.

[0032] As used herein the term "antioxidants"/"radical"/"scavengers" suitable for use in compositions of the present invention include: ascorbic acid (vitamin C) and its salts, ascorbyl esters of fatty acids, ascorbic acid derivatives (e.g., magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl sorbate); tocopherol (vitamin E) and its
esters, including tocopherol sorbate, tocopherol acetate; butylated hydroxybenzoic acids and their salts; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; gallic acid and its alkyl esters, especially propyl gallate; uric acid and its salts and alkyl esters; sorbic acid and its salts; lipoic acid; amines (e.g., N,N-diethylhydroxylamine, amino-guanidine); sulfhydryl compounds (e.g., glutathione); coenzyme Q10 and its analogues, including without limitation, idebenone; dihydroxyfumaric acid and its salts; lycine pilolate; arginine pilolate; nordihydroguaiaretic acid; bioflavonoids; curcumin; lysine; 1-methionine; proline; superoxide dismutase; silymarin; tea extracts; Vitis vinifera (grape) skin/seed extracts; melanin; and Rosmarinus officinalis (rosemary) extracts.

[0033] As used herein the term "skin soothing" and/or "healing agents" suitable for use in the present invention include: allantoin, aloe vera and its derivatives, betulinic acid, bisabolol, dipotassium glycyrrhizinate, oleonolic acid, panthenol and derivatives, pantothentic acid and its derivatives, and ursolic acid.

[0034] As used herein the term agents that help decrease the appearance of signs of aging, include mucopolysaccharides (including hyaluronic acid), aldosamines (including n-acetyl glucosamines) and biologically-active short-chain peptides (e.g., tri-, terra-, penta-, and hexapeptides, and mixtures thereof).

[0035] In some embodiment of the present invention, one or more of the cosmetic ingredients are botanically-derived (e.g., extracts). Preferred botanically-derived antibacterial agents include, but are not limited to, extract of Laurus nobilis (bay laurel), extract of Larrea divaricata (chapparal), extract of Rosa canina (rose hips) and extract of Scutellaria galericulata (skullcap). Preferred botanically-derived anti-fungal agents include, but are not limited to, extract of Laurus nobilis (bay laurel), extract of Commiphora myrrha (myrrh) and extract of Melaleuca alternifolia (tea tree oil). Preferred botanically-derived anti-inflammatory agents include, but are not limited to, extract of Iris versicolor (blue flag), extract of Calendula officinalis (calendula), extract of Chamomilla recutita (chamomile), extract of Tussilago farfara (coltsfoot), extract of Symphytum officinale (comfrey) leaves, extract of Tanacetum parthenium (feverfew), extract of Panax ginseng (ginseng), extract of Gynostemma pentaphyllum (southern ginseng), extract of Aesculus hippocastanum (horse chestnut), extract of Camellia oleifera (Japanese green tea), extract of Tilia cordata (linden tree), extract of Althea officinalis (marsh mallow), extract of Viola tricolor (pansy), extract of Mentha pulegium
(pennyroyal), extract of Vinca minor (periwinkle), extract of Chaenomeles japonica (quince) seed, extract of Anthemis nobilis (roman chamomile), extract of Valeriana officinalis (valerian) and extract of Viola odorata (violet). Preferred botanically-derived agents that help decrease the appearance of signs of aging include those that stimulate production of collagen. Non-limiting examples of these ingredients include asiatic acid, madecassic acid and asiaticoside. Botanically-derived antioxidants include, but are not limited to, extracts of Camellia oleifera (Japanese green tea), extracts of Vitis vinifera (grape) seed, extracts of Punica granatum (pomegranate), extracts of Citrus grandis (grapefruit), bioflavonoids, extracts of Panax ginseng (ginseng), extracts of Gynostemma pentaphyllum (southern ginseng), resveratrol, anthocyanidines, monoterpenoids, diterpenoids and triterpenoids. Preferred botanically-derived astringent agents include, but are not limited to, extract of Citrus medica limonum (lemon), extract of Citrus aurantifolia (lime), extract of Artium lappa (burdock), extract of Nasturtium officinale (watercress), extract of Hedera helix (ivy), extract of Hamamelis virginiana (witch hazel), extract of Myrica cerifera (bayberry) rootbark, extract of Quercus alba (oak gall), extract of Echinacea purpurea (coneflower), extract of Echinacea augustifolia (native coneflower), extract of Eugenia caryophyllus (clove oil), extract of Capsicum annuum (cayenne pepper), extract of Mentha piperita (peppermint oil), and extract of Melaleuca alternifolia (tea tree oil). Preferred botanically-derived moisturizing agents include, but are not limited to, pectin, disaccharides, oligosaccharides, and polysaccharides extracted from Aloe barbedensis, algae, seaweed and sea grass. Preferred botanically-derived skin bleaching/lightening agents include, but are not limited to, arbutin and glabradin. The above-listed botanical extracts are commercially-available from Active Organics LP (Lewisville, Texas).

[0036] Sunscreen actives may also be used in combination with the M. miehei and M. rouxi extract composition of the present invention. These include the sunscreens listed by the U.S. Food and Drug Administration in the Sunscreen Drug Products for Over-The-Counter Human use Final Monograph published in 64 Federal Register pp. 27666-27693 (May 21, 1999) but is not limited thereto. Other sunscreen active ingredients are accepted for use in countries outside the U.S. and are also considered to be within the scope of the present invention.
[0037] Dermatopharmaceutical ingredients that can be used in combination with the protease extract and the amino sugar extract of the present invention as disclosed in U.S. Patent No. 6,277,892, in Kerdel et al, Dermatologic Therapeutics (2005), and in Hardman et al., Goodman & Gilman's: The Pharmacological Basis of Therapeutics (10th Edition, 2001). Further examples of cosmetic and/or dermatopharmaceutical ingredients which are suitable for use in the delivery system of the present invention are disclosed in U.S. Patent Nos. 6,492,326.

[0038] The following examples are further illustrative of the present invention. The components and specific ingredients are presented as being typical, and various modifications can be derived in view of the foregoing disclosure within the scope of the invention. All percentages, ratios and proportions herein are by weight, unless otherwise specified. All temperatures are in degrees Celsius unless otherwise specified.

Toner Formulation Example

Deionized Water 88.190%
Methyl Gluceth-20 1.000%
Potassium Sorbate 0.100%
Sodium Benzoate 0.100%
Phenoxyethanol 0.600%
Citric acid 0.010%
M. Miehei/M. Miehei Extract, Butylene Glycol, and N-Acetylglucosamine 10.000%

Add ingredients sequentially in order listed. Mix until clear.

[0039] The present invention is a composition that exfoliates a surface and includes a protease extract and an amino sugar extract. The composition is formed from a mixture of fungal extracts, combining peptides derived from Mucor miehei with amino polysaccharides derived from cell wall components from Mucor miehei. In addition to the combination, extracts from Mucor miehei and Mucor rouxii or Mucor miehei compositions may be used independently from each other as an exfoliant. Both proteinaceous extracts Mucor miehei and amino sugar extracts from Mucor rouxii or Mucor miehei independently have modest exfoliating activity. However, the inventors realized that the combination of proteinaceous extracts from Mucor miehei and amino sugar extracts from Mucor rouxii or Mucor miehei acted with a synergistic effect to
provide an irritation-free intense exfoliating effect. In addition, the present invention includes an amino sugar extract composition that has a synergistic effect with the natural enzymes of the surface to add additional benefit to the exfoliation. As such, stratum corneum replacement rates are increased more than 25%. Cells are exfoliated as single cells, as opposed to larger flakes or clumps typically seen with other exfoliants like AHAs and BHAs.

Exfoliation has been shown to provide both short term and long term cosmetic benefits. The present invention provides an immediate improvement in skin smoothness, clarity, brightness and skin hydration. With continued use, skin firmness is improved and lines and wrinkles are reduced. The present invention, provided a synergistic effect that allowed the use of reduced concentrations of active agents to provide an irritation-free intense exfoliating effect. The present invention provides an increase in the activity of endogenous proteases and increases desmosomal breakdown. Over time, the Mucor extracts upregulate the production of cathepsin D and other stratum corneum proteases.

The composition of the present invention reduces the appearance of the signs of aging by a primary mode of action that is not based on exfoliation. Thus, there remains a long-felt but as yet unmet need to increase Collagen I by upregulating the gene(s) that codes for the synthesis of Collagen I, and/or decrease levels of MMP1, either by upregulating the gene(s) that codes for TIMP1 or downregulating the gene(s) that codes for the expression of MMP1.

These needs are met by the protease extract and the amino sugar extract composition and specifically by the Mucor miehei and Mucor rouxii or Mucor miehei extracts of the present invention. Specifically, the composition combining Mucor miehei extract and Mucor rouxii or Mucor miehei extract of the present invention upregulates expression not only of the aforementioned genes, but also notably upregulates the expression of genes that code for fibronectin and vimentin (extracellular matrix glycoproteins involved in cell adhesion, differentiation, and migration) as well as procollagen-lysine 2-oxoglutarate 5-dioxygenase (an enzyme involved in the crosslinking of procollagen to form mature bundled collagen fibers).

In one embodiment, the composition of the present invention provides a composition having 1-45% in M. miehei and M. rouxii or M. miehei extracts designed as
anti-aging treatments for the face and body. In addition, the present invention may contain one or more additives for even greater effects through the addition of retinoids, peptides, and other exfoliants. Numerous embodiments of the compositions of the present invention have been examined both in vitro and in vivo and have demonstrated multifaceted effects on skin properties in both short and long term. These effects include changes in rates of exfoliation and improved characteristics of exfoliated squame cells. In addition dramatic improvements in measurable skin properties were observed.

[0044] The effectiveness of the compositions of the present invention were examined on a variety of skin gene products involved in the aging process. Testing was conducted with real time RT-PCR utilizing cultured fibroblasts or keratinocytes.

[0045] The compositions of the present invention provide a dramatic increase in expression of CTGF (Connective Tissue Growth Factor) which has recently been shown to be under expressed in aged skin and fibroblasts. CTGF has been directly related to levels of procollagen 1, an important enzyme in dermal integrity (Table 1 below).

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Biological Function</th>
<th>Change in Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTGF</td>
<td>collagen production</td>
<td>Upregulated 156%</td>
</tr>
<tr>
<td>MMP 9</td>
<td>hydrolyses dermal components</td>
<td>Downregulated 45%</td>
</tr>
<tr>
<td>MMP 13</td>
<td>hydrolyses dermal components</td>
<td>Downregulated 51%</td>
</tr>
<tr>
<td>TIMP 1</td>
<td>inhibits MMP</td>
<td>Upregulated 44%</td>
</tr>
<tr>
<td>TIMP 2</td>
<td>inhibits MMP</td>
<td>Upregulated 37%</td>
</tr>
<tr>
<td>TIMP 3</td>
<td>inhibits MMP</td>
<td>Upregulated 50%</td>
</tr>
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</table>

[0046] In addition, the compositions of the present invention reduce the expression of MMP 9 and 13 and an increase in expression of TIMP 1, 2, and 3. MMP and TIMP which are involved in the breakdown of the dermis via proteolysis during acute inflammatory responses (Table 1).

[0047] The standard dansyl chloride method was used to assess the effectiveness of the compositions of the present invention to increase turnover rates compared to positive controls. The compositions of the present invention was assessed at 1-20% in a simple gel versus 10% lactic acid pH 3 which was used as a positive control and results with comparable concentrations (1-20%) of lactic acid.

[0048] In addition, the compositions of the present invention were evaluated for the neurosensory (NS) irritation potential of each treatment. Neurosensory irritation potential
was defined as the stinging response on the nasal fold area and is the subject of a number of publications. Briefly, panelists applied various treatments on the nasal fold area and a sting response was recorded by the panelists based upon a 1 (no sting)-5 (painful itching and burning) scale. Irritation was assessed over a 15 minute period and the average sting response was recorded. An efficacy index was established by taking the ratio of % increase in cell renewal and sting response. As seen below, the dose response was observed with increasing rates of cell replacement seen at increasing concentrations for both lactic acid and the protease extract and the amino sugar extract (e.g., the *M. miehei* and *M. rouxii* or *M. miehei* composition) of the present invention. In fact at similar concentrations similar rates of exfoliation were observed with both lactic acid and the present invention. However, significant differences were noted with respect to neurosensory irritation. With increasing concentrations lactic acid irritation increased dramatically from 2.2 at 1% to more than 4 at 20%. On the other hand the *M. miehei* and *M. rouxii* or *M. miehei* composition of the present invention showed only modest increases in irritation even at 20% concentration showing an irritation score of 1.8. That is less than what was observed for 1% lactic acid.

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Sting Score</th>
<th>Turnover Time</th>
<th>% Change</th>
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<tr>
<td>Gel only</td>
<td>1.1</td>
<td>20.6 days</td>
<td></td>
</tr>
<tr>
<td>0.5% proteinaceous <em>M. Miehei</em> I 0.5% amino sugar <em>M. Miehei</em> composition (1% lactic acid)</td>
<td>1.3</td>
<td>18.9 days</td>
<td>9%</td>
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<tr>
<td>2.5% proteinaceous <em>M. Miehei</em> I 2.5% amino sugar <em>M. Miehei</em> composition (5% lactic acid)</td>
<td>1.5</td>
<td>16.1 days</td>
<td>22%</td>
</tr>
<tr>
<td>5%, proteinaceous <em>M. Miehei</em> I 5% amino sugar <em>M. Miehei</em> composition</td>
<td>1.6</td>
<td>14.7 days</td>
<td>29%</td>
</tr>
<tr>
<td>10% lactic acid</td>
<td>3.2</td>
<td>14.1 days</td>
<td>32%</td>
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</table>
10% proteinaceous *M. Miehei* I
amino sugar *M. Miehei* composition
(20% lactic acid)

When efficacy indices were determined a dramatic difference between the
two test materials was observed. While the present invention can achieve the substantial
exfoliation rates observed with a low pH lactic acid solution, it presented none of the
irritation seen with this material. When each extract was used separately at 10% rates of
turnover of only 5% amino sugar extract (*M. Miehei*) and for 12% proteinaceous extract
(*M. Miehei*). This clearly shows the synergistic effect of the combination of an amino
sugar extract of *M. Miehei* and a proteinaceous extract of *M. Miehei* in the composition
of the present invention.

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Sting Score</th>
<th>% Increase</th>
<th>Efficacy Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel only</td>
<td>1.1</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>
| 1%, proteinaceous *M. Miehei* I
1% amino sugar *M. Miehei* composition
(1% lactic acid) | 2.2         | 5%         | 2.4           |
| 5% proteinaceous *M. Miehei* I
5% amino sugar *M. Miehei* composition
(5% lactic acid) | 2.7         | 20%        | 7.4           |
| 10%, proteinaceous *M. Miehei* I
10% amino sugar *M. Miehei* composition | 1.6 | 29% | 18.1 |
10% lactic acid & 3.2 & 32% & 10 \\

20% proteinaceous *M. Miehei I* \\
20% amino sugar *M. Miehei* & 1.8 & 39% & 21.6 \\
composition \\
(20% lactic acid) & 4.2 & 43% & 10.2 \\
10% *M. miehei* amino sugar & 2.4 & 19% & 8.1 \\
10% *M. miehei* proteinaceous & 2.5 & 5% & 2 \\

[0050] Determination of the efficacy indices also demonstrates the synergistic effect. Morphology of desquamated squames. Loosely adhering stratum corneum skin cells were examined under microscopy before and after treatment with the above exfoliants. Cell samples were taken from the lower outer calf with sticky tape, and examined via low and high power microscopy. Images were graded by trained clinicians with respect to size of exfoliated clumps (1-10). In addition, some samples were examined with an Image J processing program to determine an average clump size.

[0051] The lower leg is typically dry and cells tend to exfoliate as clumps. Prior to treatment clinicians assessed average clump size as 6.6; this reflects a relatively large group of cells. After two weeks of treatment with either 8% lactic acid or 10% composition of the present invention clump sizes of 6.3 for lactic acid (essentially no change), and 3.4 for the composition of the present invention were observed. The reduced clump size suggests exfoliation occurs in more likely a single cell manner with the composition of the present invention. This was confirmed via image analysis (data on file but not shown). A reduced squame size results in the perception of a smoother skin surface.

[0052] The compositions of the present invention were evaluated in clinical studies. A four week clinical study was conducted to evaluate the changes in skin properties with repeated use of a 10% composition of the present invention in a gel base. Twenty subjects, average age 54.5 participated in this half-face placebo controlled study, conducted in the early winter in Dallas, Texas. All subjects were subject to dry skin and showed a significant number of fine lines and wrinkles. Skin properties such as
smoothness, firmness and sagging, lines and wrinkles, and texture were examined before and after treatment. Results are summarized below in individual data tables.

[0053] Firmness. Skin firmness was assessed by extensibility characteristics of the skin with the Cutometer MPA 580. Ue measure for immediate deformation or extension as defined by Pierre Agache was used as a measure of skin firmness. Prior to treatment an average extension Ue (average) was approximately 0.35mm. Compared to baseline measurements after four weeks the placebo side showed no change in skin elasticity while the side treated by the compositions of the present invention showed a 21% increase (p < .05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-Treatment</th>
<th>Four week's</th>
<th>% Change</th>
</tr>
</thead>
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<tr>
<td>Placebo</td>
<td>0.254</td>
<td>0.256</td>
<td>1%</td>
</tr>
<tr>
<td>10% proteinaceous M. Miehei I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% amino sugar M. Miehei composition</td>
<td>0.257</td>
<td>0.205</td>
<td>21%</td>
</tr>
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[0054] Lines and Wrinkles. Via clinical grading a 47% reduction in lines and wrinkles was observed on the side of the face treated with the composition of the present invention, in contrast the control side showed only an 11% (non-significant) decrease. All twenty subjects using the composition of the present invention, showed at least a 10% reduction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-Treatment</th>
<th>Four week's</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>11.5</td>
<td>10.3</td>
<td>11%</td>
</tr>
<tr>
<td>10% proteinaceous M. Miehei I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% amino sugar M. Miehei composition</td>
<td>11.3</td>
<td>5.6</td>
<td>48%</td>
</tr>
</tbody>
</table>

[0055] Skin sagging was measured by measuring the "dropping" of the skin in the lower cheek area when skin was stressed by the attachment of a small weight (ranging from 20-50 gms) to a small section of skin. The less the skin deflects under stress of the weight, the better the skin condition. The amount of deformation with a fixed weight
correlates with chronological age. As the following Table shows after treatment with the composition of the present invention a small (9.9%) but definite decrease in deformation was observed. This contrasted to the control which showed no changes. A before and after photo, showing a representative, but not average change is attached.

<table>
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<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>After treatment</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>4.43 mm</td>
<td>4.45 mm</td>
<td>0.50%</td>
</tr>
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</table>

10% proteinaceous *M. Miehei* I 10%>

amino sugar *M. Miehei* composition 4.48 mm 4.05 mm 9.9% (p < .05)

[0056] Panelist Assessment. Each panelist was asked to assess how each product (placebo and the composition of the present invention) influenced a variety of skin properties. While panelists responded that they observed a small improvement in skin properties with the placebo, the side treated with the composition of the present invention showed dramatic improvements. Especially telling were the improvements panelists noted with respect to skin smoothness, skin glow, brightness, and reduction in lines and wrinkles. The percent improvements are listed in parenthesis for the appropriate test.

<table>
<thead>
<tr>
<th>Parameter Examined</th>
<th>Placebo</th>
<th><em>M. Miehei</em> I</th>
<th><em>M. Miehei</em> composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoothness</td>
<td>4.4</td>
<td>5.3 (21%)</td>
<td>4.4</td>
</tr>
<tr>
<td>Brightness/Glow</td>
<td>3.5</td>
<td>3.6 (3%)</td>
<td>3.8</td>
</tr>
<tr>
<td>Lines/Wrinkles</td>
<td>5.6</td>
<td>5.3 (5%)</td>
<td>5.5</td>
</tr>
<tr>
<td>Overall appearance</td>
<td>3.5</td>
<td>3.7 (6%)</td>
<td>3.6</td>
</tr>
</tbody>
</table>

[0057] The visible signs of aging (e.g., fine lines and wrinkles) are correlated with a decrease in the level of collagen in the skin. This can be attributed both to decreased
synthesis as well as increased enzymatic degradation by collagenases, in particular Collagenase I also known as Matrix Metalloprotease 1 (MMP1). The degradative activity of MMP1 is regulated by the concentration of an endogenous protease inhibitor, Tissue Inhibitor of Matrix Metalloprotease-1 (TIMP1).

The present invention provides for the combination of two extracts, a protease extract and an amino sugar extract having a synergistic interaction to promote non-irritating exfoliation. In one embodiment, the extracts from M. miehei and M. rouxii or M. miehei respectfully.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The use of the word "a" or "an" when used in conjunction with the term "comprising" 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." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the
inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0063] 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.

[0064] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0065] 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. 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.
What is claimed is:

1. A dermatocosmetic composition for reducing the signs of aging comprising
   a dermatologically-acceptable carrier;
   a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable carrier; and
   a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition.

2. The dermatocosmetic composition according to claim 1, wherein the isolated and purified proteinaceous extract is from *M. Miehei*.

3. The dermatocosmetic composition according to claim 1, wherein the isolated and purified proteinaceous extract has protease activity.

4. The dermatocosmetic composition according to claim 1 wherein the isolated and purified proteinaceous has an acid protease specific activity of from less than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 100, 150, 200, 300, 400, 500 or more than 500 HUT units/mg.

5. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract is derived from a cell wall.

6. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract is from *M. rouxii or M. miehei*.

7. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract comprises one or more selected from galactosamine, mannosamine, glucosamine, allosamine, altrosamine, ribosamine, arabinosamine, gulosamine, idosamine, fucosamine, talosamine, xylosamine, lyxosamine, sorbosamine, tagatosamine, psicosamine and fructosamine.
8. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract comprises one or more selected from D-mannosamine, D-glucosamine, D-allosamine, D-altrosamine, D-ribosamine, D-arabinosamine, L-gulosamine, L-idosamine, L-galactosamine, L-fucosamine, L-talosamine, L-xylosamine and L-lyxosamine.

9. The dermatocosmetic composition according to claim 1, further comprising one or more additives for even greater effects through the addition of retinoids, peptides, and other exfoliants.

10. The dermatocosmetic composition according to claim 1, further comprising one or more selected from botanically-derived anti-bacterial agents, botanically-derived anti-fungal agents, botanically-derived anti-inflammatory agents, botanically-derived agents that help decrease the appearance of signs of aging, botanically-derived antioxidants, botanically-derived astringent agents, botanically-derived moisturizing agents, botanically-derived skin bleaching/lightening agents, botanical extracts or combinations thereof.

11. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract is present in an amount from about 0.01 percent to about 25.0 percent of the weight of the composition.

12. A method for treating or reducing the appearance of fine lines and wrinkles on the skin comprising the step of administering to a person in need thereof the composition of claim 1.

13. A method for treating a skin condition comprising the step of administering to a person in need thereof the composition of claim 1.

14. A method for exfoliating a skin surface comprising the steps of:

   administering to a person in need thereof an exfoliating composition comprising a dermatologically-acceptable exfoliating carrier, a therapeutically-effective amount of an
isolated and purified proteinaceous extract disposed in the dermatologically-acceptable
exfoliating carrier and a therapeutically-effective amount of an isolated and purified
amino sugar extract disposed in the dermatologically-acceptable exfoliating carrier to
form an exfoliating composition.

15. The method of claim 14, further comprising the step of applying the exfoliating
composition at least two times a day.

16. The method of claim 14 further comprising the step of applying the exfoliating
composition weekly.

17. The method of claim 14, further comprising the step of applying the exfoliating
composition for a period of at least 3 months.

18. The method of claim 14, wherein the isolated and purified proteinaceous extract
is from *M. Miehei*.

19. The method of claim 14, wherein the isolated and purified proteinaceous extract
has protease activity.

20. The method of claim 14, wherein the isolated and purified proteinaceous has an
acid protease specific activity of from less than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35,
40, 45, 50, 55, 60, 65, 70, 75, 100, 150, 200, 300, 400, 500 or more than 500 HUT
units/mg.

21. The method of claim 14, wherein the isolated and purified amino sugar extract is
derived from a cell wall.

22. The method of claim 14, wherein the isolated and purified amino sugar extract is
from *M. rouxii or M. miehei*. 
23. The method of claim 14, wherein the isolated and purified amino sugar extract comprises one or more selected from galactosamine, mannosamine, glucosamine, allosamine, altrosamine, ribosamine, arabinosamine, gulosamine, idosamine, fucosamine, talosamine, xylosamine, lyxosamine, sorbosamine, tagatosamine, psicosamine, fructosamine, D-mannosamine, D-glucosamine, D-allosamine, D-altrosamine, D-ribosamine, D-arabinosamine, L-gulosamine, L-idosamine, L-galactosamine, L-fucosamine, L-talosamine, L-xylosamine and L-lyxosamine.

24. A dermatocosmetic composition for reducing the signs of aging comprising a dermatologically-acceptable carrier; and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition.

25. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract is from *M. rouxii or M. miehei*.

26. A composition for increasing the endogenous protease activity comprising a dermatologically-acceptable carrier; and a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition having a synergistic effect with one or more endogenous proteases.

27. The dermatocosmetic composition according to claim 1, wherein the isolated and purified amino sugar extract is from *M. rouxii or M. miehei*.

28. A composition for increasing the protease activity comprising a dermatologically-acceptable carrier; a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable carrier; and
a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier to form a dermatocosmetic composition.

29. The composition of claim 28, wherein the isolated and purified proteinaceous extract is from *M. Miehei*.

30. The composition of claim 28, wherein the isolated and purified amino sugar extract is from *M. rouxii or M. miehei*.

31. A method for increasing the protease activity on the skin comprising the steps of: administering to a skin surface an activating composition comprising a dermatologically-acceptable exfoliating carrier, a therapeutically-effective amount of an isolated and purified amino sugar extract disposed in the dermatologically-acceptable carrier, wherein the activating composition increases the protease activity on the surface of the skin.

32. The method of claim 31, further comprising the step of applying the exfoliating composition at least two times a day.

33. The method of claim 31, wherein the isolated and purified amino sugar extract is from *M. rouxii or M. miehei*.

34. An exfoliating composition comprising:

   a dermatologically-acceptable exfoliating carrier;

   a therapeutically-effective amount of an isolated and purified proteinaceous extract disposed in the dermatologically-acceptable exfoliating carrier; and

   a therapeutically-effective amount of an isolated and purified amino sugar extract derived disposed in the dermatologically-acceptable exfoliating carrier to form an exfoliating composition.
35. The dermatocosmetic composition of claim 34, wherein the isolated and purified proteinaceous extract is from *M. Miehei*.

36. The dermatocosmetic composition of claim 34, wherein the isolated and purified amino sugar extract is from *M. rouxii* or *M. miehei*.

37. The dermatocosmetic composition of claim 34, wherein the isolated and purified proteinaceous extract has protease activity.

38. The dermatocosmetic composition of claim 1, wherein the isolated and purified amino sugar extract is derived from a cell wall.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K  A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practically, search terms used)

EPO-Internal , WPI Data, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C.

See patent family annex.

**Date of the actual completion of the international search**

31 August 2012

**Date of mailing of the international search report**

19/09/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer

Sala-Jung, Nathalie
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<td>PALVANNAN T ET AL: &quot;Extrathermostabi li zati on of aspartyl protease from Rhi zomucor pusil lus by sugars: stabil izi ng rol e of the C-2. alpha. hydroxyl group of sugars&quot;, JOURNAL OF SCI EN TIFIC AND INDUSTRIAL RESEARCH, vol. 57, 1 January 1998 (1998-01-01), pages 625-628, XP009162345, SCIENTIFIC PUBLISHERS, INDIA ISSN: 0022-4456 page 626; table 1</td>
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<td>paragraph [0137] - paragraph [0139]; examples 3,4,5,7</td>
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