

COMPOSITION FOR TREATING SKIN CELL DAMAGE CAUSED BY FINE DUST, FOR STRENGTHENING SKIN BARRIER, AND FOR ANTI-AGING OR ANTI-INFLAMMATION, CONTAINING MENTHA ARVENSIS EXTRACT

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a composition for caring damages of skin cells by microdust, particularly a composition comprising *Mentha arvensis* extract and which takes care of damages of skin cells by significantly changing biomarkers, etc. which are skin cell genes whose expression levels are changed by microdust as compared with the normal state.

Description of the Related Art

The skin is a part of the body that is directly exposed to the external environment. It not only serves as a protective layer for protecting important organs of our body, but also regulates moisture evaporation and protects the body from external infections. However, even though the skin is meant to prevent virus invasion from the outside, excessive exposure of the skin to ultraviolet rays or pollutants causes skin irritation. Particularly, the skin is damaged by Asian dust accompanied by strong wind and dirt.

Asian dust is a phenomenon in which small sands or red clays float from the inland deserts of China, Mongolia, etc., carried far away by the upper winds and then drops near the ground. In Korea, Asian dust occurs periodically every spring. Asian dust is a combination of organic and inorganic materials, and its physical characteristics and constituents are very diverse depending on the time and place of occurrence. It also includes metals that can give a biological effect. Large

particles of Asian dust usually stay in their source or surroundings, and small particles thereof flow even into Korea. It is reported that when inhaled, this dust is deposited in the lower bronchial tubes and even in the gas exchange part of the lungs, which may cause damage to the respiratory system. In addition, it was found that damages of skin cells increased in the skin of people living in dusty areas or areas with a lot of Asian dust.

Meanwhile, among the layers of the skin, the epidermis plays an important role in preventing evaporation of the moisture within the human body. The layers of the epidermis in order from the outside in are: the stratum corneum, the stratum granulosum, the stratum spinosum and the stratum basale. Cells of the stratum corneum act like bricks, and the intercellular lipids between stratum corneum cells act like mortar to constitute the skin barrier. In addition, stratum corneum cells of healthy humans have a high concentration of natural moisturizing factor (NMF), which helps to retain moisture in the skin. For example, substances such as amino acids are water-soluble and thus effectively combines with moisture to prevent skin's moisture from drying.

However, nowadays, due to various reasons such as artificial temperature control of cooling/heating due to changes of the environment or life patterns, skin stresses due to stresses from social life and environmental pollution, frequent washing caused by makeup habits, and natural skin aging due to an age increase, moisture of the stratum corneum decreases, so that the skin becomes dry, the skin surface becomes rough, and the skin becomes loose and looks rusty due to lack of moisture, etc. Thus, there is an increasing need for skin moisturizers. Further, excessive physical and chemical

stimuli from the outside, ultraviolet rays, stress and nutritional deficiencies deteriorate the normal functions of the skin and promote phenomena such as loss of elasticity, keratinization and wrinkle formation. Particularly, the dermis-epidermis border is severely damaged by ultraviolet rays.

IL-36G is known as a useful biomarker in psoriasis caused by skin barrier weakness (see Non-Patent Literature 1). S100A7 is also known as an indicator of atopic dermatitis and psoriasis caused by impairment of skin barrier functions (see Non-Patent Literature 2). LCE3D is also known as an indicator of psoriasis risk genes (see Non-Patent Literature 3).

Interleukin-1 is classified as a senescence-associated secretory phenotype (SASP). In particular, interleukins 1a and 1b (IL-1a and IL-1b), which are representative aging markers, are known to be involved in cellular senescence (see Non-Patent Literature 4). It is also known that IL-1a, IL-1b and PTGS2 are involved in skin inflammation, and that XDH indicates oxidative stress induced by prolonged exposure to an irritation source and thus is an indicator of extrinsic skin aging (see Non-Patent Literature 5). With regard to skin inflammation, it is also known that the expression levels of PTGS2 (= COX-2) and IL-1a increase in skin cells exposed to an irritation source (see Non-Patent Literature 6). It is also known that XDH (xanthine dehydrogenase), which is an indicator of oxidative stress, is involved in skin aging.

Citation List

Non-Patent Literature

Non-Patent Literature 1:

AM D'Erme et al, "IL-36c (IL-1F9) Is a Biomarker for

Psoriasis Skin Lesions", Journal of Investigative Dermatology, Volume 135, 2015.

Non-Patent Literature 2:

Son et al, "S100A7 (psoriasin) inhibits human epidermal differentiation by enhanced IL-6 secretion through I κ B/NF- κ B signalling", Experimental Dermatology, John Wiley & Sons A/S, 2016.

Non-Patent Literature 3:

Bergboer et al, "Psoriasis Risk Genes of the Late Cornified Envelope-3 Group Are Distinctly Expressed Compared with Genes of Other LCE Groups", The American Journal of Pathology, Vol. 178, No. 4, April 2011.

Non-Patent Literature 4:

Jean-Philippe Coppe, et al, "The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression", Annual Review of Pathology: Mechanisms of Disease, volume 5, 2010.

Non-Patent Literature 5:

Kim, H.J., et al, "Transcriptome analysis of airborne PM_{2.5}-induced detrimental effects on human keratinocytes", Toxicology Letters 273, 26-35, 2017.

Non-Patent Literature 6:

Natalia D Magnani, et al, "Skin Damage Mechanisms Related to Airborne Particulate Matter Exposure", toxicological sciences, 149(1), 2016, 227-236.

SUMMARY OF THE INVENTION

The present inventors have found that microdust has harmful effects on the skin, which in turn affects the expression of skin cell genes, causing symptoms such as damages of skin cells.

Thus, one aspect of the present invention is to provide a composition for caring damages of skin cells by microdust.

In order to achieve the above object, one aspect of the present invention provides a composition for external application to the skin for caring skin damages by microdust, comprising *Mentha arvensis* extract as an active ingredient, and which regulates the expression level of at least one selected from the group consisting of IL-1a (NM_000575), IL-1B (NM_000576), IL-36G (NM_019618), S100A7 (NM_002963), LCE3D (NM_032563), PTGS2 (NM_000963), and XDH (NM_000379), which are genes in skin cells whose expression levels are affected by microdust, to the normal level.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the cell viabilities when treated with microdust extract. Here, ADSP, which represents Asian dust storm particle, refers to Asian dust, PM10 (particulate matter 10) refers to microdust having a particle size of 10 μm , and PM2.5 (particulate matter 2.5) refers to microdust having a particle size of 2.5 μm ;

FIG. 2A shows that the mRNA expression level of S100A7 genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2B shows that the mRNA expression level of IL-1A genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2C shows that the mRNA expression level of IL-1B genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2D shows that the mRNA expression level of IL-36G genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2E shows that the mRNA expression level of PTGS2 genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2F shows that the mRNA expression level of LCE3D genes increases in skin cells irritated by PM2.5 microdust;

FIG. 2G shows that the mRNA expression level of XDH genes increases in skin cells irritated by PM2.5 microdust;

FIG. 3 shows that the changed expression level of genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3A shows that the changed expression level of S100A7 genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3B shows that the changed expression level of IL-1A genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3C shows that the changed expression level of IL-1B genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3D shows that the changed expression level of IL-36G genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3E shows that the changed expression level of PTGS2 genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract;

FIG. 3F shows that the changed expression level of LCE3D genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract; and

FIG. 3G shows that the changed expression level of XDH genes in skin cells irritated by microdust is returned to the normal level by treatment with *Mentha arvensis* extract.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention will be described in detail.

Mentha arvensis is a perennial herb of the family Lamiaceae of the order Tubiflorae. It is also referred to as mint or peppermint. *Mentha arvensis* is characterized by a refreshing and cool flavor. Its leaves and stems have a deep fragrance when grown in a cool climate. It mainly contains menthol and thus emits a unique fragrance. It also contains nutritional ingredients such as vitamins.

In one aspect of the present invention, the composition comprises *Mentha arvensis* extract.

In one aspect of the present invention, the leaves or stems of *Mentha arvensis* may be used, although not limited thereto.

Mentha arvensis added with the microorganism may be fermented specifically at 22-30°C for 24-72 hours. In one embodiment, the extract is fermented at 26°C for about 48 hours.

In one aspect of the present invention, the *Mentha arvensis* may be extracted with a specific extraction solvent to form *Mentha arvensis* extract.

In one aspect of the present invention, the *Mentha arvensis* extract may be prepared by extracting *Mentha arvensis* leaves or stems with water or an organic solvent. Specifically, it may be prepared by extracting the leaves or stems of *Mentha arvensis* with at least one extraction solvent selected from the group consisting of water, C₁-C₆ anhydrous or hydrated lower alcohol, acetone, butylene

glycol, ethyl acetate, diethyl acetate, diethyl ether, benzene, chloroform, and hexane.

In one aspect, the *Mentha arvensis* extract may be extracted at room temperature.

In one aspect, the *Mentha arvensis* extract may be obtained by extracting with the extraction solvent and then further performing at least one of the following steps: filtration, concentration, separation, and drying. In particular, the *Mentha arvensis* extract may be subjected to at least one filtration step. In one embodiment, it is subjected to two filtration steps.

In one embodiment, the separation step may include a centrifugation step.

Specifically, the extraction may use at least one of: polar solvents including water, a C₁-C₆ anhydrous or hydrated lower alcohol, acetone, and butylene glycol and low-polarity solvents including ethyl acetate, diethyl acetate, diethyl ether, benzene, chloroform, and hexane.

More specifically, the solvent may be a 50-90% ethanol aqueous solution and may be a 60-80% or 65-75% ethanol aqueous solution. When the solvent is a 50-90% ethanol aqueous solution, the active ingredient can be effectively extracted from the *Mentha arvensis*. In one embodiment, the solvent may be an about 70% ethanol aqueous solution.

In one aspect, the extract may be concentrated under reduced pressure at an appropriate temperature in a distillation apparatus equipped with a cooling condenser after extraction.

However, the *Mentha arvensis* extract according to the present invention can be obtained by extraction according to a conventional method in the art, and the extraction method is not limited to the above-described methods.

In one aspect of the present invention, the composition may comprise 0.000001 to 30% by weight of *Mentha arvensis* extract based on the total weight of the composition. When the content thereof is 0.000001 to 30% by weight, the *Mentha arvensis* extract obtained an excellent effect of caring skin damages by microdust.

Specifically, the content may be 0.0000001% by weight or more, 0.0000005% by weight or more, 0.0000007% by weight or more, 0.0000009% by weight or more, 0.000001% by weight or more, 0.000002% by weight or more, 0.000004% by weight or more, 0.000006% by weight or more, 0.000008% by weight or more, 0.00001% by weight or more, 0.00003% by weight or more, 0.00005% by weight or more, 0.00007% by weight or more, 0.00009% by weight or more, 0.0001% by weight or more, 0.0003% by weight or more, 0.0005% by weight or more, 0.0007% by weight or more, 0.0009% by weight or more, 0.001% by weight or more, 0.01% by weight or more, 0.1% by weight or more, 1% by weight or more, 3% by weight or more, 5% by weight or more, 7% by weight or more, 9% by weight or more, 10% by weight or more, 13% by weight or more, 15% by weight or more, 17% by weight or more, 19% by weight or more, 21% by weight or more, 23% by weight or more, 25% by weight or more, 27% by weight or more, 29% by weight or more, 30% by weight or more, or 31% by weight or more and 32% by weight or less, 31% by weight or less, 30% by weight or less, 29% by weight or less, 28% by weight or less, 26% by weight or less, 24% by weight or less, 22% by weight or less, 20% by weight or less, 18% by weight or less, 16% by weight or less, 14% by weight or less, 12% by weight or less, 10% by weight or less, 9% by weight or less, 8% by weight or less, 6% by weight or less, 4% by weight or less, 2% by weight or less, 1% by weight or less, 0.1% by weight or less, 0.09% by weight or less, 0.04% by weight or less, 0.01% by

weight or less, 0.006% by weight or less, 0.001% by weight or less, 0.0009% by weight or less, 0.0007% by weight or less, 0.00005% by weight or less, 0.00003% by weight or less, 0.00001% by weight or less, 0.000009% by weight or less, 0.000007% by weight or less, 0.000005% by weight or less, 0.000003% by weight or less, 0.000001% by weight or less, 0.0000009% by weight or less, 0.0000007% by weight or less, 0.0000005% by weight or less, or 0.0000003% by weight or less, 0.0000002% by weight or less, 0.0000001% by weight or less, 0.00000009% by weight or less, although not limited thereto.

Another aspect of the present invention includes the use of the composition for caring skin damages by microdust.

Another aspect of the present invention provides a method for caring skin damages by microdust in a subject, comprising administering an effective amount of *Mentha arvensis* extract to a subject in need thereof.

Another aspect of the present invention provides the use of *Mentha arvensis* extract in the manufacture of compositions for caring skin damages by microdust.

Another aspect of the present invention provides *Mentha arvensis* extract for use in caring skin damages by microdust.

As used herein, the term "microdust" refers to a very small material that is invisible to the naked eye. It is a particulate matter that floats or flutters in the air for a long time and has a diameter of 10 μm or less. Especially, a particulate matter having a diameter of 2.5 μm or less is called "ultrafine particles". In the present invention, "microdust" is intended to include "ultrafine particles".

As used herein, the term "care" refers to effective protection of skin cells from irritation and inhibition,

prevention or restoration (recovery) of a change of the expression level of a specific gene by the irritation.

In one aspect, the present invention provides a composition for inhibiting damages of skin cells by microdust by regulating the expression level of a specific gene in skin cells damaged by microdust to the normal level.

Specifically, in the present invention, the genes in skin cells whose expression levels are affected by microdust include: IL-1a (NM_000575), IL-1B (NM_000576), IL-36G (NM_019618), S100A7 (NM_002963), LCE3D (NM_032563), PTGS2 (NM_000963), and XDH (NM_000379). IL-1a (NM_000575), IL-1B (NM_000576), IL-36G (NM_019618), S100A7 (NM_002963), LCE3D (NM_032563), PTGS2 (NM_000963) and XDH (NM_000379) are genes whose expression levels are increased by microdust. Thus, it is possible to inhibit damages of skin cells by suppressing the expression levels of these genes to the normal levels.

The genes used in the present invention and whose expression levels are increased by microdust are shown in Table 1. Table 1 shows genes whose expression levels are increased by microdust. In this table, the term "name" denotes genebank accession ID of NCBI, the term "gene symbol" denotes official gene symbol, and the term "gene title" denotes the name of each gene. These are described in Non-Patent Literature 1.

Table 1

Increased genes		
Name	Gene Symbol	Gene title
NM_002963	S100A7	S100 calcium binding protein A7
NM_032563	LCE3D	late cornified envelope 3D
NM_019618	IL36G	interleukin 36, gamma
NM_000576	IL1B	interleukin 1, beta

NM_000575	IL1A	interleukin 1, alpha
NM_000963	PTGS2	Cyclooxygenase-2 (COX-2)
NM_000379	XDH	xanthine dehydrogenase

The expression levels of the genes or proteins may be analyzed using various analysis methods known in the art, such as microarray, PCR, NGS (next generation sequencing), western blot, northern blot, ELISA, radioimmunoassay, radioimmunodiffusion, immunohistochemical staining, immunoprecipitation assay, etc.

Microdust causes damage to skin cells, which leads to inflammation and thus further damage to skin cells. The skin condition can be improved by taking care of the vicious cycle of the damages of skin cells with *Mentha arvensis* extract.

Another aspect of the present invention includes the use of the composition of the present invention for anti-aging and anti-inflammation.

Another aspect of the present invention provides a method for anti-aging of a subject, comprising administering an effective amount of *Mentha arvensis* extract to a subject in need thereof.

Another aspect of the present invention provides the use of *Mentha arvensis* extract in the manufacture of compositions for anti-aging.

Another aspect of the present invention provides *Mentha arvensis* extract for use in anti-aging.

Another aspect of the present invention provides a method for anti-inflammation of a subject, comprising administering an effective amount of *Mentha arvensis* extract to a subject in need thereof.

Another aspect of the present invention provides the use of *Mentha arvensis* extract in the manufacture of compositions for anti-inflammation.

Another aspect of the present invention provides *Mentha arvensis* extract for use in anti-inflammation.

In one aspect, the present invention provides a composition for inhibiting inflammation or aging by regulating the expression level of a specific gene in skin cells damaged by irritation of inflammation or aging to the normal level.

In one aspect of the present invention, genes in skin cells whose expression levels are affected by irritation of inflammation or aging specifically include: IL-1a (NM_000575), IL-1B (NM_000576), PTGS2 (NM_000963), and XDH (NM_000379). IL-1a (NM_000575), IL-1B (NM_000576), PTGS2 (NM_000963), XDH (NM_000379) are genes whose expression levels are increased by irritation of inflammation or aging. Thus, it is possible to inhibit the inflammation or aging of skin cells by suppressing the expression levels of these genes to the normal levels.

In one aspect, the genes used in the present invention and whose expression levels are increased by irritation of inflammation or aging are shown in Table 2. In this table, the term "name" denotes genebank accession ID of NCBI, the term "gene symbol" denotes official gene symbol, and the term "gene title" denotes the name of each gene.

Table 2

Increased genes		
Name	Gene Symbol	Gene title
NM_000576	IL1B	interleukin 1, beta
NM_000575	IL1A	interleukin 1, alpha
NM_000963	PTGS2	Cyclooxygenase-2 (COX-2)
NM_000379	XDH	xanthine dehydrogenase

Another aspect of the present invention includes the use of the composition of the present invention for enhancing the skin barrier.

In one aspect, the present invention provides a composition for enhancing the skin barrier by regulating the expression level of a specific gene in skin cells damaged by irritation inducing skin barrier weakness to the normal level.

In one aspect of the present invention, genes in skin cells whose expression levels are affected by irritation inducing skin barrier weakness specifically include: S100A7 (NM_002963), IL-36G (NM_019618) and LCE3D (NM_032563). S100A7 (NM_002963), IL-36G (NM_019618) and LCE3D (NM_032563) are genes whose expression levels are increased by irritation inducing skin barrier weakness. Thus, it is possible to enhance the skin barrier by suppressing the expression levels of these genes to the normal levels.

In one aspect, the genes used in the present invention and whose expression levels are increased by irritation inducing skin barrier weakness are shown in Table 3. In this table, the term "name" denotes genebank accession ID of NCBI, the term "gene symbol" denotes official gene symbol, and the term "gene title" denotes the name of each gene.

Table 3

Increased genes		
Name	Gene Symbol	Gene title
NM_002963	S100A7	S100 calcium binding protein A7
NM_019618	IL36G	interleukin 36, gamma
NM_032563	LCE3D	late cornified envelope 3D

Another aspect of the present invention provides a method for enhancing the skin barrier, comprising administering an effective amount of *Mentha arvensis* extract to a subject in need thereof.

Another aspect of the present invention provides the use of *Mentha arvensis* extract in the manufacture of

compositions for enhancing the skin barrier.

Another aspect of the present invention provides *Mentha arvensis* extract for use in enhancing the skin barrier.

In one aspect of the present invention, the composition may be a cosmetic composition, a pharmaceutical composition, or a health functional food composition.

Examples of the cosmetic composition include cosmetics such as various creams, lotions, and toners and cleansing products, cleansers, soaps, and cosmetic solutions.

In one aspect, the cosmetics comprising the composition containing the *Mentha arvensis* extract of the present invention may be in the form of a solution, an emulsion, a viscous mixture or the like.

That is, in one aspect, the formulation of the cosmetics of the present invention is not particularly limited and may be, for example, an emulsion, a cream, a toner, an essence, a pack, a gel, a powder, a makeup base, a foundation, a lotion, an ointment, a patch, a cosmetic solution, a cleansing foam, a cleansing cream, a cleansing water, a body lotion, a body cream, a body oil, a body essence, a shampoo, a rinse, a body cleanser, a soap, a hair dye, or a spray.

Ingredients other than the *Mentha arvensis* extract may be selected and added to the cosmetic composition of each formulation without difficulty by those skilled in the art according to the desired formulation or purpose.

In addition, in one aspect, the cosmetics of the present invention may comprise a composition selected from the group consisting of water-soluble vitamins, oil-soluble vitamins, polypeptides, polysaccharides, sphingolipids, and seaweed extracts.

In addition, in one aspect, the cosmetics of the present invention may contain ingredients that are generally used in cosmetics in addition to the essential ingredient, if necessary.

Examples of the additional ingredients include oil and fat ingredients, moisturizers, emollients, surfactants, organic and inorganic pigments, organic powders, UV absorbers, preservatives, sterilizers, antioxidants, plant extracts, pH adjusters, alcohols, colorants, fragrance, blood circulation stimulants, skin coolers, antiperspirants, purified water, etc.

However, the ingredients that may be contained in the cosmetics are not limited thereto. Also, the amount of any of the ingredients may be determined within a range not negatively affecting the purpose and effect of the present invention.

In one aspect, the pharmaceutical composition comprising the *Mentha arvensis* extract of the present invention may further comprise a suitable carrier, excipient and diluent conventionally used for preparation of pharmaceutical compositions.

The pharmaceutical composition comprising the *Mentha arvensis* extract may be formulated into any form suitable for pharmaceutical preparations, including oral formulations such as tablets, capsules, powders or syrups and agents for external application to the skin such as ointments, gels, creams, patches and sprays, according to a conventional method.

In general, it is to be understood that the actual dosage of the active ingredient administered by the pharmaceutical composition should be determined in light of various relevant factors such as the severity of the symptoms, the administration route, the age, gender, body weight and health condition of the subject. In general,

the dosage of the active ingredient may be 0.0001 mg/kg/day to 3000 mg/kg/day, for example, 10 mg/kg/day to 500 mg/kg/day.

In the health functional food composition according to one aspect of the present invention, the health food may refer to a food manufactured from a nutrient which is likely to be deficient in normal diets or a raw material or an ingredient (functional raw material) with a function useful for the human body, and which maintains and improves health by maintaining the normal function of the human body or activating the physiological functions, although not limited thereto. The health food may be manufactured and processed into the form of a tablet, a capsule, a powder, a granule, a liquid, a pill, etc. However, the formulation is not limited thereto, and it may be manufactured and processed into any form under the law.

Specifically, a health beverage composition has no particular limitation on ingredients other than the above compound contained in the predetermined ratio as an essential ingredient. It may contain various flavoring agents or natural carbohydrates as additional ingredients as in conventional beverages. Examples of natural carbohydrates are conventional sugars such as monosaccharide, polysaccharide and cyclodextrin and sugar alcohols such as xylitol, sorbitol, and erythritol. Also, natural flavoring agents (thaumatin, stevia extract (e.g., rebaudioside A, glycyrrhizin, etc.)) and synthetic flavoring agents (e.g., saccharin, aspartame, etc.) may be used as the flavoring agent.

In general, it is to be understood that the actual dosage of the active ingredient administered by the health functional food composition should be determined in light of various relevant factors such as the severity

of the symptoms, the administration route, the age, gender, body weight and health condition of the subject. In general, the dosage of the active ingredient may be 0.0001 mg/kg/day to 1000 mg/kg/day, for example, 0.02 mg/kg/day to 6 mg/kg/day.

Hereinafter, the constitution and effects of the present invention will be described in more detail with reference to examples. However, the following examples are provided for illustrative purposes only to facilitate understanding of the present invention, and the scope of the present invention is not limited thereto.

Example 1: Preparation of *Mentha arvensis* extract

Mentha arvensis leaves and *Acetobacter aceti* were prepared and fermented at 26°C for 48 hours. Then, the fermented product was extracted at room temperature using an extraction solvent obtained by mixing purified water and ethanol at a ratio of 3: 7, that is, 70% ethanol, as the extraction solvent. After extraction at room temperature, primary filtration was performed to remove the solid material contained in the extract. Then, the extract was concentrated to remove ethanol, followed by separation and purification. Then, the resultant was subjected to centrifugation and secondary filtration, and then dried to obtain *Mentha arvensis* extract.

Example 2: Microdust collection and extraction

Microdust was collected using a low volume air sampler (Sensidyne, Gillian, FL, U.S.A.). The filter and denuder of the filter pack were replaced at around 10:00 am on each measurement day to collect a sample for about 24 hours. Microdust was collected daily for 28 days in the downwind area of Seoul (Hankuk University of Foreign Studies, Center for International Studies, Residence Hall, 6th floor rooftop, Cheoin-gu, Yongin-si, Gyeonggi-do). The measurement time was measured by starting a timer

when turning on a vacuum pump and recording the time of the timer when turning off the vacuum pump. The collection flow rate was set at 16.7 L/min. The flow rate was measured with a flowmeter (Model 4143, TSI Inc.) at the start of the measurement and measured again at the end of the measurement. The Teflon filter in the filter pack was weighed before and after collection. The Teflon filter was dried to a constant weight in a desiccator (NIKKO, Japan) at a relative humidity of 40% for 24 hours before measuring the weight of the Teflon filter, and then weighed twice on a five decimal place electronic balance (DVG215CD, Ohaus). The mean value was recorded. After the sample was collected, the Teflon filter was again dried to a constant weight in a desiccator for 24 hours before measuring the weight, and then weighed twice. Then, the mass concentration was calculated by comparing the measured weight with the weight measured before collection. Microdust was extracted by wetting the Teflon filter with 1 mL of ethanol and putting 14 mL of DW so that the aerosol collector surface of the filter reached the water surface, closing the lid and then applying ultrasonic waves to the filter with an ultrasonic cleaner for 30 minutes. In order to minimize the error caused by moisture in the filtration step, moisture was completely removed from the filter for 48 hours in a desiccator. Then, the weight of the filter was measured using an ultra-precision scale that can measure down to 0.1 mg (Mettler Toledo Company) to measure the weights of the filter before and after extraction.

Example 3: Culture of keratinocyte lines (normal human)

Keratinocyte lines (normal human) were purchased from Lonza Inc. (Walkersville, Maryland, USA), subcultured, and then cultured in a CO₂ incubator under

the conditions of 37°C and 5% CO₂. The cell culture was performed in accordance with Lonza's guidelines. A KGM-2 bullet kit CC-4152 (ingredient: BPE (bovine pituitary extract)), human epidermal growth factor (hEGF), insulin, hydrocortisone, transferrin, epinephrine, and a KGM-2 bullet kit CC-3107 added with GA-1000 (gentamycin suflate + amphotericin-B) were added to 500ml of KBM-2 CC-3103 medium to prepare the medium used in the cell culture.

Example 4: Treatment of keratinocyte lines (normal human) with microdust and measurement of cytotoxicity

In order to investigate cytotoxicity of treatment with microdust, MTT assay was performed with keratinocyte lines (normal human) according to the method of Mossman et al. (J. Immunol. Methods, 65, 55-63, 1983).

Specifically, a 24-well plate was used. The microdust with a diameter of 2.5 µm obtained in Example 2 was dispersed in purified water to prepare a microdust dispersion. Then, the microdust dispersion was applied to cells cultured under the conditions of Example 3 and 2.5×10^5 cells per well, followed by culture for 24 hours. Then, the cells were mixed with 5 mg/ml of MTT (3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide) and further cultured at 37°C for 3 hours. The medium was then removed and the formazan crystal formed was dissolved in 500 µl of DMSO. The lysate was aliquoted in a 96-well plate and the OD value was measured at 540 nm. The measurement results are shown in FIG. 1.

As shown in FIG. 1, the concentration (IC₂₀) achieving an 80% survival rate in the cell line for cytotoxicity caused by a dispersion obtained by dispersing microdust of 2.5 micrometer or less was 12.5 µg/ml.

Example 5: Investigation of cell gene changes by microdust through next generation sequencing

In order to carry out RNA-base sequence data processing and analysis, reference was made to the general analysis step developed by Trapnell et al. (2012). The RNA-seq data quality was determined using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The base and adapter sequences with low accuracy were removed using FASTX (http://hannonlab.cshl.edu/fastx_toolkit/). Then, alignment was performed using Tophat (Trapnell et al., 2009) and a human genome (hg19), and the amount of data of each sample was determined using EVER-seq renamed to RSeQC (Wang et al., 2012). In addition, the expression level of transcripts was quantified using Cufflinks, and transcription levels were compared between the sample treated with a microdust dispersion and a normal sample (Trapnell et al., 2010). A stringent cut-off of ≥ 2.0 fold-change, with FDR adjusted p-value < 0.05 , was used to determine the gene that showed a significant expression difference when treated with a dispersion of microdust with a diameter of 2.5 μm . The measurement results are shown in the following Table 4 and FIG. 2A to FIG. 2G.

Table 4

Increased genes		
Name	Gene Symbol	Fold change
NM_000575	IL-1a	4.014605674
NM_000576	IL1B	11.31259177
NM_019618	IL36G	6.742761413
NM_002963	S100A7	9.515833375
NM_032563	LCE3D	11.96124722
NM_000963	PTGS2	2.284968542
NM_000379	XDH	2.57463302

Example 6: Real-time RT-PCR quantification

Normal human keratinocytes cultured in Example 3 was treated with the microdust having a diameter of 2.5 μm

extracted in Example 2 in the amount of 12.5 µg per 1 ml of the cell culture medium. Then, the relative mRNA expression level was measured using the primers (Applied Biosystems TaqMan® Primers) of genes indicated in Table 5 below. The extract prepared in Example 1 was used as the *Mentha arvensis* extract.

Table 5

Increased genes		
Name	Gene Symbol	TaqMan® primer
NM_000575	IL-1a	Hs00174092_m1
NM_000576	IL1B	Hs01555410_m1
NM_019618	IL36G	Hs00219742_m1
NM_002963	S100A7	Hs00161488_m1
NM_032563	LCE3D	Hs00754375_s1
NM_000963	PTGS2	Hs00153133_m1
NM_000379	XDH	Hs00166010_m1

The medium was treated with 20 ppm of *Mentha arvensis* extract. After 24 hours, the culture solution was removed and the cells were washed with 2 ml of phosphate buffered saline (PBS). Then, RNA was separated from the cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The separated RNA was further purified with an RNA kit (QIAGEN RNeasy kt, QIAGEN, Valencia, CA). Then, the quality of the RNA was determined using an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). cDNA was synthesized from the RNA using a reverse transcription kit (Superscript Reverse Transcriptase (RT) kit, Invitrogen, Carlsbad, CA). The cDNA was quantitatively analyzed by a real time-reverse transcription polymerase chain reaction (Q-RT-PCR) using the primers shown in Table 5. The changes of cell genes were evaluated by real-time PCR using TaqMan gene expression assay kit (Applied Biosystems, Foster City, CA). The results are shown in FIG. 3. Both of the Q-RT-

PCR and the real-time PCR were performed according to the standard protocols distributed by Life Technologies. Specifically, 40 cycles of 95°C for 20 seconds, 95°C for 3 seconds and 60°C for 30 seconds were performed.

FIG. 3A to FIG. 3G show that there exist genes whose expression levels increase or decrease in skin cells irritated by microdust. Also, it was found that the expression levels of interleukin-1 alpha (IL-1a), interleukin 1 beta (IL-1B), interleukin 36 gamma (IL-36G), S100 calcium binding protein A7 (S100A7), late cornified envelope 3D (LCE3D), prostaglandin-endoperoxide synthase 2 (PTGS2) and xanthine dehydrogenase (XDH) genes were reduced by treatment with *Mentha arvensis* extract.

In addition, FIG. 3A to FIG. 3G show that there exist genes whose expression levels increase in skin cells irritated by irritation of inflammation or aging. Also, it was found that the expression levels of interleukin 1 alpha (IL-1a), interleukin 1 beta (IL-1B), prostaglandin-endoperoxide synthase 2 (PTGS2) and xanthine dehydrogenase (XDH) genes were reduced by treatment with *Mentha arvensis* extract.

Therefore, it was found that *Mentha arvensis* extract effectively protect skin cells from damages of skin cells due to irritation of inflammation or aging and inhibits or prevents the expression levels of the above-described specific genes from changing due to irritation of inflammation or aging, thereby achieving the normal expression levels.

In addition, FIG. 3A to FIG. 3G show that there exist genes whose expression levels increase in skin cells irritated by irritation inducing skin barrier weakness. Also, it was found that the expression levels of interleukin 36 gamma (IL-36G), S100 calcium binding protein A7 (S100A7), and late cornified envelope 3D

(LCE3D) genes are reduced by treatment with *Mentha arvensis* extract.

Therefore, it was found that *Mentha arvensis* extract effectively protect skin cells from damages of skin cells due to irritation inducing skin barrier weakness and inhibits or prevents the expression levels of the above-described specific genes from changing due to irritation inducing skin barrier weakness, thereby achieving the normal expression levels.

Therefore, it was found that *Mentha arvensis* extract effectively protect skin cells from irritation due to microdust and inhibits or prevents the expression levels of the above-described specific genes from changing due to the irritation, thereby achieving the normal expression levels.

Hereinafter, formulation examples of the compositions according to the present invention will be described. However, the cosmetic composition, pharmaceutical composition and health functional food composition may be formulated into various other forms. These examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

Formulation Example 1: Tablet

100 mg of the *Mentha arvensis* extract according to an embodiment of the present invention, 400 mg of lactose, 400 mg of corn starch, and 2 mg of magnesium stearate were mixed and subjected to a tableting process according to a conventional method for preparing tablets to prepare tablets.

Table 6

Ingredients	Content (mg)
<i>Mentha arvensis</i> extract	100
Lactose	400
Corn starch	400

Magnesium stearate	2
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Formulation Example 2: Capsule

100 mg of the *Mentha arvensis* extract according to an embodiment of the present invention, 400 mg of lactose, 400 mg of corn starch, and 2 mg of magnesium stearate were mixed and filled in a gelatin capsule according to a conventional method for preparing capsules to prepare a capsule.

Table 7

Ingredients	Content (mg)
Mentha arvensis extract	100
Lactose	400
Corn starch	400
Magnesium stearate	2

Formulation Example 3: Granule

50 mg of the *Mentha arvensis* extract according to an embodiment of the present invention, 250 mg of anhydrous crystalline glucose, and 550 mg of starch were mixed and formulated into granules using a fluidized bed granulator. The granules were then filled in a pouch.

Table 8

Ingredients	Content (mg)
Mentha arvensis extract	50
Anhydrous crystalline glucose	250
Starch	550

Formulation Example 4: Soap

Table 9

Ingredients	Content (%)
Mentha arvensis extract	5.00
Oil and fat	q.s.
Sodium hydroxide	q.s.
Sodium chloride	q.s.
Fragrance	q.s.

Purified water	Balance
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Formulation Example 5: Lotion

Table 10

Ingredients	Content (%)
<i>Mentha arvensis</i> extract	5.00
L-ascorbic acid-2-phosphate magnesium salt	1.00
Water-soluble collagen (1% aqueous solution)	1.00
Sodium citrate	0.10
Citric acid	0.05
Licorice extract	0.20
1,3-butylene glycol	3.00
Purified water	Balance

Formulation Example 6: Cream

Table 11

Ingredients	Content (%)
<i>Mentha arvensis</i> extract	3.00
Polyethylene glycol monostearate	2.00
Self-emulsifying glycerin monostearate	5.00
Cetyl alcohol	4.00
Squalene	6.00
Glyceryl tri(2-ethylhexanoate)	6.00
Sphingoglycolipid	1.00
1,3-butylene glycol	7.00
Purified water	Balance

Formulation Example 7: Ointment

Table 12

Ingredients	Content (%)
<i>Mentha arvensis</i> extract	5.00
Polyvinyl alcohol	13.00
L-ascorbic acid-2-phosphate	1.00

magnesium salt	
Lauroyl hydroxyproline	1.00
Water-soluble collagen (1% aqueous solution)	2.00
1,3-butylene glycol	3.00
Ethanol	5.00
Purified water	Balance

Formulation Example 8: Preparation of a cosmetic solution

Table 13

Ingredients	Content (%)
Mentha arvensis extract	3.00
Hydroxyethylene cellulose (2% aqueous solution)	12.00
Xanthan gum (2% aqueous solution)	2.00
1,3-butylene glycol	6.00
Concentrated glycerin	4.00
Sodium hyaluronate (1% aqueous solution)	2.00
Purified water	Balance

Formulation Example 9: Health food

Table 14

Ingredients	Content
Mentha arvensis extract	2 mg
Vitamin A acetate	70 µg
Vitamin E	1.0 mg
Vitamin B1	0.13 mg
Vitamin B2	0.15 mg
Vitamin B6	0.5 mg
Vitamin B12	0.2 µg
Vitamin C	10 mg

Biotin	10 µg
Nicotinic acid amide	1.7 mg
Folic acid	50 µg
Calcium pantothenate	0.5 mg
Ferrous sulfate	1.75 mg
Zinc oxide	0.82 mg
Magnesium carbonate	25.3 mg
Potassium phosphate monobasic	15 mg
Calcium phosphate dibasic	55 mg
Potassium citrate	90 mg
Calcium carbonate	100 mg
Magnesium chloride	24.8 mg

Formulation Example 10: Health beverage

Table 15

Ingredients	Content
Mentha arvensis extract	50 mg
Citric acid	1000 mg
Oligosaccharide	100 g
Taurine	1 g
Purified water	Balance

While the present invention has been described with respect to the specific embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the invention as defined in the following claims.

CLAIMS

1. A composition for caring damages of skin cells by microdust, comprising *Mentha arvensis* extract as an active ingredient.
2. The composition according to claim 1, wherein the caring damages of skin cells by microdust is at least one selected from the group consisting of enhancement of the skin barrier, anti-aging and anti-inflammation.
3. A composition for enhancing the skin barrier, comprising *Mentha arvensis* extract as an active ingredient.
4. A composition for anti-aging, comprising *Mentha arvensis* extract as an active ingredient.
5. A composition for anti-inflammation, comprising *Mentha arvensis* extract as an active ingredient.
6. The composition according to any one of claims 1 to 5, wherein the content of the *Mentha arvensis* extract is 0.000001 to 30% by weight based on the total weight of the composition.
7. The composition according to any one of claims 1 to 5, wherein the *Mentha arvensis* extract is extracted with at least one extraction solvent selected from the group consisting of water, C₁-C₆ anhydrous or hydrated lower alcohol, acetone, butylene glycol, ethyl acetate, diethyl acetate, diethyl ether, benzene, chloroform, and

hexane.

8. The composition according to any one of claims 1 to 5,

wherein the *Mentha arvensis* extract is extracted from the leaves of *Mentha arvensis*.

9. The composition according to any one of claims 1 to 5,

wherein the *Mentha arvensis* is fermented *Mentha arvensis*, and

the fermentation is carried out by adding a microorganism of the genus *Acetobacter* sp.

10. The composition according to claim 9,

wherein the microorganism of the genus *Acetobacter* sp. is *Acetobacter aceti*.

11. The composition according to any one of claims 1 to 2,

wherein the composition inhibits the expression of at least one selected from the group consisting of IL-1a (NM_000575), IL-1B (NM_000576), IL-36G (NM_019618), S100A7 (NM_002963), LCE3D (NM_032563), PTGS2 (NM_000963), and XDH (NM_000379).

12. The composition according to claim 3,

wherein the composition inhibits the expression of at least one selected from the group consisting of IL-36G (NM_019618), S100A7 (NM_002963), and LCE3D (NM_032563).

13. The composition according to claim 4,

wherein the composition inhibits the expression of at least one selected from the group consisting of IL-1a

(NM_000575), IL-1B (NM_000576), PTGS2 (NM_000963), and XDH (NM_000379).

14. The composition according to claim 5,
wherein the composition inhibits the expression of
at least one selected from the group consisting of IL-1a
(NM_000575), IL-1B (NM_000576), and PTGS2 (NM_000963).

15. The composition according to any one of claims 11 to
14,
wherein the composition acts on keratinocytes.

16. The composition according to any one of claims 1 to
5,
wherein the *Mentha arvensis* extract is administered
at a dose of 10 to 500 mg/kg/day.

17. The composition according to any one of claims 1 to
5,
wherein the composition is a cosmetic composition.

18. The composition according to any one of claims 1 to
5,
wherein the composition is a pharmaceutical
composition.

19. The composition according to any one of claims 1 to
5,
wherein the composition is a health functional food
composition.