

DESCRIPTION

COMPOSITION FOR INTESTINAL BARRIER FUNCTION IMPROVEMENT

5 TECHNICAL FIELD

[0001]

The present invention relates to a composition for improving intestinal barrier function. The present invention also relates to, for example, a method of improving intestinal
10 barrier function.

BACKGROUND ART

[0002]

In recent years, there has been a greater awareness of
15 intestinal health, and many intestine-related functional foods have been commercially available. Main intestinal functions include a nutrient absorption function and a barrier function (intestinal barrier function) that prevents intrusion (permeation) of toxic substances. Of these, the intestinal
20 barrier function has been identified as being deeply involved in chronic inflammatory diseases that become more common with aging.

[0003]

A large number of immune cells such as macrophages,
25 dendritic cells, T-cells, and B-cells are present underneath intestinal epithelial cells. Typically, intestinal epithelial cells are tightly joined to each other by structures called tight junctions, so that high molecular weight substances are strictly controlled so as not to permeate the
30 intercellular space. Intestinal epithelial cells also contain transporters that discharge hydrophobic foreign substances from the cells. These tight junction structures, transporters, and the like are responsible for the intestinal barrier function that prevents intrusion of foreign substances. However, when
35 the intestinal barrier is damaged by aging, neglect of healthy

lifestyle, stress, and the like, thus increasing intestinal permeability, intestinal bacteria and high molecular weight substances such as bacterial components of intestinal bacteria, which are present in the intestine, enter the body through the intercellular space and promote inflammatory cytokine release by, for example, stimulating immune cells, thereby inducing inflammation. Presumably, this induces various pathological conditions due to chronic inflammation, such as inflammatory bowel disease in the intestine, nonalcoholic fatty liver disease (NAFLD) in the liver, arteriosclerosis-induced symptoms in the blood vessel, generalized diabetes, abnormal lipid metabolism, and autoimmune disease. Assumedly, intrusion of an undigested substance from the intestine induces allergies.

[0004]

As described above, poor intestinal barrier function may cause various diseases or the like. From this point of view, attempts have been made to search for substances that can improve the intestinal barrier function. Epithelial cell growth factor (EGF) is known to promote maturation of intestinal epithelial cells and enhance a barrier function. However, EGF, which is a cytokine, is present in only a small amount in the living body, so that it is not preferable in terms of economic efficiency and safety to use EGF as a material for improving the intestinal barrier function. According to Non-Patent Literature 1, flavonoids such as quercetin promote formation of tight junctions or the like and prevent chronic inflammation.

CITATION LIST

- Non-Patent literature

[0005]

Non-Patent literature 1: Suzuki T. et al, The Journal of Nutritional Biochemistry. 2011 May, Vol. 22 (5), pp. 401-408

SUMMARY OF INVENTION

- Technical Problem

[0006]

5 An object of the present invention is to provide, for example, a novel composition for improving intestinal barrier function, the composition being capable of improving intestinal barrier function.

- Solution to Problem

10 [0007]

In order to solve the above problems, the present inventors have made intensively studies. They added inflammatory cytokines to an intestinal permeability model with cultured human intestinal cell line Caco-2 to produce a state that can cause the intestinal barrier function to fail in humans, and found a substance that can ameliorate the state. In this manner, they attempted to solve the problems. As a result, the present inventors found that ursane-type triterpenes and their derivatives are useful for improving the intestinal barrier function.

20 [0008]

Specifically, the present invention relates to, for example, the following compositions for improving intestinal barrier.

25 (1) A composition for improving intestinal barrier function, containing at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts, as an active ingredient.

(2) The composition for improving intestinal barrier function according to (1) above, wherein the compound is at least one compound selected from the group consisting of acetyl ursolic acid, ursolic acid, an ursolic acid glycoside, and their salts.

30 (3) The composition for improving intestinal barrier function according to (1) or (2) above, wherein the compound

35

is at least one of acetyl ursolic acid or its salt.

(4) The composition for improving intestinal barrier function according to (2) or (3) above, wherein the acetyl ursolic acid is 3-acetyl ursolic acid.

5 (5) The composition for improving intestinal barrier function according to any one of (1) to (4) above, wherein the composition is an oral composition.

(6) The composition for improving intestinal barrier function according to (5), wherein the oral composition is a
10 food or beverage, a pharmaceutical product, or a quasi-pharmaceutical product.

(7) The composition for improving intestinal barrier function according to any one of (1) to (6) above, wherein the composition is used for intestinal regulation.

15 (8) The composition for improving intestinal barrier function according to any one of (1) to (7) above, wherein the composition is labeled as having an intestinal regulation action.

(9) Use of at least one compound selected from the group
20 consisting of an ursane-type triterpene, its derivative, and their salts for improving intestinal barrier function.

- Advantageous Effects of Invention [0009]

25 The present invention can provide, for example, a novel composition for improving intestinal barrier function, the composition being capable of improving intestinal barrier function. The present invention can improve the intestinal barrier function. The present invention can contribute to
30 prevention or amelioration of conditions or diseases associated with intestinal barrier dysfunction by improving the intestinal barrier function.

DESCRIPTION OF EMBODIMENTS

35 [0010]

The present invention is described in detail below. The following embodiments are examples to describe the present invention, and the present invention is not intended to be limited to these embodiments. The present invention can be implemented in various forms without departing from the gist thereof.

[0011]

The composition for improving intestinal barrier function of the present invention contains at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts, as an active ingredient.

Herein, the at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts is also referred to as an "active ingredient compound used in the present invention". In the present invention, one or more of the compounds can be used as an active ingredient(s).

[0012]

Examples of the ursane-type triterpene include ursolic acid, corosolic acid, and tormentic acid. Examples of the derivative of the ursane-type triterpene include ursane-type triterpene glycosides and ursane-type triterpenes having an acetyl group.

The ursane-type triterpene glycosides are glycosides whose aglycones are ursane-type triterpenes. For example, an ursolic acid glycoside is a glycoside whose aglycone is ursolic acid.

Examples of the ursane-type triterpenes having an acetyl group include acetyl ursolic acid, acetyl corosolic acid, and acetyl tormentic acid. Preferably, the ursane-type triterpene or its derivative in the present invention is acetyl ursolic acid, ursolic acid, or an ursolic acid glycoside. Preferably, a saccharide to form the ursane-type triterpene glycoside is a monosaccharide, a disaccharide, or the like. Examples of the monosaccharide include glucose, galactose, rhamnose, fructose,

mannose, and arabinose. Examples of the disaccharide include maltose, sucrose, and lactose.

[0013]

5 The salts of the ursane-type triterpene and its derivative are not limited but are preferably those that can be orally ingested. Examples of such salts include alkali metal salts such as sodium and potassium; alkaline earth metal salts such as calcium; and ammonium salt.

[0014]

10 Preferably, the active ingredient compound used in the present invention is at least one compound selected from the group consisting of acetyl ursolic acid, ursolic acid, an ursolic acid glycoside, and their salts. Of these, acetyl ursolic acid and/or its salt are/is more preferred as the active
15 ingredient(s), because their intestinal barrier function improvement action is significant.

[0015]

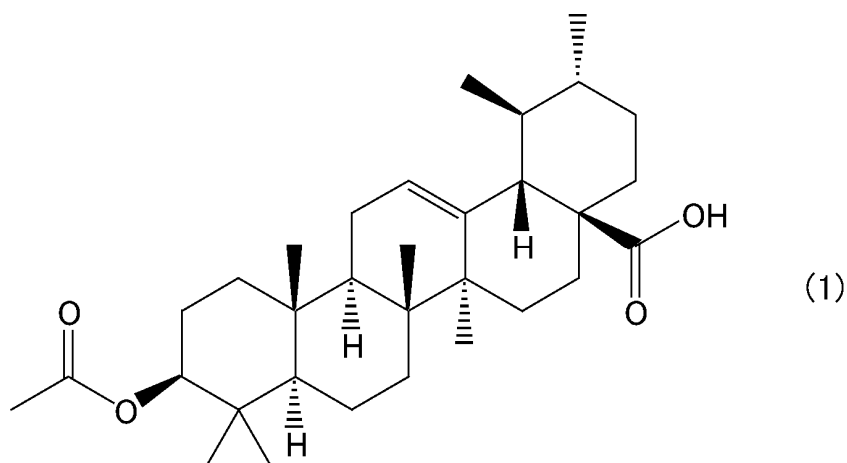
20 Preferably, the acetyl ursolic acid is 3-acetyl ursolic acid in which an acetyl group is bonded to the hydroxy group at position 3 of ursolic acid. More preferably, the composition for improving intestinal barrier function of the present invention contains 3-acetyl ursolic acid and/or its salt as active ingredient(s).

[0016]

25 3-Acetyl ursolic acid is a compound represented by the following formula (1).

[0017]

[Chem. 1]



[0018]

The ursane-type triterpene, its derivative, and their salts contained in the composition for improving intestinal barrier function of the present invention are not limited by the source or production method. For example, a plant-derived compound extracted from a plant may be used, or a synthetic compound may be used. A commercial product can also be used.

For example, the source of plant-derived acetyl ursolic acid, ursolic acid, ursolic acid glycoside, and their salts is preferably at least one plant selected from the group consisting of a plant of the genus *Aronia* of the family Rosaceae, a plant of the genus *Rubus* of the family Rosaceae, a plant of the genus *Chaenomeles* of the family Rosaceae, a plant of the genus *Malus* of the family Rosaceae, a plant of the genus *Pyrus* of the family Rosaceae, a plant of the genus *Arctostaphylos* of the family Ericaceae, a plant of the genus *Vaccinium* of the family Ericaceae, a plant of the genus *Syzygium* of the family Myrtaceae, a plant of the genus *Swertia* of the family Gentianaceae, a plant of the genus *Ternstroemia* of the family Pentaphylacaceae, a plant of the genus *Ilex* of the family Aquifoliaceae, a plant of the genus *Ficus* of the family Moraceae, a plant of the genus *Olea* of the family Oleaceae, and a plant of the genus *Eucommia* of the family Eucommiaceae.

[0019]

In the present invention, the source of 3-acetyl ursolic

acid and its salt is preferably at least one plant selected from the group consisting of a plant of the genus *Aronia* of the family Rosaceae, a plant of the genus *Rubus* of the family Rosaceae, a plant of the genus *Chaenomeles* of the family Rosaceae, a plant of the genus *Ternstroemia* of the family Pentaphylacaceae, a plant of the genus *Ilex* of the family Aquifoliaceae, a plant of the genus *Ficus* of the family Moraceae, and a plant of the genus *Eucommia* of the family Eucommiaceae.

[0020]

In addition, when the source of 3-acetyl ursolic acid and its salt is a plant of the genus *Aronia* of the family Rosaceae, the plant of the genus *Aronia* of the family Rosaceae is preferably at least one selected from the group consisting of *Aronia arbutifolia*, *Aronia melanocarpa*, and *Aronia x prunifolia*.

Aronia melanocarpa is also known as chokeberry or black chokeberry. In the case of plant extraction, for example, a raw material containing the active ingredient compound used in the present invention is extracted with water-containing ethanol, and the liquid extract is concentrated and then purified with a column, whereby the active ingredient compound used in the present invention can be obtained.

[0021]

The active ingredient compound used in the present invention is a compound present in the above plants, and is highly safe with few side effects even with long-term intake. The present invention can provide the composition for improving intestinal barrier function, the composition containing, as an active ingredient, a highly safe substance with few side effects even with long-term intake.

[0022]

In the present invention, the "intestinal barrier function" means the function that prevents intrusion (permeation) of foreign substances (e.g., toxins such as endotoxin, inflammatory substances, and undigested products)

into the body from the outside of intestinal epithelial cells (the inside of the intestine). The intestine includes the large intestine and the small intestine. A state where intrusion of foreign substances into the body from the outside of intestinal epithelial cells is promoted as compared to the normal state is referred to as a "state where intestinal epithelial cells have increased (raised) permeability to foreign substances". The "intestinal barrier function improvement" means both suppression of the increase in the permeability of intestinal epithelial cells to foreign substances and decrease in the permeability of intestinal epithelial cells to foreign substances. In the present invention, the term "intestinal barrier function improvement" is also used to mean to include suppression of the decrease in the intestinal barrier function and enhancement of the decreased intestinal barrier function. For example, the intestinal barrier function is improved by normalizing or enhancing tight junctions that bind intestinal epithelial cells together. In one embodiment, the composition for improving intestinal barrier of the present invention may be used to improve the intestinal barrier function by normalizing or enhancing tight junctions of intestinal epithelial cells.

[0023]

The intestinal barrier function improvement effect is indicated by, for example, an increase in the electrical resistance value (transepithelial electric resistance: TEER) of intestinal epithelial cells or suppression of the decrease in the TEER. A substance that increases the TEER or suppresses the decrease in the TEER has the action of normalizing or enhancing tight junctions of intestinal epithelial cells. The intestinal barrier function improvement effect is also indicated by a decrease in the amount of a substance that permeates intestinal epithelial cells from the intestinal side into the body.

A person skilled in the art can select a specific

evaluation method for the intestinal barrier function improvement effect depending on the purpose. For example, as shown in Examples (described later), a method of measuring the TEER using an intestinal permeability model with human intestinal epithelial cells (Caco-2 cells) can be used. Specifically, inflammatory cytokines (TNF- α , IL-1 β , IFN- γ , and the like) are added into Caco-2 monolayer cultured cells to produce a state that can cause the intestinal barrier function to fail in humans. If addition of a test substance suppresses the decrease in the TEER as compared to the case where the substance is not added, the test substance can be evaluated as having the intestinal barrier function improvement effect.

As shown in Examples, acetyl ursolic acid and ursolic acid suppressed the decrease in the TEER caused by the addition of the inflammatory cytokines in the intestinal permeability model with Caco-2. This indicates that these acids have an excellent intestinal barrier function improvement effect. In addition, as shown in Examples, acetyl ursolic acid showed a superior intestinal barrier function improvement effect to ursolic acid not having an acetyl group.

[0024]

The composition for improving intestinal barrier function of the present invention contains at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts, as an active ingredient, whereby the intestinal barrier function improvement effect is achieved. Thus, the composition for improving intestinal barrier function of the present invention is useful for preventing or ameliorating conditions or diseases against which improvement of the intestinal barrier function is effective in prevention, amelioration, or the like. For example, the composition is effective in prevention or amelioration of conditions or diseases associated with intestinal barrier dysfunction. The intestinal barrier dysfunction includes poor intestinal barrier function. Examples of the conditions or

diseases associated with intestinal barrier dysfunction include conditions or diseases caused by intestinal barrier dysfunction, or conditions or diseases involving intestinal barrier dysfunction. Examples of such conditions or diseases associated with intestinal barrier dysfunction include inflammatory bowel disease, irritable bowel syndrome, systemic autoimmune diseases (rheumatoid arthritis, erythematosus, and the like), allergies (food allergy, hay fever, and the like), and metabolic syndromes (obesity, type I or type II diabetes mellitus, hypertension, hyperlipidemia, nonalcoholic fatty liver disease (NAFLD), arteriosclerosis, and the like) (e.g., Camilleri et al., *Am. J Physiol Gastrointest Liver Physiol* 303: G775-G785, 2012; Mu et al., *Front. Immunol.*, Vol. 8, Article 598, 2017; Bischoff et al., *BMC Gastroenterology* 2014 14:189). [0025]

More specific examples of symptoms of the conditions or diseases associated with intestinal barrier dysfunction include symptoms such as diarrhea, constipation, and discomfort (bloating, borborygmi, abdominal pain, and the like) of the gut (abdominal part). The composition for improving intestinal barrier function of the present invention has the action of improving intestinal conditions by improving the intestinal barrier function. Thus, the composition for improving intestinal barrier function of the present invention can regulate the intestine by the intestinal barrier function improvement, and is useful for preventing or ameliorating intestinal symptoms as described above. In one embodiment, the composition for improving intestinal barrier function of the present invention may be used for intestinal regulation (in order to prevent or ameliorate, for example, diarrhea, constipation, and abdominal discomfort). The intestinal barrier dysfunction is also associated with metabolic syndromes and the like (e.g., Bischoff et al., *BMC Gastroenterology* 2014 14:189 described above). Improving the intestinal barrier function is also effective in prevention or amelioration of

metabolic syndromes. Examples of symptoms of metabolic syndromes include abnormal glucose metabolism, abnormal lipid metabolism, increase in body fat, increase in visceral fat, increase in abdominal circumference fat, and higher blood pressure. Thus, the composition for improving intestinal barrier function of the present invention can contribute to improving glucose metabolism, improving lipid metabolism, reducing fat such as body fat, visceral fat, or abdominal circumference fat or suppressing the increase of such fat, lowering higher blood pressure, and the like, by improving the intestinal barrier function.

[0026]

Herein, the phrase "prevention of conditions or diseases" refers to enhancing the resistance of a subject to conditions or diseases, delaying or preventing the onset of conditions or diseases, or the like. Herein, the phrase "amelioration of conditions or diseases" refers to helping a subject recovery from conditions or diseases, alleviating symptoms of conditions or diseases, delaying or preventing the progress of conditions or diseases, or the like. The term "recovery" includes at least partial recovery.

[0027]

The composition of the present invention is applicable for both therapeutic use (medical use) and non-therapeutic use (non-medical use).

The composition for improving intestinal barrier function of the present invention can be provided, for example, in the form of a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, but it is not limited thereto. The composition for improving intestinal barrier function of the present invention may be a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like by itself, or may be a formulation or material such as an additive used therefor. For example, the composition for improving intestinal barrier function of

the present invention can be provided in the form of an agent, but it is not limited thereto. Such an agent may be provided as a composition by itself or as a composition containing the agent. In one embodiment, the composition for improving
5 intestinal barrier function of the present invention can also be referred to as an "intestinal barrier function improving agent".

In one embodiment, the composition for improving intestinal barrier function of the present invention is
10 preferably an oral composition. The present invention can provide an oral composition having an excellent intestinal barrier function improvement action. The oral composition may be a food or beverage, a pharmaceutical product, or a quasi-pharmaceutical product, and is preferably a food or
15 beverage.

[0028]

The composition for improving intestinal barrier function of the present invention may contain one or more ingredients (other ingredients) in addition to the
20 above-described active ingredient compound used in the present invention, as long as the effect of the present invention is not impaired.

In one embodiment, the composition may contain, for example, ingredients such as lactic acid bacteria,
25 bifidobacteria, dietary fiber, and polysaccharides as the other ingredients. Lactic acid bacteria and bifidobacteria that can be orally ingested are preferred.

[0029]

The dietary fiber may be either a water-insoluble dietary
30 fiber or a water-soluble dietary fiber. Examples of the water-insoluble dietary fiber include cellulose, lignin, hemicellulose, wheat bran, apple fiber, sweet potato fiber, and chitin. The water-soluble dietary fiber is roughly divided into a high viscous dietary fiber and a low viscous dietary fiber.
35 Examples of the high viscous dietary fiber include pectin,

konjac mannan, alginic acid, sodium alginate, guar gum, and agar. Among the types of dietary fiber commonly known in Japan, the low viscous water-soluble dietary fiber refers to a dietary fiber material that contains 50% by weight or more dietary fiber and that dissolves in water at room temperature to provide a low viscous solution, i.e., an about 5% by weight aqueous solution having a viscosity of 20 mPa·s or less. Examples of the low viscous water-soluble dietary fiber include hardly digestible dextrin, polydextrose, partially hydrolyzed guar gum, and Litesse (polydextrose). Other examples of the low viscous water-soluble dietary fiber include any dietary fiber materials that satisfy low viscous and water-soluble properties. One type of the dietary fiber may be used, or two or more types thereof may be used.

15 [0030]

Examples of the polysaccharides include oligosaccharides such as galactooligosaccharides, xylooligosaccharides, mannoooligosaccharides, agarooligosaccharides, fructooligosaccharides, isomaltooligosaccharides, and raffinose. One of these oligosaccharides may be used, or two or more thereof may be used.

[0031]

The composition for improving intestinal barrier function of the present invention can contain optional additives and optional ingredients in addition to those described the above. Such additives and ingredients may be selected depending on the form of the composition for improving intestinal barrier function, for example, and those generally usable for food and beverages, pharmaceutical products, quasi-pharmaceutical products, feed, and the like can be used. Examples thereof include various additives that are acceptable as agents for oral administration for food and beverages or that are pharmaceutically acceptable, such as excipients, lubricants, stabilizers, dispersants, binders, diluents, flavors, sweeteners, flavoring agents, and colorants. For

example, when the composition for improving intestinal barrier function of the present invention is provided as an oral composition, the oral composition can appropriately contain, in addition to those describe above, ingredients that can be orally ingested, as long as the effect of the present invention is not impaired. Examples of such ingredients include vitamins, vitamin-like substances, proteins, amino acids, fats and oils, organic acids, carbohydrates, plant-derived raw materials, animal-derived raw materials, microorganisms, additives for food and beverages, and additives for pharmaceutical products.

In addition to those described above, ingredients such as materials used for food and beverages, pharmaceutical products, quasi-pharmaceutical products, feed, and the like can be appropriately added depending on the use.

[0032]

The form of the composition for improving intestinal barrier function of the present invention is not limited as long as the effect of the present invention is achieved.

In one embodiment, when the composition for improving intestinal barrier function of the present invention is provided as a food or beverage, ingredients (e.g., materials for food and beverages, and additives that are used if necessary) usable for food and beverages can be added to the active ingredient compound used in the present invention to provide various food and beverages (compositions for food and beverages). The food or beverage is not limited. Examples thereof include general food and beverages, health foods, foods with function claims, foods for specified health uses, foods for the sick, food additives, and raw materials thereof. The form of the food or beverage is also not limited. Examples thereof include various formulation forms including solid oral formulations such as tablets, coated tablets, fine granules, granules, powders, pills, capsules (including soft and hard capsules), dry syrups, and chewable tablets; and liquid oral formulations such as oral solutions and syrups.

[0033]

In one embodiment, when the composition for improving intestinal barrier function of the present invention is provided as a pharmaceutical product or quasi-pharmaceutical product, an additive such as a pharmaceutically acceptable excipient can be added to the active ingredient compound used in the present invention to provide pharmaceutical products (pharmaceutical compositions) or quasi-pharmaceutical products (quasi-pharmaceutical product compositions) of various dosage forms. Preferably, the administration form of the pharmaceutical product or quasi-pharmaceutical product is oral administration. The dosage form of the pharmaceutical product or quasi-pharmaceutical product may be one suitable to the administration form. Examples of the dosage form of the oral pharmaceutical product or quasi-pharmaceutical product include solid oral formulations such as tablets, coated tablets, fine granules, granules, powders, pills, capsules (including soft and hard capsules), dry syrups, and chewable tablets; and liquid oral formulations such as oral solutions and syrups.

[0034]

The tablets, pills, and granules may be dosage forms in which a conventional coating is applied if necessary, such as sugar-coated tablets, gelatin-coated preparations, enteric-coated preparations, and film-coated agents. The tablets can also be multiple layer tablets (e.g., double layer tablets).

[0035]

When the composition for improving intestinal barrier function of the present invention is provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, the production method is not limited, and the composition for improving intestinal barrier function can be produced by a general method using the active ingredient compound used in the present invention. The present invention also includes use of at least one compound selected from the

group consisting of an ursane-type triterpene, its derivative, and their salts in production of the composition for improving intestinal barrier function.

[0036]

5 In the composition for improving intestinal barrier function of the present invention, one or more of items of the following information may be indicated on its package, container, package insert, or the like: usage, types of active ingredients, the above-described effects, and instructions for
10 use (e.g., ingestion method or administration method). The composition for improving intestinal barrier function of the present invention may be labeled as having an intestinal barrier function improvement action or an action based on the intestinal barrier function improvement action. For example, the
15 composition may be labeled as having an intestinal regulation action.

 The intestinal regulation action is not limited as long as it is based on improvement of the intestinal barrier function. Examples of such labelling as having an intestinal regulation
20 action include "for those prone to constipation or diarrhea", "for those concerned about gut function", "for those susceptible to gut discomfort", "improvement in bowel movement", "improvement in fecal conditions", "improvement in the frequency of defecation", "better control of the amount of
25 defecation", "good feeling in the gut", "control of gut function", "regulation of the intestine", "reduction of gut discomfort", "reduction of gas generation", "reduction of abdominal bloating", and "reduction of borborygmi". The composition for improving intestinal barrier function of the
30 present invention may be labeled with one or more of these functions.

[0037]

 The amount of the active ingredient compound in the composition for improving intestinal barrier function of the
35 present invention can be appropriately set depending on the form

of the composition and the like. In one embodiment, when the composition for improving intestinal barrier function is provided as an oral composition such as a food or beverage, a pharmaceutical product, or a quasi-pharmaceutical product, the total amount of the ursane-type triterpene, its derivative, and their salts in the composition is preferably 0.0001 wt% or more, more preferably 0.01 wt% or more, and is preferably 80 wt% or less, more preferably 20 wt% or less. In one embodiment, the total amount of the ursane-type triterpene, its derivative, and their salts in the composition for improving intestinal barrier function is preferably 0.0001 to 80 wt%, more preferably 0.01 to 20 wt%.

The amount of the ursane-type triterpene, its derivative, and their salts can be measured according to known methods, such as HPLC.

[0038]

The composition for improving intestinal barrier function of the present invention can be ingested or administered by a suitable method depending on the form. Preferably, the composition for improving intestinal barrier function of the present invention is orally administered or orally ingested.

The intake (which can also be referred to as "dose") of the composition for improving intestinal barrier function of the present invention is not limited, as long as it is an amount that achieves the intestinal barrier function improvement effect. The intake may be appropriately set according to the administration form or administration method, for example. In one embodiment, in the case of oral administration to or ingestion by a human (adult), the daily intake of the composition for improving intestinal barrier function in terms of the total intake of the ursane-type triterpene, its derivative, and their salts may be 0.01 to 5000 mg, preferably 0.1 to 3000 mg, more preferably 1 to 1500 mg, still more preferably 1 to 1000 mg. Preferably, the composition in the

above amount is orally administered or ingested, for example, once daily or in two or three portions daily. In one embodiment, in the case of ingestion of the composition for improving intestinal barrier function by a human (adult) for the purpose of achieving the intestinal barrier function improvement effect, the composition for improving intestinal barrier function can be orally ingested by or administered to the subject in such a manner that the total intake of the ursane-type triterpene, its derivative, and their salts is in the above ranges. The intake may be a daily intake per 60 kg body weight. In one embodiment, the composition for improving intestinal barrier function of the present invention may be an oral composition for allowing the daily amount of the active ingredient compound used in the present invention to be ingested by or administered to an adult (60 kg body weight).

[0039]

In one embodiment, preferably, the composition for improving intestinal barrier function of the present invention contains the active ingredient compound used in the present invention in an amount that achieves a desired effect of the present invention, i.e., the effective amount, taking into account the administration form, administration method, and the like. In one embodiment, for example, when the composition for improving intestinal barrier function is an oral composition such as a food or beverage, an oral pharmaceutical product, or the like, the daily total amount of the ursane-type triterpene, its derivative, and their salts in the daily intake of the composition per adult may be 0.01 to 5000 mg, preferably 0.1 to 3000 mg, more preferably 1 to 1500 mg, still more preferably 1 to 1000 mg.

[0040]

The active ingredient compound used in the present invention is likely to increase the intestinal barrier function improvement effect by continuous intake (administration). In a preferred embodiment, the composition for improving

intestinal barrier function of the present invention is intended for continuous intake. In one embodiment of the present invention, at least one-week continuous intake of the composition for improving intestinal barrier function is preferred.

[0041]

A subject to which or by which the composition for improving intestinal barrier function of the present invention is administered or ingested (hereinafter, also simply referred to as "administration subject") is preferably a human or non-human animal, more preferably a human or non-human mammal, still more preferably a human. Preferably, the administration subject in the present invention is one who needs or wants to improve the intestinal barrier function. Preferred examples of the subject include those who have poor intestinal barrier function, those who wish to prevent or ameliorate any of the above-described conditions or diseases associated with intestinal barrier dysfunction, and those who need to prevent or ameliorate such conditions or diseases.

[0042]

The present invention also encompasses the following method of improving intestinal barrier function, and the like:

a method of improving intestinal barrier function, including administering at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts to a subject; and

use of at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts for improving intestinal barrier function.

The method and use may be therapeutic or non-therapeutic. The "non-therapeutic" is a concept that does not include medical activities, i.e., a concept that does not include methods of surgery, therapy or diagnosis of humans. The active ingredient compound used in the present invention can be used to improve the intestinal barrier function of the subject by being

administered to (ingested by) the subject. In one embodiment, the active ingredient compound used in the present invention is suitably used to improve the intestinal barrier function by normalizing or enhancing tight junctions of intestinal
5 epithelial cells.

[0043]

The dose of the at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts is not limited as long as it is the amount that
10 achieves the intestinal barrier function improvement effect, i.e., the effective amount. For example, administration of the above amount of the compound is preferred. Preferably, the administration route is oral administration. The compound may be administered directly, or a composition containing the
15 compound may be administered. For example, the above-described composition for improving intestinal barrier function of the present invention can be administered. The active ingredient compound used in the present invention, subject (administration subject), administration method, dose,
20 preferred embodiments thereof, and the like are the same as those in the composition for improving intestinal barrier function described above. In one embodiment, for example, in the case of oral administration to a human (adult), the daily total dose of the ursane-type triterpene, its derivative, and
25 their salts may be 0.01 to 5000 mg, preferably 0.1 to 3000 mg, more preferably 1 to 1500 mg, still more preferably 1 to 1000 mg. In the method and use, preferably, the active ingredient compound used in the present invention is administered continuously for one week or more.

30

EXAMPLES

[0044]

The following provides Examples that more specifically describe the present invention. The present invention is not
35 limited to these Examples.

[0045]

<Example 1>

The intestinal barrier function improvement was comparatively evaluated.

5 (Method of evaluating intestinal barrier function improvement action)

Caco-2 cells were cultured at 37°C for three weeks in DMEM (Dulbecco's modified Eagle's medium) on Transwell (available from Millicell Corporation). The medium was removed from the
10 plate of cultured Caco-2 cells, and the well was washed three times with serum-free DMEM. The well was filled with the medium. Subsequently, the transepithelial electrical resistance (TEER) of Caco-2 monolayer cells was measured by Millicell-ERS (available from Millipore Corporation). Cells (TEER $\geq$ 1000
15 $\Omega \cdot \text{cm}^2$) determined as having sufficiently tight junctions were selected, and used in the next screening. Then, a sample (described later), TNF- α (40 ng/mL), IL-1 β (20 ng/mL), and IFN- γ (10 ng/mL) were added to test solutions (media) on both apical and basolateral membrane sides and cultured for 48 hours. The
20 sample was dissolved in dimethyl sulfoxide (DMSO) before being added to the test solutions. At this point, a well to which inflammatory cytokines (TNF- α , IL-1 β , and IFN- γ) and the sample were not added was provided as normal. A well to which inflammatory cytokines were added but the sample was not added
25 was provided as control. After culturing, the TEER was measured again to evaluate whether the sample suppressed the reduction (decrease) in the TEER by the inflammatory cytokines.

[0046]

The TEER reduction suppression ratio (%) by the sample
30 was determined according to the following formula from the TEER values of the well to which the sample was added, normal, and control.

(Calculating formula of TEER reduction suppression ratio)

TEER reduction suppression ratio (%) = $100 \times ((\text{TEER of well to which sample is added}) - (\text{TEER of control})) / ((\text{TEER of normal})$
35

- (TEER of control))

In this evaluation system, a higher TEER reduction suppression ratio (%) indicates a more significant intestinal barrier function improvement action.

5 [0047]

Samples used were commercially available standards of 3-acetyl ursolic acid (available from Ark Pharm), 3-acetyl oleanolic acid (available from Ark Pharm), ursolic acid (available from United States Pharmacopeial Convention), and
10 oleanolic acid (available from ChromaDex). Each sample was added to each test solution to obtain a concentration of 5 µg/mL. Quercetin was evaluated as positive control. Quercetin used was a commercially available standard (available from Funakoshi Co., Ltd.). Quercetin was added to each test solution to obtain
15 a concentration of 9 µg/mL.

[0048]

Table 1 shows the results. 3-Acetyl ursolic acid and ursolic acid each had the intestinal barrier function improvement action. 3-Acetyl ursolic acid was found to have
20 a more significant intestinal barrier function improvement action than the ursolic acid not having an acetyl group. 3-Acetyl ursolic acid had the intestinal barrier function improvement action comparable to that of quercetin known as an agent to protect intestinal barrier, at a lower concentration
25 than that of quercetin.

[0049]

[Table 1]

Samples	Concentration ($\mu\text{g/mL}$)	TEER reduction suppression ratio (%)
3-Acetyl ursolic acid	5	24
Ursolic acid	5	18
3-Acetyl oleanolic acid	5	-13
Oleanolic acid	5	-23
Quercetin	9	25

INDUSTRIAL APPLICABILITY

[0050]

- 5 The composition for improving intestinal barrier function of the present invention is useful in the fields of food and beverages, medicines, and the like.

CLAIMS

Claim 1. A composition for improving intestinal barrier function, comprising:

5 at least one compound selected from the group consisting of an ursane-type triterpene, its derivative, and their salts, as an active ingredient.

Claim 2. The composition for improving intestinal barrier
10 function according to claim 1,

 wherein the compound is at least one compound selected from the group consisting of acetyl ursolic acid, ursolic acid, an ursolic acid glycoside, and their salts.

Claim 3. The composition for improving intestinal barrier
15 function according to claim 1 or 2,

 wherein the compound is at least one of acetyl ursolic acid or its salt.

Claim 4. The composition for improving intestinal barrier
20 function according to claim 2 or 3,

 wherein the acetyl ursolic acid is 3-acetyl ursolic acid.

Claim 5. The composition for improving intestinal barrier
25 function according to any one of claims 1 to 4,

 wherein the composition is an oral composition.

Claim 6. The composition for improving intestinal barrier
function according to claim 5,

30 wherein the oral composition is a food or beverage, a pharmaceutical product, or a quasi-pharmaceutical product.

Claim 7. The composition for improving intestinal barrier
function according to any one of claims 1 to 6,

wherein the composition is used for intestinal regulation.

Claim 8. The composition for improving intestinal barrier
5 function according to any one of claims 1 to 7,
wherein the composition is labeled as having an
intestinal regulation action.

Claim 9. Use of at least one compound selected from the
10 group consisting of an ursane-type triterpene, its derivative,
and their salts for improving intestinal barrier function.