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(54) Title: NEW ASSOCIATION OF ACTIVE INGREDIENTS OF PLANT ORIGIN, COMPOSITIONS CONTAINING IT AND USE THEREOF IN THERAPY

(57) Abstract: The present invention relates to a new association of active ingredients of plant origin, to the pharmaceutical, nutraceutical and food compositions containing it and to their uses in therapy, in particular for the treatment and prevention of autoimmune diseases whose physiopathology is associated with an increased intestinal permeability and dysbiosis.

WO 2024/252335 A1

NEW ASSOCIATION OF ACTIVE INGREDIENTS OF PLANT ORIGIN, COMPOSITIONS CONTAINING IT AND USE THEREOF IN THERAPY

The present invention relates to a new association of active ingredients of plant origin, to the pharmaceutical, nutraceutical and food compositions containing it and to the uses thereof in therapy, in particular for the treatment and prevention of autoimmune diseases, preferably autoimmune diseases whose physiopathology is associated with an alteration of the intestinal barrier, characterised by an increased permeability and dysbiosis. These factors favour the passage of toxins and pathogenic agents from the intestinal lumen to the submucosa, where they come into contact intraepithelial lymphocytes, which trigger an inflammatory cascade.

It is known that some alterations of intestinal physiology can lead to the development of numerous gastrointestinal pathologies. For example, in patients with IBD, it has been demonstrated that an increased permeability triggers an immunological response which leads to intestinal inflammation.

It has recently been hypothesised that imbalances in the intestinal microflora and alterations of intestinal permeability can also trigger the onset of various diseases extraneous to the gastrointestinal tract. It has in fact been demonstrated that a compromised intestinal barrier can lead to the translocation of bacterial metabolites, such as lipopolysaccharides (LPS), from the intestinal lumen to the submucosa, where the cells of the immune system reside, and from here to the bloodstream, from where it can spread to different sites.

Recent studies have demonstrated that anti-cyclic citrullinated peptide (anti-CCP) antibodies are detectable several years before the onset of the arthritis in humans, suggesting that rheumatoid arthritis (RA) originates in the mucosae, such as in the gut and the oral cavity.

The idea that the onset of autoimmunity may be correlated to the gastrointestinal tract is supported not only by the fact that the microbiota composition in individuals with RA differs from the controls, but also by the observation that the altered microbiome can be partially restored after the administration of anti-rheumatic drugs which treat the disease.

In addition to this, it has been hypothesised that psoriatic arthritis could also derive from some models of gastrointestinal dysbiosis, which can cause intestinal inflammation and a consequent increase in intestinal permeability, allowing bacterial antigens and their metabolites to move into the systemic circulation and trigger an immune response in different sites, including the skin.

Moreover, other autoimmune diseases, such as lupus erythematosus and Sjögren's syndrome, have been studied with the aim of understanding the link between alterations of intestinal permeability, microflora and the physiopathology of autoimmune diseases.

The experimental results at the disposal of the community suggest that restoring the intestinal barrier could represent a new strategy for attenuating various autoimmune diseases and/or prevent them from progressing to advanced stages in patients in whom the increase in permeability and intestinal dysbiosis play a role in the pathogenesis and severity of the disease.

Thus, there exists a need to find alternative therapeutic solutions to be used in the early stages of autoimmune diseases whose physiopathology is associated with an increased intestinal permeability and dysbiosis.

In addition to this, in a social context which sees a constant increase in the interest of consumers in natural therapeutic solutions, there exists a need for said therapeutic solutions to be of natural and preferably plant origin to the extent possible.

WO2021119810 discloses a system of oral administration comprising an active ingredient dispersed in a homogeneous dry mixture of a protein powder and a polysaccharide powder. The protein powder can derive from legume proteins, whereas the polysaccharide powder comprises or consists of natural polysaccharides. In particular, the protein/polysaccharide complex disclosed in WO2021119810 imparts gastric protection and/or modified release to an active ingredient dispersed therein and can be used in therapy. The therapeutic effect is thus mediated by the active ingredient present within the protein/polysaccharide complex.

However, use for the treatment of autoimmune diseases is not disclosed, nor is use for the treatment of diseases characterised by intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

Carpentier Jeremy et al., *"Complex coacervation of pea protein isolate and tragacanth gum: Comparative study with commercial polysaccharides"*, Innovative Food Science & Emerging Technologies, Vol. 69 (2021), discloses the ability of pea protein isolates (PPI) to form complex coacervates with tragacanth gum. The coacervate formation was compared to three other PPI-polysaccharide interaction models: arabic gum and sodium alginate (known to form coacervates with PPI) and tara gum, a galactomannan.

However, use for the treatment of autoimmune diseases is not disclosed, nor is the use for the treatment of diseases characterized by intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

Scuderi et al., "*Efficacy of a Product Containing Xyloglucan and Pea Protein on Intestinal Barrier Function in a Partial Restraint Stress Animal Model*", International Journal of Molecular Sciences, vol. 23, no. 4 (2022), discloses the efficacy of a product containing xyloglucan and pea protein in restoring the normal intestinal barrier function in the event of functional abdominal bloating and distension.

However, use for the treatment of autoimmune diseases is not disclosed, nor is the use for the treatment of diseases characterized by intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

It is an aim of the present invention to provide a new association or combination of active ingredients of plant origin capable of restoring an intact intestinal barrier.

It is another aim of the present invention to provide a new association or combination of active ingredients of plant origin also capable of treating autoimmune diseases; in particular, capable of treating autoimmune diseases physiologically associated with intestinal membrane damage and/or an increased intestinal permeability and/or dysbiosis.

It is a further aim of the present invention to provide a new association or combination of active ingredients of plant origin capable of treating dysbiosis.

In the context of the present invention, the expression "diseases physiopathologically associated with intestinal membrane damage" is meant to indicate conditions characterised by structural and/or functional alterations or modifications of the intestinal membrane, which lead to the development of a disease.

In particular, "physiopathologically associated" is here meant to indicate that the aetiology of such diseases is at least partially connected with the presence of a non-intact intestinal membrane, preferably with an increased intestinal permeability and/or in the presence of dysbiosis. Such diseases include or, alternatively, consist of:

- rheumatoid arthritis and psoriatic arthritis
- lupus (for example systemic lupus erythematosus -SLE)
- Sjögren's syndrome.

It is another object of the present invention to provide pharmaceutical compositions, compositions for medical devices as per Regulation (EU) 2017/745, nutraceutical and food compositions, and a food for special medical purposes (FSMP) which comprise the association or the combination of the invention.

It is a further aim of the present invention to provide a use of the association and compositions of the invention in therapy, in particular, but not only, in the prevention and treatment of diseases whose physiopathology is associated with an increased intestinal permeability and dysbiosis.

After a lengthy and laborious research and development activity, the Applicant has demonstrated that an association of specific active ingredients of plant origin is particularly effective in solving the problems associated with a compromised intestinal barrier and dysbiosis. It has been found that such characteristics are involved in the aetiology of a vast range of disorders such as, for example, diarrhoea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), colorectal cancer and autoimmune diseases.

Thus, according to one of the aspects thereof, the invention relates to an association or combination of active ingredients of plant origin which comprises or, alternatively, consists of at least one active ingredient selected from the group comprising or, alternatively, consisting of:

(i) pea proteins, and/or
(ii) karaya gum, and/or
(iii) *Prunus cerasus* gum, and/or
(iv) *Prunus armeniaca* gum.

The object of the present invention relates to an association of active ingredients of plant origin which comprises or, alternatively, consists of:

- (i) pea proteins, and
- at least one other active ingredient of plant origin selected from the group comprising or, alternatively, consisting of:
- (ii) karaya gum,
- (iii) *Prunus cerasus* gum, e
- (iv) *Prunus armeniaca* gum, or mixtures thereof,

said association being for use in a method for the prevention and treatment of autoimmune diseases.

Said autoimmune diseases include or, alternatively, consist of:

- rheumatoid arthritis and psoriatic arthritis
- lupus (for example systemic lupus erythematosus -SLE)
- Sjögren's syndrome.

Preferably, the association or combination of active ingredients of plant origin comprises, in addition to the components (i)-(iv), at least one further active ingredient selected from the group comprising or, alternatively, consisting of (v) pectin, (vi) acacia gum, (vii) xanthan gum, (viii) tara gum, (ix) guar gum, and (x) carrageenan, or mixtures thereof; preferably, said association or combination being for use in the treatment of autoimmune diseases; in particular, for the treatment of autoimmune diseases physiologically associated with intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

Preferably, the active ingredient (ii) present in the mixture (i) + (ii) + (iii) + (iv) can be replaced, in whole or in part, by at least one further active ingredient selected from the group comprising or, alternatively, consisting of (v) pectin, (vi) acacia gum, (vii) xanthan gum, (viii) tara gum, (ix) guar gum, and (x) carrageenan, or mixtures thereof; preferably, said association or combination being for use in the treatment of autoimmune diseases; in particular, for the treatment of autoimmune diseases physiologically associated with intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

Preferably, the active ingredient (iii) present in the mixture (i) + (ii) + (iii) + (iv) can be replaced, in whole or in part, by at least one further active ingredient selected from the group comprising or, alternatively, consisting of (v) pectin, (vi) acacia gum, (vii) xanthan gum, (viii) tara gum, (ix) guar gum, and (x) carrageenan, or mixtures thereof; preferably, said association or combination being for use in the treatment of autoimmune diseases; in particular, for the treatment of autoimmune diseases physiologically associated with intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

Preferably, the active ingredient (iv) present in the mixture (i) + (ii) + (iii) + (iv) can be replaced, in whole or in part, by at least one further active ingredient selected from the group comprising or, alternatively, consisting of (v) pectin, (vi) acacia gum, (vii) xanthan gum, (viii) tara gum, (ix) guar gum, and (x) carrageenan, or mixtures thereof; preferably, said association or combination being for use in the treatment of autoimmune diseases; in particular, for the treatment of autoimmune diseases physiologically associated with intestinal membrane damage and/or increased intestinal permeability and/or dysbiosis.

According to another of the aspects thereof, the invention relates to an association or combination of active ingredients of plant origin which comprises or, alternatively, consists of (i) pea proteins in combination or association with at least one other selected active ingredient of plant origin selected from the group comprising or, alternatively, consisting of: (ii) karaya gum, (iii) *Prunus cerasus* gum, and/or (iv) *Prunus armeniaca* gum, or mixtures thereof.

According to the invention, said association or combination of active ingredients of plant origin, or composition which comprises it, is for use in a method for the prevention and treatment of autoimmune diseases.

Preferably, said autoimmune diseases are diseases physiopathologically associated with intestinal membrane damage and/or ad increased intestinal permeability and/or dysbiosis.

According to one of the aspects thereof, the association or combination according to the invention, or a composition which comprises it, is for use in a method for the prevention and treatment of autoimmune diseases physiopathologically associated with intestinal membrane damage, an increased intestinal permeability and/or dysbiosis.

Preferably, said association or combination comprises or, alternatively, consists of:

(i) pea proteins + (ii) karaya gum; or

(i) pea proteins + (iii) *Prunus cerasus* gum; or

(i) pea proteins + (iv) *Prunus armeniaca* gum;

(i) pea proteins + (ii) karaya gum + (iii) *Prunus cerasus* gum; or

(i) pea proteins + (ii) karaya gum + (iv) *Prunus armeniaca* gum; or

(i) pea proteins + (iii) *Prunus cerasus* gum + (iv) *Prunus armeniaca* gum; or

(i) pea proteins + (ii) karaya gum + (iii) *Prunus cerasus* gum + (iv) *Prunus armeniaca* gum. The term "active ingredients" indicates at least one of the four components (i), (ii), (iii), and/or (iv), just as it indicates the other components (v), (vi), (vii), (viii), (ix) and (x) of the association of the invention.

In the association or combination of the present invention, the PP (i) are present, relative to the natural gums (ii) + (iii) + (iv), in an amount by weight comprised from 1:5 to 5:1, preferably from 1:4 to 4:1, more preferably from 1:3 to 3:1, even more preferably 1:1. For example, the PP (i) are present, relative to the natural gums (ii) + (iii) + (iv), in an amount by weight of about 4:3, for example 200 mg of PP and 150 mg of (ii) + (iii)+ (iv), in a weight ratio of 1:1:1.

The pea proteins (hereinafter indicated as PP) (or isolated pea proteins) (i) appear as a light cream-coloured powder, derived from *Pisum sativum.*

PP are reserve proteins mainly composed of albumin, legumin and vicilin (for example, globulin protein associated with legumin), besides being known as a source of essential amino acids and fibre and for being highly digestible. PP are characterised by high solubility at an alkaline pH, minimal solubility at the isoelectric point and moderate solubility in an acidic environment. Moreover, globulins and vicilin impart gelling properties to the PP.

Plant protein-based products are approved as food additives according to the FDA (21CFR§170.3) and PP received *Generally Recognized As Safe* (GRAS) status in 2015 (GRN 608 Axiom Foods GRN 581 and GRN 788) after an assessment of absorption, distribution, excretion studies, bioavailability, toxicity and mutagenicity.

According to a preferred embodiment, the PP according to the invention are high quality and non-GMO (genetically modified organisms).

For example, one type of PP (*Pisum sativum L.*) is isolated at least at 80% on a dry basis, preferably has a density of about 0.5 g/ml, is water soluble, gives rise to a solution having a pH value comprised from 6.5 to 8.5 and can have a particle size of 100% through 100 mesh. For example, 80% pea protein is obtained from golden or yellow peas by means of dry and liquid processes. In the dry phase, the pea shell is mechanically removed and ground. Subsequently, pea flour is obtained and, thanks to its properties of water solubility, the proteins are separated and collected by centrifugation.

Karaya gum (ii) is obtained as an exudate from *Sterculia urens*, a large bushy tree belonging to the family *Sterculiaceae*, and is well known as a food additive (for example with the number E-416). The gum mainly consists of high molecular weight acetylated polysaccharides, which, by hydrolysis, yield galactose, rhamnose, and galacturonic acid together with a small amount of glucuronic acid.

Karaya gum appears as a fine powder, with a greyish pink colour and a slightly acetic flavour and odour. Karaya gum has very low water solubility and produces solutions with a very low concentration (<0.02% in cold water and

0.06% in hot water), due to the acetyl groups in the structure of the gum. The viscosity of karaya gum in 0.5% dispersions, for example, has a value close to 120-400 centipoises (cPs) and in a 3% dispersion, for example, about 10000 cPs. By virtue of its complex branched structure, it has emulsifying, cohesive and adhesive (film-forming) properties and is capable of forming a mucilaginous gel in contact with water. Karaya gum was recognised as safe (GRAS) by the FDA after the completion toxicologic, teratogenic and mutagenic studies.

For example, one type of karaya gum (ii) in powder form can be the one with CAS No 9000-36-6, EC 232-539-4.

Karaya gum is neither digested nor degraded by enteric microflora and is not absorbed by humans.

It has been demonstrated that karaya gum powder, if applied on an open wound, has considerable cicatrising properties.

Thanks to its mass forming properties, it has also been used as a laxative, and also as a binder and vehicle for drugs in various pharmaceutical preparations.

Prunus cerasus gum (iii) consists of branched polysaccharide chains containing various monosaccharides such as D-xylose, D-galactose, L-arabinose, L-rhamnose, D-mannose and D-glucuronic acid, which derives from glucose. In solution, *Prunus cerasus* shows a viscous behaviour rather than a tendency to form a gel.

For example, one type of *Prunus cerasus* (iii) in the form of an extract can be the one with CAS No 89997-53-5, EC 289-688-3.

Prunus armeniaca L. gum (iv) belongs to the family *Rosaceae*. The gum exudate obtained from branches of *P. armeniaca* trees is used as a food additive and possesses antioxidant capacities. *Prunus armeniaca* gum is used as a matrix for extended drug release and does not seem to exert bactericidal activity.

For example, one type of *Prunus armeniaca* (iv) in the form of an extract can be the one with CAS No 68650-44-2, EC 272-046-1.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise pectin (v).

Pectin consists mainly of partial methyl esters of polygalacturonic acid and their ammonium, sodium, potassium and calcium salts. It is obtained by extraction in an aqueous medium of strains of appropriate edible plant material, usually citrus fruits or apples. No organic precipitants are used for its extraction other than methanol, ethanol and propan-2-ol.

Pectin is preferably characterised by a galacturonic acid content of no less than 65% on an ash-free and anhydrous basis after washing with acid and alcohol. It appears in the form of a white, yellowish or brown powder, is soluble in water and insoluble in ethanol and gives rise to an opalescent colloidal solution.

It has a pH comprised from 2.8 to 3.8 in solution (2% solution). Preferably, the pectin (v) is sugar beet pectin. An example of pectin extracted from sugar beet is the product Sugarbeetpectin E 440 sold by C.E. Roeper GmbH.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise an acacia gum (vi).

Acacia gum (vi) is a natural gum also known as gum arabic as it is extracted from two species of sub-Saharan acacia: *Acacia senegal* and *Acacia seyal*. Acacia gum consists mainly of complex carbohydrates and glycoproteins, which make it an emulsifier and a stabilising agent that is useful in various applications. Preferably, an acacia gum powder is used. It is insoluble in alcohol and 1g of powder in 2 ml of water forms a solution that flows easily and is acidic to litmus. The pH (25% solution in water) is comprised from 4.1 to 5.8. For example, one type of (vi) acacia

gum in the form of a dried gummy exudate of trunks and branches of Acacia senegal (Linné) Willdenow or of other related African species can be the one with a CAS No 9000-01-5, EC 232-519-5.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise a xanthan gum (vii).

Xanthan gum (vii) is a high molecular weight polysaccharide gum produced by fermentation in a pure culture with *Xanthomonas campestris*, purified by recovery with ethanol or isopropyl alcohol, dried and ground. It is prepared as a sodium, potassium or calcium salt. It is prepared in the form of a sodium, potassium or calcium salt and appears as a cream-coloured water-soluble powder. The viscosity (1% solution in 1% KCl) is comprised from 1200 to 1600 mPas. The xanthan gum that can be present in the association or combination of the invention has the CAS No 11138-66-2 EC 234-394-2.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise a tara gum (vii).

Tara gum (viii) consists mainly of high molecular weight polysaccharides, essentially galactomannan, and is produced by grinding the endosperm of the seed of the variety *Caesalpinia spinosa* (family *Legum*inosae). It appears as a powder that is white to yellow in colour. The viscosity (1%, 20 U/min, 10 min. 87°C) is 5000 mPas. Tara gum E417 is well known as a food additive in powder form (for example, with the number E-417). An example of the tara gum that can be present in the association or combination of the invention is the product CEROTA Tara Gum Type 5000, sold by C.E. Roeper GmbH.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise a guar gum (ix).

Guar gum (ix) is a gum consisting mainly of a high molecular weight hydrocolloidal polysaccharide. In particular, the main constituent is a galactomannan, a trisaccharide formed by mannose and galactose units, specifically polymerised to form α-D-mannopyranosyl chains joined with a β-D-(1-4) glycosidic bond and having a molecular weight of around 200,000-300,000 dalton to form a 1-4 linear chain with 1-6 short side branches of galactose. Guar gum is obtained by grinding the endosperm of seeds of guar (*Cyamopsis tetragonoloba*), a herbaceous plant of the legume family, typical of India and Pakistan. Preferably, it appears as a white to yellowish white water-soluble powder, almost odourless, with a pH (1% solution) of 5.5 to 7. The guar gum that can be present in the association or combination of the invention has the CAS No 9000-30-0 EC 232-536-8.

Preferably, the association or combination (i) + (ii) + (iii) + (iv) can further comprise a carrageenan (x).

Carrageenan (x) is a gelatine widely used in food, medicine and industry and consisting essentially of calcium, potassium sodium and magnesium salts of sulphate esters of polysaccharides which yield galactose and 3,6-anhydrogalactose by hydrolysis. It appears in the form of a yellowish to colourless powder and is practically odourless, with a particle size of about 160 microns. It is stable in a neutral or alkaline environment and insoluble in organic solvents.

On food labels it is indicated with the code E 407.

Preferably, the carrageenan (x) present in the association of the invention is kappa or iota carrageenan, more preferably kappa. Kappa carrageenan forms strong, stiff gels in the presence of potassium ions and reacts with milk protein. It originates mainly from *Kappaphycus alvarezii*.

An example of the carrageenan that can be present in the association or combination according to the invention is the product CEROGEL Carrageenan RCK 580, sold by C.E. Roeper GmbH, which can have, for example, a pH

(1.5% w/w at 60 °C) comprised from 8 to 11, and a viscosity (1.5% w/w at 75°C, Spindle 2 60rpm. LVT Brookfield Viscometer) that has a minimum value of 20 – 70 mPas.

All the active ingredients of the association or combination of the invention are known in the art and commercially available.

All the active ingredients of the association of the invention are preferably in solid form, more preferably in the form of a powder, or granules or flakes.

As mentioned, the Applicant has been able to observe that the association or combination of the invention has demonstrated to be effective in the prevention and treatment, preferably early treatment, of autoimmune diseases physiopathologically associated with intestinal membrane damage, preferably with an increased intestinal permeability and/or in the presence of dysbiosis.

"Physiopathologically associated" is here meant to indicate that the aetiology of such diseases is at least partially connected with the presence of a non-intact intestinal membrane, preferably with an increased intestinal permeability and/or in the presence of dysbiosis.

Such diseases include or, alternatively, consist of:

- rheumatoid arthritis and psoriatic arthritis
- lupus (for example systemic lupus erythematosus -SLE)
- Sjögren's syndrome.

For the administration thereof, the association of the invention is preferably formulated in a pharmaceutical, nutraceutical or food composition.

According to another of the aspects thereof, the invention relates to a composition which comprises the association or combination of the present invention, together, optionally, with at least one conventional vehicle and/or excipient and/or a technological additive or excipient, said composition being for use in a method for the prevention and treatment of autoimmune diseases; preferably, for the treatment of autoimmune diseases physiopathologically associated with intestinal membrane damage, an increased intestinal permeability and/or dysbiosis.

The composition of the present invention can be a pharmaceutical, nutraceutical or food composition which comprises the association of the invention, together, optionally, with at least one conventional vehicle and/or excipient (briefly, the composition(s) of the present invention).

The composition of the invention is for oral use.

Preferably, the association or combination of the present invention is present in the composition in an amount by weight comprised from 10% to 90%, preferably comprised from 25% to 75%, more preferably comprised from 40% to 60%, whereas said at least one conventional vehicle and/or excipient are present in an amount by weight comprised from 90% to 10%, preferably from 75% to 25%, more preferably comprised from 60% to 40% by weight, relative to the total weight of the composition.

Preferably, the pea proteins (i) are present in the mixture (i) + (ii) + (iii) + (iv) in an amount by weight comprised from 40% to 70%, preferably comprised from 50% to 60%, relative to the total weight of the mixture.

Preferably, the active ingredients (ii), (iii) and (iv) are present in the mixture (i) + (ii) + (iii) + (iv) in an amount by weight comprised from 30% to 60%, preferably comprised from 40% to 50%, relative to the total weight of the

mixture.

Preferably, the active ingredients (v), (vi), (vii), (viii), (ix) and (x) are present in the mixture (i) + (v) + (vi) + (vii) + (viii) + (ix) + (x) in an amount by weight comprised from 30% to 60%, preferably comprised from 40% to 50%, relative to the total weight of the mixture. For example, the active ingredients (v), (vi), (vii), (viii), (ix) and (x) are present in a weight ratio of 1:1:1:1:1:1. The individual to be treated with the composition of the invention is preferably a mammal, more preferably a human being.

The composition of the invention is formulated for oral use, for example in the form of pharmaceutical, nutraceutical or food compositions, such as, for example, tablets, possibly coated, granules, fine granules, powders, hard capsules, soft capsules, syrups, emulsions, suspensions and solutions, suitable for oral administration.

According to a preferred embodiment, the composition of the invention is in a solid oral form, preferably selected from tablets, capsules, powders and granules. For example, the tablets and the capsules can have a weight comprised from 200 mg to 1,200 mg, preferably from 400 mg to 1,000 mg, more preferably from 600 mg to 800 mg.

The types of pharmaceutical additives used to prepare the composition of the invention, the ratios of the additive contents relative to the active ingredient and the methods for preparing the pharmaceutical composition can be appropriately selected by the person skilled in the art. Organic or inorganic substances, or solid or liquid substances can be used as excipients and vehicles, provided that they are pharmaceutical or food grade.

Examples of excipients used to prepare solid pharmaceutical compositions include, for example, lactose, sucrose, starch, talc, cellulose, dextrin, kaolin, calcium carbonate, stearic acid or magnesium stearate, lactose, polyethylene glycol, mannitol, sorbitol, chelating agents, anti-agglomerant agents, sweetening agents, preservative agents and flavouring agents.

For the preparation of liquid compositions for oral administration, use can be made of a conventional inert diluent such as water or an oil, for example a vegetable oil. The liquid composition can contain, in addition to the inert diluent, auxiliaries such as wetting agents, suspension agents, sweeteners, flavourings, colourings and preservatives. The liquid composition can be enclosed in capsules made of an absorbable material, such as gelatine.

The sweeteners can be one or more natural sugars, reduced as desired, such as, for example sucrose, dextrose, xylitol, mannitol or sorbitol, or a synthetic product, such as, for example, sodium saccharine, aspartame, acesulfame K or the sucralose. Acidifying agents can also be added.

The flavouring agents are pharmaceutically acceptable flavours and tastes of synthetic oils or natural oils, the latter extracted from plants, flowers, fruits and combinations thereof, such as, for example, cinnamon, mint, anise, and citrus fruit leaves, bitter almonds, citrus fruits, in particular orange and/or lemon oils, linden, vanilla, chocolate and grapefruit. Chocolate, vanilla or eucalyptus flavourings and fruit essences, in particular apple, pear, peach, strawberry, apricot, orange, lemon and grape, can also be advantageously used.

The composition of the invention can also comprise other components, useful for the use according to the invention. The mixtures and compositions subject-matter of the present invention are prepared using methods and equipment known to an expert in the nutraceutical or pharmaceutical sector.

The composition of the invention can be packaged in dosage units or in multidose packages, according to the conventional methods well known to the person skilled in the art.

Preferably, the composition of the invention is in the form of dosage units.

According to a preferred embodiment, the invention relates to a pharmaceutical composition, a composition for a medical device as per Regulation (EU) 2017/745, a nutraceutical or food composition or food for special medical purposes (FSMP) for use according to the invention, which comprises or, alternatively, consists of:

- from 50 mg to 500 mg, preferably from 75 mg to 450 mg, more preferably from 100 mg to 400 mg, even more preferably from 125 mg to 350 mg, for example 150 mg, or 175 mg, or 200 mg, or 250 mg, or 300 mg, of pea proteins,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of karaya gum,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of *Prunus cerasus*, and
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of *Prunus armeniaca* gum,

together, optionally, with at least one conventional food or pharmaceutical grade vehicle and/or excipient.

Preferably, said pharmaceutical composition, composition for a medical device as per Regulation (EU) 2017/745, nutraceutical or food composition or food for special medical purposes (FSMP) for use according to the invention comprises or, alternatively, consists of:

- from 50 mg to 500 mg, preferably from 75 mg to 450 mg, more preferably from 100 mg to 400 mg, even more preferably from 125 mg to 350 mg, for example 150 mg, or 175 mg, or 200 mg, or 250 mg, or 300 mg, of pea proteins,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of pectin,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of acacia gum,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of xanthan gum,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of tara gum,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of guar gum, and
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of carrageenan gum,

together, optionally, with at least one conventional food or pharmaceutical grade vehicle and/or excipient.

Preferably, the natural gums (ii) karaya gum, (iii) *Prunus cerasus* gum and (iv) *Prunus armeniaca* gum can for

example be present, in said composition, in a weight ratio of 1:1:1, for example 50 mg (ii) + 50 mg (iii) + 50 mg (iv); or also 75 mg (ii) + 50 mg (iii) + 50 mg (iv); or also 50 mg (ii) + 75 mg (iii) + 50 mg (iv); or also 50 mg (ii) + 50 mg (iii) + 75 mg (iv); or also 100 mg (ii) + 50 mg (iii) + 50 mg (iv); or also 50 mg (ii) + 100 mg (iii) + 50 mg (iv); or 50 mg (ii) + 50 mg (iii) + 100 mg (iv).

Preferably, the active ingredients pectin (v), acacia gum (vi), xanthan gum (vii), tara gum (viii), guar gum (ix) and carrageenan (x) can for example be present in said association or combination in a weight ratio, for example, of 1:1:1:1:1:1, for example 25 mg (v) + 25 mg (vi) + 25 mg (vii) + 25 mg (viii) + 25 mg (ix) + 25 mg (x).

According to another of the aspects thereof, the invention relates to the association and the pharmaceutical or nutraceutical composition according to the invention for use in therapy.

According to another of the aspects thereof, the invention relates to the association and the pharmaceutical or nutraceutical composition according to the invention for use in the prevention and treatment, preferably early treatment, of diseases physiopathologically associated with intestinal membrane damage, preferably with an increased intestinal permeability and/or dysbiosis.

Such diseases are selected from the group comprising or, alternatively, consisting of:

- rheumatoid arthritis and psoriatic arthritis
- lupus (for example systemic lupus erythematosus)
- Sjögren's syndrome.

More preferably, the association of the invention and/or the composition comprising that association are advantageously used for the treatment of systemic lupus erythematosus.

In fact, the Applicant has observed that the association comprising pea proteins and one or more of the tested active ingredients (see examples 1 and 2) is capable of attenuating and/or treating damage to the intestinal membrane in a mouse model of systemic lupus erythematosus. The association and compositions of the invention are well tolerated and do not give significant side effects.

The association and compositions of the invention thus offer a natural, nontoxic, alternative solution to conventional therapies for the prevention and early treatment of autoimmune diseases aetiologically correlated with an altered intestinal permeability and/or with dysbiosis.

According to another of the aspects thereof, the invention relates to a method for the prevention and treatment, preferably the early treatment, of diseases physiopathologically associated with intestinal membrane damage, preferably with an increased intestinal permeability and dysbiosis, which comprises administering, to an individual in need thereof, an effective dose of the association and/or composition according to the invention.

Experimental section

Example 1 - Evaluation of pea proteins combined with polysaccharides (ii) – (iv) on the intestinal characteristics of systemic lupus erythematosus (SLE) using an MRL/lpr genetic mouse model

1. Evaluation of the synergistic effect of the combination of pea proteins in combination with various polysaccharides (*Prunus cerasus* gum (iii), *Prunus armeniaca* gum (iv) and karaya gum (ii)) in restoring intestinal permeability in a mouse model of systemic lupus erythematosus (SLE) using an MRL/lpr genetic mouse model.

2. The test was performed in the laboratories of the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina. Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina.

3. The experiments conducted on animals comply with Italian legislation on the protection of animals used for experimental purposes and other scientific purposes (Ministerial Degree 116192) and Directive 2010/63/EU, as amended by Regulation (EU) 2019/1010, as well as ARRIVE guidelines.

4. The animals were fed on a complete standard diet in the form of pellets supplied by the authorised breeder. Filtered tap water from the local mains supply was provided ad libitum.

5. The pea proteins (PP), polysaccharides and various combinations thereof were administered orally once a day starting from 16 weeks of age until 18 weeks of age using an oral gavage. The following doses were tested: 150 mg of polysaccharides (a total of 3: *Prunus cerasus*, *Prunus armeniaca* and karaya gum; preferably in a weight ratio of 1:1:1) and 200 mg of pea proteins, preferably with a PP content of at least 80% by weight. The polysaccharides were tested individually and in three different combinations, such as, for example: Polysaccharide 1 + PP; Polysaccharide 2 + PP; Polysaccharide 3 + PP). The dose administered to the animals was defined by suitable conversion of the dose envisaged for humans based on the proportion of body surface. The vehicle was saline solution.

6. For the experiment, MRL/lpr female mice were used (3 weeks of age; 20-25 g; Jackson Laboratory, USA) and C57BL/6 female mice as control groups (CTR) (3 weeks of age; 20-25 g; Envigo, Italy). The animals were housed in a controlled environment (22 ± 2 °C, 55 ± 15% relative humidity, light/dark cycle of 12 hours) with a standard diet and water ad libitum. The experimental protocols began when the animals reached sixteen weeks of age. The experiments on the animals were conducted in compliance with Italian legislation on the protection of animals used for experimental purposes and other scientific purposes (Ministerial Degree 116192) and Directive 2010/63/EU, as amended by Regulation (EU) 2019/1010, as well as ARRIVE guidelines.

7. The MRL/lpr mouse is a genetic model that shares many pathological characteristics with SLE (Katzav A. et al., *Treatment of MRL/lpr mice, a genetic autoimmune model, with the Ras inhibitor, farnesylthiosalicylate (FTS)*. Clin Exp Immunol. 2001 Dec;126(3):570-7); in fact, the MRL/lpr strains spontaneously develop characteristics similar to lupus with an onset of disease at around 8 weeks of age and an evolution of around 16 weeks. In this regard, it was verified that the natural compounds tested are effective in restoring the intestinal barrier damaged by the progression of lupus. In particular, the oral treatments were administered for two weeks when both mouse strains (C57BL/6 and MRL/lpr) reached sixteen weeks of age. In this regard, it was verified that the natural compounds are effective in restoring the intestinal barrier damaged by the progression of lupus.

8. Experimental groups

The mice were randomly divided into the following experimental groups, using the C57BL/6 strain for the CTR group and the MRL/lpr strain for the SLE group.

Group 1: CTR + vehicle: sixteen-week-old C57BL/6 mice to which the vehicle was administered orally once a day for 2 weeks (N = 8).

Group 2: CTR + PP: sixteen-week-old C57BL/6 mice to which PP was administered orally once a day for 2 weeks (N = 8).

Group 3: CTR + Polysaccharide 1: sixteen-week-old C57BL/6 mice to which polysaccharide 1 was administered orally once a day for 2 weeks (N = 8).

Group 4: CTR + Polysaccharide 2: sixteen-week-old C57BL/6 mice to which polysaccharide 2 was administered orally once a day for 2 weeks (N = 8).

Group 5: CTR + Polysaccharide 3: sixteen-week-old C57BL/6 mice to which polysaccharide 3 was administered orally once a day for 2 weeks (N = 8).

Group 6: CTR + Polysaccharide 1 + PP: sixteen-week-old C57BL/6 mice to which polysaccharide 1 was administered orally in combination with PP once a day for 2 weeks (N = 8).

Group 7: CTR + Polysaccharide 2 + PP: sixteen-week-old C57BL/6 mice to which polysaccharide 2 was administered orally in combination with PP once a day for 8 weeks (N = 8).

Group 8: CTR + Polysaccharide 3 + PP: sixteen-week-old C57BL/6 mice to which polysaccharide 3 was administered orally in combination with PP once a day for 2 weeks (N = 8).

Group 9: SLE + vehicle: sixteen-week-old MRL/lpr mice to which the vehicle was administered orally once a day for 2 weeks (N = 8).

Group 10: SLE + PP: sixteen-week-old MRL/lpr mice to which PP was administered orally once a day for 2 weeks (N = 8).

Group 11: SLE + Polysaccharide 1: sixteen-week-old MRL/lpr mice to which polysaccharide 1 was administered orally once a day for 2 weeks (N = 8).

Group 12: SLE + Polysaccharide 2: sixteen-week-old MRL/lpr mice to which polysaccharide 2 was administered orally once a day for 2 weeks (N = 8).

Group 13: SLE + Polysaccharide 3: sixteen-week-old MRL/lpr mice to which polysaccharide 3 was administered orally once a day for 2 weeks (N = 8).

Group 14: SLE + Polysaccharide 1 + PP: sixteen-week-old MRL/lpr mice to which polysaccharide 1 was administered orally in combination with PP once a day for 8 weeks (N = 8).

Group 15: SLE + Polysaccharide 2 + PP: sixteen-week-old MRL/lpr mice to which polysaccharide 2 was administered orally in combination with PP once a day for 8 weeks (N = 8).

Group 16: SLE + Polysaccharide 3 + PP: sixteen-week-old MRL/lpr mice to which polysaccharide 3 was administered orally in combination with PP once a day for 8 weeks (N = 8).

9. The mice were sacrificed by cervical dislocation 2 weeks after treatment, when they had reached 18 weeks of age. The blood was collected for biochemical analyses of inflammatory and autoimmune markers and for the FITC-dextran test.

10. Intestinal permeability

The FITC-dextran method was used to measure intestinal permeability in the C57BL/6 and MRL/lpr animals. The mice were fasted for 6 hours, after which FITC-dextran was administered by gavage (500 mg/kg of body weight, 125 mg/mL). Subsequently, 100 µL of blood were collected from the caudal vein after 1 h and 4 h. The blood was

centrifuged at 12,000× g for 5 min at 4° C. The plasma concentration of FITC-dextran was measured with a microplate reader. The values were compared with a standard curve created by diluting FITC-dextran diluted with phosphate-buffered saline (1:1, v/v).

11. Evaluation of inflammatory and autoimmune markers in serum

The quantities of cytokines such as IL-1β, IL-6 and TNF-α were measured in serum at the end of the experiment. Similarly, lipocalin-2 (LCN2), an indicator of the severity of lupus, was measured with an ELISA test. Furthermore, in order to evaluate the autoimmune parameters, autoantibodies such as antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) were also evaluated. All the ELISA tests were carried out following the instructions of the kit manufacturer.

The first preliminary results are very promising and indicate that the association and the composition comprising said association, both forming the subject matter of the present invention, could have valid application in the prevention and treatment of autoimmune diseases, for example where said autoimmune diseases are selected from those physiopathologically associated with intestinal membrane damage, or broadly, in the case of rheumatoid arthritis and psoriatic arthritis; lupus, preferably systemic lupus erythematosus and/or Sjögren's syndrome).

In particular, the Applicant has observed that the association of active ingredients (i), (ii), (iii) and (iv) is capable of attenuating and/or treating damage to the intestinal membrane, in particular in mice having autoimmune diseases in which such damage is present.

Example 2 - Evaluation of pea proteins combined with polysaccharides (v) – (x) on the intestinal characteristics of systemic lupus erythematosus (SLE) using an MRL/lpr genetic mouse model

1. Evaluation of the synergistic effect of the combination of pea proteins i) in combination with various polysaccharides (pectin (v), acacia gum (vi), xanthan gum (vii), tara gum (viii), guar gum (ix) and carrageenan (x)) in restoring intestinal permeability in a mouse model of systemic lupus erythematosus (SLE) using an MRL/lpr genetic mouse model.

2. The test was performed in the laboratories of the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina. Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina.

3. The experiments conducted on animals comply with Italian legislation on the protection of animals used for experimental purposes and other scientific purposes (Ministerial Degree 116192) and Directive 2010/63/EU, as amended by Regulation (EU) 2019/1010, as well as ARRIVE guidelines.

4. The animals were fed on a complete standard diet in the form of pellets supplied by the authorised breeder. Filtered tap water from the local mains supply was provided ad libitum.

5. The pea protein (PP), polysaccharides and various combinations thereof were administered orally once a day starting from 16 weeks of age until 18 weeks of age using an oral gavage. The following doses were tested: 150 mg of polysaccharides (a total of 6: sugar beet pectin, acacia gum, xanthan gum, tara gum, guar gum and carrageenan) and 200 mg of pea proteins.

The dose administered to the animals was defined by suitable conversion of the dose envisaged for humans based on the proportion of body surface. The vehicle was saline solution.

6. For the experiment, MRL/lpr female mice were used (3 weeks of age; 20-25 g; Jackson Laboratory, USA) and C57BL/6 female mice as control groups (CTR) (3 weeks of age; 20-25 g; Envigo, Italy). The animals were housed in a controlled environment (22 ± 2 °C, 55 ± 15% relative humidity, light/dark cycle of 12 hours) with a standard diet and water ad libitum. The experimental protocols began when the animals reached sixteen weeks of age.

7. The MRL/lpr mouse is a genetic model that shares many pathological characteristics with SLE (Katzav A. et al., *Treatment of MRL/lpr mice, a genetic autoimmune model, with the Ras inhibitor, farnesylthiosalicylate (FTS)*. Clin Exp Immunol. 2001 Dec;126(3):570-7); in fact, the MRL/lpr strains spontaneously develop characteristics similar to lupus with an onset of disease at around 8 weeks of age and an evolution of around 16 weeks. In particular, the oral treatments were administered for two weeks when both mouse strains (C57BL/6 and MRL/lpr) reached sixteen weeks of age. In this regard, it was verified that the natural compounds are effective in restoring the intestinal barrier damaged by the progression of lupus.

8. Experimental groups

The mice were randomly divided into the following experimental groups, using the C57BL/6 strain for the CTR group and the MRL/lpr strain for the SLE group.

Group 1: CTR + vehicle: sixteen-week-old C57BL/6 mice to which the vehicle (saline solution) was administered orally every day for 2 weeks (N = 5).

Group 2: CTR + PP: sixteen-week-old C57BL/6 mice to which PP was administered orally every day for 2 weeks (N = 5).

Group 3: CTR + tara gum: sixteen-week-old C57BL/6 mice to which tara gum was administered orally every day for 2 weeks (N = 5).

Group 4: CTR + guar gum: sixteen-week-old C57BL/6 mice to which guar gum was administered orally every day for 2 weeks (N = 5).

Group 5: CTR + xanthan gum: sixteen-week-old C57BL/6 mice to which xanthan gum was administered orally every day for 2 weeks (N = 5).

Group 6: CTR + acacia gum: sixteen-week-old C57BL/6 mice to which acacia gum was administered orally every day for 2 weeks (N = 5).

Group 7: CTR + sugar beet pectin: sixteen-week-old C57BL/6 mice to which sugar beet pectin was administered orally every day for 2 weeks (N = 5).

Group 8: CTR + carrageenan: sixteen-week-old C57BL/6 mice to which carrageenan was administered orally every day for 2 weeks (N = 5).

Group 9: CTR + tara gum + PP: sixteen-week-old C57BL/6 mice to which tara gum plus PP was administered orally every day for 2 weeks (N = 5).

Group 10: CTR + guar gum + PP: sixteen-week-old C57BL/6 mice to which guar gum plus PP was administered orally every day for 2 weeks (N = 5).

Group 11: CTR + xanthan gum + PP: sixteen-week-old C57BL/6 mice to which xanthan gum and PP was administered orally every day for 2 weeks (N = 5).

Group 12: CTR + acacia gum + PP: sixteen-week-old C57BL/6 mice to which acacia gum plus PP was administered orally every day for 2 weeks (N = 5).

Group 13: CTR + sugar beet pectin + PP: sixteen-week-old C57BL/6 mice to which sugar beet pectin plus PP was administered orally every day for 2 weeks (N = 5).

Group 14: CTR + carrageenan + PP: sixteen-week-old C57BL/6 mice to which carrageenan plus PP was administered orally every day for 2 weeks (N = 5).

Group 15: SLE + vehicle: sixteen-week-old MRL/lpr mice to which the vehicle (saline solution) was administered orally every day for 2 weeks (N = 5).

Group 16: SLE + PP: sixteen-week-old MRL/lpr mice to which PP was administered orally every day for 2 weeks (N = 8).

Group 17: SLE + tara gum: sixteen-week-old MRL/lpr mice to which tara gum was administered orally on a daily basis for 2 weeks (N = 8).

Group 18: SLE + guar gum: sixteen-week-old MRL/lpr mice to which guar gum was administered orally on a daily basis for 2 weeks (N = 8).

Group 19: SLE + xanthan gum: sixteen-week-old MRL/lpr mice to which xanthan gum was administered orally every day for 2 weeks (N = 8).

Group 20: SLE + acacia gum: sixteen-week-old MRL/lpr mice to which acacia gum was administered orally every day for 2 weeks (N = 8).

Group 21: SLE + sugar beet pectin: sixteen-week-old MRL/lpr mice to which sugar beet pectin was administered orally every day for 2 weeks (N = 8).

Group 22: SLE + carrageenan: sixteen-week-old MRL/lpr mice to which carrageenan was administered orally every day for 2 weeks (N = 8).

Group 23: SLE + tara gum + PP: sixteen-week-old MRL/lpr mice to which tara gum plus PP was administered orally every day for 2 weeks (N = 8).

Group 24: SLE + guar gum + PP: sixteen-week-old MRL/lpr mice to which guar gum plus PP was administered orally every day for 2 weeks (N = 8).

Group 25: SLE + xanthan gum + PP: sixteen-week-old MRL/lpr mice to which xanthan gum plus PP was administered orally every day for 2 weeks (N = 8).

Group 26: SLE + acacia gum + PP: sixteen-week-old MRL/lpr mice to which acacia gum plus PP was administered orally every day for 2 weeks (N = 8).

Group 27: SLE + sugar beet pectin + PP: sixteen-week-old MRL/lpr mice to which sugar beet pectin plus PP was administered orally every day for 2 weeks (N = 8).

Group 28: SLE + carrageenan + PP: sixteen-week-old MRL/lpr mice to which carrageenan plus PP was administered orally every day for 2 weeks (N = 8).

9. The mice were sacrificed 2 weeks after the oral treatments, by cervical dislocation. Then the colon, kidneys and liver were surgically removed and treated for histological analyses. Furthermore, blood was collected for biochemical analyses of inflammatory and autoimmune markers and for the FITC-dextran test.

10. Histological analyses

For the histological evaluations, colon, kidney and liver tissues were embedded in paraffin, sectioned into 7µm thick slices and stained with H&E for microscopic analyses.

11. Immunohistochemical analyses of the tight junctions (TJs) in colon samples

At the end of the experiment, immunohistochemistry was used to analyse the function of the intestinal barrier by evaluating the TJs in the colon samples.

In particular, the paraffin-embedded tissue samples were sectioned (7 µm), deparaffinised and treated according to the immunohistochemical protocol. The sections were incubated overnight at 4°C with the following primary antibodies: zonula occludens-1 (ZO-1) (Santa Cruz Biotechnology sc-33725, 1:100 in PBS, v/v) and anti-occludin (Santa Cruz Biotechnology sc-13).

The slices were then incubated with a secondary antibody (Vector Laboratories). Subsequently, the antigen-antibody complexes were detected using an avidin-biotin complex detection system (Vectastain ABC Kit, Vector Laboratories) and a 3,3'-diaminobenzidine (DAB) substrate kit (Vector Laboratories). The sections were examined using a Nikon Eclipse Ci-L microscope.

12. Intestinal permeability

The FITC-dextran method was used to measure intestinal permeability in the C57BL/6 and MRL/lpr animals. Briefly, the mice were fasted for 6 hours, after which FITC-dextran was administered by gavage (500 mg/kg of body weight, 125 mg/mL). After 1 h and 4 h, 100 µL of blood were collected from the caudal vein. The blood was centrifuged at 12,000× g for 5 min at 4° C. The plasma concentration of FITC-dextran was measured with a microplate reader. The values were compared with a standard curve created by diluting FITC-dextran in untreated plasma diluted with phosphate-buffered saline (1:1, v/v).

13. Evaluation of inflammatory and autoimmune markers in serum

At the end of the experiment, cytokines such as IL-1β, IL-6 and TNF-α were measured in serum. Lipocalin-2 (LCN2), an indicator of the severity of lupus, was measured with an ELISA test. Furthermore, in order to evaluate the autoimmune parameters, autoantibodies such as antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) were also evaluated. All the ELISA tests were carried out following the instructions of the kit manufacturer.

The first results indicate that the association comprising pea proteins and one or more of the tested active ingredients (tara gum, guar gum, xanthan gum, acacia gum, sugar beet pectin, carrageenan) seems to be able to attenuate and/or treat damage to the intestinal membrane in mice having autoimmune diseases in which such damage and/or an increased intestinal permeability and/or dysbiosis is present, in particular in a mouse model of systemic lupus erythematosus.

CLAIMS

1. An association of active ingredients of plant origin which comprises or, alternatively, consists of:

- (i) pea proteins, and

- at least one other active ingredient of plant origin selected from the group comprising or, alternatively, consisting of:

- (ii) karaya gum,

- (iii) *Prunus cerasus* gum,

- (iv) *Prunus armeniaca* gum, or mixtures thereof,

said association being for use in a method for the prevention and treatment of autoimmune diseases.

2. The association for use according to claim 1, wherein said autoimmune diseases are diseases physiopathologically associated with intestinal membrane damage, increased intestinal permeability and/or dysbiosis.

3. The association for use according to claim 1 or 2, wherein said autoimmune diseases are diseases associated with dysbiosis.

4. The association for use according to any one of the preceding claims, wherein said autoimmune diseases are selected from the group comprising or, alternatively, consisting of: rheumatoid arthritis and psoriatic arthritis; lupus, preferably systemic lupus erythematosus and/or Sjögren's syndrome, even more preferably systemic lupus erythematosus.

5. The association for use according to any one of the preceding claims, wherein said association comprises or, alternatively, consists of:

- (i) pea proteins + (ii) karaya gum; or

- (i) pea proteins + (iii) *Prunus cerasus* gum; or

- (i) pea proteins + (iv) *Prunus armeniaca* gum; or

- (i) pea proteins + (ii) karaya gum + (iii) *Prunus cerasus* gum; or

- (i) pea proteins + (ii) karaya gum + (iv) *Prunus armeniaca* gum; or

- (i) pea proteins + (iii) *Prunus cerasus* gum + (iv) *Prunus armeniaca* gum; or

- (i) pea proteins + (ii) karaya gum + (iii) *Prunus cerasus* gum + (iv) *Prunus armeniaca* gum.

6. The association for use according to any one of the preceding claims, wherein in said association said (i) pea proteins are present, relative to said at least one other active ingredient of plant origin (ii) and/or (iii) and/or (iv), in an amount by weight comprised from 1:5 to 5:1, preferably from 1:4 to 4:1, more preferably from 1:3 to 3:1, even more preferably 1:1.

7. The association for use according to any one of claims 1 to 4, wherein said association comprises (i) pea proteins and, as a replacement for the active ingredients (ii) – (iv), at least one further active ingredient selected from the group comprising or, alternatively, consisting of:

- (v) pectin,

- (vi) acacia gum,

- (vii) xanthan gum,

- (viii) tara gum,
- (ix) guar gum, and
- (x) carrageenan, or mixtures thereof.

8. A composition which comprises the association according to any one of claims 1-7, together, optionally, with at least one conventional vehicle and/or excipient, for use in a method for the prevention and treatment of autoimmune diseases, preferably autoimmune diseases physiopathologically associated with intestinal membrane damage, an increased intestinal permeability and/or dysbiosis.

9. The composition for use according to claim 8, wherein said association is present in said composition in an amount by weight comprised from 10% to 90%, preferably comprised from 25% to 75%, more preferably comprised from 40% to 60%, whereas said at least one conventional vehicle and/or excipient are present in an amount by weight comprised from 90% to 10%, preferably from 75% to 25%, more preferably comprised from 60% to 40% by weight, relative to the total weight of the composition.

10. The composition for use according to claim 8 or 9, wherein said composition comprises or, alternatively, consists of:

- from 50 mg to 500 mg, preferably from 75 mg to 450 mg, more preferably from 100 mg to 400 mg, even more preferably from 125 mg to 350 mg, for example 150 mg, or 175 mg, or 200 mg, or 250 mg, or 300 mg, of pea proteins,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of karaya gum,
- from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of *Prunus cerasus*, and

from 10 mg to 200 mg, preferably from 25 mg to 150 mg, more preferably from 50 mg to 125 mg, even more preferably from 75 mg to 100 mg of *Prunus armeniaca* gum,

together, optionally, with at least one conventional vehicle and/or excipient.

11. The composition for use according to any one of claims 8-10, wherein said composition is for oral use; it is preferably in the form of dosage units.

12. The composition for use according to claim 11, in the form of tablets, possibly coated, granules, fine granules, powders, hard capsules, soft capsules, syrups, emulsions, suspensions and solutions.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/055559

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K36/185 A61K36/48 A61K36/73 A61K38/16 A23L33/105
A23L33/185 A23L33/21
ADD.

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

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 practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCUDERI SARAH ADRIANA ET AL: "Efficacy of a Product Containing Xyloglucan and Pea Protein on Intestinal Barrier Function in a Partial Restraint Stress Animal Model", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, [Online] vol. 23, no. 4, 1 February 2022 (2022-02-01), page 2269, XP093112052, Basel, CH ISSN: 1661-6596, DOI: 10.3390/ijms23042269 [retrieved on 2023-12-14] section 2.2 Effect of XG and PP on Gut Permeability; figure 2 ----- -/--	1-12

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2024	01/10/2024

Name and mailing address of the ISA/	Authorized officer
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2

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/055559

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MA LONGHUAN ET AL: "Loss of Gut Barrier Integrity In Lupus", FRONTIERS IN IMMUNOLOGY, [Online] vol. 13, 20 June 2022 (2022-06-20), XP093206980, Lausanne, CH ISSN: 1664-3224, DOI: 10.3389/fimmu.2022.919792 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9250981/pdf/fimmu-13-919792.pdf> [retrieved on 2024-09-20] the whole document -----	1-12
A	WO 2017/207223 A1 (NOVINTETHICAL PHARMA SA [CH]) 7 December 2017 (2017-12-07) the whole document -----	1-12
X,P	WO 2023/110855 A1 (DEVINTEC SAGL [CH]) 22 June 2023 (2023-06-22) page 7, lines 4-9; claims 1-8, 11 page 7, lines 34-37 page 12, line 38 - page 13, line 1 -----	1-12

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/055559

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		EP 3463418 A1	10-04-2019
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