Abstract: The present patent of invention aims to the use of muilaca/camapú (Physalis angulata L.) extract and/or physalins obtained from extraction in supercritical carbon dioxide as anti-inflammatory, immunoregulatory, anti-irritant, antioxidant and anti-senescent agent by mechanisms that involve the reduction on the production of pro-inflammatory cytokines, modulation of the production of immunosuppressive cytokines, reduction on the production of inflammatory mediators, reduction on the production of free radicals, increase on the production of antioxidant enzymes, regulation of the production of tissue growth factors and decrease on the production of histamine, being also used in pure form or mixed with other oils, extracts and/or ingredients in cosmetics and dermatological preparations for specific skin imperfections such as sensitive skin, cosmetic intolerance syndrome, skin allergies, allergic or irritant contact dermatitis and/or atopic dermatitis.
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, Published: ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
"USE OF Physalis angulata (MULLACA/CAMAPU) EXTRACT AND/OR PHYSALINS"

Field of Invention

The present patent of invention aims the use of mullaca/camapù (Physalis angulata L.) extract and/or physaliras obtained from the extraction in supercritical carbon dioxide as anti-inflammatory, immunoregulatory, anti-irritant, antioxidant and anti-senescence agent due to mechanisms involving the decreasing of pro-inflammatory cytokines production, modulation of immunosuppressive cytokines production, reduction on the production of inflammatory mediators, reduction on the free radicals production, increase on the antioxidant enzymes production, production regulation of tissue growth factors and production decreasing of histamine, pure or mixed with other oils, extracts and/or ingredients in cosmetic and dermatological products formulations for the face or body treatment of specific skin changes such as sensitive skin and cosmetics intolerance syndrome, allergic and irritant contact dermatitis and atopic dermatitis.

Invention background / Prior Art

With the claim for the use of Physalis angulata and its isolated active ingredients, some products have been developed:

- PI 9904635-0 - Process for isolating physalins from plants and pharmaceutical compositions with antiprotozoal activity containing physalins.
- US 20020103386 - Process for isolating physalins from plants and pharmaceutical compositions containing physalins.
WO 200303951 - Process for preparing dry extracts from a fluid extract, and at least, one additional substance by means of drying process through atomization (spray dryer).

PI 0404635-8 – Process for obtaining dry steroids derived from ergostane such as Physalins.

US 541 1733 - Antiviral agent containing crude drugs derived from plants, including the Physalis angulata species.

For the treatment of sensitive skin and atopic dermatitis, some compounds have been developed:

US 7008627 – Use of complexes for the preparation of compositions for the treatment of sensitive skin, preparation process and hypoallergenic compositions,

US 6241993 - Therapeutic/ cosmetic compositions comprising bradykinin antagonists for treating sensitive human skin.

US 4444780 - Method for treating atopic dermatitis with cyproheptadine or its salt added to an acid.

WO2005034901 - Use of a combination of cromoglicic acid and/or one of the salts and/or esters thereof and a gelling agent, and cosmetic or pharmaceutical applications herof - relates new compositions containing chromoglicic acid as active ingredient, with cosmetic or preparation purposes for sensitive, hypersensitive or intolerant skin care.

WO2004082583 - use of oligogalacturonides for cosmetic, dermatological or pharmaceutical compositions preparation used for sensitive skin care, by limiting the propagation of inflammatory response.

Some patents describe the extraction process by supercritical carbon dioxide:

US 7250374 - System and method for processing a substrate using supercritical carbon dioxide process.

US 4308200 - Extraction of coniferous woods with fluid carbon dioxide and other supercritical fluids.

US 5073267 - Process of volatile compounds extraction with supercritical carbon dioxide, according to percolation of the supercritical gas, and compounds obtained through this process.

US 6248797 – Supercritical carbon dioxide extraction of contaminants from ion exchange resings - extraction of ion exchange contaminants by Supercritical carbon dioxide from
resins.

* WO 2005 116049 - selective separation or extraction of steroidal glycosides by extraction with supercritical fluid using carbon dioxide.

BRIEF DESCRIPTION OF FIGURES

Figure 1 is a graph that represents the evaluation of the production of IL-1α in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulaia L. obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). # P<0.05, in relation to basal control; @P<0.05, in relation to LPS – Control Group (ANOVA, Tukey).

Figure 2 is a graph showing the evaluation of the production of IL-6 in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). # P<0.05, in relation to basal control; @P<0.05, in relation to LPS – Control Group (ANOVA, Tukey).

Figure 3 is a graph showing the evaluation of the production of TNF-α in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycoSic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 4 is a graph showing the evaluation of the production of IFN-γ in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).
Figure 5 is a graph showing the evaluation of the production of IL-10 in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 6 is a graph showing the evaluation of the production of PLA₂ in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS – Control Group (ANOVA, Tukey).

Figure 7 is a graph showing the evaluation of the production of CGX-2 in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS – Control Group (ANOVA, Tukey).

Figure 8 is a graph showing the evaluation of the production of LOX in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 9 is a graph showing the evaluation of the production of PGE₁ in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).
Figure 10 is a graph showing the evaluation of the production of PGE$_2$ in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 \textit{Physalis angulata} L. extract obtained by extraction in supercritical carbon dioxide, PAE HG \textit{Physalis angulata} L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 11 is a graph showing the evaluation of the production of LTB$_4$ in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 \textit{Physalis angulata} L. extract obtained by extraction in supercritical carbon dioxide, PAE HG \textit{Physalis angulata} L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 12 is a graph showing the evaluation of the production of Histamine in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 \textit{Physalis angulata} L. extract obtained by extraction in supercritical carbon dioxide, PAE HG \textit{Physalis angulata} L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 13 is a graph showing the evaluation of the production of TGF-β in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 \textit{Physalis angulata} L. extract obtained by extraction in supercritical carbon dioxide, PAE HG \textit{Physalis angulata} L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 14 is a graph showing the evaluation of the production of GM-CSF in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 \textit{Physalis angulata} L. extract obtained by extraction in supercritical carbon dioxide, PAE HG \textit{Physalis angulata} L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).
Figure 15 is a graph showing the evaluation of the production of SOD in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

Figure 16 is a graph showing the evaluation of the production of CAT in human keratinocytes cell culture after 48 hours of incubation with PAE CO2 (Physalis angulata L. extract obtained by extraction in supercritical carbon dioxide), PAE HG (Physalis angulata L. extract obtained through hydroglycolic extraction) or Hydrocortisone. All groups were evaluated in the presence and absence of lipopolysaccharide (LPS). #P<0.05, in relation to basal control; @P<0.05, in relation to LPS - Control Group (ANOVA, Tukey).

DETAILED DESCRIPTION OF THE INVENTION

Emalis anuulata

Physalis angulata belongs to the family Solanaceae and it is popularly known as "mullaca", "camapû", "jua", "Joã", "jua de capote" and others. It is a native plant of Brazil that grows mainly as a weed in the Northern and Northeastern regions of the country, but it also occurs in other tropical regions of Africa, America and Asia.

This plant has been employed in the popular Brazilian medicine against rheumatism, once its fruits and leaves are considered detoxifiers, diuretic and resolutes, against liver problems, inflammation, coughing and body pains, otitis, malaria, jaundice and kidney problems. The species is still employed in Brazil and Colombia against skin diseases, because parts of this plant present anti-inflammatory and antiseptic activity.

A large number of chemicals have already been described for this species, among them alkaloids - mainly ftrigrin -, flavonoids, in which only a glycoside of mirecetin was isolated from the leaves of the plant - the terpenoids - including β-Phellandrene and β-damascenone obtained from the essential oil of the leaves, oleanoic acid, hydrocarbons and other unknown terpenoids, which were obtained from the fruit and steroids that represent the major constituents of this plant. The steroids have already been isolated from leaves,
such as vamonolide and physangulide, and from the dry fruits, such as physagulmes A, B, C₅D₅F, H, I, J, K, vitamins and physagulmines E, F and G. Sitosterol, physalins B, D, E, F, G, H, I, J, K and whitanguatine were isolated from the branches. From the constituents detected in this species, physalins A and B are the most important, and physalins A and B are the major compounds in Physalis angulata L..

An important range of pharmacological activities has been described for Physalis angulata L.. In pre-clinical studies, animals demonstrated the following properties: anti-inflammatory - comparable to the effects of hydrocortisone -, antimicrobial, anti-tumoral for whitangulatina. physalin B and F, miricetine, immunomodulatory, hypotensive, molluscidic, trypanosomicide and tuberculostatic activities.

The immunoregulatory action of this plant is the main activity of the plant contents which act as corticoid-like. A group of substances named vitasteroids has blastogenic activity on human lymphocytes, in addition to important immunosuppressive activity in rats observed for the production of NOS (nitric oxide synthase) by immunocytes of the spleen. Moreover, the immunomodulatory activity was detected in fractions of the whole plant, by increasing the proliferation of B cells.

Physalins have important effects, as described in literature. Physalins A and B, like F and G, have potent immunomodulatory activity, inhibiting the production of nitric oxide from macrophages. These effects are similar to those produced by glucocorticoids (corticoid-like activity), but they occur by different mechanisms from those described for the dexamethasone. As the same way than other glucocorticoids, physalins show inhibitory activity in the production of TNF-α and IL-6 in peritoneal macrophages activated by lipopolysaccharide, however, physalins are steroids 30 times more potent than corticosteroids such as dexamethasone. A recent study developed by the same research group shows that physalins also prevent the rejection of transplants, clearly suggesting their uses as immunosuppressive agents.

According to the literature, physalins can be isolated by high-performance liquid chromatography (HPLC). However, the use of more sophisticated techniques, such as CO2 supercritical extraction, favors isolation, in particular the attainment of higher concentrations of more purified compounds.
**Extraction by Supercritical Carbon Dioxide**

The extraction with supercritical fluids presents advantages in relation to conventional processes for obtaining active principles from the herbal matrix, because most of these processes are based on extraction with chemical solvents and present inconvenience such as high temperatures of operation. In this context, there is the possibility of thermal degradation of the active compounds, presence of toxic residues in the final product and possibility of human and environmental contamination due to the use of high-risk organic solvents.

Due to these advantages, the chemical, food, pharmaceutical and cosmetics industries have shown interest in the use of this new technology in processes that prioritize the maximum quality of the products yielded. The supercritical extraction of natural products already occurs industrially worldwide, particularly with regard to the extraction of caffeine from coffee beans. The technique is also used in the decaffeinating of tea, extraction, fractionation and refinement of fats and oils, removal of alcohol from beverages, extraction of fragrance, among others.

Studies have shown the importance of this methodology in the extraction of vegetal chemicals, and the reference to the quality of this extraction method is common and unquestionable, when compared to extraction methods with organic solvents. A recent study compared the isolation of vegetal chemicals using four different extraction techniques, including CO2 supercritical extraction and showed that for some compounds, this methodology is more efficient in terms of final product yield. Another study showed that the CO2 supercritical extraction compared to the extraction with organic solvents was more effective in obtaining pure compounds with antioxidant activity.

Studies using this method in the extraction of constituents from vegetal species of great economic value for the cosmetics industry, demonstrated the suitability of this method to obtain compounds of interest and with the desired quality in the development of products in the cosmetics and pharmaceutical areas.

The extraction by supercritical carbon dioxide has been proposed as a non-contaminant method of vegetal products extraction in substitution of extraction methods using organic solvents, which obtain low-quality vegetal compounds, requiring many
refining and purification processes in order to obtain a final product completely free from chemical contaminants and effectively purified with significant active compounds contents.

Thus, the use of this technique is essential for the development of products aimed to consumers with sensitive or reactive skin, people with atopic and contact dermatitis and other skin hypersensitivity reactions, since that besides eliminating the presence of solvents in the final product, the active compounds are obtained in a more concentrated form and with better quality, thus decreasing the dose used in the final product.

*Sensitive Skin and Cosmetics Intolerance Syndrome*

Scientifically, sensitive skin is defined in dermatology and cosmetology as any skin discomfort, sensory or clinical, from the use of a cosmetic product.

In recent years, an increased number of individuals have developed some kind of subjective discomfort related to skin sensitivity. The main complaints relate to the particular susceptibility to apply products commonly used for skin care such as soaps, sunscreens and cosmetics in general without the traditional visible signs of irritation, contact allergy, phototoxicity or photoallergy.

Some authors claim that the diagnosis of skin sensitivity is extremely difficult, since the data are based only on subjective perceptions of patients. The most common sensory events are burning, itching and skin dryness sensation. The clinical signs observed by the dermatologist, regardless of user subjective perception are mainly redness, scaling and several other signs, such as eye puffiness and increase in wrinkles in the periorbital region. These signs are usually arising from the change in the permeability of the skin and vascular barrier and may appear minutes, hours or days after application. Moreover, an increase on the stratum corneum permeability and an acceleration of the skin nervous response are observed.

Epidemiological data show that approximately 40% of the population classifies their skin type as sensitive, and that 50% of these individuals do not present visible signs of inflammation, which confirms the difficulty of the diagnosis. Accordingly, other epidemiological studies have been made to assess the number of people with sensitive skin. In the UK, for example, a study based on self-assessment questionnaires showed that 51.4% of women and 38.2% of men reported sensitive skin. Similar data were obtained in studies
in the American female population, where 52% of the respondents reported sensitive skin. More recently, studies have been conducted among the Italian population, where it was found that 54.9% responded positively to the sensitive skin diagnosis clinical test, called stinging test, which uses lactic acid as a mean of inducing the sensitivity.

An extreme variant of sensitive skin is the cosmetics intolerance syndrome (status cosmeticus), which was firstly described by Fisher in 1980, who used the term to define the condition in which the patient could not tolerate any kind of cosmetics; however, without the classic signs of an inflammatory response. The main chemicals commonly used in cosmetics that can lead to this phenomenon are benzoic acid, bronopol, cinnamic acid and derivatives, ammonium quaternary products and related compounds, formaldehyde, lactic acid, ethoxylated emulsifiers, glycols, sodium lauryl sulfate, sorbic acid and urea, among others.

When in contact with the skin, these chemical agents initiate an immune and inflammatory cascade that ends up by increasing the skin sensitivity, as well as their susceptibility to extrinsic attacks. Many of the individual steps in the inflammation cascade are controlled by cytokines or other soluble regulatory molecules, known as inflammatory mediators. A determined mediator not only produces a direct effect, but also stimulates the production of other one by inducing subsequent steps, resulting in an integrated response.

Contact Dermatitis

Contact dermatitis is a frequent inflammatory dermatosis in industrialized countries leading to a large socioeconomic impact because it is one of the most common occupational diseases. Since the skin is the most external barrier of the human body, it is the First to get in contact with chemical and physical factors from the environment. According to the physiopathological mechanisms involved, two types of contact dermatitis can be distinguished: allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). ICD is a result of toxic and pro-inflammatory effects of xenobiotic agents capable of activating the skin innate immunity. ACD requires the activation of the antigen-specific acquired immunity leading to the development of T cells, which mediate the skin inflammation. It is characterized by erythema, papules and vesicles, followed by dryness and scaling. ACD, also known as contact hypersensitivity (CHS) is a skin inflammatory reaction mediated by
T cells resulting repeated skin contact with non-protein chemical compounds, called haptens. Unlike what happens with the classic delay hypersensitivity (DHS), which requires the intradermal injection of exogenous protein, the initiation of the CHS is generated by the topical application of sensitizing haptens on the epidermis (such as nickel, chromium, DNFB, TNCB and oxazollne).

**Atopic Dermatitis**

The term atopic dermatitis or atopic eczema describes skin inflammatory manifestations associated to atopy. Nowadays, atopy would be the hereditary predisposition of the immune system to promote hypersensitivity reactions mediated by IgE in response to common antigens in food and in the intra and extra home environment, and this concept classifies atopic dermatitis as one of the manifestations of atopic triad diseases (atopic dermatitis, asthma, allergic rhinitis). It is characterized as a chronic and pruritic inflammation of the skin upper layers that often affects individuals who exhibit fever of hay, asthma and individuals who have relatives with these diseases. Individuals with atopic dermatitis commonly have many other allergic disorders. The relationship between these disorders and dermatitis is not fully understood yet. Some individuals may have inherited tendency to produce excessive antibodies (eg immunoglobulin E) in response to several different stimulus. Many conditions may worsen the atopic dermatitis, including emotional stress, changes in temperature or humidity, bacterial skin infections and contact with irritant clothes. In some infants, food allergies can cause atopic dermatitis.

Sometimes, atopic dermatitis manifests in the first few months after birth. The infants may present hyperemic and secreting rashes with erythema and secretion, that form crusts on the face, scalp, perineal area, hands, upper limbs, feet or lower limbs. Dermatitis often disappears around 3 to 4 years old, although the recurrence is common. In mature children and adults, the rash often occurs in only one of the elbows or behind the knees.

Although color, intensity and location of the eruption may vary, it is always itchy. Often the itching makes the individual scratch his/herself in an uncontrollable way, triggering a cycle that exacerbates the problem. Scratching and frictioning can also cause laceration of the skin, allowing the entry of bacteria and subsequent infection. For unknown reasons, individuals with chronic atopic dermatitis sometimes have cataracts between 20
and 40 years old. In individuals with atopic dermatitis, the herpes simplex, which is usually light, and usually affects a small area, it can produce a serious illness with high fever and eczema, called eczema herpeticum.

The diagnosis is considered difficult and requires a lot of clinical research for the establishment of the diagnosis, since there is no specific examination or test for atopic dermatitis. The diagnosis is established based on the typical pattern of the rash and, often, on the history of other allergy cases in the family.

**Immune and Inflammatory Skin Responses**

The skin acts as a semi permeable barrier that prevents both the transepidermat water loss and the penetration of toxic substances in the body. It is well specified in literature that the epidermal homeostasis is vulnerable to various environmental factors such as climate change, ultraviolet radiation, free radicals, toxins, allergens, and endogenous factors such as genetic predispositions, immunologic and hormonal balance. Under the effect of these stressing agents, there is a skin barrier disruption, which immediately stimulates the increase of pro-inflammatory cytokines production in the skin, causing imbalances in the barrier function and in other skin structures, improving the severity of the skin diseases such as a contact dermatitis, atopic dermatitis and other skin hypersensitivity reactions.

The inflammatory response can be beneficial or harmful. The occurrence of an increased in the local vascular infusion, the extravasation of fluids and the formation of clot can act to facilitate the flow of leukocytes, dilute or inactivate the pathogen and promote healing. On the other hand, inflammation is harmful when causes temporary or permanent injury of tissues and interfere in the normal functions; these harmful effects are known as allergy or hypersensitivity. Vascular congestion resulting from this process, if not contained, could lead, to an increase of proteolytic enzymes activity, causing the degradation of connective tissue and the extracellular matrix (ECM), as well as a decline in the production of antioxidant enzymes, resulting in the loss of elasticity and firmness, imbalance of the skin barrier and consequent increase on the susceptibility of the skin to allergic reactions and dermatitis, installation of opportunistic microorganisms and formation of wrinkles, a process that comes with aging.
The damage to skin tissue initiates a cascade of events including inflammatory process, formation of new tissue and tissue repair. This process is started immediately to the damage by the release of various growth factors, cytokines and endothelial factors. Keratinocytes participate actively in the skin immune response, producing a large amount of pro-inflammatory cytokines, such as interleukin-1 alpha (IL-1α), IL-6, IL-12, TNF-α (tumor necrosis factor alpha) and IFN-γ (interferon-gamma) in response to various stimuli. They also produce chemokines and other immunoregulatory cytokines mainly in response to IL-1α and TNF-α. These factors have important effects on immune cells of the skin such as mast cells, dendritic cells and Langerhans cells, resulting in the regulation of the expression of other mediators and recruitment of additional cells from blood flow, culminating in an integrated response.

IL-1α and TNF-α are cytokines that, although with structural differences, presenting many biological effects. They are produced by all nucleated cells, including members of the monocyte-macrophage strain, NK cells (natural-killer), T lymphocytes, keratinocytes, dendritic cells, fibroblasts, neutrophils, endothelial and smooth muscle cells, and its secretion can be stimulated by immunological complexes, toxins, physical injury among other inflammatory process. In the endothelium, they induce a series of changes by increasing the production of adhesion molecules and other chemical mediators (cytokines, chemokines, growth factors), thus promoting the margination and migration of leukocytes to the inflammation site.

The IL-6, despite serving as pro-inflammatory and chemostatic cytokine for neutrophils, it also plays an important role in the proliferation of keratinocytes, aiding in the tissue reepithelization process. The IL-12 is known for the action inducing the production of IFN-γ by T lymphocytes and NK cells. This cytokine also seems to be capable of inducing the production of IFN-γ by macrophages, resulting in a positive feedback capable of activating these cells in different situations. The IL-1α, produced by mast cells, eosinophils and keratinocytes has a key role in the initiation and modulation of the innate immune response and the generation of adaptive response, influencing in the development of allergic reactions, synthesis of histamine, leukotriene B4 (LTB4), LTC4, platelet activating factor (PAF) and synthesis of IgE.
Inflammatory mediators such as LTB4 and prostaglandin E2 (PGE2) are secreted by the various cell types that serve to intensify the outbreak or specific aspects of the inflammatory reaction. These mediators can be called eicosanoids or metabolites of the arachidonic acid (AA), and are derived from the phospholipids components of the cytoplasmatic membrane. Catalyzed by phospholipase A2 (PLA2), the AA is released from the cell membrane and is metabolized by two enzymes: lipoxigenase (LOX) and/or cyclooxygenase (COX). From the metabolism of AA by LOX, leukotrienes are originated. When suffers the action of COX5 the AA is metabolized into prostaglandin and thromboxanes (TXA). Leukotrienes, especially LTB4, are potent chemostatic agents, which increase the migration of leukocytes into the extravascular environment by binding to endothelial cells. Along with PGEl and PGE2, these mediators are responsible for the vasodilation and increase on vascular permeability, with subsequent formation of erythema and edema, common in inflammatory allergic reactions, besides promoting the degradation of ECM components such as collagen, elastin and glycosaminoglycans.

**Detailed description of the Invention**

The present patent of invention aims to the use of miliaca/camapâ (Physalis angulata L.) and/or physalin as anti-inflammatory, immunoregulatory, anti-irritant, antioxidant and anti-senescence agent in dermatological and cosmetic formulations.

These effects are supported by mechanisms involving the reduction of the production of pro-inflammatory cytokines, modulation of the production of immunosuppressive cytokines, reduction on the production of inflammatory mediators, reduction on the production of free radicals, increase on the production of antioxidant enzymes, regulation of the production of tissue growth factors and reduction on the production of histamine.

The plant extract and the isolated physaiins, obtained by extraction in supercritical carbon dioxide, can be used in pure form or mixed with other oils, extracts and / or ingredients in cosmetics and dermatological formulations for the specific skin deficency such as sensitive skin and cosmetics intolerance syndrome, skin allergies and allergic or irritant contact dermatitis and atopic dermatitis.
These assertions are supported by *in vitro* efficacy tests, as exemplified below.

**Example 1**

Evaluation of the Immunoregulatory activity of *Physalis angulata* L. extracted by Supercritical Carbon Dioxide:

These tests evaluated the immunoregulatory activity of *Physalis angulata* extracts obtained by extraction in Supercritical Carbon Dioxide (PAE CO2)\textsubscript{S} comparing to *Physalis angulata* extract obtained by hydroglycolic extraction (PAE HG) and to Hydrocortisone, a topical standard corticoid, in order to verify the effectiveness of this extraction method. All samples tested were incubated in concentrations of 0.5, 0.25, 0.12, 0.06 and 0.03 mg/mL in human keratinocytes culture, with the objective of measuring the production of pro-inflammatory cytokines IL-1\(\alpha\) to, IL-6, IFN-\(\gamma\) TNF-\(\alpha\), and the anti-inflammatory cytokine IL-10 in the cell culture supernatant. For this process, the cell culture was incubated with the samples for a period of 48 hours. The inflammatory process was induced by the addition of lipopolysaccharide (LPS) to the cell culture, a component of bacterial origin experimentally used as standard induction of immune and inflammatory response. The quantitative analysis for the production of the parameters above was performed through quantitative immunoenzymatic test (ELISA). NOTE: To evaluate the possible influence of the fluid extractor in the attainment of the PAE HG\textsubscript{S} a control containing only the same solvent at the same extraction proportions was used. In the graph, it is found with abbreviation CHG.

The results obtained are discribed in the represented graphs from 1 to 16.

Conclusion: The results observed in graphs from 1 to 5 showed the *in vitro* effects of the immunoregulatory activity PAE CO2 through quantification of pro-inflammatory and anti-inflammatory cytokines. The tests were conducted in a comparative way in order to verify that the product obtained through this extraction method is more effective than that obtained through the hydroglycolic method traditionally generally used by cosmetic and dermatological products industries. Moreover, the evaluation was conducted in comparison, at the same doses, with Hydrocortisone, a corticosteroid widely used in the topic treatment of dermatitis and other allergic skin reactions. Based on these results, it was concluded that
the PAE CO2 has an immunoregulatory effect comparable to that of the Hydrocortisone, since it reduced the levels of all mediators increased during the inflammatory process in a dose-dependent manner (groups treated with LPS). The reduction on the production of these substances promoted the reduction of deleterious effects caused by the allergic and inflammatory process such as disruption and degradation of connective tissue that ended up by breaking of the skin barrier. Moreover, it is observed that the results obtained with PAE HG was considerably lower than those found with PAE CO2, allowing to conclude that the extraction declared in this patent is significantly superior to the most traditional extraction forms.

Example 2
Assessment of the anti-inflammatory activity of Physalis angulata L. extracted through Supercritical Carbon Dioxide:

The anti-inflammatory activity of the Physalis angulata extract obtained through Supercritical Carbon Dioxide extraction (PAE CO2) was evaluated comparing to the Physalis angulata extract obtained through hydroglycolic extraction (PAE HG) and to Hydrocortisone, a topic use standard corticosteroid in order to verify the effectiveness of this extraction method. All samples tested were incubated in concentrations of 0.5, 0.25, 0.12, 0.06 and 0.03 mg/mL in human keratinocytes culture with the objective of measuring the production of enzymes and other inflammatory mediators such as Phospholipase A2 (PLA2), Cyclooxygenase 2 (COX-2), Lipoxigenase (LOX), Prostaglandins E1 and E2 (PGE1 and PGE2) and Leukotriene B4 (LTB4). All these parameters were evaluated in supernatant or lysate of cell cultures incubated with the samples for a period of 48 hours. The inflammatory process was induced by the addition of lipopolysaccharide (LPS) in the cell culture, a component of bacterial origin experimentally used for induction of immune and inflammatory response. The quantitative analysis for the production of the parameters above was performed through quantitative immunoenzymatic test (ELISA). NOTE: To evaluate the possible influence of the fluid extractor in the attainment of the PAE HG, a control containing only the same solvent at the same extraction proportions was used. In the graph, it is found with abbreviation CHG.
Conclusion: The results observed in graphics from 6 to 11 showed the *in vitro* effects of the anti-inflammatory activity of the PAE CO2 through the quantification of enzymes and other inflammatory mediators. The tests were made in a comparative way in order to verify that the product obtained by this extraction method is more effective than that obtained through the traditional hydroglycolic method generally used by cosmetic and dermatological products industries. Moreover, the evaluation was performed in comparison, at the same doses, with Hydrocortisone, a corticosteroid widely used in the topic treatment of dermatitis and other allergic and/or inflammatory skin reactions. Based on these results, we conclude that the PAE CO2 presents anti-inflammatory effect comparable to Hydrocortisone, once it reduced in a dose-dependent way and significantly the levels of all initial and Final mediators of the inflammatory cascade, mimicked by the use of LPS. The reduction on the production of these substances promotes the reduction on the deleterious effects caused by allergic and inflammatory process such as the disruption and degradation of connective tissue that ended up by breaking of the skin barrier. Moreover, it is observed that the results obtained with PAE HG was considerably lower than those found with PAE CO2, which concluded that the extraction declared in this patent is significantly superior to the more traditional extraction forms. It is interesting to mention that in the parameter PGEl, no pronounced effects for the PAE CO2 were observed, when compared to Hydrocortisone. This result shows the cytoprotective effect of this extract, since PGE1 is expressed as a constituent, regardless the installation of an inflammatory process, playing an important role in cell protection.

Examrle_3

Evaluation of the anti-histaminic activity (antiallergic) of *Physalis angulata* extracted through Supercritical Carbon Dioxide:

These tests evaluated the anti-histaminic activity of the *Physalis angulata* extract obtained through Supercritical Carbon Dioxide extraction (PAE CO2) compared with *Physalis angulata* extract obtained through hydroglycolic extraction (PAE HG) and with Hydrocortisone, a topic use standard corticosteroid in order to verify the effectiveness of this extraction method. All samples tested were incubated in concentrations of 0.5, 0.25, 0.12, 0.06 and 0.03 mg/mL in human keratinocytes culture with the objective of measuring
the production of enzymes of the production of histamine in cell culture lysate incubated for 48 hours. The allergic response was induced by the addition of lipopolysaccharide (LPS) to the cell culture, a component of bacterial origin experimentally used as standard induction of immune and inflammatory response. The quantitative analysis for the production of the parameters above was performed through quantitative immunoenzymatic test (ELISA). NOTE: To evaluate the possible influence of the fluid extractor in the attainment of the PAE HG₃ a control containing only the same solvent at the same extraction proportions was used. In the graph, it is found with abbreviation CHG.

Conclusion: The result observed in graphic 12 showed the in vitro effects of the antihistamine activity of PAE CO₂ through histamine quantification. The tests were conducted in a comparative way in order to verify that the product obtained through this extraction method is more effective than that obtained through the hydroglycolic method traditionally generally used by cosmetic and dermatological products industries. Moreover, the evaluation was conducted in comparison, at the same doses, with Hydrocortisone, a corticosteroid widely used in the topic treatment of dermatitis and other allergic skin reactions. Based on these results, one concludes that the PAE CO₂ presents anti-inflammatory effect comparable to Hydrocortisone, once it reduced in a dose-dependent way and significantly the levels of all initial and final mediators of the inflammatory cascade, mimicked by the use of LPS. The reduction on the production of these substances promotes the reduction on the deleterious effects caused by allergic and inflammatory process such as the disruption and degradation of connective tissue that ended up by breaking of the skin barrier. Moreover, it is observed that the results obtained with PAE HG was considerably lower than those found with PAE CO₂, which concluded that the extraction declared in this patent is significantly superior to the more traditional extraction forms.

Example 4
Evaluation of the modulatory activity Physalis angulata extracted through Supercritical Carbon Dioxide on the production of tissue growth factors:

These tests evaluated the modulatory activity of Physalis angulata extract obtained through Supercritical Carbon Dioxide extraction (PAE CO₂) on the production of tissue
growth factors GM-CSF and TGF-β compared to Physalis angulata extract obtained through hydroglycolic extraction (PAE HG) and Hydrocortisone, a topic use standard corticoid in order to verify the effectiveness of this extraction method. All samples tested were incubated at concentrations of 0.5, 0.25, 0.12, 0.06 and 0.03 mg/mL in human keratinocytes culture with the objective of measuring the production of GM-CSF and TGF-β in the cell culture supernatant incubated for 48 hours. The tissue damage during inflammatory response was mimicked by the addition of iipopolysaccharide (LPS) to the cell culture, a component of bacterial origin experimentally used as induction standard of the immune and inflammatory response. The quantitative analysis for the production of the parameters above was performed through quantitative immunoenzymatic test (ELISA).

NOTE: To evaluate the possible influence of the fluid extractor in the attainment of the PAE HG, a control containing only the same solvent at the same extraction proportions was used. In the graph, it is found with abbreviation CHG.

Conclusion: The results observed in graphs 13 and 14 showed the in vitro effects of the tissue protective and restorative activity of PAE CO2 through TGF-β and GM-CSF quantification. The tests were performed on a comparative way in order to verify that the extract obtained through this extraction method is more effective than that obtained through the traditional hydroglycolic method generally used by cosmetic and dermatological products industries. Moreover, the evaluation was conducted in comparison, at the same doses, with Hydrocortisone, a corticosteroid widely used in the topic treatment of dermatitis and other allergic and/or inflammatory skin reactions. As could be evaluated in results obtained with the Hydrocortisone, corticosteroids are in general potent topic anti-inflammatory agents; however they generate considerable tissue damage, and cause disarrangement in the extracellular matrix due to the reduced on the production of macromolecules (collagen, elastin and glycosaminoglycans) in the dermis. These effects result, in part, from the significant reduction on the production of the tissue growth factors TGF-β and GM-CSF, caused by Hydrocortisone, that even in the absence of an inflammatory response, generates a reduction on the synthesis of these factors. Unlike, the reduction on the production of TGF-β and GM-CSF caused by PAE CO2 was significantly lower than effects observed with Hydrocortisone. Based on this result, we concluded that the PAE CO2 has a tissue protective effect, since during the inflammatory process, as
mimicked by the use of LPS, it promoted only a partial reduction on the production of these factors, so that they would return to basal levels produced in cultures not treated with LPS.

Example 5

**Evaluation of the antioxidant activity of Physalis anguiata** extracted through **Supercritical Carbon Dioxide:**

These tests evaluated the antioxidant activity of *Physalis anguiata* extract obtained through Supercritical Carbon Dioxide extraction (PAE CO2) compared to *Physalis anguiata* extract obtained through hydrogiycoiic extraction (PAE HG) and with Hydrocortisone, a topic use standard corticosteroid in order to verify the effectiveness of the compound through this extraction method. All samples tested were incubated in concentrations of 0.5, 0.25, 0.12, 0.06 and 0.03 mg/mL in human keratinocytes culture with the objective of measuring the production of natural defense antioxidant enzymes Superoxide Dismutase (SOD) and Catalase (CAT) in the cell culture supernatant incubated for 48 hours. The oxidative tissue damage that occurs during the inflammatory response was mimicked through the addition of lipopoiysaccharide (LPS) to the cell culture, a component of bacterial origin experimentally used as induction standard of immune and inflammatory response, unchaining the formation of oxygen reactive species (free radicals). The quantitative analysis of the SOD and CAT production was performed through quantitative enzymatic test (ELISA). NOTE: To evaluate the possible influence of the fluid extractor in the attainment of the PAE HG, a control containing only the same solvent at the same extraction proportions was used. In the graph, it is found with abbreviation CHG.

**Conclusion:** The results observed in graphs 15 and 16 show the *in vitro* effects of the antioxidant activity of *Physalis anguiata* extract obtained by means of Supercritical Carbon Dioxide extraction (PAE CO2) through SOD and CAT quantification. The tests were conducted in a comparative way in order to verify that the *Physalis anguiata* extract obtained through this extraction method is more effective than that obtained through the hydrogiycoic method traditionally generally used by cosmetic and dermatological products industries. Moreover, the evaluation was conducted in comparison, at the same doses, with Hydrocortisone, a corticosteroid widely used in the topic treatment of dermatitis and other allergic skin reactions. During the evolution of these skin changes a large formation of free
radicals occurs, as a response to the harmful stimulus. As an attempt to reverse the harmful oxidative process, a mechanism called redox homeostasis (RH) is used by cells as endogenous defense. This control system is composed of substances capable, even in low concentrations, to compete with oxidizable cellular substrates, and then to reduce or significantly inhibit the oxidation, preserving its structure and function. This includes some enzymes, among them the superoxide dismutase (SOD) and catalase (CAT). Biological potent antioxidant compounds have the ability to stimulate the synthesis of these enzymes by the cells, consequently, neutralizing or scavenging excessive radical species. Corticosteroids, as could be observed in results obtained with the Hydrocortisone, are potent topic anti-inflammatory agents; however, they generate considerable tissue damage responsible, in part, for the reduction on the production of SOD and CAT by skin cells. Unlike, the PAE CO2 promoted, even before the induction of the inflammatory response generated by the addition of LPS, a considerable increase on the production of SOD and CAT at all doses evaluated, in a dose-dependent way. Based on this result, it was concluded that the PAE CO2 has a potent biological antioxidant effect. Moreover, it has been observed that the results obtained with PAE HG was considerably lower than those found with PAE CO2, which enabled concluding that the extraction method declared in this patent is significantly superior to most traditional extraction forms.

Final Conclusion

Considering the following:

- During the development of hypersensitivity skin reactions such as allergic and irritant contact dermatitis, atopic dermatitis and cosmetic intolerance syndrome, the development of an immune and inflammatory response occurs, and the *Physostis angulata* Extract obtained through Supercritical Dioxide Carbon extraction (PAE CO2) was able to significantly reduce the production of key enzymes and Inflammatory mediators, in addition to regulating the immune response of the skin by reducing the production of pro-inflammatory cytokines and modulating the production of anti-inflammatory cytokines;

- During the development of these and other skin alterations, an increase on the production of free radicals or reactive oxygen species occur, in addition to a
considerable reduction on the production of natural defense antioxidant enzymes, and this extract (PAE CO2) stimulated significantly the production of these enzymes;

It could be concluded that:

- The *Physalis angulata* Extract obtained through Supercritical Carbon Dioxide extraction (PAE CO2) presented more expressive effects than the corresponding hydroglycolic extract and according to all the results shown above, attend to the claims requested.
Claims:

1. Use of Mullaca/Camapú (*Physalis angulata* L.) extract and/or physalins obtained from this extract in dermatological and cosmetic formulations for the facial or body treatment of specific skin alterations.

2. The extract claimed in item 1 is characterized by being obtained from aerial parts (stems, leaves, branches and fruits) of Mullaca/Camapú (*Physalis angulata* L.) through supercritical carbon dioxide extraction (CO2 supercritical).

3. The extract claimed in item 2 is characterized by the use, in any proportion of mixture and their parts separately, in dermatological and cosmetic applications for specific skin imperfections such as sensitive skin, cosmetics intolerance syndrome, skin allergies, allergic or irritant contact dermatitis and atopic dermatitis.

4. The extract claimed in item 3 is characterized by presenting as one of the action mechanisms, the reduction on the production of pro-inflammatory cytokines (IL-1α, IL-6, TNF-α IFN-γ), favoring the restoration of normal skin conditions.

5. The extract claimed in item 4 is characterized by having the modulation of the production of immunosuppressive cytokines (IL-10) as one of the action mechanisms, which are fundamental in the regulation of the immune and inflammatory response.

6. The extract claimed in item 5 is characterized by having the reduction on the production of inflammatory mediators (PLA2, COX-2, LOX, PGE1, PGE2, LTB4) as one of the action mechanisms, favoring the restoration of normal skin conditions and reducing the signs of inflammation (heat, pain, erythema and edema).

7. The extract claimed in item 6 is characterized by having the reduction on the production of histamine as one of the action mechanisms, favoring the anti-allergic action and reducing the probability of the development of hypersensitivity reactions, dermatitis and the appearance of the cosmetic intolerance syndrome.

8. The extract claimed in item 7 is characterized by having the modulation of the production of tissue growth factors (TGF-b and GM-CSF) as one of the action mechanisms, which are essential for the maintenance of the cellular homeostasis, tissue regeneration and synthesis of proteins of the extracellular matrix.
9. The extract claimed in item 8 is characterized by having the increase on the production of skin natural antioxidant enzymes (superoxide dismutase and catalase) as one of the action mechanisms, favoring the antioxidant action and avoiding tissue damage and cellular senescence.
FIGURE 1 - IL-1α (Interleukin-1 α)

FIGURE 2 - IL-6 (Interleukin-6)
**FIGURE 5** - IL-10 (Interleukin-10)

![Graph showing IL-10 levels with Basal Condition and + LPS conditions.](image)

**FIGURE 6** - Phospholipase A2 (PLA2)

![Graph showing PLA2 levels with Basal Condition and + LPS conditions.](image)
FIGURE 7 - Cyclooxygenase-2 (COX-2)

FIGURE 8 - Lipoxigenase (LOX)
FIGURE 9 - Prostaglandin E₁ (PGE₁)

[Graph showing Basal Condition and + LPS for PGE₁ levels across different conditions]

FIGURE 10 - Prostaglandin E₂ (PGE₂)

[Graph showing Basal Condition and + LPS for PGE₂ levels across different conditions]
FIGURE 11 - Leukotriene B₄ (LTB₄)

FIGURE 12 - Histamine
FIGURE 13 - TGF-β (Tissue Growth Factor-beta)

FIGURE 14 - GM-CSF (Granulocytes and Macrophages Colony Stimulating Factor)
FIGURE 15 - SOD (Superoxide Dismutase)

FIGURE 16 - CAT (Catalase)
INTERNATIONAL SEARCH REPORT

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

A61K36/81

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 practical, search terms used)

EPO-Internal, FSTA, BIOSIS, EMBASE, WPI Data

<table>
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