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(54) **KRILL AND/OR MARINE EXTRACTS FOR PREVENTION AND/OR TREATMENT OF CARDIOVASCULAR DISEASES ARTHRITIS, SKIN CANCER DIABETES, PREMENSTRUAL SYNDROME AND TRANSDERMAL TRANSPORT**

(76) Inventor: **Tina Sampalis**, Laval (CA)

Correspondence Address:
CROWELL & MORING LLP
INTELLECTUAL PROPERTY GROUP
P.O. BOX 14300
WASHINGTON, DC 20044-4300 (US)

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(57) **ABSTRACT**

The present invention relates to a method of treatment and/or prevention of cardiovascular disease, rheumatoid arthritis, skin cancer, premenstrual syndrome, diabetes and transdermal transport enhancement. The method comprises the administration of a therapeutically effective amount of krill and/or marine oil to a patient. The present invention also relates to a composition for the treatment and/or prevention of these diseases.

KRILL AND/OR MARINE EXTRACTS FOR PREVENTION AND/OR TREATMENT OF CARDIOVASCULAR DISEASES ARTHRITIS, SKIN CANCER DIABETES, PREMENSTRUAL SYNDROME AND TRANSDERMAL TRANSPORT

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] This invention relates to multi-therapeutic extracts derived from krill and/or marine, which can prevent and/or treat several diseases.

[0003] 2. Description of Prior Art

[0004] Krill is the common name for small, shrimp-like crustaceans, however not shrimp, that swarm in dense shoals, especially in Antarctic waters. It is one of the most important food source for fish, some kind of birds and especially for baleen whales as being an important source of protein. Krill is also a good source of omega-3 fatty acid, which are well known for their health benefits.

[0005] It is known in the art to use krill and/or marine enzymes for the treatment of a great variety of diseases in human and animals such as infections, inflammations, cancers, HIV/AIDS, pain, polyps, warts, hemorrhoids, plaque, wrinkles, thin hairs, allergic itch, anti-adhesion, eye disease, acne, cystic fibrosis and immune disorders including autoimmune disease and cancer.

[0006] It is also known in the art that krill and/or marine oil may be used for the treatment of autoimmune murine lupus and other autoimmune diseases and can also be used for treating cardiovascular diseases.

[0007] However, the krill and/or marine oil used for these treatments has only conserved its omega-3 fatty acids as active ingredients, which is a very small part of all the active ingredients of the krill and/or marine itself. This fact reduces the potential of the krill and/or marine oil as a treatment for these diseases.

[0008] There is an increasing demand for treatments using products derived from a natural source, therefore, it would be highly desirable to be provided with a krill and/or marine extract having an enhanced potential for prevention and/or treatment and/or management of disease.

SUMMARY OF THE INVENTION

[0009] In accordance with the present invention there is provided a method of prevention, therapy and/or treatment of several disease, the method comprising the administration of a therapeutically effective amount of krill and/or marine oil to a patient.

[0010] In a preferred embodiment of the present invention the krill and/or marine oil is obtained from a process comprising the steps of:

[0011] (a) placing krill and/or marine material in a ketone solvent, preferably acetone to achieve extraction of the soluble lipid fraction from the marine and/or aquatic animal material;

[0013] (c) recovering a first lipid rich fraction from the liquid contents by evaporation of the solvent present in the liquid contents;

[0014] (d) placing the solid contents in an organic solvent selected from the group of solvents consisting of alcohol, preferably ethanol, isopropanol or t-butanol and esters of acetic acid, preferably ethyl acetate to achieve extraction of the remaining soluble lipid fraction from the marine and/or aquatic material;

[0015] (e) separating the liquid and solid contents;

[0016] (f) recovering a second lipid rich fraction by evaporation of the solvent from the liquid contents; and

[0017] (g) recovering the solid contents.

[0018] In a preferred embodiment of the present invention, the krill and/or marine oil comprises Eicosapentanoic acid, Docosahexanoic acid, Phosphatidylcholine, Phosphatidylinositol, Phosphatidylserine, Phosphatidylethanolamine, Sphingomyelin, a-tocopherol, all-trans retinol, Astaxanthin and flavonoid.

[0019] In another embodiment of the present invention, the krill and/or marine oil comprises Eicosapentanoic acid, Docosahexanoic acid, Linolenic acid, Alpha-linolenic acid, Linoleic acid, Arachidonic acid, Oleic acid, palmitic acid, palmitoleic acid, stearic acid, nervonic acid, Phosphatidylcholine, Phosphatidylinositol, Phosphatidylserine, Phosphatidylethanolamine, Sphingomyelin, Cholesterol, Triglycerides, Monoglycerides, a-tocopherol, all-trans retinol, Astaxanthin, Canthaxanthin, β-carotene, flavonoid, Zinc, Selenium, sodium, potassium and calcium.

[0020] In another embodiment of the present invention, the krill and/or marine oil comprises Eicosapentanoic acid, Docosahexanoic acid, Linolenic acid, Alpha-linolenic acid, Linoleic acid, Arachidonic acid, Oleic acid, palmitic acid, palmitoleic acid, stearic acid, Phosphatidylcholine, Phosphatidylinositol, Phosphatidylserine, Phosphatidylethanolamine, Sphingomyelin, Cholesterol, Triglycerides, Monoglycerides, a-tocopherol, all-trans retinol, Astaxanthin, Canthaxanthin, β-carotene, Zinc and Selenium.

[0021] The diseases that can be treated and/or prevented by the method of the present invention are cardiovascular diseases, arthritis, skin cancer, diabetes, premenstrual syndrome and transdermal transport enhancement.

[0022] In accordance with the present invention there is also provided a composition for the treatment and/or prevention and/or therapy of the previously mentioned diseases, the composition comprising a therapeutically effective amount of krill and/or marine oil in association with a pharmaceutically acceptable carrier.

[0023] In accordance with the present invention, it is further provided the use of krill and/or marine oil for the treatment and/or prevention and/or therapy of the previously mentioned diseases.

[0024] In accordance with the present invention, it is also provided the use of krill and/or marine oil for the manufac-

DETAILED DESCRIPTION OF THE INVENTION

[0025] In accordance with the present invention, there is provided krill and/or marine extract for prevention and/or treatment and/or therapy of several diseases.

[0026] A multi-therapeutic oil extract free of enzyme is derived from krill and/or marine, found in any marine environment around the world, for example, the Antarctic ocean (euphasia superba), the Pacific ocean (euphasia pacifica), the Atlantic ocean, the Indian ocean, in particular coastal regions of Mauritius Island and/or Reunion Island of Madagascar, Canadian West Coast, Japanese Coast, St-Lawrence Gulf and Fundy Bay, and this oil extract is a free fatty acid lipid fraction.

[0027] The extraction process can be described as the following:

[0028] (a) Placing marine and/or aquatic krill and/or marine in a ketone solvent, preferably acetone, to achieve the extraction of grease from the krill and/or marine;

[0029] (b) Separating the liquid and the solid phases;

[0030] (c) Recovering a lipid rich fraction from the liquid phase obtained at step (b) by evaporation of the solvent present in the liquid phase;

[0031] (d) Placing the solid phase in an organic solvent, which can be alcohol, preferably ethanol, isopropanol or t-butanol, or esters of acetic acid, preferably ethyl acetate. This in order to extract the remaining soluble lipid fraction from the solid phase;

[0032] (e) Separating the liquid and the solid phases; and

[0033] (f) Recovering a lipid rich fraction from the liquid phase obtained at step (e) by evaporation of the solvent present in the liquid phase.

[0034] The active components of the enzyme-free krill and/or marine oil extract are:

[0035] Lipids

[0036] i) Omega-3:

[0037] i. Eicosapentanoic acid: >8 g/100 g

[0038] ii. Docosahexanoic acid: >2 g/100 g

[0039] iii. Linolenic acid: >0.10 g/100 g

[0040] iv. Alpha-linolenic acid: >0.3 g/100 g

[0041] In the preferred embodiment of the present invention, the Omega-3 are found in more than 30 g/100 g.

[0042] ii) Omega-6: i. Linoleic acid: >0.9 g/100 g

[0043] ii. Arachidonic acid: <0.45 g/100 g, preferably <0.6 g/100 g

[0044] iii) Omega-9: i. Oleic acid: >5 g/100 g

[0045] iv) palmitic acid: >10 g/100 g

[0046] v) palmitoleic acid: 0.08 g/100 g

[0048] Phospholipids

[0049] Phosphatidylcholine: >4.5 g/100 g

[0050] Phosphatidylinositol: >107 mg/100 g

[0051] Phosphatidylserine: >75 mg/100 g

[0052] Phosphatidylethanolamine: >0.5 g/100 g

[0053] Sphingomyelin: >107 mg/100 g

[0054] Neutral Lipids

[0055] Cholesterol: <3 g/100 g

[0056] Triglycerides: <55 g/100 g

[0057] Monoglycerides: >0.5 g/100 g

[0058] In another embodiment of the present invention, the neutral lipids of the krill and/or marine extract also comprises:

[0059] Diglycerides: >0.5 g/100 g

[0060] Antioxydants

[0061] α-tocopherol (vitamin E): >1.0 IU/100 g

[0062] all-trans retinol (vitamin A): >1500 IU/100 g

[0063] β-carotene: >3000 μg/100 ml

[0064] Pigments

[0065] Astaxanthin: >20 mg/100 g

[0066] Canthaxanthin: >2 mg/100 g

[0067] Metals

[0068] Zinc: >0.1 mg/100 g

[0069] Selenium: >0.1 mg/100 g

[0070] In another embodiment of the present invention, the krill and/or marine extract also comprises:

[0071] Flavonoids: >0.5 mg/100 g

[0072] Sodium: <500 mg/100 g

[0073] Calcium: >0.1 mg/100 g

[0074] Potassium: >50 mg/100 g

[0075] Aluminum: <8.5 mg/100 g

[0076] Protein: >4 g/100 g

[0077] Moisture and volatile matter: <0.8%

[0078] After characterization of the krill and/or marine oil extract, it was determined that the extract contains less than 25 ppm of solvent residue from the extraction process.

[0079] The oil has the following stability indexes:

[0080] Peroxide value: <0.1 (mEq/kg)

[0081] Oil Stability index: <0.1 after 50 hours at 97.8° C.

[0082] Saponification index: 70-180

[0083] Iodine value:60-130%

[0084] The present invention will be more readily under-

EXAMPLE 1

Cardiovascular Disease Prevention and/or Treatment

[0085] Krill and/or marine oil has been shown to decrease cholesterol in vivo. It also inhibits platelet adhesion and plaque formation and reduces vascular endothelial inflammation in a patient. It can offer hypertension prophylaxis. It prevents oxidation of low-density lipoprotein. It may have an inhibitory effect on the secretion of VLDL due to increased intracellular degradation of apo B-100. It also offers a post-myocardial infarction prophylaxis because of its ability to decrease CIII apolipoprotein B, to decrease CIII non-apolipoprotein B lipoproteins and to increase antithrombin III levels. Krill and/or marine oil is suitable for prophylactic usage against cardiovascular disease in human where cardiovascular disease relates to coronary artery disease, hyperlipidemia, hypertension, ischemic disease (relating to angina, myocardial infarction, cerebral ischemia, shock without clinical or laboratory evidence of ischemia, arrhythmia)

[0086] To evaluate the effects of krill and/or marine oil on the course of arteriosclerotic coronary artery disease and hyperlipidemia, a study was performed (prospective clinical trial, statistical significance $p<0.05$) with patients with known hyperlipidemia.

[0087] A group of 13 patients took krill and/or marine oil concentrate gelules. Both fish oil and krill and/or marine oil contained equal amounts of omega-3 fatty acids. Recommended dosage is of 1 to 6 capsules per day, each capsule containing 800 mg of oil. In this study, each patient took 6 capsules per day.

[0088] The patients were tested for LDL, HDL, Triglycerides, vital signs, CBC, SGOT/SGPT, γ-GT, ALP, Urea, Creatine, Glucose, K^+, Na^+, Ca^{2+} and total indirect bilirubin cholesterol before treatment and also at 2 months.

[0089] Table 1 is showing the results obtained from the previously described tests:

TABLE 1

	Paired Samples Test							
	Paired Differences							
				95% Confidence Interval of the Difference				
Parameter tested	Mean	SD.	Std. Error Mean	Lower	Upper	t-value	df	Sig. (2-tailed)
Cholesterol	.4954	.55800	.15476	.1582	.8326	3.201	12	.008
Triglycerides	.3538	.54543	.15127	.0242	.6834	2.339	12	.037
HDL	−.2108	.29859	.08281	−.3912	−.0303	−2.545	12	.026
LDL	.2846	.47333	.13128	−.0014	.5706	2.168	12	.051
Chol/HDL	.3600	.53446	.14823	.0370	.6830	2.429	12	.032

[0090] From the above, it was shown that a daily uptake of 1 to 4.8 g of krill extract was providing to the patients a cholesterol decrease in the range of 15%, a triglycerides decrease in the range of 15%, a HDL increase in the range of 8%, a LDL decrease in the range of 13% and a Choles-

[0091] This shows that an uptake of krill extract has a beneficial effect on patient suffering from hyperlipidemia, which is known to be the primary causative factor of atherosclerosis.

EXAMPLE 2

Arthritis Treatment

[0092] Krill and/or marine oil offers symptomatic relief for Arthritis where arthritis relates to adult arthritis, Still's disease, polyarticular or pauciarticular juvenile rheumatoid arthritis, rheumatoid arthritis, osteoarthritis because it has been shown that it provides a clinical improvement in decreasing the number of tender joints and of analgesics consumed daily by decreasing the production of Interleukin-8 and Interleukin-1 in human patients. Patients with a bleeding tendency or severe psychiatric disease were excluded from the study.

[0093] To evaluate the effects of krill and/or marine oil supplementation on the clinical course of osteoarthritis, a study was performed (prospective clinical trial, statistical significance $p<0.05$) with patients diagnosed with and treated for osteoarthritis which is Active class I, II or III and having treatment with NSAIDs and/or analgesics for at least 3 months before enrollment.

[0094] A group of 13 patients took krill and/or marine oil concentrate capsules at a daily rate of 6 capsules of 800 mg krill oil per capsule. The recommended dosage varies between 1 and 4.8 grams of pure krill extract per day. Patients were asked to follow a normal healthy diet consisting of 20% fat (less than 10% animal fat), 40% protein and 40% carbohydrates.

[0095] The inclusion criteria for the study are being aged between 50 and 65 years, both genders being admissible, having a clinical diagnosis of primary osteoarthritis (mild to moderate) 6 to 12 months prior to study enrollment including pain and stiffness, radiographic confirmation of illness prior to enrollment. It also include evidence of measurable symptoms of OA for at least 3 months prior to study enrollment requiring the use of acetaminophen, anti-inflammatory agents or opioid analgesics. Patients were asked to stop the use of all "pain-killers" the week prior to initiation of the trial for wash-out purposes.

medicines for anti-inflammatory use, use of topical analgesics within 4 weeks of randomization visit, steroid injection into either knee within past 3 months, initiation of physical therapy or muscle conditioning within 3 months, seafood allergies, use of anticoagulants or salicylates, alcohol consumption exceeding 3 mixed drinks per day, concurrent medical/arthritic disease that could confound or interfere with the evaluation of pain, prior surgery (including arthroscopy) of either knee, a known "secondary" cause of osteoarthritis.

[0097] Evaluation was based on daily dose of NSAIDs and/or analgesics and/or SAARDs, number of painful joints, number or swollen joints, duration of morning stiffness, visual analog scale (0-100) WOMACscale and SF36. Preliminary results have been obtained after 2 months. The number of NSAIDs and/or analgesics and/or SAARDs required for daily functioning has been recorded at initiation and at 2 months after initiation.

[0098] Results shown at Table 2 demonstrate the effect of an uptake of krill extract on the relief of arthritis.

TABLE 2

	Frequency	%	Valid %	Cumulative %
No change	3	23.1	23.1	23.1
Pain relief	10	76.9	76.9	100.0
Total	13	100.0	100.0	

[0099] This shows that ten out of 13 (76.9%) people reported a significant pain relief and improvement of flexibility of large joints (lower back, knees, shoulders)

EXAMPLE 3

Skin Cancer Prophylaxis

[0100] Krill and/or marine oil has been shown to be a skin cancer prophylactic because of its retinol anti-carcinogenic effect, Astaxanthin anti-carcinogenic effect and its phospholipid anti-carcinogenic effect.

[0101] To evaluate the photoprotective potential of krill and/or marine oil against UVB-induced skin cancer, a study was performed on nude mice, preferably on C57BL6 Nude Congenic Mice-B6NU-T (heterozygotes) because of their proven susceptibility to skin cancer.

[0102] Groups were formed as follows: 48 fish oil: 16 with oral supplementation (po) 16 with local application, 16 with po and local application; 48 krill and/or marine oil: 16 with po, 16 with local application, 16 with po and local application. In order to establish efficacy of krill and/or marine oil for the prevention of skin cancer, the test was conducted as a randomized blind controlled trial (statistical significance $p<0.05$). Half of the mice have been treated orally or topically or both with oil containing 100% by weight krill and/or marine oil and the other half have been treated the same way with fish oil.

[0103] Nutrition was fat-free chow for the first week and was modified accordingly with the assigned group as

[0104] The mice were divided in six groups as follows:

[0105] Group A: fat-free chow with supplementation of fish oil (20% of total calories)

[0106] Group B: fat-free chow (100% of calories)+local application of fish oil 2 times per day

[0107] Group C: fat free chow with supplementation of fish oil (20% of total calories)+local application of soy oil 2 times per day

[0108] Group D: fat-free chow with supplementation of krill and/or marine oil (20% of total calories)

[0109] Group E: fat free chow (100% of calories)+local application of krill and/or marine oil 2 times per day

[0110] Group F: fat-free chow with supplementation or krill and/or marine oil (20% of total calories)+local application of krill and/or marine oil 2 times per day

[0111] The mice had been submitted to UVB radiation using a fluorescent test lamp, emission spectrum 270400 nm during weeks 2-20. The essay were performed during 30 minutes of UVB exposure per day and the test lamp was at a distance of 30 cm from the mice. At the end of the 20 weeks, or when malignant tumors had formed, mice were anesthetized with ether and sacrificed. Skin was examined blind by pathologists for signs of carcinogenesis.

[0112] The following tables (Tables 3-8) are showing the results obtained about the incidence of cancer when ultraviolet radiations are administered to mice's skin during 5 weeks.

TABLE 3

Krill extract Oral uptake

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Benign	14	87.5	87.5	87.5
	Cancer	2	12.5	12.5	100.0
	Total	16	100.0	100.0	

[0113]

TABLE 4

Control Oral uptake

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Benign	14	87.5	87.5	87.5
	Cancer	2	12.5	12.5	100.0
	Total	16	100.0	100.0	

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