(57) Abrégé/Abstract:
The invention relates to compositions that include an oil extract rich in fatty acid amide compounds including N-acylethanolamine (NAE) compounds such as N-palmitoylethanolamine (PEA) and N-arachidonoylethanolamide (AEA). The composition may be derived from marine materials including mussel meat.
(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2008 (26.06.2008)

(10) International Publication Number
WO 2008/075978 A3

(51) International Patent Classification:
A61K 31/164 (2006.01) A61P 29/00 (2006.01)
A61K 31/202 (2006.01) A61P 9/14 (2006.01)
A61P 25/28 (2006.01) A61P 11/06 (2006.01)
A61P 19/02 (2006.01) A61P 25/02 (2006.01)

(21) International Application Number:
PCT/NZ2007/000370

(22) International Filing Date:
18 December 2007 (18.12.2007)

(25) Filing Language:
English

(26) Publication Language:
English

(30) Priority Data:
552238 20 December 2006 (20.12.2006) NZ

(71) Applicant (for all designated States except US):
SEPEREX NUTRITIONALS LIMITED [NZ/NZ]; c/- TD Scott & Co Limited, Level 6, Otago House, 481 Moray Place, Dunedin (NZ).

(72) Inventors; and
(75) Inventors/Applicants (for US only): WILLIAMS, Charles, Edward [ZA/NZ]; 96 Campbells Road, Pine Hill, Dunedin (NZ). SANSOM, Andrew, John [NZ/NZ]; 5 Northview Crescent, Belleknowes, Dunedin (NZ).

(54) Title: AN EXTRACT

(57) Abstract: The invention relates to compositions that include an oil extract rich in fatty acid amide compounds including N-acyltylthanolamine (NAA) compounds such as N-palmitoylethanolamide (PEA) and N-arachidonoylethanolamide (AEA). The composition may be derived from marine materials including mussels.
OIL EXTRACT FROM A BIVALVE MOLLUSC
THAT IS RICH IN N-ACYLETHANOLAMIDES

TECHNICAL FIELD

The invention relates to an extract. More specifically, the invention relates to oil extracts from marine based raw materials rich in fatty acid amide compounds including N-acylethanolamide (NAE) compounds such as N-palmitoylethanolamide (PEA) and N-arachidonoylethanolamide (anandamide, AEA).

BACKGROUND ART

N-palmitoylethanolamide (PEA) is an endogenous fatty acid amide belonging to the family known as N-acylthanolamines (NAE’s; or N-acylethanolamides), including ‘cannabis-like’ compounds such as N-arachidonoylethanolamide (anandamide, AEA). PEA and AEA, have the potential to treat a range of human/animal conditions involving abnormal inflammatory and/or immune responses and associated pain (see Lambert et al., 2002, for a review).

History and Scientific Literature Overview of PEA and other NAE’s

a) Origin

PEA is a naturally occurring lipid found in many different cells of animal, marine and plant origin (see Lambert et al., 2002, for a review). In living organisms, PEA synthesis is rapidly induced in response to cellular stressors, such as tissue damage or pathological insults, which are often accompanied by inflammation and pain (Darmani, et al., 2005). While the biological functions of PEA in humans are not completely understood, it has been hypothesized that PEA constitutes one of several natural anti-inflammatory and analgesic chemicals (Darmani, et al., 2005).

The crude lipid extracted from the freeze-dried flesh of Perna canaliculus using chloroform and methanol consists of a complex mixture of triglycerides, sterol esters, sterols, polar lipids, free fatty acids and their derivatives, such as NAEs (Sepe et al., 1998; Murphy et al.,
2002; Murphy et al., 2003). PEA and N-stearoyl ethanolamide are the most abundant NAEs in blue mussel lipid extracts along with much smaller amounts of N-myristoyl (C14:0)-, N-oleoyl (C18:1)-, N-linoleoyl (C18:2)-, and N-arachidonoyl (C20:4; anandamide/ AEA)-ethanolamides. The nature and number of NAE's present in a crude or purified extract may have therapeutic implications since the prior art has revealed the existence of complex synergistic interactions between NAE's in whole animals and animal tissues. For example, data from animal studies indicates that some actions of PEA may be mediated via AEA and possibly other NAEs. Furthermore, evidence of synergistic effects between NAEs have been reported (see below for details).

b) Chemistry

PEA is a saturated 16 carbon fatty acid ethanolamide (C16:0), with the structure:

![PEA Structure](image1)

AEA is a polyunsaturated 20 carbon fatty acid ethanolamide (C20:4) with the structure:

![AEA Structure](image2)

c) Historical Perspective

Interest in the anti-inflammatory properties of PEA was first noted in the early 1950's by Coburn et al. (1954) who found that feeding guinea pigs a diet high in egg yolk protected them from experimental allergy. Subsequent studies isolated and purified PEA from egg yolk and identified the anti-inflammatory properties in animals (see Lambert et al., 2002, for a review). Research interest in NAEs was revived in the 1990's following the discovery of another endogenous NAE, AEA, with similar properties to the active component of cannabis. The cloning of the cannabinoid receptors (designated CB1 and CB2) and the generation of selective CB receptor ligands provided the tools to further accelerate research activity (Devane et al., 1992). Since then a substantial body of evidence gathered from animal and
human studies has shown that PEA has anti-inflammatory and analgesic properties when given via different routes of administration.

d) Pre-Clinical Studies

A number of studies have shown that synthetic PEA has anti-inflammatory and analgesic properties in a range of animal models. Typically, an inflammatory substance, such as carrageenan, collagen or phorbol ester, is injected beneath the skin and the resulting pathological and behavioural changes are measured within hours (acute model) or several days (chronic model). To date, the vast majority of these studies have been conducted in acute animal models of inflammation (e.g., Aloe et al., 1993; Mazzari et al., 1996; Conti et al., 2002; Costa et al., 2002).

e) Clinical Trials

In comparison to animal studies, there have only been a small number of human clinical trials to investigate the anti-inflammatory effects of PEA. During the early 1970's several trials were conducted in Czechoslovakia using an oral formulation given the trade name Impulsin™ (N-2-Hydroxyethyl palmitamide, SPOFA United Pharmaceutical Works). The first set of trials evaluated the efficacy of PEA (3 times/day/12 days) to reduce the incidence and severity of respiratory tract infections in 1345 adult volunteers (either young male soldiers or employees of the Skoda Car Co.; Masek et al., 1974). Results indicated that Impulsin™ helped to prevent viral infections when given before an infectious episode, but did not reduce the duration of infectious symptoms. A further series of similar trials conducted between 1973 and 1975, on a total of 1864 young male soldiers (same dose regime), confirmed that prophylactic Impulsin™ significantly reduced the incidence of acute respiratory infections in this population (Kahlich et al., 1979). The incidence of unwanted effects during the 12 week trial was particularly low (just a few percent; Kahlich et al., 1979).

The apparent success of these trials led to the use of Impulsin™ in the former Czechoslovakia for acute respiratory disease. After several years on the market, the drug was withdrawn for unknown reasons which do not appear to be related to toxicity (see Lo Verme et al., 2005b, for a review). Two subsequent clinical trials have been initiated to investigate the efficacy of PEA for chronic back pain (lumbosciatalgia) and multiple sclerosis (see Lambert et al., 2002, for a review).

A cream containing "structured natural lipids" with PEA for topical administration has also been developed (Physiogel™ A.I., Stiefel Laboratories) and evaluated in two small-scale
clinical trials. The first was an observational study in which 19 adult patients diagnosed with anal eczema were instructed to apply the cream to the affected area for between 6 and 63 days (Rohde & Ghyczzy, 2003). After 4 weeks of the trial, 68% of patients reported a reduction in pain, burning sensation and itch, with 21% recording a worsening of symptoms. The cream was reportedly well tolerated by 95% patients. In the second trial, 21 adult patients with end-stage kidney failure and suffering from uremic itch, applied the cream twice daily for three weeks (Szepietowski et al., 2005). At the conclusion of the 3 week test period there was a statistically significant reduction in itch (completely absent in just under 40% patients) and 81% of patients reported an elimination of dryness in the affected area (xerosis). The cream was well tolerated by all patients with no adverse effects reported. These results provide an encouraging sign that a PEA-containing cream may provide an alternative therapeutic option in the treatment of inflammatory skin conditions.

f) Mode of Action

The precise mechanism(s) by which PEA exerts its anti-inflammatory and analgesic effects are not completely understood. It is generally accepted that PEAs effects are not mediated via classical cannabinoid receptors, a finding that may account for PEAs lack of psychotropic effects. In contrast, the majority of AEA actions appear to be mediated via CB1 and/or CB2 receptors in the brain and periphery. One problem with the use of AEA is its psychotropic side effects, which are thought to be mediated by CB1 receptors. However, there is evidence that such unwanted effects may be reduced or eliminated by co-administering low (sub-therapeutic) doses of AEA and another NAE (Calignano et al., 1998; Di Marzo et al., 2001; De Petrocellis et al., 2001; Lo Verme et al., 2005b). For example, sub-analgesic doses of PEA or AEA provide analgesia when given in combination at the same low doses (Calignano et al., 2001).

At the molecular and behavioural levels, PEA interacts with a number of important targets in the body. Evidence gathered from in vitro and in vivo studies indicates that PEA reduces oedema, mast cell proliferation, neutrophil infiltration and a number of endogenous mediators of inflammation including: mast cell degranulation (preventing release of histamine and serotonin), cyclo-oxygenase-2 (COX-2) activity, endothelial nitric oxide synthase activity, nitric oxide production from macrophages and lipid peroxidation during acute hypoxia (Gulaya et al., 1998). In addition, PEA reduces hyperalgesia in animal models of inflammatory pain (Jaggar et al., 1998; Farquhar & Smith, 2001; Conti et al., 2002).
Recent data suggests that peroxisome proliferator-activated receptor alpha (PPAR-α) is of key importance to the anti-inflammatory actions of PEA (Lo Verme et al., 2005a).

**Standard Therapeutic Approaches to the Control of Inflammatory Conditions**

**A. Diseases Involving Chronic Inflammation**

Inflammation is part of the body’s normal response to injury and infection and is characterised by the classic signs of localised redness, swelling, heat and pain. A normal inflammatory response is an acute process that resolves following removal of the stimulus and the initiation of repair and tissue healing. In some circumstances, acute inflammation can progress to chronic inflammation, which is a key component of a large number of pathologies affecting bone and joint, respiratory, skin, gastrointestinal tract, cardiovascular and neural systems. Importantly, the processes underlying acute and chronic inflammation are to a large distinct.

The popularity of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of inflammation and pain is largely due to their more favourable risk profiles compared to other anti-inflammatory drugs. However, there are several major limitations to long term NSAID use including: their propensity to cause gastric damage (peptic ulcers and gastrointestinal bleeding), renal impairment and an increased risk of bleeding. Furthermore, since NSAIDs do not alter disease progression, sufferers may require an additional class of drugs, the Disease Modifying Anti-inflammatory Drugs (DMARDs; e.g., methotrexate, etanercept, corticosteroids). Unfortunately, most of these agents have narrow margins of safety and are characterised by frequent unwanted effects that negatively impact upon quality of life.

**B. Atopic dermatitis/ Eczema**

Atopic dermatitis/ eczema (AD) is a chronic skin condition classically presenting as reddening, itch and the formation of vesicles which may lead to weeping and crusting. AD affects at least 15% of the developed world and is often associated with other forms of allergy, such as asthma and hay fever (Lee, Y-A., et al., 2000).

Topical corticosteroids are the gold standard of drug treatment for AD. However, one of the main drawbacks to long-term topical corticosteroid use is the risk of adverse effects such as skin shrinkage, oral and allergic contact dermatitis, acne, decreased skin pigmentation and excessive hair growth within the treatment area. Although a number of non-steroidal products are available (e.g., pimecrolimus, tacrolimus, antibiotics, cyclosporine,
methotrexate, etc), these are often less efficacious and none are without adverse effects that are occasionally serious (see Abramovits, 2005, for a review). For example, pimecrolimus and tacrolimus recently received FDA Black Box warnings regarding a possible cancer risk. Clearly, there is a need for effective anti-inflammatory products with fewer adverse effects.

**Anti-inflammatory effects of Perna Canaliculus**

“Stabilized” lipid extracts of the New Zealand Green-lipped mussel (*Perna canaliculus*, NZGLM) have demonstrated beneficial effects in several different animal models of inflammation as well as chronic inflammatory conditions in humans (Whitehouse et al., 1997; Whitehouse et al., 1999; Shiels & Whitehouse, 2000; Tenikoff et al., 2005; Gibson & Gibson, 1998; Emelyanov et al., 2002; Cho et al., 2003; Gruenwald, et al., 2004). Stabilization typically involves the addition of an organic acid, such as tartaric acid, to reduce oxidation of PUFA prior to processing (WO85/05033; NZ211928). However not all human studies in which stabilized mussel lipid extracts were used to treat inflammation have reported positive outcomes (Lau et al., 2004; see Cobb and Ernst, 2006, for a review) or have been able to demonstrate a reduction in blood markers of inflammation in healthy volunteers (Murphy et al., 2006).

**Mussel Patents**

Mussel patents in the art are related to various aspects of mussel extract formulation, for example:

**NZ211928** describes a formulation for stabilising green shell mussel extract by adding an organic acid (acetic acid, citric acid, tartaric acid, lactic acid) and/or a metal salt to the mussel flesh suspended in saline once harvested.

**NZ270754** describes a combination of finely ground mussel extract suspended within fish oil.

**NZ314867** describes a protein extract from green shell mussel combined with glycosaminoglycans.

**NZ514389** describes delivery of a green shell mussel extract to a pet in a pet food at a rate of 0.18 to 114 mg/kg of animal body weight per day.
Other patents describe extraction processes to enrich the extract in selected components, for example:

**NZ329018** describes a process for extracting glycogen from mussels by treating with proteolytic enzyme in water, separating the solid residue and recovering the glycogen from the aqueous solution.

**NZ510407** describes an extract of green shell mussel containing carbohydrate and lipids and with the protein fraction removed.

**NZ328489** describes a protein extract from green shell mussel as well as a method of producing the extract by stirring the flesh for 45 minutes in phenol solution then centrifuging / aspirating the upper layer and precipitating out the protein containing product using ethanol.

None of the above patents refer to producing an enriched NAE compound extract nor recognize the usefulness of these compounds in various treatments.

**Commercial Mussel Products**

Two widely available commercial preparations of the NZGLM are Seatone™ and Lyprinol™. Seatone consists of a stabilised freeze-dried powder obtained from whole mussel flesh, whereas Lyprinol™ is an oil extracted from stabilized freeze-dried flesh (via supercritical CO₂ fluid) with the addition of olive oil and vitamin E and formulated into capsules (Pharmalink International Ltd., Cayman Islands). Lyprinol™ contains five major classes of lipid: free fatty acids, triglycerides, sterol esters, sterols and phospholipids (Sinclair et al., 2000; Wolnyiak et al., 2005). The most abundant free fatty acids in Lyprinol™ are: palmitic (C16:0), linoleic (C18:2n-6), EPA (C20:5n-3), DHA (C22:6n-3), palmitoleic (C16:1n-7), C16:1n-9,7,5 and myristic (C14:0) (Sinclair et al., 2000; Wolnyiak et al., 2005). Although a large number of other fatty acids have also been identified (some 91 in all), individually these are present in only small quantities (below 5% w/w total lipid; Wolnyiak et al., 2005). The omega-3 PUFA’s comprise 40% of the total fatty acids and EPA and DHA are the most abundant (Wolnyiak et al., 2005). There is evidence to suggest that most of the anti-inflammatory activity of Lyprinol™ resides in the fatty acid fraction (Whitehouse et al., 1999; Treschow et al., 2007).

Following a course of Lyprinol™ human subjects had reduced levels of several pro-inflammatory compounds including thromboxane B₂, prostaglandin E₂ and interleukin-1β,
similar to those observed following low-dose omega-3 polyunsaturated fatty acid
supplements (Sinclair et al., 2000). This suggests that a significant component of
Lyprinol's™ activity may be due to its omega-3 content, a possibility that is consistent with
recent in vitro evidence (McPhee et al., 2007; Treschow et al., 2007). The hypothesized
mode of action of Lyprinol™ is via inhibition of both the 5-lipoxygenase and COX pathways.

Given the above evidence outlining the therapeutic benefits of PEA and other NAE
compounds, it should be appreciated that a product rich in these compounds may be
advantageous.

It is an object of the present invention to address the foregoing problems or at least to
provide the public with a useful choice.

No admission is made that any reference constitutes prior art. The discussion of the
references states what their authors assert, and the applicants reserve the right to challenge
the accuracy and pertinency of the cited documents. It will be clearly understood that,
although a number of prior art publications are referred to herein; this reference does not
constitute an admission that any of these documents form part of the common general
knowledge in the art, in New Zealand or in any other country.

It is acknowledged that the term 'comprise' may, under varying jurisdictions, be attributed
with either an exclusive or an inclusive meaning. For the purpose of this specification, and
unless otherwise noted, the term 'comprise' shall have an inclusive meaning - i.e. that it will
be taken to mean an inclusion of not only the listed components it directly references, but
also other non-specified components or elements. This rationale will also be used when the
term 'comprised' or 'comprising' is used in relation to one or more steps in a method or
process.

Further aspects and advantages of the present invention will become apparent from the
ensuing description which is given by way of example only.

**DISCLOSURE OF INVENTION**

For the purposes of this specification, the term 'marine based' refers to shellfish
organisms living in or around the seawater or freshwater.
The term 'N-acylethanolamine' (NAE) is used but includes other names common to the art for this group of compounds including 'N-acylethanolamides' or fatty acid ethanolamides.

According to a first aspect of the present invention, there is provided a composition containing an oil extract from a marine based organism that is rich in fatty acid amide compounds.

According to a further aspect of the present invention, there is provided a composition containing an oil extract from a marine based organism that is rich in N-acylethanolamine (NAE) compounds.

Preferably, the extract is rich in N-palmitoylethanolamide (PEA). It should be appreciated by those skilled in the art that PEA also has a number of different names including but not limited to palmidrol, and N-(2-hydroxyethyl) - hexadecanamide.

According to a further aspect of the present invention, there is provided a composition containing an oil extract from a marine based organism containing at least 0.10 µg/g of PEA as measured in the wet weight of the marine organism flesh prior to extraction.

According to a further aspect of the present invention, there is provided a composition containing an oil extract from a marine based organism that is rich in NAE compounds including at least 0.10 µg/g of PEA as measured in the wet weight of the marine organism flesh prior to extraction.

As noted above, the composition is rich in a range of NAE compounds. By way of illustration, the composition includes (but is not limited to) having quantities of the NAEs: N-stearoylethanolamide (18:0), 70 ng/ g; N-oleoylethanolamide (C18:1), 5 ng/ g; N-linoleoylethanolamide (C18:2), 5 ng/ g; and N-arachidonoylethanolamide (C20:4, anandamide), 8 ng/ g in the wet flesh prior to extraction. This should not be seen as limiting as the composition may also contain other NAEs and may also include NAEs in amounts varying above and below that disclosed.

Preferably, the marine based organism is a bivalve mollusc. More preferably, the organism is a mussel species. Most preferably, the organism is a mussel of the species Perna or Mytilus (green or blue mussel respectively). This should not be
seen as limiting as other species may also be included for example, clam and oyster species.

Preferably, the fatty acid amide compounds include NAE compounds.

Preferably, the NAE compounds include: N-myristoyl ethanolamide, C14:0; N-palmitoyl ethanolamide (PEA), C16:0; N-stearoyl ethanolamide, C18:0; N-oleoyl ethanolamide, C18:1; N-linoleoyl ethanolamide, C18:2; N-arachidonoyl ethanolamide (anandamide, AEA), C20:4; N-eicosaenoyl ethanolamide, C20:1, and combinations thereof.

In preferred embodiments, the level of PEA in the oil extract is at least 0.10 µg/g as measured in the wet tissue of the marine organism prior to any extraction. More preferably, the level is at least 0.50 µg/g. In selected embodiments the level of PEA may be greater than 3.0 µg/g.

Preferably, the level of AEA in the oil extract is at least 0.008 µg/g wet tissue of the marine organism prior to any extraction. More preferably, the level is greater than 0.01 µg/g wet tissue of the marine organism prior to any extraction. In selected embodiments the level of AEA may be greater than 0.05 µg/g.

Preferably, the composition includes PEA in the oil extract at a level greater than 1.0 µg/g as measured in the dried tissue of the marine organism prior to any further extraction.

Preferably, the composition includes AEA in the oil extract at a level greater than 0.09 µg/g as measured in the dried tissue of the marine organism prior to any further extraction.

It should be appreciated that the above levels of at least PEA and AEA are significantly higher in the oil extract of the present invention than that of the prior art which either does not teach of PEA levels at all or does not teach the levels described. In the closest prior art, Sepe et al teaches of producing only 0.053 ± 0.0039 µg/g of PEA and 0.0018 ±0.003 µg/g of AEA in wet tissue from Mediterranean mussels.

It is emphasised that the above values refer to PEA and AEA concentration in wet flesh. It should be appreciated that methods which reduce the water content (such
as drying) also concentrate the level of active compounds including PEA and AEA. The extract of the present invention is already substantially more concentrated before such water removal processes are completed.

According to a further aspect of the present invention, the fatty acid amide levels in the raw marine material substantially as described above may be enriched.

For the purposes of this specification, the term 'enriched' refers to an increase in the concentration/amount of fatty acid amide compounds in the marine material before any concentration occurs via a drying process.

In one preferred embodiment, enrichment occurs by harvesting, crushing and then holding the marine material in a crushed state at between 4 and 10°C for a time period of at least 24 hours. In one embodiment, the material may be held for up to 144 hours.

The inventors have found that by completing the above enrichment step, the concentration/amount of fatty acid amide compounds increases. This is thought to be due to biochemical reactions occurring post-mortem in the marine material. Unexpectedly, this process resulted in a substantial enrichment of mussel meat fatty acid amide levels. Also unexpectedly, the inventors discovered that by using the parameters described, microbial contamination does not occur to an extent detrimental to the extract being suitable for human consumption. As may be appreciated, normal handling procedures for marine based materials requires steps to be taken as quickly as possible to prevent microbial growth such as freezing or drying. Allowing the material to be held for several days at 10°C goes against current practice and yet the level of microbial contamination was unexpectedly low for this time and temperature.

As noted above, the composition is rich in fatty acid amides including PEA and AEA but may also include other compounds (both fatty acid amides and other compounds). In fact, it is the inventors understanding that it is highly desirable to have multiple NAE compounds present besides PEA as, based on prior art experience with NAEs (published literature), synergies between NAEs are common and therefore, the potency of a product containing multiple NAEs may be substantially increased compared to that containing only one NAE.
In a further embodiment, the composition also includes at least one polyunsaturated fatty acid (PUFA) compound. More preferably, the PUFA compound or compounds include omega-3 PUFAs. It may be appreciated that having an oil extract that includes both fatty acid amide compounds and PUFA compounds is desirable. What may also be appreciated is that as fatty acid amide compounds and PUFA compounds have different chemical properties, obtaining an extract with significant levels of both types of compound is unexpected. It should however be appreciated that PUFA compounds are not essential to the composition of the present invention and the presence of PUFA compounds should not be seen as limiting.

Preferably, the oil extract includes at least one PUFA compound. More preferably, the PUFA or PUFAs are of the omega-3 class. Preferably, the PUFA or PUFAs are selected from: 4,7,10,13,16,19-docosahexaenoic acid (DHA; 22:6n3), 5,8,11,14,17-eicosatetraenoic acid (EPA, 20:5n3), 6,9,12,15-octadecatetraenoic acid (OTA, 18:4n3), 9,12,15-octadecatrienoic acid (ALA, 18:3n3), 7,10,13,16,19-docosapentaenoic acid (DPA, 22:5n3), 11,14,17-eicosatrienoic acid (ETA, 20:3n3), 8,11,14,17-eicosatetraenoic acid (20:4n3) and combinations thereof. In preferred embodiments, the DHA content is greater than 3g/100g as measured in the extract. Preferably, the EPA content is greater than 5g/100g as measured in the extract.

Preferably, the composition is formulated as an orally administered powder, solution, suspension, emulsion, oil, tablet or capsule. The composition may alternatively be formulated for topical administration, for example as a cream, lotion, ointment or oil. In a further embodiment, the composition is a solid or liquid food for administration as a ‘functional food’.

According to a further aspect of the present invention there is provided a formulation for oral or topical administration containing a therapeutically effective amount of an oil extract from a marine based organism that is rich in fatty acid amide compounds.

According to a further aspect of the present invention there is provided a formulation for oral or topical administration containing a therapeutically effective amount of an oil extract from a marine based organism that is rich in NAEs.

According to a further aspect of the present invention there is provided a formulation for oral or topical administration containing a therapeutically effective amount of an oil extract rich in PEA.
According to a further aspect of the present invention there is provided a formulation for oral or topical administration containing a therapeutically effective amount of an oil extract from a marine based organism containing at least 0.10 µg/g of PEA as measured in the wet weight of the marine organism flesh prior to extraction.

According to a further aspect of the present invention there is provided a formulation for oral or topical administration containing a therapeutically effective amount of an oil extract from a marine based organism that is rich in NAE compounds including at least 0.10 µg/g of PEA in the wet weight of the marine organism flesh prior to extraction.

Preferably, the oil extract also includes at least 0.008 µg/g of AEA in the wet weight of the marine organism flesh prior to extraction.

Preferably, the formulation as described above also includes at least one PUFA. More preferably, the PUFA or PUFAs include omega-3 fatty acids.

In embodiments envisaged by the inventors, the formulation includes carrier substances and may also include accepted food-grade antioxidants to assist in long-term stability of the extracted active compounds.

In one preferred embodiment, the formulation is a capsule for oral administration wherein the capsule is filled with oil extract sourced from mussel meat.

In an alternative preferred embodiment, the formulation is a cream or lotion for topical administration wherein the cream/ lotion includes an oil extract sourced from mussel meat.

According to a further aspect of the present invention there is provided a method of treatment of inflammation and associated pain by oral or topical administration of a composition or formulation substantially as described above.

In one embodiment, the inflammation is of a chronic rather than acute nature. By way of example, disease conditions involving chronic inflammation include: eczema/ atopic dermatitis, asthma, inflammatory bowel disease (including Crohn's disease
and ulcerative colitis), rheumatoid and osteoarthritis, glomerulonephritis, atherogenesis, Alzheimer's disease and adult respiratory distress syndrome. By way of second example, disease conditions involving chronic pain include neuropathic and arthritic pain.

In an alternative embodiment, the inflammation is of an acute nature and includes treatment of a soft tissue injury by topical application of a cream containing a composition or formulation of the present invention to the exterior of the injury site.

According to a further aspect of the present invention there is provided a method of treatment of a disease associated with inflammation by oral or topical administration of a composition or formulation substantially as described above.

According to a further aspect of the present invention there is provided a method of ameliorating the development of inflammation by oral or topical administration of a composition or formulation substantially as described above.

According to a further aspect of the present invention there is provided a method of treatment of a skin disease by topical administration of a composition or formulation substantially as described above.

According to a further aspect of the present invention there is provided a method of treatment of a skin disease by oral or topical administration of a composition or formulation substantially as described above.

According to a further aspect of the present invention there is provided a method of ameliorating the symptoms of a skin disease by oral or topical administration of a composition or formulation substantially as described above.

In preferred embodiments, the skin diseases to be treated include atopic dermatitis/eczema and contact dermatitis.

In preferred embodiments, the symptoms ameliorated include itch, dryness, swelling and reduced proliferation of human keratinocytes.

In one embodiment, the treatments substantially as described above may be incorporated with at least one non-steroidal anti-inflammatory drug (NSAID). It is envisaged that the extract may enhance the activity of even low (sub-therapeutic)
doses of NSAID thereby reducing the severity of side effects. In addition, the compositions and formulations of the present invention may also act to enhance the NSAID activity therefore allowing for the advantage of either better efficacy and/or reduced dosage of NSAID required.

According to a yet further aspect of the present invention there is provided the use of an oil extract substantially as described above in the manufacture of a formulation for treatments as described above.

From the above description it should be appreciated that there is provided an oil extract containing substantially elevated levels of active compounds that according to the art, are beneficial for at least joint mobility. Associated oral and topical formulations are also provided as well as treatments for various inflammation-related disorders and the symptoms thereof.

There is an urgent need for efficacious anti-inflammatory products that can be used long-term with fewer unwanted effects compared to current pharmaceuticals. The extract of the present invention may address this need. It is envisaged that daily administration of a food or formulation containing an NAE-enriched marine material extract represents an ideal treatment modality for the prevention or treatment of inflammatory symptoms.

**BRIEF DESCRIPTION OF DRAWINGS**

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

*Figure 1* LC/MS chromatogram of a representative *Perna canaliculus* extract showing the peak containing PEA (arrowhead, lower trace) at time = 10 minutes. The relative size of the peak demonstrates that PEA makes up an appreciable portion of the total ion current (top trace) suggesting a significant amount of PEA is present in the extract.

**BEST MODES FOR CARRYING OUT THE INVENTION**

Examples are now provided showing formulations produced and envisaged by the inventors relating to NAE enriched oil extracts.
EXAMPLE 1

An experiment was carried out to determine PEA levels in Blue mussels (Mytilus edulis) and Green-lipped mussels (Perna canaliculus) harvested commercially from New Zealand waters on a laboratory scale (Trial 1). A second experiment was conducted to determine PEA and AEA levels in Perna canaliculus on a simulated industrial scale (Trial 2).

Methods

PEA and AEA levels were measured by LC/MS in the crude lipid extracted from the dried meat of freshly harvested Blue and Green mussels and reported as µg/g in wet flesh (Table 1 below; Giuffrida et al., 2000).

The presence of PEA in the lipid extract of P. canaliculus was confirmed by LC/MS as shown in Figure 1 where the arrowhead indicates the PEA peak.

Results

<table>
<thead>
<tr>
<th>Wet Mussel Homogenate (µg/g)</th>
<th>PEA</th>
<th>AEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. galloprovincialis</td>
<td>Sepe et al.¹</td>
<td>0.021 - 0.053</td>
</tr>
<tr>
<td>M. edulis</td>
<td>Trial 1¹</td>
<td>0.15 – 0.39</td>
</tr>
<tr>
<td>P. canaliculus</td>
<td>Trial 1¹</td>
<td>0.3 - 0.61</td>
</tr>
<tr>
<td></td>
<td>Trial 2²</td>
<td>0.54 - 0.97</td>
</tr>
</tbody>
</table>

1. NAE levels were determined by back-calculation from lipid extract data.

2. NAE levels were estimated using a conversion factor based upon trial 1 data (Table 1).

Conclusions - Trials 1 and 2

1) PEA levels in P. canaliculus were higher than both Mytilus species. Since P. canaliculus contains slightly more meat on an equal weight basis and therefore a slightly higher proportion of dry matter, this at least partly accounts for the higher PEA levels relative to Mytilus.
2) AEA was also present at higher levels in *P. canaliculus* compared to *M. galloprovincialis*, although the absolute levels were low.

**EXAMPLE 2**

A method to enrich the endogenous levels of NAEs in the flesh of *P. canaliculus* prior to extraction is described. In this example, homogenised intact mussel meat from commercially harvested mussels was incubated under various conditions, as described below, in a laboratory. Incubation times ranged from 0 to 144 hours at 4 or 10 deg C in either aerobic or anaerobic conditions at atmospheric pressure. Tissue pH was monitored at intervals throughout the trial. At the end of the incubation period, the tissue was frozen and subsequently freeze-dried. The experimental conditions were tightly controlled to minimise potential confounding factors such as differences between batches of mussels, freezing or freeze-drying conditions, etc. NAE and omega-3 levels were measured in the freeze-dried mussel.

Results from the experiment showed that dried meat prepared from homogenised mussels incubated at 10°C under aerobic conditions for up to 144 hours had 5 to 10-fold more PEA and AEA relative to dried meat prepared from the same batch of mussels frozen immediately after homogenisation.

The inventors also unexpectedly found that levels of the major PUFA omega-3s (DHA, EPA and ALA) in the same dried mussel samples were retained even after 144 hours incubation.

In addition, microbial levels in the dried product were unexpectedly low given the time and temperatures involved.

**EXAMPLE 3**

As noted above in the description, it is understood that the invention oil extract is rich in NAE compounds including but not limited to PEA. By way of example, the invention extract contains PEA, AEA and/or PUFAs. A concentration profile within the lipid extract is anticipated to be: 3.0 – 57.0 μg/g PEA or more; 0.1-5.2 μg/g anandamide or more and/or at least 3g/ 100g DHA and at least 5g/ 100g EPA.

**EXAMPLE 4**

In one embodiment of the invention an oral formulation is described. By way of example, a
typical oral formulation includes mussel lipid extract (oil) of the present invention, with or without carrier lipid and antioxidant(s), contained within a gel capsule. The dosage envisaged for an adult human is approximately 1-4 capsules taken 1-2 times per day. It is anticipated that this administration regime will provide relief of pain and swelling due to chronic inflammation, particularly involving joints (arthritis).

EXAMPLE 5

In a further embodiment, the invention oil extract is used in a topical formulation. By way of example a topical formulation may include a cream or lotion containing the lipid extract (oil) of the present invention, with or without carrier lipid and antioxidant(s). Other substances that may be used in the cream/ lotion, include, but are not limited to: propylene glycol, water, glycerin, glycerides, hydrogenated lecithin, betaine, hydroxyethylcellulose, sodium carbomer, squaline, xanthan gum.

The cream is applied topically to affected areas 1-3 times per day for the relief of dry skin, redness, swelling, itch and the reduction of dermal thickening.

EXAMPLE 6

In a further example, an oral or topical formulation, as described above, is used for the treatment of acute inflammation. In one example, the extract could be administered within 4 hours of the onset of inflammation (e.g., soft tissue injury) in order to reduce the severity of the symptoms and possibly recovery time.

EXAMPLE 7

In a further example, the invention oil extract is utilised in a functional food. In one such embodiment, the food may be eaten in order to reduce the risk of developing an inflammatory condition including those described above.

By way of example, the product may be the invention oil extract in a ready to use state to be added (e.g. mixed into) various food products either by the consumer or the product manufacturer.

In one example, the invention oil extract is atomized to produce microspheres and added to a range of food products by manufacturers.
EXAMPLE 8

The invention oil extract may be co-administered with an NSAID. For example, a low dose of NSAID and an oral dose of the invention extract are used to treat chronic inflammation (e.g. rheumatoid or osteoarthritis; adult dose: 1-4 capsules taken at the same time and frequency as the NSAID). The invention extract then potentiates the anti-inflammatory effect of a low dose of NSAID and thus reduce the risk of unwanted effects.

EXAMPLE 9

The invention extract was further investigated by comparison to a prior art commercial product (P. canaliculus product; described in US 6,083,536 and US 6,346,278). The invention extract is rich in fatty acid amides including NAE compounds such as PEA and AEA. The invention extract may also include PUFA compounds such as EPA and DHA. The relative amounts of each key component in the extract of the present invention are compared to the commercial P. canaliculus product (Table 2 below).

<table>
<thead>
<tr>
<th>Test Material</th>
<th>NAEs (µg/ g oil)¹</th>
<th>Omega 3 Fatty Acids (g/100g oil)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEA</td>
<td>AEA</td>
</tr>
<tr>
<td>Invention Extract</td>
<td>57.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Commercial P. canaliculus product³</td>
<td>3.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

¹ Ethanolamides were quantified by liquid chromatography and mass spectrometry (LC/MS; triple quadruple MS).

² Omega-3 Fatty acids were converted to their FAME and quantified by gas chromatography according to a validated method (AOAC # 963.22).

³ Each capsule contains: 50 mg P. canaliculus extract, 100 mg olive oil and 0.225 mg d-alpha-tocopherol.
Data presented in Table 2 reveal that the extract of the present invention contains substantially higher levels of NAEs (17 fold higher PEA and 26 fold higher AEA) and Omega-3s (particularly the DHA and EPA) relative to the commercial *P. canaliculus* product.

It should be appreciated that formulations using the composition of the present invention may simply be an undiluted extract or may be mixed with other ingredients, therefore diluting the components above, but to a different extent than in the commercial *P. canaliculus* product.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the claims.

REFERENCES


WHAT WE CLAIM IS:

1. An oil extract from a bivalve mollusc that is rich in NAE compounds wherein the extract comprises PEA at a level greater than 1.0 µg/g as measured in the dried weight of bivalve mollusc flesh prior to extraction.

2. The extract of claim 1 further comprising at least one additional NAE compound comprising lauroylethanolamide, C12:0; N-myristoylethanolamide, C14:0; N-stearoylethanolamide, C18:0; N-oleoylethanolamide, C18:1; N-linoleylethanolamide, C18:2; N-arachidonylethanolamide (AEA), C20:4; or N-eicosaenylethanolamide, C20:1, or a combination thereof.

3. An oil extract from a bivalve mollusc that is rich in NAE compounds wherein the extract comprises AEA at a level greater than 0.09 µg/g as measured in the dried weight of bivalve mollusc flesh prior to extraction.

4. The extract as claimed in any one of claims 1-3 wherein the bivalve mollusc is a mussel from the genus *Perna*.

5. The extract of any one of claims 1-3 wherein the bivalve mollusc is a mussel from the genus *Mytilus*.

6. A composition comprising the extract of any one of claims 1-5 and at least one polyunsaturated fatty acid (PUFA) compound comprising DHA or EPA or a combination thereof.

7. The composition as claimed in claim 6 wherein the PUFA is DHA and the DHA content is greater than 4g/100g in a crude extract.

8. The composition as claimed in claim 6 wherein the PUFA is EPA and the EPA content is greater than 6g/100g in a crude extract.

9. A composition comprising an oil extract from a marine based organism that is rich in NAE compounds comprising at least 1 microgram per gram of PEA and at least one omega-3 type fatty acid compound as measured in the dried weight of the flesh of said organism prior to extraction.
10. The composition as claimed in claim 9 wherein the omega-3 type fatty acid compound comprises an N-acylethanolamine (NAE) compound.

11. The composition as claimed in claim 9 wherein the composition comprises at least one additional NAE compound comprising: lauroylethanolamide, C12:0; N-myristoylethanolamide, C14:0; N-stearoylethanolamide, C18:0; N-oleoylethanolamide, C18:1; N-linoleoylethanolamide, C18:2; N-arachidonoylethanolamide (AEA), C20:4; or N-eicosaenoylethanolamide, C20:1, or a combination thereof.

12. The composition as claimed in any one of claims 9 to 11 wherein the marine based organism is a bivalve mollusc.

13. The composition as claimed in any one of claims 9 to 11 wherein the marine based organism is a mussel from the genus *Perna*.

14. The composition as claimed in any one of claims 9 to 11 wherein the marine based organism is a mussel from the genus *Mytilus*.

15. The composition of any one of claims 9-14 further comprising at least one polyunsaturated fatty acid (PUFA) compound comprising DHA or EPA or a combination thereof.

16. The composition as claimed in claim 15 wherein the PUFA compound is DHA and the DHA content is greater than 4g/100g in a crude extract.

17. The composition as claimed in claim 15 wherein the PUFA compound is EPA and the EPA content is greater than 6g/100g in a crude extract.

18. The extract of any one of claims 1-5 or the composition of any one of claims 6-19 formulated for oral administration.

19. The extract of any one of claims 1-5 or the composition of any one of claims 6-18 formulated for topical administration.

20. A formulation for oral or topical administration comprising the extract of any one of claims 1-5 or the composition of any one of claims 6-18 and at least one carrier or excipient.
21. A formulation for oral or topical administration comprising at least 0.09 \mu g/g of AEA as measured in the dried weight of the bivalve mollusc flesh prior to extraction and at least one carrier or excipient.

22. The formulation as claimed in any one of claims 20 or 21 wherein the formulation is a capsule for oral administration.

23. The formulation as claimed in any one of claims 20 or 21 wherein the formulation is a cream for topical administration.

24. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating inflammation.

25. The use as claimed in claim 24 wherein the inflammation is chronic.

26. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating asthma.

27. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating inflammatory bowel disease.

28. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating rheumatoid arthritis.

29. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating osteoarthritis.

30. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating glomerulonephritis.

31. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating atherogenesis.
32. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating Alzheimer’s disease.

33. Use of the extract, composition or formulation of any one of claims 1-23 in the manufacture of a medicament for oral or topical administration for treating neuropathic pain.

34. The use as claimed in claim 24 wherein the inflammation involves a disease of the skin.

35. The use as claimed in claim 34 wherein the skin disease is atopic dermatitis/eczema or contact dermatitis.

36. The use as claimed in claim 24 wherein the inflammation is acute.

37. A method of enriching the fatty acid amide compounds in a marine material comprising the steps of:

   a) harvesting tissue from a marine based organism (MBO);

   b) holding the tissue at between substantially 4-10 °C for at least substantially 24 hours up to 144 hours.

38. A method as claimed in claim 37 comprising the further step of: c) drying the tissue.

39. A composition comprising a mixture of NAE compounds wherein at least one of the NAE compounds in the mixture has been obtained as an extract from a marine based organism (MBO) via the method steps of:

   a) harvesting tissue from the MBO; and

   b) holding the tissue at between substantially 4-10 °C for at least substantially 24 hours up to 144 hours.

40. The composition as claimed in claim 39 wherein the composition is in powder form.

41. The composition as claimed in claim 39 wherein the composition is oil.
42. The composition as claimed in claim 41 wherein the composition is contained within a capsule for oral consumption.

43. The composition as claimed in any one of claims 39 to 42 wherein one of the NAE compounds is PEA at a level at least 57.0 ug/g oil.

44. The composition as claimed in any one of claims 39 to 42 wherein one of the NAE compounds is AEA at a level at least 5.2 ug/g oil.

45. An oil comprising a mixture of NAE compounds wherein at least one of the NAE compounds in the mixture has been obtained as an extract from a marine based organism (MBO) via the method steps of:

   a) harvesting tissue from the MBO; and

   b) holding the tissue at between substantially 4-10 °C for at least substantially 24 hours up to 144 hours

and wherein one of the NAE compounds is PEA at a level at least 57.0 ug/g oil.

46. An oil comprising a mixture of NAE compounds wherein at least one of the NAE compounds in the mixture has been obtained as an extract from a marine based organism (MBO) via the method steps of:

   a) harvesting tissue from the MBO; and

   b) holding the tissue at between substantially 4-10 °C for at least substantially 24 hours up to 144 hours

and wherein one of the NAE compounds is AEA at a level at least 5.2 ug/g oil.

47. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating inflammation.

48. The use as claimed in claim 47 wherein the inflammation is chronic.

49. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating asthma.
50. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating inflammatory bowel disease.

51. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating rheumatoid arthritis.

52. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating osteoarthritis.

53. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating glomerulonephritis.

54. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating atherogenesis.

55. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating Alzheimer’s disease.

56. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating neuropathic pain.

57. Use of the composition as claimed in any one of claims 39 to 44 in the manufacture of a medicament for oral or topical administration for treating a disease/pathology involving inflammation of the skin.

58. The use as claimed in claim 57 wherein the skin disease to be treated is atopic dermatitis/eczema or contact dermatitis.

59. The use as claimed in claim 47 wherein the inflammation is acute.

60. Use of the extract, composition, formulation or oil of any one of claims 1-23 or 39 to 46 for treating inflammation, asthma, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, glomerulonephritis, atherogenesis, Alzheimer’s disease, neuropathic pain, atopic dermatitis/eczema or contact dermatitis.