Title: COMPOSITIONS OF MONOTERPENOIDS FOR STIMULATING THE IMMUNE SYSTEM

Abstract: The invention concerns use of a composition for stimulating the immune system in a mammal. The composition comprises a mixture of 30% - 80% of at least one compound having the formula (I) and 10% to 40% of at least one compound of the formula (II) wherein the substituents are as defined in the description. The invention also relates to use of the composition in the preparation of a medicament for stimulation of the immune system in a mammal.
COMPOSITIONS OF MONOTERPENOIDS FOR STIMULATING THE IMMUNE SYSTEM

FIELD OF THE INVENTION

The present invention relates to a composition for modulation of the human immune system. The present invention also relates to methods of treating patients having compromised immune systems and patients suffering from an immune system disease.

BACKGROUND OF THE INVENTION

The importance of the immune system in disease protection is widely studied, although not fully understood. Immunodeficient or immunocompromised persons are particularly vulnerable to opportunistic infections. Immunodeficiency can be caused by a number of factors such as stress, age, insufficient sleep, insufficient exercise, diabetes, obesity, alcohol and drug use, chemotherapy and certain medications. There are also a number of hereditary conditions in which certain parts of the immune system malfunction. Conventional medical response to immunocompromised patients includes prescribing antibiotics and immunoglobulin or interleukin treatment. A significant disadvantage of the latter is toxicity at high doses and side effects. There are numerous herbal remedies with purported immune boosting or immunostimulatory activity. However, there is little, if any, scientific validation for such remedies.

It is therefore an object of the present invention to provide a composition and method for use in modulating the immune system.

SUMMARY OF THE INVENTION

According to a first broad from of the present invention there is provided a composition comprising;

(a) 30% - 80% of at least one compound having the formula 1
wherein ———— is —— or ———, but consecutive ———— cannot be ————;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ———— attached to the ring is ———— the ring carbon to which the ———— is attached is unsaturated;

one of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ or R⁹ is OH, and each of the remaining R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ is H;

o is 0 when the ———— to which R¹, R⁴, R⁵ or R⁹ is attached is ——— and o is 1 when the ———— to which R¹, R⁴, R⁵ or R⁹ is ———;

(b) 10% to 40% of at least one compound of formula 2
wherein ~ is —— or ——, but consecutive ——— cannot be ——— or ——;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ——— attached to the ring is — or

_ the ring carbon to which the ——— is attached is unsaturated;

X is -O- or -O-O-;

Y is -O- or -O-O-

n is 0 or 1, m is 0 or 1, but n and m cannot both be 1 and neither n or m can be 1 if the —— —— attached to the ring is ——— or —

R^1, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28} or R^{29} are each independently selected from H, OH, OOH, OC=OR, OR; or an adjacent pair of R^1, R^{22}, R^{23}, R^{24}, R^{27}

or R^{28} may join to form an epoxide or

wherein any one of R^{22}, R^{23}, R^{27}, R^{28} or R^{29} may further be =O, provided that =O is attached to an unsaturated carbon; o is 0 when the ——— to which R^1, R^{24} or R^{26} is

attached is ——— or —— and o is 1 when the ——— to which R^1, R^{24} or R^{26} is

R is a C_3 to C_3 alkyl;

wherein the compound contains at least two Oxygen atoms; and

(c) from 0 to 20% of at least one compound selected from the group consisting of a sesquiterpene, a sesquiterpene alcohol, a sesquiterpene epoxide, p-cymene, an oxygenated cyclohexane, an oxygenated cyclohexene, and a monoterpene, wherein the amount of any
monoterpenes in the fraction does not exceed 5% of the total composition.

for use in modulating the immune system in a patient.

5 In the present specification and claims, the term % refers to the percentage as determined by gas chromatographic analysis, unless indicated otherwise. Gas chromatographic methods for the analysis of mixtures of terpenes and related compounds are well known in the art of natural product chemistry and aroma chemistry.

10 The present inventor has surprisingly and unexpectedly discovered that the above composition is able to modulate the immune system.

The term "modulate an immune response" as used herein refers to the suppression, or elevation of one or more immune parameters. Such parameters are known to those of skill in the art and include levels of immune cell types such as th1 and th2 cells, cytotoxic T cells, mature T cells, B cells, Activated t and B cells and macrophages and levels of cytokines including IL-1, IL-3, IL-6, IL-10, GM-CSF, IFNγ and TNF-α.

The compounds of formulas 1 and 2 include oxygenated derivatives of monoterpenes having a p-menthane skeleton. Monoterpenes are widely found in natural products and have the formula C₁₀H₁₆. The compounds of formulas 1 and 2 may be obtained or derived from known natural sources of monoterpenes or may be synthesized. Sources of monoterpenes and their oxygenated derivatives include but are not limited to eucalyptus oil, oil of cajeput, oil of camphor, oil of cardamom, tea tree oil, oil of cedar and oil of cypress. Suitable methods of extraction are known to those of skill in the art.

25 The oxygenated compounds of formula 1 and formula 2 may be obtained by oxidation of parent monoterpenes. Suitable starting monoterpenes include, α pinene, β pinene, sabinene, myrcene, δ phellandrene, β phellandrene, α terpinene, β terpinene, γ terpinene, limonene, δ limonene diepoxide and
terpinolene. These compounds are naturally occurring and may be isolated from a variety of plant sources.

Many of these compounds are commercially available in an essentially pure form. Oxidation of monoterpenes is used to produce compounds for use in the flavour and perfumery industry. Oxidation of monoterpenes is well known in the field of organic flavour chemistry.

α-terpinene is a suitable starting material as it may readily be oxidised with molecular oxygen via a diels alder cyclization of the 1,3-diene to produce the menth-2-ene, 1,4-endoperoxide (reaction 1 in Scheme 1 below). The peroxide is a useful intermediate as it may undergo further reaction with water to produce 1-hydroperoxy-4-hydroxy-menth-2-ene (reaction 2). Subsequent reduction with lithium hydroxide for example yields 1,4-dihydroxymenth-2-ene (reaction 3).

\[
\text{Scheme I}
\]

α-terpinene may also be oxidized with t-butyl chromate according to the procedure described by Suga et al. "Stereochemical Studies of monoterpen compounds III. Stereochemistry and intramolecular hydrogen bonding of 1-hydroxy-p-menth-3-en-2-one and its reduction products," Bulletin of the Chemical Society of Japan, 41, 944-048 (1968). The reaction produces 1-hydroxy- p-menth-3-en-2-one (reaction 4) which may subsequently be hydrogenated (reaction 5) and reduced (reaction 6) or reduced (reaction 7) and hydrogenated (8) as shown in scheme 2 below.
Scheme 2

Terpinene-4-ol is also a suitable starting product and may be oxidised to 1,2,4-trihydroxy menthane by a number of reactions, one of which is as shown below in scheme 3 (Cristea, et al. "Stereoselective frans-dihydroxylation of terpinen-4-ol: synthesis of some sterosomers of 1^\text{st}menthane-1,2,4-trior, Tetrahedron: Asymmetry, 13, (9) 915-918.

Scheme 3

1,4-dihydroxymenth-2-ene can be prepared from ascaridole by first hydrolysing ascaridole to menth-2-ene-1,4-endoperoxide followed by reduction to give the product compound.
Scheme 4

Oxidation of monoterpenes often results in a mixture of compounds. In the compositions and methods of the present invention, mixtures of compounds of the invention may be tolerated where components of the mixture either are inactive and non-toxic or present in very low concentrations. In some cases, it may not be necessary for the parent monoterpenes to be chemically or chirally pure.

Preferred compounds of formula 1 include a-terpineol (1), β-terpineol (2), γ-terpineol (3), terpinene-4-ol (4), menthol (5), thymol (6), carvacrol (7), carveol (8), isopipertinol (9), perillyl alcohol (10), 8-hydroxy-p-cymene (11), isopulegol (12), limonene-10-ol (13) and dihydrocarceol (14) and 4-isopropyl-1-methylcyclohex-e-enol (15)
A preferred composition contains terpinene-4-ol as the major constituent. Terpinene-4-ol is found in nature and is a major constituent of the tea tree oil. Terpinene-4-ol is also available commercially in the racemic form, and as the R and S isomers. In tea tree oil the chiral purity has been found to be (+)-(4R)-\( \chi \) Terpineol 75% and (-)-4S-oc-Terpinen4-ol 25% (Burfield and Sheppard Hanger", "super Clone 88 - Melaleuca Alternifolia - what is its value?", http://atlanticinstitute.com/teatree.pdf). Preferably, terpinene-4-ol is present from about 40% to about 70%, preferably between about 45% to about 65%,
preferably between about 48% to about 60% of the composition. Preferably the chiral purity of the terpinene-4-ol reflects that found in nature.

Preferably, the composition includes at least two compounds of formula 1. Preferably, the second compound is \( \alpha \)-terpineol. A typical composition comprises between about 40% to about 70% preferably between about 45% to about 65%, preferably between about 48% to about 60% terpinene-4-ol and about 2% to about 15%, preferably between about 4% to about 12%, most preferably between about 5% to about 10% \( \alpha \)-terpineol.

In one embodiment, the compounds of formula 1 consist essentially of terpinene-4-ol and \( \alpha \)-terpineol. As used herein, the phrase "consisting essentially of" limits the scope of the related disclosure or claim to the specified compounds, plus those that do not materially affect the basic and novel characteristic(s) of the disclosed and/or claimed subject matter. For example, a composition in which the compounds of formula 1 "consist essentially of terpinene-4-ol and \( \alpha \)-terpineol means that the recited compounds together represent at least 80%, or at least 85%, or at least 90% or at least 95% or at least 97.5% or at least 99% of the compounds of formula 1.

Suitable compounds of formula 2 include the following:
Preferably, the composition includes between about 10% to about 40%, preferably between about 15% to about 35%, preferably between about 20% to about 30% of di and tri-oxygenated compounds of formula 2.

Preferably the ratio of di-oxygenated to tri-oxygenated (i.e. compounds that contain 2 or 3 oxygen atoms) compounds is between about 1:1 to 5:1, preferably between about 1.5:1 to about 4:1, most preferably between about 2:1 to about 3:1.

An especially preferred composition comprises a fraction of compounds of formula 2 that comprises between about %1 to about 4%, preferably between
about 2% to about 3% 2-hydroxy-1,4-cineole, between about 5% to about 15%, preferably between about 6% to about 12%, preferably between about 8% to about 10% 1,4-dihydroxy-menth-2-ene, between about 0.5% to about 5%, preferably between about 1% to about 4% 1,2-dihydroxy-menth-3-ene and between about 1% to about 10%, preferably between about 2% to about 8%, preferably between about 3% to about 6% 1,2,4-trihydroxy-menthane.

Some of these compounds are naturally occurring and are found in essential oils. 2-hydroxy-1,4-cineole may be found in extracts from *Hibiscus sabdariffa* L. It is also available commercially. Cymenol is found in sage essential oil (*Salvia officinalis*) and essential oils of *Juniperus genus* plants, 4,6-dihydroxy-p-menth-1-ene is found in oil of cumin, 1,4-dihydroxy-p-menth-2-ene in peppermint oil, 1,2-dihydroxy-p-menth-3-ene is found in *Ferula jaeschkeana* and 1,2,4-trihydroxy-p-menthane is found in the oil of *Zanthoxylum bUndruga* fruits.

The composition may also contain up to 20% of compounds other than those of formulas 1 and 2. These other compounds may include sesquiterpenes and oxygenated derivatives thereof, p-cymene, an oxygenated cyclohexane, an oxygenated cyclohexene, and low levels (less than 5%) of monoterpenes. It will be appreciated that any additional compounds should be non-toxic or be present in below toxic levels. In one embodiment, the composition can contain up to 15wt% p-cymene.

The term oxygenated cyclohexene refers to any compound having a cyclohexene skeleton that is substituted with one or more oxygen groups such as =O, -OOH, or OH. The cyclohexene may further be substituted with a methyl or ethyl. A preferred compound is 4-methyl - 4-hydroxy - cyclohex-3-enone.

The term oxygenated cyclohexane refers to any compound having a cyclohexane skeleton that is substituted with one or more oxygen groups such as =O, -OOH, or OH. The cyclohexane may further be substituted with a methyl or ethyl.

The composition may also contain up to 5% of a compound of formula 3;
Formula 3

wherein \( \cdots \) is \( = \), \( \equiv \) or \( \bigtriangleup \), but consecutive \( \cdots \) cannot be \( = \) or \( \bigtriangleup \);

\( R^{31} \) and \( R^{33} \) are each independently selected from \( H, OH, OOH, OC=OR, \) OR, \( R^{31} \) may further be \( =O \) provided that the \( =O \) is not attached to an unsaturated carbon; \( R^{32} \) is selected from the group consisting of CO, COOH, COH, COOR, COR; \( R^{31} \) and \( R^{32} \) may join to form a lactone;

\( o \) is 0 when the \( \cdots \) to which \( R^{10} \) is \( = \) or \( \bigtriangleup \) and \( o \) is 1 when the carbon to which \( R^{33} \) is \( = \) and

\( R \) is a \( C_1 \) to \( C_3 \) alkyl.

The compounds of formula 3 may be formed by an oxidative ring opening reaction of a parent monoterpene. This may occur as a by-product of the oxidation reaction that produces compounds of formula 1. Especially preferred compounds include cis and/or trans 6-oxo-3-isopropyl-hept-2-enal and 6-oxo-3-isopropylheptenoic acid.

**Stereoisomers:**
Certain compounds of the invention contain chiral centres. Both racemic and diasteromeric mixtures, as well as the individual optical isomers isolated or synthesized, substantially free of their enantiomeric or diastereomeric partners,
are all within the scope of the invention. The racemic mixtures may be separated into their individual, substantially optically pure isomers through well-known techniques, such as the separation of diastereomeric salts formed with optically active adjuncts, e.g., acids or bases followed by conversion back to the optically active substances. The desired optical isomer may be synthesized by means of stereospecific reactions, beginning with the appropriate stereoisomer of the desired starting material.

**Prodrugs:**

Prodrugs of the compounds of the invention are also contemplated. The terms "pro-drug" and "prodrug" are used interchangeably herein and refer to any compound which releases an active parent compound according to Formula 1, 2 or 2a in vivo when such prodrug is administered to an animal. Prodrugs may be prepared by modifying one or more functional group(s) present in the compound of Formula 1 or 2 in such a way that the modification(s) may be cleaved in vivo to release the parent compound.

Prodrugs include compounds of Formulas 1, 2 or 3 wherein a hydroxy, carboxy or carbonyl group in a compound of Formulas 1, 2 or 3 is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl group. Examples of prodrugs include, but are not limited to, esters (e.g., acetate, dialkylaminoacetates, formates, phosphates, sulfates, and benzoate derivatives) of hydroxy functional groups and esters groups (e.g. ethyl esters, morpholinoethanol esters) of carboxyl functional groups, oximes, acetals, ketals and enol esters of ketone and aldehyde functional groups in compounds.

The pharmaceutical compositions of the present invention can be manufactured by methods well known in the art such as conventional mixing, dissolving, encapsulating, lyophilizing or emulsifying, among others. The compositions can be in the form of, for example, granules, powders, tablets, capsules, syrup, suppositories, injections, emulsions, elixirs, suspensions or solutions. The instant compositions can be formulated for various routes of administration, for example, by oral administration, by transmucosal administration, by rectal administration, or subcutaneous administration as well as intrathecal, intravenous, intramuscular, intraperitoneal, intranasal, intraocular or
intraventricular injection. The compound or compounds of the instant invention can also be administered in a local rather than a systemic fashion, such as injection as a sustained release formulation. The following dosage forms are given by way of example and should not be construed as limiting the instant invention.

For oral, buccal, and sublingual administration, powders, suspensions, granules, tablets, pills, capsules, gelcaps, and caplets are acceptable as solid dosage forms. These can be prepared, for example, by mixing one or more compounds of the instant invention, or pharmaceutically acceptable salts or tautomers thereof, with at least one additive or excipient such as a starch or other additive. Suitable additives or excipients are sucrose, lactose, cellulose sugar, mannitol, maltitol, dextran, sorbitol, starch, agar, alginates, chitins, chitosans, pectins, tragacanth gum, gum arabic, gelatins, collagens, casein, albumin, synthetic or semi-synthetic polymers or glycerides, methyl cellulose, hydroxypropylmethylcellulose, and/or polyvinylpyrrolidone. Optionally, oral dosage forms can contain other ingredients to aid in administration, such as an inactive diluent, or lubricants such as magnesium stearate, or preservatives such as paraben or sorbic acid, or anti-oxidants such as ascorbic acid, tocopherol or cysteine, a disintegrating agent, binders, thickeners, buffers, sweeteners, flavoring agents or perfuming agents. Additionally, dyestuffs or pigments may be added for identification. Tablets and pills may be further treated with suitable coating materials known in the art.

Liquid dosage forms for oral administration may be in the form of pharmaceutically acceptable emulsions, syrups, elixirs, suspensions, slurries and solutions, which may contain an inactive diluent, such as water. Pharmaceutical formulations may be prepared as liquid suspensions or solutions using a sterile liquid, such as, but not limited to, an oil, water, an alcohol, and combinations of these. Pharmaceutically suitable surfactants, suspending agents, emulsifying agents, may be added for oral or parenteral administration.

For nasal or buccal administration, the pharmaceutical formulations may be a spray or aerosol containing and appropriate solvents and optionally other compounds such as, but not limited to, stabilizers, antimicrobial agents,
antioxidants, pH modifiers, surfactants, bioavailability modifiers and combinations of these. A propellant for an aerosol formulation may include compressed air, nitrogen, carbon dioxide, or a hydrocarbon based low boiling solvent. The compound or compounds of the instant invention are conveniently delivered in the form of an aerosol spray presentation from a nebulizer or the like.

Injectable dosage forms generally include aqueous suspensions or oil suspensions which may be prepared using a suitable dispersant or wetting agent and a suspending agent. Injectable forms may be in solution phase or in the form of a suspension, which is prepared with a solvent or diluent. Acceptable solvents or vehicles include sterilized water, Ringer's solution, or an isotonic aqueous saline solution. Alternatively, sterile oils may be employed as solvents or suspending agents. Preferably, the oil or fatty acid is non-volatile, including natural or synthetic oils, fatty acids, mono-, di- or tri-glycerides.

For injection, the formulations may optionally contain stabilizers, pH modifiers, surfactants, bioavailability modifiers and combinations of these. The compounds may be formulated for parenteral administration by injection such as by bolus injection or continuous infusion. A unit dosage form for injection may be in ampoules or in multi-dose containers.

For rectal administration, the pharmaceutical formulations may be in the form of a suppository, an ointment, an enema, a tablet or a cream for release of compound in the intestines, sigmoid flexure and/or rectum. Rectal suppositories are prepared by mixing one or more compounds of the instant invention, or pharmaceutically acceptable salts or tautomers of the compound, with acceptable vehicles, for example, cocoa butter or polyethylene glycol, which is present in a solid phase at normal storing temperatures, and present in a liquid phase at those temperatures suitable to release a drug inside the body, such as in the rectum. Oils may also be employed in the preparation of formulations of the soft gelatin type and suppositories. Water, saline, aqueous dextrose and related sugar solutions, and glycerols may be employed in the preparation of suspension formulations which may also contain suspending agents such as
pectins, carbomers, methyl cellulose, hydroxypropyl cellulose or carboxymethyl cellulose, as well as buffers and preservatives.

The compounds may also be administered dermally. It has been observed that the compounds are readily absorbed through the skin such that dermal uptake directly into the lymphatic system by dermal application about the lymph nodes is possible.

The terms "effective amount" and "therapeutically effective amount" of a compound as used herein mean a sufficient amount of the compound or a mixture of one or more thereof, to provide the desired therapeutic or physiological effect or outcome. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

The most preferred form of administration is oral. Preferably the oral formulation is designed for absorption is the stomach in preference to the small intestine.

DETAILED DESCRIPTION OF THE INVENTION

Example 1

A composition was prepared having the following composition and allocated the reference MJR-2

2-hydroxy 1,4-cineole - 4%

\[
\text{1a, 4p-dihydroxy-menth-2-ene} - 5\%
\]
1α, 2β dihydroxy menth-3-ene - 2%

1α, 2β, 4-trihydroxy - menthane - 5%

1α, 4cc-dihydroxy-menth-2-ene - 5%

terpinene-4-ol - 56.4 %
a-terpineol - 6%

\[
\begin{align*}
\text{p-cymene 8%}
\end{align*}
\]

The balance of the composition contains minor amounts of compounds of formula 1 and formula 2 and also minor amounts of sesquiterpenes.

1. **Formulations**

   The following formulations will be prepared

   **Oral Formulation 1**
   
   300mg composition
   
   500mg microcrystalline cellulose
   
   Total - 800mg

   **Oral Formulation 2**
   
   150 mg composition
   
   500 mg microcrystalline cellulose
   
   Total - 650mg

2. **Phase 1 Trials**

   **Study Design**

   Twenty nine subjects were divided into three equal dosing groups of 5 males and 4-5 females. The subjects were aged between 19 to 53 years of age with normal body weight. The three groups were subjected to the following dosage regimes of an oral capsular dose of the composition of Example 1; Group 1 - a
single 600mg dose after a meal; a single 750mg dose after a meal and three 300 mg doses at 8 hour intervals for 5 days.

Results

The effect of a single dose on the vital body parameters, systolic and diastolic blood pressures and heart rate at one and two hours after administration was observed. No clinically significant changes were observed.

Urine chemistry, urine sediments, blood chemistry and haematological profiles were measured prior to dosing and at 14 days post dosing. The results showed that after all dosage regimes, urine sediments, levels of urine, blood chemistries and haematological profiles were in normal values. These findings show that there was no apparent harm to the liver where the total levels of total and direct biliruben, SGOT and alkaline phopshatase in the blood were in normal ranges and there was no biliruben nor urobilin found in urine. Similar results were obtained for the kidneys where the levels of total protein albumin, blood urea nitrogen (BUN) and creatinine in blood were normal and no protein, blood cells nor leucocytes were found in the urine. The absence of glucose and ketone bodies in the urine also indicted that 600mg of the inventive composition did not affect glycogeniesis as well. Hematological profiles (particularly the levels of white and red blood cells, haemoglobin, hematocrtite, thrombocyte (platelets) and coagulation time of the subjects were also unaffected by the doses. Further there was no production of Ca oxalate, triphosphate or uric acid crystals in the urine.

The conclusion that can be made from this trial is that the composition of Example 1 is well tolerated in subjects and is not likely to affect vital organs such as liver, kidney and hematology and it did not cause crystaluria.

Experimental Design

Four groups of specific pathogen free (SPF) mice received different treatments throughout this study. Each group comprised 2 mice. Mice in groups 1 and 3 were fed 2x 25μl doses of a 40% solution of the composition of Example 1 in sunflower oil every day for 5 days. This dosage was equivalent to 800mg/kg per mouse. Mice in groups 2 and 4 were fed 2x 25μl sunflower oil alone for 5 days as a control.
To stimulate the immune system, mice in groups 3 and 4 also received 20μg lippolysaccharide (LPS) dissolved in 100μl PBS interperitoneally (i.p) on day 5 and 100μg LPS in 100μlPBS i.p. 2.5 hours prior to immune cell harvest. Mice in grouped 1 and 3 received 100μl PBS alone i.p. on day 5 and 2.5 hours prior to immune cell harvest.

Immune parameters were analysed ex vivo from the peritoneum, spleen and blood. The results are summarised in the following Table 1

Table 1:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peritoneum</th>
<th>Spleen</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ Th1 and Th2 Cells</td>
<td>↑</td>
<td>↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>CD8+ Cytotoxic T cells</td>
<td>↑</td>
<td>↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>CD3+ Mature T cells</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>CD80+ B cells, costimulator</td>
<td>↑</td>
<td>-</td>
<td>Not analysed</td>
</tr>
<tr>
<td>CD40+ B cells macrophages</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>CD25+ Activated T and B cells</td>
<td>↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F4/80+ Macrophages</td>
<td>↑↑</td>
<td>-</td>
<td>↑↑</td>
</tr>
<tr>
<td>CD11b Macrophages</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

*Actions (Producer Cell type)*

IL-1α T cell and macrophage activation (macrophages) | ↑↑↑ |

IL-1β T cell and macrophage activation (macrophages) | ↑↑↑ |

IL-3 Hematopoises (T cells) | ↑↑ |

IL-6 T & B cell growth differentiation (T cells, macrophages) | ↑↑↑ |

IL-10 Macrophage func. Supressant (T cells, macrophages) | ↑ |

GM-CSF DC growth and differentiation (T cells, macrophages & MHC) | - |

IFN-γ Stim. Macrophages & MHC molecules (T & NK cells) | ↑ |

TNF-α Local inflammation (macrophages, NK & T cells) | ↑ |

**Key**

↑ indicates a slight increase compared to the control
Conclusion

Feeding high doses of the composition of the Example 1 increased the percentage of several of the immune cell types especially in the peritoneum and the blood of SPF mice. Feeding the composition also significantly increased many of the cytokines analysed in the blood serum.

It may also be seen that there TNF-α is only slightly increased indicating that the inflammatory immune response is not necessarily activated. This means that the composition of the invention may be used to modulate the immune response without incurring inflammation. This is especially important for treatment of patients suffering from conditions such as rheumatoid arthritis where the use of immune stimulating or enhancing drugs would be contra-indicated.

It will be appreciated that various changes and modifications may be made to the invention as described and claimed therein without departing form the spirit and scope thereof.
CLAIMS

1. A composition comprising;

(a) 30% - 80% of at least one compound having the formula 1

wherein ——— is ——— or ———, but consecutive ——— cannot be ———;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ——— attached to the ring is ——— the ring carbon to which the ——— is attached is unsaturated;

one of R₁, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ or R⁹ is OH, and each of the remaining R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ is H;

o is 0 when the ——— to which R¹, R⁴, R⁵ or R⁹ is attached is ——— and o is 1 when the ——— to which R¹, R⁴, R⁵ or R⁹ is ———;

(b) 10% to 40% of at least one compound of formula 2
Formula 2

wherein ——— is ——— or \( \bigtriangleup \), but consecutive ——— cannot be

——— or \( \bigtriangleup \).

5

the cyclohexane ring may be saturated or unsaturated with any degree of
unsaturation provided that when a ——— attached to the ring is ——— or

\( \bigtriangleup \) the ring carbon to which the ——— is attached is unsaturated;

\( X \) is -O- or -0-0-,

\( Y \) is -O- or -0 -O-

10

\( n \) is 0 or 1, \( m \) is 0 or 1, but \( n \) and \( m \) cannot both be 1 and neither \( n \) or \( m \) can
be 1 if the ——— attached to the ring is ——— or \( \bigtriangleup \)

\( R^1, R^2, R^3, R^4, R^5, R^6, R^7 R^8 \) or \( R^9 \) are each independently selected
from \( H, OH, OOH, OC=OR, OR; \) or an adjacent pair of \( R^1, R^2, R^3, R^4, \)

\( R^7 \) or \( R^8 \) may join to form an epoxide or \( \bigtriangleup \); wherein any one of
R²², R²³, R²⁷, R²⁸ or R⁹ may further be =0, provided that =0 is attached to an
unsaturated carbon; o is 0 when the \[\ldots\] to which R¹, R²⁴ or R²⁶ is
attached is \[\ldots\] or \[\ldots\] and o is 1 when the \[\ldots\] to which R²¹, R²⁴ or
R²⁶ is \[\ldots\] and

5  R is a C₁ to C₃ alkyl;

wherein the compound contains at least two Oxygen atoms; and

(c) from 0 to 20% of at least one compound selected from the group
consisting of a sesquiterpene, a sesquiterpene alcohol, a sesquiterpene
epoxide, p-cymene, an oxygenated cyclohexane, an oxygenated
cyclohexene, and a monoterpane, wherein the amount of any
monoterpenes in the fraction does not exceed 5% of the total
composition.

for use in stimulating the immune system in a mammal.

2. The composition of claim 1, characterized in that the at least one
compound of formula 1 is selected from the group consisting of a-terpineol, a-
terpineol, γ-terpineol terpinene-4-ol, menthol, thymol, carvacrol, carveol, perillyl
alcohol, isopulegol, limonene-10-ol, and dihydrocarveol.

3. The composition of claim 1 or claim 2, characterized in that the at least
one compound of formula 1 is terpinene-4-ol.

4. The composition of claim 3, characterized in that the composition
comprises from 40% to 70% terpinene-4-ol.

5. The composition of claim 1, having at least two compounds of formula 1.

6. The composition of claim 5, characterized in that there compounds of
formula 1 consist essentially of terpinene-4-ol and α-terpineol.

7. The composition of claim 6 comprising between about 40 and about 70%
terpinene-4-ol and about 4 to about 15% terpinene-4-ol.

8. The composition of any one of claims 1 to 7, characterized in that the at
least one compound of formula 2 is selected from the following compounds;
9. The composition of any one of claims 1 to 8, characterized in that the composition comprises at between about 10% to about 40%, preferably between about 15% to about 35%, preferably between about 20% to about 30% of at least one compound of formula 2.

10. The composition of claim 9, characterized in that the composition comprises at least two compounds of formula 2, and at least one compound has 2 oxygen atoms and at least one compound has three oxygen atoms, wherein the ratio of the at least one compound having two oxygen atoms to the at least one compound having three oxygen atoms is between about 1:1 to 5:1, preferably between about 1.5:1 to about 4:1, most preferably between about 2:1 to about 3:1.

11. The composition of claim 9, characterized in that the at least one compound of formula 2 comprises between about 1 to about 4%, preferably between about 2 to about 3% 2-hydroxy-1,4-cineole; between about 5% to about 15%, preferably between about 6% to about 12%, 1,4-dihydroxy-menth-2-en; between about 0.5% to about 5%, preferably between about 1% to about 4% 1,2-dihydroxy-menth-3-ene and between about 1 to about 10%, preferably between about 3% to about 6% 1,2,4-trihydroxy-menthane.

12. The composition of any one of claims 1 to 8, characterized in that the composition comprises between about 7% to about 15% p-cymene.

13. The composition of any one of claims 1 to 12, characterized in that the composition comprises at least one sesquiterpene.
14. The composition of claim 13, characterized in that the at least one sesquiterpene is selected from the group consisting of isoledene, calamene, ledene, allo-aromadendrene, aromadendrene.

15. The composition of any one of claims 1 to 14, characterized in that the composition further comprises up to 5% of a compound of formula 3;

\[
\begin{align*}
\text{Formula 3} \\
\text{wherein} \quad \begin{array}{c}
\text{is} \quad \begin{array}{c}
\text{or} \quad \bigtriangleup \text{, but consecutive} \quad \bigtriangleup \text{cannot be} \\
u \quad \begin{array}{c}
\text{or} \quad \bigtriangleup \text{;}
\end{array}
\end{array}
\end{array}
\end{align*}
\]

\( R^{31} \) and \( R^{33} \) are each independently selected from \( \text{H, OH, OOH, OC=OR, OR} \), \( \text{OR, } R^{31} \) may further be \( = \text{O} \) provided that the \( = \text{O} \) is not attached to an unsaturated carbon; \( R^{32} \) is selected from the group consisting of \( \text{CO, COOH, COH, COOR, COR;} \ R^{31} \) and \( R^{32} \) may join to form a lactone;

\[ o \text{ is} \ 0 \text{ when the} \quad \begin{array}{c}
\text{to which} \quad R^{10} \text{ is} \quad \bigtriangleup \quad \begin{array}{c}
\text{and} \quad o \text{ is} \ 1 \text{ when the} \\
\text{carbon to which} \quad R^{33} \text{ is} \quad \bigtriangleup \text{and}
\end{array}
\end{array}
\]

\[ R \text{ is a} \ C_1 \text{ to} \ C_3 \text{ alkyl.} \]

16. The composition of any one of claims 1 to 15, characterized in that the composition is formulated for oral administration.
17. The composition of claim 16, characterized in that the composition is administered in an amount of about 150mg to about 900mg preferably between about 150 to about 400mg and most preferably between about 150 to about 300 mg per day for a 70kg human.

18. The composition of any one of the preceding claims, characterized in that the mammal is a human having a compromised immune system.

19. The composition of claim 18, characterized in that the human is suffering from stress, is over the age of 50, is diabetic, suffering from alcohol or drug abuse or is obese.

20. A method of stimulating the immune system in a mammal, the method administering to the a mammal an effective amount of a composition comprising:

(a) 30% - 80% of at least one compound having the formula 1

```
R^9
```

```
(R^1)_o
```

```
R^2
```

```
R^3
```

```
(R^4)_o
```

```
R^5
```

```
(R^6)_o
```

```
R^6
```

```
R^7
```

```
R^8
```

```
R^9
```

wherein ———— is ———— or ————, but consecutive ———— cannot be ————;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ———— attached to the ring is ———— the ring carbon to which the ———— is attached is unsaturated;

one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 or R^9 is OH, and each of the remaining R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 and R^9 is H;
o is 0 when the = to which R₁, R₄, R⁵ or R⁹ is attached is and o is 1 when the = to which R₁, R₄, R⁵ or R⁹ is ;

(b) 10% to 40% of at least one compound of formula 2

Formula 2

wherein = is , = or , but consecutive = cannot be = or ;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a = attached to the ring is = or the ring carbon to which the = is attached is unsaturated;

X is -O- or -O-O-;

Y is -O- or -O-O-

n is 0 or 1, m is 0 or 1, but n and m cannot both be 1 and neither n or m can be 1 if the = attached to the ring is = or
R², R²2, R²3, R²4, R²5, R²6, R²7, R²8 or R²9 are each independently selected from H, OH, OOH, OC=OR, OR; or an adjacent pair of R²1, R²2, R²3, R²4, R²7 or R²8 may join to form an epoxide or \( \text{O} \), wherein any one of R²2, R²3, R²7, R²8 or R²9 may further be =O, provided that =O is attached to an unsaturated carbon; o is 0 when the \( \text{O} \) to which R²1, R²4 or R²6 is attached is \( \text{O} \) or \( \text{O} \) and o is 1 when the \( \text{O} \) to which R²1, R²4 or R²6 is \( \text{O} \) and

R is a C₁ to C₃ alkyl;

wherein the compound contains at least two Oxygen atoms; and

(c) from 0 to 20% of at least one compound selected from the group consisting of a sesquiterpene, a sesquiterpene alcohol, a sesquiterpene epoxide, \( \beta \)-cymene, an oxygenated cyclohexane, an oxygenated cyclohexene, and a monoterpen, wherein the amount of any monoterpenes in the fraction does not exceed 5% of the total composition.

21. The method of claim 20, wherein the at least one compound of formula 1 is selected from the group consisting of oc-terpineol, \( \beta \)-terpineol, \( \gamma \)-terpineol terpinene-4-ol, menthol, thymol, carvacrol, carveol, perillyl alcohol, isopulegol, limonene-10-ol, and dihydrocarveol.

22. The method of claim 20 or claim 21, characterized in that the at least one compound of formula 1 is terpinene-4-ol.

23. The method of claim 22, characterized in that the composition comprises from 40 % to 70% terpinene-4-ol.

24. The method of claim 20, having at least two compounds of formula 1.

25. The method of claim 24, wherein the compounds of formula 1 consist essentially of terpinene-4-ol and oc-terpineol.
26. The method of claim 25, wherein the composition comprises between about 40 and about 70% terpinene-4-ol and about 4 to about 15% terpinene-4-ol.

27. The method of any one of claims 20 to 26, wherein the at least one compound of formula 2 is selected from the following compounds:
28. The method of any one of claims 20 to 26, wherein the at least one compound of formula 2 consists essentially of 2-hydroxy 1,4-cineole, dihydroxymenth-2-ene, dihydroxy menth-3-ene, 4-trihydroxy menthane and dihydroxymenth-2-ene.

29. The method of any one of claims 20 to 28, where in the composition comprises from about 7 to about 15% p-cymene.

30. The method of any one of claims 20 to 29 wherein the composition comprises at least one sesquiterpene.

31. The method of claim 30, wherein the at least one sesquiterpene is selected from the group consisting of isoledene, calamene, ledene, alloaromadendrene, aromadendrene.

32. The method of any one of claims 20 to 31, wherein the composition further comprises up to 5% of a compound of formula 3;
Formula 3

$$\text{R}^3_1$$ and $$\text{R}^3_2$$ are each independently selected from H, OH, OOH, OC=OR, OR,

$$\text{R}^3_1$$ may further be =O provided that the =O is not attached to an unsaturated carbon; $$\text{R}^3_2$$ is selected form the group consisting of CO, COOH, COH, COOR, COR; $$\text{R}^3_1$$ and $$\text{R}^3_2$$ may join to form a lactone;

$$\text{o is 0 when the}$$ to which $$\text{R}^1_0$$ is $$\text{or}$$ and $$\text{o is 1 when the}$$

carbon to which $$\text{R}^3_3$$ is $$\text{and}$$

$$\text{R}$$ is a C1 to C3 alkyl.

33. The method of any one of claims 20 to 32, wherein the mammal is a human having a compromised immune system.

34. The method of claim 33 wherein the human is suffering from stress, is over the age of 50, is diabetic, suffering from alcohol or drug abuse or is obese.

35. Use of a composition comprising;

(a) 30% - 80% of at least one compound having the formula 1
wherein ---- is ------ or ------, but consecutive ------ cannot be -------;

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ------ attached to the ring is = the ring carbon to which the ------ is attached is unsaturated;

one of $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$, $R_8$ or $R_9$ is OH, and each of the remaining $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$, $R_8$ and $R_9$ is H;

$\circ$ is 0 when the ------- to which $R_1$, $R_4$, $R_5$ or $R_9$ is attached is = and $\circ$ is 1 when the ------- to which $R_1$, $R_4$, $R_5$ or $R_9$ is -------;

(b) 10% to 40% of at least one compound of formula 2
wherein ——— is ——, —— or □, but consecutive ——— cannot be □ —— or □.

the cyclohexane ring may be saturated or unsaturated with any degree of unsaturation provided that when a ——— attached to the ring is = or □ the ring carbon to which the ——— is attached is unsaturated;

X is -O- or -0-0-;

Y is -O- or -O-O-

n is 0 or 1, m is 0 or 1, but n and m cannot both be 1 and neither n or m can be 1 if the ——— attached to the ring is ——— or □.

R\textsuperscript{1}, R\textsuperscript{2}, R\textsuperscript{3}, R\textsuperscript{4}, R\textsuperscript{5}, R\textsuperscript{7}, R\textsuperscript{8} or R\textsuperscript{9} are each independently selected from H, OH, OOH, OC=OR, OR; or an adjacent pair of R\textsuperscript{1}, R\textsuperscript{2}, R\textsuperscript{3}, R\textsuperscript{4}, R\textsuperscript{7} or R\textsuperscript{8} may join to form an epoxide or □; wherein any one of R\textsuperscript{2}, R\textsuperscript{3}, R\textsuperscript{4}, R\textsuperscript{7} or R\textsuperscript{9} may further be =O, provided that =O is attached to an unsaturated carbon; o is 0 when the ——— to which R\textsuperscript{1}, R\textsuperscript{4} or R\textsuperscript{6} is attached is ——— or □ and o is 1 when the ——— is □

R is a C\textsubscript{1} to C\textsubscript{3} alkyl;

wherein the compound contains at least two Oxygen atoms; and

(c) from 0 to 20% of at least one compound selected from the group consisting of a sesquiterpene, a sesquiterpene alcohol, a sesquiterpene epoxide, p-cymene, an oxygenated cyclohexane, an oxygenated cyclohexene, and a monoterpene, wherein the amount of any
monoterpenes in the fraction does not exceed 5% of the total composition.

for the preparation of a medicament for stimulating the immune system in a mammal.

36. The use of claim 35, wherein the at least one compound of formula 1 is selected from the group consisting of α-terpineol, β-terpineol, γ-terpineol terpinene-4-ol, menthol, thymol, carvacrol, carveol, perillyl alcohol, isopulegol, limonene-10-ol, and dihydrocarveol.

37. The use of claim 35 or claim 36, wherein the at least one compound of formula 1 is terpinene-4-ol.

38. The use of claim 37, wherein the composition comprises from 40% to 70% terpinene-4-ol.

39. The use of claim 35, wherein the composition comprises at least two compounds of formula 1.

40. The use of claim 39, wherein the compounds of formula 1 consist essentially of terpinene-4-ol and α-terpineol.

41. The use of claim 40, wherein the composition comprises between about 40% and about 70% terpinene-4-ol and about 4% to about 15% terpinene-4-ol.

42. The use of any one of claims 35 to 41, wherein the at least one compound of formula 2 is selected from the following compounds;
43. The use of any one of claims 35 to 42, characterized in that the composition comprises at between about 10% to about 40%, preferably between about 15% to about 35%, preferably between about 20% to about 30% of at least one compound of formula 2.

44. The use of claim 43, characterized in that the composition comprises at least two compounds of formula 2, and at least one compound has 2 oxygen atoms and at least one compound has three oxygen atoms, wherein the ratio of the at least one compound having two oxygen atoms to the at least one compound having three oxygen atoms is between about 1:1 to 5:1, preferably between about 1.5:1 to about 4:1, most preferably between about 2:1 to about 3:1.

45. The use of claim 43, characterized in that the at least one compound of formula 2 comprises between about 1 to about 4%, preferably between about 2 to about 3% 2-hydroxy-1,4-cineole; between about 5% to about 15%, preferably between about 6% to about 12%, 1,4-dihydroxy-menth-2-en; between about 0.5% to about 5%, preferably between about 1% to about 4% 1,2-dihydroxy-menth-3-ene and between about 1 to about 10%, preferably between about 3% to about 6% 1,2,4-trihydroxy-menthane.

46. The use of any one of claims 35 to 45, wherein the composition comprises between about 7 to about 15% p-cymene.
47. The use of any one of claims 35 to 46 wherein the composition comprises at least one sesquiterpene.

48. The use of claim 47, wherein the at least one sesquiterpene is selected from the group consisting of isoleadene, calamene, ledene, allo-aromadendrene, aromadendrene.

49. The use of any one of claims 35 to 48, wherein the composition further comprises up to 5% of a compound of formula 3;

![Formula 3 Diagram]

wherein \( \cdots \) is \( \cdots \), \( \cdots \) or \( \text{\( =O \)} \), but consecutive \( \cdots \) cannot be \( \cdots \) or \( \text{\( =O \)} \);

\( R^{31} \) and \( R^{33} \) are each independently selected from \( H, \text{OH, OOH, OC}=\text{OR, OR, } R^{31} \) may further be \( =O \) provided that the \( =O \) is not attached to an unsaturated carbon; \( R^{32} \) is selected from the group consisting of \( \text{CO, COOH, COH, COOR, COR} \); \( R^{31} \) and \( R^{32} \) may join to form a lactone;

\( o \) is 0 when the \( \cdots \) to which \( R^{10} \) is \( \cdots \) or \( \text{\( =O \)} \) and \( o \) is 1 when the carbon to which \( R^{33} \) is \( \cdots \) and

\( R \) is a \( \text{C}_1 \) to \( \text{C}_3 \) alkyl.

50. The use of any one of the claims 35 to 49, wherein the mammal is a human having a compromised immune system.
51. The use of claim 50 wherein the human is suffering from a condition selected from the group consisting of stress, over the age of 50, diabetes, or suffering from alcohol or drug abuse or is obese.
**INTERNATIONAL SEARCH REPORT**

**International application No**

PCT/GB2011/001237

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**A. CLASSIFICATION OF SUBJECT MATTER**

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

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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>MIKHAELI BOTROS R ET AL: &quot;Chemistry and immunomodulatory activity of frankincense oil &quot;., ZEITSCHRIFT FOR NATURFORSCHUNG. C, JOURNAL OF BIOSCIENCES 2003 MAR-APR LNK: PUBMED: 12710734, vol. 58, no. 3-4, March 2003 (2003-03), pages 230-238, XP009159715, ISSN: 0399-5075 abstract; figure 1; table 1 page 231, right-hand col umn, line 19 - page 232, left-hand col umn, line 11 ----</td>
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* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
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"Z" document member of the same patent family

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**Date of the actual completion of the international search**

1 June 2012

**Date of mailing of the international search report**

08/05/2012

**Name and mailing address of the ISA/Authorized officer**

European Patent Office, P.B. 5818 Patentlaan 2 NL-2380 HV Rijswijk
Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Madal inska, K

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