(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2011 (19.05.2011)

PCT

(10) International Publication Number
WO 2011/057327 A1

(51) International Patent Classification:
A61K 36/77 (2006.01) *A61K 31/34* (2006.01)
A61K 31/37 (2006.01) *A61P 29/00* (2006.01)

(21) International Application Number:
PCT/AU2010/001497

(22) International Filing Date:
10 November 2010 (10.11.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2009905499 10 November 2009 (10.11.2009) AU

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: ANTI-INFLAMMATORY EXTRACT

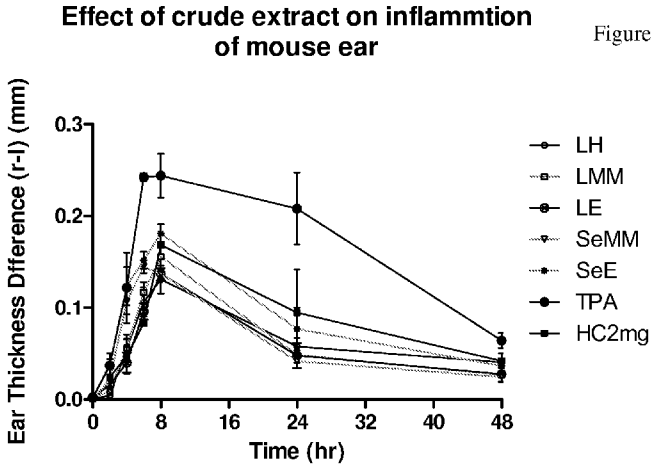

Figure 2

(57) Abstract: The present invention provides anti-inflammatory extracts derived from plants of the *Dodonaeoideae* subfamily. The present invention also provides a method for preparing an anti-inflammatory extracts. Other aspects of the present invention also relate to pharmaceutical compositions comprising anti-inflammatory extracts and methods of treating or preventing inflammation in a subject.

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ANTI-INFLAMMATORY EXTRACT

PRIORITY CLAIM

This patent application claims priority to Australian provisional patent application 2009905499, filed 10 November 2009, the content of which is hereby incorporated by reference.

FIELD

The present invention relates to anti-inflammatory extracts derived from plants of the *Dodonaeoideae* subfamily.

BACKGROUND

Inflammation is a complex biological process that occurs in response to stimuli including, for example, infection, damage to cells and/or tissue, irritants, etc. While inflammation is vital for healing and combating infection, abnormal or excessive inflammation can adversely affect the health, comfort and/or mobility of a subject.

A wide range of anti-inflammatory agents are known including steroids (such as glucocorticoids) and non-steroidal anti-inflammatory drugs (such as aspirin, ibuprofen, naproxen, etc). However, these drugs may be ineffective at treating some inflammatory conditions and/or may be associated with adverse side effects.

For example, some current anti-inflammatory agents have adverse side effects which include any one or more of gastrointestinal tract damage, renal damage, photosensitivity, hepatic stimulation, headaches, dizziness, Crushing's syndrome, hypertension, hypokalemia, hypernatremia, etc. Furthermore, due to adverse reactions

some anti-inflammatory agents may not be suitable for some subjects including, for example, pregnant subjects and subjects with an inflammatory bowel disease. Adverse side-effects of anti-inflammatory agents may result from topical, oral or other forms of administration.

Due to the limitations of many current anti-inflammatory drugs, there is a continual need to develop new anti-inflammatory agents.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

SUMMARY

In a first aspect the present invention provides an anti-inflammatory extract derived from a plant of the *Dodonaeoideae* subfamily.

In some embodiments, the extract may be a crude extract, a semi-purified extract or a purified extract.

In some embodiments, the extract may comprise a compound that belongs to the clerodane class. In some embodiments, the extract may comprise a diterpenoid. In some embodiments, the extract may comprise a flavonoid.

A second aspect of the present invention provides a method for preparing an anti-inflammatory extract of the first aspect of the invention, the method including:

i) mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent;

ii) collecting the solvent containing the extract from step (i); and

iii) removing at least a portion of the solvent.

A third aspect of the present invention provides an anti-inflammatory extract produced according to the method of the second aspect of the invention.

A fourth aspect of the present invention provides a pharmaceutical or cosmetic composition comprising an anti-inflammatory extract of the first aspect of the invention or the third aspect of the invention together with a pharmaceutically acceptable carrier or cosmetically acceptable carrier.

A fifth aspect of the present invention provides a method of treating or preventing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of an anti-inflammatory extract of the first aspect of the invention or the third aspect of the invention.

A sixth aspect of the present invention provides a method of treating or preventing inflammation in a subject, the method comprising administering to the subject a pharmaceutical composition of the fourth aspect of the invention.

A seventh aspect of the present invention provides use of an anti-inflammatory extract of the first aspect of the invention or the third aspect of the invention, in the preparation of a medicament for the treatment, prevention or alleviation of inflammation

DESCRIPTION OF EXEMPLARY EMBODIMENTS

It is to be understood that the following description is for the purpose of describing particular embodiments only, and is not intended to be limiting with respect to the above description.

A first aspect of the present invention provides an anti-inflammatory extract derived from a plant of the *Dodonaeoideae* subfamily.

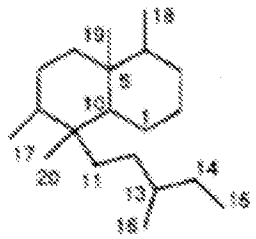
Dodonaeoideae is a subfamily of flowing plants in the Sapindaceae family and includes, for example, the genera *Dodonaea* and *Koelreuteria*. The *Koelreuteria* genus comprises three species, namely, *Koelreuteria bipinnate*, *Koelreuteria elegans*, and *Koelreuteria paniculate*.

In some embodiments, the plant is of the *Dodonaea* genus. The *Dodonaea* genus comprises about 70 species including, for example, *Dodonaea adenophora, Dodonaea amblyophylla, Dodonaea angustifolia, Dodonaea angustissima, Dodonaea aptera, Dodonaea attenuate, Dodonaea baueri, Dodonaea biloba, Dodonaea boroniifolia, Dodonaea bursariifolia, Dodonaea caespitose, Dodonaea camfieldii, Dodonaea ceratocarpa, Dodonaea concinna, Dodonaea coriacea, Dodonaea cuneata, Dodonaea divaricate, Dodonaea ericifolia, Dodonaea ericoides, Dodonaea falcate, Dodonaea filifolia, Dodonaea filiformis, Dodonaea glandulosa, Dodonaea hackettiana, Dodonaea heteromorpha, Dodonaea hexandra, Dodonaea hirsute, Dodonaea humifusa, Dodonaea humilis, Dodonaea inaequifolia, Dodonaea intricate, Dodonaea lanceolata, Dodonaea larreoides, Dodonaea lobulate, Dodonaea macrossanii, Dodonaea madagascariensis, Dodonaea megazyga, Dodonaea microzyga, Dodonaea multijuga, Dodonaea oxyptera, Dodonaea pachyneura, Dodonaea peduncularis, Dodonaea petiolaris, Dodonaea physocarpa, Dodonaea pinifolia, Dodonaea pinnata, Dodonaea platyptera. Dodonaea polyandra, Dodonaea polyzyga, Dodonaea procumbens, Dodonaea ptarmicifolia, Dodonaea rhombifolia, Dodonaea rigida, Dodonaea rupicola, Dodonaea serratifolia, Dodonaea sinuolata, Dodonaea spatulate, Dodonaea stenophylla, Dodonaea stenozyga, Dodonaea subglandulifera, Dodonaea tenuifolia, Dodonaea tepperi, Dodonaea triangularis, Dodonaea trifida, Dodonaea triquetra, Dodonaea truncatiales. Dodonaea uncinate, Dodonaea vestita, and Dodonaea viscose.*

In some embodiments, the plant is of the species *Dodonaea polyandra*.

In some embodiments, the extract comprises a compound that belongs to the clerodane

class. Compounds belonging to the clerodane class comprise a clerodane carbon skeleton. In some embodiments, the compound may comprise a clerodane diterpenoid. For example, a clerodane diterpenoid may comprise the following carbon skeleton:

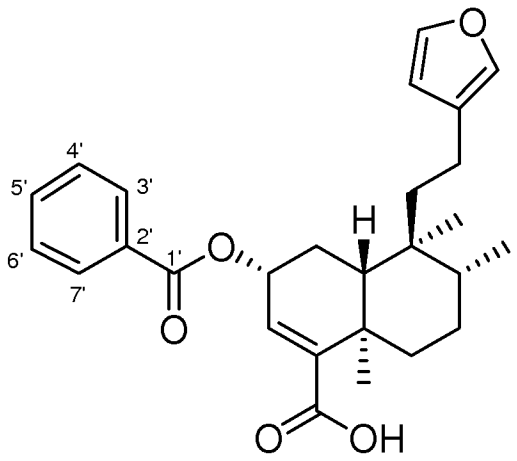


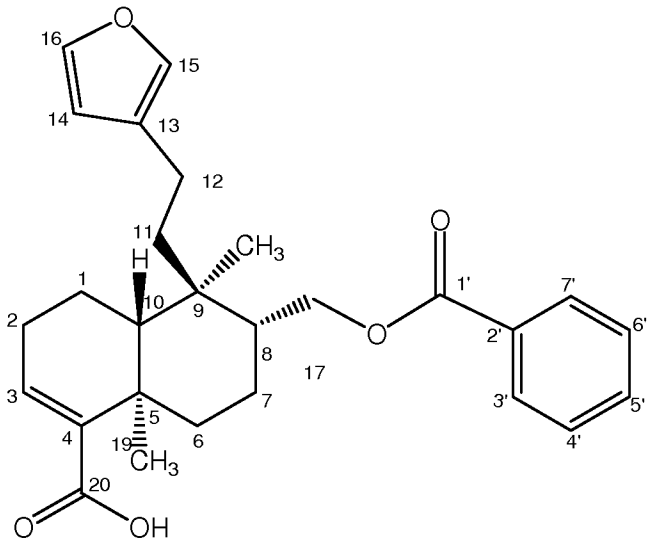
The clerodane class of diterpenoids are comprised of a continuous 20 carbon skeleton which encompasses a bicyclic ring system. As indicated in the diagram above, positions 4, 5 and 8 coincide with the branching of a single carbon containing group (eg. methyl (CH_3) group) while position 9 bears the attachment of a single carbon group and a 6 carbon chain. These sites form the basis of extensive biosynthetic modification.

In some embodiments, the extract comprises a diterpenoid. Diterpenoids may be characterized by the continuous connectivity of 20 carbon atoms formed from the head to tail joining of isoprene subunits. Biogenetically, this leads to the formation of geranylgeranyl pyrophosphate. Thus, while such compounds may be linear, they more commonly undergo some degree of intra-cyclization, giving rise to different classes of diterpenoids.

In some embodiments, the extract may comprise a benzoyl ester clerodane diterpenoid.

In some embodiments, the extract comprises one or more of the following compounds:





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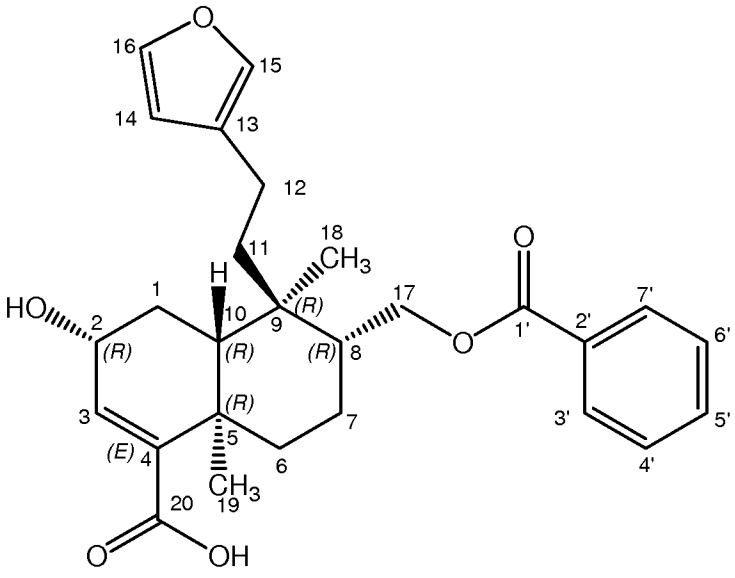
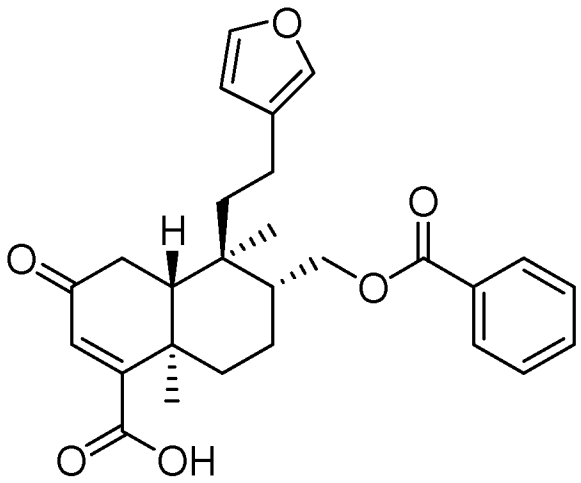




In some embodiments, the extract may comprise any one or more of: 15, 16-epoxy-3-clerodaene-8(α)-methylbenzoate-18-oic acid; 15, 16-epoxy-2(α)-hydroxy-3-clerodaene-8(α)-methylbenzoate-18-oic acid; 15, 16-epoxy-3-clerodaene-2-one-8(α)-methylbenzoate-18-oic acid; and 15, 16-epoxy-3-clerodaene-2(α)-benzoate-18-oic acid.

The extract may be a crude extract, a semi-purified extract or a purified extract. The term "crude extract" as used herein is intended to mean an extract that has undergone minimal purification of components in the extract. For example, a crude extract may be an extract obtained from a biomass of a plant by solvent extraction without further processing of the extract other than the removal of some or all of the solvent.

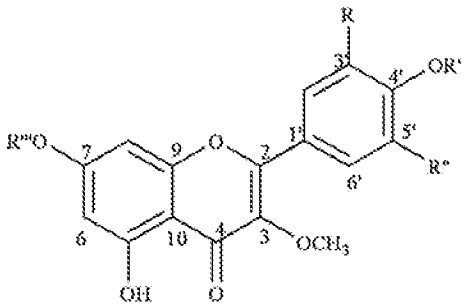
The term "semi-purified extract" or "fractionated extract" as used herein is intended to mean an extract that has undergone some processing to remove, concentrate or isolate one or more components of the extract. A semi-purified extract is likely to include multiple components. Methods of semi-purification of extracts are generally known in the art. For example, a semi-purified extract may include fractionation of the extract based on charge, molecular weight or other properties of components in the extract.

The term "purified extract" as used herein is intended to mean an extract that has been processed to isolate a chemical compound from the extract. Methods of preparing a purified extract are generally known in the art and include, for example, thin-layer chromatography or high-pressure liquid chromatography.

Exemplary examples of "semi-purified extracts", "fractionated extracts" and "purified extracts" include the "LH" fractions (e.g. LH1-LH13) and the compounds (e.g. compounds C, D, E and F) referred to in the examples herein. In some embodiments, one or more semi-purified, fractionated and/or purified extracts may be combined.

As indicated above, in some embodiments, the various extracts may include multiple compounds. In some embodiments, the extracts may comprise one or more flavonoids including, for example, one or more prenylated flavonoids (e.g. 3′(γ,γ –dimethylallyl)-5,7,4′-trihydroxy-3-methoxy flavone, 3′(γ,γ –dimethylallyl)-5,7-dihydroxy-3,4′-dimethoxy flavone or 3′,5′(γ,γ –dimethylallyl)-5,7,4′trihydroxy-3-methoxy flavones).

In some embodiments, the extract may comprise any one or more of the following compounds:



i) R= H; R'=H; R''=H, R'''=H

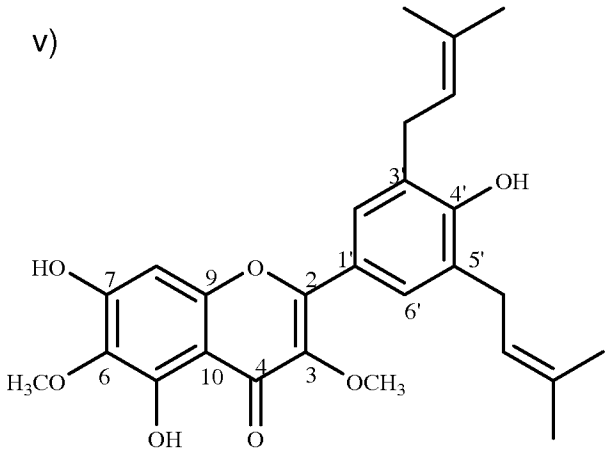
ii) R= R'=CH3; R''=H; R'''=H

iii) R= R'=H; R''= R'''=H

iv) R=H, R'=H, R''=H, R'''=OCH3

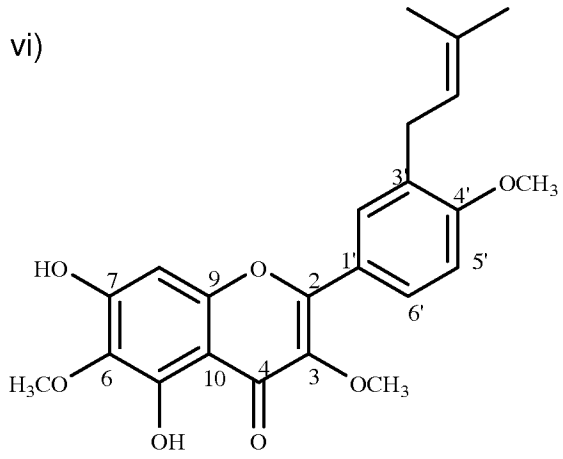
or

v)



or

vi)



In some embodiments, the extract may comprise any one or more flavonoids described above and any one or more diterpenoids described above.

A second aspect of the invention provides a method for preparing the anti-inflammatory extract of the first aspect of the invention, the method including:

i) mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent;

ii) collecting the solvent containing the extract from step (i); and

iii) removing at least a portion of the solvent.

The one or more anti-inflammatory agents extracted from the biomass into the solvent

may comprise any one or more of the compounds described above in relation to the first aspect of the invention. In some embodiments, the method includes preparing a crude extract, a semi-purified extract or a purified extract.

The biomass of the plant may be provided by different parts of the plant and may comprise fresh or dried plant material. In some embodiments, the biomass comprises dried plant material.

In some embodiments, the biomass may comprise any one or more of leaves, stems, or bark. In some embodiments, the biomass may comprise flowers, roots, branches or a trunk.

In some embodiments, the biomass may be crushed, torn, broken, macerated, blended or shredded prior to or concurrent with mixing with the solvent. Alternatively, the biomass may be used in a substantially undamaged state.

As set out above, the method of the second aspect of the invention includes mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent. As will be appreciated by those skilled in the art, the appropriate conditions may vary depending on the solvent used and the anti-inflammatory agents to be extracted.

In some embodiments, the appropriate conditions may comprise a temperature between 4°C and 30°C. In some embodiments, the appropriate conditions may comprise a temperature between 20°C and 30°C. In some embodiments, the appropriate conditions may comprise a temperature between 20 °C and 40 °C.

In some embodiments, the appropriate conditions comprise agitation of the plant material and solvent. Agitation may be performed by a number of different methods including, for example, stirring, shaking, inversion, etc. In some embodiments, the

agitation may be sufficient to rupture one or more cells in, or otherwise damage, the plant biomass, which may be advantageous for the extraction of some compounds from the plant biomass.

The second aspect of the invention contemplates any suitable extraction time. In some embodiments, mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent may be performed for longer than approximately 1 , 4 , 6 , 8 , 10 , 12 , 16, 20 or 24 hours. In some embodiments, mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent may be performed for less than 1 hour.

In some embodiments, collecting the solvent containing the extract from the mixture of the biomass of the plant and the solvent involves separating the solvent containing the extract from the biomass of the plant (e.g. by filtration). In some embodiments, the extract may be contained in only a portion or a component of the solvent (e.g. in an aqueous phase or organic phase), in which case collecting the solvent containing the extract may comprise separating one or more portions or components of the solvent. Collection or separation methods include those known in the art including, for example, decanting, filtering, density gradient separation, centrifugation, etc.

As set out above, the method for preparing the anti-inflammatory extract of the first aspect of the invention also includes removing at least a portion of the solvent. While removing at least a portion of the solvent may be performed prior to the collection of the solvent containing the extract, it is envisaged that this step will normally be performed after collection of the solvent containing the extract. In some embodiments, removing at least a portion of the solvent may comprise removing a component of the solvent (e.g. removal of ethanol from an aqueous ethanol solvent) or may comprise removing at least a portion or volume of the solvent as a whole (e.g. removing 40% of the volume of the solvent). In some embodiments, only a portion of the solvent may be

removed thereby concentrating the extract in the remaining solvent or changing the concentration of components in the solvent. In some embodiments, substantially all the solvent may be removed to prepare a dry or solid extract, or all of one or more components of the solvent may be removed.

Methods for removing solvents or components of solvents are known in the art and include, for example, precipitation of the extract, evaporation of the solvent or components of the solvent, chromatography, density gradient separation and/or centrifugation. Evaporation may be promoted by increasing/decreasing the temperature and/or pressure (e.g. freeze drying, baking, rotary evaporation, etc). Which components of a solvent and how much of the solvent is removed may depend on the desired form and use of the extract and the particular solvent that is used.

In some embodiments, the solvent comprises an alcohol. As will be appreciated, a range of different alcohols may be used including, for example, primary (e.g. ethanol), secondary (e.g. isopropyl alcohol) or tertiary (e.g. tert-butyl alcohol) alcohols. In some embodiments, the alcohol may be mixed with water.

In some embodiments, the solvent comprises ethanol or methanol. The alcohol may be either the pure alcohol or aqueous alcohol comprising 50-99 % alcohol by volume.

In some embodiments, the solvent comprises an ester including, for example, ethyl acetate (ethyl ethanoate).

In some embodiments, the solvent comprises a hydrocarbon. The hydrocarbon may comprise a linear hydrocarbon, a branched hydrocarbon or a cyclic hydrocarbon. In some embodiments, the hydrocarbon may be a substituted hydrocarbon.

In some embodiments, the solvent comprises an alkane hydrocarbon. The alkane hydrocarbon may comprise, for example, a pentane, a hexane or an octane

hydrocarbon.

In some embodiments, the alkane hydrocarbon comprises hexane.

In some embodiments, the solvent comprises methylene chloride. Methylene chloride (aka dichloromethane) is a chlorohydrocarbon with the formula CH_2Cl_2. In some embodiments, the methylene chloride may be mixed with other organic compounds. Accordingly, in some embodiments, the solvent may comprise a mixture of methylene chloride and methanol. While different ratios of methylene chloride to methanol may be used depending on the extract to be extracted and the extraction conditions, in some embodiments, the ratio of methylene chloride to methanol is approximately 1:1.

The method for preparing the anti-inflammatory extract of the first aspect of the invention may comprise a single extraction step with a single solvent or may comprise two or more sequential extraction steps with two or more different solvents or solvents of different concentrations. Thus, in some embodiments, the method comprises a sequential extraction with different solvents. In some embodiments, the method may comprise a single extraction step with multiple solvents.

The term "sequential extraction" as used herein is meant that the biomass is mixed with a first solvent under appropriate conditions and for an appropriate duration before the first solvent is removed and replaced with a second solvent. Further solvents may then be used once the second solvent has been removed. The different solvents may be used in a specific order to remove different compounds from the plant biomass, which may allow extracts to be selected which include a first compound but which exclude a second compound. For example, a first solvent, which is suitable for extracting a first compound but not a second compound from a plant biomass, may be used on the plant biomass prior to a second solvent which would otherwise extract both compounds. As the first solvent has already extracted the first compound from the plant biomass, the second solvent will be able to extract the second compound from

the plant biomass with minimal amounts of the first compound being extracted.

It will be appreciated that a range of different solvents may be used for the sequential extraction including, for example, any of the solvents previously mentioned herein. In some embodiments, the different solvents comprise hexane, methylene chloride/methanol (1:1), and ethanol. In some embodiments, the different solvents may be used in any order. In some embodiments, the different solvents are used in the order hexane, then methylene chloride/methanol (1:1), and then ethanol. In some embodiments, two or more solvents may be pooled at the end of extraction.

In some embodiments, the solvent extraction of the biomass of the plant may be repeated. The solvent containing the extract collected from the repeated solvent extraction may be combined with the solvent containing the extract collected from the original solvent extraction, or it may be processed separately.

A third aspect of the invention provides an anti-inflammatory extract produced according to the method of the second aspect of the invention. The anti-inflammatory extract may be a crude extract, a semi-purified extract or a purified extract.

A fourth aspect of the invention provides a pharmaceutical or cosmetic composition comprising the anti-inflammatory extract of the first or third aspects of the invention together with a pharmaceutically acceptable carrier or a cosmetically acceptable carrier.

In some embodiments, the pharmaceutical or cosmetic composition comprises a topical composition.

The pharmaceutical composition may also include one or more pharmaceutically acceptable additives, including pharmaceutically acceptable salts, amino acids, polypeptides, polymers, solvents, buffers, excipients and bulking agents, taking into consideration the particular physical and chemical characteristics of the anti-

inflammatory extract to be administered.

The preparation of such pharmaceutical compositions is known in the art, for example as described in Remington's Pharmaceutical Sciences (18th ed., Mack Publishing Co., Easton, Pa., 1990) and U.S. Pharmacopeia: National Formulary (Mack Publishing Company, Easton, Pa., 1984).

For example, the anti-inflammatory extract may be prepared into a variety of pharmaceutical compositions in the form of, e.g., an aqueous solution, an oily preparation, a fatty emulsion, an emulsion, a gel, etc., and these preparations may be administered as intramuscular or subcutaneous injection or as injection to an organ, or as an embedded preparation or as a transmucosal preparation through nasal cavity, rectum, uterus, vagina, lung, etc. The composition may be administered in the form of oral preparations (for example solid preparations such as tablets, capsules, granules or powders; liquid preparations such as syrup, emulsions or suspensions). Compositions containing the anti-inflammatory extract may also contain a preservative, stabiliser, dispersing agent, pH controller or isotonic agent. Examples of suitable preservatives include glycerin, propylene glycol, phenol or benzyl alcohol. Examples of suitable stabilisers include dextran, gelatin, a-tocopherol acetate or alpha-thioglycerin. Examples of suitable dispersing agents include polyoxyethylene (20), sorbitan mono-oleate (Tween 80), sorbitan sesquioleate (Span 30), polyoxyethylene (160) polyoxypropylene (30) glycol (Pluronic F68) or polyoxyethylene hydrogenated castor oil 60. Examples of suitable pH controllers include hydrochloric acid, sodium hydroxide and the like. Examples of suitable isotonic agents are glucose, D-sorbitol or D-mannitol.

The composition may also contain other constituents or additives such as a pharmaceutically or cosmetically acceptable carrier, diluent, excipient, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbent, preservative, surfactant, colorant, flavorant or sweetener, taking into

account the physical and chemical properties of the anti-inflammatory extract being administered.

The composition may be administered orally, parenterally, by inhalation spray, adsorption, absorption, topically, rectally, nasally, bucally, vaginally, intraventricularly, via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, or by any other convenient dosage form. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, and intracranial injection or infusion techniques.

When administered parenterally, the composition may be in a unit dosage, sterile injectable form (solution, suspension or emulsion) which is preferably isotonic with the blood of the recipient with a pharmaceutically acceptable carrier. Examples of such sterile injectable forms are sterile injectable aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable forms may also be sterile injectable solutions or suspensions in non-toxic parenterally-acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, saline, Ringer's solution, dextrose solution, isotonic sodium chloride solution, and Hanks' solution. In addition, sterile, fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides, corn, cottonseed, peanut, and sesame oil. Fatty acids such as ethyl oleate, isopropyl myristate, and oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated versions, are useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

The carrier may contain minor amounts of additives, such as substances that enhance

solubility, isotonicity, and chemical stability, for example anti-oxidants, buffers and preservatives.

When administered orally, the anti-inflammatory extract may be formulated into unit dosage forms such as tablets, cachets, powder, granules, beads, chewable lozenges, capsules, liquids, aqueous suspensions or solutions, or similar dosage forms, using conventional equipment and techniques known in the art. Such formulations typically include a solid, semisolid, or liquid carrier. Exemplary carriers include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, mineral oil, cocoa butter, oil of theobroma, alginates, tragacanth, gelatin, syrup, methyl cellulose, polyoxyethylene sorbitan monolaurate, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, and the like.

A tablet may be made by compressing or moulding the anti-inflammatory extract optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active, or dispersing agent. Moulded tablets may be made by moulding in a suitable machine, a mixture of the powdered active ingredient and a suitable carrier moistened with an inert liquid diluent.

The administration of the anti-inflammatory extract in the various embodiments of the present invention may also utilize controlled release technology. The anti-inflammatory extract may also be administered as a sustained-release pharmaceutical. To further increase the sustained release effect, the anti-inflammatory extract may be formulated with additional components such as vegetable oil (for example soybean oil, sesame oil, camellia oil, castor oil, peanut oil, rape seed oil); middle fatty acid triglycerides; fatty acid esters such as ethyl oleate; polysiloxane derivatives; alternatively, water-soluble high molecular weight compounds such as hyaluronic acid or salts thereof (weight average molecular weight: ca. 80,000 to 2,000,000),

carboxymethylcellulose sodium (weight average molecular weight: ca. 20,000 to 400,000), hydroxypropylcellulose (viscosity in 2% aqueous solution: 3 to 4,000 cps), atherocollagen (weight average molecular weight: ca. 300,000), polyethylene glycol (weight average molecular weight: ca. 400 to 20,000), polyethylene oxide (weight average molecular weight: ca. 100,000 to 9,000,000), hydroxypropylmethylcellulose (viscosity in 1% aqueous solution: 4 to 100,000 cSt), methylcellulose (viscosity in 2% aqueous solution: 15 to 8,000 cSt), polyvinyl alcohol (viscosity: 2 to 100 cSt), polyvinylpyrrolidone (weight average molecular weight: 25,000 to 1,200,000).

In some embodiments, the anti-inflammatory extract may be incorporated into a hydrophobic polymer matrix for controlled release over a period of days. The anti-inflammatory extract may then be moulded into a solid implant, or externally applied patch, suitable for providing efficacious concentrations of the anti-inflammatory extract over a prolonged period of time without the need for frequent re-dosing. Such controlled release films are well known to the art. Other examples of polymers commonly employed for this purpose that may be used include nondegradable ethylene-vinyl acetate copolymer a degradable lactic acid-glycolic acid copolymers which may be used externally or internally. Certain hydrogels such as poly(hydroxyethylmethacrylate) or poly(vinylalcohol) also may be useful, but for shorter release cycles than the other polymer release systems, such as those mentioned above.

The carrier may also be a solid biodegradable polymer or mixture of biodegradable polymers with appropriate time release characteristics and release kinetics. The anti-inflammatory extract may then be moulded into a solid implant suitable for providing efficacious concentrations of the anti-inflammatory extract over a prolonged period of time without the need for frequent re-dosing. The anti-inflammatory extract can be incorporated into the biodegradable polymer or polymer mixture in any suitable manner known to one of ordinary skill in the art and may form a homogeneous matrix with the biodegradable polymer, or may be encapsulated in some way within the

polymer, or may be moulded into a solid implant.

In some embodiments, the composition may be adapted for ocular administration. For example the composition may take the form of eye drops, an eye ointment or the like. Compositions for ocular administration include active components (e.g. extracts as claimed herein) and non active components (e.g. ophthalmically acceptable carriers). In many instances, the composition may be an aqueous composition. Non active components for ocular compositions are generally known in the art and may include, for instance, buffering agents, isotonicity agents, surfactants and/or chelating agents. Examples of buffering agents include phosphoric acid salts, boric acid salts and organic bases. Examples of isotonicities include sodium chloride, potassium chloride, boric acid and sodium borate. Examples of surfactants include polysorbate 80 and polyoxyethylene-hydrogenated castor oil 60. In addition, examples of chelating agents include sodium edetate and sodium citrate.

In some embodiments, the composition may be a topical composition. For topical administration, the composition of the present invention may be in the form of a solution, spray, lotion, cream (for example a non-ionic cream), gel, paste, ointment or lozenge. Alternatively, the composition may be delivered via a liposome, nanosome, or nutri-diffuser vehicle.

In some embodiments, the topical composition is adapted for administration to skin or gums. For example the composition may be provided in the form of a cream, a lotion, a paste, an ointment, a gel, etc.

"Creams" may be formulations that contains water and oil and is stabilized with an emulsifier. Lipophilic creams are called water-in-oil emulsions, and hydrophilic creams oil-in-water emulsions. The cream base for water-in-oil emulsions may be absorption bases such as vaseline, ceresin or lanolin. The bases for oil-in-water emulsions may be mono-, di- and triglycerides of fatty acids or fatty alcohols with soaps, alkyl sulphates

or alkyl polyglycol ethers as emulsifiers.

"Lotions" may be opaque, thin, non-greasy emulsion liquid dosage forms for external application to the skin, which may contain a water-based vehicle with greater than 50% of volatiles and sufficiently low viscosity that it may be delivered by pouring. Lotions are usually hydrophilic, and contain greater than 50% of volatiles as measured by LOD (loss on drying). A lotion tends to evaporate rapidly with a cooling sensation when rubbed onto the skin.

"Pastes" may be opaque or translucent, viscous, greasy emulsion or suspension semisolid dosage forms for external application to the skin, which may contain greater than 50% of hydrocarbon-based or a polyethylene glycol-based vehicle and less than 20% of volatiles. A paste may contain a large proportion (20-50%) of dispersed solids in a fatty or aqueous vehicle. An ointment tends not to evaporate or be absorbed when rubbed onto the skin.

"Ointments" may be opaque or translucent, viscous, greasy emulsion or suspension semisolid dosage forms for external application to the skin, which may contain greater than 50% of hydrocarbon-based or a polyethylene glycol-based vehicle and less than 20% of volatiles. An ointment is usually lipophilic, and contains > 50% of hydrocarbons or polyethylene glycols as the vehicle and < 20% of volatiles as measured by LOD. An ointment tends not to evaporate or be absorbed when rubbed onto the skin.

"Gels" may be translucent, non-greasy emulsion or suspension semisolid dosage forms for external application to the skin, which contain a gelling agent in quantities sufficient to impart a three-dimensional, cross-linked matrix. A gel is usually hydrophilic, and contains sufficient quantities of a gelling agent such as starch, cellulose derivatives, carbomers, magnesium-aluminum silicates, xanthan gum, colloidal silica, aluminium or zinc soaps.

Compositions for topical administration may further include drying agents, anti-foaming agents; buffers, neutralizing agents, agents to adjust pH; colouring agents and decolouring agents; emollients; emulsifying agents, emulsion stabilizers and viscosity builders; humectants; odorants; preservatives, antioxidants, and chemical stabilizers; solvents; and thickening, stiffening, and suspending agents, and a balance of water or solvent. In some embodiments, the topical formulation may also be in the form of a spray. Examples of suitable spray formulations include nasal sprays, mouth or throat sprays and skin sprays.

As described above, the composition comprising the anti-inflammatory extract may be a cosmetic composition also comprising a cosmetically acceptable carrier. In some embodiments, the form of the composition may the same or similar to the pharmaceutical compositions described above. The cosmetically acceptable carrier may also be the same or similar to pharmaceutically acceptable carriers described above.

In some embodiments, the cosmetic composition may comprise a cream, gel, serum, lotion, paste, powder, perfume, etc, which may be used as make-up, make-up remover, moisturiser, anti-aging products, suncream, artificial tan, shampoo, conditioner, hair colour or other hair products, nail polish, lipstick, lip balm or other cosmetics for lips, contact lenses, toothpaste or other teeth or gum related products, etc.

The cosmetic composition may also contain additives customarily used in cosmetics, such as water-absorbent or lipophilic gelling agents, water-absorbent or lipophilic active ingredients, preservatives, antioxidants, solvents, perfumes, fillers, screens, and coloring substances. The quantities of these various additives may include those conventionally used in cosmetics; for example, from 0.01% to 10% of the total weight of the composition. As can be appreciated, the selection of additives and quantities will depend on the form of the cosmetic composition.

Oils that may be used in the cosmetic composition may include, for example, mineral oils (e.g. vaseline oil), vegetable oils (e.g. liquid fraction of karite nut butter, sunflower oil), animal oils (e.g. perhydrosqualene), synthetic oils (e.g. Purcellin oil), silicone-containing oils (e.g. cyclomethicone), and fluorinated oils (e.g. perfluoropolyethers). Fatty alcohols and fatty acids (e.g. stearic acid) can be added to these oils.

Emulsifiers that may be used in the cosmetic composition may include, for example, glycerol stearate, polysorbate 60, and the PEG-6/PEG-32/glycol stearate mixture sold under the trade name TefoseR 63 by the Gattefosse Company.

Solvents that may be used in the cosmetic composition may include the lower alcohols, in particular ethanol and isopropanol.

Water-absorbent gelling agents that may be used in the cosmetic composition may be made of carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkylacrylate copolymers, polyacrylamides, polysaccharides such as hydroxylpropylcellulose, natural gums, and clays. Lipophilic gelling agents and modified clays such as bentonites and the metallic salts of fatty acids, such as aluminum stearates and hydrophobic silica may also be used.

Water-absorbent active ingredients that may be used in the cosmetic composition include, for example, proteins and protein hydrolyzates, amino acids, polyalcohols, urea, allantoin, sugars and sugar derivatives, vitamins, and hydroxy acids.

The cosmetic composition may also include lipophilic active ingredients including, for example, retinol (vitamin A) and the derivatives thereof, tocopherol (vitamin E) and the derivatives thereof, essential fatty acids, ceramides, essential oils, and salicylic acid and the derivatives thereof.

A fifth aspect of the invention provides a method of treating or preventing

inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of the anti-inflammatory extract according to the first aspect of the invention or the third aspect of the invention.

A sixth aspect of the invention provides a method of treating or preventing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of the fourth aspect of the invention.

In embodiments whereby a therapeutically effective amount of the anti-inflammatory extract is used, the extract may be a crude extract, a semi-purified extract or a purified extract.

The term "inflammation" as used herein is intended to mean the process by which a subject's immune system coordinates a response to tissue damage, infection, antigenic challenge, etc. Inflammation may be associated with increased blood supply to the tissue, increased capillary permeability in the tissue and increased leukocyte migration to the tissue.

The term "treating" as used herein in relation to inflammation in a subject is intended to mean that the extract or pharmaceutical composition reduces or abrogates the symptoms and/or cause of the inflammation.

The term "preventing" as used herein in relation to inflammation in a subject is intended to mean that the extract or pharmaceutical composition substantially prevents an inflammatory response and/or reduces the symptoms of the inflammatory response that would otherwise occur had the subject not been treated with the extract or pharmaceutical composition.

In some embodiments, the inflammation comprises acute inflammation. In some

embodiments, the acute inflammation in the subject may be in response to any one or more of the following: a wound (e.g. a cut, bruise, burn, etc); an infection (e.g. bacterial, viral, fungal, protist, etc); exposure to a toxin or ionizing radiation; exposure to an allergen or antigen; and the presence of a foreign body (e.g. a splinter) in the subject. In some embodiments, the acute inflammation may be associated with dermatitis.

In some embodiments, the inflammation comprises chronic inflammation. In some embodiments, the chronic inflammation may be associated with a persistent form of an acute inflammation, as described above, or may be associated with an inflammatory disorder. Inflammatory disorders may comprise, for example, an autoimmune disease (e.g. rheumatoid arthritis, Crohn's disease, inflammatory bowel disease, sarcoidosis, psoriasis, multiple sclerosis, etc), a hypersensitivity reaction against innocuous environmental antigens (e.g. asthma, eczema, hay fever, urticaria, food allergy, etc), a hypersensitivity reaction against a persistent infection or a delayed hypersensitivity reaction such as contact hypersensitivity, tuberculin-type hypersensitivity or granulomatous hypersensitivity.

In some embodiments, the inflammation may associated with other diseases or conditions, including, for example, glomerulonephritis, spondylitis, osteoarthritis, vasculitis, scleroderma, Still's disease, gingivitis, etc. In some embodiments, the inflammation is associated with an immune response to a transplanted organ or tissue.

The symptoms of the inflammation will depend on the type of inflammation but may include one or more of the following: redness; increased heat; swelling; pain; and loss of function of the affected tissue.

In some embodiments, the inflammation may be mediated by any one or more of the following immune reactions or processes: T cell activation; B cell activation; dendritic cell activation; activation of the innate immune cells (i.e. phagocytic cells including, for example, monocytes, macrophages, neutrophils, etc.); release of chemotactic molecules;

release of complement; release of pro-inflammatory cytokines; abrogation of anti-inflammatory cytokines; release of antibodies; migration of immune cells to a site of injury, infection, etc.; increased blood supply to a site of injury, infection, etc.; an increase in vascular permeability near a site of injury, infection, etc; and increased expression of adhesion molecules by cells near a site of injury, infection, etc. In some embodiments, the compound, the extract or the pharmaceutical composition may inhibit any one or more of these immune reactions or processes.

In some embodiments, the inflammation may comprise acute or chronic inflammation of the eye.

Administering the anti-inflammatory extract or the pharmaceutical composition to the subject may comprise administration by any suitable method. For example, the anti-inflammatory extract or the pharmaceutical composition may be administered topically, orally, parenterally, endoscopically, by injection, systemically or by any other suitable means.

As set out above, the method according to the sixth aspect of the present invention may be used to treat or prevent inflammation in a subject. In some embodiments, the subject is an animal subject. Suitable subjects include, for example, mammalian subjects such as humans, primates, livestock animals such as horses, cattle, sheep, pigs, goats or the like, companion animals such as dogs or cats, laboratory test animals such as mice, rats, guinea pigs or birds, or animals of veterinary significance, or animals of economic significance. The subject may also include non-mammalian animal subjects such as birds including poultry birds such as chickens; reptilian subjects including companion reptiles such as turtles, tortoises and snakes; fish including wild-caught fish and fish in aquaculture.

Accordingly, in some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

A seventh aspect of the invention provides use of the anti-inflammatory extract of the first aspect of the invention or the third aspect of the invention, in the preparation of a medicament for the treatment, prevention or alleviation of inflammation.

The present invention is further described by the following non-limiting examples:

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows a table of the anti-inflammatory activities and % yields of different crude extracts obtained from leaves of *Dodonaea polyandra*.

FIGURE 2 shows a graph of the anti-inflammatory effects of the crude extracts obtained from leaves of *Dodonaea polyandra* (at 0.4 mg/ear) in a murine ear model of inflammation (LH = n-hexane extracted, LMM = methylene chloride/methanol (1:1) extracted, LE = 80% ethanol extracted, SeMM = sequential methylene chloride/methanol extracted, SeE = sequential 80% ethanol extracted, TPA = 12-O-tetradecanoylphorbol 13-acetate).

FIGURE 3 shows a graph of the average percent inhibition of inflammation due to the crude extracts obtained from leaves of *Dodonaea polyandra* in a murine ear model of inflammation (LH = n-hexane extracted, LMM = methylene chloride/methanol (1:1) extracted, LE = 80% ethanol extracted, SeMM = sequential methylene chloride/methanol extracted, SeE = sequential 80% ethanol extracted, HC=hydrocortisone hemisuccinate salt).

FIGURE 4 shows a graph of the ear thickness differences between control ears and ears treated with an extract obtained from stems of *Dodonaea polyandra* in a murine model of inflammation. The stem extract was administered at 0.4 mg, 0.04 mg and 0.004 mg per ear and croton oil was used as a positive control for inflammation.

FIGURE 5 shows a table of the anti-inflammatory activities and % yields of crude extracts obtained from stems of *Dodonaea polyandra* at 0.4 mg, 0.04 mg and 0.004 mg per ear with croton oil was used as a positive control for inflammation.

FIGURE 6 shows a chart of the fractionation of a hexane leaf extract from *Dodonaea polyandra* leaves, the yield of the fractions and the inhibitory activity of the fractions. The chart also shows the purification of compounds from the extract, the yield of the compounds and the inhibitory activity of one of the compounds (compound C).

FIGURE 7A shows the chemical structure of compound C isolated and tested for inhibitory activity from Figure 6. FIGURE 7B and 7C respectively show the chemical structures of Compound A and Compound B isolated from Figure 6.

FIGURE 8 shows a chart of the fractionation of a hexane leaf extract from *Dodonaea polyandra* leaves, the yield of the fractions and the inhibitory activity of the fractions. The chart also shows the purification of compounds from the extract, the yield of the compounds and the inhibitory activity of two of the compounds (compounds D and E).

FIGURE 9a shows the chemical structure of compound D isolated and tested for inhibitory activity from Figure 8.

FIGURE 9b shows the chemical structure of compound E isolated and tested for inhibitory activity from Figure 8.

FIGURE 10 shows a chart of the fractionation of a methylene chloride/methanol (1:1) leaf extract from *Dodonaea polyandra* leaves and the yield of the fractions. The chart also shows the purification of compounds from the extract, the yield of the compounds and the inhibitory activity of one of the compounds (compound F).

FIGURE 11 shows the chemical structure of compound F isolated and tested for inhibitory activity from Figure 10.

FIGURE 12 shows the chemical structure of compound H.

FIGURE 13 shows the percentage maximum and average inhibition of inflammation in a murine model of inflammation using semi-pure fractions of hexane leaf extracts from *Dodonaea polyandra*. The semi-purified fractions 1-13 are as provided in figure 6.

FIGURE 14 shows the percentage maximum and average inhibition of inflammation in a murine model of inflammation using isolated compound C, isolated compound D, isolated compound E and isolated compound F. Hydrocortisone and betamethasone dipropionate were used as positive controls.

FIGURE 15 shows a graph of the average percent inhibition of inflammation over 48 hours for compound C (DP5), compound D (DP9), compound E (DP6) and compound F (DP8) in the murine model of inflammation. Hydrocortisone (HC) and betamethasone dipropionate (BMS) were used as positive controls.

FIGURE 16 shows a graph of the time course of the inflammation reduction by compound C (DP5), compound D (DP9), compound E (DP6) and compound F (DP8) in the murine model of inflammation. TPA was used as a negative control. Betamethasone was used as a positive control. The graph is interpreted as the TPA line being the maximum inflammatory response and any line beneath the TPA line being indicative of some level of anti-inflammation.

FIGURE 17 shows a graph of inflammation reduction by a range of doses of compound E (DP6) in the murine model of inflammation. Betamethasone (BM) was used as a positive control. Percentage inhibition data are presented as the mean +/- SEM (n=4 for each treatment group) *$p<0.05$ compared to negative TPA control.

FIGURE 18 shows a graph of inflammation reduction by a range of doses of compound F (DP8) in the murine model of inflammation. Betamethasone (BM) was used as a positive control. Percentage inhibition data are presented as the mean +/- SEM (n≥6 for each treatment group) *p<0.05 compared to negative TPA control.

FIGURE 19 shows a graph of inflammation reduction by a range of doses of compound C/D in the murine model of inflammation. Betamethasone (BM) was used as a positive control. Percentage inhibition data are presented as the mean +/- SEM (n≥6 for each treatment group) *p<0.05 compared to negative TPA control; a,b p<0.05 a statistically significant difference exists between treated groups of the same compound.

FIGURE 20 shows a graph comparing of the activities of compound F (DP8) and its de-esterified product in the murine model of inflammation. Percentage inhibition data are presented as the mean +/- SEM (#p<0.05).

EXAMPLE 1

Collection of plant material from *Dodonaea polyandra*

Plant material from *Dodonaea polyandra* was collected by Northern Kaanju people on Northern Kaanju Kuku I'yu Homelands (at Chuula Homelands), Central Cape York Peninsula, Queensland in collaboration with ethnobotanist Mr Nick Smith who confirmed the Western scientific species names. The leaves and stems of *Dodonaea polyandra* (voucher number NMS5293) were collected from a population of 30 plant samples from both male and female types (juveniles ≤ 2m) in December 2007. Voucher specimens and details of plant location (13°07'14", 142°59'45") were recorded and lodged at Brisbane Herbarium, Queensland. Plant material was allowed to air dry in the shade and packed into paper bags for transportation. Leaves and stems were then separated from one another and stored in separate paper bags at -20°C until extraction.

EXAMPLE 2

General materials

All solvents used for plant extraction and crude separations were analytical grade (Merck, Australia and Univar, Australia) or HPLC grade (Merck, Australia and Univar, Australia) for HPLC experiments. Thin-layer chromatography (TLC) plates (reverse phase RP-18 F254 and normal phase Silica gel 60 F254) were purchased from Merck (Darmstadt). Plates were visualised under UV light (254/365 nm) using a Chromato-vue cabinet CC-60 (UVP, Australia). Waters C18 125Å (Milford, MA), Merck Silica gel 60 (70-230 mesh ASTM) and Sephadex LH-20 (Sigma) were used for column chromatography. All HPLC experiments were carried out on a Shimadzu SIL-10A with auto injector, SCL-10A system with Activon GoldPak 100 5µm ODS 25x1 cm or Activon GoldPak Silica Gel 5 µm semi-preparative HPLC columns.

EXAMPLE 3

Aqueous alcoholic solvent extraction of leaf and stem material

Leaf plant material (50 g) and stem plant material (50 g) from example 1 were separately extracted with 80 % (v/v) aqueous ethanol using a solvent to dry plant material ratio of 5:1 at 25 °C with agitation for 24 ± 1 h. After 24 h the ethanolic extract was decanted and filtered *in vacuo* through Whatman No.1 filter paper (Whatman, UK). A second equivalent amount of solvent was added and allowed to extract for a further 24 hrs before removal and filtration. The filtered extract was concentrated using Buchi Rotavap at 40-42°C to remove ethanol and subsequently freeze-dried (Christ Alpha 2-4 LD) to remove residual water. The yield for the leaf extract (LE) and stem extract (SE) was 34.4 % (17.7 g) and 14.0 % (7.02 g), respectively. Long-term storage of dried extract was at -20 °C.

EXAMPLE 4

Non-sequential and sequential extraction of leaf and stem material using different organic solvents

Extracts of the leaf material and stem material from example 1 were individually prepared using the method described in example 3 but using n-hexane and methylene chloride/methanol (1:1) as the solvents. In addition, a batch of leaf material and a batch of stem material were extracted sequentially starting with n-hexane then methylene chloride/methanol (1:1) and lastly 80 % (v/v) aqueous ethanol with plant material being extracted once only with the respective solvents. The leaf yields for n-hexane (LH) and methylene chloride/methanol (1:1) (LMM) extracts were 1.2 % (600 mg) and 22.3 % (11.1 g), respectively. Meanwhile, for the sequential method the leaf yields were 1.34 % (670 mg), 15.4 % (7.69 g) and 14.1 % (7.03 g) for the n-hexane (LSeH), methylene chloride/methanol (1:1) (LSeMM) and 80 % (v/v) aqueous ethanol (LSeE) extracts, respectively. Long-term storage of dried extracts was at -20 °C.

EXAMPLE 5

Mouse ear oedema model

Anti-inflammatory activity was measured in the mouse ear oedema model using 12-o-tetradeconoylphorbol-13-acetate (TPA) (Sigma) or croton oil as the inducer of inflammation. The mouse ear oedema model is described in detail by Sanchez and Moreno, (*Biochemical Pharmacology*, 58: 877-879, 1999). Male Balb/C mice 7-9 weeks old and weighing 20-25 g were used. Mice were housed in cages at constant room temperature (20 ± 2 °C) with access to food (standard rat/mouse pellet) and water ad libitum. A 12 hr day/night cycle was maintained in the animal holding facility (Reid building animal house, University of South Australia) and all experiments were carried out during the day phase.

Baseline measurements of ear thickness were measured using a digital micrometer

prior to the experiment. TPA (2.5 μg/ear), which was dissolved in acetone, was then applied in a volume of 20 μL to the inner surface of the right ear and 20 μL of acetone to the left ear as control. After thirty minutes the test samples, which were dissolved in ethanol, were applied to the inner surface of the right ear and ethanol on the left ear to the respective treatment groups. Hydrocortisone 21-hemisuccinate sodium salt (Sigma) (6mg/ear) was used as a positive control. At 2 h, 4 h, 6 h, 8 h, 24 h and 48 h after the application, the ear thicknesses were measured using a digital micrometer. A non-treated control group to which only croton oil or TPA were applied was used as a measure of maximum inflammation achieved with percent inhibition of inflammation of test sample being calculated relative to this group. Following completion of the experiment, mice were euthanased by inhalation of isoflurane followed by cervical dislocation.

EXAMPLE 6

Crude leaf extracts of *D. polyandra* inhibit inflammation in the mouse oedema model

The anti-inflammatory properties of the crude extracts prepared in example 3 and example 4 were tested in the TPA-induced mouse ear oedema model of acute inflammation as described in example 5.

As shown in Figure 1, each of the crude extracts significantly inhibited inflammation in the mouse oedema model. The levels of inhibition were comparable to or exceeded those of hydrocortisone, which was used as a positive control for inhibition of inflammation. As shown in Figure 2, the inhibitory effects of the crude extracts followed a similar time course trend as the positive controls. The average inhibition of inflammation by the crude extracts exceeded that of the control or was comparable to the control as indicated in Figure 3.

EXAMPLE 7

Stem extracts of *D. polyandra* inhibit inflammation in the mouse oedema model

The anti-inflammatory properties of the crude stem extracts of *D. polyandra* were tested in the TPA-induced mouse ear oedema model of acute inflammation as described in example 5.

As shown in Figure 4 and Figure 5, the stem extracts of *D. polyandra* provided strong inhibition of inflammation at 0.004 mg/ear and 0.4 mg/ear.

EXAMPLE 8

Fractionation of *D. polyandra* leaf extract

The crude leaf extract (LH) obtained from the n-hexane extraction (example 4) was further fractionated by liquid chromatography.

The initial separation carried out on leaf hexane (LH) extract (7 g) was normal phase flash chromatography. The first separation was carried out using a glass column (Ø 45 mm, packing height 120 mm). The crude sample was applied to the column using the pre-adsorbtion method. The mass ratio of silica gel to crude extract adsorbed was 5:2 (i.e. 2.5 g silica/g sample). The separation was carried out under inert conditions using nitrogen. The eluent used was n-hexane with increasing amounts of CH_2Cl_2 up to 100 % CH_2Cl_2 to which MeOH was introduced beginning with a 99:1 ratio. The separation was ceased once the column had been flushed with CH_2Cl_2/MeOH (90:10). Approximately 400 mL of eluent was used for each different composition throughout the separation whilst collecting 20 mL fractions.

Every second fraction was analysed on TLC and pooled into larger fractions based on the observed TLC profile. A total of 13 main fractions were obtained from the initial separation with each subsequently tested in the TPA-induced mouse ear oedema

model. A chart showing the fractionation of the LH extract, the yields of the fractions and the level of inhibition of inflammation is provided in Figure 6. The maximum and percentage inhibition of inflammation of each fraction is also provided in the table of Figure 13. As shown in the figures, a number of the fractions inhibited inflammation.

A major yellow spot was observed on TLC from fraction (Fr) LH10 which initiated further purification of this fraction. Fraction LH10 (1036mg) was chromatographed under gravity using lipophillic sephadex LH-20 (Ø 30 mm, packing height 480 mm) with a CH_2Cl_2/MeOH (3:1) mobile phase. Five fractions were obtained from this step with Fr-LH10.3 containing the yellow spot of interest. Subsequently the semi-pure Fr-LH10.3 was separated isocratically by reverse phase (C18) HPLC using 80% MeOH/H_2O as eluent and 2 mL/min flowrate. Concentration of the 2 mL fractions and RP-TLC analysis revealed the presence of two yellow spots not previously observed on normal phase TLC. Therefore, the remainder of Fr-LH10.3.2 was reinjected onto RP-HPLC with the fraction size collected reduced to 1 mL in volume. Reducing the fraction volume resolved the two compounds from one another to afford compounds A (4.8 mg) and B (31 mg) both as yellow gummy solids. The chemical formulas of compound A and compound B are shown in Figure 7B and Figure 7C, respectively.

The equally most active fraction LH11 was separated into five subfractions using low-pressure reverse phase (C18) column chromatography. The separation was carried out on a glass column (Ø 30 mm, 150 mm packing height) using isocratic elution with 90% MeOH/H_2O. Approximately 5 mL fractions were collected and the separation was continuously monitored by TLC analysis. The spot of interest from this sample was green/blue in appearance upon spraying with anisaldehyde reagent. The remaining components were flushed off with 100% MeOH followed by isopropanol. The bulk of the separation was contained within Fr-LH11.3 (606 mg) consisting of 3 individual components on the basis of TLC. The components present in Fr-LH11.3 showed greater resolution on normal phase TLC compared to reverse phase TLC. Therefore, Fr-LH11.3 (75 mg) was further purified using normal phase HPLC with hexane/ethyl

acetate (8:2) isocratic elution whilst collecting 0.5 mL fractions. The separation gave 44 mg of compound C (see Figure 6) as a white amorphous solid. Figure 6 provides further details of the fractionation and purification of compounds A, B and C. The chemical structure of compound C is shown in Figure 7A. The yield of purified compound C was 44 mg.

Figure 8 shows a chart of the further fractionation and purification of compounds from the LH extract fractions. Purification of Fr-LH9 was conducted in an identical manner to Fr-LH11 using low-pressure reverse phase (C18) column chromatography (Ø 30 mm, 150 mm packing height) with 90% MeOH/H_2O isocratic elution. The fraction also contained a major green/blue spot with an Rf different to that of compound C obtained from Fr-LH11.3. The reverse phase column separation gave three fractions with Fr-LH9.2 containing the green/blue spot. During the preparation of a sample of Fr-LH9.2 for normal phase HPLC purification it was observed that the majority the sample was not soluble in the dissolvent being used (hexane/ethyl acetate (8:2)). The yellow colour of the sample moved into solution, with a white solid remaining at the bottom of the vial. Based on this observation a larger portion of the sample (65mg) was carefully washed with cold hexane/ethyl acetate (8:2). The supernatant was withdrawn using a glass pasteur pipette and the process was repeated several times, leaving a white amorphous solid following evaporation of remaining solvent. Development of a sample applied to normal phase TLC revealed a single blue/green spot. Completion of the washing process yielded 40 mg of compound E (Figure 8). The chemical structure of compound E is shown in Figure 9b.

Subsequent purification of Fr-LH12 was carried out on a reverse phase column (Ø 30 mm, 150 mm packing height) under low pressure with an isocratic elution using 85% MeOH/H2O. The separation was monitored by TLC until the spot of interest (green/blue) had eluted from the column. The column was then washed with 100 % MeOH followed by isopropanol. This separation gave three fractions (LH12.1-LH12.3. Fraction LH12.2 underwent further separation under normal phase conditions using a

glass column (Ø 5 mm, 125 mm packing height). The column was initially eluted with CH_2Cl_2 switching to CH_2Cl_2/MeOH (99:1) after a visible yellow band had migrated halfway down the column. This step gave two fractions to which Fr-LH12.2.2 (38mg) was subjected to normal phase semi-preparative TLC (using analytical TLC plate). A 5 mg sample in MeOH was applied to the baseline of the plate. The mobile phase used was CH_2Cl_2/MeOH (95:5) and the plate was developed over a distance of 8 cm. After allowing the plate to dry the plate was placed under UV 254 nm light and the silica within the region containing the band of interest was cut from the plate. The extracted silica obtained from subsequent developments was pooled together and loaded dry into a glass Pasteur pipette (mini glass column) plugged with glass wool to prevent silica from escaping. The component was then desorbed from the silica by flushing with ethyl acetate. The eluate was collected into a round bottom flask and solvent evaporated under reduced pressure on a rotorvap. The process gave 20 mg of compound D (Figure 8). The chemical structure of compound D is shown in Figure 9A. As is evident by comparing Figure 7A and Figure 9A, compounds C and D were shown to have the same chemical structure.

Precipitation was observed from fractions obtained during the initial separation of the crude LH extract which were consequently grouped to give Fr-LH6. As a result of this it suggested that the precipitate was a major component of the extract and potentially responsible for some of the anti-inflammatory activity. Hence this observation was further investigated by redissolving Fr-LH6 into a minimal amount of CH_2Cl_2/hexane (25:75) in a glass beaker. The beaker was placed into a sealed secondary container to reduce the rate of evaporation. The sample was left for approximately 24hrs and the supernatant was poured off, the contents carefully washed and filtered under gravity using cold CH_2Cl_2hexane (25:75) to give compound G (27mg), a white amorphous solid (Figure 8).

The active fraction LH13 contained a small green/blue spot with Rf 0.06 when developed on normal phase TLC (CH_2Cl_2MeOH (95:5)). However, the task of isolating

the component in significant quantities was deemed impractical from this fraction considering the intensity (lack of) of the spot, limited quantity of Fr-LH13 and material loss at each purification step. The same spot however was prominent in the sequential methylene chloride/methanol (SeMM) crude extract which was also significantly active in the mouse ear oedema model. Hence the SeMM extract was used as a means of isolating the component of interest rather than Fr-LH13. The flow chart shown in Figure 10 summarises the processes involved in the purification of this constituent from SeMM extract.

An initial clean-up step was conducted by dissolving 15 g of SeMM extract in 150 mL 70 % MeOH/H_2O and partitioning between 150 mL n-hexane. The aqueous layer was retained and subsequently extracted with 150 mL CH_2Cl_2. The CH_2Cl_2 layer which contained the component of interest was evaporated on a rotavap at 37 °C under reduced pressure to give Fr-SeMMMc (7.00 g). Fraction SeMM2 (2.24 g) was subjected to repeated low-pressure reverse phase (C18) column chromatography (Ø 30mm, 150 mm packing height) under isocratic elution with 85 % MeOH/H2O. The separation was monitored by normal phase TLC until the blue/green spot of interest had eluted from the column. The column was washed with MeOH followed by isopropanol to remove remaining components from the column. Five fractions (SeMM2.1-SeMM2.5) were pooled together on the basis of TLC analysis with Fr-SeMM2.4 containing the blue/green spot. The SeMM2.4 fraction (255 mg) was purified further under low pressure normal phase conditions (Ø 10 mm, packing height 240 mm) and CH_2Cl_2/MeOH (99:1) eluent. This process was repeated twice to yield compound F (50mg) as a white amorphous solid. The chemical structure of compound F is shown in Figure 11.

The isolation of compounds from the n-hexane crude leaf extract of *D. polyandra* was based on a partial activity-guided fractionation process. The rationale for this was due to the *in vivo* approach used to measure pharmacological activity. The statistically meaningful data obtained from testing semi-purified fractions and the chemical

simplicity of these fractions meant that it was probable the activity was due to the most abundant component(s). Therefore further separation of the most active semi-purified fractions was guided based on the most prominent spots observed on TLC.

An extraction of *D. polyandra* stems was prepared from CH_2Cl_2/MeOH (1:1) using the same procedure as described above for leaf extract preparation. The extract (DPS) yield was 34.9 g (9.8 %) and was cleaned up by liquid/liquid partitioning between CH_2Cl_2 and 70 % aqueous methanol (350 mL each, 700 mL total). The organic layer was removed and dried using Buchi Rotavap at 40-42 °C giving a yield of 14.4 g. An initial separation on 10.1 g of the CH_2Cl_2 fraction (DPS1) was subjected to normal phase glass column chromatography eluting with n-hexane/ CH_2Cl_2 with increasing amounts of CH_2Cl_2, ceasing with CH_2Cl_2/MeOH (95/5). Fifteen mililitre fractions were collected and grouped into three major fractions based on their TLC profiles.

Fraction DPS1A (4.1 g) was separated under reverse phase conditions eluting with 75 % aqueous methanol with increasing amounts of methanol to afford 3 major fractions (DPS1A1, DPS1A2 and DPS1A3). Fraction DPS1A1 (685 mg) was passed through a Sephadex LH-20 column eluting with CH_2Cl_2/MeOH (3:1) giving two fractions (DPS1A1a and DPS1A1b). Fraction DPS1A1a (40 mg) was separated using normal phase preparative TLC (CH_2Cl_2/MeOH 95/5). The region of interest was cut out and component of interest recovered by passing ethyl acetate through the sample which had been placed into a miniature glass column. Upon drying of the sample under nitrogen, 1 mg of compound H was obtained as an off-white solid. The formula for compound H (15, 16-epoxy-3-clerodaene-2-one-8(α)-methylbenzoate-18-oic acid) is shown in Figure 12.

EXAMPLE 9

Semi-purified leaf extracts of *D. polyandra* inhibit inflammation in the mouse oedema model

Semi-purified fractions LH1-LH13 prepared as per example 8 were used in the mouse oedema model to determine the anti-inflammatory effect of the fractions. As shown in Figure 13, fractions LH11, LH12 and LH13 showed a significant average % inhibition oedema of 41.7, 37.6 and 35.6 %, respectively when tested at 0.4 mg/ear.

EXAMPLE 10

Compounds purified from leaf extracts of *D. polyandra* inhibit inflammation in the mouse oedema model

Compounds C, D, E and F, which were purified from leaf extracts of *D. polyandra*, as set out in example 8, were used in the mouse oedema model to determine the anti-inflammatory effect of the compounds. As shown in Figure 14, each compound resulted in a significant average inhibition of inflammation at a concentration of 0.9 μmol/ear. Compounds D and F were the most active and resulted in an average % inhibition of inflammation of 46.3 and 46.0 %, respectively. This was comparable to betamethasone, a potent fluorinated corticosteroid (at the single concentration tested) which gave an average inhibition of 55.3 %. The inhibitory effects of the compounds are also shown in Figures 15 and 16.

Figures 17 and 18 demonstrate dose responses of compounds E and F respectively in the TPA-induced mouse ear oedema model. Whilst compound E showed some significant level of activity when compared to TPA control at the highest concentration tested (1.83 μmol/ear, $p<0.05$), there was no observable effect at lower concentrations. Compound F showed a linear dose-response over the dose range 0.0055 – 1.77 μmol. At 8 hrs post application, 0.22 μmol dose gave a maximum of 70.2 ± 10.0 % (mean ± SEM) inhibition with no improvement in activity observed for the two higher doses.

Compounds C and D were shown to have the same chemical structure and therefore will be referred to herein as compound C/D. Figure 19 demonstrates a dose response of compound C/D in the TPA-induced mouse ear oedema model. Compound C/D showed equally potent activity as F with maximum inhibition of 76.4 ± 7.3 % achieved at a dose of 0.91 μmol, however the dose-response relationship was non-linear. The relationship was characterized by a U-shaped dose-response over the experimental range, featuring significant activities at low and high doses. The shape of this dose-response is characteristic of some steroid compounds with estrogenic activity, which appear to act through two opposing pathways within the same process. Hence, the effects of such phenomena may be more obvious at one dose and not another due to the sensitivity each pathway has for the compound being applied. The activities of F and C/D after 8 hours were comparable to the positive control betamethasone dipropionate (0.90 μmol).

EXAMPLE 11

Removal of the benzoyl ester moiety from benzoyl ester clerodane diterpenoids purified from leaf extracts of *D. polyandra* reduces the anti-inflammatory capacity of the compounds in the mouse oedema model

To determine whether the benzoyl ester moiety was important for the observed activity, compound F was subjected to mild alkaline hydrolysis and subsequently tested *in vivo*. The mild alkaline ester hydrolysis was carried out using a previously described method (Khurana, *Monatshefte für Chemie*: 135, 83-87, 2004). Briefly, potassium hydroxide was reacted with the compound using a mol ratio of 3:1 in 100 μL methanol at 37°C. The progress of the reaction was monitored and terminated at 2 hr by addition of 300 μL water, followed by 100 μL of 1M HCl. The component of interest was recovered by liquid-liquid extraction with 500 μL CH_2Cl_2 and the organic layer subsequently removed from aqueous layer, dried over sodium sulphate and centrifuged (14,000 rpm for 5 min) to remove solid particulates. The dried organic

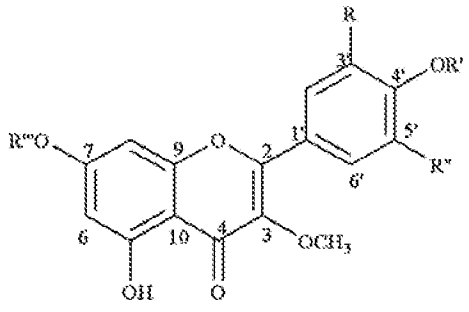
layer containing the hydrolysed component of interest was purified by preparative TLC under normal phase conditions (92.5/7.5 CH2Cl2/MeOH). The structure of the hydrolysis product was confirmed by NMR.

As shown in figure 20, removal of this functional group significantly reduced ($p<0.05$) the ability of the compound to inhibit inflammation in the mouse oedema model. This result indicates that the benzoyl ester may be involved in interactions between the ligand and target or may play a role in improving bioavailability. For example, the increased lipophilicity imparted by the group suggests that it may be important for absorption into the skin.

EXAMPLE 12

Leaf and Stem Extracts from *D. polyandra* contain flavonoids

Thin layer chromatography was performed on leaf and stem extracts from *D. polyandra*. Results (not shown) demonstrated the presence of the following flavonoids in both leaf and stem extracts:

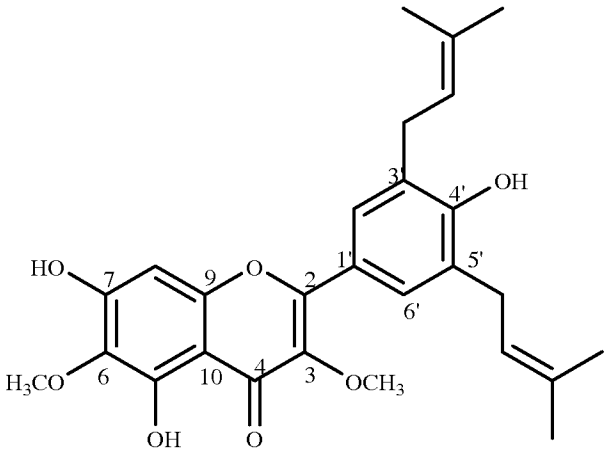


i) R= H; R'=H, R''=H, R'''=H

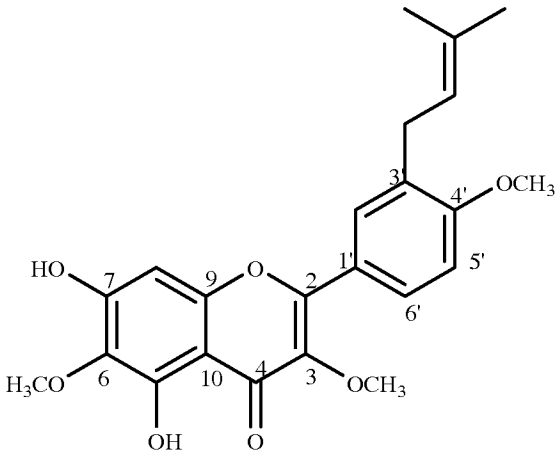
ii) R= R'=CH_3; R''=H; R'''=H

iii) R= R'=H; R''= R'''=H

iv) R=H, R'=H, R''=H, R'''=OCH_3


HO
7
9
O
2
1'
3'
4'
OH
5'
6'
H_3CO
6
10
4
3
OCH_3
OH
O

and


HO
7
9
O
2
1'
3'
4'
OCH_3
5'
6'
H_3CO
6
10
4
3
OCH_3
OH
O

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An anti-inflammatory extract derived from a plant of the *Dodonaeoideae* subfamily.

2. The anti-inflammatory extract of claim 1, wherein the plant is of the *Dodonaea* genus.

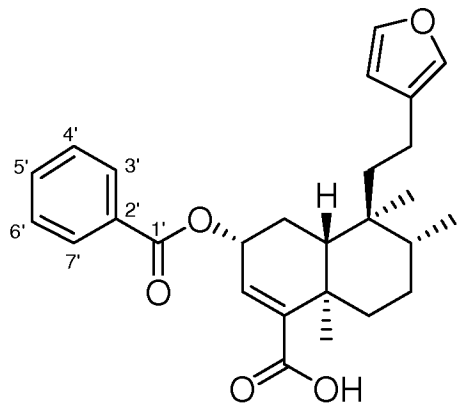
3. The anti-inflammatory extract of claim 1 or claim 2, wherein the plant is of the *Dodonaea polyandra* species.

4. The anti-inflammatory extract of any one of claims 1 to 3, wherein the extract comprises a compound that belongs to the clerodane class.

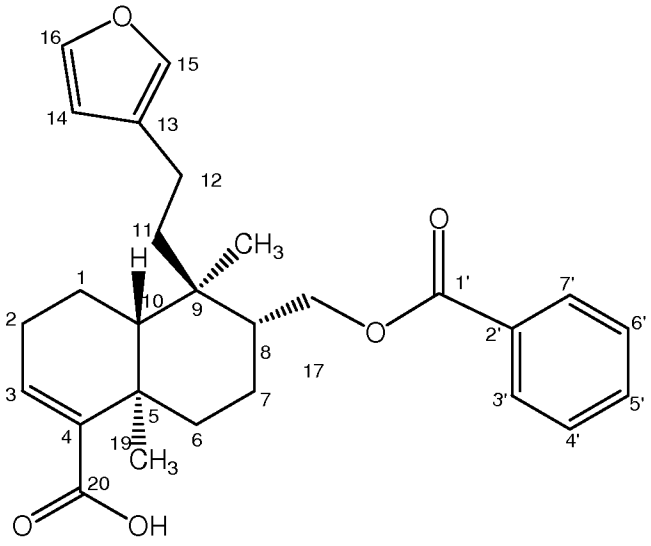
5. The anti-inflammatory extract of any one of claims 1 to 4, wherein the extract comprises a diterpenoid.

6. The anti-inflammatory extract of any one of claims 1 to 5, wherein the extract comprises a benzoyl ester clerodane diterpenoid.

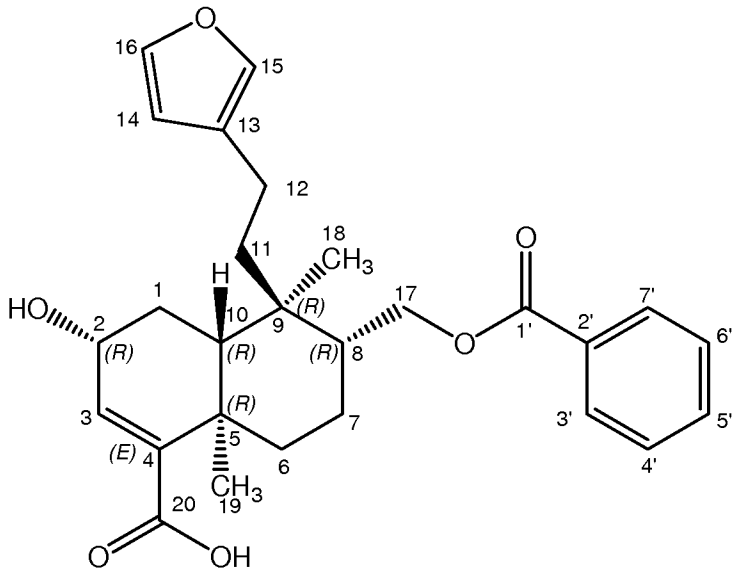
7. The anti-inflammatory extract of any one of claims 1 to 6, wherein the extract comprises the following compound:



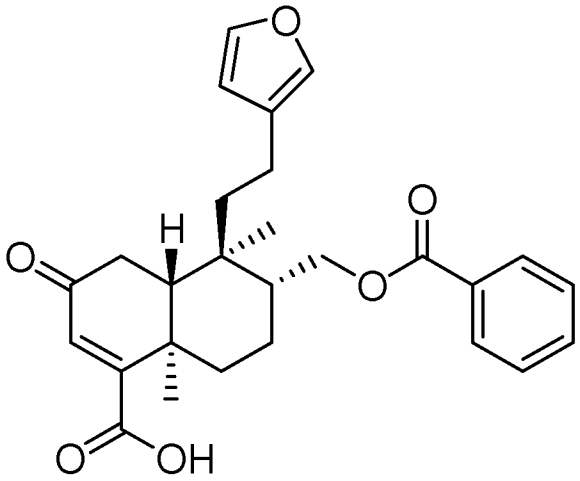
8. The anti-inflammatory extract of any one of claims 1 to 7, wherein the extract comprises the following compound:



9. The anti-inflammatory extract of any one of claims 1 to 8, wherein the extract comprises the following compound:

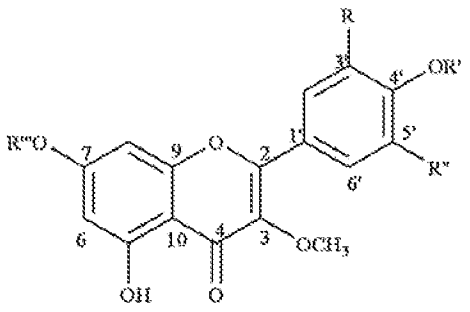


10. The anti-inflammatory extract of any one of claims 1 to 9, wherein the extract comprises the following compound:



11. The anti-inflammatory extract of any one of claims 1 to 10, wherein the extract comprises any one or more of: 15, 16-epoxy-3-clerodaene-8(α)-methylbenzoate-18-oic acid; 15, 16-epoxy-2(α)-hydroxy-3-clerodaene-8(α)-methylbenzoate-18-oic acid; 15, 16-epoxy-3-clerodaene-2-one-8(α)-methylbenzoate-18-oic acid; and 15, 16-epoxy-3-clerodaene-2(α)-benzoate-18-oic acid.

12. The anti-inflammatory extract of any one of claims 1 to 11, wherein the extract comprises any one or more of the following compounds:



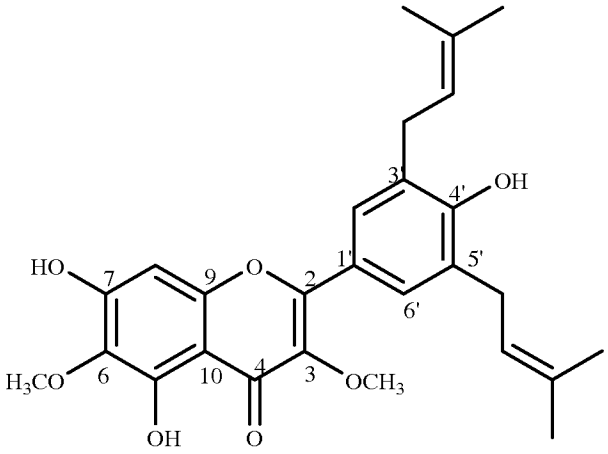
i) R= H; R'=H, R"=H, R"'=H

ii) R= R'=CH_3; R"=H, R"'=H

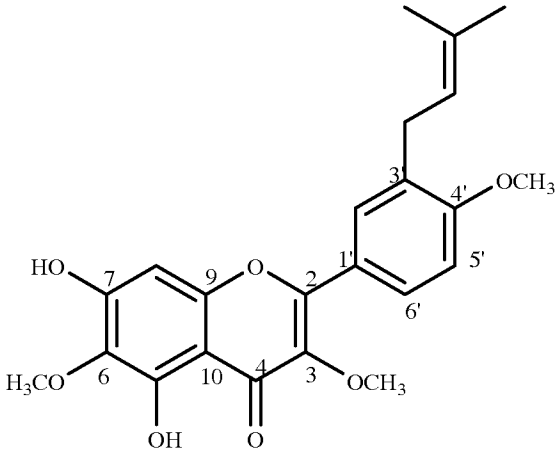
iii) R= R'=H; R"= R"'=H

iv) R=H, R'=H, R"=H, R"'=OCH_3

or



or



13. A method for preparing an anti-inflammatory extract of any one of claims 1 to 12, the method including:

i) mixing biomass of the plant with a solvent under appropriate conditions to extract one or more anti-inflammatory agents from the biomass into the solvent;

ii) collecting the solvent containing the extract from step (i); and

iii) removing at least a portion of the solvent.

14. The method of claim 13, wherein the biomass comprises dried plant material.

15. The method of claim 13 or claim 14, wherein the biomass comprises leaves.

16. The method of any one of claims 13 to 15, wherein the biomass comprises stems.

17. The method of any one of claims 13 to 16, wherein the biomass comprises bark.

18. The method of any one of claims 13 to 17, wherein the appropriate conditions comprise a temperature between 20°C and 30°C.

19. The method of any one of claims 13 to 18, wherein the appropriate conditions comprise agitation of the plant material and solvent.

20. The method of any one of claims 13 to 19, wherein the solvent comprises an alcohol.

21. The method of any one of claims 13 to 20, wherein the solvent comprises ethanol.

22. The method of any one of claims 13 to 21, wherein the solvent comprises an alkane hydrocarbon.

23. The method of claim 22, wherein the alkane hydrocarbon comprises hexane.

24. The method of any one of claims 13 to 23, wherein the solvent comprises methylene chloride.

25. The method of any one of claims 13 to 24, wherein the solvent comprises a mixture of methylene chloride and methanol.

26. The method of claim 25, wherein the ratio of methylene chloride to methanol is approximately 1:1.

27. The method of any one of claims 13 to 26, wherein the method comprises a sequential extraction with different solvents.

28. The method of claim 27, wherein the different solvents comprise hexane, methylene chloride/methanol (1:1), and ethanol.

29. The method claim 28 wherein the solvents are used in the order hexane then methylene chloride/methanol (1:1), and then ethanol.

30. An anti-inflammatory extract produced according to the method of any one of claims 13 to 29.

31. A pharmaceutical or cosmetic composition comprising the anti-inflammatory extract of any one of claims 1 to 12 or claim 30 together with a pharmaceutically acceptable carrier or a cosmetically acceptable carrier.

32. The pharmaceutical or cosmetic composition of claim 31 wherein the composition comprises a topical composition.

33. A method of treating or preventing inflammation in a subject, the method comprising administering to the subject a therapeutically effective amount of the anti-inflammatory extract of any one of claims 1 to 12 or claim 30.

34. A method of treating or preventing inflammation in a subject, the method comprising administering to the subject the pharmaceutical composition of claim 31 or 32.

35. The method of claim 33 or 34, wherein the inflammation comprises acute inflammation.

36. The method of claim 35, wherein the acute inflammation is in response to a wound.

37. The method of claim 35, wherein the acute inflammation is in response to an infection.

38. The method of claim 35, wherein the acute inflammation is in response to exposure to a toxin or ionizing radiation.

39. The method of claim 35, wherein the acute inflammation is in response to the presence of a foreign body in the subject.

40. The method of claim 35, wherein the acute inflammation is associated with dermatitis.

41. The method of claim 33 or claim 34, wherein the inflammation comprises chronic inflammation.

42. The method of claim 41, wherein the chronic inflammation is associated with a persistent form of the acute inflammation of any one of claims 35 to 40.

43. The method of claim 42, wherein the chronic inflammation is associated with an inflammatory disorder.

44. The method of claim 43, wherein the inflammatory disorder comprises a hypersensitivity disorder.

45. The method of any one of claims 33 to 44, wherein the subject is a mammal.

46. The method of any one of claims 33 to 45, wherein the subject is a human.

47. Use of the anti-inflammatory extract of any one of claims 1 to 12 or claim 30, in the preparation of a medicament for the treatment, prevention or alleviation of inflammation.

Figure 1

Extract	% inhibition (mean ± SEM) (p-value)				Yield (%)
	Maximum		Average		
n-hexane (n=4)	46.4 ± 6.2*	< 0.001	57.8 ± 4.4*	< 0.001	1.2
80% ethanol(n=4)	44.0 ± 2.8*	< 0.001	61.8 ± 4.3*	< 0.001	17.7
Methylene chloride/methanol (1:1) (n=4)	36.2 ± 5.1*	< 0.001	58.9 ± 4.0*	< 0.001	22.3
80% ethanol (sequential) (n=4)	25.9 ± 4.2*	< 0.001	44.7 ± 8.1*	< 0.001	15.4
Methylene chloride/methanol (1:1) (sequential) (n=4)	44.0 ± 2.3*	0.008	60.8 ± 2.9*	< 0.001	14.1
Hydrocortisone (2 mg) (n=2)	30.9 ± 1.4*	0.006	44.9 ± 11.0*	0.001	n/a

*statistically significant relative to TPA control at α = 0.05

Figure 2

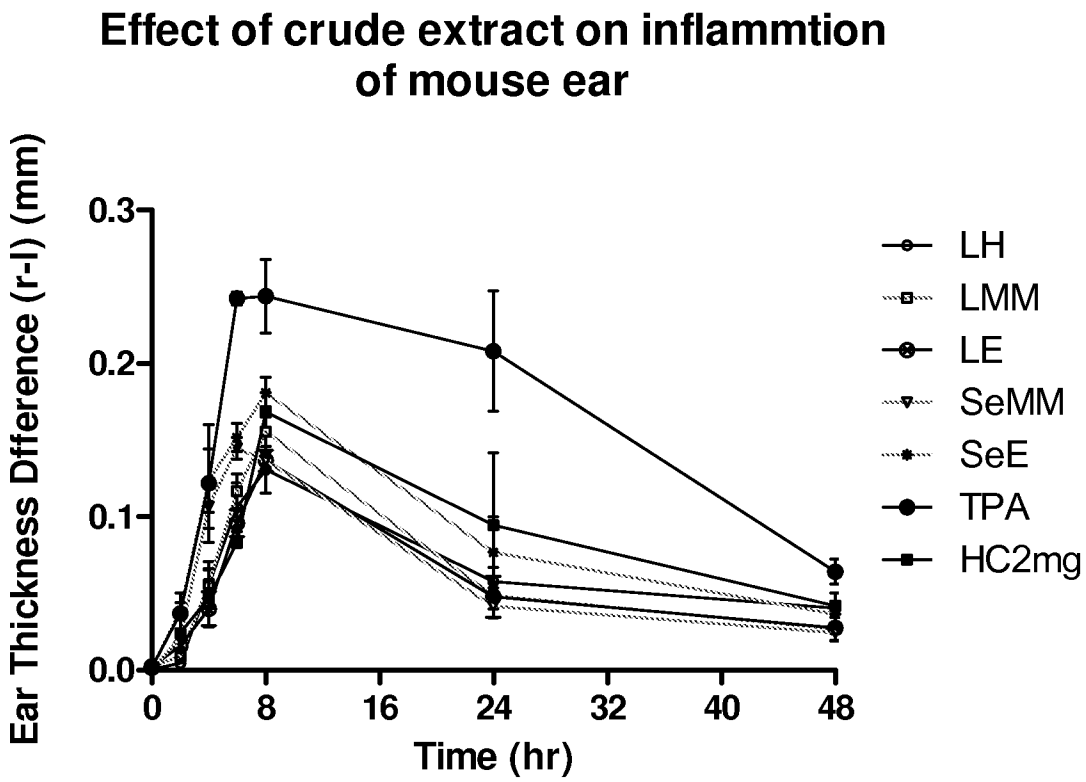

Effect of crude extract on inflammtion of mouse ear
Ear Thickness Dfference (r-l) (mm)
0.3
0.2
0.1
0.0
0
8
16
24
32
40
48
Time (hr)
LH
LMM
LE
SeMM
SeE
TPA
HC2mg

Figure 3

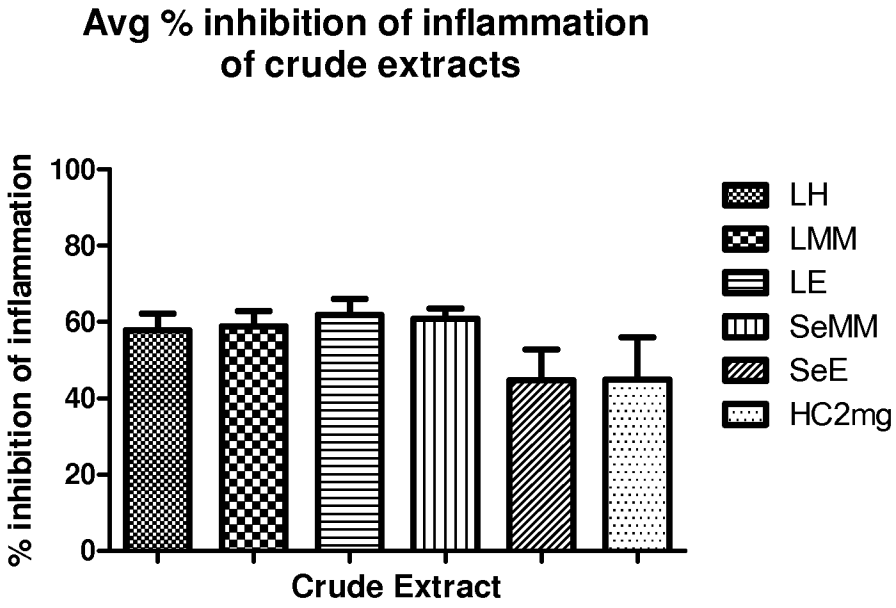

Avg % inhibition of inflammation of crude extracts
% inhibition of inflammation
100
80
60
40
20
0
Crude Extract
LH
LMM
LE
SeMM
SeE
HC2mg

Figure 4

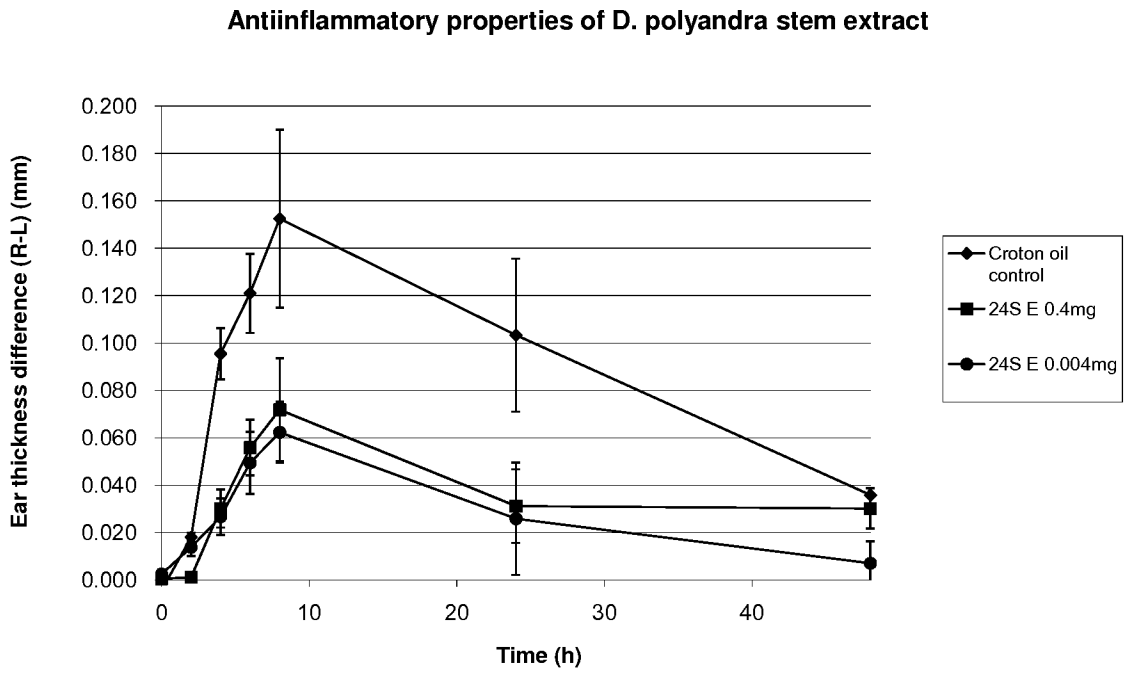

Antiinflammatory properties of D. polyandra stem extract
Ear thickness difference (R-L) (mm)
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
10
20
30
40
Time (h)
Croton oil control
24S E 0.4mg
24S E 0.004mg

Figure 5

Extract/concentration	% inhibition (mean ± SEM) (p-value)			
	Maximum		Average	
Stem 0.4 mg/ear (n=3)	52.9 ± 11.9	0.074	52.3 ± 11.7	0.064
Stem 0.04 mg/ear (n=3)	9.6 ± 9.9	0.674	15.0 ± 3.7	0.562
Stem 0.004 mg/ear (n=3)	59.1 ± 8.5*	0.047	62.1 ± 14.8*	0.030
*statistically significant relative to croton oil control at α = 0.05				

Figure 6

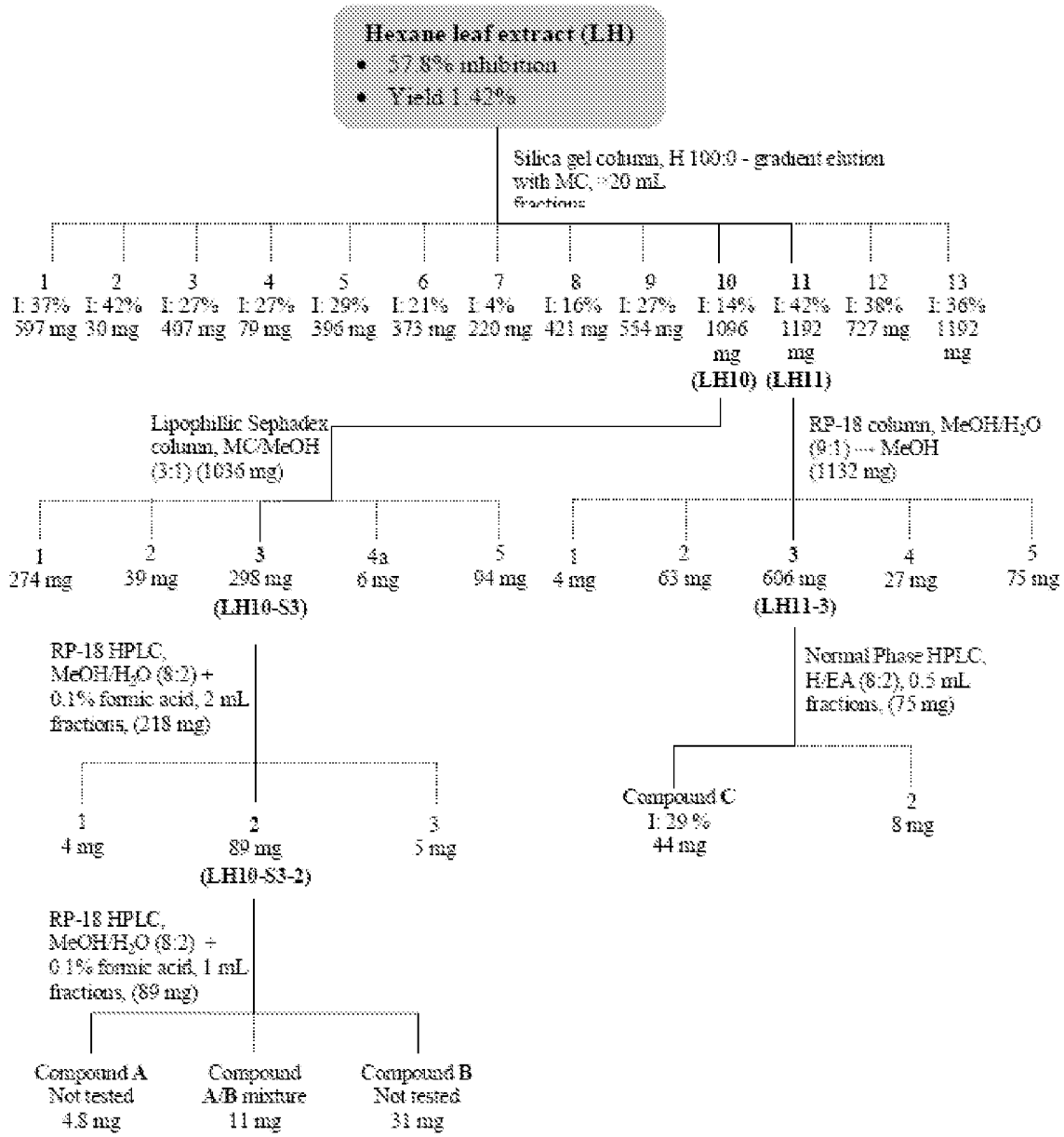
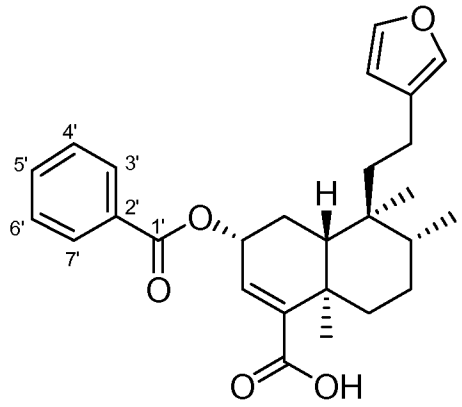
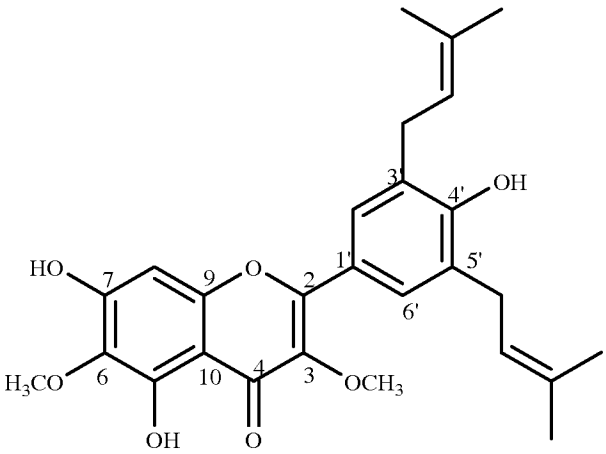

Hexane leaf extract (LH)
• 57.8% inhibition
• Yield 1.42%
Silica gel column, H 100:0 - gradient elution with MC, ~20 mL fractions
1 I: 37% 597 mg
2 I: 42% 30 mg
3 I: 27% 407 mg
4 I: 27% 79 mg
5 I: 29% 396 mg
6 I: 21% 373 mg
7 I: 4% 220 mg
8 I: 16% 421 mg
9 I: 27% 554 mg
10 I: 14% 1096 mg (LH10)
11 I: 42% 1192 mg (LH11)
12 I: 38% 727 mg
13 I: 36% 1192 mg
Lipophilic Sephadex column, MC/MeOH (3:1) (1036 mg)
RP-18 column, MeOH/H2O (9:1) → MeOH (1132 mg)
1 274 mg
2 39 mg
3 298 mg (LH10-S3)
4a 6 mg
5 94 mg
1 4 mg
2 63 mg
3 606 mg (LH11-3)
4 27 mg
5 75 mg
RP-18 HPLC, MeOH/H2O (8:2) + 0.1% formic acid, 2 mL fractions, (218 mg)
Normal Phase HPLC, H:EA (8:2), 0.5 mL fractions, (75 mg)
1 4 mg
2 89 mg (LH10-S3-2)
3 5 mg
Compound C I: 29 % 44 mg
2 8 mg
RP-18 HPLC, MeOH/H2O (8:2) + 0.1% formic acid, 1 mL fractions, (89 mg)
Compound A Not tested 4.8 mg
Compound A/B mixture 11 mg
Compound B Not tested 31 mg

Figure 7

A


O
4'
5'
3'
2'
6'
1'
7'
O
H
O
OH

B


3'
4'
OH
HO
7
9
O
2
1'
5'
6'
H3CO
6
10
4
3
OCH3
OH
O

C

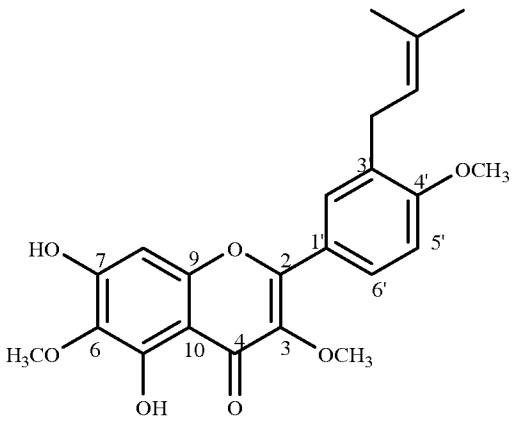

3'
4'
OCH3
HO
7
9
O
2
1'
5'
6'
H3CO
6
10
4
3
OCH3
OH
O

Figure 8

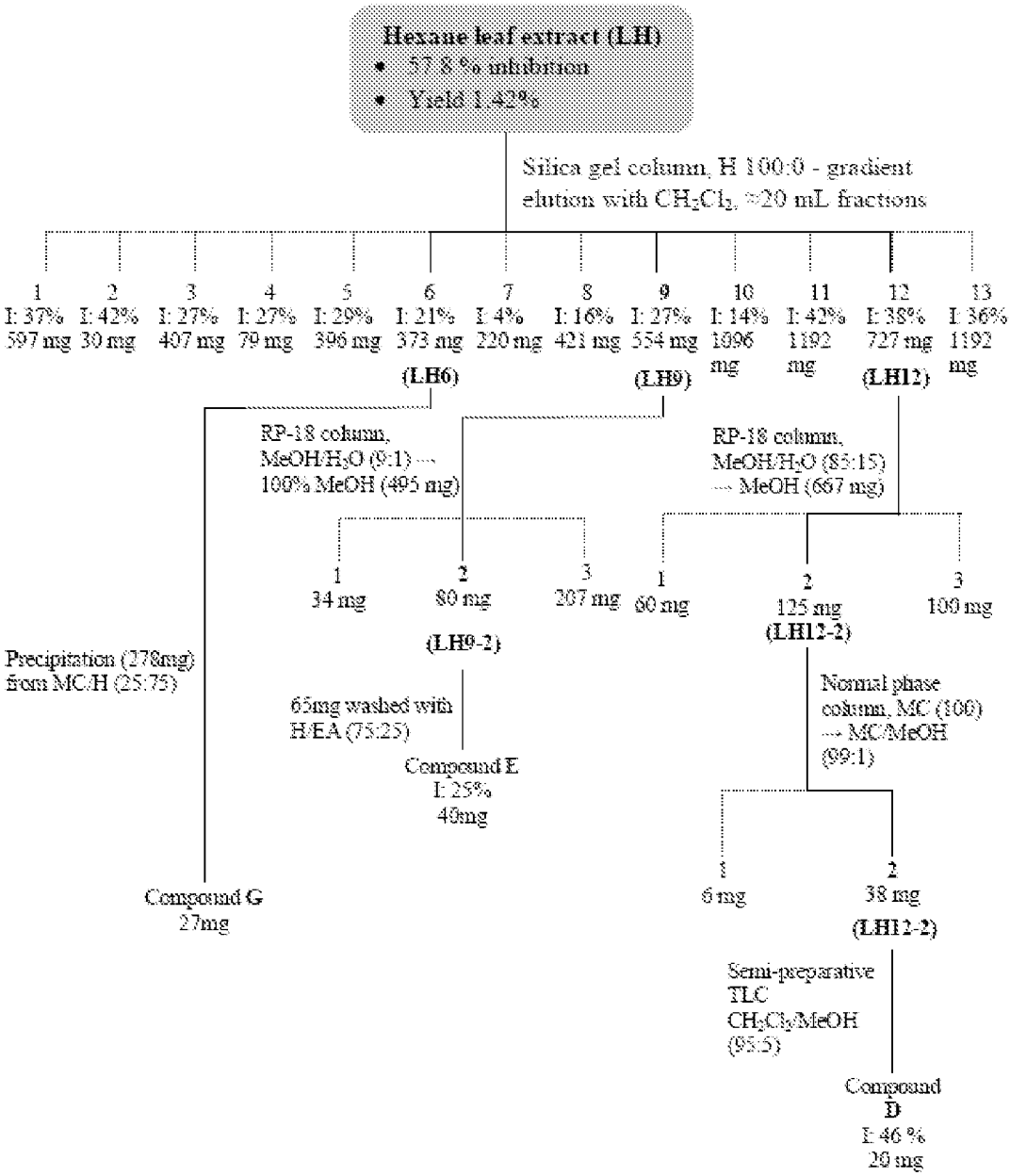
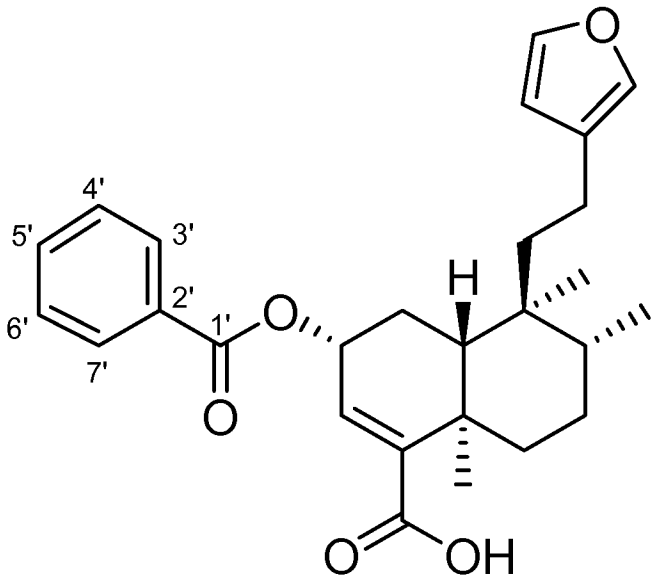

Hexane leaf extract (LH)
• 57.8 % inhibition
• Yield 1.42%
Silica gel column, H 100:0 - gradient elution with CH_2Cl_2, ~20 mL fractions
1 I: 37% 597 mg
2 I: 42% 30 mg
3 I: 27% 407 mg
4 I: 27% 79 mg
5 I: 29% 396 mg
6 I: 21% 373 mg (LH6)
7 I: 4% 220 mg
8 I: 16% 421 mg
9 I: 27% 554 mg (LH9)
10 I: 14% 1096 mg
11 I: 42% 1192 mg
12 I: 38% 727 mg (LH12)
13 I: 36% 1192 mg
RP-18 column, MeOH/H_2O (9:1) → 100% MeOH (495 mg)
RP-18 column, MeOH/H_2O (85:15) → MeOH (667 mg)
1 34 mg
2 80 mg (LH9-2)
3 207 mg
1 60 mg
2 125 mg (LH12-2)
3 100 mg
Precipitation (278mg) from MC/H (25:75)
65mg washed with H/EA (75:25)
Compound E I: 25% 40mg
Normal phase column, MC (100) → MC/MeOH (99:1)
Compound G 27mg
1 6 mg
2 38 mg (LH12-2)
Semi-preparative TLC CH_2Cl_2/MeOH (95:5)
Compound D I: 46 % 20 mg

Figure 9

a)


O
4'
5'
3'
2'
6'
1'
7'
O
H
O
O
OH

b)

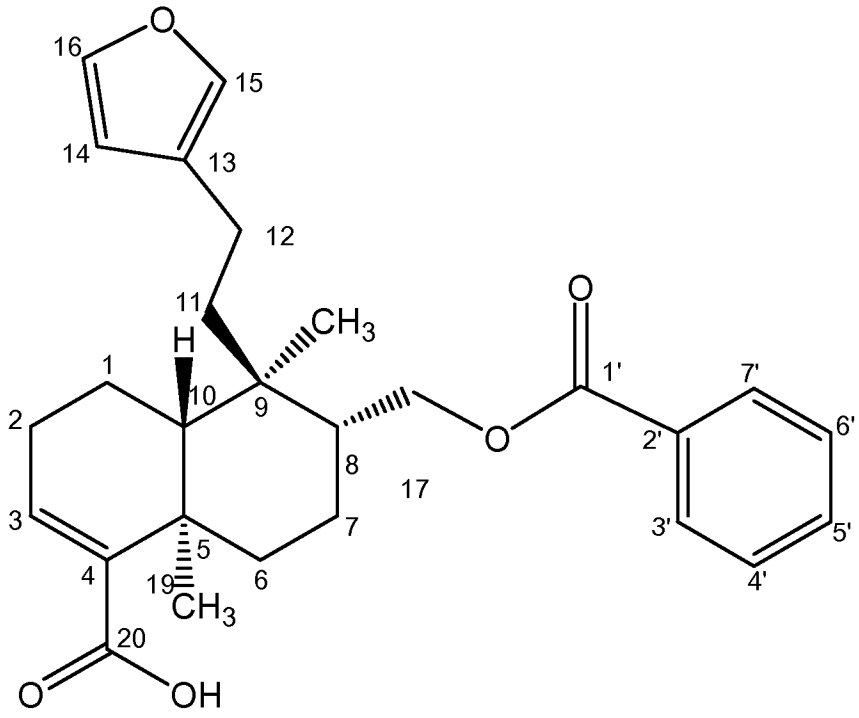

O
16
15
14
13
12
11
H
CH3
1
10
9
1'
7'
2
O
2'
6'
8
17
3
7
3'
5'
5
4
19
6
4'
CH3
20
O
OH

Figure 10

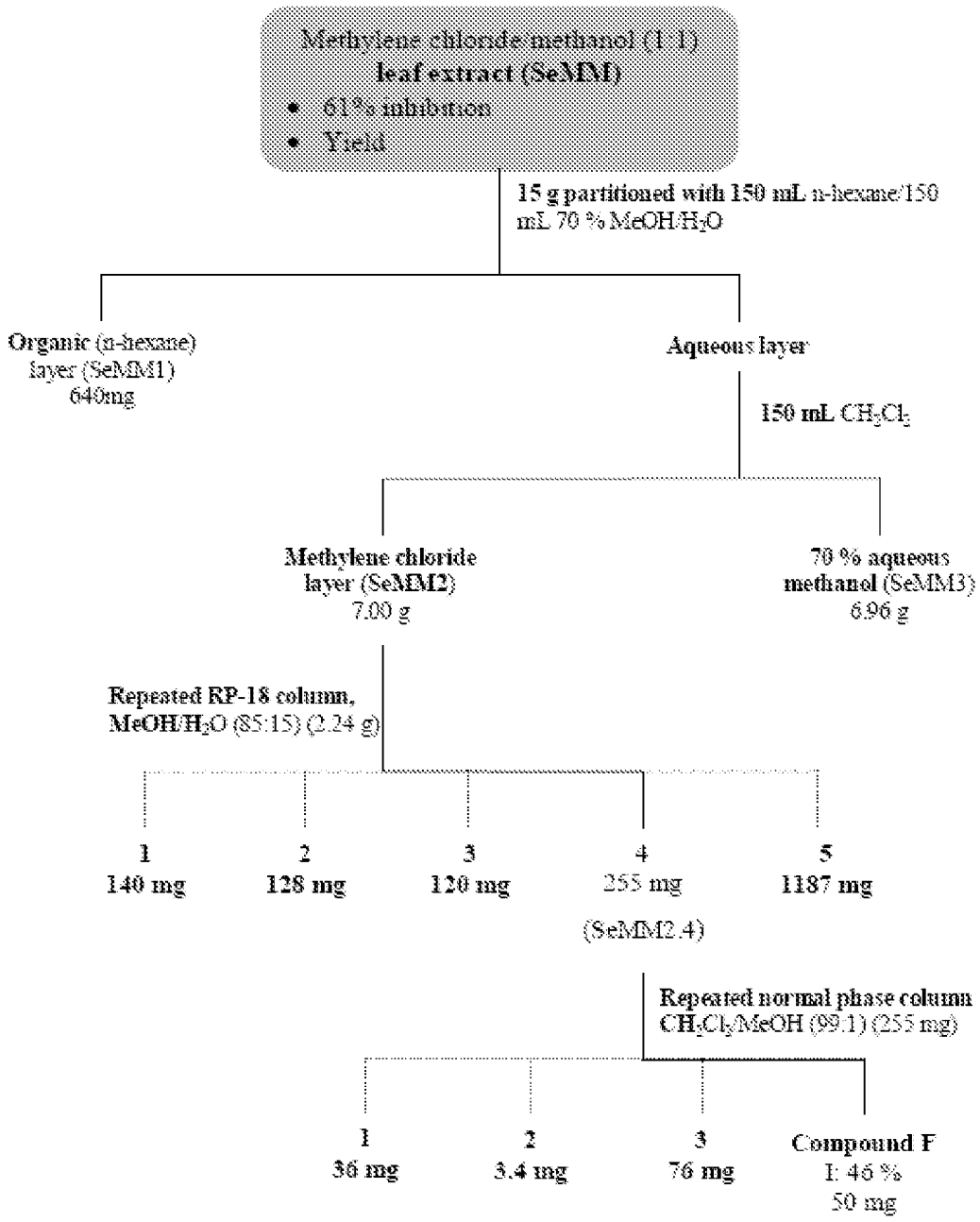

Methylene chloride-methanol (1:1) leaf extract (SeMM)
• 61 % inhibition
• Yield
15 g partitioned with 150 mL n-hexane/150 mL 70 % MeOH/H2O
Organic (n-hexane) layer (SeMM1) 640mg
Aqueous layer
150 mL CH2Cl2
Methylene chloride layer (SeMM2) 7.00 g
70 % aqueous methanol (SeMM3) 6.96 g
Repeated RP-18 column, MeOH/H2O (85:15) (2.24 g)
1 140 mg
2 128 mg
3 120 mg
4 255 mg (SeMM2.4)
5 1187 mg
Repeated normal phase column CH2Cl2/MeOH (99:1) (255 mg)
1 36 mg
2 3.4 mg
3 76 mg
Compound F I: 46 % 50 mg

Figure 11

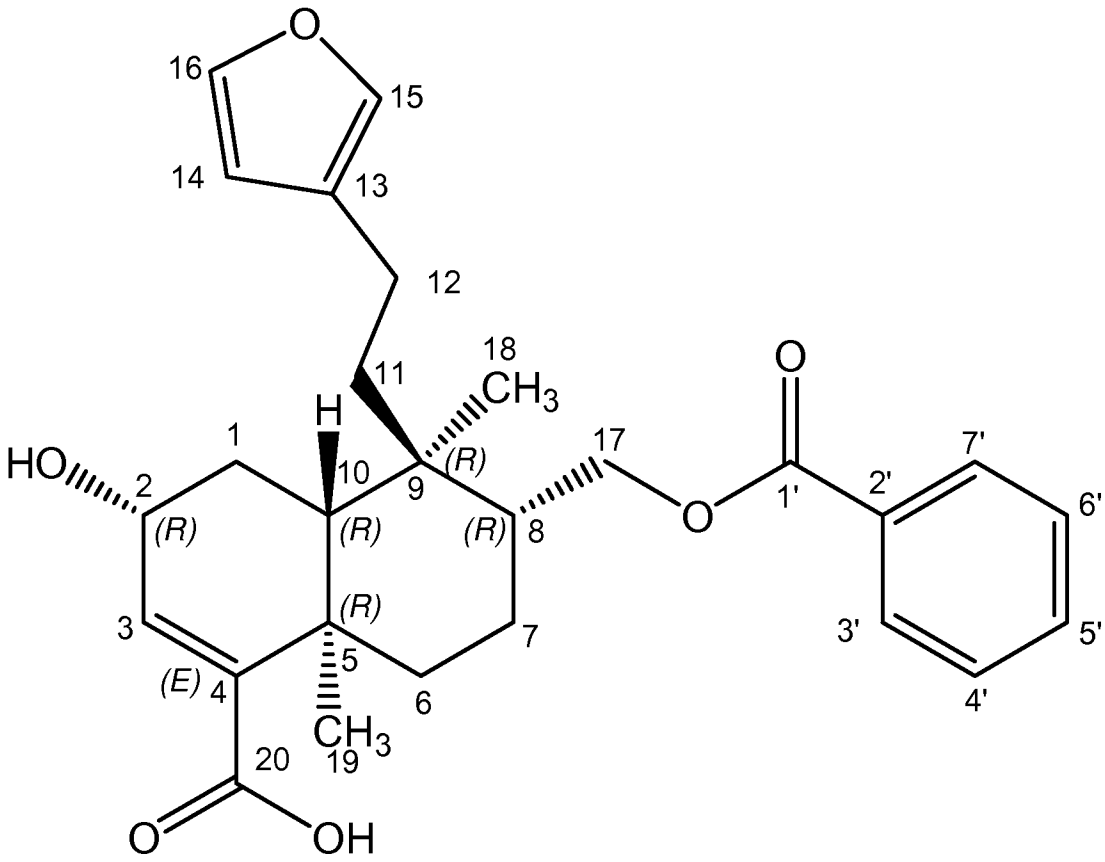

O
16
15
14
13
12
18
11
CH3
H
1
HO
2
10
9
(R)
17
O
O
7'
1'
2'
6'
(R)
(R)
(R)
8
(R)
3
7
3'
5'
(E)
4
5
6
4'
CH3
20
19
O
OH

Figure 12

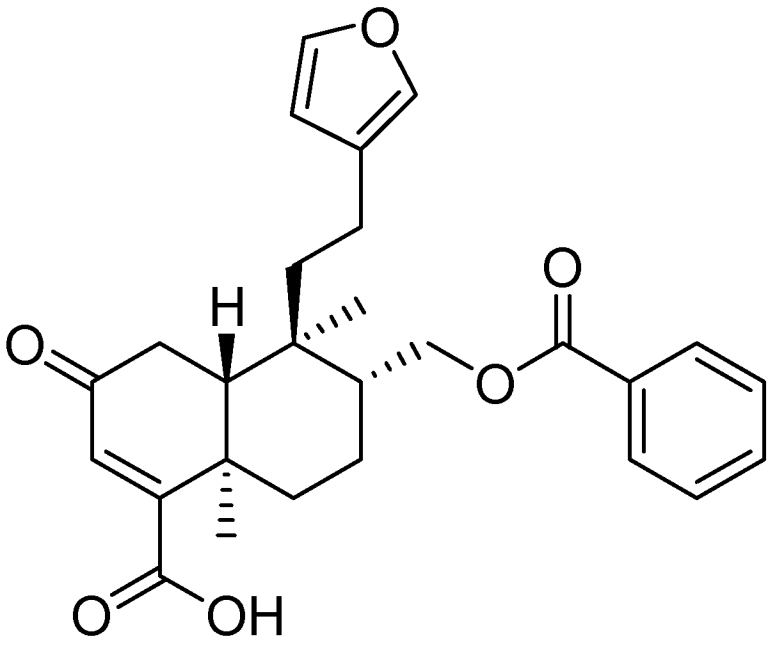


Figure 13

Semi-pure fraction	% inhibition (mean ± SEM) (p-value)			
	Maximum		Average	
LH1 (n=3)	46.3 ± 7.1*	0.028	37.6 ± 5.5*	0.025
LH2 (n=3)	75.5 ± 6.5*	<0.001	42.1 ± 11.0*	0.010
LH3 (n=3)	27.4 ± 5.6	0.310	27.4 ± 8.8	0.147
LH4 (n=3)	41.0 ± 8.9	0.062	27.4 ± 2.7	0.147
LH5 (n=3)	29.5 ± 9.5	0.252	29.1 ± 8.0	0.113
LH6 (n=3)	11.2 ± 6.4	0.824	21.2 ± 1.2	0.335
LH7 (n=3)	25.4 ± 12.5	0.372	4.1 ± 6.6	0.933
LH8 (n=3)	15.9 ± 11.1	0.693	16.3 ± 7.0	0.541
LH9 (n=3)	32.1 ± 15.1	0.190	27.3 ± 13.0	0.149
LH10 (n=3)	28.9 ± 15.0	0.268	14.4 ± 14.7	0.620
LH11 (n=3)	64.0 ± 27.4*	0.001	41.7 ± 19.5*	0.011
LH12 (n=3)	60.0 ± 7.8*	0.003	37.6 ± 2.5*	0.025
LH13 (n=3)	51.0 ± 26.6*	0.013	35.6 ± 16.3*	0.036

*statistically significant relative to TPA control at α = 0.05

Figure 14

Isolated compound	% inhibition (mean ± SEM) (p-value)			
	Maximum		Average	
Compound C (DP5) (n=4)	52.5 ± 15.9*	0.001	29.3 ± 10.8*	0.025
Compound D (DP9) (n=4)	87.6 ± 9.5*	< 0.001	46.3 ± 8.7*	0.001
Compound E (DP6) (n=4)	38.4 ± 9.2*	0.017	24.7 ± 3.8	0.060
Compound F (DP8) (n=4)	70.4 ± 9.3*	< 0.001	46.0 ± 6.6*	0.001
Hydrocortisone (6 mg) (n=4)	85.2 ± 3.6*	< 0.001	46.7 ± 2.0*	0.001
Betamethasone dipropionate (0.9 µmol/ear) (n=4)	89.6 ± 3.0*	< 0.001	55.3 ± 3.2*	< 0.001
*statistically significant relative to TPA control at α = 0.05				

Figure 15

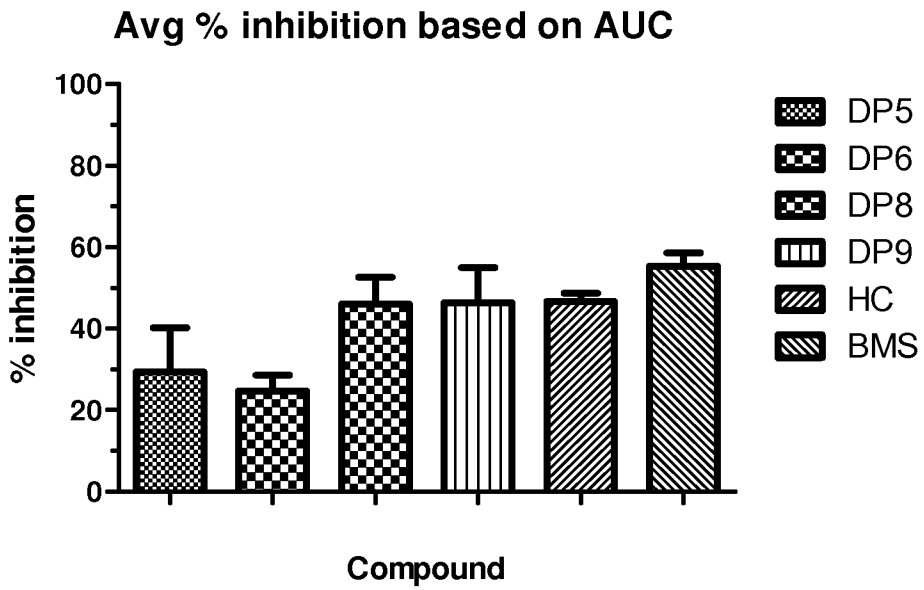

Avg % inhibition based on AUC
% inhibition
100
80
60
40
20
0
Compound
DP5
DP6
DP8
DP9
HC
BMS

Figure 16

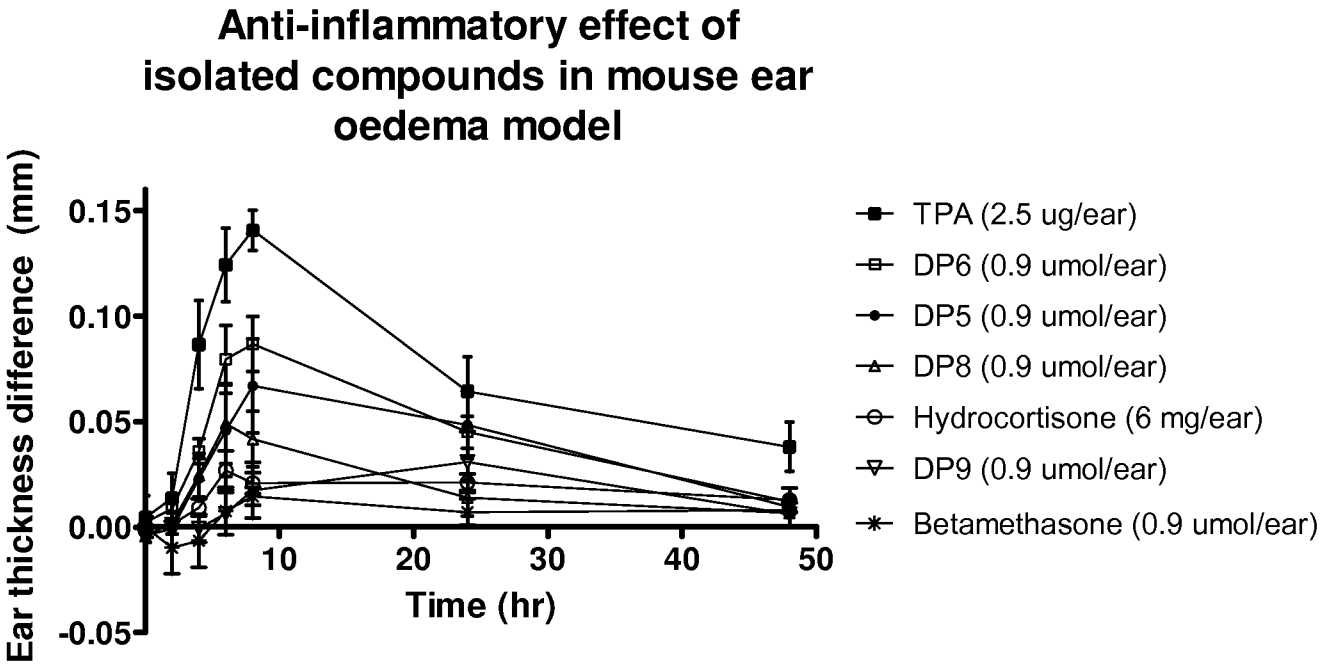

Anti-inflammatory effect of isolated compounds in mouse ear oedema model
Ear thickness difference (mm)
0.15
0.10
0.05
0.00
-0.05
10
20
30
40
50
Time (hr)
TPA (2.5 ug/ear)
DP6 (0.9 umol/ear)
DP5 (0.9 umol/ear)
DP8 (0.9 umol/ear)
Hydrocortisone (6 mg/ear)
DP9 (0.9 umol/ear)
Betamethasone (0.9 umol/ear)

Figure 17

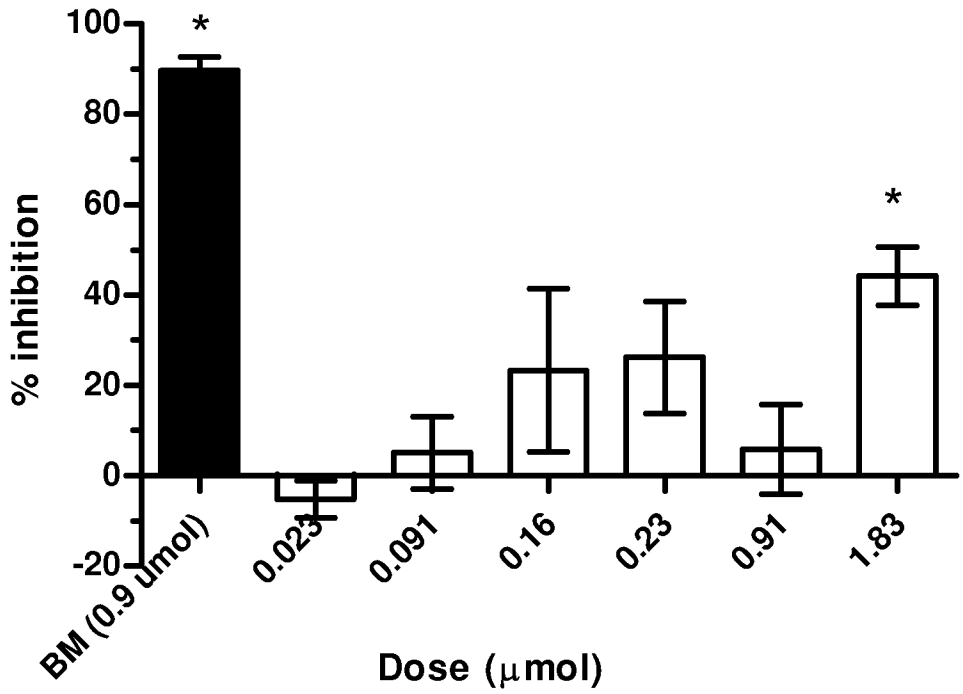

100
80
60
40
20
0
-20
% inhibition
*
*
BM (0.9 umol)
0.023
0.091
0.16
0.23
0.91
1.83
Dose (μmol)

Figure 18

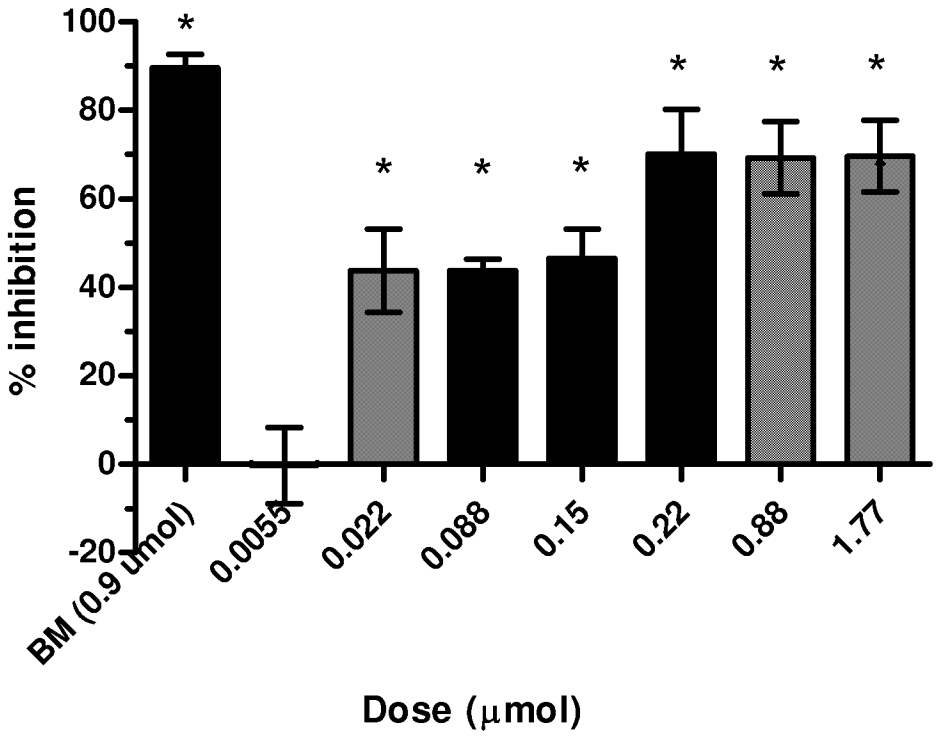

100
80
60
40
20
0
-20
% inhibition
*
*
*
*
*
*
*
BM (0.9 umol)
0.0055
0.022
0.088
0.15
0.22
0.88
1.77
Dose (μmol)

Figure 19

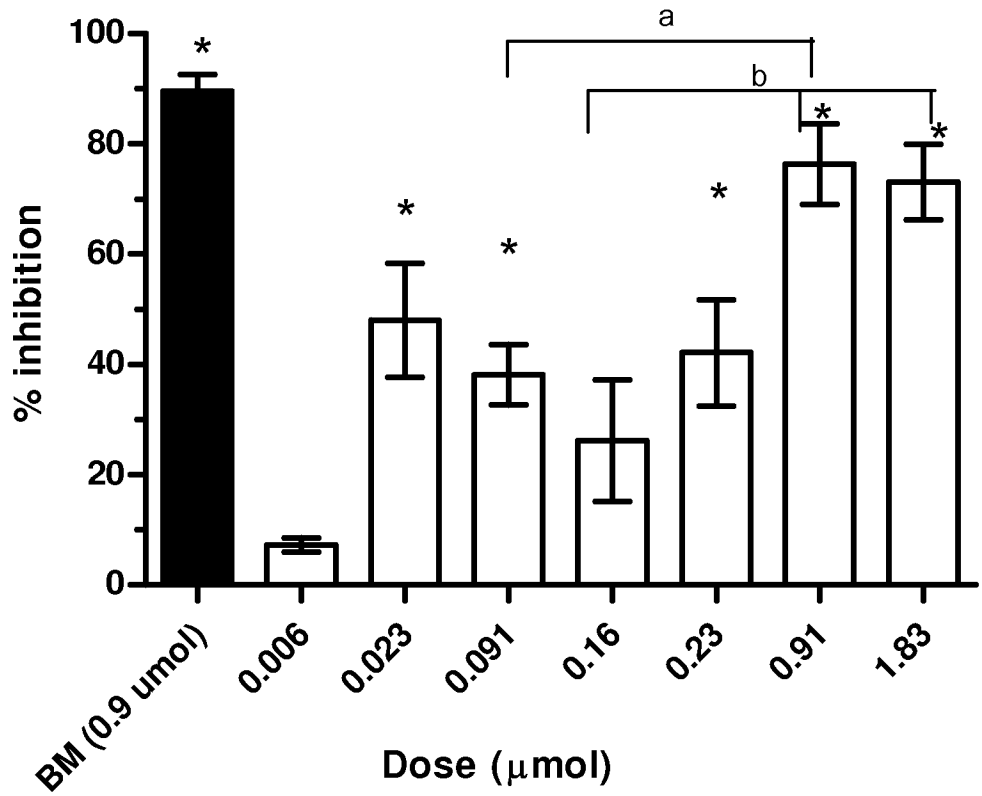
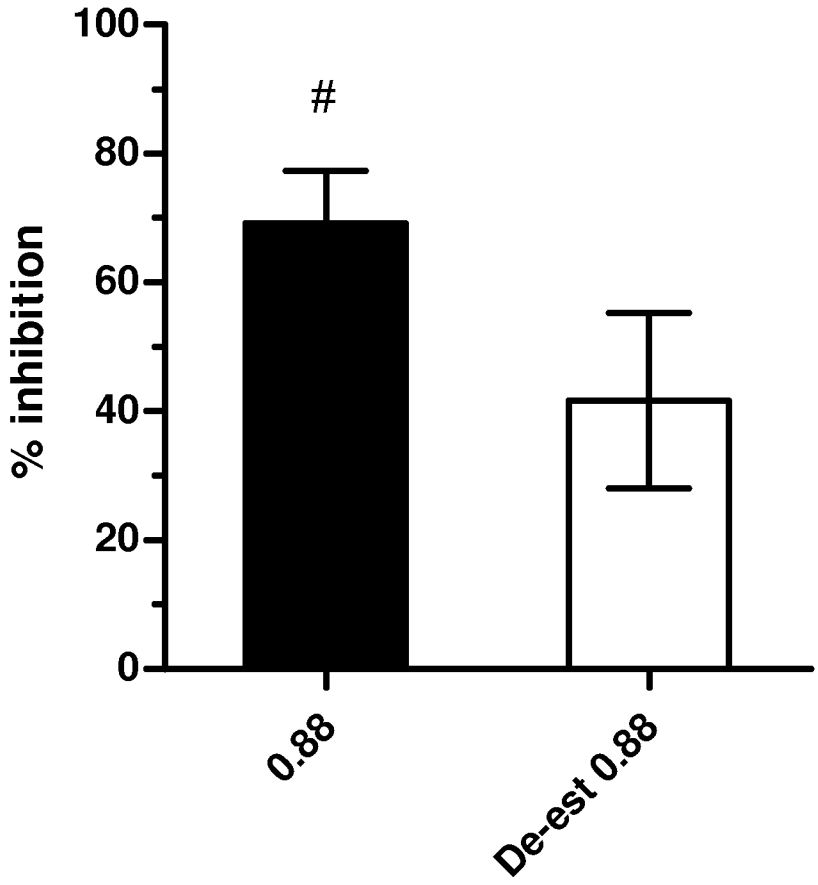

a
b
*
*
*
*
*
*
100
80
60
40
20
0
% inhibition
BM (0.9 umol)
0.006
0.023
0.091
0.16
0.23
0.91
1.83
Dose (μmol)

Figure 20



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2010/001497

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 36/77 (2006.01) ***A61K 31/37*** (2006.01) ***A61K 31/34*** (2006.01) ***A61P 29/00*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, EPODOC, WPI, XPTK, CAPLUS, BIOSIS, BIOTECHABS, AGRICOLA (keywords: dodonaea, hob bush, extract, anti-inflammatory, pain, tooth-ache and related terms)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6, 143, 303 A (JANAKIRAM, C. AND KHALILULLAH, M.) 7 November 2000 See abstract; col. 1, lines 50 – 56; col. 5, lines 4 – 18; examples 1 – 4.	1, 2, 13–15, 17, 20–36, 38, 40–47
X	ISAACS, J., "Bush Food – Aboriginal Food and Herbal Medicine", Weldon Publishing, Australia, 1989, ISBN 0-947116-90-7. See pg. 234, appendix: table of herbal medicines.	1–12, 30–39, 45–47
X	RANI, S. ET AL., "*Dodonaea viscosa* Linn. – An Overview", JOURNAL OF PHARMACEUTICAL RESEARCH AND HEALTH CARE, 2009, July, vol. 1, no. 1, pp. 97 – 112. See abstract; pg. 99, paragraph 3; Table, pg. 101.	1, 2, 5, 12, 31–37, 41–43, 45–47

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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"E" earlier application or patent but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2011	17 FEB 2011
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **CATHERINE GRAY** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2637

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THRING T.S.A. ET AL., "Antimicrobial Activities of Four Plant Species from the Southern Overberg Region of South Africa", AFRICAN JOURNAL OF BIOTECHNOLOGY, 2007, Vol. 6, no. 15, pp. 1779 – 1784. See pg. 1780, left column, paragraph 3 and right column, paragraph 1)	1, 2, 5, 13–16, 18–21, 30–34, 41, 43, 45–47
X	JEFFERIES, P.R. ET AL., "The Chemistry of *Dodonaea* spp. VIII* Isolation and Crystal Structure of a Diterpene Acid from *Dodonaea petiolaris*", AUSTRALIAN JOURNAL OF CHEMISTRY, 1981, vol. 34, no. 5, pp. 1001 – 1007. See introduction, pg. 1001.	1, 2, 4 – 6
X	JEFFERIES, P.R. ET AL., "Structure Elucidation of Some *ent*-Clerodane Diterpenes from *Dodonaea boroniaefolia* and *Cyanostegia augustifolia*", AUSTRALIAN JOURNAL OF CHEMISTRY, vol. 26, no. 10, pp. 2199 – 2211. See abstract; pg. 2201.	1, 2, 4, 5
X	PENGALLY, A., "Medicinal Activity of *Dodonaea viscosa* – A Preliminary Study", Rural Industries Research and Development Corporation Publication No. 08/172, November 2008. See pg. vii, paragraphs 1 and 7; pg. 3, last paragraph – pr. 4, paragraph 4; pg. 26, paragraph 3.	1, 2, 5, 12–15, 18–21, 30–34, 45–47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2010/001497

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
US 6143303	NONE

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX