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(71) **Applicant** *(for all designated States except US)*: **BIO-PHARMACOPAE DESIGN INTERNATIONAL INC.** [CA/CA]; 350 Franquet St., Entrance #10, Sainte-Foy, Québec G1P 4P3 (CA).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **GUAY, Johane** [CA/CA]; 3116 René Street, Saint-Augustin-de-Desmaures, Québec G3A 2L7 (CA). **GENDRON, Nathalie** [CA/CA]; 171 Rang Ste-Anne, Saint-Basile-de-Portneuf, Québec G0A 3G0 (CA). **PAGÉ, Brigitte** [CA/CA]; 9171 Carré Richard, Québec, Québec G2B 3P7 (CA).

(74) **Agent**: **MBM INTELLECTUAL PROPERTY LAW LLP**; 270 Albert Street, 14th Floor, Ottawa, Ontario K1P 5G8 (CA).

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(54) **Title**: EXTRACTS FROM PLANTS OF THE TSUGA GENUS AND USES THEREOF IN THE TREATMENT OF SKIN DISORDERS

(57) **Abstract**: Extracts derived from plants of the *Tsuga* genus having comedolytic and/or anti-acne activity are provided. The *Tsuga* extracts are suitable for the treatment of skin disorders associated with the formation of comedones, such as acne. The use of the *Tsuga* extracts in the prevention of acne and/or comedones is also provided. The *Tsuga* extracts also possess anti-inflammatory properties which allow the extracts to additionally combat the irritant or inflammatory effects of other acne treatment compounds (such as retinoids) and/or be incorporated into dermatological formulations for sensitive skins. In addition, the *Tsuga* extracts possess wound healing properties that can facilitate the healing of, and/or reduce the scarring from, acneic lesions.

EXTRACTS FROM PLANTS OF THE TSUGA GENUS AND USES THEREOF IN THE TREATMENT OF SKIN DISORDERS

FIELD OF THE INVENTION

The present invention relates to fields of pharmaceuticals and cosmetics and, in particular, to extracts from plants of the *Tsuga* genus for use to treat acne and other skin disorders associated with the formation of comedones.

BACKGROUND OF THE INVENTION

Acne is a common multifactor pathology which affects skin rich in sebaceous glands (such as the face, scapula region, arms and inter-triginous regions). Various pathogenic factors play a determining role in the formation of acne including genetic predisposition, overproduction of sebum (seborrhea), androgens, follicular keratinization disorders (comedogenesis) and bacterial colonization and inflammatory factors.

The clinical manifestations of acne include non-inflammatory closed (blackhead) or open (whitehead) comedones, as well as inflammatory lesions, including papules, pustules, cysts and nodules.

Acne can be divided into mild, moderate and severe based on the number of lesions and the surface of skin involved. Mild acne is characterized by open and closed comedones sometimes accompanied by few superficial inflammatory lesions, moderate acne is characterized by increasing largely superficial inflammatory lesions with pustules that have the tendency to scar with time. Nodules and cysts with marked scarring characterize severe acne.

The comedo is the primary lesion of acne vulgaris and results from the obstruction by cells of the wall of the follicle of the canal, preventing the sebum produced by sebocytes (cells of the sebaceous glands) from reaching the surface of the skin. The mixture of sebum and cells forms a plug, referred to as a comedo. Proliferation of bacteria which normally live on the skin, such as *P. acnes* and *P. granulosum*, in the plugged pore can bring about the inflammation of the surrounding tissue.

The treatment and prevention of acne includes various topical and systemic therapies and is guided by the type of clinical lesions present. Topical therapy is often preferred because of its safety compared with others forms of treatment. Current topical therapies include comedolytic agents such as tretinoin, adapalene, azelaic acid, tazarotene and salicylic acid; antimicrobial agents such as benzoyl peroxide; antibiotics such as clindamycin, erythromycin and tetracycline; and anti-inflammatory agents such as sodium sulfacetamide. Oral antibiotics are often added to the treatment regimen when acne does not respond satisfactorily to topical therapy. Other systemic treatments for more severe, recalcitrant acne include estrogens, antiandrogens, and isotretinoin.

International Patent Application No. PCT/CA04/02007 (WO 2006/053415) describes a large number of plant extracts that are useful for the preparation of dermatological formulations and uses of these formulations for ameliorating the effects of ageing and for the routine care of skin, hair and/or nails.

International Patent Application No. PCT/CA2009/000379 (WO 2009/121168) describes extracts from plants of the *Tsuga* genus and their use in the amelioration of inflammation, irritation and/or infection.

The *Tsuga* genus is a genus of conifers in the family Pinaceae. Plants in this genus are known under the common name of "hemlock." Catechol tannins extracted from *Tsuga* or hemlock have been described for the treatment of burns (U.S. Patent No. 2,276,241; GB Patent No. 544,615 and Canadian Patent No. 406,408) due to their tanning action. As further described in these patents, tannins are not germicidal and as such the burn treatment compositions further comprise an effective germicide, specifically a phenolic compound, which is compatible with the tannin.

Tsuga extracts have been described for their deodorant properties. For example, Japanese Patent Application Publication No. 2002087973 describes extracts from *Tsuga* as part of cosmetic compositions for suppressing human body odour; Japanese Patent Application Publication No. 4030855 describes a mousse-like deodorant containing several plant extracts including a *Tsuga* extract, and U.S. Patent No. 4,898,727 describes a deodorant containing several plant extracts including a *Tsuga* extract, a filter using same and a method of producing the deodorant.

European Patent Application Publication No. 0 870 507 describes a synergistic anti-bacterial composition that includes an extract of botanical materials and an essential oil. The essential oil is described as having anti-microbial activity, whereas the extract of botanical materials has significantly lower activity, or no anti-microbial activity, when used alone. A variety of potential botantical materials are described in the application including *Tsuga*, with the preferred material being a combination of Plantago, Hypericum, Echinacea and Propolis.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

It is an object of the invention to provide extracts from plants of the *Tsuga* genus and uses thereof in the treatment of skin disorders. In accordance with one aspect of the present invention, there is provided a method of reducing the number of acneic lesions, such as comedones, on the skin such of a subject comprising applying to the skin of the subject an effective amount of an extract from a plant of the *Tsuga* genus.

In accordance with another aspect of the invention, there is provided a method of treating acne comprising administering to the skin of a subject in need thereof an effective amount of an extract from a plant of the *Tsuga* genus.

In accordance with another aspect of the invention, there is provided a dermatological formulation comprising an extract from a plant of the *Tsuga* genus and optionally one or more anti-acne compounds. The dermatological formulation is optionally formulated for sensitive skins and/or is optionally formulated as a cosmetic

In certain embodiments, the one or more anti-acne compounds are selected from antibiotics, antimicrobials, comedolytic agents and anti-inflammatory agents. The antibiotics may optionally be selected from: erythromycin, clindamycin and tetracyclines. The antimicrobials may optionally be selected from: chlorexidine, benzoylperoxide, 1-pentadecanol, derivatives of 1-pentadecanol, cedrene, caryophyllene, longifolene and thujopsene. The comedolytic agents may optionally be selected from: tretinoin, isotretinoin,

adapalene, azelaic acid, tazarotene, salicylic acid and salicylic acid derivatives. The anti-inflammatory agents may optionally be selected from NSAIDs, steroidal anti-inflammatory agents, cetylsalicylic acid, ibuprofen, naproxen, sulfacetamide and hydrocortisone. In certain embodiments, the dermatological formulation may be for sensitive skins or a cosmetic.

In certain embodiments, the plant of the *Tsuga* genus is a *Tsuga canadensis, Tsuga diversifolia* or *Tsuga heterophylla* plant.

In certain embodiments, the extract is prepared by solvent extraction of needles, twigs, small branches, bark, or a combination thereof, from the plant of the *Tsuga* genus.

In certain embodiments, wherein the *Tsuga* extract is an aqueous-alcoholic extract, aqueous-glycolic extract or aqueous-glycerine extract.

In certain embodiments, the extract is prepared using a glycol, an alcohol or a glycerine as the solvent.

In certain embodiments, wherein the extract is a supercritical CO_2 extract.

In certain embodiments, the extract is prepared using a ratio of solvent:plant material between about 5:1 and about 50:1; about 15:1 and about 25:1; is about 70:30 by weight; is about 80:20 by weight; is about 30:70 by weight; is about 20:80 by weight.

In certain embodiments, the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is between about 60:40 and about 40:60; is about 70:30 by weight; is about 30:70 by weight; is about 80:20 by weight or is is about 20:80 by weight.

BRIEF DESCRIPTION OF THE FIGURES

These and other features of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings.

Figure 1 presents the results demonstrating the anti-inflammatory effect of a *Tsuga canadensis* extract in human skin keratinocytes after UV irradiation % inhibition of interleukin (IL)-6 release.

Figure 2 presents the results of an evaluation of the antimicrobial effect of *Tsuga canadensis* extract 207-20156 at 2% and 5% against **(A)** *Staphyloccocus epidermidis* and **(B)** *Propionibacterium acnes* (results in CFU).

Figure 3 presents the results of an *in vitro* a wound healing assay demonstrating the effects of a *Tsuga canadensis* extract on wound healing.

Figure 4 presents results from a cosmetologist clinical evaluation of the effect of a cream containing a *Tsuga canadensis* extract ("Product B") on acneic lesions in human volunteers; **(A)** evolution of the number of lesions after 28 days of application of Product B; **(B)** evolution of the number of lesions after 42 days of application of Product B; **(C)** comparison of the number of lesions after 28 days of application of Product B and a placebo ("Product A"), and **(D)** comparison of the number of lesions after 42 days of application of Product B and a placebo.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the newly identified activity of extracts derived from plants of the *Tsuga* genus ("*Tsuga* extracts") in the treatment of comedones and/or acne. In one aspect, therefore, the present invention provides for the use of the *Tsuga* extracts to treat skin disorders associated with the formation of comedones, such as acne.

In one embodiment, the present invention provides for the use of *Tsuga* extracts to decrease comedones in a subject. In another embodiment, the present invention provides for the use of *Tsuga* extracts to treat acne. In accordance with this embodiment, the extracts may decrease comedones and/or other skin lesions in the subject under treatment.

In certain embodiments, the present invention also provides for the use of the *Tsuga* extracts in the prevention of acne and/or comedones. In accordance with these embodiments, the *Tsuga* extracts may be used in the treatment of skin having a tendency toward acne in order to combat the appearance of skin lesions, such as comedones, papules or pustules.

The *Tsuga* extracts also possess anti-inflammatory properties which allow the extracts to additionally combat the irritant or inflammatory effects of other acne treatment compounds (such as retinoids) and/or be incorporated into dermatological formulations for sensitive skins.

The ability of the *Tsuga* extracts to combat the irritant effects of retinoids and other anti-acne compounds allows for formulations that comprise the extract and higher than standard amounts of these agents. In one embodiment, therefore, the present invention provides for dermatological formulations that comprise a *Tsuga* extract and one or more of a retinoid or other anti-acne compound with irritant effects, wherein the retinoid or other anti-acne compound is present in a higher than standard amount.

The *Tsuga* extracts also possess healing properties as shown herein, which allow the extracts to additionally facilitate the healing of, and/or reduce the formation of scars that may result from, acneic lesions, such as comedones, papules or pustules.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "plant material," as used herein, refers to any part or parts of a specified plant taken either individually or in a group. Examples include, but are not limited to, leaves, needles, roots, bark, stems, buds, twigs, cones, branches and the like.

The term "extract," as used herein with reference to a specified plant, refers to a composition prepared by mixing plant material with a solvent following the procedures described herein. The extract can optionally be subjected to one or more separation and/or purification steps.

The term "attenuate," as used herein, means to reduce or inhibit, wherein the inhibition may be complete or partial inhibition.

A "dermatological formulation," as used herein, refers to a pharmaceutical composition, a cosmeceutical composition or a cosmetic formulated for topical administration to the skin.

The term "ameliorate," as used herein, means to make more tolerable (for example by reducing the incidence or severity), to heal or to cure.

The term "treatment," as used herein, refers to an intervention performed with the intention of improving a recipient's status. The improvement can be subjective or objective and is related to the amelioration, either temporary or long-term, of one or more of the symptoms associated with a condition being treated. In some embodiments, treatment includes the

prevention of the development of the condition. Thus, in various embodiments, the term treatment includes the prevention (prophylaxis), moderation, reduction, and/or curing of a condition at various stages. In certain embodiments, prevention of deterioration of a recipient's status is also encompassed by the term. Those in need of treatment thus may include those already having the condition as well as those prone to, or at risk of developing, the condition and those in whom the condition is to be prevented.

The term "subject," as used herein, refers to an individual in need of treatment or who would otherwise benefit from the use of a dermatological formulation in accordance with the invention.

As used herein, the term "about" refers to approximately a +/-10% variation from a given value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

Unless otherwise specified, when ranges are provided herein, it is understood that the range includes both the upper and lower limits specified for the range.

TSUGA EXTRACTS

The present invention provides for extracts from plants of the *Tsuga* genus ("*Tsuga* extracts") suitable for dermatological use. In accordance with the present invention, the *Tsuga* extracts have comedolytic and/or anti-acne activity.

In accordance with the present invention, the *Tsuga* extracts are solvent-based extracts obtained by solvent extraction of plant material from a selected *Tsuga* plant. The selected *Tsuga* plant can be, for example, *Tsuga canadensis; Tsuga caroliniana; Tsuga chinensis; Tsuga diversifolia; Tsuga dumosa; Tsuga forrestii; Tsuga heterophylla; Tsuga mertensiana* or *Tsuga sieboldii*. In one embodiment of the present invention, the *Tsuga* plant is a plant native to North America, *i.e. Tsuga canadensis; Tsuga caroliniana; Tsuga heterophylla* or *Tsuga mertensiana*. In another embodiment, the *Tsuga* plant is a plant native to Asia, *i.e. Tsuga chinensis; Tsuga diversifolia; Tsuga dumosa; Tsuga forrestii* or *Tsuga sieboldii*. In another embodiment, the *Tsuga* plant is *Tsuga canadensis; Tsuga heterophylla* or *Tsuga diversifolia*.

In one embodiment of the present invention, the *Tsuga* plant is *Tsuga canadensis*.

The solvent used for the preparation of the extract can be an aqueous solvent (such as water or a buffer), or it can be a liquid organic compound, or a combination of an aqueous solvent and a liquid organic compound. Examples of solvents that may be used in the preparation of the *Tsuga* extracts are described in detail below. In some embodiments, the solvent may be a supercritical or sub-critical fluid. In one embodiment of the invention, the *Tsuga* extract is an aqueous, alcoholic or aqueous-alcoholic extract. In another embodiment, the *Tsuga* extract is an aqueous, glycolic or aqueous-glycolic extract.

PREPARATION OF THE *TSUGA* EXTRACTS

The *Tsuga* extracts in accordance with the invention are obtained by solvent extraction of plant material from a selected *Tsuga* plant.

Plant Material

The plant material is derived from one or a combination of the species of *Tsuga* noted above, *i.e. Tsuga canadensis; Tsuga caroliniana; Tsuga chinensis; Tsuga diversifolia; Tsuga dumosa; Tsuga forrestii; Tsuga heterophylla; Tsuga mertensiana* and/or *Tsuga sieboldii*. The plant material employed in the extraction process can be the entire plant (tree), or it can be one or more distinct tissues from the plant or plants, for example, leaves (needles), cones, roots, branches, bark, stems, twigs, buds or various combinations thereof. In one embodiment of the invention, the *Tsuga* extract is prepared from needles, twigs, small branches, bark, or any combination thereof. In another embodiment, the *Tsuga* extract is prepared from needles and optionally other parts of the plant.

The plant material can be fresh, dried or frozen. In one embodiment, the plant material used in the preparation of the *Tsuga* extracts is dried. The plant material may be used immediately after harvesting or it can be stored for a period of time prior to being subjected to the extraction process. If the plant material is stored, it can be treated prior to storage, for example, by drying, freezing, lyophilizing, or some combination thereof, as is known in the art. The storage time may be of various durations, for example, the storage period may be between a few days and a few years. Typically storage times range between less than one week and about one year in duration.

If desired, the plant material can be treated prior to the extraction process in order to facilitate the extraction process. Typically such treatment results in the plant material being fragmented

by some means such that a greater surface area is presented to the solvent. For example, the plant material can be crushed or sliced mechanically, using a grinder or other device to fragment the plant parts into small pieces or particles, or the plant material can be frozen in liquid nitrogen and then crushed or otherwise fragmented into smaller pieces.

If desired and when practicable, the plant material can be derived from a *Tsuga* plant that was subjected to a stress treatment. A stress treatment comprises contacting or treating the plant, or material from the plant, with one or more stressor with the aim of inducing or eliciting increased production of one or more chemicals. The stressor can be a chemical compound or a physical treatment. Examples of chemical stressors include, but are not limited to, organic and inorganic acids including fatty acids; glycerides; phospholipids; glycolipids; organic solvents; amino acids; peptides; monosaccharides; oligosaccharides; polysaccharides; lipopolysaccharides; phenolics; alkaloids; terpenes; terpenoids; antibiotics; detergents; polyamines; peroxides; ionophores, and the like. Examples of physical stress treatments include, but are not limited to, ultraviolet radiation, sandblasting, low and high temperature stress, and osmotic stress induced by salt or sugars. Nutritional stress is another example of a physical stress and is defined as depriving the plant of essential nutrients (*e.g.* nitrogen, phosphorus or potassium) in order to induce or elicit increased production of one or more chemicals. The one or more stressor (*i.e.* chemical compound(s), physical treatment(s), or combination thereof) may be applied continuously or intermittently to the plant material. Various stressors and procedures for stressing plants prior to extract preparation have been described previously (see International Patent Application WO 02/06992) and are suitable for use in accordance with the present invention.

Solvent extraction

Various extraction processes are known in the art and can be employed in the process of the present invention (see, for example, International Patent Application WO 02/06992). The solvent extraction process employed in the preparation of the *Tsuga* extracts typically employs as solvent an aqueous solvent (such as water or a buffer), a liquid organic compound, or a combination thereof. Exemplary liquid organic compounds that can be used as solvents in the extraction process to prepare the *Tsuga* extracts include, but are not limited to, alcoholic solvents, which include primary alcohols such as methyl alcohol (methanol), ethyl alcohol (ethanol), 1-propanol and 1-butanol; secondary alcohols such as 2-propanol and 2-butanol; tertiary alcohols such as 2-methyl-2-propanol, and liquid polyhydric alcohols such

as glycerine (glycerol) and glycols. Suitable glycols include, for example, ethylene glycol (1,2-ethandiol), propylene glycol (1,2-propanediol), trimethylene glycol (1,3-propanediol), 1,3-butylene glycol, pentylene glycol (1,2-pentanediol), hexylene glycol (2-methyl-2,4-pentanediol), diethylene glycol, dipropylene glycol and lower molecular weight polyethylene glycols. Other known organic solvents for plant extraction include acetone, tetrahydrofuran, acetonitrile, 1,4-dioxane, pyridine, dimethylsulfoxide, N,N-dimethyl formamide, acetic acid, diethyl ether, hexane, heptane, dichloromethane and ethyl acetate. Supercritical or sub-critical fluids, such as water or carbon dioxide, are also suitable solvents for the preparation of the *Tsuga* extracts.

In one embodiment of the invention, the solvent employed to prepare the *Tsuga* extracts comprises an alcohol. In another embodiment, the solvent employed to prepare the *Tsuga* extracts comprises a primary alcohol or a liquid polyhydric alcohol. In another embodiment, the solvent employed to prepare the *Tsuga* extracts comprises a supercritical or sub-critical fluid.

When the extraction process is carried out using a solvent that comprises a mixture of an aqueous solvent and a liquid organic compound, the content of the liquid organic compound ranges from about 5% to about 95% by volume. In certain embodiments of the invention, the extraction process is carried out using a solvent that comprises a mixture of an aqueous solvent and a liquid organic compound in which the content of the liquid organic compound ranges from about 10% to about 95% by volume, from about 15% to about 95% by volume, from about 10% to about 90% by volume, from 20% to about 95% by volume, from about 20% to about 90% by volume, from about 10% to about 85% by volume and from about 20% to about 85% by volume.

In one embodiment, a solvent that is compatible with mammalian skin is used in the extraction. This can, for example, allow for the extract to be incorporated directly into a dermatological formulation with little, or no, further processing. Examples of such solvents include, but are not limited to, water, an aqueous buffer, a combination of water/buffer with a lower alcohol or an anhydrous lower alcohol. In the context of the present invention, a lower alcohol refers to an alcohol having 1 to 6 carbon atoms, such as a primary, secondary, tertiary or liquid polyhydric alcohol. In one embodiment of the present invention, the solvent for the preparation of the *Tsuga* extract is selected from water, a lower alcohol or a combination thereof. In another embodiment, the solvent for the preparation of the *Tsuga* extract

comprises a lower alcohol selected from the group of: methyl alcohol (methanol), ethyl alcohol (ethanol), 1-propanol, 1-butanol, 2-propanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, glycerine, ethylene glycol, propylene glycol, diethylene glycol, dipropylene glycol, 1,3-propanediol and 1,3-butylene glycol. In one embodiment, the solvent for the preparation of the *Tsuga* extract is a glycol or a glycol-water mixture.

When the extraction process employs a combination of an aqueous solvent and a lower alcohol as solvent, the lower alcohol content of the solvent typically ranges from about 5% to about 95% by volume, for example from about 10% to about 95% by volume. In one embodiment of the invention, the extraction process is carried out using a solvent that comprises a mixture of an aqueous solvent and a lower alcohol in which the content of the lower alcohol ranges from about 15% to about 95% by volume. In another embodiment of the invention, the extraction process is carried out using a solvent that comprises a mixture of an aqueous solvent and a lower alcohol in which the content of the lower alcohol ranges from about 20% to about 95% by volume. In other embodiments, the extraction process is carried out using a solvent that comprises a mixture of an aqueous solvent and a lower alcohol in which the content of the lower alcohol ranges from about 20% to about 90% by volume, and from about 20% to about 85% by volume.

For example, when the extraction process employs a solvent that is an aqueous solvent/primary alcohol mixture, the primary alcohol can be present in an amount between about 20% to about 90% by volume, for example from about 30% to about 90% by volume, whereas when the extraction process employs a solvent that is an aqueous solvent/glycol mixture, the glycol can be present in an amount between about 10% to about 80% by volume, for example from about 10% to about 60% by volume. Similarly, when the extraction process employs a solvent that is an aqueous solvent/glycerine mixture, the glycerine can be present in an amount between about 10% to about 80% by volume, for example from about 10% to about 60% by volume.

In certain embodiments, the extraction process employs a solvent that is an aqueous solvent/primary alcohol mixture in which the primary alcohol is present in an amount between about 35% to about 90% by volume, between about 40% to about 90% by volume, between about 45% to about 90%, between about 45% to about 85%, and between about 50% to about 85% by volume.

In some embodiments, the extraction process employs a solvent that is an aqueous solvent/glycol mixture in which the glycol is present in an amount between about 15% to about 80% by volume, for example, between about 15% to about 70% by volume, between about 15% to about 60% by volume, between about 15% to about 55% by volume, between about 20% to about 55% by volume, and between about 20% to about 50% by volume.

A number of standard extraction techniques known in the art can be employed to prepare the plant extracts. In general, the extraction process entails contacting solid plant material with a solvent with adequate mixing and for a period of time sufficient to ensure adequate exposure of the solid plant material to the solvent such that activity present in the plant material can be taken up by the solvent.

An appropriate amount of the solvent to be used in the extraction can be determined by the skilled worker based on the amount of plant material being employed in the extraction. In one embodiment of the invention, the w/v (g/100mL) of plant material to solvent used in the extraction process is between about 1/2 and about 1/50. In another embodiment, the w/v (g/100mL) of plant material to solvent used in the extraction process is between about 1/5 and about 1/50. In another embodiment, the w/v (g/100mL) of plant material to solvent used in the extraction process is between about 1/10 and about 1/50. In other embodiments, the w/v (g/100mL) of plant material to solvent used in the extraction process is between about 1/10 and about 1/40; between about 1/10 and about 1/30; and between about 1/10 and about 1/25.

A variety of conditions can be employed for the extraction process. Typically, the extraction procedures are conducted over a period of time between about 10 minutes and about 72 hours at a temperature between about 4°C and about 50°C. However, temperatures between about 4°C and about 90°C, for example between about 4°C and about 70°C can be employed. Higher temperatures are also contemplated, with or without increased pressure, when certain extraction techniques are employed, for example, pressurized liquid extraction, sub-critical fluid extraction (for example, sub-critical water extraction (SWE)) or supercritical fluid extraction. Similarly, the extraction time may be varied depending on other extraction conditions, such as the solvent and temperature employed, for example, the extraction time can range from several minutes to several days. For example, in one embodiment, the extraction time is at least one hour. In another embodiment, the extraction time is between

about one hour and about 72 hours. Determination of appropriate extraction temperatures and times is within the ordinary skills of a worker in the art.

Adequate contact between the solvent and the plant material can be encouraged by shaking, stirring, percolating and/or macerating the suspension. Alternatively, an extraction device equipped with, for instance, a stirring machine, or a Soxhlet or other device known in the art can be employed which may improve the extraction efficiency. The extraction can be carried out at ordinary pressure, under pressure or at reduced pressure established by, for example, aspiration. Appropriate extraction conditions can readily be determined or selected by one skilled in the art taking into consideration the production conditions such as production facilities and yields.

In one embodiment, the present invention also provides for the use of supercritical fluid extraction for the preparation of the *Tsuga* extracts. Supercritical fluid extraction involves the use of a supercritical fluid (SCF) as a solvent. A SCF is a liquid or a gas at atmospheric conditions, but becomes supercritical when it is compressed above its critical pressure and heated above its critical temperature. Supercritical fluids have increased dissolving power in their supercritical regions. A supercritical fluid exhibits properties between those of a gas and a liquid, and has the capacity to dissolve compounds that may only dissolve poorly or not at all in the gas or liquid state. Most components extracted from the plant material, once dissolved, can quickly and cleanly be precipitated or removed from the supercritical fluids by lowering the pressure and/or temperature to achieve separation. Using the method of post-extraction fractionation with a column designed to allow for temperature and pressure drops at different levels to gain the desired results may effect further concentration and purification when desirable.

Supercritical fluid extraction processes are well known in the art, for example, see Martinez, J.L. (*Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds* (2007, CRC Press, Boca Raton, FL) and Herrero. M. *et al.* (2005, *Food Chem*, 98:136-148).

In general, the starting plant material is placed in an extractor device together with the supercritical fluid, with or without a chemical modifier, at specified pressure and temperature conditions to extract the desired components from the plant material. After extraction, the fluid and the compound are passed through a separator which changes the pressure and

temperature, thereby reducing the dissolving power of the supercritical fluid and causing the separation or fractionation of the dissolved components.

Examples of suitable supercritical fluids for the preparation of the *Tsuga* extracts include water and carbon dioxide. Carbon dioxide has a critical temperature of 31.06°C, a critical pressure of 73.83 bar, and a critical density of 0.460 g/cm^3, which allows the use of relatively low temperatures for the extraction process. An exemplary SCF extraction process utilizing carbon dioxide as the SCF is as follows. Comminuted plant material is combined with the carbon dioxide with one or more modifiers in an extractor device. The extraction is conducted at a pressure between about 270 to about 320 bar, and a temperature of about 40°C to about 60°C. The ratio of supercritical fluid solvent to starting plant material is typically between about 5:1 and about 80:1 by weight, for example between about 5:1 and about 60:1 by weight, between about 5:1 and about 50:1 by weight or between about 10:1 and about 40:1 by weight.

Preparation of the *Tsuga* extracts using subcritical fluids, with or without a co-solvent, is also contemplated. Examples of suitable subcritical fluids for preparation of the *Tsuga* extracts include water and carbon dioxide.

As noted above, in some embodiments, one or more co-solvents (or modifiers) are included in the supercritical fluid or subcritical fluid. Modifiers generally possess volatility between that of the supercritical or subcritical fluid and of the components being extracted, and must be miscible with the supercritical/subcritical fluid. Suitable modifiers include, for example, ethanol, methanol, propanol, acetone, ethyl acetate, methylene chloride, and the like. Water is also a suitable modifier when the supercritical/subcritical fluid is carbon dioxide. Ethanol, for example, can be used as a modifier in a ratio of 35 to 75 kg ethanol solvent per kg of plant material.

Following a typical extraction process, whether using standard pressure or sub- or supercritical fluids, the liquid fraction (the *Tsuga* extract) can be separated from the solid (insoluble) matter. Separation of the liquid and solid fractions can be achieved by one or more standard separation processes known to those skilled in the art, such as various centrifugation or filtration processes. In one embodiment of the invention, the *Tsuga* extract is separated from solid matter after the extraction by one or more filtration steps. In another

embodiment, the *Tsuga* extract is separated from solid matter after the extraction by a series of filtration steps.

Once the *Tsuga* extract has been isolated, its activity can be tested directly or after being diluted in a suitable solvent, or it may be subjected to further procedures. For example, the *Tsuga* extract can be subjected to one or more additional steps to further purify or concentrate the extract. For example, the extract may be subjected to solid-liquid extraction, liquid-liquid extraction, solid-phase extraction (SPE), membrane filtration, ultrafiltration, dialysis, electrophoresis, solvent concentration, centrifugation, ultracentrifugation, liquid or gas phase chromatography (including size exclusion, affinity, and the like) with or without high pressure, lyophilization, evaporation, precipitation with various "carriers" (including PVPP, carbon, antibodies, and the like), the use of supercritical fluids (such as CO_2), or various combinations thereof. In one embodiment, the *Tsuga* extract is subjected to procedures to remove fatty acids or chlorophyll components that may interfere with its activity. Various procedures known in the art may be employed. In one embodiment, one or more additional partitioning steps using an organic solvent, such as hexane, heptane or ethyl acetate, are included. The liquid *Tsuga* extract can be concentrated and solubilised in an appropriate solvent prior to the one or more partitioning steps, if desired.

In one embodiment of the present invention the *Tsuga* material is subjected to an extraction process that entails contacting the solid plant material with a solvent with adequate mixing over a period of time between about 10 minutes and about 72 hours at a temperature between about 4°C and about 50°C. The liquid fraction (the *Tsuga* extract) is then separated from the solid (insoluble) matter by one or more standard processes known to those skilled in the art.

In one embodiment of the invention, the *Tsuga* extract is prepared by extracting plant material with an alcoholic solvent alone or in combination with an aqueous co-solvent for a time period between about 4 hours and about 48 hours, for example between about 4 hours and about 24 hours, at a temperature between about 4°C to about 32°C, for example between about 4 °C to about 25°C. In one embodiment, the *Tsuga* extract is prepared by extracting plant material using a combination of ethanol and water as the solvent, wherein the range of ethanol:water is between about 50:50 and about 85:15, and wherein the extraction is conducted for a time period between about 4 hours and about 48 hours, for example between about 4 hours and about 24 hours, at a temperature between about 4°C to about 32°C, for example between about 4°C to about 25°C. In another embodiment, the *Tsuga* extract is

prepared by extracting plant material using a combination of a glycol and water as the solvent, wherein the range of glycol:water is between about 100:0 and about 20:80, for example between about 80:20 and about 40:60 or between about 60:40 and about 40:60, and wherein the extraction is conducted for a time period between about 4 hours and about 48 hours, for example between about 4 hours and about 24 hours, at a temperature between about 4°C to about 32°C, for example between about 4°C to about 25°C.

In certain embodiments, the ratio of solvent to starting plant material for the extraction is between about 5:1 and about 50:1 by weight, for example, between about 10:1 and about 50:1 by weight, between about 5:1 and about 40:1 by weight, between about 10:1 and about 40:1 by weight, between about 5:1 and about 30:1 by weight, between about 10:1 and about 30:1 by weight, between about 5:1 and about 25:1 by weight, between about 10:1 and about 25:1 by weight, between about 15:1 and about 25:1 by weight, and about 20:1 by weight.

The present invention contemplates that the extraction process and any subsequent purification steps may be carried out on various scales including known large, medium and small-scale methods of preparing extracts.

In one embodiment of the invention, the *Tsuga* extract is prepared on a large-scale. For example, the *Tsuga* extract can be prepared on a commercial scale by using the extraction process employed in the initial analytical scale preparation of the extract. The small-scale extraction procedure is simply scaled-up and additional steps of quality control can be included to ensure reproducible results.

Also contemplated are modifications to the small-scale procedure as may be required during scale-up for industrial level production of the *Tsuga* extract. Such modifications include, for example, alterations to the solvent being used or to the extraction procedure employed in order to compensate for variations that occur during scale-up and render the overall procedure more amenable to industrial scale production, or more cost effective. Modifications of this type are standard in the industry and would be readily apparent to those skilled in the art.

TESTING THE *TSUGA* EXTRACTS

The *Tsuga* extracts in accordance with the present invention have comedolytic and/or anti-acne activity. These properties can be assessed using standard techniques known in the art. Exemplary techniques are provided below and in the Examples section.

Determination of Anti-Microbial Activity in vitro

The ability of the *Tsuga* extracts to inhibit the growth of microbial cells can be assessed using standard *in vitro* methods known in the art. In general, these methods involve contacting a culture of the microbial cells with various concentrations of the extract and monitoring the growth of the cell culture relative to an untreated control culture. A second control culture comprising cells contacted with a known anti-microbial agent may also be included in such tests, if desired. An exemplary test for determining the ability of the *Tsuga* extract to inhibit a variety of bacterial species is provided in the Examples (see Example IX).

The ability of the *Tsuga* extracts to inhibit the growth of bacterial cells can also be determined by measurement of the minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration that inhibits growth of the organism to a pre-determined extent. For example, a MIC_{100} value is defined as the lowest concentration that completely inhibits growth of the organism, whereas a MIC_{90} value is defined as the lowest concentration that inhibits growth by 90% and a MIC_{50} value is defined as the lowest concentration that inhibits growth by 50%. MIC values are sometimes expressed as ranges, for example, the MIC_{100} for a compound may be expressed as the concentration at which no growth is observed or as a range between the concentration at which no growth is observed and the concentration of the dilution which immediately follows.

Typically, anti-bacterial MICs are measured using a broth macro- or microdilution assay (see Amsterdam, D. (1996) "Susceptibility testing of antimicrobials in liquid media," pp.52-111. In Loman, V., ed. *Antibiotics in Laboratory Medicine*, 4th ed. Williams and Wilkins, Baltimore, MD). A standardised anti-bacterial susceptibility test is provided by the National Committee for Clinical Laboratory Standards (NCCLS) as NCCLS, 2000; document M7-A58.

In the classical broth microdilution method, the *Tsuga* extract would be diluted in culture medium in a sterile, covered 96-well microtiter plate. An overnight culture of a single bacterial colony is diluted in sterile medium such that, after inoculation, each well in the microtiter plate contains an appropriate number of colony forming units (CFU)/ml (typically,

approximately 5 x 10^5 CFU/ml). Culture medium only (containing no bacteria) is also included as a negative control for each plate and known antibiotics are often included as positive controls. The inoculated microtiter plate is subsequently incubated at an appropriate temperature (for example, 35°C – 37°C for 16-48 hours). The turbidity of each well is then determined by visual inspection and/or by measuring the absorbance, or optical density (OD), at 595nm or 600nm using a microplate reader and is used as an indication of the extent of bacterial growth.

In vivo *Activity*

The *Tsuga* extracts may undergo additional testing on human volunteers to assess their ability to exert the desired dermatological effect(s).

For example, the effect of the extracts on skin lesions can be assessed by visual examination. For example, the effect of the *Tsuga* extract on the skin can be evaluated by formulating the extract such that it is suitable for external application to the skin and subsequently sensory tests can be conducted on the formulation using a panel of human volunteers. A sensory test typically involves application of the formulation to the skin of the panelists on a regular basis, such as once or twice a day, over a period of several weeks. The effect of the formulation on the skin lesions can be evaluated by inspecting the skin of the panelists and assessing visually the effect on the number and/or size of the lesions.

The plant extracts may also undergo one or more safety, stability and/or bioavailability test prior to testing on human volunteers, for example, the Human Repeat Insult Patch Test (HRIPT).

Other Tests

Safety Testing

The *Tsuga* extracts may be submitted to other standard tests to evaluate safety, cytotoxicity, stability, bioavailability and the like, as necessary.

The ability of the *Tsuga* extract to penetrate the skin may also be assessed, of desired, for example, by *in vitro* release tests (see, for example, the U.S. Center for Drug Evaluation and Research guidance document entitled "*Guidance for Industry. Nonsterile Semisolid Dosage Forms. Scale-up and post-approval changes: in vitro release testing and in vivo*

bioequivalence documentation"). Typically, such testing is conducted using an open chamber diffusion cell, such as a Franz cell, fitted with an appropriate membrane. The test extract is placed on the upper side of the membrane and kept occluded to prevent solvent evaporation and compositional changes. A receptor fluid, such as aqueous buffer or hydro-alcoholic medium, is placed on the other side of the membrane in a receptor cell. Diffusion of the active component across the membrane is monitored by assay of sequentially collected samples of the receptor fluid. For the *Tsuga* extracts in accordance with the invention, the assay could comprise, for example, testing the ability of the collected sample to inhibit MMP-9 activity, as the *Tsuga* extracts have been shown to have this activity. The membrane can be a synthetic membrane, for example polysulphone, cellulose acetate or nitrate, or polytetrafluoroethylene, or it can be a skin sample, such as a sample taken from a cadaver.

Other tests are known in the art (for example, see U.S. Pharmacopoeia XXII (1990)) and are suitable for testing the stability and/or safety of the *Tsuga* extracts.

As will be readily apparent to one skilled in the art, a selected extract may need to meet certain criteria in order to meet regulatory requirements for human use. Conducting tests such as those described above, therefore, allows the suitability of an extract for human use to be assessed.

DERMATOLOGICAL FORMULATIONS

The present invention further provides for formulations comprising a *Tsuga* extract suitable for dermatological, including cosmetic, applications. The formulations can optionally comprise other active agents, such as therapeutic or cosmetic agents and/or other plant extracts. The formulations are prepared by standard techniques such that they have acceptable toxicity and stability.

The formulations are prepared by mixing a suitable amount of the *Tsuga* extract with a physiologically acceptable carrier or diluent. In one embodiment of the invention, the formulation comprises only the *Tsuga* extract and a diluent or carrier. In another embodiment of the invention, the formulation comprises the *Tsuga* extract and one or more thickener, excipient, binder or the like, and optionally one or more other active agent.

Suitable amounts of the *Tsuga* extract for incorporation into the dermatological formulation are generally in the range of between about 0.01% (v/v) and about 20% (v/v). In certain embodiments, the dermatological formulation comprises between about 0.01% (v/v) and about 18% (v/v) of the *Tsuga* extract, between about 0.01% (v/v) and about 16% (v/v), between about 0.01% (v/v) and about 15% (v/v), between about 0.01% (v/v) and about 14% (v/v), between about 0.01% (v/v) and about 12% (v/v), between about 0.01% (v/v) and about 10% (v/v), between about 0.01% (v/v) and about 8% (v/v), and between about 0.01% (v/v) and about 6% (v/v) of the *Tsuga* extract.

In other embodiments, the dermatological formulation comprises between about 0.1% (v/v) and about 20% (v/v), between about 0.1% (v/v) and about 10% (v/v), between about 0.2% (v/v) and about 20% (v/v), between about 0.4% (v/v) and about 20% (v/v), between about 0.5% (v/v) and about 20% (v/v), between about 0.5% (v/v) and about 18% (v/v), between about 0.6% (v/v) and about 15% (v/v), between about 0.8% (v/v) and about 12% (v/v), and between about 1.0% (v/v) and about 10% (v/v) of the *Tsuga* extract.

The amount of *Tsuga* extract for incorporation into the dermatological formulation can also be defined as % by weight. For example, suitable amounts of the *Tsuga* extract for incorporation into the dermatological formulation are generally in the range of between about 0.01% and about 20% by weight, for example, between about 0.01% and about 15% by weight, or between about 0.01% and about 10% by weight. In various embodiments of the invention, the dermatological formulation comprises between about 0.1% and about 18% by weight, between about 0.1% and about 16% by weight, between about 0.1% and about 15% by weight, between about 0.1% and about 14% by weight, between about 0.1% and about 12% by weight, between about 0.1% and about 10% by weight, between about 0.1% and about 8% by weight, between about 0.1% and about 6% by weight, between about 0.2% and about 20% by weight, between about 0.4% and about 20% by weight, between about 0.5% and about 20% by weight, between about 0.5% and about 18% by weight, between about 0.6% and about 15% by weight, between about 0.8% and about 12% by weight, and between about 1.0% and about 10% by weight of the *Tsuga* extract.

In one embodiment of the present invention, the dermatological formulations comprising the *Tsuga* extract are formulated for topical administration. Such formulations may be presented as, for example, aerosol sprays, powders, sticks, granules, creams, liquid creams, mousses, pastes, gels, lotions, ointments, on sponges or cotton applicators, or as a solution or a

suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion.

Various physiologically acceptable carriers are known in the art. Examples of such carriers include, but are not limited to, hydroxypropyl cellulose, starch (corn, potato, rice, wheat, quinoa), pregelatinized starch, gelatine, sucrose, acacia, alginic acid, sodium alginate, guar gum, ethyl cellulose, carboxymethylcellulose sodium, carboxymethylcellulose calcium, polyvinylpyrrolidone, methylcellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, polyethylene glycol, powdered cellulose, glucose, croscarmellose sodium, crospovidone, polacrilin potassium, sodium starch glycolate, tragacanth, calcium carbonate, dibasic calcium phosphate, tribasic calcium phosphate, kaolin, mannitol, talc, cellulose acetate phthalate, polyethylene phthalate, shellac, titanium dioxide, carnauba wax, microcrystalline wax, calcium stearate, magnesium stearate, castor oil, mineral oil, light mineral oil, glycerine, sorbitol, mannitol, stearic acid, sodium lauryl sulfate, hydrogenated vegetable oil (for example. peanut, cottonseed, sunflower, sesame, olive, corn, soybean), zinc stearate, ethyl oleate, ethyl laurate, agar, calcium silicate, magnesium silicate, silicon dioxide, colloidal silicon dioxide, calcium chloride, calcium sulfate, silica gel, castor oil, diethyl phthalate, glyercine, mono- and di-acetylated monoglycerides, propylene glycol, triacetin, alamic acid, aluminum monostearate, bentonite, bentonite magma, carbomer 934, carboxymethylcellulose sodium 12, carrageenan, hydroxyethyl cellulose, magnesium aluminum silicate, pectin, polyvinyl alcohol, povidine, sodium alginate, tragacanth, xanthan gum, silicones and various combinations thereof.

Exemplary thickeners are cross-linked polyacrylate materials available under the trademark Carbopol™ (B. F. Goodrich Company), xanthan gum, carrageenan, gelatine, karaya, pectin and locust bean gum. Under certain circumstances the thickening function may be accomplished by a moisturiser component of the formulation. For instance, silicone gums and esters such as glycerol stearate have dual functionality. A thickener will usually be present in amounts from 0.1 to 20% by weight of the formulation.

The formulations can optionally include one or more moisturising agents, *i.e.* an agent that facilitates hydration of the skin by inhibiting or preventing loss of water from the skin, that absorbs water from the atmosphere and hydrates the skin, and/or that enhances the skin's ability to absorb water directly from the atmosphere. Moisturising agents generally minimise

or prevent the skin from drying and cracking. Moisturisers, when used, are typically present in an amount from about 0.01 to 20% by weight of the formulation.

Examples of moisturising agents include, but are not limited to, 2-hydroxyacetic acid (glycolic acid); 2-hydroxypropanoic acid (lactic acid); 2-methyl 2-hydroxypropanoic acid; 2-hydroxybutanoic acid; phenyl 2-hydroxyacetic acid; phenyl 2-methyl 2-hydroxyacetic acid; 3-phenyl 2-hydroxyacetic acid; 2,3-dihydroxypropanoic acid; 2,3,4-trihydroxybutanoic acid; 2,3,4,5,6-pentahydroxyhexanoic acid; 2-hydroxydodecanoic acid; 2,3,4,5-tetrahydroxypentanoic acid; 2,3,4,5,6,7-hexahydroxyheptanoic acid; diphenyl 2-hydroxyacetic acid; 4-hydroxymandelic acid; 4-chloromandelic acid; 3-hydroxybutanoic acid; 4-hydroxybutanoic acid; 2-bydroxyhexanoic acid; 5-hydroxydodecanoic acid; 12-hydroxydodecanoic acid; 10-hydroxydecanoic acid; 16-hydroxyhexadecanoic acid; 2-hydroxy-3-methylbutanoic acid; 2-hydroxy-4-methylpentanoic acid; 3-hydroxy-4-methoxymandelic acid; 4-hydroxy-3-methoxymandelic acid; 2-hydroxy-2-methylbutanoic acid; 3-(2-hydroxyphenyl) lactic acid; 3-(4-hydroxyphenyl) lactic acid; hexahydromandelic acid; 3-hydroxy-3-methylpentanoic acid; 4-hydroxydecanoic acid; 5-hydroxydecanoic acid; aleuritic acid; 2-hydroxypropanedioic acid; 2-hydroxybutanedioic acid; tannic acid; salicylic acid; erythraric acid; threaric acid; arabiraric acid; ribaric acid; xylaric acid; lyxaric acid; glucaric acid; galactaric acid; mannaric acid; gularic acid; allaric acid; altraric acid; idaric acid; talaric acid; 2-hydroxy-2-methylbutanedioic acid; citric acid, isocitric acid, agaricic acid, quinic acid, glucoronic acid, glucoronolactone, galactoronic acid, galactoronolactone, uronic acids, uronolactones, ascorbic acid, dihydroascorbic acid, dihydroxytartaric acid, tropic acid, ribonolactone, gluconolactone, galactonolactone, gulonolactone, mannonolactone, citramalic acid; pyruvic acid, hydroxypyruvic acid, hydroxypyruvic acid phosphate and esters thereof; methyl pyruvate, ethyl pyruvate, propyl pyruvate, isopropyl pyruvate; phenyl pyruvic acid and esters thereof; methyl phenyl pyruvate, ethyl phenyl pyruvate, propyl phenyl pyruvate; formyl formic acid and esters thereof; methyl formyl formate, ethyl formyl formate, propyl formyl formate; benzoyl formic acid and esters thereof; methyl benzoyl formate; ethyl benzoyl formate; propyl benzoyl formate; 4-hydroxybenzoyl formic acid and esters thereof; 4-hydroxyphenyl pyruvic acid and esters thereof; and 2-hydroxyphenyl pyruvic acid and esters thereof. It should be understood that one or more derivatives of the above compounds, including esters or lactones or pharmaceutically acceptable salts thereof, may also be used.

Further examples of moisturising agents include, but are not limited to, beeswax, shea butter, ceramide, borage oil (linoleic acid), tocopherol linoleate, dimethicone, glycerine, hyaluronic acid, sodium peroxylinecarbolic acid (sodium PCA), wheat protein (such as laurdimonium hydroxypropyl hydrolyzed wheat protein), primrose oil, flax seed oil and mixtures thereof.

The formulations may also comprise one or more preservatives. Exemplary preservatives include, but are not limited to, benzoic acid and its derivatives with benzyl alcohol, benzalkonium chloride, sodium benzoate, bronopol, chlorhexidine, chlorocresol and its derivatives, ethyl alcohol, phenethyl alcohol, phenoxyethanol, potassium sorbate, diazolidinylurea, parabens such as propylparaben or methylparaben.

The formulations may additionally comprise one or more additives normally used in the cosmetics or pharmaceutical field, such as neutralizing agents, sunscreens, antioxidants, fillers, electrolytes, colorants, normal inorganic or organic bases or acids, fragrances, essential oils, cosmetic active principles, moisturizing agents, vitamins, essential fatty acids, sphingolipids, self-tanning compounds, such as DHA (with or without erythrulose), whitening agents such as kojic acid and arbutin, soothing and skin-protecting agents, propenetrating agents or a mixture of these. Amounts of these materials can range from 0.001% up to 20% by weight of the formulation.

As noted above, the dermatological compositions may optionally include one or more other active agents and/or plant extracts that are intended to impart additional beneficial properties to the formulation, for example components that have auxiliary action in the treatment of acne or that provide skin benefits. In one embodiment, the dermatological formulation includes one or more additional anti-acne components, such as anti-seborrheics and anti-infectives. Exemplary anti-acne components include, but are not limited to, antibiotics (such as erythromycin, clindamycin and tetracyclines), antimicrobials (such as chlorexidine and benzoylperoxide), synthetic or natural substances which have been described as possessing antimicrobial activity (such as 1-pentadecanol and derivatives thereof, cedrene, caryophyllene, longifolene and thujopsene), comedolytic agents (such as tretinoin, isotretinoin, adapalene, azelaic acid, tazarotene, salicylic acid and derivatives thereof), anti-inflammatory agents (such as NSAIDs (for example, acetylsalicylic acid, ibuprofen, naproxen and sulfacetamide) and steroidal anti-inflammatory agents (for example, hydrocortisone)), vitamins (such as retinoic acid and derivatives thereof), and oil or sebum control agents (such as clay silicones). The anti-acne components, when used, can each be incorporated into the

formulations of the present invention in an amount between about 0.001% and about 10 % by weight, for example, between about 0.001% and about 8 % by weight or between about 0.001% and about 5 % by weight.

As noted above, in certain embodiments of the invention, the *Tsuga* extracts can be used to combat the irritant effects of retinoids and other anti-acne compounds and thus allow formulations to be prepared that comprise the extract in combination with a higher than standard amount of one or more of these compounds. Retinoids are generally included in anti-acne formulations in an amount between about 0.1% and about 0.5% by weight, with the upper limit of this range being considered "high." Accordingly, in the context of the present invention a "higher than standard amount" of a retinoid is considered to be 0.3% by weight or greater. In certain embodiments, a "higher than standard amount" of the retinoid can be considered to be 0.4% by weight or greater; 0.5 % by weight or greater, or 0.6% by weight or greater.

In another embodiment, the dermatological formulation comprises one or more other plant extracts that can provide a benefit to the skin. Examples of suitable plant extracts that can be included in the dermatological formulations include those described in International Patent Application No. PCT/CA02/00285 (Publication No. WO02/069992) and include, for example, extracts from *Abelmoschus esculentus, Abies balsamea, Abies cephalonica, Abies firma, Abies lasiocarpa, Acer campestre, Acer mandshurica, Acer palmaturn "burgundy," Acer tataricum, Acer truncatum, Achillea millefolium, Achillea ptarmica, Achillea tomentosa, Acolypha hispida, Aconitum napellus, Aconitum spp., Acorus calamus, Actaea racemosa, Actinidi colonicta, Actinidia arguta, Actinidia chinensis, Actinidia colomicta, Adansonia digitata, Adianthum radiatum, Adianthum trapezieformis, Adiantum pedatum, Adiantum tenerum, Aechmea luddemoniana, Aesculus hypocastanum, Aesculus waertilensis, Aesculus woerlitzenis, Aessopteria crasifolia, Aframomum melegueta, Agaricus bisporus, Agastache foeniculum, Agastache mexuicana, Agatis robusta, Ageratum conizoides, Aglaonema commutatus, Agrimonia eupatora, Agropyron cristatum, Agropyron repens, Agrostis alba, Agrostis stolonifera, Ailantus altissima, Ajuga reptans, Alcea rosea, Alchemilla mollis, Alchemilla sp., Alium cernum, Alkanna tinctoria, Allium ampeloprasum, Allium cepa, Allium fistulosum, Allium grande, Allium nutans, Allium porrum, Allium sativum, Allium schoenoprasum, Allium sp., Allium tuberosum, Allium victorialis, Aloe vera, Alpinia officinarum, Althaea officinalis, Alum japonica, Amaranthus caudatus, Amaranthus*

retroflexus, Amaranthus tricolor, Ambrosia artemisiifolia, Amelanchier alnifolia, Amelanchier canadensis, Amelanchier sanguinea, Amelanchier sanguinea x A. laevis, Amelanchier spicata, Amigdalus nana, Amsonia tabernaemontana, Ananas comosus, Anaphalis margaritacea, Anemona japonica, Anethum graveolens, Angelica archangelica, Angelica dahurica, Angelica sinensis, Antericum ramosum, Anthemis tinctoria, Anthoxanthum odoratum, Anthriscus cerefolium, Anthurium altersianum, Anthurium andreanum, Anthurium elegans, Anthurium guildingii, Anthurium hookeri, Anthurium magnificurn, Anthyrium filis-femina, Anthyrium nopponicum, Apium graveolens, Apocynum cannabinum, Arachis hypogaea, Aralia cordata, Aralia nudicaulis, Aralis mandshurica, Archirantus bidentata, Arctium lappa, Arctium minus, Arctostaphylos uva-ursi, Armoracea rusticana, Armoraica ristica, Aronia melanocarpa, Aronia x prunifolia, Arrhenatherum elatius, Artemisia abrotanum, Artemisia absinthium, Artemisia dracunculus, Artemisia ludoviciana, Artemisia vulgaris, Asarum europaeum, Asclepias incarnata, Asclepias tuberosa, Asimina triloba, Asorum canadensis, Asparagus officinalis, Asplenium australasicum, Aster spp, Aster-Nova anglicae, Astilbe chinensis, Astilbe x arendsii, Astilboides tabularis, Astragulus sinicus, Athyrium asperum, Atriplex hortensis, Atropa belladonna, Austolachia australis, Avena sativa, Averrhoa carambola, Bactisia australis, Baptisia tinctoria, Barbaric sp., Beckmannia eruciformis, Begonia convolvulacea, Begonia eminii, Begonia glabra, Begonia mannii, Begonia polygonoides, Bellis perennis, Berberis thungergi, Berberis vulgaris, Bergenia crassifolia, Bergenia x schmidtii, Beta vulgaris, Betula alba, Betula alleghaniensis, Betula daurica, Betula glandulosa, Betula nigra, Betula pendula, Bocconia cordata, Boechimeria boloba, Boesenbergia rotunda, Boletus edulis, Borago officinalis, Boxus sempervirens, Brassica cepticepa, Brassica chinensis, Brassica juncea, Brassica napa, Brassica napus, Brassica nigra, Brassica oleracea, Brassica rapa, Bromelia balansae, Bromus inermis, Brugmansi graveolens (ralf), Brugmansia suaveolens, Brugmansia suaveolens, Buddleja davidii, Bupleurum falcatum, Butomus umbellatus, Buxus microphilla "japonica", Buxus microphylla,Cachris alpina, Cactus officinalis, Caladium spp., Calamagrostis arundiflora, Calamintha nepeta, Calathea zebrina, Calendula officinalis, Calicatus floridus, Camellia sinensis, Campanula carpatica, Campanula rapunculus, Canna indica, Cantharellus cibarius, Capparis spinosa inemis, Capsella bursa-pastoris, Capsicum annuum, Capsicum frutescens, Carex morrowii, Carica papaya, Carlina acaulis, Carpinus caroliniana, Carthamus tinctorius, Carum capsicum, Carum carvi, Carya cordiformis, Caryota ureus, Casia hebecarpa, Castanea sativa, Castanea spp., Celosia cristata, Celtis occidentalis, Centaurea dealbata, Centaurea solstitialis, Centauria maculata, Cerastium

tomentosum, Cerasus japonica, Cerasus maghabab, Ceratoramia mexicana, Chaenomeles x superba, Chaernomelis superba, Chaerophyllum bulbosum, Chamaemelum nobile, Charnaechrista fasciculata, Charnaeciparis pisifera, Chelidonium majus, Chenopodium album, Chenopodium bonus-henricus, Chenopodium quinoa, Chrysanthemum coronarium, Cicer arietinum, Cichorium endivia subsp. endivia, Cichorium intybus, Cinnamomum verum, Cirsium arvense, Cissus discolor, Cistus incanus, Citinis coggriaria, Citrullus colocynthis, Citrullus lanatus, Citrus limettoides, Citrus limon, Citrus reticulata, Citrus sinensis, Citrus x paradisi, Clematis alpina, Clematis armandii, Clematis chiisanensis, Clematis rectae, Clerodendrurn speciossicum, Cobiaeum varilarturn, Coccoloba caracasana, Cocculus laurifolius, Cocos nucifera, Coix lacryma-jobi, Colocasia spp., Comus mass, Convalaria majalis, Conyza canadensis, Corchorus olitorius, Coreopsis verticillata, Coriandrum sativum, Cornus alba, Cornus canadensis, Cornus mas, Cornus sericea, Coronolla varia, Coryllus avelana, Corylus maxima, Cosmos sulphureus, Cotinus coggygria, Cotoneaster fangianus, Cotoneaster horisontalis, Cotynus cogygria, Crambe cordifolia, Cramble cardifolia, Crataegus praegophyrum, Crataegus sanguinea, Crataegus spp., Crataegus submollis, Crategus macrophyllum, Crithmum maritimum, Cryptotaenia canadensis, Crytomium fortunei, Cucumis anguria, Cucumis melo, Cucumis metuliferus, Cucumis sativus, Cucurbita maxima, Cucurbita moschata, Cucurbita pepo, Cullen corylifolium, Cuminum cyminum, Cupress lusitanica, Cupressus sempervirens, Curcuma longa, Curcuma zedoaria, Cycas cirinalis, Cydonia oblonga, Cymbopogon citratus, Cymbopogon martinii, Cynara cardunculus subsp. cardunculus, Cynnamonum zeylonicum, Cyperus alternifolius, Cyperus esculentus, Dactylis glomerata, Dahlia spp., Darura stramonium, Datisca cannabina, Datura metel, Datura stramonium, Daucus carota, Deutria scabra, Dieffenbachia leopoldii, Dieffenbachia segiunae, Digitalis lutea, Digitalis purpurea, Dimocarpus longan, Diopiros kaka, Dioscorea batatas, Diospyros kaki, Dipsacus sativus, Dirca palustris, Dolichos lablab, Dracaena fragrans, Dracaena sp., Dryopteris filis-max, Dryopteris filix-mas, Echinacea purpurea, Echinochloa frumentacea, Echinops sphae, Eleagnus angustifolia, Eleagnus cemutata, Eleusine coracana, Encephalaris horridum, Epilobium augustifolium, Equisetum hyemale, Equisetum variegatum, Erigeron speciosus, Eriobotria japonica, Eriobotrya japonica, Eruca vesicaria, Erungium campestre, Erysimum perofskianum, Erythrinia caffra, Erythrinia crista, Erythrinia glabeliferus, Eschscholzia californica, Eucaliptus rudis, Eucomia ulurifolia, Euonimus elata, Euonomus europea, Euonomus verrucosa, Euphorbia amygdaloides, Fagopyrum esculentum, Fagopyrum suffruticosum, Fagopyrum tataricum, Fagus silvatica, Fautenousus qualiqualia, Festuca rubra, Feucrium hamedris, Ficus

benjaminii, Ficus elastica, Ficus purnila, Ficus religiosa, Ficus sp., Ficus triangularis, Filipendula rubra, Filipendula ulmaria, Filipendula vulgaris, Foeniculum vulgare, Foenix zeulonica, Forsithsia suspensa, Forsitsia europea, Forsythia x intermedia, Fortunella spp., Fragaria x ananassa, Frangula alnus, Fraxinus exelsior, Fuchsia magellanica, Fuchsia spp., Fucus vesiculosus, Fumaria officinalis, Galinsoga quadriradiata, Galium aparine, Galium odoratum, Gallium sporium, Gardenia jasminoides, Gaultheria hispidula, Gaultheria procumbens, Genista multibracteata, Gentiana cruciata, Gentiana littorala, Gentiana lutea, Gentiana macrophylla, Gentiana tibetica, Geranium maculata, Geranium phaeum, Geranium pratense, Geranium sanguineum, Geranium x cantabrigiense, Geum fanieri, Geum macrophyllum, Geum rivale, Gingko biloba, Glaux maritima, Glechoma hederacea, Glyceria maxima, Glycine max, Glycyrrhiza glabra, Gnetum guemon, Gossypium herbaceum, Gratiola officinalis, Gravilea robusta, Guizotia abyssinica, Haemanthus katharina, Hamamelis mollis, Hamamelis virginiana, Haser trilobum, Hedeoma pulegioides, Hedychium coronarium, Hedychium spp., Helenium spp., Helianthus annus, Helianthus strumosus, Helianthus tuberosus, Helichrysum angustifolium, Helichrysum thianschanicum, Heliotropium arborescens, Helleborus niger, Heraclelum pubescens, Herba schizonepetae, Hernerocalis spp., Hibiscus cannabinus, Hissopus zeraucharicus, Hiuga reptans, Hordeum hexastichon, Hordeum vulgare, Hordeum vulgare subsp. vulgare, Hosta fortuna, Hosta fortunaea, Hosta lancefolia, Hosta sieboldiana, Hosta zibalda, Houttuynia cordata, Humulus lupulus, Hydrangea quercifolia, Hydrastis canadensis, Hydrocotile asiatica, Hylotelephium spp., Hymenoxys hoopesii, Hyoscyamus niger, Hypericum henryi, Hypericum perforatum, Hypericum spp., Hypomyces lactifluorum, Hyppoach rhamnoides, Hyssopus officinalis, Iberis amara, Iberis sempervirens, Ilex agnifolium, Ilex cornuta, Inula helenium, Ipomea tricolor, Ipomoea aquatica, Ipomoea batatas, Iris alida, Iris pseudocarpus, Iris versicolor, Isatis tinctoria, Jacobinia sp., Jasminum frutocarus, Jeffersonia diphylla, Juca sp., Juglands regia, Juglans nigra, Juniperus "blue pacific", Juniperus communis, Keyleiteria paniculata, Kochia scoparia, Koeleria glauca, Kolkwitzia amabilis, Korria japonica, Krameria lappacea, Lactuca sativa, Lactuca serriola, Lal lab purpurea, Lamiastrum galeobdolon, Lapia dulcis, Laportea canadensis, Larix dedidua, Laserpitium latifolium, Lathyrus sativus, Lathyrus sylvestris, Laurus nobilis, Lavandula angustifolia, Lavandula latifolia, Lavandula officinalis, Ledum groenlandicum, Lens culinaris subsp. culinaris, Lentinus edodes, Leontopodium alpinum, Leonurus cardiaca, Lepidium sativum, Leucanthemum vulgare, Levisticum officinale, Liatris spinata, Liclum barbatum, Ligularia dentata, Ligustrum vulgare, Linaria vulgaris, Lindera benzoin, Linium hirsutum, Linum usitatissimum, Lippa dulcis, Litchi

chinensis, Livistona fragrans, Lobelia siphitica, Lolium multiflorum, Lolium perenne, Lonicera ramosissima, Lonicera syringantha, Lotus corniculatus, Lotus tetragonolobus, Luglands nigra, Lunaria annua, Lupinus luteaus, Lupinus polyphyllus, Luzula sylvatica, Lychnis chalcedonica, Lycodium japonicum, Lycopersicon esculentum, Lycopersicon pimpinellifolium, Lysimachia clethroides, Lythrum salicaria, Madia sativa, Magnolia agrifolia, Magnolia cobus, Magnolia loebheril, Magnolia stellata, Magnolia x loebneri, Malus hupehensis, Malus prunifolia, Malus spp., Malva moschata, Malva sylvestris, Malva verticillata, Mangifera indica, Manihot esculenta, Marrubium vulgare, Matricaria recutita, Matricaria spp., Matteuccia pensylvanica, Matteucia strutioptoris, Medicago sativa, Melaleuca alternifolia, Melilotus albus, Melilotus officinalis, Melissa officinalis, Mentha arvensis, Mentha pulegium, Mentha spicata, Mentha suaveolens, Mentha x piperita, Menyanthes trifoliata, Mespilus germanica, Metasequoia glyptotrobioldes, Metrosideros excelsa, Microbiata decussata, Microlepia platphylla, Microlepia platyphylla, Microsorium punctatum, Minispermum dauricum, Mirica certifera, Miscanthus sacchariflorus, Miscanthus sinensis, Momordica charantia, Monarda didyma, Monarda fistulosa, Monarda spp., Monstera deliciosa, Monstera pertusa, Montia perfoliata, Morus alba, Murraya exotica, Musa textilis, Musa x paradisiaca, Myrica pensylvanica, Myrthus communis, Nasturtium officinale, Nepeta cataria, Nicodemia diversifolia, Nicotiana rustica, Nicotiana tabacum, Nigella sativa, Ocimum Basilicum, Ocimum tenuiflorum, Oenothera biennis, Oenothera fruticosa subsp fruticosa, Olea europaea, Olea olcaster, Onobrychis viciifolia, Onoclea sensibilis, Ophiopogon japonicus, Opuntia spp., Oreopanax capitata, Origanum majorana, Origanum vulgare, Oryza sativa, Osmanthus spp., Osmunda regalis, Osmundastrum claytonionum, Ostrea carpinifolia, Ostrea connote, Oxalis deppei, Oxobachus nictogenea, Oxyria digyna, Pachyra affinis, Paeonia daurica, Paeonia lactiflora, Paeonia rubra, Paeonia spp., Paeonia suffructicisa, Panax quinquefolius, Panicum miliaceum, Parrotia persica, Parthenosicus tricuspidata, Passiflora caerulea, Passiflora spp., Pastinaca sativa, Pegamun hamalis, Pelargonium zonale, Pennisetum alopecuroides, Penstemon digitalis, Pentaphylloides fruticosa, Perilla frutescens, Persea americana, Petasites japonicus, Petroselinum crispum, Peucedanum cervaria, Peucedanum oreaselinum, Pfaffia paniculata, Phacelia tanacetifolia, Phalaris arundinacea, Phalaris canariensis, Phaseolus acutifolius, Phaseolus coccineus, Phaseolus vulgaris, Phebodium aureum, Philadelphus coronarius, Philodendron amurense, Phleum pratense, Phlox paniculata, Phoenix dactylifera, Phylidendron speciosus, Phyllanthus grandifolium, Phyllitis scolopendrium, Phymatosorus scolopendria, Physalis alkekengi, Physalis creticola, Physalis grisea, Physalis philadelphica,

Physalis spp., Physostegia virginiana, Phytolacca americana, Picea schrenkiana, Pieras japonica, Pigelia pennata, Pimpinella anisum, Pinus bungiana, Pinus cembra, Pinus mugo, Pinus pinea, Pinus pumila, Pinus salinifolia, Pinus silvestris, Pinus sirtrobus, Pinus strobus, Piper chaba, Piper nigrum, Pisum sativum, Pithecelobium unguis, Pittisporum tibica, Plantago coronopus, Plantago major, Plantago minor, Platanus acidentalis, Platicada grandiflora, Plectranthus fruticosus, Plectranthus spp., Pleurotus spp., Plumbago zeylanica, Poa compressa, Poa pratensis, Podocarpus spinulosus, Podophyllum amodii, Podophyllum peltatum, Poligonum aviculare, Poligornun latifolia, Polygonium odoratum, Polygonum aviculare, Polygonum chinense, Polygonum cuspidatum, Polygonum pensylvanicum, Polygonum persicaria, Polymonium ceruleum, Polyschium braunii, Pongamia pinnata, Pontederia cordata, Populus incrassata, Populus tremula, Populus x petrowskyana, Portulaca oleacea, Potentilla alba, Potentilla anserina, Potentilla fruticosa, Poterium sangiusorba, Primula veris, Princepia sp., Prunella vulgaris, Prunus armeniaca, Prunus cerasifera, Prunus cerasus, Prunus persica, Prunus serotica, Prunus spp., Prunus tomentosa, Prunus xocane, Psathyrostachys juncea, Pseudotsuga menzisia, Psidium guajava, Psidium spp., Psychotria metbacteriodomasica, Psychotria nigropunctata, Pteridium aquilinum, Pterigota alata, Puansetia sp., Pulmonaria molissima, Pulmonaria officinalis, Pulmonaria saccharata, Punica granatum, Pyrus communis, Pyrus pyrifolia, Quercus castanufolia, Quercus imbricaria, Quercus nigra, Quercus robur "fastigiata," Quercus rubra, Quercus trojana, Raphanus raphanistrum, Raphanus sativus, Ratibiunda columnus-Fera, Rauwolfia tetraphylla, Rehmannia glutinosa, Reseda luteola, Reseda odorata, Rheum officinale, Rheum palmatum, Rheum x hybridum, Rhododendron spp., Rhus aromatica, Rhus toxicodenta, Rhus trilobata, Ribes americanum, Ribes grossularia, Ribes nigrum, Ribes sylvestre, Ribes uva-crispa, Ribes x nidigrolaria, Ricinus communis, Rimula japonica, Rodgersia podophylla, Rodgersia spp., Rosa cocanica, Rosa multiflora, Rosa rugosa, Rosmarinus officinalis, Rubus allegheniensis, Rubus arcticus, Rubus canadensis, Rubus idaeus, Rubus occidentalis, Rubus phoenicolasius, Rubus pubescens, Rubus thibetanus, Rudbeckia maxima, Rumex acetosa, Rumex acetosella, Rumex crispus, Rumex patientia, Rumex scutatus, Ruschia indurata, Ruta graveolens, Saccharum officinarum, Salis babilonics, Salix purpurea, Salix tamarisifolia, Salvia elegans, Salvia officinalis, Salvia sclarea, Salvia sylvestris, Sambucus canadensis, Sambucus ebulus, Sambucus nigra, Sanchezia nobilis, Sanguisorba minor, Sanguisorba officinalis, Santolina chamaecyparissus, Saponaria officinalis, Satureja hortensis, Satureja montana, Satureja repandra, Schisandra chinensis, Scolymus hispanicus, Scorzonera hispanica, Scotch pine, Scrophularia nodosa, Scutellaria certicola, Scutellaria lateriflora,

Scutellarian altissima, Secale cereale, Sechium edule, Sedum album, Sedum telchium, Sempervivum tectorum, Senecio platifilla, Senecio vulgaris, Senseviera sp., Serenoa repens, Seringa josiceae, Serratula tinctoria, Seruginea suffruticisa, Sesamum indicum, Sesbania exaltata, Sesbania speciosa, Setaria italica, Sibirea altaiensis, Sidalcea spp., Silene vulgaris, Silybum marianum, Sinapis alba subsp. alba, Siringa vulgaris, Sium sisarum, Sluffera sp., Solanum dulcamara, Solanum melongena, Solanum scabrum, Solanum tuberosum, Soleirolia soleirolii, Solidago caesia, Solidago canadensis, Solidago spp., Solidago virgaurea, Solidago x hybrida, Sonchus oleraceus, Sorbocotoneaster sp., Sorbus aucuparia, Sorbus cominicta, Sorghum bicolor, Sorghum x drummondii, Spartina potentiflora, Spathiphyllum cochlearispaturn, Spathiphyllum grandiflorum, Spinacia oleracea, Stachis lanata, Stachys affinis, Stachys byzantina, Stachys macrantha, Staphylea trifolia, Stellaria graminea, Stellaria media, Stephanandra incisa, Stepochlaena tenuifolia, Sterulia elata, Stevartia coreana, Stewartia pseudocamellia, Stipa capillata, Strelitzia reginae, Sulda sanganea, Sundapsis spp., Symphitium officinalis, Symphoricarpos albus, Symphoricarpos orbiculatus, Symphytum officinale, Syngoniurn aurutum, Syngoniurn podophyllum, Taccus bacata, Tagetes minuta, Talictrum minus, Talictrum sp., Tamarindus india, Tamarindus indica, Tanacetum balsamita, Tanacetum balsamita subsp. balsamita, Tanacetum cinerariifolium, Tanacetum parthenium, Tanacetum vulgare, Tapeinochilos spectabilis, Taraxacum officinale, Taraxacum officinalis, Taxodium dixticum, Taxus cuspidata, Taxus hiksii, Taxus media, Taxus x media, Tetraclinis articulata hinensis, Tetradenia riparia, Teucrium chamaedrys, Thalictrum aquilegiifolium, Thalictum flavum, Thlaspi arvense, Thuja occidentalis, Thymus camosus, Thymus cretaceus, Thymus cytridorus "aureus, Thymus fragantissimus, Thymus herba-barona,Thymus lemabarona, Thymus portugalense, Thymus praecox, Thymus praecox subsp. arcticus, Thymus pseudolamginosus, Thymus pseudolanuginosus, Thymus puleglodes "lemons", Thymus puliglodes, Thymus serphylum, Thymus speciosa, Thymus thrasicus, Thymus vulgaris, Thymus vulgaris "argenteus," Thymus vulgaris "oregano," Thymus wooly, Thymus x citriodorus, Tiarella cordifolia, Tiarella spp., Tragopogon porrifolius, Tragopogon spp., Trambe pontica, Trevesia sungaica, Trichosanthes kirilowii, Trifolium hybridum, Trifolium incarnatum, Trifolium pannonicum, Trifolium pratense, Trifolium repens, Trigonella foenum-graecum, Triticum aestivum, Triticum aestivum subsp. spelta, Triticum turgidum, Trollius x cultorum, Tropaeolum majus, Tsuga canadensis "penola", Tsuga diversifolia, Tsuga mertensiana, Thuja orientalis "eligantissima", Tula ocidentalis "columbia," Tulip tree, Turnera ulmifolia, Tussilago farfara, Typha latifolia, Ulmus americana, Ulmus pumila, Urtica dioica, Uschusa sp., Uvularia perfoliata, Vaccinium

angustifolium, Vaccinium corymbosum, Vaccinium macrocarpon, Valeriana officinalis, Valerianella locusta, Veratrum nigrum, Veratrum viride, Verbascum thapsus, Verbena officinalis, Verium oleander, Vernonia gigantea, Veronica austriaca ssp teucrium, Veronica beccabunga, Veronica officinalis, Viburnum opulus, Viburnum plicatum, Vicia faba, Vicia sativa, Vicia villosa, Vigna angularis, Vigna mungo, Vigna unguiculata, Vinca minor, Vincetocsicum officinalis, Vitis labrissa, Vitis spp., Weigela coraeensis, Weigela hortensis, Withania somnifera, x Triticosecale spp., Xanthium sibiricum, Xanthium strumarium, Xanthosoma sagittifolium, Xeupressocyparis deylandii, Yucca elephantipes, Yucca filamentosa, Zea mays, Zelcova, and *Zingiber officinale.*

In one embodiment of the invention, the dermatological compositions comprise one or more other plant extracts selected from the group of: *Aconitum napellus, Acorus calamus, Alchemilla mollis, Allium cepa, Allium sativum, Allium tuberosum, Ambrosia artemisiifolia, Anethum graveolens, Anthemis tinctoria, Aronia melanocarpa (Michx.) Ell., Arctostaphylos uva-ursi, Aronia x prunifolia, Artemisia dracunculus, Avena sativa, Beta vulgaris, Beta vulgaris L. subsp. Vulgaris, Borago officinalis, Brassica napus, Brassica oleracea, Brassica oleracea L. var. italica Plenck, Brassica rapa, Bromus inermis, Capsicum annuum L. var. annuum, Cerastium tomentosum, Chaerophyllum bulbosum, Chenopodium quinoa, Chenopodium quinoa subsp. Quinoa, Chenopodium quinoa Willd., Chichorium endivia, Chichorium endivia subsp. Endivia, Circium arvense, Citrullus lanatus, Cornus canadensis, Cornus sericea, Cynara cardunculus subsp. Cardunculus, Daucus carota, Daucus carota subsp carota L., Dolichos lablab, Euphorbia amygdaloides, Fagopyrum tataricum, Foeniculum vulgare, Frangula alnus, Galinsoga quadriradiata, Gentiana lutea, Geranium sanguineum, Geranium x cantabrigiense, Glycyrrhiza glabra, Hamamelis virginiana, Helianthus strumosus, Heliotropium arborescens, Hordeum vulgare subsp. Vulgare, Hypomyces lactifluorum, Juniperus communis L., Lentinus edodes, Lotus corniculatus, Manihot esculenta, Matricaria recutita, Melilotus albus, Melilotus alba Medik., Melissa officinalis, Mentha x piperita, Oenothera biennis, Pastinaca sativa L., Petroselinum crispum, Phaseolus vulgaris, Physalis philadelphica, Phytolacca decandra, Phytolacca decandra syn. P. americana, Pimpinella anisum, Pisum sativum, Potentilla anserina L., Potentilla fruticosa, Poterium sanguisorba, Pyrus communis, Raphanus raphanistrum, Rheum x hybridum, Rhus typhina L., Ribes nigrum L., Ribes sylvestre, Rodgersia spp., Rosmarinus officinalis, Rubus occidentalis, Rubus thibetanus, Rumex crispus, Rumex scutatus, Ruta graveolens, Salvia officinalis, Sambucus canadensis L., Setaria italica, Solanum melongena L., Sorghum dochna*

bicolor gr technicum, Stellaria media, Tanacetum cinerariifolium, Taraxacum officinale, Teucrium chamaedrys, Thymus fragantissimus, Thymus x citriodorus, Trifolium incarnatum, Triticosecale spp., Tropaeolum majus L., Tsuga canadensis, Tsuga diversifolia, Vaccinium angustifolium, Vaccinium angustifolium Ait., Vitia sp., x Triticosecale spp., Zea mays L. and *Zingiber officinale.*

In one embodiment of the invention, the dermatological compositions comprise one or more other plant extracts selected from the group of: *Caspicum annuum, Chenopodium quinoa, Geranium cantabrigiens, Glycyrrhiza glabra, Juniperus communis, Lotus corniculatus, Melilotus albus, Melissa officinalis, Pastinaca sativa L, Potentilla anserina, Rhus thyphina, Triticosecale sp., Vaccinium sp, Zea mays* and *Zingiber officinale.*

Other plant derived components or extracts that may be added to the dermatological formulations in certain embodiments of the invention include jojoba oil, tea tree oil or extract, aloe, *Spirea spp* and *Salix spp.*

The dermatological formulations intended for topical application can be packaged in a suitable container to suit the viscosity and intended use. For example, a lotion, fluid cream, foam or mousse can be packaged in a bottle or a roll-ball applicator, a capsule, a propellant-driven aerosol device, a spray or a container fitted with a pump suitable for finger operation. When the composition is a cream or paste, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar.

USES

The present invention provides for the use of the *Tsuga* extracts and formulations comprising same to treat comedones and/or acne present on the skin of a subject. The skin can be facial skin or non-facial skin (for example, arms, legs, hands, chest, back, buttocks and other areas prone to lesions).

In one embodiment, the present invention provides for the use of *Tsuga* extracts to decrease comedones in a subject. In another embodiment, the present invention provides for the use of *Tsuga* extracts to treat acne. In accordance with this embodiment, the extracts may decrease comedones and/or other skin lesions in the subject under treatment. Examples of acne that may be treated in accordance with certain embodiments of the present invention include acne

vulgaris, comedonal acne, papulopustular acne, papulocomedonal acne, nodulocystic acne, acne conglobata, acne keloid of the back of the neck, recurrent acne miliaria, acne necrotica, acne neonatorum, occupational acne, acne rosacea, senile acne, solar acne and acne medicamentosa.

In one embodiment, the present invention provides for the use of the *Tsuga* extracts to treat acne vulgaris.

In certain embodiments, the present invention also provides for the use of the *Tsuga* extracts in the prevention of acne and/or comedones. In accordance with these embodiments, the *Tsuga* extracts may be used in the treatment of skin having a tendency toward acne in order to combat the appearance of skin lesions, such as comedones, papules or pustules.

In another embodiment, the invention provides for the use of the *Tsuga* extracts in formulations comprising anti-acne compounds that can exert irritant or inflammatory effects on the skin of the user, for example, retinoids, in order to ameliorate the irritant/inflammatory effects of these compounds. In another embodiment, the invention provides for the use of the *Tsuga* extracts in dermatological formulations for sensitive skins.

The *Tsuga* extracts also possess healing properties as shown herein, which allow the extracts to additionally facilitate the healing of, and/or reduce the formation of scars that may result from, acneic lesions, such as comedones, papules or pustules.

KITS

The present invention additionally provides for pharmaceutical or cosmetic packs or kits comprising the *Tsuga* extracts. The kit comprises the *Tsuga* extract or a formulation comprising the *Tsuga* extract, as described above, in a suitable container or containers.

The *Tsuga* extract or formulation thereof can be provided in a format or container that facilitates its application to the affected area of a subject. For example, the *Tsuga* extract or formulation can be provided as lotion or fluid cream packaged in a squeezable container, a container equipped with a nozzle or roll-ball applicator, a spray bottle or aerosol, or a container fitted with a pump suitable for finger operation. The kit can optionally further comprise one or more suitable implements to facilitate application of the *Tsuga* extract or formulation.

In certain embodiments, the kit can comprise sufficient amounts of the *Tsuga* extract or formulation for application to the subject for a prescribed length of time, for example, for a length of time between one and twelve months.

The kit can further provide an appropriate usage regimen over a prescribed period of time for the *Tsuga* extract or formulation, for example in the form of a set of instructions, generally written instructions.

There may also be associated with the kit a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical or cosmetic products, which notice reflects approval by the agency of manufacture, use or sale for human or animal administration.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

EXAMPLE I: *Preparation of Extracts from* Tsuga canadensis

Analytical Scale Preparation

In general, five grams of the dried plant material to be extracted was placed in a beaker and a sufficient amount of solvent was added to allow moderate agitation with a stirring bar. The solvents used in this Example were: butylene glycol (100%), butylene glycol/water (50/50, v/v), butylene glycol/water (20/80, v/v); ethanol (100%), ethanol/water (85/15, v/v), ethanol/water (50/50, v/v); water (100%); 1,3-propanediol (100%); 1,3-propanediol/water (50/50, v/v) and 1,3-propanediol/water (20/80, v/v).

Several different extraction times were employed for each solvent: after mixing for periods of 4 to 24 hours at room temperature, the suspension was filtered through a 60mesh filter and then filtered through 40, 20, 11 and 1.2 micron paper filter consecutively. For the filtered butylene glycol mixtures, the solvent was then evaporated at 120°C and the residual matter was weighed to determine the yield of extraction at each time point. For the filtered ethanol

mixtures, the solvent was removed under reduced pressure at a temperature of less than 45°C in order to determine the yield of extraction at each time point.

The above protocol is suitable for the preparation of extracts that are to be employed in dermatological formulations. Glycol extracts such as butylenes glycol or 1,3-propanediol extracts, for example, can be included directly into formulations intended for topical application. Ethanol extracts may undergo one or more additional steps, such as CO_2 extraction, prior to incorporation into formulations intended for topical application.

The following is an example of a specific protocol that was employed to prepare a *Tsuga canadensis* extract.

To 5 g of dried needles and small branches of *Tsuga canadensis* lot 20252, 100ml of a 50:50 butylene glycol:water was added. The mixture was thoroughly stirred and macerated for 4 hours at room temperature. The resulting mix was filtered through 40µ to 1.2µ filters to obtain a suitable extract for further analysis.

The *Tsuga canadensis* extracts 207-20252, 207-20156, 207-20376 and 207-20010 tested in the following examples are all extracts prepared from needles and small branches of *Tsuga canadensis* using 50:50 butylene glycol:water as the extraction solvent following the protocol outlined above. The extracts differed in the individual *Tsuga canadensis* tree that the plant material came from and in the time that the plant material was harvested.

The *Tsuga canadensis* extract 207/2-20376 also tested in the following examples is an extract prepared from needles of *Tsuga canadensis* using 50:50 1.3-propanediol:water as the extraction solvent following the protocol outlined above.

Large Scale Preparation

Analysis of the results from the analytical scale preparation allows for the selection of appropriate plant materials for the large-scale extraction. The selection includes a decision regarding part of the *Tsuga* tree and quantity of dried material needed to obtain a sufficient yield on a large scale. The selection also involves a choice of solvent system for an active extract.

The extraction protocol is essentially the same as the procedure for the analytical preparation except for the filtration process. The dried and pulverized material (2-3 Kg for large scale) is

extracted repeatedly (maceration / percolation) with solvent (3 x 2 - 20 l) at room temperature for 24-72 h, based on the analytical scale yield of extraction results. The resulting extract is then passed through a 60 mesh filter and then may be filtered through 10 to 100kDa ultrafiltration column or on a 0.1 to 1µ ceramic column.

Non-limiting examples of large scale extraction preparations of extracts from *Tsuga canadensis* are provided below.

A. To 3kg of dried needles and small branches from *Tsuga canadensis* lot 20252, 60L of a 50:50 Butylene glycol:water was added. The mixture was thoroughly stirred and macerated for 24 hours. The resulting mix was filtered through a 65mesh filter and then filtered on a 1µ ceramic column to obtain an extract suitable for further analysis.

B. To 3kg of dried needles and small branches from *Tsuga canadensis* lot 20252, 60L of a 50:50 Butylene glycol:water or 1,3-propanediol:water was added. The mixture was thoroughly stirred and macerated for 24 hours, then decanted for another 18-24hours. The resulting mix was filtered through a 65mesh filter and then filtered by ultrafiltration at 100kDa to obtain an extract suitable for further analysis.

EXAMPLE II: ***Anti-inflammatory effect of*** **Tsuga canadensis** ***Extracts***

This example demonstrates the potential of *Tsuga canadensis* extracts to reduce a UV-induced inflammatory response in human skin keratinocytes after irradiation with a Solar Light Simulator. The anti-inflammatory effect was evaluated by the inhibition of pro-inflammatory cytokines found in the media after the irradiation.

Keratinocytes were first grown in 175cm^2 to reach about 80% confluence then trypsinized and plated at 1X10^5 cells/well in a 24-well plate. After 24 to 48 hours (80% confluence), the extracts or controls were added to cells at a non-cytotoxic concentration. After 24 hours, media was removed, cells were washed with 1ml of HBSS and irradiated with 10 J/cm^2 of UVB. A corresponding non-irradiated plate was prepared at the same time and was submitted to all the same steps.

After irradiation, the HBSS was removed and media added with the corresponding samples, (1 ml). Cells were incubated for another 24 hours at 37°C, 5% CO_2. Media from each well was recovered for ELISA testing, and Alamar Blue was added to the cells to evaluate

viability. The protocol of each ELISA was performed according to the instructions provided in each kit.

The results for *Tsuga canadensis* extract 207-20376 are presented in Figure 1 and are expressed as the % inhibition of IL-6 release after UV irradiation.

EXAMPLE III: ***Non- Irritating Behaviour of*** **Tsuga** ***Extracts***

This example provides results showing the non-irritating quality of the *Tsuga* extracts. The amount of interleukin-8 (IL-8) released in the following assay is used to quantify a possible irritating reaction after exposure of keratinocytes to the *Tsuga* extract.

Release of IL-8 was evaluated on human skin keratinocytes (Cascade Biologics, Portland, OR, catalog number C-005) and measured using the Quantikine hIL8 ELISA kit (R&D Systems, Minneapolis, MN, catalog number D8000C). Keratinocytes were first grown in a 96-well plate using complete medium M154 (M154 + HKGS cat S-001 from Cascade Biologics). This medium was used as a negative (non-irritating) control, while 2.5µM phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich Canada, Oakville, Ontario) was used as a positive (irritating) control. All extracts and controls were diluted in complete medium M154. Cells were seeded into 96-well plates at a concentration of 8 X 10^3cells/well in complete M154 medium and plates were incubated for 48 hours at 37°C in a humidified 5% CO_2 atmosphere. The medium was removed and 200µl of *Tsuga* extract or control were added to the wells (all performed in duplicate) and then the plates were incubated for 48 hours at 37°C in a humidified 5% CO_2 atmosphere. ELISA was performed using following the manufacturer's recommendations (R&D Systems). The absorbance was read at 450 nm on the Spectrafluor Plus plate reader (Tecan).

Controls treated with M154 medium showed the lowest IL-8 release and this amount was taken as the minimum IL-8 release. PMA induced a strong inflammatory response, which was taken as the highest irritating level (highest IL-8). The evaluation of cytokine release stimulated by a *Tsuga* extract in this experiment enables a maximum concentration of the extract to be set for further *in vivo* studies.

Following the above protocol, *Tsuga canadensis* extract 207-20252 stimulated 2.2pg/ml of IL-8 release at a concentration of 200µg/ml, while PMA stimulated 282pg/ml of IL-8 release at a concentration of 5µM. The basal level was evaluated at 18pg/ml.

EXAMPLE IV: ***Wound Healing Effect of*** **Tsuga** ***Extracts*** **in vitro**

This example demonstrates the increase in wound healing by *Tsuga canadensis* extract in the "scratch assay." The increase in wound healing is evaluated by the increased migration rate of keratinocytes and/or reduced area of scratch evaluated each day for four days.

Keratinocytes were first grown in 175cm^2 to reach about 80% confluence, then trypsinized. 70µl of a 5X10^5 cells/ml suspension were plated in each side of a culture insert (Ibidi GmbH, München, Germany) which were placed in a 24-well plate (one insert per well). After 24 hours (confluent layer), the insert was removed from the well and 1 ml of the extract or control was added at a non-cytotoxic concentration. A digital picture of the "scratch" that was generated was then taken and the area of the scratch calculated using ImagePro software. Media was replaced with fresh media containing extract or control each day and a digital picture taken in order to calculate the % of migration of the keratinocytes. This was repeated for 3 more days.

The results for *Tsuga canadensis* extract 207-20376 are presented in Figure 3 and are expressed as the % migration of the keratinocytes for each day of the experiment.

EXAMPLE V: ***Anti-inflammatory Effect of*** **Tsuga** ***Extracts in Humans: A Case Report***

Materials and methods

Four creams were prepared using a 50:50 butylene glycol:water *Tsuga canadensis* extract prepared as described in Example I. The creams were as follows (all % are w/w):

Cream #1: 0.5% retinol

Cream #2: 0.5% retinol and 5% *Tsuga canadensis* extract

Cream #3: 1% retinol

Cream #4: 1% retinol and 5% *Tsuga canadensis* extract

The patient was a healthy female volunteer age 39. She applied all four creams (#1 to #4, above) twice a day in a blinded manner to her forearms. She noted any sign of irritation. The study was stopped when the redness due to irritation was too strong and discomfort appeared.

Results

The results after 21 days of treatment were as follows. An irritating effect of retinol at 1% (Cream #3) was observed starting at day 10 of the experiment. The redness was intense at day 18 to 21, when the patient was told to stop the study. The *Tsuga* extract at 5% (Cream #4) clearly helped to diminish the level of inflammation caused by retinol from an assessment of "3" to an assessment of "1" based on qualitative evaluation of the redness of the skin. The intensity of inflammation was less when the 0.5% retinol cream (Cream #1) was used. However, addition of the *Tsuga* extract at 5% (Cream #2) also helped to diminish the level of inflammation caused by this lower amount of retinol.

EXAMPLE VI: ***Determination of Irritation or Sensitization by*** **Tsuga** ***Extracts on Human Skin I***

This example and Example VII below demonstrate that the *Tsuga* extracts are non-irritating to human skin. The test employed was the HRIPT (Human Repeat Insult Patch Test), which determines if a topical agent has the potential to induce irritation or sensitization of any kind.

52 volunteers were selected for the study. The volunteers were men and women of ages 23 to 57. The agent, *Tsuga canadensis* extract 207-20156 10% (v/v) in petroleum jelly, was applied to the skin of the volunteers repeatedly using 10 patches over a period of 3 weeks. The patches used in the study were TruMed® semi-occlusive, cotton "Nova® net embossed" with "Avery® 4750U tape" adhesive backing. After a rest period (incubation phase) varying from 10 to 14 days, a challenge phase was conducted. The patch was applied for 48 hours and removed. The test sites were cleaned and examined for any signs of intolerance or irritation by a dermatologist.

Results:

Under the conditions of the study, *Tsuga canadensis* extract 207-20156 produced no signs of cutaneous irritation or skin sensitization in either the induction phase or the challenge phase of the test. The extract is therefore considered non-irritant and potentially hypo-allergenic. In

addition, given the control provided by a dermatologist, the test product may bear the claim "Tested under control of a dermatologist."

EXAMPLE VII: ***Determination of Irritation or Sensitization by*** **Tsuga** ***Extracts on Human Skin II***

54 volunteers were selected for the study. The volunteers were men and women of ages 20 to 53. The agent, *Tsuga canadensis* extract 207/2-20376 10% (v/v) in a base cream, was applied to the skin of the volunteers repeatedly using 10 patches over a period of 3 weeks. The patches used in the study were TruMed® semi-occlusive, cotton "BBA149-129 Absorbent" with "3M1530 tape" adhesive backing. After a rest period (incubation phase) varying from 10 to 14 days, a challenge phase was conducted. The patch was applied for 48 hours and removed. The test sites were cleaned and examined for any signs of intolerance or irritation by a dermatologist.

Results:

Under the conditions of the study, *Tsuga canadensis* extract 207/2-20376 10% (v/v) produced no signs of cutaneous irritation or skin sensitization in either the induction phase or the challenge phase of the test. The extract is therefore considered non-irritant and potentially hypo-allergenic. In addition, given the control provided by a dermatologist, the test product may bear the claim "Tested under control of a dermatologist."

EXAMPLE VIII: ***Exemplary Dermatological Formulations Comprising a*** **Tsuga** ***Extract***

The following formulations comprising a *Tsuga canadensis* extract are non-limiting examples of suitable formulations for human efficacy studies (such as those described in Example X). The formulations can be modified for commercial use as is known in the art. All % are w/w.

Cream:

Beeswax	1-2%
Glycerin	2-4%
Xanthan Gum	0.5-1%
PPG-15 stearyl ether	6-10%
Stearic acid	1-2%
Kaleol 8670	1-4%

EDTA	0.25-0.5%
Shea butter	1-3%
Extract 20252	0.1-5%
Preservatives	0.2-1%
Water	q.s. 100%

Gel :

Demineralized water	52.62%
Carbopol Ultrez-21	0.40%
Xantham gum	0.20%
Propylene glycol	5.00%
Cetiol CC	10.00%
PEG-400	5.00%
Jojoba oil	3.00%
Frescolat ML	0.50%
Triethanolamine 99%	0.45%
Tsuga canadensis extract	5.00%
Triethanolamine (sol. 25%)	0.10%
Germall Plus liquid	0.60%
Demineralized water	q.s. 100%

Cream :

Demineralized water	52.62%
Carbopol Ultrez-21	0.35%
Xantham gum	0.20%
Euxyl PE0910	0.9%
Glycerine	3.0%
Benzoic acid	0.2%
Lanette 18	3.0%
Beewax	2.0%
Cetiol CC	2.0%
Drakeol 7	6.0%
Frescolat ML	1.0%
Squalane	2.0%
Olive butter	1.0%

Petrolatum	2.0%
Eumulgin B1	1.8%
Brij 72	0.8%
Coviox T70	0.02%
Myritol 312	10%
Dehydol LS3 Deo-N	0.4%
Triethanolamine 99%	q.s. ad pH 4.50
Demineralized water	5.0%
Biovera Aloes 200X	0.4%
Tsuga canadensis extract	5.00%
Sodium ascorbyl phosphate	0.3%
Demineralized water	q.s. 100%
Sodium hydroxide (sol 20%)	q.s. pH 5.5

Other examples of formulations comprising *Tsuga canadensis* extracts include those in which the extract is added in an amount between 0.1 and 10% (w/w) into a pre-made skin formulation. For example, a cream base, a lotion, an aftershave lotion, a soothing mask, or petrolatum jelly.

EXAMPLE IX: ***Anti-microbial Activity of*** **Tsuga** ***Extracts II***

To determine the antimicrobial effect of *Tsuga* extracts, *Staphyloccocus epidermidis* (ATCC 12228) and *Propionibacterium acnes* (ATCC 6919) were used to challenge different concentrations of *Tsuga canadensis* extract 207-20156 (diluted in water). *S. epidermidis* was inoculated at 1.5 X 10^6 bacteria/ml in 10ml of diluted extract and *P. acnes* was inoculated at 1 X 10^6 bacteria/ml in 10ml of diluted extract. All samples of extract or vehicle (50:50 butylene glycol:water) were incubated at 37°C and evaluated at 0, 7, 14 and 28 days. Experiments involving *P. acnes* were conducted under anaerobic conditions. The efficacy was analyzed by determining the colony forming units (CFU) per g on tryptic soy agar.

The results are summarized in Figure 2 (Figure 2A shows the results against *S. epidermidis* and Figure 2B shows the results against *P. acnes*). The results demonstrate that when the *Tsuga* extract was challenged with 2 different species of bacteria related to acne, it showed a significant inhibition of growth even after the first incubation period. In these experiments, it

can be seen that the vehicle (solvent) also showed some antimicrobial effects due to the toxicity of the solvent at high concentrations.

EXAMPLE X: ***Clinical Trials to Evaluate the Effect of the*** **Tsuga** ***Extracts on Skin Lesions***

The efficacy of a *Tsuga canadensis* extract in decreasing acneic lesions in volunteers was evaluated in a small clinical trial.

Extract

Tsuga canadensis extract 207-20376 was used in this trial and was prepared as described in Example I with respect to extract 207-20156. The extract was formulated into a cream having the following composition:

Demineralized water	52.62%
Carbopol Ultrez-21	0.35%
Xantham gum	0.20%
Euxyl PE0910	0.9%
Glycerine	3.0%
Benzoic acid	0.2%
Lanette 18	3.0%
Beewaxes	2.0%
Cetiol CC	2.0%
Drakeol 7	6.0%
Frescolat ML	1.0%
Squalane	2.0%
Olive butter	1.0%
Petrolatum	2.0%
Eumulgin B1	1.8%
Brij 72	0.8%
Coviox T70	0.02%
Myritol 312	10%
Dehydol LS3 Deo-N	0.4%
Triethanolamine 99%	q.s. ad pH 4.50

Demineralized water	5.0%
Tsuga canadensis extract	5.00%
Sodium ascorbyl phosphate	0.3%
Demineralized water	q.s. 100
Sodium hydroxide (sol 20%)	q.s. pH 5.5

Study Design

The objective of this study was to evaluate the *in vivo* efficacy of a product for skins with acneic tendency in comparison with its placebo. The products tested were coded Product A and Product B.

Product A = cream formulation without any extract (control or placebo).

Product B = cream formulation comprising the *Tsuga canadensis* extract.

The study was carried out as a "double blind test." Neither the participating subjects nor the investigator were aware of the type of product being applied throughout the study. The study was a comparative study in which the results obtained at one treated area by one of the products were compared with those obtained at another treated area with the other product. The subjects served as their own reference and results obtained at various assessment times were compared with those obtained at T0.

Evaluation was performed using a cosmetologist clinical evaluation. The study lasted 42 days following the first application of the product(s).

Test subjects and Inclusion Criteria

12 subjects were selected for the study.

Inclusion criteria: Standard

- Female (50%) or male (50%).
- Healthy.
- Between 18 and 45 years of age.
- Skin at assessed area is healthy (free of psoriasis, eczema, erythema, oedema, scars, wounds).

Inclusion criteria: Specific

- Having an oily skin with acneic tendency
- Having inflammatory lesions (papules and pustules) on the face
- Having a shiny skin
- Displaying dilated pores on the cheeks

- *Non-inclusion criteria: Standard*
- Failing to meet the aforementioned inclusion criteria.
- Being in remanence, at the beginning of the study, on the studied area(s), following another cosmetic, dermatological, or medical test.
- Having undergone any major surgery in the previous year.
- Having undergone plastic surgery on the studied area(s).
- Taking part or intending to take part in another study liable to interfere with this study.
- Being diabetic.
- Being asthmatic.
- Having participated in skin or peri-ocular tolerance testing in the past two weeks and/or in sensitization trials in the past four months.
- The refusal to give their assent by signing the consent form.
- *For female subjects:* Being pregnant or breastfeeding in the past three months
- *For female subjects:* Intending to become pregnant during the study.
- Having sun-tanned skin
- Intending to expose themselves to artificial UV light and/or to the sun during the study
- Having changed their cosmetic habits in the 14 days preceding the start of the study or intending to change them during the study.
- Having cutaneous hypersensitivity or a skin allergy to cosmetic products.
- Following or intending to follow a chronic medicinal treatment comprising any of the following products taken orally: aspirin-based products, anti-inflammatories, anti-histamines, corticotherapy (the only medication permitted is paracetamol/tylenol).
- Having applied a product and/or make up, including the usual cleanser (face cleaned with water only) to the studied area the day of the measurements.

Non-inclusion criteria: Specific

- Having applied cosmetic products with anti-seborrheic aims or cosmetics for oily skin in the 2 weeks before the start of the study.
- Intending to apply cosmetic products with anti-seborrheic aims or cosmetics for oily skin during the study.
- Having had beauty treatment (e.g. skin cleansing, exfoliation, peeling) in the 4 weeks preceding the start of the study.
- Intending to have beauty treatment (e.g. skin cleansing, exfoliation, peeling) during the study.
- Having started, changed or stopped a hormonal treatment (Hormone Replacement Therapy, thyroid, oral contraception) in the past three months.
- Intending to start, change or stop a hormonal treatment (Hormone Replacement Therapy, thyroid, oral contraception) during the study.
- *For female subjects:* Having taken "Diane" oral contraception in the past three months.
- *For female subjects:* Intending to take "Diane" oral contraception during the study.
- Having taken an "androcure" treatment in the past three months.
- Intending to start an "androcure" treatment during the study.
- Having had oral or local antibiotic treatment for acne in the past 2 weeks.
- Intending to start an oral or local antibiotic treatment for acne during the study.
- Having had local benzoyl-peroxide-based treatment in the past month.
- Intending to start a local benzoyl-peroxide-based treatment during the study.
- Having had local or oral corticoid-based treatment in the past month.
- Intending to a start local or oral corticoid-based treatment during the study.
- Having had an oral treatment with a base of cimetidine, zinc or spironolactone in the past month.
- Intending to start an oral treatment with a base of cimetidine, zinc or spironolactone during the study.
- Having had an oral retinoid-based treatment in the past 6 months or local retinoid-based treatment in the past 2 months.
- Intending to start an oral or local retinoid-based treatment during the study.
- Having had PUVA therapy in the past 4 weeks.
- Intending to have PUVA therapy during the study.

Product application

The application of the products was carried out by the subjects themselves. Quantities of application corresponded to normal conditions of use.

Product	Application area(s)	Frequency of application	Application duration	Conservation
A	One randomized half face	2 times/day	42 days	At an ambient temperature
B	The other half face	2 times/day	42 days	At an ambient temperature

The selection of the half-face to be treated by one or the other product was determined at random for each subject. This randomization was carried out using software designed for this purpose.

Cosmetologist clinical evaluation

Acquisition of source data

Principle: The evaluation was based on a visual evaluation by a cosmetologist at T0, T+28 days and T+42 days, of the following parameters:

- Counting of the number of retentional and inflammatory lesions
- Grading of the pores dilatation

Acquisition methodology

Environmental conditions: The evaluation was carried out under a controlled temperature and relative humidity (temperature: 21±1°C, hygrometry: 45±5 %). The lighting was provided by a ceiling lamp as well as a movable lamp to avoid shadows on the face.

Subject: Each subject was evaluated sitting on a chair facing the cosmetologist. The subject was wearing a paper cap and a cloak. A 20-minute period of acclimatisation in the air-conditioned room was respected.

Studied areas: each half-face

Counting of the number of retentional and inflammatory lesions

The cosmetologist evaluated in detail the number of retentional lesions (closed comedones and opened comedones) and/or inflammatory lesions (papules, pustules and excoriated lesions) and the number obtained for each type of lesion was noted. In cases where a large number of retentional lesions were observed, the evaluation of these lesions was made on a part of the face. This area was chosen at the discretion of the cosmetologist and specified in the observations record.

Grading of the pores dilatation

The evaluation of the descriptor "pores dilatation" by the cosmetologist was carried out using an analogical scale defined by the references "no dilatation" (limit 0) and "maximum dilatation" (limit 10). The result is given in term of a mark (/10).

Results

The results obtained on visual evaluation of the hemi-faces demonstrated that treatment with Product B (containing the *Tsuga canadensis* extract) significantly ($p<0.05$) reduced retentional lesions (closed and open comedones) by 38% and the total number of lesions (including inflammatory lesions) by 31% following 42 days of application compared to T=0; while application of the placebo had no significant effect.

Comparison in time for each product:

Verification of the normality of the distributions was evaluated using the Shapiro-Wilk test, threshold at 1%, for each product. The statistical analysis of the evolution of the measured parameters during the study for each product was performed using the Student test (normality of distributions checked) or with the Wilcoxon test (normality of the distributions rejected). The significance threshold is fixed at 5%.

The results of the cosmetologist clinical evaluation for Product B (containing the *Tsuga canadensis* extract) are shown in Figure 3A and B. Figure 3A shows the evolution of lesions after 28 days of application of Product B, and Figure 3B shows the evolution of lesions after 42 days of application of Product B. The results show a statistically significant decrease in the amount of closed comedones after just 28 days of application of Product B.

Comparison of the two products:

Verification of the normality of the distributions was evaluated using the Shapiro-Wilk test, threshold at 1%, for the comparison of the two products at T0 and at Tn-T0. The statistical

comparison of the two products at T0 and on the differences (Tn-T0), for each of the measured parameters, was performed with the Student test (normality of distributions checked) or with the Wilcoxon test (normality of the distributions rejected). The significance threshold is fixed at 5%.

The results of the comparison between the cosmetologist clinical evaluation for Product A and Product B are shown in Figure 3C and D. Figure 3C shows difference in the evolution of lesions after 28 days of application of either Product A or Product B, and Figure 3B shows the difference in the evolution of lesions after 42 days of application of either Product A or Product B. Although the results of the comparison fail to show a statistically significant difference in the reduction of the number of lesions between the two products, it is worth noting that this result is not unexpected. It is well known in the art that application of a cream to the face by massage and rubbing will have an effect even if the cream is a placebo (see, for example, Johann W. Wiechers "*Much Ado About Nothing: Cosmetics Testing with a Placebo*" February 2009, available online from the website of *Cosmetics & Toiletries*).

This exploratory study, although based on a small number of volunteers, demonstrates that the *Tsuga canadensis* extract has a significant effect against acne and is a good candidate as an active ingredient for formulations dedicated to the reduction of acneic lesions.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSUVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of reducing the number of acneic lesions on the skin of a subject comprising applying to the skin of the subject an effective amount of an extract from a plant of the *Tsuga* genus.

2. The method according to claim 1, wherein the skin lesions are comedones.

3. The method according to claim 1 or 2, wherein said extract is incorporated into a dermatological formulation for topical administration.

4. The method according to claim 3, wherein the dermatological formulation is for sensitive skins.

5. The method according to claim 3 or 4, wherein the dermatological formulation is a cosmetic.

6. The method according to any one of claims 1 to 5, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis, Tsuga diversifolia* or *Tsuga heterophylla* plant.

7. The method according to any one of claims 1 to 5, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis* plant.

8. The method according to any one of claims 1 to 7, wherein the extract is an aqueous-alcoholic extract, aqueous-glycolic extract or aqueous-glycerine extract.

9. The method according to any one of claims 1 to 7, wherein the extract is an aqueous-glycolic extract.

10. The method according to claim 9, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is between about 60:40 and about 40:60.

11. The method according to any one of claims 1 to 7, wherein the extract is a supercritical CO_2 extract.

12. The method according to any one of claims 1 to 11, wherein the extract is prepared by solvent extraction of needles, twigs, small branches, bark, or a combination thereof, from the plant of the *Tsuga* genus.

13. The method according to any one of claims 1 to 12, wherein the extract is prepared using a ratio of solvent:plant material between about 5:1 and about 50:1 by weight.

14. The method according to any one of claims 1 to 12, wherein the extract is prepared using a ratio of solvent:plant material between about 15:1 and about 25:1 by weight.

15. A method of treating acne comprising administering to the skin of a subject in need thereof an effective amount of an extract from a plant of the *Tsuga* genus.

16. The method according to claim 15, wherein treating acne comprises reducing the number of comedones on the skin of said subject.

17. The method according to claim 15 or 16, wherein said extract is incorporated into a dermatological formulation for topical administration.

18. The method according to claim 17, wherein the dermatological formulation is for sensitive skins.

19. The method according to claim 17 or 18, wherein the dermatological formulation is a cosmetic.

20. The method according to any one of claims 15 to 19, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis, Tsuga diversifolia* or *Tsuga heterophylla* plant.

21. The method according to any one of claims 15 to 19, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis* plant.

22. The method according to any one of claims 15 to 21, wherein the extract is an aqueous-alcoholic extract, aqueous-glycolic extract or aqueous-glycerine extract.

23. The method according to any one of claims 15 to 21, wherein the extract is an aqueous-glycolic extract.

24. The method according to claim 23, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is between about 60:40 and about 40:60.

25. The method according to any one of claims 15 to 21, wherein the extract is a supercritical CO_2 extract.

26. The method according to any one of claims 15 to 25, wherein the extract is prepared by solvent extraction of needles, twigs, small branches, bark, or a combination thereof, from the plant of the *Tsuga* genus.

27. The method according to any one of claims 15 to 26, wherein the extract is prepared using a ratio of solvent:plant material between about 5:1 and about 50:1 by weight.

28. The method according to any one of claims 15 to 26, wherein the extract is prepared using a ratio of solvent:plant material between about 15:1 and about 25:1 by weight.

29. A dermatological formulation comprising an extract from a plant of the *Tsuga* genus and optionally one or more anti-acne compounds.

30. The dermatological formulation according to claim 29, wherein the one or more anti-acne compounds are selected from antibiotics, antimicrobials, comedolytic agents and anti-inflammatory agents.

31. The dermatological formulation according to claim 30, wherein the antibiotics are selected from: erythromycin, clindamycin and tetracyclines.

32. The dermatological formulation according to claim 30 or 31, wherein the antimicrobials are selected from: chlorexidine, benzoylperoxide, 1-pentadecanol, derivatives of 1-pentadecanol, cedrene, caryophyllene, longifolene and thujopsene.

33. The dermatological formulation according to any one of claims 30 to 32, wherein the comedolytic agents are selected from: tretinoin, isotretinoin, adapalene, azelaic acid, tazarotene, salicylic acid and salicylic acid derivatives.

34. The dermatological formulation according to any one of claims 30 to 33, wherein the anti-inflammatory agents are selected from: NSAIDs and steroidal anti-inflammatory agents.

35. The dermatological formulation according to any one of claims 30 to 34, wherein the anti-inflammatory agents are selected from: cetylsalicylic acid, ibuprofen, naproxen, sulfacetamide and hydrocortisone.

36. The dermatological formulation according to claim 29, wherein the one or more anti-acne compounds comprise a retinoid and wherein the retinoid is present in a higher than standard amount.

37. The dermatological formulation according to claim 36, wherein the retinoid is present in an amount of about 0.5% by weight or greater.

38. The dermatological formulation according to any one of claims 29 to 37, wherein the extract is present in an amount between about 0.01% and about 20% by weight.

39. The dermatological formulation according to any one of claims 29 to 38, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis, Tsuga diversifolia* or *Tsuga heterophylla* plant.

40. The dermatological formulation according to any one of claims 29 to 38, wherein the plant of the *Tsuga* genus is a *Tsuga canadensis* plant.

41. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is an aqueous-alcoholic extract, aqueous-glycolic extract or aqueous-glycerine extract.

42. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is an aqueous-glycolic extract.

43. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent.

44. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is a supercritical CO_2 extract.

45. The dermatological formulation according to any one of claims 29 to 44, wherein the extract is prepared by solvent extraction of needles, twigs, small branches, bark, or a combination thereof, from the plant of the *Tsuga* genus.

46. The dermatological formulation according to any one of claims 29 to 45, wherein the extract is prepared using a ratio of solvent:plant material between about 5:1 and about 50:1 by weight.

47. The dermatological formulation according to any one of claims 29 to 45, wherein the extract is prepared using a ratio of solvent:plant material between about 15:1 and about 25:1 by weight.

48. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is prepared using a ratio of solvent:plant material of about 70:30 by weight.

49. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is prepared using a ratio of solvent:plant material of about 80:20 by weight.

50. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is prepared using a ratio of solvent:plant material of about 30:70 by weight.

51. The dermatological formulation according to any one of claims 29 to 40, wherein the extract is prepared using a ratio of solvent:plant material of about 20:80 by weight.

52. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is between about 60:40 and about 40:60 by weight.

53. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the glycol:water is about 70:30 by weight.

54. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the glycol:water is is about 30:70 by weight.

55. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the glycol:water is about 80:20 by weight.

56. The dermatological formulation according to claim 42, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the glycol:water is is about 20:80 by weight.

57. The dermatological formulation according to claim 42, wherein the extract is prepared using a a glycol, an alcohol or a glycerine as the solvent.

58. The dermatological formulation according to any one of claims 29 to 57, wherein the dermatological formulation is for sensitive skins.

59. The dermatological formulation according to any one of claims 29 to 58, wherein the dermatological formulation is a cosmetic.

60. The method of claim 1 or 15, wherein the extract is prepared using a ratio of solvent:plant material of about 70:30 by weight.

61. The method of claim 1 or 15, wherein the extract is prepared using a ratio of solvent:plant material of about 80:20 by weight.

62. The method of claim 1 or 15, wherein the extract is prepared using a ratio of solvent:plant material of about 30:70 by weight.

63. The method of claim 1 or 15, wherein the extract is prepared using a ratio of solvent:plant material of about 20:80 by weight.

64. The method of claim 1 or 15, wherein the extract is prepared using a combination of a glycol and water as the solvent.

65. The method of claim 1 or 15, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is about 70:30 by weight.

66. The method of claim 1 or 15, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is is about 30:70 by weight.

67. The method of claim 1 or 15, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is about 80:20 by weight.

68. The method of claim 1 or 15, wherein the extract is prepared using a combination of a glycol and water as the solvent, wherein the range of glycol:water is is about 20:80 by weight.

69. The method of claim 1 or 15, wherein the extract is prepared using a glycol, an alcohol or a glycerine as the solvent.

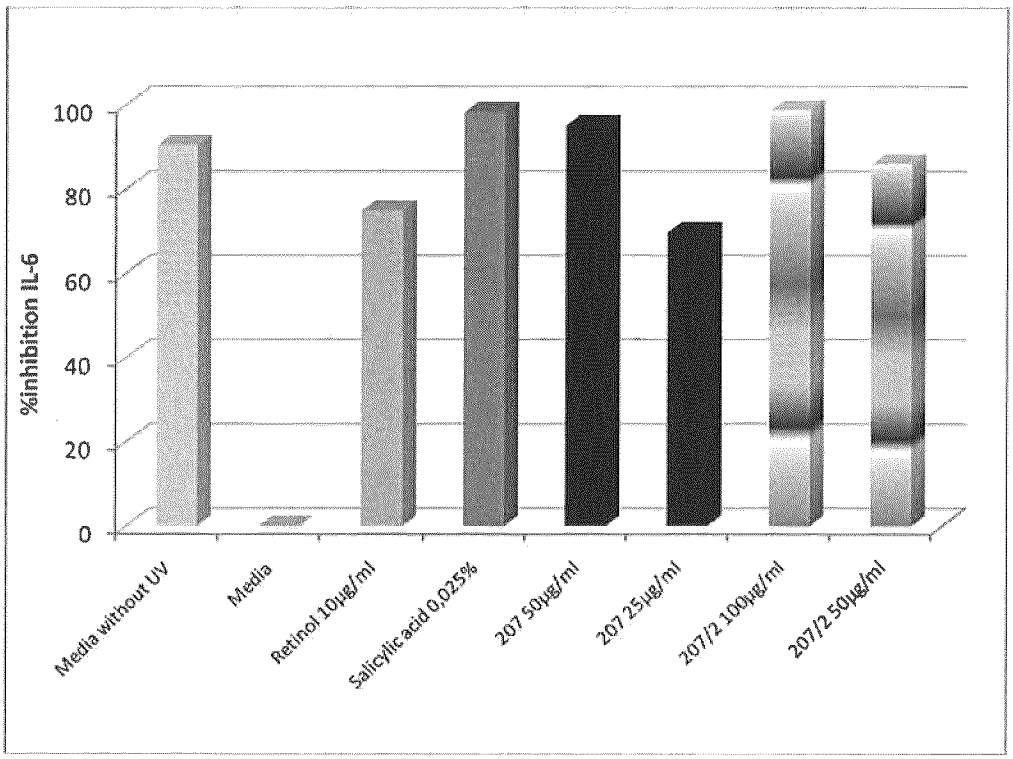
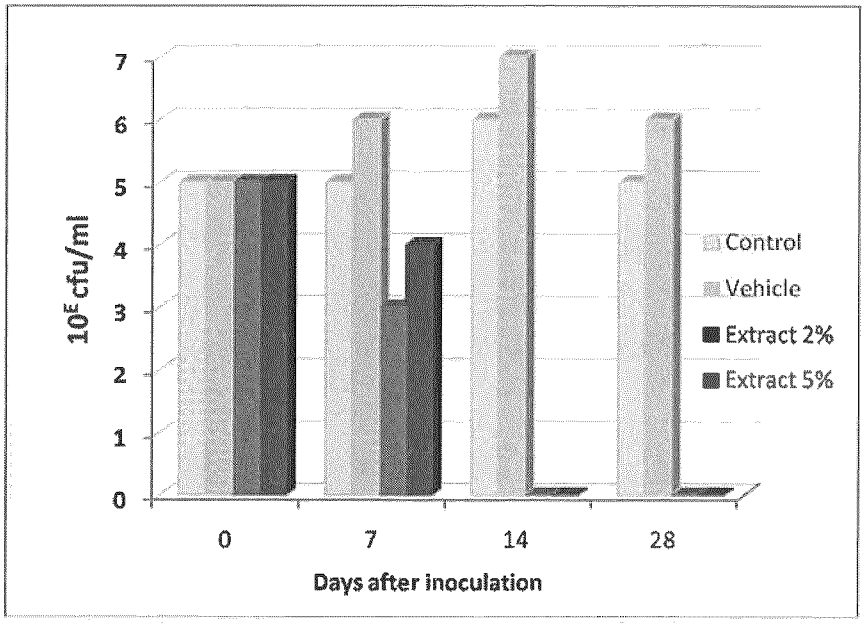

%inhibition IL-6
100
80
60
40
20
0
Media without UV
Media
Retinol 10µg/ml
Salicylic acid 0,025%
207 50µg/ml
207 25µg/ml
207/2 100µg/ml
207/2 50µg/ml

Figure 1

A.


7
6
5
4
3
2
1
0
10^E cfu/ml
0
7
14
28
Days after inoculation
Control
Vehicle
Extract 2%
Extract 5%

B.

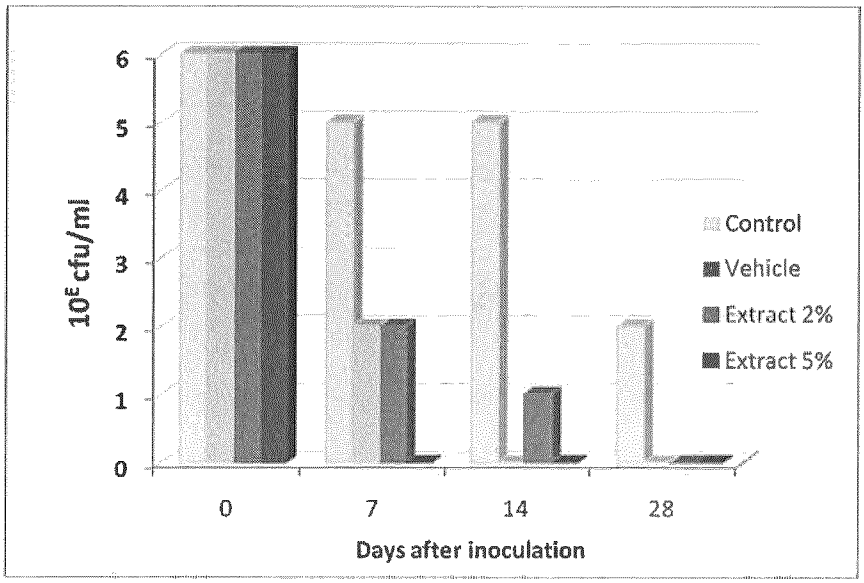

6
5
4
3
2
1
0
10^E cfu/ml
0
7
14
28
Days after inoculation
Control
Vehicle
Extract 2%
Extract 5%

Figure 2

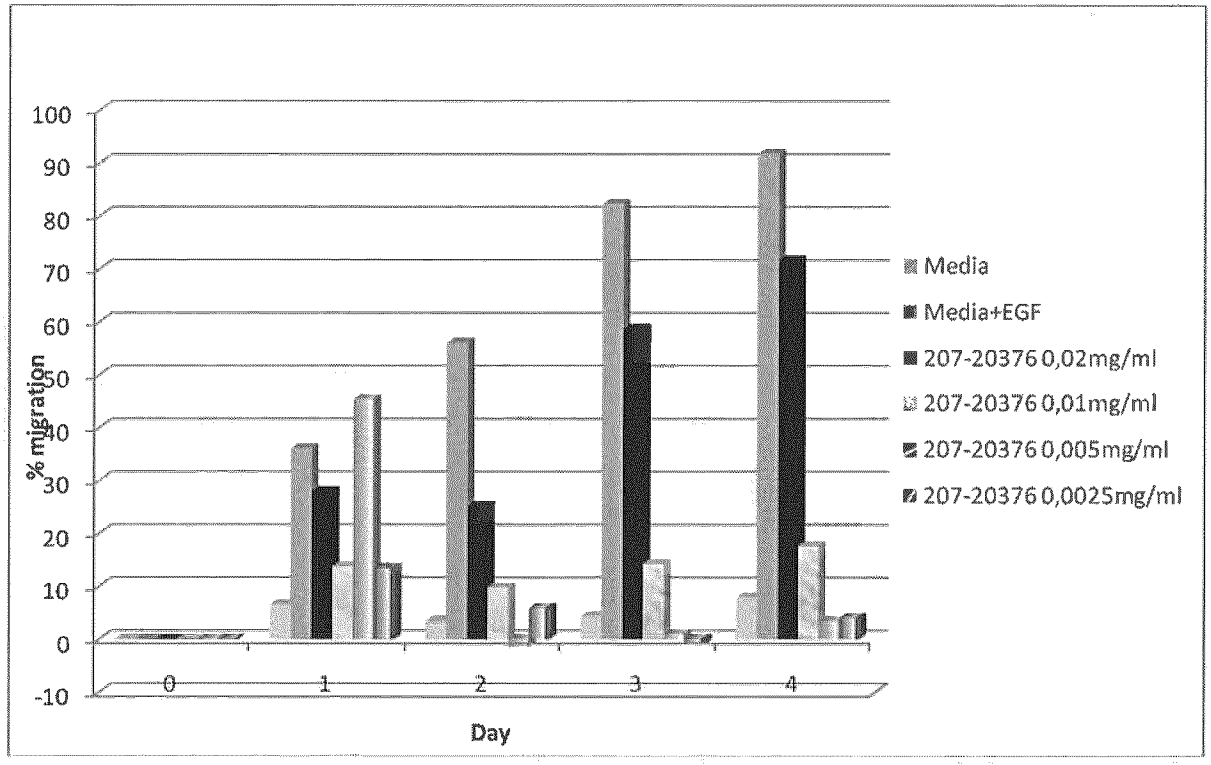
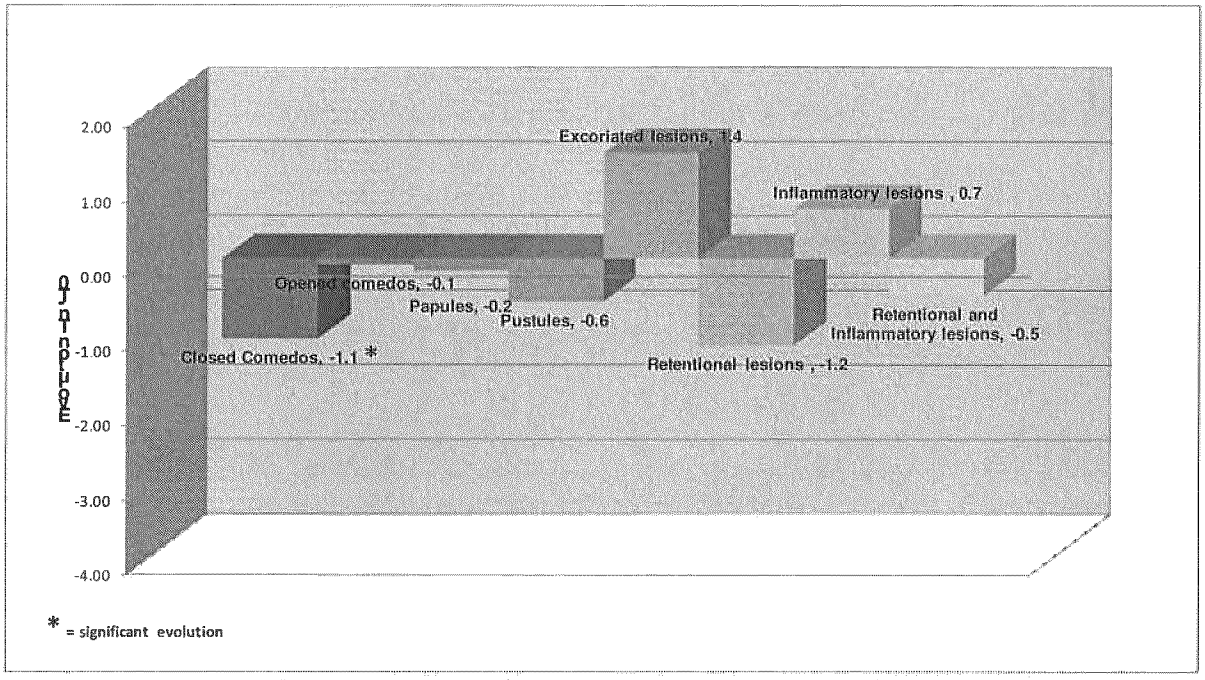

100
90
80
70
60
50
40
30
20
10
0
-10
% migration
0
1
2
3
4
Day
Media
Media+EGF
207-20376 0,02mg/ml
207-20376 0,01mg/ml
207-20376 0,005mg/ml
207-20376 0,0025mg/ml

Figure 3

A.


2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
Excoriated lesions, 1.4
Inflammatory lesions , 0.7
Opened comedos, -0.1
Papules, -0.2
Pustules, -0.6
Retentional and
Inflammatory lesions, -0.5
Closed Comedos, -1.1 *
Retentional lesions , -1.2
* = significant evolution

B.

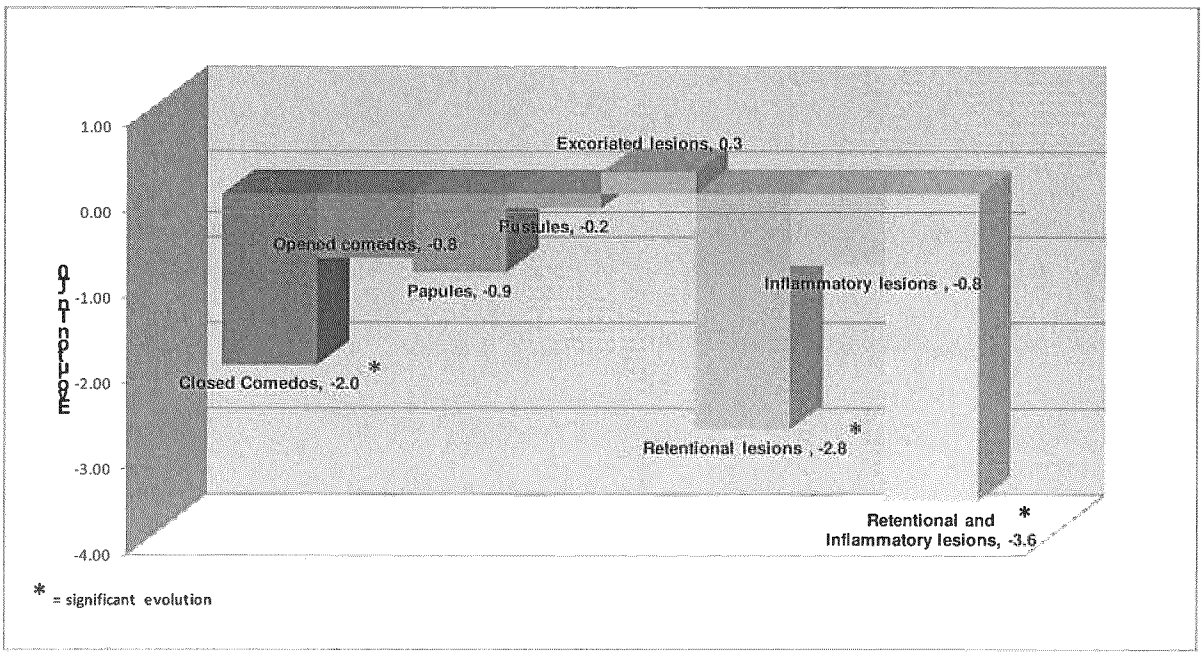
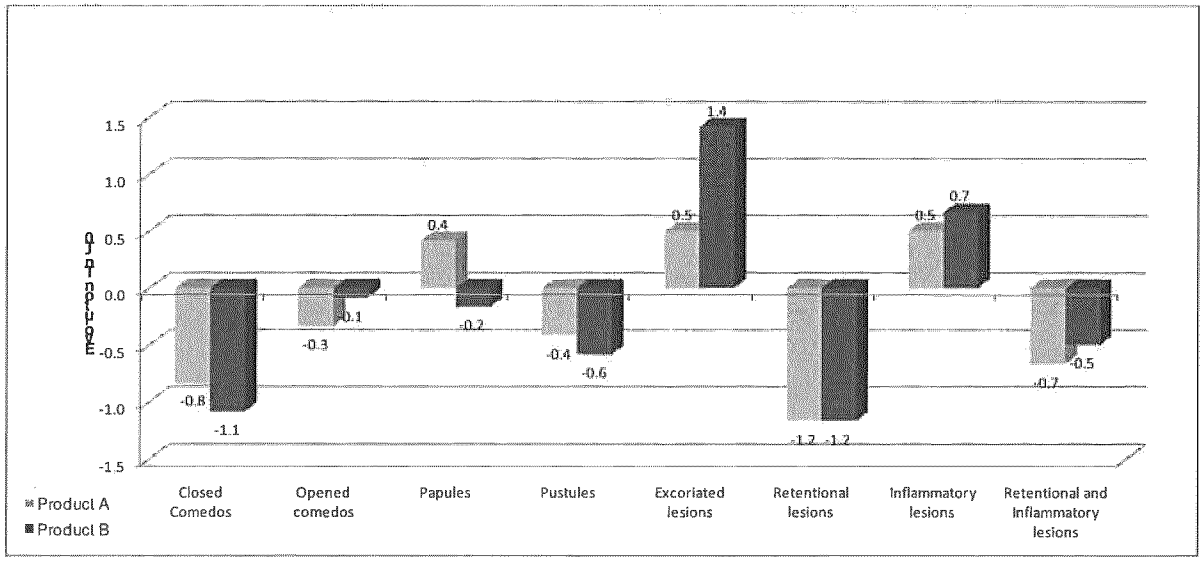

1.00
0.00
-1.00
-2.00
-3.00
-4.00
Excoriated lesions, 0.3
Pustules, -0.2
Opened comedos, -0.8
Papules, -0.9
Inflammatory lesions , -0.8
Closed Comedos, -2.0 *
Retentional lesions , -2.8 *
Retentional and *
Inflammatory lesions, -3.6
* = significant evolution

Figure 4

C.



D.

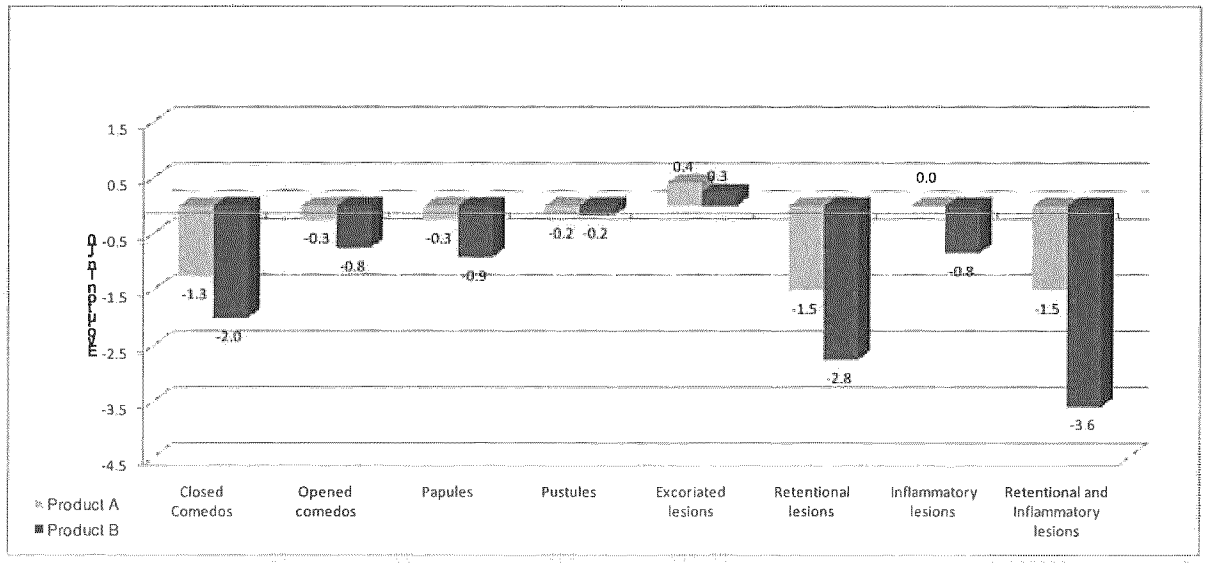


Figure 4 (con.)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050161

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 36/15*** (2006.01) , ***A61P 17/10*** (2006.01) , *A61K 8/97* (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)
A61K 36/15 (2006.01) , ***A61P 17/10*** (2006.01) , *A61K 8/97* (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
NativeAmerican Ethnobotany Database, TotalPatents, CPDP (Intellect), Agricola, Pubmed, Scopus with keywords: Tsuga, acne, skin, dermatol*, topical, hemlock, antibiotic.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2009/121168 A1 (GUAY Johane and CYR Benoit) 08 October 2009 (08-10-2009) the entire document	29, 30, 33 - 59 31
X	WO 2006/039807 (CYR B. et al.) 20 April 2006 the entire document	29, 30, 32, 34 - 43, 45, 52, 53, 57 - 59

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"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
05 June 2012 (05-06-2012)	18 July 2012 (18-07-2012)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Kalie Gossen (819) 956-9973

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050161

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1 to 28 and 60 to 69
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1 to 28 and 60 to 68 are directed to methods for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim 29.

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BLACK M.J., Algonquin Ethnobotany: an interpretation of Aboriginal Adaptation in South Western Quebec, National Museums of Canada, Ottawa, Mercury Series Number 65, 1980, page 125.	29, 39, 40 and 45
X	TURNER N.J. and EFRAT B.S., Ethnobotany of the Hesquiat Indians of Vancouver Island, British Columbia Provincial Museum, Victoria. 1982, pp 44-45.	29, 39 and 45
Y	KERI J. and SHIMAN M.,, An update on the management of acne vulgaris, Clinical, Cosmetic and Investigational Dermatology, vol. 2, 2009 pp 105-110.	31

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2012/050161

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2009121168A1	08 October 2009 (08-10-2009)	CA2757342A1	08 October 2009 (08-10-2009)
		EP2280718A1	09 February 2011 (09-02-2011)
		US2011177014A1	21 July 2011 (21-07-2011)
WO2006039807A1	20 April 2006 (20-04-2006)	AU2003264192A1	19 March 2004 (19-03-2004)
		CA2536604A1	11 March 2004 (11-03-2004)
		CA2626049A1	20 April 2006 (20-04-2006)
		EP1539204A1	15 June 2005 (15-06-2005)
		EP1816996A1	15 August 2007 (15-08-2007)
		EP1816996A4	12 August 2009 (12-08-2009)
		JP2008516899A	22 May 2008 (22-05-2008)
		US2006228426A1	12 October 2006 (12-10-2006)
		US2009004302A1	01 January 2009 (01-01-2009)
		US2009263516A1	22 October 2009 (22-10-2009)
		US2010323041A1	23 December 2010 (23-12-2010)
		WO2004019961A1	11 March 2004 (11-03-2004)