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(54) Title: ANTISEBUM AND ANTIOXIDANT COMPOSITIONS

(57) Abstract

Gugulipid (a lipophilic ethyl acetate extract from C. mukul or C. wightii) or an alcoholic fraction of gugulipid or a low molecular weight fraction of gugulipid as an antisebum and/or antioxidant active in cosmetic skin care compositions and methods are described. Gugulipid from C. mukul and its alcoholic and low molecular weight fractions are capable of delivering dual benefit to the skin: controlling or preventing sebum secretion (oily skin conditions) and protecting the skin from free radical damage.

ANTISEBUM AND ANTIOXIDANT COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to methods and compositions for controlling or preventing sebum secretion from sebocytes, for controlling or preventing oily skin conditions, and also for protecting skin from free radical activity.

BACKGROUND OF THE INVENTION

Sebum is skin oil which is produced by sebocytes (cells of the sebaceous glands in the skin) and is then secreted to the skin surface. Excessive amount of sebum on the skin surface results in the condition known as "oily skin." Oily skin is associated with a shiny, undesirable appearance and a disagreeable tactile sensation. Many methods and compositions exist which attempt to control the excessive sebum secretion, but none have proved totally satisfactory.

Formation of free radicals in the skin does not appear to be related to the sebum secretion. Low levels of free radicals are formed in the skin as part of the natural metabolic pathways. The level of free radicals is increased in response to UV radiation and other environmental oxidants, e.g. pollution and cigarette smoke. Increased concentration of free radicals leads to lipid peroxidation in skin cells and cellular damage, which in turn results in a premature ageing of the skin with an accompanying loss of firmness and elasticity, wrinkles, discoloration, age spots, and dryness. Antioxidants, such as vitamin E (alpha-tocopherol), decrease the level of free radicals in the skin.

Cosmetic actives which provide more than one benefit are highly desirable, both from the manufacturer's and consumer's perspective.

Guggal is obtained from a gum/resin of the plant Commiphora mukul or Commiphora wightii. Guggal contains a complex mixture of terpenes, sterols, esters and higher alcohols. The ethyl acetate extract of the resin is an oily resinous material known as "gugulipid" or "guggal lipid." Gugulipid has been used medicinally in the treatment of obesity and elevated cholesterol levels. The medicinal activity of gugulipid is attributed to two known ketonic steroids (guggulsterones).

Bombardelli et al. (U.S. Patent 5,273,747) discloses the anti-inflammatory activity of gugulipid and a guggulsterone-enriched fraction thereof and their use in the treatment of benign prostatic hypertrophy and in the treatment of acne. In this regard it is important to note that although increased sebum production may be one of the many factors that lead to the formation of acne, an anti-acne agent does not necessarily possess antisebum activity. For instance, benzoyl peroxide and salicylic acid are well-established anti-acne agents, but they do not decrease sebum output. See Cunliffe, et al., "Topical Benzoyl Peroxide Increases The Sebum Excretion Rate In Patients With Acne", British Journal of Dermatology (1983) 109, 577-579; William J. Cunliffe, "Acne", p. 256, Martin Dunitz Ltd. (1989). See also Comparative Example 3 below. Furthermore, the guggulsterone-enriched fraction described by Bombardelli was obtained with ethyl acetate and did not separate compounds by molecular weight. By contrast, in the present invention preferably an alcoholic fraction and/or a low molecular weight fraction is employed.

Bissett et al. (U.S. Patents 4,847,071 and 4,847,069) and Piazza et al. (U.S. Patent 5,521,223) disclose photoprotective and anti-wrinkle compositions containing guggal as a natural anti-inflammatory. Although some compounds may be anti-inflammatory through antioxidant pathways, not all anti-inflammatory mechanisms are antioxidant mediated, nor are all antioxidant anti-inflammatory. Put another way, anti-inflammatory and antioxidants effects do not necessarily follow each other.

WO 96,03033 describes a cosmetic composition that comprises an extract of the resin of commiphora mukul, made by extraction with ethyl acetate and subsequent dilution with ethanol.

US 5587176 describes a method of reducing oily skin by applying a composition optionally including guggal extracted from commiphora mukul.

The art discussed above does not address the need for an agent which contains both antisebum and antioxidant activities. The art does not disclose either antisebum or antioxidant activity of guggal or gugulipid or fractions thereof. Furthermore, as far as fractions of gugulipid are concerned, the art discloses the preparation and use of only a guggulsterone-enriched (ethyl acetate) fraction. The preparation, use and activities of an alcoholic fraction or a low molecular weight fraction are not disclosed.

SUMMARY OF THE INVENTION

The present invention includes in its first aspect a cosmetic method of controlling or preventing an oily skin condition, especially in the facial area, by applying to the skin a composition comprising gugulipid and/or alcoholic fraction thereof and/or a low molecular weight fraction thereof in a cosmetically acceptable vehicle.

The second aspect of the present invention includes a cosmetic method of reducing, preventing or controlling sebum secretion from sebocytes by applying to the skin a composition comprising gugulipid and/or alcoholic fraction thereof and/or

a low molecular weight fraction thereof in a cosmetically acceptable vehicle.

Another aspect of the invention is a cosmetic method of protecting the skin from free radical activity (i.e., relieving the oxidative stress in the skin) by applying to the skin a composition comprising gugulipid and/or alcoholic fraction thereof and/or a low molecular weight fraction thereof in a cosmetically acceptable vehicle.

Still another aspect of the invention is a cosmetic method of simultaneously controlling or preventing sebum secretion while also protecting the skin from free radical damage, by the use of a single active agent: gugulipid and/or an alcoholic fraction thereof and/or a low molecular weight fraction thereof.

The invention further provides the use of gugulipid and/or alcoholic fraction thereof and/or a low molecular weight fraction thereof, and also provides the use of cosmetic compositions comprising such agents, in controlling or preventing an oily skin condition, in reducing, preventing or controlling sebum secretion from sebocytes in skin and/or in protecting the skin from free radical activity (i.e. relieving the oxidative stress in the skin).

The invention further provides the use of gugulipid and/or alcoholic fraction thereof and/or a low molecular weight fraction thereof, and also provides the use of cosmetic compositions comprising such agents, in reducing or preventing oily skin conditions while also protecting the skin from free radical damage.

Yet another aspect of the invention is a method for the manufacture of an alcoholic fraction of gugulipid.

Still another aspect of the invention is a cosmetic composition for care of the skin, the composition comprising an alcoholic fraction or a low molecular weight fraction of gugulipid in a cosmetically acceptable vehicle.

The inventive methods and compositions provide control of sebum secretion from sebocytes, improved oil control and improved skin feel, prevent shine and stickiness, while also protecting the skin from damaging free radical activity, which results in reduced appearance of wrinkles and aged skin, improved skin color, improved appearance of photoaged skin, improvement in skin's radiance and clarity and finish, and an overall healthy and youthful appearance of the skin.

DETAILED DESCRIPTION OF THE INVENTION

The term "skin" as used herein includes skin on the face, neck, chest, back, and scalp.

Gugulipid and non-guggulsterone fraction thereof:

The term "gugulipid" as used herein means an ethyl acetate extract of gum/resin guggal from the tree C. mukul or C. wightii. The term "alcoholic fraction" as used herein means a highly polar, non-aqueous fraction of gugulipid. Preferably, the alcoholic fraction is of non-pet ether fraction of gugulipid.

The inventive methods of controlling sebum secretion, controlling oily skin and/or protecting skin from free radicals employs gugulipid and/or an alcoholic fraction thereof and/or a low molecular weight fraction thereof. It

has been found, as part of the present invention that gugulipid and an alcoholic and/or a low molecular weight fractions thereof possess a rare quality of providing a dual benefit for skin care, i.e. both antisebum and antioxidant activity. It has been found that an aqueous extract of guggal from C. mukul has little or none of antisebum and/or antioxidant activity observed with the ethyl acetate extract of guggal from C. mukul. It has also been found that the antisebum and/or antioxidant activity of gugulipid is concentrated in an alcoholic fraction of gugulipid or a low molecular weight fraction, rather than an ethyl acetate guggul-sterone enriched fraction described by Bombardelli.

In addition, it has been found that a low molecular weight fraction of gugulipid is easier to formulate, due to its lower viscosity and better color than any one of gugulipid, the alcoholic fraction or a high molecular weight fraction. The low molecular weight fraction can also be obtained in a higher yield than the alcoholic fraction.

Gugulipid may be obtained from the following suppliers:
C. Mukul extract:
Indena (80 E Route 4, Paramus, NJ, 07652)
Pt. Cosmetique Java, Bogar (Campo R&D, Singapore)(C. wightii extract also available).

The inventive methods and compositions may employ from 0.0001 to 10 wt. %, preferably from 0.001 to 3 wt. %, and most preferably from 0.01% to 2 wt. % of gugulipid or an alcoholic fraction and/or a low molecular weight fraction of gugulipid.

When an alcoholic fraction is employed, it may advantageously be included in a lower amount than gugulipid to

provide the same activity as may be obtained with higher amounts of gugulipid itself.

The alcoholic fraction may be obtained by subjecting gugulipid or, preferably, the non-pet ether fraction of gugulipid to separation (e.g., by extraction or eluting with a highly polar, non-aqueous solvent. Typical suitable solvents are alcohols, preferably low chain (i.e., less than 6 carbons) alcohols, i.e. methanol, ethanol, propanol, isopropanol, butanol, 2-butanol, 1-pentanol, 2-pentanol, 3-pentanol, 1-hexanol. The obtained fraction is preferably dried, e.g. by evaporation or freeze-drying to concentrate the active amount. The alcoholic fraction employed in the inventive methods or compositions generally contains less than 99% alcohol, by weight of the fraction, preferably less than 25%, most preferably less than 5%.

The alcoholic fraction contains little or no of cis guggulsterone (also known as guggulsterone E) described by Bombardelli. It has been unexpectedly found, as part of the present invention, that cis-guggulsterone does not provide either antioxidant or antisebum activity. Consequently, the antisebum and/or antioxidant activity of gugulipid is due to active compounds other than cis-guggulsterone and is found concentrated in an alcoholic fraction of gugulipid. The alcoholic fraction according to the present invention contains less than 0.1% of cis-guggulsterone by weight of the fraction, and preferably less than 0.05% % of cis-guggulsterone. The guggulsterone amount in a fraction may be checked by HPLC, mass spectroscopy or TLC.

The low molecular weight fraction is obtained by dispersing or dissolving gugulipid in a polar solvent. Typical suitable solvents being alcohols, preferably low chain (i.e., less than 6 carbons) alcohols, i.e. methanol, ethanol,

propanol, isopropanol, butanol, 2-butanol, 1-pentanol, 2-pentanol, 3-pentanol, 1-hexanol. This may then be separated by ultrafiltration to obtain a fraction of 1,000 Da or less, preferably 800 Da or less and optimally of 500 Da or less. The solvent may then be evaporated under nitrogen by e.g. gentle heat/steam bath.

In a preferred embodiment of the present invention, the low molecular weight fraction of gugulipid is employed to obtain greater ease of formulation (improved color and lower viscosity). The low molecular weight fraction is also obtained in a higher yield than the alcoholic fraction.

Cosmetically Acceptable Vehicle:

The compositions according to the invention also comprise a cosmetically acceptable vehicle to act as a dilutant, dispersant or carrier for gugulipid and/or the alcoholic fraction and/or the low molecular weight fraction thereof in the composition, so as to facilitate its distribution when the composition is applied to the skin.

The vehicle may be aqueous, anhydrous or an emulsion. Preferably, the compositions are aqueous or an emulsion, especially water-in-oil or oil-in-water emulsion. Water when present will be in amounts which may range from 5 to 99%, preferably from 20 to 70%, optimally between 35 and 60% by weight.

Besides water, relatively volatile solvents may also serve as carriers within compositions of the present invention. Most preferred are monohydric C_1-C_3 alkanols. These include ethyl alcohol, methyl alcohol and isopropyl alcohol. The amount of monohydric alkanol may range from 1 to 70%, preferably from 10 to 50%, optimally between 15 to 40% by

weight. The alcohol content of the alcoholic fraction of the gugulipid is not included in these amounts.

Emollient materials may also serve as cosmetically acceptable carriers. These may be in the form of silicone oils and synthetic esters. Amounts of the emollients may range anywhere from 0.1 to 50%, preferably between 1 and 20% by weight.

Silicone oils may be divided into the volatile and non-volatile variety. The term "volatile" as used herein refers to those materials which have a measurable vapor pressure at ambient temperature. Volatile silicone oils are preferably chosen from cyclic or linear polydimethylsiloxanes containing from 3 to 9, preferably from 4 to 5, silicon atoms. Linear volatile silicone materials generally have viscosities less than 5 centistokes at 25°C while cyclic materials typically have viscosities of less than 10 centistokes. Nonvolatile silicone oils useful as an emollient material include polyalkyl siloxanes, polyalkylaryl siloxanes and polyether siloxane copolymers. The essentially non-volatile polyalkyl siloxanes useful herein include, for example, polydimethyl siloxanes with viscosities of from 5 to 100,000 centistokes at 25°C. Among the preferred non-volatile emollients useful in the present compositions are the polydimethyl siloxanes having viscosities from 10 to 400 centistokes at 25°C.

Among the ester emollients are:

(1) Alkenyl or alkyl esters of fatty acids having 10 to 20 carbon atoms. Examples thereof include isoarachidyl neopentanoate, isononyl isonanonoate, oleyl myristate, oleyl stearate, and oleyl oleate.

(2) Ether-esters such as fatty acid esters of ethoxylated fatty alcohols.

(3) Polyhydric alcohol esters. Ethylene glycol mono and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, polyethylene glycol (200-6000) mono- and di-fatty acid esters, propylene glycol mono- and di-fatty acid esters, polypropylene glycol 2000 monooleate, polypropylene glycol 2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol poly-fatty esters, ethoxylated glyceryl monostearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol distearate, polyoxyethylene polyol fatty acid ester, sorbitan fatty acid esters, and polyoxyethylene sorbitan fatty acid esters are satisfactory polyhydric alcohol esters.

(4) Wax esters such as beeswax, spermaceti, myristyl myristate, stearyl stearate and arachidyl behenate.

(5) Sterols esters, of which cholesterol fatty acid esters are examples thereof.

Fatty acids having from 10 to 30 carbon atoms may also be included as cosmetically acceptable carriers for compositions of this invention. Illustrative of this category are pelargonic, lauric, myristic, palmitic, stearic, isostearic, hydroxystearic, oleic, linoleic, ricinoleic, arachidic, behenic and erucic acids.

Humectants of the polyhydric alcohol-type may also be employed as or as part of cosmetically acceptable carriers in compositions of this invention. The humectant aids in

increasing the effectiveness of the emollient, reduces scaling, stimulates removal of built-up scale and improves skin feel. Typical polyhydric alcohols include glycerol, polyalkylene glycols and more preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, hexylene glycol, 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycerol, propoxylated glycerol and mixtures thereof. For best results the humectant is preferably propylene glycol. The amount of humectant may range anywhere from 0.5 to 30%, preferably between 1 and 15% by weight of the composition.

Thickeners may also be utilized as part of the cosmetically acceptable carrier of compositions according to the present invention. Typical thickeners include crosslinked acrylates (e.g. Carbopol 982), hydrophobically-modified acrylates (e.g. Carbopol 1382), cellulosic derivatives and natural gums. Among useful cellulosic derivatives are sodium carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, ethyl cellulose and hydroxymethyl cellulose. Natural gums suitable for the present invention include guar, xanthan, sclerotium, carrageenan, pectin and combinations of these gums. Amounts of the thickener may range from 0.0001 to 5%, usually from 0.001 to 1%, optimally from 0.01 to 0.5% by weight.

Collectively the water, solvents, silicones, esters, fatty acids, humectants and/or thickeners will constitute the cosmetically acceptable carrier in amounts from 1 to 99.9%, preferably from 80 to 99% by weight.

Optional Skin Benefit Materials and Cosmetic Adjuncts:

An oil or oily material may be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

Surfactants may also be present in cosmetic compositions of the present invention. Total concentration of the surfactant will range from 0.1 to 40%, preferably from 1 to 20%, optimally from 1 to 5% by weight of the composition. The surfactant may be selected from the group consisting of anionic, nonionic, cationic and amphoteric actives. Particularly preferred nonionic surfactants are those with a C_{10}-C_{20} fatty alcohol or acid hydrophobe condensed with from 2 to 100 moles of ethylene oxide or propylene oxide per mole of hydrophobe; C_2-C_{10} alkyl phenols condensed with from 2 to 20 moles of alkylene oxide; mono- and di- fatty acid esters of ethylene glycol; fatty acid monoglyceride; sorbitan, mono- and di- C_8-C_{20} fatty acids; block copolymers (ethylene oxide/ propylene oxide); and polyoxyethylene sorbitan as well as combinations thereof. Alkyl polyglycosides and saccharide fatty amides (e.g. methyl gluconamides) are also suitable nonionic surfactants. Preferred anionic surfactants include soap, alkyl ether sulfate and sulfonates, alkyl sulfates and sulfonates, alkylbenzene sulfonates, alkyl and dialkyl sulfosuccinates, C_8-C_{20} acyl isethionates, acyl glutamates, C_8-C_{20} alkyl ether phosphates and combinations thereof.

Various types of other active ingredients may be present in cosmetic compositions of the present invention. Actives are defined as skin benefit agents other than emollients and other than ingredients that merely improve the physical characteristics of the composition. Although not limited to

this category, general examples include anti-acne agents, additional anti-sebum agents, and sunscreens.

Anti-acne agents include but are not limited to benzoyl peroxide (up to 20 wt.% may be included), retinoids (typically 0.025% - 0.05%), salicylic acid (typically up to 2 wt. %), and sulphur (up to 8 wt.%).

Other antisebum actives may be included, most preferably tridecyl salicylate (or zinc pyriphione) in an amount up to 10 wt. %.

Retinol or esters thereof may be included to provide various skin benefits. Suitable retinol esters include retinol palmitate, retinol acetate, and retinol linoleate. Retinol linoleate is especially preferred to provide additional antisebum activity and anti-aging benefits. The amounts of retinol and/or retinol esters are in the range of from 0.001 wt.% to 3 wt.% by weight of the composition, preferably from 0.001 wt.% to 0.5 wt.%.

An especially preferred combination of an active (gugulipid and/or an alcoholic fraction and/or a low molecular weight fraction thereof) is with an optional ingredient selected from the group consisting of tridecyl salicylate, retinyl linoleate and mixtures thereof, in order to provide maximum antisebum activity, anti-aging and/or optimum healthy-looking skin.

Sunscreens include those materials commonly employed to block ultraviolet light. Illustrative compounds are the derivatives of PABA, cinnamate and salicylate. For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available

under the trademarks, Parsol MCX and Benzophenone-3, respectively. The exact amount of sunscreen employed in the compositions can vary depending upon the degree of protection desired from the sun's UV radiation.

Preservatives may be included in composition of the present invention in order to protect them against the growth of potentially harmful microorganisms. Suitable preservatives include alkyl esters of p-hydroxybenzoic acid, hydantoin derivatives, propionate salts, and a variety of quaternary ammonium compounds. Particularly preferred preservatives of this invention are methyl paraben, propyl paraben, phenoxyethanol and benzyl alcohol. Preservatives will usually be employed in amounts ranging from 0.1% to 2% by weight of the composition.

Powders may be incorporated into the cosmetic composition of the invention. These powders include chalk, talc, kaolin, starch, smectites clays, chemically modified magnesium aluminum silicate, organically modified montmorillonite clay, hydrated aluminum silicate, fumed silica, aluminum starch octenyl succinate and mixtures thereof.

Other adjunct minor components may also be incorporated into the cosmetic compositions. These ingredients may include coloring agents, opacifiers and perfumes. Amounts of these other adjunct minor ingredients may range anywhere from 0.001% up to 20% by weight of the composition.

Use of the Composition:

The composition according to the invention is intended primarily as a product for topical application to human skin, especially as an agent for controlling or preventing oily skin,

for improving skin's radiance and clarity and finish, and for preventing or reducing the appearance of wrinkled, dry, aged or photoaged skin.

In use, a quantity of the composition, for example from 1 to 100 ml, is applied to exposed areas of the skin, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device.

Product Form and Packaging:

The topical skin composition of the invention can be in any form, e.g. formulated as a lotion, a fluid cream, or a cream. The composition can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or fluid cream can be packaged in a bottle or a roll-ball applicator or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar. The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

The composition may also be included in capsules such as those described in U.S. Patent No. 5,063,057.

The following specific examples further illustrate the invention, but the invention is not limited thereto.

All parts, percentages and proportions referred to herein and in the appended claims are by weight of the composition unless otherwise indicated.

EXAMPLE 1

This example demonstrates the procedure for the fractionation of gugulipid into fractions of increasing polarity (Procedure A) and the procedure for obtaining a low molecular weight fraction of gugulipid (Procedure B).

PROCEDURE A

Materials:

Gugulipid (Lot No. 42941, Lipo Chemicals Inc. Paterson NJ)
Medium pressure column (5cm i.d x 62 cm in length).
Silica (gel, Merck), Aldrich, Cat no. 22,719-6, Grade 9385,230,400, mesh 60 A.
TLC plates, LHP-kk 20x10 cc Lot#004966, Cat# 4805-711 Whatman.
Hexane, HPLC grade (Fisher)
Ethyl Acetate, HPLC grade (Fisher)
Methanol, HPLC grade (Fisher)
Chloroform, HPLC grade (Fisher)
Phosphoric Acid (Fisher)
Cupric Sulfate $CuSO_4$ (Fisher)
Petroleum Ether (Fisher)
24 Beakers (400ml)
Scintillation Vials
Capillary tubes (5 µl)
2 Graduated cylinders (2000ml)
1 Developing dish
1 Oven (Napco Model 420)
1 Hot/stir plate
2 Round bottom Flasks (500ml)
Rotary Evaporator
Water (Milli-Q Water)

Method:

1. Weighed 5.0672g of Gugulipid into a 500ml round bottom flask.

   (A) Dissolved Gugulipid in 100ml of Ethyl Acetate (to make a slurry).
   (B) The Ethyl Acetate was pulled off using the Rotary Evaporator.
   (C) Added 350ml of Pet Ether to the flask.
   (D) Mixture was allowed to stir overnight.

2. Filled medium pressure column up to 75% with Silica (Merck), and prepared the column by washing with 700ml of Hexane/Ethyl Acetate (10:1 ratio).

3. Removed flask from stir plate and decanted the Pet Ether into a 500ml round bottom flask.

   (A) The Pet Ether was pulled off using the rotavapor until approx. 3ml remained.
   (B) The remaining 3ml was quantitatively transferred to a scintillation vial for which a tare weight had been previously recorded.
   (C) Sample was evaporated to dryness under the hood.
   (D) Final tare weight was recorded, and sample labeled (Pet Ether non-polar fraction#1)

4. Dissolved the remaining residue from the Pet Ether extraction in 50ml of Ethyl Acetate, and transferred it to the medium pressure column using a glass Pasteur pipet.

5. Fraction 2 was eluted from the column with 800ml of Hexane/Ethyl Acetate, 7:1 ratio.

6. Fractions 3-5 were eluted with Hexane/Ethyl Acetate at a 5:1 ratio. Each fraction was approximately 200ml.

7. Fractions 6-12 were eluted with 1500ml Hexane/Ethyl Acetate at a 2:1 ratio.

8. Fractions 13-19 were eluted from the column with 2000ml Hexane/Ethyl Acetate, 1:1 ratio.

9. Fractions 20-29 were eluted with 3000ml Hexane/Ethyl Acetate, 1:2 ratio.

10. Fractions 30-37 were eluted with 2000ml Hexane/Ethyl Acetate, 1:3 ratio.

11. Fractions 38-41 were eluted with 100% Ethyl Acetate.

12. Fraction 42 was eluted with 100% methanol (1000ml).

13. Fractions 43 and 44 were eluted with 100% methanol (1000ml).

14. All fractions were evaporated down to approximately 10ml using a steam bath and transferred into scintillation vials.

15. Fractions 1 - 44 were analyzed by Thin Layer Chromatography (TLC).

(A) TLC plates were developed using Chloroform/Methanol 9:1 ratio.

(B) TLC plates were stained with 10% Copper Sulfate in 8% Phosphoric Acid.

16. Due to poor TLC results fractions 26 - 39 were concentrated and reanalyzed.

17. Fractions which were similar in TLC profile were pooled. The following fractions were pooled.

Fractions 9, 10, and 11
Fractions 13, and 14
Fractions 16, 17, and 18
Fractions 19, and 20
Fractions 22, 23, and 24
Fractions 40, 41, and 42 (but these contained 99% of fraction 42, due to the extremely small sample size of fractions 40 and 41)

18. Fractions 26 - 39, and the pooled fractions were reanalyzed by TLC.

19. Fractions that were similar in TLC profile and which were combined:

Fractions 26, and 27
Fractions 30, 31, and 32
Fractions 34, and 35

20. The remaining fractions were evaporated to dryness under the hood. A total of eighteen sample fractions resulted.

21. Fractions which did not show any spots by TLC were discarded.

Results:

Starting with 5.06g of gugulipid (350ml pet ether), fraction 1 (1.7469g) was obtained as the pet ether soluble

fraction. The fraction not soluble in pet ether was dissolved in 50ml of Ethyl Acetate and transferred onto the silica column (5cm i.d x 62cm in length) and eluted with solvent portions of increasing polarity. This resulted in 43 further fractions. Based on similar TLC profiles, several fractions were combined. Fractions 26-29 contained very little material and had to be concentrated before TLC results could be obtained. From the multiple bands on the TLC plates it was clear that most fractions contain several compounds. Some fractions were more concentrated than others as apparent from the intense bands on the TLC plates. 18 fractions resulted.

Fractions 43 and 44 were the only fractions that contained solely methanolic extract. Fraction 42 was eluted with methanol, but was combined with fractions 40 and 41 (which were eluted with ethyl acetate, but were very small in size) . Fraction 42 actually contained about 99% of fraction 42 and about 1% of fractions 40 and 41. Fraction 44 contained substantially less active ingredient than fractions 42 and 43, because it was an end portion of the alcoholic fraction.

PROCEDURE B

In preparation of low molecular weight fraction of gugulipid, complete gugulipid was passed through a 50,000 MW filter. The filtrate was collected and passed through a 10,000 MW filter. This was followed by filtration through 5000 MW and 500 MW filters. Analysis of each fraction by high performance thin layer chromatography revealed carryover in each fraction. In other words, compounds present in the 500 MW filtrate were seen in the other fractions. However, components present in the 50,000 MW filtrate were not seen in the lower MW filtrates. It is believed that entrapment of the smaller MW components by

larger, possibly polymeric, components interfered with filtrate efficiency.

In a second experiment, complete gugulipid was filtered through a 500 MW filter only. The second experiment is described in a greater detail hereinbelow. The low molecular weight fraction obtained from the second experiment was used in the Examples below.

Materials

Gugulipid (Lot # 42941-38285) standardized to 10% total guggalsterone was from Indena® (Seattle, WA) and HPLC grade methanol was from Fischer Scientific (Fair Lawn, NJ). The Ultrafiltration unit was a 50 mL Amicon 8050 Ultrafiltration cell and the membrane was a YC05 500 Da cutoff filter (Cat. No. 13022) , both from Millipore (Bedford, MA). The air tank used to supply pressure to the filtration cell was a T (tall) size nitrogen tank fitted with a Fischer Scientific FS-700 nitrogen pressure gauge. The pressure gauge was always preset to deliver a maximum pressure of 35 psi and then increased as needed. The filtration unit itself cannot be operated safely at pressures above 75 psi.

It should be noted that a 500 Da cutoff filter may potentially have an error or +300 Da. Thus the filter employed may have passed molecules up to about 800 Da.

Filter Preparation:

The YC05 filter was prepared by floating it for 30 min.(shiny side down, which is the side that will eventually face the sample) in a 1.0 L beaker containing 1.0 L of Milli-Q water. The filter was then placed in the filter

support of the filtration unit (shiny side up) and the sealing rubber "O" ring put in place. The solvent holding chamber of the filtration unit was placed on top of the filter support, and then the locking plate attached to finger tightness.

The membrane was rinsed with 25 to 30 mLs of the HPLC grade methanol prior to filtering the gugulipid samples. The alcohol was added by a graduated 50 mL transfer pipette and the magnetic stir assembly placed inside the solvent holding area. The top of the filtration unit was then affixed to allow proper movement of the stir assembly, and the entire unit placed inside the metal holding bracket. The entire apparatus was then placed on a magnetic stir plate and the line from the nitrogen tank attached.

The main valve to the nitrogen tank was opened (preset to deliver 35 psi) and the magnetic stir plate was put on setting 4 (on a setting scale of 1[slow] through 6 [fast]). As the methanol began to flow through the membrane and out the effluent line, the pressure was slowly increased to a maximum of 55 psi so that a final flow rate of 3 to 4 mLs/ min was attained. As it is not advised to run the membranes to complete dryness, the methanol rinse was terminated at a point to leave 1 to 2 mLs of solvent above the filter membrane. This was done by closing the main valve to the tank at the time the solvent had decreased to the desired point, and then venting the pressure from the filtration cell by way of it's release valve.

Filtration of gugulipid:

The gugulipid extract was dissolved to a final concentration of 6.5% (w/v) gugulipid using the HPLC grade methanol specified. After 10 -15 min., gugulipid was

dissolved in the solvent yielding a clear, medium brown solution (similar to ice tea). Upon standing, a fine talc-like, white precipitate was observed to make a thin coating at the bottom of the container. Gentle swirling of the solution caused the precipitate to slightly opacify the solution, but this would again clear if the solution was allowed to stand unaggitated for a few minutes.

The same process used to prepare the filter was also used to filter the gugulipid. The solution was vigorously stirred during sample removal so that a representative aliquot of the solution and precipitate could be removed. A total volume of 50 mL was delivered to the solvent holding chamber by way of a graduated transfer pipette, and the unit reassembled as outlined above. The maximal pressure used to filter the gugulipid did not exceed 55 psi. Once the sample had been decreased to 1-2 mLs, the system was vented and 25 mLs of clean methanol was introduced to the solvent holding chamber. This was then passed through the filter to the same point and repeated again with 15 mls of methanol.

All effluent was collected in the same container (approximately 90 mLs) and represented the low molecular weight fraction. The retentate material (still residing in the solvent holding chamber or on the filter itself) was collected by extensive rinsing of the filter and holding chamber with methanol. This material was then collected together and represented the high molecular weight fraction material.

The containers containing both fractions were placed on a Pierce Reacti-therm III heating module equipped with a Reacti-vap III nitrogen drying assembly. Under a constant nitrogen stream of 2 psi, the heat was slowly increase over

20 min. to a maximum setting of 4 -5 (setting range 1 [low] to 10 [high]). These conditions were maintained until the samples reached complete dryness. After allowing the containers to cool to room temperature, samples were redissolved in small amounts of methanol and quantitatively transferred to pre-tarred test tubes for mass determinations.

84% of the gugulipid was recovered in a low molecular weight fraction.

Different Characteristics of the Fractions Recovered:

The physical characteristics of the alcoholic, the high molecular weight and the low molecular weight fractions were found to be quite different. The high molecular weight fraction is more similar to the starting gugulipid material in that it still has a black tar-like color and is a solid at room temperature. The alcoholic fraction was a dark brown color and is also solid at room temoerature. The low molecular weight fraction has a golden/amber color and, although it is quite viscous, it does have a certain fluid character at room temperature.

EXAMPLE 2

This example reports an in vitro analysis of sebum suppression of gugulipid and various fractions thereof.

In Vitro Sebocyte Lipogenesis Assay:

Human sebaceous glands were isolated from the nose of a male (age 60) and cultured using submerged tissue culture techniques (Bajor et al, J. Invest. Dermatol. 102: 1994, P.

564). These sebocytes accumulate intracellular lipid droplets characteristic of mature human sebum.

Harvested and passaged sebocytes were added to each well of a 48 well tissue culture plate and incubated at 37°C in the presence of 7.5% CO_2 for 10 days. On the day of experimentation, the growth medium was removed and the sebocytes washed three times with phosphate buffered saline (PBS). Fresh PBS in 0.5 ml amount was added to each well and 10 µl of a test agent, at various concentrations as indicated in Table 1. Triplicate wells were utilized for each sample. Controls consisted of PBS, dimethyl sulfoxide (DMSO) used to solubilize the lipophilic compounds, and phenol red, a compound which possesses estrogen-like activity (Phenol Red decreases sebum secretion and was used as a control to verify the integrity of the sebocyte assay). The cultures were incubated at 37°C/7.5% CO_2 for 30 minutes. Radioactive label was prepared by adding 100 µl of ^{14}C labelled acetic acid (Amersham, sodium salt, specific activity of 56 mCi/mmol) to 10 ml of 50 mM sodium acetate buffer. Then, 50 µl was added to each well containing the sebocytes and test agents. The cultures were returned to the incubator for four hours. Thereafter, the sebocytes were rinsed three times with fresh PBS to remove unbound active and radioactive label. Radioactive label remaining in the cultured sebocytes was counted using a Beckman scintillation counter. The results were expressed as % reduction compared to control (DMSO).

To determine whether a fraction contained cis-guggulsterone, a sample of each fraction was applied to the lower left corner of a separate 10x10 cm high performance silica gel plate. Samples were first developed to 9 cm in a running phase of hexane:ethyl acetate (50:50), air died, turned 90 degrees, then developed to 9 cm in chloroform:methanol

(95:5). Plates were dried, immersed into a Solution of 10 %copper sulfate/8% phosphoric acid, and heated to 185°C for 10 minutes. A standard of cis-Guggulsterone (Steraloids, Inc.) was used as a reference.

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The results that were obtained are summarized in Table 1.

TABLE 1

Fraction #	Guggul-sterone presence	0.005% % Red'n (SD)	0.01% % Red'n (SD)	0.05% % Red'n (SD)	0.10% % Red'n (SD)
Complete Gugulipid	++	37.8* (8.1)	52.8* (7.2)	62.6* (6.0)	66.3* (4.0)
0 (Pet Ether)	++	-1.8 (13.6)	47.1* (16.3)	63.6* (1.4)	78.2* (0.9)
10	++	-	-	-3.8 (3.5)	19.2* (4.5)
14	++	-	-	18.1 (4.3)	13.1 (8.1)
15	+++	-	-	17.8 (5.6)	20.0* (4.3)
16	++++	-	-	39.9* (0.1)	38.6* (2.3)
20	+	-	-	27.5* (3.3)	32.1* (3.0)
21	?[1]	23.9* (3.9)	26.3* (4.3)	62.6* (5.8)	64.3* (6.6)
22	?	31* (4.9)	46.1* (4.9)	65.9* (3.8)	-
24	?	35.5* (3.8)	43.9* (9.1)	50.6* (5.2)	67.5* (1.1)
27	-	38.7* (6.6)	36.0* (11.1)	63.5* (4.6)	74.0* (2.3)
28	-	38.4* (11.4)	59.6* (6.0)	-	69.0* (2.5)
32	-	39.3* (4.4)	50.2* (4.9)	65.4* (0.4)	78.3* (0.6)
33	-	29.1* (9.7)	34.3* (8.2)	65.9* (3.8)	68.5* (2.9)
35	-	27.8 (13.7)	32.3* (13.4)	70.9* (2.3)	-
40-42 (99% of 42)	-	56* (5.2)	60.1* (12.2)	64.3* (6.6)	71.8* (8.6)
43	?	49.4* (5.5)	68.5* (5.5)	64.9* (6.1)	78.3* (0.6)
44	-	25* (7.7)	44.6* (9.2)	75.1* (6.1)	73.1* (0.9)

Phenol Red	-	-	-	-	40.9* (2.5)

statistically significant results compared to control (DMSO) at $p < 0.01$ (calculated using student T-test)

[1] "?" indicates migration to a similar area in the chromatogram but unclear if it is the cis-guggulsterone (it charred a different color). The level present was extremely low.

The analysis of the results in Table 1 is most meaningful at the lowest concentration tested, because at higher concentrations in vitro sebocyte viability may be compromised. Also, at higher concentrations, even a poor antisebum active which may be present in a non-alcoholic fraction may deliver a relatively high activity. It is evident from the results in Table 1 that at the lowest concentration tested (0.005%) fractions 42 and 43 (alcoholic fractions) were the most active in suppressing sebum production. These fractions do not appear to contain any cis-guggulsterone. The reduced activity of fraction 44 compared to fractions 42 and 43 is probably due the reduced concentration of any active material, because fraction 44 was the end portion of the alcoholic fraction.

The in-vitro sebocyte assay was repeated for oil soluble extract of C. wightii. The results that were obtained are summarized in Table 1A.

TABLE 1A

Treatment	Concentration	% Reduction	STD DEV
C. wightii extract (Oil Soluble)	0.01%	40.6	9.4
	0.10%	44.9	4.4

It can be seen from Table 1A that oil soluble C. wightii extract also decreased sebum secretion.

For further experimentation with the low molecular weight fraction ("low MW fraction"), the following changes to the *in vitro* sebocyte protocol were made. The low molecular weight fraction was added at 1 µl and 5 µl to each well containing the sebocytes. Quadruplicate wells were utilized for each test sample. Controls consisted of DMSO and complete gugulipid as an internal positive control. The results that were obtained are summarized in Table 1B.

TABLE 1B

Treatment	Concentration	% Reduction	STD DEV
EXPERIMENT 1			
Gugulipid	0.01%	50.9	11.7
Low MW fraction	0.01%	47.9	8.6
Low MW fraction	0.04%	54.5	10.5
EXPERIMENT 2			
Gugulipid	0.01%	62	6.9
Low MW fraction	0.01%	53.6	7.0
Low MW fraction	0.04%	61.4	4.2

It is evident that the low molecular weight fraction has excellent anti-sebum activity. When a high molecular weight fraction was tested, a similar reduction in sebum secretion was obtained. The use of a low molecular weight fraction is advantageous, however, in that it is easier to formulate than either the gugulipid or the alcoholic fraction or the high molecular weight fraction: the low molecular weight fraction is less viscous and has a gloden/amber rather than dark brown/black color. The low molecular weight fraction is further advantageous compared to the alcoholic fraction because it can be obtained in a higher yield than the alcoholic fraction.

COMPARATIVE EXAMPLE 3

The sebocyte assay described in Example 2 was repeated with various compounds as indicated in Table 2. All compounds in Table 2 are outside the scope of the invention.

The results that were obtained are summarized in Table 2. Negative values indicate increase in sebum production.

TABLE 2

Treatment	Concentration	% Reduction	STD DEV
Estradiol	0.0028% (100 μM)	39.7	7.9
Dihydrotestosterone	0.00003% (1 μM)	-28.8	4.1
Salicyclic Acid	0.14% (10.0 mM)	3.6	7.4
	0.10%	-46.6	12.4
cis-guggalsterone	0.05%	2.7	8.1
	0.01%	2.7	8.8

The results in Table 2 demonstrate that the sebocyte assay is a valid and reliable test for measuring sebum suppression, because estradiol (estrogen-like compound) provided sebum suppression, as predicted from the other sources, whereas dihydrotestosterone (androgen) actually increased sebum production, as also predicted from other sources. Salicylic acid, a known anti-acne agent did not inhibit sebum output, demonstrating that an antiacne agent does not necessarily have antisebum activity. Cis-guggulsterone did not reduce sebum secretion, indicating that an active in gugulipid and in the alcoholic or low molecular weight fraction thereof which provides antisebum activity is not cis-guggulsterone.

EXAMPLE 4

This example reports a chemical assay and an in vitro analysis of antioxidant activity of gugulipid and various fractions thereof.

Chemical Assay:

Chemical assay measures the antioxidant activity of various test compounds indicated in Table 3 (each tested at a concentration of 0.08%, except the low MW fraction, which was tested at 1.67% and 0.17%). 2,2'azino-di-[3-ethylbenzthialoine sulphonate] (6.1 µmol/l) and metmyoglobin (610 µmol/l) were solubolized in phosphate buffered saline (5mmol/l, pH 7.4). Test materials were then added and absorbance was measured at 734 nm before and after addition of the substrate, hydrogen peroxide (250 µmol/l). The initial absorbance was subtracted from the substrate containing absorbance. This prevents discrepancies in absorbance due to the test compound itself. The absorbance changes with time, thus multiple time points were examined. Results were expressed as % oxidation relative to a control containing all assay components but deionized water instead of test reagent (100% oxidation). A high number means no prevention of oxidation, a poor antioxidant. The antioxidant activity of Trolox (registered trademark of Hoffman-LaRoche), a water soluble form of vitamin E was measured to establish the validilty of the test. Trolox was purchased from Aldrich (2.5 mmol/l). C. wightii (lipophilic extract) was obtained from Campo.

The results that were obtained are summarized in Table 3.

TABLE 3

TEST MATERIAL	% *oxidation (STD DEV) at 3 minutes relative to water control*	% *oxidation (STD DEV) at 6 minutes relative to water control*	% *oxidation (STD DEV) at 9 minutes relative to water control*
Trolox (water soluble vitamin E)	-0.70 (0.5)*	15.8 (2.1)*	44.6 (2.1)
Total Guggulipid	2.8 (1.1)*	4.4 (0.08)*	5.1 (0.6)*
Fraction #0	60.7 (8.3)	72.5 (4.7)	73.8 (0.9)
Fraction #10	82.9 (3.5)	88.7 (2.3)	88.2 (0.2)
Fraction #14	98.4 (1.0)	99.4 (0.9)	111.1 (16.7)
Fraction #15	94.6 (0.6)	96.5 (0.3)	94.4 (2.0)
Fraction #16	97.6 (4.7)	93.8 (5.2)	87.9 (8.4)
Fraction #20	65.8 (14.7)	71.2 (8.3)	70.1 (1.7)*
Fraction #21	47.7 (0.54)*	49.9 (0.6)*	50.5 (2.6)
Fraction #24	46.2 (6.9)	56.9 (6.2)	60.9 (3.6)*
Fraction #27	79.8 (7.1)	78.0 (4.6)	77.2 (2.1)*
Fraction #40-42 (99% of 42)	18.7 (3.1)*	10.6 (1.7)*	9.2 (0.7)*
Fraction #43	-33.2 (21.2)*	-4.5 (2.2)*	-3.2 (1.6)*
Fraction #44	-12.6 (8.6)*	-6.5 (4.9)*	-4.4 (3.4)*
cis-guggulsterone	100 (10.0	97.9 (4.1)	97.4 (6.1)
C. Wightii	85.6 (0.0)	98.0 (5.3)	99.9 (7.1)

* p-value < 0.05 (statistically significant compared to water control): p-value was determined using Lotus 1-2-3 students t-test.

The results in Table 3 demonstrate that total gugulipid and fractions 42 - 44 had the best anti-oxidant activity, with raction 43 having the highest activity. Cis-guggulsterone had a very inferior anti-oxidant activity, proving that it is other actives, not cis-guggulsterone, that impart anti-oxidant activity to gugulipid or to the alcoholic fraction of gugulipid. Neither C. wightii nor the aqueous extract of C. mukul had significant antioxidant activity. The chemical assay outlined above measures antioxidant activity obtained via direct free radical quenching, not via antiinflammatory pathway. The assay establishes that gugulipid and the alcoholic fraction thereof act as antioxidants via direct free radical quenching.

The chemical assay above was repeated in a separate experiment to test the low molecular weight fraction. The results that were obtained are summarized in Table 3A.

TABLE 3A

TEST MATERIAL	% *oxidation at 3 minutes relative to water control*	% *oxidation at 6 minutes relative to water control*	% *oxidation at 9 inutes relative to water control*
Ethanol	98.79	91.16	87.5
Low MW fraction (at 0. 167 %)	49.70	44.86	42.59
Low MW fraction (at 1.67 %)	-43.83	-28.06	-21.37

It is evident from the results in Table 3A that the low molecular weight fraction has excellent anti-oxidant activity, at both concentrations tested.

In general, the antioxidant capability measured chemically correlates with in vitro keratinocyte results (see Table 4 below).

In vitro antioxidant assay:

Lipid peroxidation is a well established mechanism of cellular injury, which occurs in both plants and animals. Cleavage of unsaturated fatty acids, including membrane lipids, leads to the production of byproducts such as aldehydes. The aldehydic byproducts malondialdehyde (MDA) and 4-hydroxyalkenals serve as convenient markers of lipid peroxidation.

Cell culture:

Human keratinocytes, isolated from neonatal foreskin, were grown on a feeder layer of mytomycin treated T3T mouse fibroblasts in Complete Media (DME + f-12, 1 M Hepes, Adenine, Hydrocortisone, Cholera Toxin, Insulin, EGF, FBS, and Penn-Strep) and in the presence of 0.09 mM calcium. Following treatment with trypsin, keratinocytes were seeded at 15 x 10^3 cells/well in 12 well tissue culture plates (Costar) in 1000µL of complete KSFM (keratinocyte serum-free medium from Life Technologies) containing 0.09 mM calcium.

After 96 hours of incubation, the medium was removed, and fresh medium was added along with 0.01% of a test agent, as indicated in Table 4, within KSFM. 48 hours after incubation with the test agent the medium was again removed and 1 ml of phosphate buffered saline containing 1 mM hydrogen peroxide was added to each well. 1mM of ferrous sulfate was then added to begin the oxidation. After 90 minutes of incubation with this

oxidant mixture, oxidation was quenched by the addition of BHT. Plates were then stored at -70°C awaiting further analysis.

Lipid peroxidation products were measured as a marker of oxidative stress and normalized to the amount of cell DNA to account for proliferation.

DNA Assay:

Plates were removed from the freezer, media was aspirated. Plates were freeze fractured three times. Hoechst dye (10 g/mL final concentration) was added to each well and plated were incubated in the dark. After 15 minutes of incubation, plates were read in a fluorimeter (excitation 360 nm and emission 460 nm).

Lipid Peroxidation:

Culture wells containing the cells oxidized in PBS were scraped and 200 L of sample was taken. The chromagen N-methyl-2-phenylindole reacts with the test sample in acid at 45°C. Under the given conditions, one molecule of either MDA or 4-hydroxyalkenal will react with 2 molecules of the reagent to produce a stable chromophore with maximal absorbance at 586 nm. Results were expressed as (absorbance of untreated cells = 100% lipid oxidation)/DNA absorbance. Untreated cells (no antioxidant) had peroxidation of 100% +/- 10.1. Alpha-tocopherol was used as a positive control.

The results that were obtained are summarized in Table 4.

TABLE 4

TEST MATERIAL *(0.01%)*	*% lipid oxidation/DNA (STD DEV) relative to water control [100 %(10.1)]*
alpha-tocopherol	61.4 (8.9)
Total Guggulipid	85.7 (15.8)
Fraction #0	85.2 (3.7)
Fraction #10	155.4 (19.9)
Fraction #14	205.9 (13.9)
Fraction #15	118.7 (19.8)
Fraction #21	159.8 (22.7)
Fraction #24	82.4 (5.7)
Fraction #42	53.2 (21.3)
Fraction #43	74.1 (2.7)*
Fraction #44	84.2 (3.8)
C. Wightii	167.2 (46.0)

p-value was determined using Lotus 1-2-3 students t-test.
* p-value < 0.05 (statistically significant compared to untreated cells)

The results in Table 4 indicate that gugulipid and fractions 42-44 (alcoholic fractions) had antioxidant activity as predicted by the chemical assay. Alcoholic fractions 42-44 had the highest antioxidant activity, approaching the antioxidant activity of alpha-tocopherol. C. wightii still did not have antioxidant activity. Some of the other fractions had good antioxidant activity, even though they had only marginal activity in the chemical assay. It should be noted that the concentration tested in the in-vitro peroxidation assay was relatively high, i.e. twice as high as the lowest concentration

tested in a sebocyte assay. It is believed that at the lower concentration, more differences in the activities of various fractions would have been observed. The reduced activity of fraction 44 compared to fractions 42 and 43 is probably due the reduced concentration of any active material, because fraction 44 was the end portion of the alcoholic fraction. In any event, as noted above, gugulipid and the alcoholic fractions thereof did exhibit antioxidant activity.

The results in Example 4 demonstrate the criticality of using gugulipid from C. mukul, rather than guggal from C. wightii, in order to deliver antioxidant activity.

In Examples 1-4, when fractions of gugulipid were tested, alcoholic and low molecular weight fractions had the highest antisebum and antioxidant activity. Gugulipid from C. mukul and alcoholic and low molecular weight fractions of gugulipid from C. mukul had optimum dual antisebum and antioxidant activity.

EXAMPLE 5

TEST PRODUCT:

A toner containing gugulipid was formulated as follows:

INGREDIENT	% w/w
Water	to 100%
Alcohol (SDA 40-B)	35.00
Green Tea	1.00
PEG-40 Hydrogenated Castor Oil	1.00
PPG-5-ceteth 20 (ethoxylated cetyl alcohol)	1.00
Gugulipid	0.20

SUBJECTS: Panelists who were self-proclaimed to have oily skin were screened for participation using the Sebumeter. The women ranged between the ages of 18 and 55. From this group, 11 women who met requirements of having oily skin as defined by a site score average (3 Sebumeter readings) $\geq$ 150, were chosen to participate in the five week product evaluation.

Use of the topical acne medications (e.g., Retin A, Alpha Hydroxy/Fruit Acids products, alcoholic toners, and masks/exfoliators) were suspended beginning one week prior to the study and continuing throughout the duration of the study.

TEST CONDITIONS: The test environment was set to maintain a room temperature of 8.5°C ± 1°C (73 ± 1EF), and relative humidity of 45 ± 10%.

PROCEDURE: Panelists were evaluated, once weekly, twice (except week one) each day over the five week period. After the initial evaluation which was used for baseline results, panelists were instructed to use a test product on half of their face for a total of three (3) weeks, followed by one (1) week of regression (no treatment). During the evaluation days, panelists were required to cleanse and NOT use test toner at least three (3) hours prior to AM evaluation periods. Panelists were also required NOT to wear make-up on foreheads throughout the day during evaluation periods. Panelists were required to be available for all evaluation times and agree not to touch, rub, or disturb the test site in anyway throughout the duration of the test day.

At the beginning of each evaluation period (8:30-11:30), panelists came having washed their foreheads, at least three (3) hours prior, with their regular facial cleanser/soap. Test site areas measuring approximately 18 cm^2 were located on the panelist's forehead immediately above each eyebrow line. Utilizing the split forehead design, morning values for each panelist were established through three Sebumeter (manufactured by Courage and Kahazaka, Koln, Germany, model #SM810) readings done within each test site area. The Sebumeter cassette was held in contact with the skin for 30 seconds while a constant pressure of 10 N was applied. After the 30 seconds have counted down, the device beeps for the cassette to be placed back into it for a reading. In 1 second, the measured value flashes on the instrument. Two additional readings per site were acquired, with the average used as the panelist's AM score. Panelists were then allowed to apply a product, with the left/right site application balanced based on degree of

oiliness across panelists. Test site areas were treated with Code 426, 881 or 502 as per the usage instructions. Additional Sebumeter readings were obtained at five (5) hours after treatment.

TREATMENT: Applications of test product occurred one to two times daily. They involved dispensing an ad lib amount of product using a cotton ball in a randomized half-face design.

ANALYSIS: The decoded and sorted data was analyzed using Lotus 123 and SAS software. Paired t-Tests (Treated - Untreated) were run on the data sets from each treatment group, at each evaluation time.

The results that were obtained are summarized in Table 5 below.

TABLE 5

DIFFERENCE VALUES					
PANELIST#	BASE	WEEK ONE		WEEK TWO	
	AM TR-UNT	AM TR-UNT	PM TR-UNT	AM TR-UNT	PM TR-UNT
1	23.7	46.0	-25.7	-6.3	3.7
2	-6.7	-61.7	-23.7	074.0	-11.3
3	-39.0	33.7	-4.7	33.0	8.0
4	27.7	17.3	11.7	-68.3	-29.0
5	22.3			-28.7	-63.7
6	23.3	-39.0	-84.7	-26.7	-24.3
7	52.0	-79.3	-65.0	-70.0	-52.3
8	-19.7	-19.3	6.0	-30.3	-39.3
9	-12.3	4.7	-18.0	4.7	13.7
10	-37.3	-29.3	-10.7	30.7	10.0
11	-17.0	-18.3	8.7	-37.0	-64.0
AVG	**1.5**	**-14.5**	**-20.6**	**-24.8**	**-22.6**

TABLE 5 - CONTINUED

DIFFERENCE VALUES				
PANELIST#	WEEK THREE		REGRESSION	
	AM TR-UNT	PM TR-UNT	AM TR-UNT	PM TR-UNT
1	-8.0	-121.0	6.0	-51.0
2	-25.0	-20.0	-25.3	-56.0
3	0.3	10.0	-30.7	-11.0
4	-79.0	1.3	-24.0	3.7
5	-45.0	19.0	30.0	0.0
6	-7.0	-4.3	-26.3	3.0
7	31.0	39.0	24.3	-29.7
8	-15.0	-89.0	-19.0	63.0
9	54.7	33.3	-2.0	0.0
10	44.3	44.7	-17.0	-44.0
11	-96.0	-9.7	15.0	-12.0
AVG	**-13.2**	**-8.8**	**-6.3**	**-12.2**

RESULTS: The sites treated with Gugulipid scored lower than the untreated sites at all time periods. Average scores for the sites treated with Gugulipid ranged between 183 and 242 (baseline = 227), while the average scores for the untreated sites ranged between 197 to 263 (baseline = 225). According to a paired comparison of the treated minus untreated readings each evaluation time, only the Week 2 PM value for the group using toner with gugulipid was statistically ($p < 0.05$) significantly different from zero, while the Week 1 PM value exhibited marginal ($p < 0.10$) significance. The lack

of statistical significance may be attributed to the high degree of variability in the data and the small sample size.

CONCLUSION: The application to the skin of women with increased sebum production of a toner with gugulipid led to consistently lower oil levels for treated sites compared to the untreated control sites.

Examples 6-9 illustrate topical compositions according to the present invention. The compositions can be processed in conventional manner. They are suitable for cosmetic use. In particular the compositions are suitable for application to oily, wrinkled, aged and/or photodamaged skin, to improve the appearance (radiance, clarity, finish) and feel thereof as well as for application to healthy skin to prevent or retard oiliness or deterioration thereof.

EXAMPLE 6

The following is a typical antisebum and anti-oxidant composition within the scope of the claims defining the present invention:

Oil Controlling Lotion

INGREDIENT	% W/W
DI Water	QS
Propylene Glycol	1.000
Xanthan Gum	0.200
Disodium EDTA	0.100
Methylparaben	0.300
Polysorbate 20	1.500
Octyl Methoxycinnamate	2.000
Retinyl Linoleate	0.100
Tridecyl Salicylate	2.000
Low MW fraction of Gugulipid	0.001
Cetyl Alcohol	1.500
PEG-165 Glycerol Stearate	3.000
Propylparaben	0.100
Cyclomethicone	15.000
Dimethicone	2.000
Dimethiconol	0.500
Micronized Titanium Dioxide	0.500
Sodium Hyaluaronate 1% sln	3.000
Triethanolamine 99%	0.200
Salicyclic Acid	0.200
Phenoxyethanol	0.350

EXAMPLE 7

A leave-on facial emulsion composition is prepared by combining the following components utilizing conventional mixing techniques.

INGREDIENT	% WEIGHT
Water	qs
Glycerin	3.00
Cetyl Palmitate	3.00
Cetyl Alcohol	1.26
Glyceryl Monohydroxy Stearate	0.74
Dimethicone	0.60
Stearic Acid	0.55
Octyldodecyl Myristate	0.30
Potassium Hydroxide	0.20
Carbomer 1342	0.125
Tetrasodium EDTA	0.125
DMDM Hydantoin and Iodopropynyl	0.10
Gugulipid	0.10
Carbomer 951	0.075

This emulsion is useful for providing control of sebum secretion and protecting the skin from free radical damage.

EXAMPLE 8

A leave-on facial emulsion composition is prepared by combining the following components utilizing conventional mixing techniques.

INGREDIENT	% WEIGHT
Water	qs
Glycerin	3.00
Cetyl Palmitate	3.00
Cetyl Alcohol	1.26
Quaternium-22	1.00
Glyceryl Monohydroxy Stearate	0.74
Dimethicone	0.60
Stearic Acid	0.55
Cyclomethicone and Dimethiconol	0.50
Octyldodecyl Myristate	0.30
Potassium Hydroxide	0.20
Carbomer 1342	0.125
Tetrasodium EDTA	0.10
DMDM Hydantoin and Iodopropynyl	0.10
Low MW fraction of gugulipid	0.05
Carbomer 951	0.075

EXAMPLE 9

The following are additional examples of typical antisebum and anti-oxidant composition within the scope of the claims defining the present invention:

EXAMPLE 9A: Skin Cream (Oil in Water type)

Chemical	% w/w
Water	qs
Disodium EDTA	0.100
Polysorbate 40	2.000
Butylene Glycol	3.000
Glycerin	5.000
Methylparaben	0.300
Retinyl Palmitate	0.300
Gugulipid	0.100
Isopropyl Palmitate	2.000
Isostearyl Isostearate	3.000
Dimethicone, 200 cst	2.000
Cyclomethicone	10.00
Imidazolidinyl Urea	0.200
Polyacrylamide	3.000

EXAMPLE 9B: Skin Cream (Oil in Water type)

Chemical	% w/w
Water	qs
Carbopol 1382	0.300
Disodium EDTA	0.100
Tween 40	5.000
Propylene Glycol	1.000
Glycerin	3.000
Methylparaben	0.300
Triethanolamine 99%	0.300
Tridecyl Salicylate	1.200
Retinol	0.100
Squalane	1.000
Alcoholic fraction of Gugulipid	0.500
Shea Butter	0.500
Cetyl Alcohol	1.500
Octyl Palmitate	2.000
C12-15 Alkyl Benzoate	5.000
Octyl Stearate	2.000
Silicone 344 Fluid (Cyclomethicone)	2.000
Imidazolidinyl Urea	0.200

EXAMPLE 9C: Skin Cream (Oil in Water type)

Chemical	% w/w
Water	qs
Carbopol 1382	0.250
Disodium EDTA	0.100
Butylene Glycol	2.000
Glycerin	3.000
Methylparaben	0.250
Triethanolamine 99%	0.250
Capric/Caprylic Triglyceride	5.000
Shea Butter	0.500
Cetyl Alcohol	1.000
PEG-100 Glycerol Monostearate	4.000
C12-15 Alkyl Benzoate	6.000
Tocopheryl Linoleate	0.500
Low MW fraction of Gugulipid	0.250
Silicone 200 Fluid (Dimethicone)	2.000
Imidazolidinyl Urea	0.200

EXAMPLE 9D: Micro Emulsion

Chemical	% w/w
PPG-5-Ceteth-20	4.000
PEG-40 Hydrogenated Castor Oil	1.750
Polyglyceryl-10 Decaoleate	10.00
PEG-8 Caprylic/Capric Glycerides	10.00
SDA Alcohol 40B	12.00
Isodecyl Neopentanoate	16.00
Glyceryl Trioctanoate	8.000
Cyclomethicone (DC 344 Fluid)	8.000
Propylparaben	0.100
Isostearic Acid	2.500
Tridecyl Salicylate	2.500
Low MW fraction of gugulipid	0.300
Phenoxyethanol	0.300
Deionized Water	QS

EXAMPLE 9E: Skin Cream (Water in Oil type)

Chemical	% w/w
Cyclomethicone (DC 344 Fluid)	12.000
Dimethicone (DC 200/10 fluid)	2.000
Dimethicone Copolyol	2.500
Cetyl Dimethicone	0.500
C12-15 Alkyl Benzoate	3.000
Tridecyl Salicylate	5.000
Gugulipid	0.500
Glycerin	3.000
Propylene Glycol	2.000
Disodium EDTA	0.100
Methylparaben	0.250
Sodium Chloride	1.200
Phenoxyethanol	0.200
Deionized Water	QS

EXAMPLE 9F: Anhydrous Serum

Chemical	% w/w
SD Alcohol 40 B (200 proof)	20.00
Cyclomethicone (DC 344 Fluid)	2.500
Squalene	1.000
Octyl Isononanoate	2.500
Dimethicone (DC 200 Fluid)	5.200
Isononyl Isononanoate	30.00
PEG-7 Glyceryl Cocoate	1.000
Polyglycerol Ricinoleate	3.000
Gugulipid	1.000
Butylene Glycol	1.000
Propylparaben	0.100
Dimethiconol	2.750

EXAMPLE 9G: Sunscreen Lotion (Oil in Water type)

Chemical	% w/w
Water	qs
Disodium EDTA	0.100
Butylene Glycol	2.000
Glycerin	5.000
Methylparaben	0.250
Octyl Methoxycinnamate	7.500
Benzophenone-3	2.500
Shea Butter	0.500
Cetyl Alcohol	1.500
Octyl Palmitate	2.000
C12-15 Alkyl Benzoate	2.000
Silicone 200 Fluid (Dimethicone)	1.000
Low MW fraction of Gugulipid	2.000
Imidazolidinyl Urea	0.200
Laureth-7, Polyacrylamide	3.500

It should be understood that the specific forms of the invention herein illustrated and described are intended to be representative only. Changes, including but not limited to those suggested in this specification, may be made in the illustrated embodiments without departing from the clear teachings of the disclosure. Accordingly, reference should be made to the following appended claims in determining the full scope of the invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

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

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A cosmetic composition for skin care comprising

(a) from 0.0001 wt. % to 10 wt. % of low molecular weight fraction of 1000 Da or less of gugulipid; and

(b) a cosmetically acceptable vehicle.

2. A cosmetic method of reducing, preventing or controlling sebum secretion from sebocytes, the method comprising applying to the skin a composition comprising

(a) from 0.0001 wt. % to 10 wt. % of a low molecular weight fraction of 1000 Da or less of gugulipid; and

(b) a cosmetically acceptable vehicle.

3. A cosmetic method of protecting skin from free radical activity, the method comprising applying to the skin a composition comprising

(a) from 0.0001 wt. % to 10 wt. % of a low molecular weight fraction of 1000 Da or less of gugulipid; and

(b) a cosmetically acceptable vehicle.

4. The method of claims 2 or 3 wherein the low molecular weight fraction of gugulipid is obtained from Commiphora mukul.

5. A cosmetic use of an antioxidant/anti-sebum agent, selected from a low molecular weight fraction of 1000 Da or less of gugulipid, in simultaneously reducing or preventing oily skin conditions whilst also protecting skin from free radical activity.

6. A cosmetic composition as hereinbefore described with reference to the examples.

DATED THIS 11th day of March, 2002.

UNILEVER PLC
By Its Patent Attorneys
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