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(54) **Title:** IMPROVED METHODS FOR BOTANICAL AND/OR ALGAE EXTRACTION

(57) **Abstract:** The present disclosure relates to methods for extraction of biomass. Biomass of most interest is that which contains biologically active, extracts suitable for the skin care market. The biomass of interest includes botanicals (plant extracts and bioferments thereof), algae (red, brown, green and red, including bioferments thereof), fungi and even animal extracts (insect, crustacean) origin. Further the use of said extracts in cosmetic preparations prepared by the disclosed method is envisioned.

Improved Methods for Botanical and/or Algae Extraction

This application takes the benefit of U.S. Provisional Application No. 61/656,221 filed June 6, 2012, the contents herein incorporated entirely by reference.

BACKGROUND OF THE INVENTION

A. Field of the Invention

The present invention relates to methods for extraction of biomass. Biomass of most interest is that which contains biologically active extracts suitable for the skin treatment market. The biomass of interest includes botanicals (plants and bioferments thereof), algae (red, brown, green and red, including bioferments thereof), fungi and even animal (insect, crustacean) origin and the use of said extracts in cosmetic preparations prepared by the disclosed method.

These natural product hydrophobic extracts are often used in cosmetic and pharmaceutical application. Natural products, particularly botanically, fungi and algae derived such as flavonoids or flavonoid derivatives, have demonstrable beneficial properties on the skin and hair. For example, these extracts have demonstrated antimicrobial, antiseptic, anti-inflammatory, antioxidant, enzyme stimulation or inhibition, pigmentation enhancement or control, photoprotective, treatment of skin aging, skin imperfections, dry skin, photodamaged skin, wrinkles, age spots, acne, skin lightening, psoriasis, and atopic dermatosis.

The botanical, algae and fungal species are for example any botanical, algae or fungal species which may server as a source for flavonoid or flavonoid derivatives.

More particularly for example the biomass may be selected from but is not limited to the group consisting of: *Acacia senegal*, *Achillea millefolium*, *Aloe barbadensis*, *Ananus sativus*, *Argania spinosa*, *Avena sativa*, *Cassia alata*, *Cocoa Callus*, *Cocos nucifera*, *Bupleurum falcatum*, *Butyrospermum parkii*, *Calluna vulgaris*, *Camellia sinensis*, *Chondrus crispus*, *Centella asiatica*, *Ceratonia siliqua*, *Cestrum latifolium*, *Cinnamomum cassia*, *Citrus limon*, *Coffea Arabica*, *Cola acuminata*, *Cucumis sativus*, *Durio zibethinus*, *Glycine soja* (soybean),

Glycyrrhiza glabra, Gymnema sylvestre, Haslea ostrearia, Heliantus annuus, Hibiscus abelmoschus, Humulus lupulus, Laminaria digitata, Lepidium meyenii, Linum usitatissimum, Macadamia ternifolia, Malva sylvestris, Melissa officinalis, Morus alba, Morus bombycis, Nereocystis luetkeana, Olea europaea, Orthosiphon stamineus, Palmeria palmate, Peucedanum graveolens, Peumus boldus, Pisum sativum, Pueraria lobata, Punicia granatum, Pyrus malus, Rheum palmatum, Rhodiola crenulata, Rosmarinus officinalis, Saxifraga sarmentosa, Sarrcodiotheca gaudichaudii, Scutellaria baicalensis, Serenoa serrulata, Spirulina platensis, Theobroma cacao, Tuber magnatum, Uncaria tomentosa, Vitis vinifera, Ptychopetalum olacoides, Zea mays and Zingiber officinale.

In particular extracts from algae are of special interest. For example, extraction of *Chondrus crispus, Nereocystis luetkeana* and *Sarrcodiotheca gaudichaudii, Fucus vesiculosus, Sarrcodiotheca gaudichaudii, Ulva lactuca, Laminaria longicuris, Nannochloropsis oculata, Tetraselmis suecica* are of special interest.

Extracts from plants such as *Cassia alata, Argania spinosa* and *Cocoa Callus* are also of preferred interest.

Description of Related Art

Extracts may be obtained from the whole plant, algae or fungi (i.e., the entire plant is used to prepare the extract) or from a part of the plant (e.g., leaf, stem, root, flower, seed, sap, bark, etc.). The extracts can also be derived by fermentation which helps to break down the cellular tissue of the algae or plant, thereby possibly increasing the bioavailability of the naturally occurring bioactive targets.

During extraction typically more than one compound is extracted from the botanical or algae and will frequently range from at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 of differing extractants in a single composition.

The skin active targets of particular interest are the flavonoids and flavonoid derivatives.

Typically the plant (or any part of the plant such as the leaves, stems, bark, roots, fruit, flowers or flower buds, seeds, seed pods, sap, whole plant, etc.) is disrupted by mechanical means which results in a puree. The puree is then processed to be substantially free of impurities or

undesired solids. The puree can then be poured into a shallow vessel and quickly exposed to low temperature, i.e., flash frozen, for example at -20 degrees centigrade or lower, preferably under a vacuum for removal of water content (lyophilization).

Aqueous, alcoholic, or oil based extraction techniques, or combinations thereof, have also been used in the past and are used on the whole plant or any part thereof of (e.g., leaves, stems, bark, roots, fruit, flowers or flower buds, seeds, seed pods, sap, whole plant, etc.) to produce an extract. In such a process, the desired part of the plant or the whole plant is crushed up (e.g., blender) and then extracted with the desired solvent (e.g., water, alcohol, water/alcohol, or oil based solvents) to obtain the desired extract. The extract can then be stored in liquid form, lyophilized, or subject to further processing techniques (e.g., heating, cooling, etc.). Extraction processes are well-known to those having ordinary skill in the extract field (e.g., maceration, infusion, percolation, digestion, decoction, hot continuous extraction, aqueous-alcoholic extract, counter current extract, microwave assisted extraction, ultrasound extraction, supercritical fluid extracts, phytonic extract (e.g., with hydro-fluoro-carbon solvents), etc.

It is known that certain surfactant water based systems may be used for extraction of various hydrocarbons. For example, Calvert, T. L. et al, *AIChE Journal*, (1994), Vol, 40, # 9, p1449-1457; Hurter, P.N. et. al. *Langmuir* 1992, 8, 1291-1299 teach the extraction of hydrocarbons from water waste systems.

Additionally, it is also known that some ethanol derived plant extracts can be solubilized in aqueous solutions containing poly(ethylene-oxide)-poly(propylene oxide) copolymers (V Dinou. et al, *Revista de Chimie*, 62/4, 396-400).

Further, U.S. Serial No. 2012/0010390 discloses the use of aqueous two phase systems for isolation of biomolecules or target compounds such as an antibody or a protein from a fermentation broth using ethylene oxide and propylene oxide random copolymers dissolved in one of the liquid phases. PCT application No. WO2012011589 discloses a method for the manufacturing of fat-soluble bioactive substances extracted from microbial cells using an organic solvent in combination with a surfactant.

There are however numerous disadvantages to extracting with solvents or even water especially when the extracts of interest are lipophilic, hydrophobic or amphiphilic. Although

water is environmentally friendly, it is limited as an effective extracting solvent when the desired extractants are hydrophobic. Organic solvents are less desirable because of their possible flammability and environmental drawbacks. Furthermore, when the extractants are desired for use in cosmetics, in particular skin active cosmetics such as moisturizers etc., solvents are most undesirable because of their irritating or drying effects on skin. The organic solvent can usually be removed by evaporation but this is an additional step with related economic and environmental costs.

Further there is a need to retrieve more complex targeted biomass extracts which can be obtained through traditional aqueous, glycol or ethanol extractions and preferably accompanied by higher overall concentrations of the targeted extractants. It is of special importance that highly active skin benefit agents are extracted from the biomass such as for example flavonoids and flavonoid derivatives. These actives have been shown to be particularly effective in preventing radical damage on skin incurred from UV exposure and oxidation reactions.

Even though certain skin active targets are known to be secondary metabolites in numerous plants, algae and fungi, prediction of the activity of the total extracts on skin, for example activity in protection against UV and radical induced skin damage is much harder to predict. Changing the extraction medium may give rise to increased amounts of known protectants such as flavonoids but this change will also be impacted by the increase or decrease in unknown co-extractants which in turn may alter the activity of the total extractant on skin. Thus, the need for alternative extraction methods which not only increase the extraction of skin actives but do not negatively impact the overall effect of the total extractants is desired. It would also be highly desirable if the total composition of the extracts gives overall better performance in protection of skin from the adverse effects of known skin harming agents such as ultraviolet rays, especially UV-B rays. In particular, it would be highly desirable to effectively partition the skin active target molecules without reliance on organic solvents and via an extraction method which achieves higher concentrations of targeted molecules. It would also be desirable for the partitioning matrix itself be suitable for cosmetic applications without further processing and for the partitioning matrix to protect the target molecules from oxidation and light degradation.

For certain biomass materials, extraction processes can retrieve cell toxic components as well as skin beneficial components as the partitioning occurs based on for example, solubility parameters. Thus typical extraction processes frequently do not adequately distinguish between the two (beneficial vs. toxic). The present inventors have found that the present process using

the particular nonionic surfactants of interest in many instances excludes the toxic extractants from the total extractant.

Further, it is also highly desirable that the extraction process retrieves relatively low color extractants but still retrieves the important target molecules and that the total extracted materials have high beneficial activity on skin.

Additionally, the extraction process for biomass can be quite time consuming especially in relation to the filtering step (removal of the biomass). For example, aqueous extract of an algae biomass may take as long as three days and use multiple filters to remove the solid biomass from the extracting liquid. It would be highly desirable to improve this filtering step by speeding up the filtering process and the elimination of multiple filters.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a comparison of extraction fingerprints from *Nereocystis luetkeana* using HPLC and different extraction mediums.

Bottom: 5% algae extraction medium with butylene glycol

Fourth: 5% algae extraction medium with DI water.

Third: 5% algae extraction medium with 10 wt. % Pluronic® L44 in water.

Second: 5% algae extraction medium with 10 wt. % Pluronic® F68 in water.

Top: 5% algae extraction medium with 10 wt. % Pluronic® F127 in water.

Figure 2 is picture of *Fucus vesiculosus* extraction medium with:

Left – water extraction only.

Right -water and 10 wt. % Pluronic® F127 extraction.

SUMMARY OF THE INVENTION

The Applicants have devised a solution to many of the above difficulties by extracting the biomass using aqueous solutions of nonionic surfactants. The algae, fungi or botanical biomasses are easily extracted gaining significant efficiency benefits and overall improvements in the biologically active compounds extracted.

Other non-trivial advantages are apparent from the improved process such as color and odor improvements of the extractants. See Figure 2. The avoidance of solvents such as alcohols and glycols can be eliminated entirely in the present process and the extractant medium presently employed is even milder than those currently in use containing glycols and/or ethanol. Glycols in particular are not popular with 'green' and sensitive skin companies.

Quite significantly the discovered method allows for much faster filtration and a reduction in the number of filtrations necessary to remove the extracted biomass from the aqueous extractant and surfactant solution. This equates to huge manufacturing cost savings.

Accordingly, the Applicants claim:

A method of extraction of at least one target skin bioactive from algae, fungi or botanical biomass, which method comprises

- a) contacting the algae, fungi or botanical biomass with an aqueous liquid to form a slurry, wherein the aqueous liquid comprises about 0.01 to about 20, preferably 0.1 to about 10, and most preferably about 0.5 to about 5 wt. percent, especially 0.5 to about 2 or about 3 wt. % of a nonionic surfactant selected from the group consisting of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides, and the wt. percent is based on the total weight of the slurry,

and

- b) optionally, separating the biomass from the aqueous slurry, wherein the target skin bioactive is a flavonoid or flavonoid derivative.

Use of a nonionic surfactant in an aqueous medium to increase flux during a filtration process of a solid biomass from an aqueous extract,

wherein the nonionic surfactant is a surfactant selected from the group consisting of of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides and the aqueous extract comprises at least a flavonoid or flavonoid derivative.

The solid biomass is selected from algae, fungi or botanical biomass, preferably flavonoid rich biomass.

Use of a nonionic surfactant in an aqueous medium to increase the flavonoid extraction from a flavonoid rich plant, wherein the nonionic surfactant is a surfactant selected from the group consisting of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides.

DETAILED DESCRIPTION OF THE INVENTION

All percentages and ratios used herein are by weight of the total composition unless otherwise designated, and all temperatures are in degrees Celsius unless otherwise designated.

The term "safe and effective amount" as used herein means an amount of a compound or composition sufficient to induce a positive benefit as described herein, but low enough to avoid serious side effects in the judgment of the skilled artisan.

The term "slurry" as used herein means a suspension of plant, algae or fungi matter in water.

The term "molecular weight" as used herein refers to weight average molecular weight unless otherwise specified.

The term "comprising" for purposes of the invention is open ended, that is may include other components.

Plant, algae or fungi for purposes of this disclosure means any plant, algae or fungi, especially flavonoid rich plant, algae or fungi. For example the plant, algae or fungi, preferably flavonoid rich plant, algae or fungi may be selected for example from the group of genus species consisting of *Acacia senegal*, *Achillea millefolium*, *Aloe barbadensis*, *Ananus sativus*, *Argania spinosa*, *Avena sativa*, *Cocos nucifera*, *Bupleurum falcatum*, *Butyrospermum parkii*, *Calluna vulgaris*, *Camellia sinensis*, *Cassia alata*, *Cocoa Callus*, *Chondrus crispus*, *Centella asiatica*, *Ceratonia siliqua*, *Cestrum latifolium*, *Cinnamomum cassia*, *Citrus limon*, *Coffea Arabica*, *Cola acuminata*, *Cucumis sativus*, *Durio zibethinus*, *Fucus vesiculosus*, *Glycine soja* (soybean), *Glycyrrhiza glabra*, *Gymnema sylvestre*, *Haslea ostrearia*, *Heliantus annuus*, *Hibiscus abelmoschus*, *Humulus lupulus*, *Laminaria digitata*, *Lepidium meyenii*, *Linum usitatissimum*, *Macadamia ternifolia*, *Malva sylvestris*, *Melissa officinalis*, *Morus alba*, *Morus bombycis*, *Nereocystis luetkeana*, *Olea europaea*, *Orthosiphon stamineus*, *Palmeria palmate*, *Peucedanum graveolens*, *Peumus boldus*, *Pisum sativum*, *Pueraria lobata*, *Punica granatum*, *Pyrus malus*, *Rheum palmatum*, *Rhodiola crenulata*, *Rosmarinus officinalis*, *Saxifraga*

sarmentosa, *Sarrcodiotheca gaudichaudii*, *Scutellaria baicalensis*, *Serenoa serrulata*, *Spirulina platensis*, *Theobroma cacao*, *Tuber magnatum*, *Uncaria tomentosa*, *Vitis vinifera*, *Ptychopetalum olacoides*, *Zea mays* and *Zingiber officinale*.

Any of the plants, algae or fungi used in the present disclosure may be natural occurring, cultivated or genetically modified organisms (GMO). For industrial cosmetic applications in the case of algae, it may be preferred to use cultivated algae, since cultivation reduces the risk that supplies will become limited in the case of algae as aquaculture expands and marine environmental conditions change.

The plant, algae or fungi source may be pre-treated in a fermentation process to further breakdown the plant, algae or fungi biomass pulp possibly making any bioactives present in the biomass more readily available for extraction.

For example, *Nereocystis* is genus of edible sea kelp that forms thick beds of up to 74 meter plants on rocks in what are known as kelp forests. This sea kelp may be fermented with *Lactobacillus*, the same bacterium that produces yogurt. Fermentation breaks down the cellular tissue of the kelp leaf, thereby increasing the bioavailability of the naturally occurring phytonutrients that are abundant in kelp.

Fermentation and subsequent reduction of the kelp extract may result in the concentration of these vital nutrients for use in topical formulations. In skincare products it acts as excellent oil-free moisturizer.

The term "biomass" as used herein means a pulp or puree derived from the algae, plant or fungi. Preferably the term biomass refers to an algae or plant. The pulp or puree may be derived from the entire plant, algae or fungi or from a part of the plant (e.g., leaf, stem, root, flower, seed, sap, bark, etc.).

The marine algae may for example be preferably selected from the group consisting of *Chondrus crispus*, *Nereocystis luetkeana* and *Sarrcodiotheca gaudichaudii*, *Fucus vesiculosus*, *Sarrcodiotheca gaudichaudii*, *Ulva lactuca*, *Laminaria longicuris*, *Nannochloropsis oculata*, *Tetraselmis suecica*

The hydrophobic extracts from algae are especially interesting because the extracts are known to moisturize skin, have firming and anti-irritant properties which make the extracts highly suitable for aging skin. The algae extracts contain polysaccharide sugars such as alginates,

fucoidanes, polyphenols and fucosterol. Alginates are compounds responsible for moisture retention and elasticity of the skin. Fucoidanes are sulfated polysaccharides that encourage circulation and polyphenols have antiseptic, anti-inflammatory and anti-oxidant properties. Fucosterol functions as a emollient, moisturizer and blood stimulant

The term “extract” for purposes of this application means any skin active which has been extracted from the plant, algae or fungi biomass. In the present case, the extract is present in the extracting water/surfactant mixture. It is this mixture which is directly added to the cosmetic, topical or pharmaceutical product compositions. Indeed this is one of the advantages of the extracting method. The presence of the surfactant, in particular the Pluronic® does not need to be removed. It's presence in the final cosmetic, topical or pharmaceutical composition is desirable.

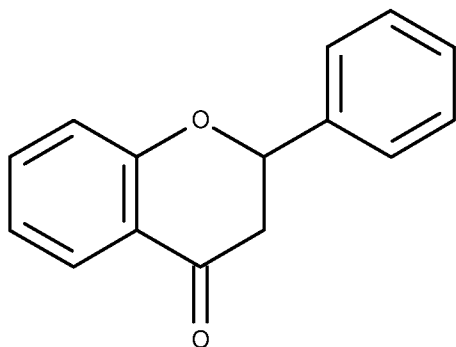
The extract will be present in the aqueous surfactant mixture liquid phase after extraction from the biomass.

Extraction for purposes of this application means treatment or exposure of the biomass (pulp or puree) with a liquid. Target bioactive moiety or moieties (at least one skin active) are retrieved from the solid biomass via solubilization in the liquid phase.

As explained previously the plant, algae or fungi is flavonoid rich. Extraction according to the presently disclosed methods of the flavonoid rich plant, algae or fungi will retrieve “target skin actives”.

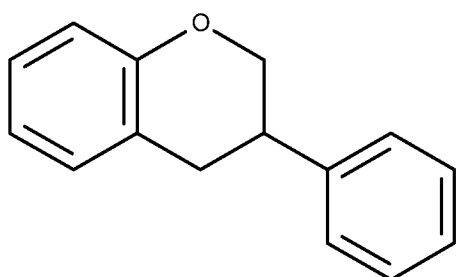
The term “target skin actives” to be extracted includes compounds containing the flavone backbone (2-phenyl-1,4-benzopyrone), isoflavone and the neoflavonoids backbones or derivatives thereof.

The flavone backbone is:



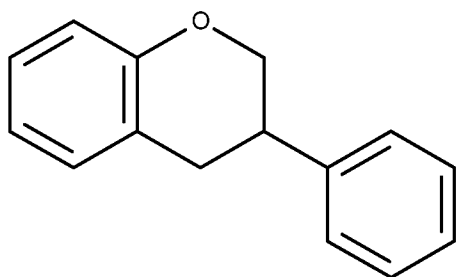
(A)

The isoflavan core is



(B)

The neoflavanoid core is



(C)

These above cores will normally be substituted by hydroxyl groups at various positions on the aromatic rings. In some cases such as myricitrin the oxygen containing ring is substituted by a sugar moiety. Accordingly the flavonoid or flavonoid derivative (the target skin actives) are selected from compounds which contain cores represented by (A), (B) and (C), the cores may be further substituted.

In terms of this disclosure what is meant by flavonoid-rich would preferably include plant, algae or fungi extract which contains flavonoids aglycones and or glycosides of flavones, of flavanones, of 3-hydroxyflavones (flavonoles), of aurones, and of isoflavones. Also preferred are biflavonoids constructed from two flavonoid units, for example, those occurring in ginkgo species. Further preferred flavonoids are the chalcones, especially phloricin, hesperidin methyl chalcone, and neohesperidin dihydrochalcone.

Accordingly a preferred listing of flavonoids would include abyssinone I, abyssinone V, afzelechin, ampelopsin, aromadendrin, asebogenin, auriculoside, betagarin, broussin, broussonin C, butin, butrin, (+)-catechin, catechin 7-O- β -xyloside, davidigenin, diffutin, 7,4'-dihydroxyflavan, 2,6-dihydroxy-4'-methoxydihydro-chalcone, 7,3'-dihydroxy-4-methoxy-8-methylflavan, 7,4'-dihydroxy-8-methylflavan, 6,8-diprenylnaringenin, dracorubin, (-)-epicatechin, ent-epicatechin, epigallo catechin 3-gallate, eriocitrin, eriodictyol, farrerol, fisetinidol, fisetinidol-4-ol, fustin, garbanzol, glabranin, glepidotin β , glycyphyllin, hesperetin, hesperidin, homoeriodictyol, 7-hydroxyflavan, isochamaejasmin, isosakuranetin, isourietin, kazinol a, kolaflavanone, liquiritigenin, manniflavanone, 6, methocyaromadendrin 3-O-acetate, 6-methoxytaxifolin, 2'-O-methylodoratol, naringenin, naringin, narirutin, neoastilbin, neoeriocitrin, neohesperidin, odoratol, phloretin, phellamurin, phloretin, phloridzin, pinobanksin, pinocembrin, pinocembrin 7-rhamnosyl-glucoside, piperaduncin β , poncirin, 5'-prenyl, naringenin, pruning, sakuranetin, sanggenon C, sanggenon D, silandrin, silybin, silychristin, sophoranone, strobopinin, taxifolen, taxifolin-3-O-acetate, tephrowatsin, theasinensin A, 2',4',6'-trihydroxy-3'-formyldihydrochalcone and uvaretin.

A more preferred list would include the flavonoids and flavonoid derivatives selected from the group consisting of naringin (aurantiin, naringenin 7-rhamnoglucoside), α -glucosylrutin, α -glucosylmyricetin, α -glucosylisoquercetin, α -glucosylquercetin, dihydroquercetin (taxifolin), hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone 7-rhamnoglucoside, hesperitin 7-O-rhamnoglucoside), neohesperidin, rutin (3,3',4',5,7-pentahydroxyflavone 3-rhamnoglucoside, quercetin 3-rhamnoglucoside), troxerutin (3,5-dihydroxy-3',4',7-tris(2-hydroxyethoxy)flavone-3-(6-O-(6-deoxy- α -L-mannopyranosyl) β -D-glucopyranoside)), monoxerutin (3,3',4',5-tetrahydroxy-7-(2-hydroxyethoxy)flavone-3-(6-O-(6-deoxy- α -L-mannopyranosyl) β -D-glucopyranoside)), diosmin (3',4',7-trihydroxy-5-methoxyflavanone 7-rhamnoglucoside),

eriodictin, and apigenin 7-glucoside (4',5,7-trihydroxyflavone 7-glucoside), kaempferol, quercitrin, avicularine, myricitin, epicatechins and catechins.

Extraction Method

As explained above the puree or pulp biomass is added to a water based medium to form a slurry.

The slurry will normally comprise primarily water, that is, comprise for example over 50 wt. %, more typically over 75 wt. %, most typically over 85 wt. % water but may further contain organic solvents.

The organic solvents may be hydrocarbons, fatty acid esters, ethers, alcohols, fatty acids or ketones. However, if organic solvents are used alcohols or polyalcohols are preferred and may be selected from the group consisting of methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, tert-butyl alcohol, 1,2-ethanediol, 1,2-propanediol, 1,3-propandiol, butylene glycol and glycerin. Preferably, no organic solvent is present in the extraction slurry.

Thus the slurry of step a) may comprise an organic solvent from about 1 to about 10 wt. percent of the slurry.

But the slurry is preferably essentially organic solvent free. By essentially organic solvent free, it is meant that the slurry contains minor amounts of organic solvent, that is about 0.1 to about 5 wt. percent, or 0.1 to about or less than 1 wt. percent organic solvent.

The slurry will contain about 0.01 to about 20, preferably 0.1 to about 10, and most preferably about 0.5 to about 5 wt. %, especially 0.5 to about 2 or about 3 wt. % of a nonionic surfactant, preferably the nonionic surfactant is a copolymer, preferably a block copolymer of poly(ethylene oxide) and poly(propylene oxide), or an alkyl polyglucoside and more preferably the nonionic surfactant is a triblock copolymer of poly(ethylene oxide) and poly(propylene oxide) or an alkyl polyglucoside.

The weight percent of the nonionic surfactant is based on the total weight of the slurry.

The term "hydrophobic" as used herein meant a component of the natural product (biomass) which is more soluble in a nonpolar solvent than in water.

Since many bioreactive or target molecules of the biomass are located within the structure of the cell wall or other organelles within the cell, a suitable process is required to extract the desired components from the cell. Accordingly, the cell wall barrier must be perturbed or ruptured sufficiently to allow diffusion to occur into the extraction liquid. A method is therefore needed to rupture cell walls and membranes to maximize the removal of the active. Examples of such process conditions include the use of heat, high shear mixing, ultrasonic waves, microwaves, high pressure and prolonged exposure to the extractant medium.

Thus the biomass is generally in the form of a pulp or puree which has been mashed or broken up to rupture cell walls and membranes.

The amount of pulp or puree biomass which is added to the extractant medium (water and nonionic surfactant) to form a slurry will vary from about 1 to about 50 wt. %, preferably about 2 to about 25 wt. % and most preferably about 3 to about 15 wt. % of the total weight of the slurry.

The pulp or puree biomass wt. % is normally a dry weight. For example, the pulp or puree is normally dried before extraction and most or all of the water content is removed. The weight percent of the dried biomass varies from about 1 to about 50 wt. % of the total weight of the slurry.

Therefore, the dried biomass contains, if any, only small amounts of water, for example .01 to 5 wt. %, preferably .01 to about 2 wt. % water.

Non-ionic Surfactants

By "surfactant," it is meant any of those molecules that are commonly known in the art to provide a reduction in surface tension (such as being able to reduce the surface tension of water to 60 dynes/cm or less, and, more preferably 50 dynes/cm or less when added to pure deionized water, and measured at ambient temperature i.e., 20 ° C.). Furthermore, it is preferred that the surfactant or surfactants have a water solubility of at least about 1% in deionized water at ambient temperature. As such, the term surfactant can also include those molecules that are also commonly referred to as oil in water emulsifiers. In one embodiment, the non-ionic surfactant has a Hydrophile-Lipophile Balance (HLB) that is from about 8 to >24, and more preferably from about 10 to about >24.

By "non-ionic surfactant," it is meant a surfactant that does not ionize in aqueous media. In a preferred embodiment, the non-ionic surfactant is liquid at ambient temperature.

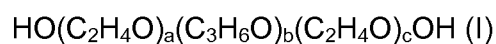
The function of the surfactant is to provide one or more of the following: emulsification or solubilization of hydrophobic compounds, wetting and surface tension reduction.

Non-ionic surfactants, which can be used in the presently disclosed extraction process, include those broadly defined as compounds produced by the condensation of alkylene oxide groups (hydrophilic in nature) with an organic hydrophobic compound, which may be aliphatic or alkyl aromatic in nature. Examples of preferred classes of nonionic surface-active agents are: the long chain alkanolamides; the polyethylene oxide condensates of alkyl phenols; the condensation product of aliphatic alcohols having from about 8 to about 18 carbon atoms, in either straight chain, block or branched chain configuration, with ethylene oxide; the long chain tertiary amine oxides; the long chain tertiary phosphine oxides; the long chain dialkyl sulfoxides containing one short chain alkyl or hydroxy alkyl radical of from about 1 to about 3 carbon atoms; and the alkyl polysaccharide (APS) surfactants such as the alkyl polyglycosides; the polyethylene glycol (PEG) glyceryl fatty esters and copolymers of ethylene oxide and propylene oxide including block copolymers of polyethylene oxide and polypropylene oxide.

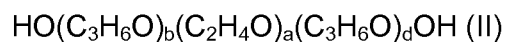
The preferred non-ionic surfactants are the condensation product of aliphatic alcohols having from about 8 to about 18 carbon atoms, in either straight chain, block or branched chain configuration, with ethylene oxide. For example, copolymers of ethylene oxide and propylene oxide are preferred.

Of special interest however are the block copolymers of polyethylene oxide and polypropylene oxide. These block copolymers are often referred to as poloxamers. These poloxamer surfactants are polymeric and comprise blocks of alternating hydrophobic and hydrophilic blocks. The hydrophobic blocks comprise polypropylene oxide while the hydrophilic blocks comprise polyethylene oxide blocks.

The poloxamers of interest in this application are diblock or triblock, preferably triblock poloxamers of the structures below and are known under the tradename Pluronic® supplied by BASF Corporation.



or



wherein *a* and *c* are independently 3 to 200,

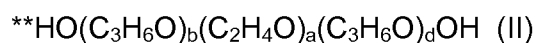
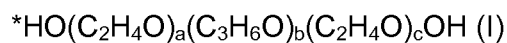
and *b* and *d* are independently is 5 to 100.

For example, some typical triblock polymers which are envisioned in the present application are:

Pluronic®	Molecular Weight
L10	3200
L31	1100
L35	1900
L38	4700
L42	1630
L43	1850
L44	2200
L61	2000
L62	2500
L63	2650
L64	2900
P65	3400
F68	8400
L72	2750
P75	4150
F77	6600
L81	2750
P84	4200
P85	4600
F87	7700
F88	11400
L92	3650
F98	13000
L101	3800
P104	5900
P105	6500
F108	14600
L121	4400
L122	5000
P123	5750
F127	12600

RPE 1050	1950
RPE 1720	2150
RPE 1740	2650
RPE 2520	3100
RPE 2540	3600

Particularly preferred Pluronics® are Pluronic L35, L44, L43, P105, F68, F87, F108, RPE 1050, RPE 1720, RPR 1740 and F127 (NF Grades- BASF maintains a Drug Master File for some of these products).



Pluronic®	Poloxamer	a and c ¹	b and d ²	Mw ³	HLB
L35*	101	11	16	1900	19
L43*	123	7	21	1850	7-12
L44 *	124	12	20	2090-2360	12-18
F68*	188	75	30	7680-9510	>24
F87*	237	62	39	6840-8830	>24
F108*	338	128	54	12700 – 17400	>24
P105*	335	38	54	6500	12-18
F127*	407	98	58	9840 – 14600	18-23
RPE 1050**	NA	22	8	~1950	15

RPE 1720**	NA	10	15	~2150	6
RPE 1740**	NA	24	15	~2650	12

Note: ** and * designate whether formula (I) or (II) of the non-ionic surfactant.

1. The number given in the table represents the number of repeating units in a and the number of repeating units in b. Accordingly for F108 of structure (I), a is 141 and c is 141.
2. The number given in the table represents the number of repeating units of b and d. Accordingly for RPE 1050 of structure (II), a is 22 and b is 8 and d is 8.
3. Mw represents weight average molecular weight.

The first step in synthesizing these Pluronic® surfactants is the creation of a hydrophobe of desired molecular weight by the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol. Ethylene oxide is then added to sandwich this hydrophobe between hydrophilic groups, controlled by length to constitute from 10% to 80% (by weight) of the final molecule.

In the RPE (or reverse Pluronic® surfactants) the structure is as in the formula II above. Thus the build up of the block copolymer is reversed. The central block is polyethylene oxide sandwiched between two polypropylene blocks.

A range of Pluronic® surfactants are available where the hydrophobe or center block (polypropylene oxide) varies from about 900 to 4000 weight average molecular weight and the ethylene oxide blocks make up from 10 to 80, preferably 30 to 95, most preferably 40 to 90 weight percent of the total block copolymer.

The reverse Pluronics® RPE surfactants are available where the center block (polyethylene glycol) weight average molecular weight varies from about 300 to about 1000 average molecular mass and the total weight average molecular weight for the polypropylene oxide terminal blocks range from about 1000 to about 3800.

Accordingly the triblock polymers typically range from about 1000 to about 16000 average molecular weight. The triblock polymers are liquid, solid or paste depending upon the molecular weight and the weight ratio of the hydrophobic (PP) and hydrophilic blocks (PE).

The inventors have discovered that a range of triblock copolymers of differing molecular weights and PP and PE ratios function when dissolved in aqueous solution are highly effective solubilizers for the target molecules from various plant, fungi and algae sources.

These particular block nonionic surfactants are known to form micelles in water. Thus they may function as solubilizing agents for target molecules.

Other non-ionic surfactants of particular interest are the alkyl polyglucoside.

Alkyl polyglucosides (APGs) are well known in the art and may be purchased under the tradename Plantaren® from BASF SE.

The terms alkyl polyglucoside and alkyl glucoside are interchangeable.

An alkyl polyglycoside is formed from the reaction of glucose and fatty alcohol. An alkyl polyglycoside compound has a hydrophobic portion (carbon chain) and a hydrophilic portion (glycoside unit or group). When describing an alkyl polyglycoside, the average degree of polymerization (DP) is mentioned. For example, in an alkyl polyglycoside or alkyl glycoside a compound with a DP of about 1.4, there are, on average, 1.4 units of glucose for each alkyl group. An alkyl polyglucoside or alkyl glucoside is normally a mixture of varying amounts of glucose units on the molecule. It is to be understood that a DP of 1.4 does not mean that each molecule has 1.4 glucose units.

Alkyl polyglucosides may be represented by the following general formula: $R_1-O-(R_2O)_b-(Z)_a$ wherein R_1 is a monovalent organic radical having from about 6 to about 30 carbon atoms, R_2 is a divalent alkylene radical having from 2 to 4 carbon atoms, and Z is a saccharide residue having 5 or 6 carbon atoms, b is a number from 0 to about 12, and a is a number of from 1 to 6.

Additional suitable alkyl polyglucosides include, but are not limited to GLUCOPON® 225DK, in which the alkyl group contains 8 to 10 carbon atoms and has an average DP of 1.7; GLUCOPON® 625UP, in which the alkyl group has 12 to 16 carbon atoms and has an average

DP of 1.6; APG® 325N, in which the alkyl group has 9 to 11 carbon atoms and has an average DP of 1.5; GLUCOPON® 600UP, in which the alkyl group has 12 to 16 carbon atoms and has an average DP of 1.4; PLANTAREN 2000®, in which the alkyl group has 8 to 16 carbon atoms and has an average DP of 1.5; and PLANTAREN 1300®, in which the alkyl group has 12 to 16 carbon atoms and an average DP of 1.6.

The alkyl polyglucosides is typically formed by reacting a sugar with a higher alcohol in the presence of an acid catalyst, or by reacting a sugar with a lower alcohol (for example, methanol, ethanol, propanol, butanol) to thereby provide a lower alkyl glycoside, which is then reacted with a higher alcohol. The higher alcohol generally has the formulation $R_1O(R_2O)_xH$, wherein R_1 represents a straight or branched alkyl or alkenyl group having from 8 to 22 carbon atoms, R_2 represents an alkylene group having from 2 to 20 carbon atoms, and x is a mean value that is 0 to 10.

Specific non-limiting examples of the higher alcohol are straight or branched alkanol such as hexanol, heptanol, octanol, nonanol, decanol, dodecanol, tridecanol, tetradecanol, pentadecanol, hexadecanol, heptadecanol, octadecanol, methylpentanol, methylhexanol, methylheptanol, methyloctanol, methyldecanol, methylundecanol, methyltridecanol, methylheptadecanol, ethylhexanol, ethyloctanol, ethyldecanol, ethyldodecanol, 2-heptanol, 2-nonanol, 2-undecanol, 2-tridecanol, 2-pentadecanol, 2-heptadecanol, 2-butyloctanol, 2-hexyloctanol, 2-octyloctanol, 2-hexyldecanol and/or 2-octyldecanol; an alkenol such as hexenol, heptenol, octenol, nonenol, decenol, undecenol, dodecenol, tridecenol, tetradecenol, pentadecenol, hexadecenol, heptadecenol and octadecenol. These alcohols may be used either alone or a mixture of two or more of them.

Preferred alkyl glucosides comprise from about 1 to about 6 glucose residues per molecule, preferably 1 to 4. Preferred alkyl polyglucoside are decyl glucoside, caprylyl/capryl glucoside, coco glucoside and lauryl glucoside which are the condensation product of the corresponding alcohol with a glucose polymer or single glucose residue and is available commercially from BASF Corporation of Florham Park, NJ under the trade name, Plantaren®.

The cosmetic compositions presently disclosed may further comprise various additives utilized in the cosmetic field. The CTFA Cosmetic Ingredient Handbook and Personal Care Product Counsels ingredient buyers guide describe a wide variety of nonlimiting cosmetic ingredients

commonly used by those skilled in the art and which are suitable for use in the cosmetic compositions of the present invention. Examples of these ingredient classes included: abrasives, emulsifiers, absorbents, gelling agents, antifoaming agents, buffering agents, colorants, film formers, pH adjusters, humectants, thickeners and pigments. It is further recognized that additional cosmetic active ingredients, such as anti-acne actives (for example, salicylic acid or benzoyl peroxide), anti-wrinkle actives (for example, retinoids or beta-hydroxy acids), antioxidants (for example, ascorbic acid and its derivatives or tea extracts), chelators (for example, furildioxime), anti-inflammatory agents (for example, corticosteroids), slimming agents (for example, caffeine), skin lightening agents (for example, mulberry extract or kojic acid), or sunscreens (for example, those commercially available under the name PARSOL), may be utilized in the cosmetic compositions of the present invention based on the desired overall benefits intended to be conferred by the composition.

The upper and lower limits for the quantity of the extract according to the present invention in any given formulation for a cosmetic composition is based both on the desired effect of the cosmetic compositions, the other components of the formulation, the type of composition, cost and practicality. However, the plant, algae or fungi extract preferably is included in a quantity between about 0.01% and about 5% and more preferably between about 1% and 3% based on the final weight of the composition. For purposes of this application, this wt. % includes the extract, surfactant and water mixture.

Filtering of the Fungi, Algae or Biomass

Extracting with aqueous solutions of Pluronic® is faster and offers higher yields of extractant.

In order to remove the biomass, algae or fungi from the slurry it is often necessary to filter using increasingly smaller pore filters. For example, it is typical to require multiple filtrations starting from a large pore size of about 150 micron moving to progressively smaller pore sizes. For example, five filtrations may be necessary starting with 150 micron, followed by 11 micron, to 2.5 micron and finishing with 0.45 and 0.22 micron filters.

Another typical way of removing the extracted biomass is to centrifuge then filter the supernatant.

These consecutive filtrations are very time consuming. The filtration becomes progressively slower as the pore size decreases.

While the speed of filtration is to a certain extent biomass determined, if the extractant medium is water/Pluronic or an alkyl glucoside, it has been discovered that some of the intermediate filtrations can be eliminated. For example, one can move from a 2.5 micron filtration directly to a 0.45 filtration without going through an intermediate say 8.0 micron filtration. Of course, the elimination of extra filtration steps is highly desirable and one of the unexpected advantages of the present process.

Thus it has surprisingly been found that when the extractant medium is water/non-ionic surfactant or preferably water/Pluronic® or alkyl glucoside, the filtration process is significantly accelerated.

For example, when the inventive extractant medium is used, the filtering process is normally 2 or 3 time faster than an extractant medium comprising water only.

Accordingly the present method may embody the step of separating the plant, algae or fungi biomass from the aqueous liquid copolymer slurry by filtration and the filtration is carried out using a filter having a pore size ranging from about 0.1 to about 0.5 microns, preferably about 0.1 to about 0.4, most preferably about 0.1 to about 0.3 microns and the speed of filtration is at least twice as fast as separation of the plant, algae or fungi biomass from the same water or water/organic solvent slurry.

Use of the Extractant

The present extracts are particularly suitable for body care products, in particular for use in skin-care products, as bath and shower products, preparations containing fragrances and odoriferous substances (perfumes, after-shave lotions), hair-care products, deodorizing and antiperspirant preparations, decorative preparations, light protection formulations (sunscreens) and skin preparations containing active ingredients (vitamins, hormones or antimicrobials).

The extract is intended primarily for topical application to human skin or hair. The composition according to the invention is particularly useful as an agent for conditioning and smoothing the skin or hair, and preventing or reducing the appearance of wrinkled or aged skin and can thus

be formulated into a topical skin treatment formulation for use in cosmetic applications. The topical skin treatment formulation of the invention is thus useful in the removal of oxidants from the skin and in the dermatological treatment of the skin including, but not limited to, skin imperfections, dry skin, photodamaged skin, wrinkles, age spots, acne, skin lightening, psoriasis, and atopic dermatosis.

Suitable skin-care products are, in particular, body oils, body lotions, body gels, treatment creams, skin protection ointments, shaving preparations, such as shaving foams or gels, skin powders, such as baby powder, moisturizing gels, moisturizing sprays, revitalizing body sprays, cellulite gels and peeling preparations.

Preparations containing fragrances and odoriferous substances are in particular scents, perfumes, toilet waters and shaving lotions (aftershave preparations).

Suitable hair-care products are, for example, shampoos for humans and animals, in particular dogs, hair conditioners, products for styling and treating hair, perming agents, hair sprays and lacquers, hair gels, hair fixatives and hair dyeing or bleaching agents.

Suitable decorative preparations for skin are for example lipsticks, nail varnishes, eye shadows, mascaras, dry and moist make-up, rouge, powders, depilatory agents and suntan lotions.

Suitable cosmetic formulations containing active ingredients are in particular hormone preparations, vitamin preparations, vegetable extract preparations and antibacterial preparations.

The present body care products can be in the form of creams, ointments, pastes, foams, gels, lotions, powders, make-ups, sprays, sticks or aerosols. The present extractants may be present in the oil phase or in the aqueous or aqueous/alcoholic phase.

The following examples further describe and illustrate the present invention and should not be construed as limitations of the present invention.

EXAMPLES

Example 1

Genus Species: *Nereocystis luetkeana*

Nereocystis Luetkeana was provided by BC Kelp. The *N. luetkeana* is a dried, crushed pulp of all parts of the algae organism dried crushed material

The above dried algae was added to various extractant mediums at the amounts given below in

Table 1.

Table 1 – Extraction Slurry Compositions for *N. Luetkeana*

DI Water	BG	Pluronic® L44	Pluronic® F68	Pluronic® F127	Wt. % Algae
95.00					5.00
	95.00				5.00
85.00		10.00			5.00
85.00			10.00		5.00
85.00				10.00	5.00

Notes: numbers in % are w/w basis. Pluronics dissolved in water prior to addition of algae. BG refers to butylene glycol.

The above slurries are heated to 60 °C and mixed with high speed stirring for 1 hour. The slurry is cooled to room temperature and is filtered via 11 micron filter. Samples are filtered a second time through a 0.22 micron filter before analysis.

Example 2

Species: *Fucus vesiculosus*

A water only and water/Pluronic containing slurries were mixed overnight then filtered with a consecutively smaller pore size filters starting at 150 micron, 11micron, 2.5 micron, 0.45, 0.22

Table 2 –Extraction Slurry Compositions for *F. vesiculosus*

DI Water	Pluronic® F127	% Algae	% Yield ¹	Time to Filter ² (hrs)
95	-	5	30 %	6
85	10.00	5	50 %	1

1. The % yield is a measure of the weight percent of the filtered extract collected after filtration. A higher yield would indicate less extract is lost during filtration.
2. The water only extracting fluid slurry was filtered in sequence with consecutive filters used (pore size in μm) = 150, 11, 2.5 and 0.8. The series of water only filtrations took 6 hours in total.

The water/Pluronic extracting fluid slurry was filtered in sequence with consecutive filters of pore sizes in microns 150, 11, 2.5, 0.45 and 0.22. The inventive extractions took a total of 2 hours. See Figure 2 showing the difference in color of the extracting fluid.

Clearly incorporation of the Pluronic® within the aqueous media gave an improved extraction and filtering efficiency when compared with the water only. Extracting with aqueous solutions of Pluronic® is faster, of higher yield (almost double the yield at 1/3 the time).

Analytical Results:

Figure 1 is a comparison of extraction fingerprints from *Nereocystis luetkeana* using HPLC and different extraction mediums.

Pluronic extracts contained the same compounds as the 100% aqueous and butylene glycol extraction medium plus additional components.

Figure 2 indicates that the color of extractant in the water/surfactant solution is of much better color when compared to the algae extracted with only water

The extracts are thought to have antioxidant, anti-inflammatory, anti-melanogenic properties and perhaps collagen boosting properties. It is also believed that the surfactant-extract water mixtures are by themselves more oxidation resistant.

Table 3-7 Extractant Slurries for Additional Species Aqueous extraction as above is carried out for a number of different plant species. The aqueous medium extractant only is compared to the extractant via aqueous medium containing varying amounts of non-ionic surfactants.

Dried, cut *Cassia alata* or *Argania spinosa* leaves were mixed with hot water (80°C) or with the hot aqueous medium containing the non-ionic surfactants at a ratio of 1:9 (100g leaves:0,9 L water), the pH of the mixture was adjusted to pH =6 and extraction was proceeded during 1 hour at 80°C, pH6 under shaking. After cooling to room temperature the insolubles were removed by centrifugation and the supernatant filtered. The concentration of the targeted flavonoids in each of the liquid extracts was measured (mg/100g of liquid extract). The total amount of dry matter recovered in each extract was measured (g/100g of liquid extract). The amount of active matter is calculated as the difference between the dry matter of the plant extract with surfactant medium and the dry matter of the surfactant solution alone.

Quantification of Flavonoids

Flavonoid determination in the liquid extracts were performed by HPLC analysis. , the method depending of the flavonoids contained in the plant.

For *Cassia alata* leaves extracts, the column used was a C18 reversed phase column: Symmetryshield ® RP 18 WATERS 5 µm (4.6 x 250 mm) maintained at 30°C. Gradient elution of the samples and standard were performed using water (eluent A) and acetonitrile (eluent B). The gradient elution initial conditions were 20% of eluent B with linear gradient to 60% from 0 to 35 min, followed by linear gradient to 20% of eluent B at 37 min.

The flow rate was 1 ml/min and the sample injection volume was 10 µl: 2 injections were performed for each sample

Detection was performed with a Photo Diode Array Detector at 350 nm

Calibration curves were realized with a Kaempferol 3-O-sophoroside and Kaempferol standards injected at different concentrations.

Biological Testing

Antiinflammatory Properties In Vitro-UVB Light Protection (UVB-LDH and UVB-PGE2)

Cell Protection Effect Against UVB on In Vitro Cultivated Human Keratinocytes

Background: UVB rays (from 280 to 320 nm) trigger inflammation (erythema, odema) by activating an enzyme, namely phospholipase A2 or PLA2, which removes arachidonic acid from the phospholipids of the plasma membrane. Arachidonic acid is the precursor of prostaglandins, which cause inflammation and cell membrane damage; the prostaglandins E2 (=PGE2) are formed by cyclooxygenase. This membrane stress is indicated by the release of the cytoplasm enzyme lactate dehydrogenase (LDH). The effect of UVB radiation was investigated on keratinocytes in vitro by determining the release of the cytoplasm enzyme LDH (lactate dehydrogenase). This enzyme serves as a marker for cell damage.

Method: To carry out the tests, a defined medium (DMEM), which comprises 10% fetal calf serum, was inoculated with the keratinocytes and the plant extract (diluted with saline solution) was added 72 hours after inoculation.

The keratinocytes were then irradiated with a UVB dose (30 mJ/cm²-tubes: DUKE GL40E).

Following further incubation for 1 day at 37° C and at 5% CO₂, the LDH and the PGE2 content in the supernatant was determined. The content of LDH (lactate dehydrogenase) was determined by means of an enzyme reaction (kit used to investigate the LDH content from Roche). The content of PGE2 was determined using an ELISA test (ELISA kit from Roche). Following trypsin treatment, the cells were centrifuged and counted.

Anti-Radical Action Testing

The oxidative anti-stress properties were evaluated by "in tubo" and "in vitro" tests.

The group of in tubo tests includes both the initial radical-type forms of oxygen and the reactive forms introduced in vivo: radical hydroxyl (HO and anion superoxide (O₂)).

1) "Chemical" Tests in Tubo

a) AO antioxidant DPPH Test (AO-DPPH)

DPPH (diphenylpicryl hydrazyl) is a free, stable, violet-coloured radical which, in its leuco

derivative, is modified by substances which capture free radicals (neutralising effect, also described as a "scavenger effect").

The result is given as percent inhibition of DPPH⁰ in radical form relative to the control material without extract.

b) Anti-HO⁰ Test with Salicylic Acid (Fenton Reaction)

The HO⁰ (formed by H₂O₂ with Fe.sup.++ and EDTA present) hydroxylate the salicylic acid, which then forms a reddish compound.

The optical density at 490 nm corresponds to the hydroxylated salicylic acid content.

An anti-radical substance reacts with the HO⁰ radicals and reduces the formation of this red compound.

The results are given as percent inhibition of the hydroxylation content (average of 2 tests).

Thiobarbituric Acid Reactive Assay (%AO-TBARS)

An In vitro model to test relative antioxidant potential : ultraviolet-induced lipid peroxydation in liposomes, *Archives of biochemistry and biophysics*, Vol. 283, No. 2, 234-240, (1990).

An approach towards understanding the genesis of sunlight-induced skin cancer, *Indian Journal of Biochemistry and Biophysics*, Vol. 27, 254-263, (1990).

Abbreviations

APG-1 is Plantacare® 2000 UP or Decyl Glucoside (INCI)

APG-2 is Plantacare® 810 UP or Caprylyl/Capryl Glucoside (INCI)

L35 is Pluronic® L35 or Poloxamer 101(USAN)

L43 is Pluronic ® L43 or Poloxamer 123 (USAN)

L44 is Pluronic ® L44 or Poloxamer 124 (USAN)

NPE 1720 is a Pluronic® RPE 1720

P105 is Pluronic ® P105 or Poloxamer 335 (USAN)

UVB-LDH LDH or lactate dehydrogenase determination by means of an enzyme reaction

UVB-PGE2 prostaglandins E2 determined using an ELISA test (ELISA kit from Roche).

%AO-DPPH – Anti-Radical test using diphenylpicryl hydrazyl

%AO-TBARS - Thiobarbituric Acid Reactive Assay – See above.

Table 3 – Extracts of *Cassia alata* leaves with quantification of flavonoids

	Surfactant	Surfactant Wt.%.	Active Matter (g/100g)	Kaempferol-3-O-sophoroside (K3OS) (mg/100g of liquid extract)	Kaempferol (mg/100g of liquid extract)
Cassia alata extracts	None (water)	0	3.59	225	0
	APG-1	1	3.65	217	-
	APG-2	5	3.86	220	3.6
	L35	10	3.67	269	9.1
	L43	10	3.80	272	11.3
	L44	10	4.31	268	19.7
	RPE1720	10	2.35	217	0
	P105	10	4.85	286	26.6

Table 4- Comparison of anti-UVB activities between water and aqueous medium containing non-ionic surfactants extracts of *Cassia alata* leaves (all liquid extracts were tested at a 0,5% dilution)

Species	Extract	Wt.%Surf	LDH (U/mg protein)	PGE2 (pg/well)
	Control without UV		0	-4
	Control with UV		100	100
	Positive control (aspirin at 0,03%)		15	-6

<i>Cassia alata</i> extracts	Water		42	29
	APG-1	1	NT	NT
	APG-2	5	118	34
	L35	10	52	18
	L43	10	41	1
	L44	10	29	2
	NPE1720	10	140	15
	P105	10	4	-1

Table 5– Extracts of *Argania spinosa* leaves with quantification of flavonoids

Species	Surfactant	Surfactant Wt. %	Active Matter (g/100g of liquid extract)	Myricitrin (mg/100g of liquid extract)	Quercitrin (mg/100g of liquid extract)
<i>Argania spinosa</i> Leaves extracts	None (water)	0	4.27	37.9	24.4
	APG-1	1	4.08	38.2	23.9
	APG-2	5	4.63	46.9	32.8
	L35	10	3.37	55.4	35.4
	L43	10	2.16	50.4	31.0
	L44	10	2.90	53.6	33.5
	RPE1720	10	1.42	35.3	19.1
	P105	10	6.29	76.0	52.4

Table 6 - Comparison of anti-UVB activities between water and aqueous medium containing non-ionic surfactants extracts of *Argania spinosa* leaves (all liquid extracts were tested at a 0.037% dilution)

Species	Surfactant	Surfactant Wt.%..	LDH (U/mg protein)	PGE2 pg/well
	Control without UV		0	0
	Control with UV		100	100
	Positive control (aspirin at 0.03%)		0	0
<i>Argania spinosa</i> Leaves extracts	None (water)	0	79	58
	APG-1	1	86	84
	APG-2	5	48	42
	L35	10	78	55
	L43	10	56	23
	L44	10	81	33
	RPE1720	10	86	52
	P105	10	4	0

The dry weight of the plant species in all cases is 5 wt. % of the total slurry weight in Table 7. The slurries are stirred for 1- 2 hours at room temperature before measurements are taken for grams of active extractant per 100/g of liquid, flavonoid concentration and biological testing.

Table 7 – Extracts of *Cacao alata* with quantification of flavonoid and corresponding biological activity

Species	Surfactant	Wt.%Surf.	Extract ¹ (g/100g)	Kaempferol (% var. vs.H2O ext.)	UVB-LDH	UVB-PGE
<i>Cacao alata</i>	None (water)		0.22	0	42	29
	APG-1	1	0.10	neg.		
	APG-2	1	0.10	neg.	118	34
	L35	10	0.25	19.40	52	18
	L43	10	0.39	20.72	41	1
	L44	5	0.32	19.12	29	2
	NPE1720	1	0.33	neg.	140	15
	P105	1	0.15	2.7	4	neg.

Formulation Examples

Example 1A

Purifying Toner for Skin

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	89.40
	Glycerin	Glycerin	3.00
	Elestab® 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00

	D-Panthenol 75 W	Panthenol	0.50
B	Eumulgin® HPS	Coceth-7 (and) PPG-1-PEG-9 Lauryl Glycol Ether (and) PEG-40 Hydrogenated Castor Oil	1.50
	Copherol®1250 C	Tocopheryl Acetate	0.50
	Ocean Breeze 6110736 ¹	Fragrance	0.10
C	Mat-XS™ Clinical	Water (and) Butylene Glycol (and) Xanthan Gum (and) Sarcosine	2.00
	Extract according to invention	Exact (and) water (and) nonionic surfactant	.01-5
	TEA 99%	Triethanolamine	q.s.

Supplier Footnotes:

1. Bell Flavors and Fragrances

Procedure

While stirring Phase A, add premixed Phase B. Then add Phase C one by one, and mix well between additions.

Example 2A

Day Cream with Naturally Sourced UV Protection

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	q.s.
	Edeta® BD	Disodium EDTA	0.10
	D-Panthenol® 75W	Panthenol	0.75
B	Glycerin	Glycerin 99%	2.00
	Keltrol CG ¹	Xanthan Gum	0.20
	Veegum Ultra ²	Magnesium Aluminum Silicate	0.40
C	Lanette® 22	Behenyl Alcohol	2.00

	Eumulgin [®] SG	Sodium Stearoyl Glutamate	0.75
	Myritol [®] 331	Cocoglycerides	3.50
	Emulgade [®] PL 68/50	Cetearyl Glucoside (and) Cetearyl Alcohol	3.50
	Cetiol [®] SB 45	Butyrospermum Parkii (Shea) Butter	1.50
	Cetiol [®] OE	Dicaprylyl Ether	3.00
D	Z-COTE [®] LSA	Zinc Oxide (and) Triethoxycaprylsilane	12.50
	Cetiol [®] RLF	Caprylyl Caprylate/Caprates	7.00
	Cetiol [®] CC	Dicaprylyl Carbonate	7.50
E	Sensiva SC 50 ³	Ethylhexylglycerin	1.00
	Marine Filling Spheres	Pentaerytrityl Tetraistearate (and) Silica Dimethyl Sylilate (and)Sodium Chondroitin Sulfate (and) Extract according to the invention	1.00
	Ocean Breeze 6110736 ⁴	Fragrance	0.10
	Citric Acid 25% Sol.	Citric Acid	q.s.

Supplier Footnotes:

¹ C.P. Kleco³ Shulke² RT Vanderbilt⁴ Intarone

Procedure

Combine Phase A and start heating to 75-80C. Premix Phase B and add to Phase A while heating to 75-80 C. Combine Phase C and heat to 75-80C. Combine Phase D homogenize until uniform then add to Phase C and continue heating to 75-80 C. Add Phase C/D to Phase A/B while homogenizing until uniform. Transfer to sweep mixing and start cooling to 40 C. Add Phase E ingredients one by one and mix well between additions. Cool to room temperature and stop.

Example 3A

Moisturizing Makeup Removing Wipe

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	q.s. to 100
	Emulgade [®] CM	Cetearyl Isononanoate (and) Cetareth-20 (and) Cetearyl Alcohol (and) Glyceryl Stearate (and) Glycerin (and) Cetareth-12 (and) Cetyl Palmitate	6.00 – 10.00
	Extract according to the Invention	Extract according to the invention	.01 – 5.00
	Citric Acid (50% Soln)	Citric Acid	0.10
	Fragrance	Fragrance	q.s.
	Preservative	Preservative	q.s.

Viscosity [mPas]: Brook. RVF, 23°C, spindle 4, 10 rpm. pH: 4.9 – 5.1

Example 4A

Night Cream

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	65.15
	Elestab 388	Propylene glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
B	Glycerin	Glycerin	3.00
	Vanzan NF ¹	Xanthan gum	0.10
	Cosmedia [®] SP	Sodium Polyacrylate	0.70
C	Emulgade [®] Sucro	Sucrose Polystearate (and) Hydrogenated Polyisobutene	3.00
	Eumulgin [®] SG	Sodium Stearoyl Glutamate	1.00
	Cutina [®] HVG	Hydrogenated Vegetable Glycerides	4.00
	Monomuls [®] 90-O 18	Glyceryl Oleate	0.50

	Cetiol® RLF	Caprylyl Caprylate/Caprates	3.00
	Myritol® 312	Caprylic/ Capric Triglycerides	6.00
	Cetiol® J600	Oleyl Erucate	6.00
	Generol® R	Brassica Campestris (Rapeseed) Sterols	0.50
	Dow Corning 200 Fluid 350 cSt ²	Dimethicone	1.00
D	Hyalufix™ GL	Water (and) Butylene Glycol (and) Alpinia Galanga Leaf Extract (and) Xanthan Gum (and) Caprylic/Capric Triglyceride	3.00
	Extract according to the invention	Extract (and) Xanthan Gum	2.00
	Lavender Vanilla F-127981 ³	Frangrance	0.05

Viscosity: Brookfield RVT, 23°C, spindle T-E @ 5 rpm: 180,000 cps pH: 6.3

Supplier Footnotes:

¹ RT Vanderbilt, Inc ² Dow Corning Corp. ³ Intarome Fragrance and Flavor Corp.

Procedure

Premix Phase B and swell the mixture in the water Phase of Phase A. Then heat to 85 C. Heat Phase C to 85C and mix until homogeneous. While stirring add Phase C to Phase A/B. Allow the emulsion to cool while stirring in such a way that it remains in continual motion. Avoid incorporating air. If necessary homogenize with a suitable dispersion unit (like Ultra Turrax) at approx. 65-60C. When mixture cools to below 50C, add Phase D while mixing. Stir while cooling until reaches room temperature.

Example 5A

Skin Serum

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	84.7

	Glycerin	Glycerin	3.00
	Elestab 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
B	Cosmedia® SP	Sodium Polyacrylate	0.80
C	Cetiol® Sensoft	Propylheptyl Caprylate	3.00
	Eutanol® G 16 S	Hexyldecyl Stearate	2.00
	Cetiol® 868	Ethylhexyl Stearate	2.00
D	Actiwhite™ LS 9808	Water (and) Glycerin (and) Sucrose Dilaurate (and) Polysorbate 20 (and) Pisum Sativum (Pea) Extract	3.00
	Extract according to the invention	Water (and) Extract	2.00
	Bisabolol Natural	Bisabolol	0.50

Viscosity: Brookfield RVT, 23°C, spindle T-C @ 10 rpm, with Helipath: 12000 cpspH: 5.80

Procedure

Mix Phase A and while stirring, slowly add Phase B to Phase A. When Phase B completely swells, add each ingredient of Phase C one at a time into the gel Phase. When completely mixed, add Phase D ingredients one at a time.

Example 6A

After-Sun Gel for Sensitive Skin

Ph.	Trade Name	INCI Name	% wt
A	Dehyquart® F75	Distearoylethyl Hydroxyethylmonium Methosulfate (and) Cetearyl Alcohol	0.70
	Emulgade® Sucro	Sucrose Polystearate (and) Hydrogenated Polyisobutene	0.50
	Cutina® PES	Pentaerythrityl Distearate	1.00

	Cutina [®] GMS V	Glyceryl Stearate	1.00
	Cetiol [®] Sensoft	Propylheptyl Caprylate	3.00
	Myritol [®] 331	Cocoglycerides	2.00
	Cyperus Root Oil	Cyperus Esculentus Root Oil	2.00
	Cosmedia [®] Triple C	Polyquaternium-37 (and) Dicaprylyl Carbonate (and) Lauryl Glucoside	1.50
B	Glycerin	Glycerin	3.00
	Elestab [®] 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
	Deionized Water	Water	77.70
C	Extract according to the invention	Water (and) Extract	1.00
	Skinasensyl [™] PW LS 9852	Mannitol (and) Sodium Citrate (and) Acetyl Tetrapeptide-15	0.30
	Ethanol	Ethanol	5.00
D	NaOH (10%)	Sodium Hydroxide	q.s.
	Floral Plumeria F-127985 ¹	Fragrance	0.30

Viscosity: Brookfield RVT, 23°C, spindle T-E @ 5 rpm, with Helipath: 120,000 cps

pH: 4.0 - 4.5

Supplier Footnotes:

¹ Intarome Fragrance and Flavor Corp.

Procedure

Heat Phase A and B separately to 80-85C. Add Phase B to A. under moderate mixing.

Start cooling. Add Phase C at 45C or below and mix well between each addition.

Add Phase D one by one, mix well between additions, than cool to room temperature and stop.

Example 7A

No-Foam Cleanser

Ph.	Trade Name	INCI Name	% wt
A	Eumulgin® VL 75	Lauryl Glucoside (and) Polygercyl-2 Dipolyhydroxystearate (and) Glycerin	1.50
	Cutina® HVG	Hydrogenated Vegetable Glycerides	1.50
	Cutina® PES	Pentaerythrityl Distearate	1.00
	Cetiol® CC	Dicaprylyl Carbonate	3.00
	Luvitol® Lite	Hydrogenated Polyisobutane	3.00
	Myritol® 331	Cocoglycerides	2.00
	Cosmedia® SP	Sodium Polyacrylate	0.80
B	Deionized Water	Water	80.65
	Glycerin	Glycerin	3.00
	Elestab® 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
	Horse Chestnut Extract	Mannitol (and) Ammonium Glycyrrhizate (and) Caffeine (and) Zinc Gluconate (and) Aesculus Hippocastanum (Horse Chestnut) Seed Extract	1.00
	NaOH (10%)	Sodium Hydroxide (and) Water	q.s.
C	Extract according to the invention	Water (and) Extract	1.50
	Champagne Petals RU-2133 ¹		0.05

Viscosity: Brookfield RVT, 23°C, spindle T-E @ 5 rpm, with Helipath: 61,000 cps

pH: 6.0

Supplier Footnotes:

¹ Takasago International Corp.

Procedure

Combine Phase A, without Cosmedia SP and heat to 80-85C. Sprinkle Cosmedia SP into Phase A while at 80-85C and mix well. Combine Phase B and heat to 80-85C. Add Phase B to Phase A while at 80-85 C and mix well. Start cooling, and homogenize at 55-60C. Add Phase C at 40C or below one by one and mix well between additions. Cool to room temperature and stop.

Example 8A
Eye Cream

Ph.	Trade Name	INCI Name	% wt
A	Emulgade® SE-PF	Glyceryl Stearate (and) Ceteareth-20 (and) Ceteareth-12 (and) Cetearyl Alcohol (and) Cetyl Palmitate	5.00
	Emulgade® PL 68/50	Cetearyl Glucoside (and) Cetearyl Alcohol	2.00
	Cetiol® RLF	Caprylyl Caprylate/Caprates	5.00
	Cetiol® MM	Myristyl Myristate	0.50
	Cetiol® SB 45	Butyrospermum Parkii (Shea) Butter	1.00
	Cetiol® Sensoft	Propylheptyl Caprylate	2.00
	Eutanol® G	Octyldodecanol	2.00
	Covi-ox® T 70 C	Tocopherol	0.50
	Dow Corning 200 Fluid 350 cSt ¹	Dimethicone	0.50
	Cosmedia® SP	Sodium Polyacrylate	0.50
	Vanzan NF ²	Xanthan Gum	0.20
B	Deionized Water	Water	70.70
	Glycerin	Glycerin	3.00
	Elestab® 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
C	AMC™	Glycerin (and) Water (and) Sodium PCA (and) Urea, (and) Trehalose (and) Triacetin (and) Sodium Hyaluronate (and) Polyquaternium-51	3.00

Extract according to the invention	Water (and) Extract (and) Xanthan Gum	3.00
Champagne Petals RU-2133 ³		0.10

Viscosity: Brookfield RVT, 23°C, spindle T-E @ 5 rpm, with Helipath: 30,000 cps

pH: 6.0

Supplier Footnotes:

¹ Dow Corning Corporation

² RT Vnaderbilt, Inc

³ Takasago International Corp.

Procedure

Heat Phase A (without Cosmedia SP and Xanthan Gum) and Phase B to 80-85 C.

While stirring Phase A, disperse Cosmedia SP and Xanthan Gum.

Combine and heat Phase B to 80-85 C then add to Phase A, while mixing.

Start cooling. Homogenize at 55-60 C. Add Phase C one by one at 40C or below, mix well between additions, then cool to room temperature and stop.

Example 9A
Skin Firming Lotion

Ph.	Trade Name	INCI Name	% wt
A	Deionized Water	Water	68.40
	Glycerin	Glycerin	3.00
	Elestab® 388	Propylene Glycol (and) Phenoxyethanol (and) Chlorphenesin (and) Methylparaben	1.00
	Vanzan NF	Xanthan Gum	0.10
	Cosmedia® SP	Sodium Polyacrylate	1.00
B	Plantapon® LGC Sorb	Sodium Lauryl Glucose Carboxylate (and) Lauryl Glucoside	1.50
C	Dehymuls® PGPH	Polyglyceryl-2 Dipolyhydroxystearate	4.00
	Cetiol® Sensoft	Propylheptyl Caprylate	5.00
	Myritol® 331	Cocoglyceride	3.00
	Cetiol® J600	Oleyl Erucate	1.00
	Cetiol® SB 45	Butyrospermum Parkii (Shea) Butter	2.00
D	Champagne Petals RU-2133		3.00
E	Extract according to the invention	Water (and) Extract	2.00
	Baobab Extract	Hydrolyzed Adansonia Digitata Extract	5.00

Viscosity: Brookfield RVT, 23°C ,spindle #5 @ 10 rpm: 7,800 cps
pH: 5.5

Supplier Footnotes:

¹ RT Vanderbilt, Inc

² Takasago International Corp.

Procedure

Swell pre-mixed Cosmedia SP and Vanzan NF in Phase A. Add Phase B and mix homogeneously. Heat Phase C to 45-50 C—just enough to melt shea butter. Mix homogeneously and cool to room temp. Add Phase D to Phase C and mix well. Then slowly add oil Phase C/D to the water Phase A/B while stirring. Avoid incorporating air. If necessary homogenize with a suitable dispersion unit (e.g. Ultra Turrex).. Add ingredients in Phase E one by one while mixing.

Example 10A

Body Lotion

Ph.	Trade Name	INCI Name	% wt
A	Emulgade® PL 68/50	Cetearyl Glucoside (and) Cetearyl Alcohol	5.00
	Cutina® CP	Cetyl Palmitate	3.00
	Monomuls® 90-O 18	Glyceryl Oleate	0.50
	Cetiol® OE	Dicaprylyl Ether	2.00
	Myritol® 312	Caprylic/Capric Triglyceride	5.00
	Cegesoft® PS 6	Vegetable Oil	4.00
	Cegesoft® VP	Vegetable Oil (and) Hydrogenated Vegetable Oil (and) Euphorbia Cerifera (Candelilla) Wax	1.00
B	Deionized Water	Water	74.15
	Vanzan NF (RT Vanderbilt) ¹	Xanthan Gum	0.50
	Glycerin	Glycerin	3.00
	Potassium Sorbate ²	Potassium Sorbate	0.30
	Elestab® CPN	Chlorphenesin	0.25
	Eumulgin® SG	Sodium Stearoyl Glutamate	1.00
C	Extract according to the invention	Extract (and) Pluronic®	0.20
	Bergamot & Jasmine		0.10

Musk	F-127986 ³	
Citric Acid (25%)	Citric Acid	q.s.

Viscosity: Brookfield RVT, 23°C, spindle T-E @ 5 rpm: 40,000 cps

pH: 6.4

Supplier Footnotes:

¹ RT Vanderbilt, Inc

² Jeen International Corporation

³ Inatrome Fragrance and Flavor Corp

Procedure

Prepare Phase B. Hydrate the thickeners first, then add the rest of the ingredients while mixing. Heat Phase A and B to 80-85 C. Add Phase B to Phase A, then cool to 55-60 C and homogenize. Transfer to regular mixing and continue cooling. Add Phase C one by one at 40 C or below and mix well between additions. If necessary adjust pH with citric acid.

Example 11 A

Lipstick or other anhydrous product type

Phase	Ingredient	Wt. % (w/w)
A	Mineral wax	17.0
	Isostearyl isostearate	31.5
	Propylene glycol dipelargonate	2.6
	Propylene glycol isostearate	1.7
	PEG 8 beeswax	3.0
	Hydrogenated palm kernel oil, glycerides, hydrogenated palm glyceride	3.4
	Lanolin oil	3.4
	Sesame oil	1.7

	Tribehenin	1.7
	Cetyl lactate	3.0
	Mineral oil, lanolin alcohol	3.0
B	Castor oil	qsp 100
	Titanium dioxide	3.9
	CI 15850:1	.616
	CI 45410:1	.256
	CI 19140:1	.048
	CI 77491	1.048
C	Extract according to invention	.001 – 5

Claims

1. A method of extraction of at least one target skin bioactive from algae, fungi or botanical biomass, which method comprises

a) contacting the algae, fungi or botanical biomass with an aqueous liquid to form a slurry,

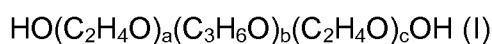
wherein the aqueous liquid comprises about 0.01 to about 20, preferably 0.1 to about 10, most preferably about 0.5 to about 5 wt. percent, especially 0.5 to about 2 or about 3 wt. % of a nonionic surfactant selected from the group consisting of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides and the wt. percent is based on the total weight of the slurry,

and

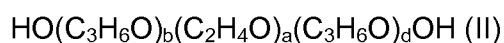
b) optionally, separating the biomass from the aqueous slurry,

and the target skin bioactive is a flavonoid or flavonoid derivative.

2. The method according to claim 1, wherein the nonionic surfactant is a triblock copolymer defined by the formula (I) or (II)



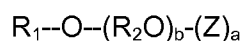
or



wherein *a* and *c* are independently 3 to 200,

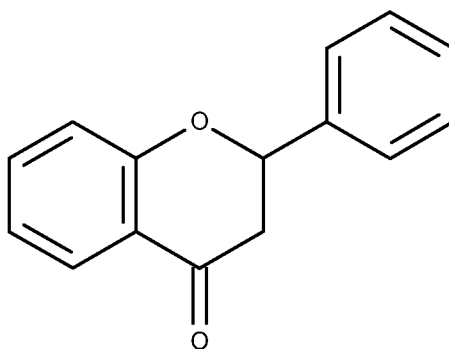
and *b* and *d* are independently 5 to 100.

3. The method according to claim 1, wherein the nonionic surfactant is the alkyl polyglucoside and is defined by the formula

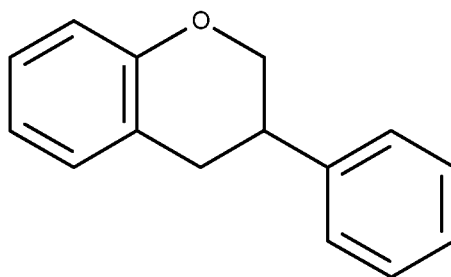


wherein R_1 is a monovalent organic radical having from about 6 to about 30 carbon atoms, R_2 is a divalent alkylene radical having from 2 to 4 carbon atoms, and Z is a saccharide residue having 5 or 6 carbon atoms, b is a number from 0 to about 12, and a is a number of from 1 to 6.

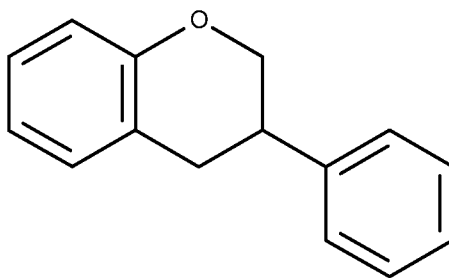
4. The method according to any one of the above claims, wherein the biomass is a flavonoid rich plant, algae or fungi.
5. The method according to any one of the above claims, wherein the flavonoid or flavonoid derivative is selected from compounds comprising the cyclic cores



(A)



(B) and



(C).

6. The method according to anyone of the above claims, wherein the flavonoid or flavonoid derivative is selected from the group consisting of abyssinone I, abyssinone V, afzelechin, ampelopsin, aromadendrin, asebogenin, auriculoside, betagarin, broussin, broussonin C, butin, butrin, (+)-catechin, catechin 7-O- β -xyloside, davidigenin, diffutin, 7,4'-dihydroxyflavan, 2,6-dihydroxyl-4'-methoxydihydro-chalcone, 7,3'-dihydroxyl-4-methoxy-8-methylflavan, 7,4'-dihydroxyl-8-methylfalvan, 6,8-diprenylnaringenin, dracorubin, (-)-epicatechin, ent-epicatechin, epigallo catechin 3-gallate, eriocitrin, eriodictyol, farrerol, fisetinidol, fisetinidol-4-ol, fustin, garbanzol, glabranin, glepidotin β , glycyphyllin, hesperetin, hesperidin, homoeriodictyol, 7-hydroxyflavan, isochamaejasmin, isosakuranetin, isourietin, kazinol a, kolaflavanone, liquiretigenin, manniflavanone, 6, methoxyaromadendrin 3-O-acetate, 6-methoxytaxifolin, 2'-O-methylodoratol, naringenin, naringin, narirutin, neoastilbin, neoeriocitrin, neoheesperidin, odoratol, phloretin, phellamurin, phloretin, phloridzin, pinobanksin, pinocembrin, pinocembrin 7-rhamnosyl-glucoside, piperaduncin β , poncirin, 5'-prenyl, naringenin, pruning, sakuranetin, sanggenon C, sanggenon D, silandrin, silybin, silychristin, sophoranone, strobopinin, taxifolen, taxifolin-3-O-acetate, tephrowatsin, theasinensin A, 2',4',6'-trihydroxyl-3'-formyldihydrochalcone and uvaretin.
7. The method according to any one of claims 1 to 5, wherein the biomass is a plant and selected from the group consisting of *Cassia alata*, *Argania spinosa* and *Cocoa Callus*.
8. The method according to any one of claims 1 to 6, wherein the slurry in step a) is essentially organic solvent free.

9. Use of a nonionic surfactant in an aqueous medium to increase flux during a filtration process of a solid biomass from an aqueous extract,

wherein the nonionic surfactant is a surfactant selected from the group consisting of of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides and the aqueous extract comprises at least a flavonoid or flavonoid derivative.
10. Use of a nonionic surfactant in an aqueous medium to increase the flavonoid extraction from a flavonoid rich plant, wherein the nonionic surfactant is a surfactant selected from the group consisting of of block copolymers of poly(ethylene oxide)/poly(propyleneoxide) and alkyl polyglucosides.

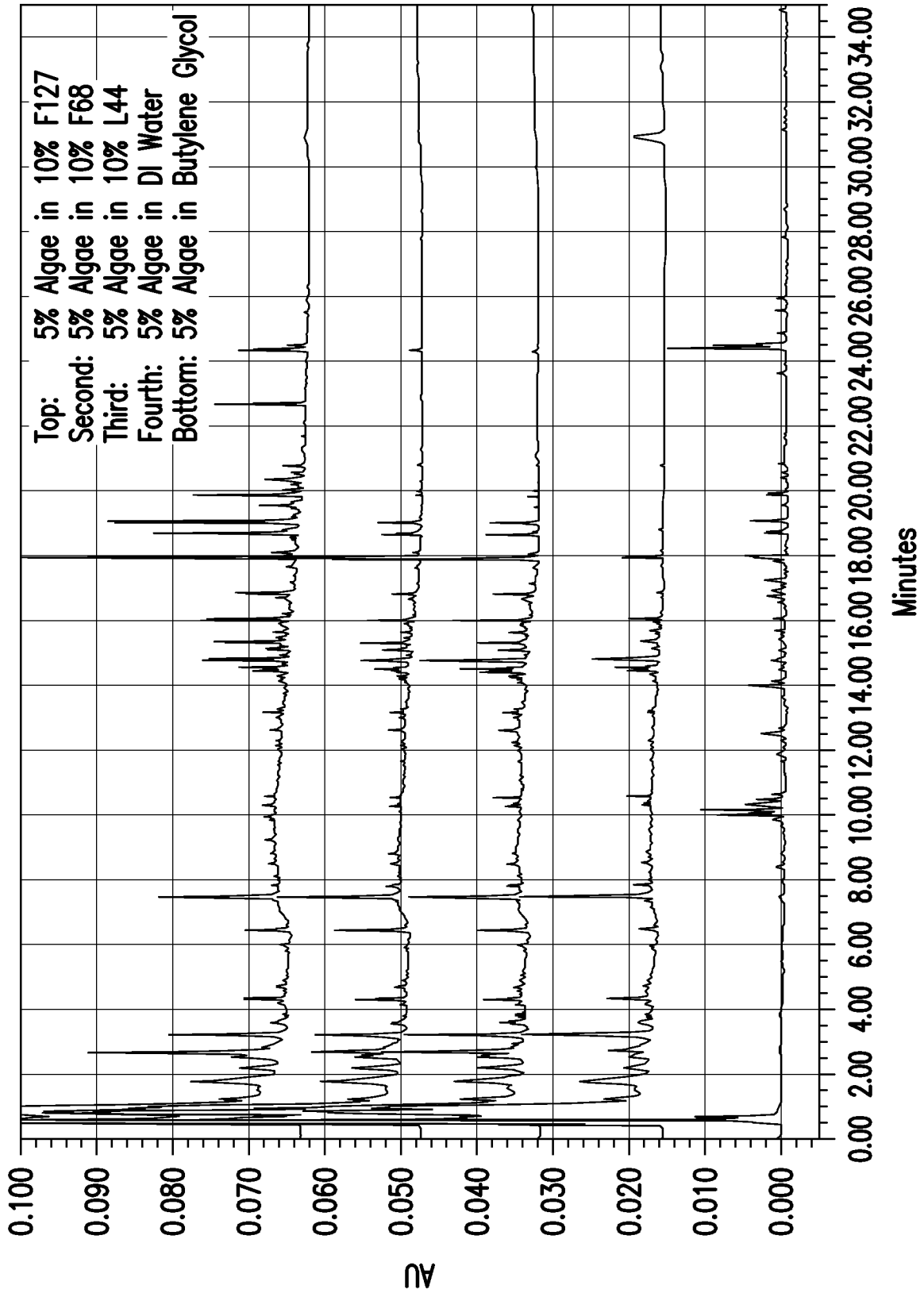
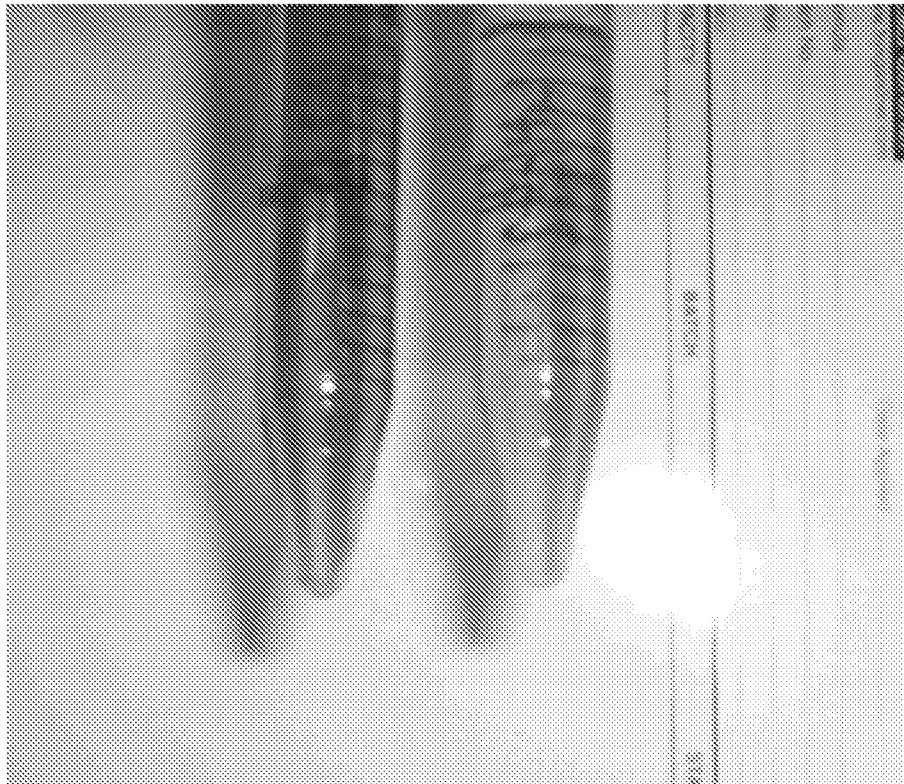


FIG. 1



5% Fucus Vesiculosus Extraction In Water On Left And
Extraction 10% Pluronic F127/Water Solution On The Right.

FIG.2

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/352(2006.01)i, A61K 31/35(2006.01)i, A61P 17/00(2006.01)i

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 31/352; C12N 1/00; A61K 31/7048; C12P 19/60; C09K 8/584; A61K 36/48; A61P 17/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: extraction, biomass, flavonoid, nonionic surfactant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006-0099690 A1 (CHANG, P. R. et al.) 11 May 2006 See abstract; paragraphs [0095]-[0113]; and claims 53-119.	1-4, 9, 10
A	MINUTH, T. et al., 'Extraction of cholesterol oxidase from Nocardia rhodochrous using a nonionic surfactant-based aqueous two-phase system', Journal of Biotechnology, 1995, Vol. 38, pages 151-164. See abstract; and pages 154-162.	1-4, 9, 10
A	KR 10-0741644 B1 (HUONS CO., LTD.) 23 July 2007 See abstract; page 4; and claims 1-5.	1-4, 9, 10
A	US 2011-0190175 A1 (STEINBRENNER, U. et al.) 04 August 2011 See abstract; paragraphs [0074]-[0104]; and claim 1.	1-4, 9, 10
A	US 2006-0078633 A1 (NA, M. K. et al.) 13 April 2006 See abstract; paragraphs [0009]-[0012]; and claims 1-13.	1-4, 9, 10



Further documents are listed in the continuation of Box C.



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

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"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

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Date of the actual completion of the international search

29 October 2013 (29.10.2013)

Date of mailing of the international search report

29 October 2013 (29.10.2013)

Name and mailing address of the ISA/KR


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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/044464

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

- 2. Claims 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. Claims Nos.: 5-8
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 an additional fee, this Authority did not invite payment of any additional fee.
- 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 claims 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 claims 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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/044464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006-0099690 A1	11/05/2006	AU 2003-269646 A1 BR 0314481 A CA 2496316 A1 CN 1685053 A EP 1546356 A2 JP 2006-502712 A WO 2004-027074 A2 WO 2004-027074 A3	08/04/2004 26/07/2005 01/04/2004 19/10/2005 29/06/2005 26/01/2006 01/04/2004 23/09/2004
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