For example, 1 mg of cold-water extract will exhibit a different therapeutic activity profile than 1 mg of supercritical fluid extract. Moreover, the SCF extract has demonstrated improved efficacy over oleandrin alone and even over the hot-water extract in treating some conditions.

[0014] Given the important therapeutic properties of oleandrin and extracts containing oleandrin and other components of the Nerium plant, a need remains for improved pharmaceutical compositions containing oleandrin and for improved methods of producing oleandrin and oleandrin-containing extracts.

SUMMARY ~~OF THE INVENTION~~

[0015] The present ~~invention~~disclosure seeks to overcome some or all of the disadvantages inherent in the art. Objects of the ~~invention~~disclosure include providing an improved process for production of oleandrin-containing extract, and an improved process for production of oleandrin. Another object of the ~~invention~~disclosure includes providing an oleandrin-containing extract and an oleandrin-containing composition. Another object of the ~~invention~~disclosure provides a sublingual or buccal dosage form comprising the extract (or composition) and at least one pharmaceutical excipient. Another object of the ~~invention~~disclosure is to provide pharmaceutical compositions, dosage forms, cosmetics, and nutraceutical products containing oleandrin or the extract(s) described herein.

[0016] The improved process(es) provide(s) a) a higher yield of oleandrin per kg of plant biomass as compared to a supercritical fluid (SCF) extraction (SCFE)-based process; b) an oleandrin-containing composition with a higher ratio of oleandrin to other components as compared to a SCFE process; c) a dual extract composition comprising an organic solvent extract and a subcritical extract; d) an organic solvent extract with a higher content of oleandrin as compared to a SCFE process obtained from the same amount of plant material; e) a subcritical CO_2 extract with a higher content of oleandrin as compared to a SCF extract obtained using the same solvent(s) and same amount of plant material.

[0017] In each ~~embodiment~~example of the organic solvent extract or organic solvent extraction, the organic solvent comprises a) one or more organic solvents; or b) water and one or more organic solvent extracts. The water may further comprise one or more salts, one or more buffers, or a combination thereof.

[0018] An ~~aspect~~ example of the ~~invention~~disclosure provides a method of producing oleandrin-containing extract, the method comprising

subjecting oleandrin-containing biomass to subcritical liquid extraction (SbCLE) employing subcritical liquid (SbCL) comprising carbon dioxide, optionally further comprising organic solvent (modifier), for a period of time sufficient to extract the oleandrin and form extraction milieu;

separating said biomass from said extraction milieu to provide oleandrin-containing SbCL; and

removing SbCL from said oleandrin-containing SbCL to provide said oleandrin-containing (OC) extract (OCE).

[0019] An ~~aspect~~ example of the ~~invention~~disclosure provides a method of producing oleandrin, the method comprising

subjecting oleandrin-containing biomass to subcritical liquid extraction (SbCLE) employing subcritical liquid (SbCL) comprising carbon dioxide, optionally further comprising organic solvent (modifier), for a period of time sufficient to extract the oleandrin to form extraction milieu;

separating said biomass from said extraction milieu to provide oleandrin-containing SbCL;

removing SbCL from said oleandrin-containing SbCL to provide oleandrin-containing extract; and

isolating oleandrin from said extract.

[0020] Another example ~~aspect~~ of the ~~invention~~disclosure provides a method of producing a combination composition, the method comprising

subjecting oleandrin-containing first biomass to organic solvent extraction to provide an oleandrin-containing organic solvent extract;

subjecting oleandrin-containing second biomass to subcritical liquid (SbCL) extraction (SbCLE) to provide an oleandrin-containing SbCL extract; and

combining said oleandrin-containing organic solvent extract and said oleandrin-containing SbCL extract to provide said combination composition.

[0020a] An aspect of the disclosure provides a method of producing the combination composition of the disclosure, the method comprising

subjecting oleandrin-containing first biomass to organic solvent extraction to provide an oleandrin-containing organic solvent extract;

subjecting oleandrin-containing second biomass to subcritical liquid (SbCL) extraction (SbCLE) to provide an oleandrin-containing SbCL extract, wherein the SbCLE is performed with SbCL comprising carbon dioxide and optionally one or more modifiers; and

combining said oleandrin-containing organic solvent extract and said oleandrin-containing SbCL extract to provide said combination composition.

[0021] In some ~~embodiment~~examples, the combination composition comprises at least oleandrin, oleanolic acid, ursolic acid, betulinic acid, kanerocin, kanerodione, oleandrigenin, Nerium F, neritaloside, odoroside (A and H), adynerin, odoroside-G-acetate, and gitoxigenin. The combination composition may further comprise one or more of neriin, folinerin, gitoxigenin, digitoxigenin, nerigoside, rutin, ursonic acid, neridienone A, adynerigenin, deacetyloleandrin, odoroside G acetate, and/or quercetin.

[0022] The composition (or the extract) may further comprise polyphenol(s), carbohydrate(s), flavonoid(s), amino acid(s), soluble protein(s), cellulose, starch, alkaloid(s), saponin(s), tannin(s), and any combination thereof.

[0023] The amino acid can be selected from the group consisting of aspartic acid, glutamic acid, asparagine, serine, glutamine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine, tryptophan, and lysine. In some ~~embodiment~~examples, the amino is selected from the group consisting of asparagine, arginine, threonine, alanine, proline, tyrosine, valine, isoleucine, leucine, phenylalanine, tryptophan, and lysine.

[0024] Another example ~~aspect~~ of the ~~invention~~disclosure provides a cosmetic (cosmeceutical) product or a nutraceutical product comprising oleandrin, oleandrin-containing composition, oleandrin-containing combination composition, or oleandrin-containing extract(s) as defined herein. An aspect of the disclosure provides a nutraceutical composition comprising the combination composition of the disclosure and one or more dietary ingredients and/or or food additives. An aspect of the disclosure provides a cosmetic composition comprising the combination composition of the disclosure and one or more cosmetically acceptable ingredients.

[0025] The cosmetic product may further comprise one or more cosmetic excipients. The cosmetic product can be a cleanse, shampoo, conditioner, body wash, face cleanser, skin toner, serum, moisturizer, balm, makeup, lotion, cream, gel, hydrogel, ointment, oil-based solution, suspension, powder, spray, foundation, primer, highlighter, eye shadow, foundation, blush, towelette, deodorant, bath oil, bubble bath, bath salt, body butter, lipstick, lip gloss, lip liner, concealer, rouge, facial mask treatment, or any other type of cosmetic product.

[0026] A nutraceutical can include a dietary ingredient, dietary supplement, bioceutical, or food additive such as may be defined by the USFDA or other regulatory agencies. The nutraceutical product may further comprise one or more nutraceutical or dietary ingredients. The nutraceutical may include a liquid, solid, powder, cream, solution, suspension, dosage form, gel, capsule, tablet, pill, prebiotic, probiotic, functional food, meal supplement, meal replacement, or other such products.

[0027] The ~~invention~~disclosure also provides an improved oleandrin-containing combination composition comprising a portion of oleandrin-containing organic solvent extract and a portion of oleandrin-containing SbCL extract. An aspect of the disclosure provides an improved oleandrin-containing combination composition comprising a portion of oleandrin-containing organic solvent extract and a portion of oleandrin-containing subcritical liquid (SbCL) extract, wherein the organic solvent extract comprises at least oleandrin and other components extracted from the biomass, and the compositional profile of extracted volatile and semi-volatile components in said organic solvent extract is different than the compositional profile of said components in said subcritical liquid extract. In some ~~embodiment~~examples, a) substantially equal portions of the two extracts are present in the combination composition; b) the content of organic solvent extract in said combination is higher than the content of SbCL extract; or c) the content of SbCL extract in said combination is higher than the content of organic solvent extract. Respective methods of preparation and methods of use are also provided substantially as described herein.

[0028] As compared to either the organic solvent extract and the subcritical liquid extract, the combination composition advantageously comprises a greater range of phytochemicals and other components, aside from oleandrin, extractable from the plant material. It is known that one or more of those other components are also therapeutically effective (pharmacologically active) ingredients.

[0029] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein a) the organic solvent extract comprises at least oleandrin and other components extracted from the biomass; b) in the organic solvent extract, the molar ratio of oleandrin to other components extracted from the biomass is greater than said molar ratio in the subcritical liquid extract; c) the SbCL extract comprises at least oleandrin and other components extracted from the biomass; d) the content of oleandrin in the ethanolic extract is greater than the content of oleandrin in the subcritical liquid extract; e) the content of extracted non-oleandrin components in the organic solvent extract is greater than the content of said components in said subcritical liquid extract; f) the compositional profile of extracted volatile and semi-volatile components in said organic solvent extract is different than the compositional profile of said components in said subcritical liquid extract; and/or g) relative to oleandrin content, the subcritical liquid extract comprises a greater proportion of extracted volatile and semi-volatile components than does the organic solvent extract.

[0030] The first and second biomass may be the same or different. The first biomass extracted with organic solvent may be used as the second biomass; alternatively, the first biomass is extracted with organic solvent, and a different second biomass is extracted with subcritical liquid.

[0031] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the subcritical liquid extraction process further comprises one or more of the following: a) separating said biomass from the extraction milieu by filtering the extraction milieu; b) adding modifier to the carbon dioxide or extraction milieu; c) heating and pressurizing the interior of a vessel containing the extraction milieu to form subcritical liquid phase extraction milieu; d) removing said SbCL from said oleandrin-containing SbCL by volatilizing said SbCL to form raw SbCL extract (SbCLE); e) diluting the raw SbCL extract with organic solvent or aqueous organic solvent; f) filtering the diluted raw or crude SbCL extract one or more times; g) treating the diluted raw or crude SbCL extract with activated carbon; h) removing most of the organic solvent from the diluted raw extract to form; and/or i) sterile filtering the raw or crude extract to obtain the SbCL extract. The SbCL extract is optionally further

diluted with organic solvent or aqueous organic solvent. The organic solvent extract is optionally further diluted with organic solvent or aqueous organic solvent. The SbCL extract may comprise 0.1 mg to 1 mg or 0.5 to 0.8 mg of oleadrin per mL of extract.

[0032] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the carbon dioxide (along with any modifier, if any is present) is in the subcritical liquid phase during about 50% or more of the extraction time period, i.e. about 50% or more of the total time for the "subjecting" step. In some ~~embodiment~~examples, the extraction milieu, comprising extraction fluid (carbon dioxide along with any modifier, if any is present) in the subcritical liquid phase and biomass, will remain in liquid form for at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of the extraction period. In some ~~embodiment~~examples, the extraction milieu will remain in liquid form throughout the extraction period.

[0033] Accordingly, the SbCLE is conducted such that the extraction milieu (comprising biomass and CO_2, and optionally further comprising organic solvent (modifier)) is under the following conditions, with respect to the phase diagram of CO_2, during about 50% or more of the extraction time period: a) at or below the critical pressure (p_c), at or above the triple point (solid-liquid-gas) temperature (t_{tp}), below the critical temperature (t_c), and above the liquid-gas phase line; b) at or below the t_c, at or above the t_{tp}, below the p_c, and above the liquid-gas phase line; c) at or above the p_c, below the t_c, at or above the t_{tp}, above the liquid-gas phase line, and below the solid-liquid phase line; or d) at a temperature and pressure falling within the liquid region bounded by the solid-liquid phase line, the liquid-gas phase line, the liquid-supercritical phase line, the t_{tp}, the triple point pressure (p_{tp}), the critical point (liquid-gas-supercritical) temperature (t_{cp}), and the critical point pressure (p_{cp}). The critical pressure and temperature are with respect to CO_2.

[0034] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein SbCLE is conducted at a temperature of about 0°C up to about 31°C and a pressure of about 5.2 bar to about 3000 bar. Other ~~embodiment~~examples of the ~~invention~~disclosure include those wherein SbCLE is conducted at a temperature of about 0°C up to about 31°C and a pressure of about 5.2 bar to about 1000 bar. In preferred ~~embodiment~~examples, SbCLE is conducted at a temperature of about 2°C to about 16°C and a pressure of about 115 bar to about 135 bar. In other preferred ~~embodiment~~examples, SbCLE is conducted at a temperature of about 5-10 °C and a pressure of about 120-130 bar. In some ~~embodiment~~examples, the SbCLE is conducted at a temperature in the range of about 5°C to about 15°C and a pressure in the range of about 50 bar to 70 bar.

[0035] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the SbCL further comprises one or more modifiers, e.g. organic solvent(s). When the one or more modifiers is(are) present, the weight ratio of carbon dioxide to modifier is about 100: about 0.01-20, about 100: about 0.01-15, about 100: about 0.01-10, or about 100: about 0.01-5.

[0036] The one or more modifiers (organic solvents) may be independently selected upon each occurrence from alcohol, organic solvent, alkyl ketone, alkyl ester, water. Particularly suitable modifiers include ethanol, methanol propanol, acetone, ethyl acetate, methyl ethyl ketone, glycol (e.g. propylene glycol), dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), or N-methylpyrrolidone (NMP)

[0037] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein a) said organic solvent extraction is conducted with organic solvent or aqueous organic solvent; b) independently upon each occurrence, said organic solvent or aqueous organic solvent comprises alcohol; c) independently upon each occurrence, said organic solvent is selected from the group consisting of methanol, ethanol, propanol, butanol, chloroform, methylene chloride, ethyl acetate, and a combination thereof; d) said organic solvent extraction comprises extracting said oleandrin-containing biomass with organic solvent or aqueous organic solvent one or more times; e) independently upon each occurrence, said aqueous organic solvent comprises up to about 20 % w/w, about 5% w/w to about 15% w/w, or about 10% w/w of water; f) said organic solvent extract is conducted at a temperature of from about 0°C to about 75°C, about 5°C to about 70°C, about 10°C to about 60°C, about 10°C to about 50°C, about 10°C to about 45°C, or about 20°C to about 40°C; g) the v/w ratio of organic solvent (or aqueous organic solvent) to biomass is in the range of about 50-1: about 1, about 40-1: about 1, about 30-1: about 1, about 20-1: about 1, about 20-5: about 1, or about 10: about 1. Where undessicated or undehydrated or unfreeze-dried biomass is used for extraction, the water content included in the biomass is accounted for as the v/w ratio of aqueous organic solvent to biomass (dry weight basis).

[0038] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the organic solvent extraction further comprises one or more of the following: a) separating said biomass from the extraction milieu by filtering the extraction milieu to form crude extract; b) adding aqueous liquid to the organic solvent or extraction milieu; c) reducing the content of organic solvent from said crude extract; d) treating the crude extract with activated carbon; e) filtering the crude extract one or more times; f) adding additional organic solvent to the crude extract; g) sterile filtering the organic solvent extract; and/or h) adding water to the

crude extract and extracting the aqueous crude extract with organic solvent. The organic solvent extract is optionally further diluted with organic solvent or aqueous organic solvent. The organic solvent extract may comprise 0.1 to 10 mg/mL, 0.5 to 8 mg/mL, 1 to 8 mg/mL, 1.5 to 8 mg/mL, or 1.5 to 5 mg/mL (mg of oleandrin per mL of extract).

[0039] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the oleandrin-containing biomass is *Nerium* sp., *Nerium oleander*, *Nerium oleander* L (Apocynaceae), *Nerium odourum*, *Nerium indicum*, white oleander, pink oleander, *Thevetia* sp., *Thevetia peruviana*, yellow oleander, *Agrobacterium tumefaciens*, or a combination thereof. In some ~~embodiment~~examples, the biomass comprises leaves, stems, flowers, bark, fruits, seeds, sap, cultured cellular mass, and/or pods.

[0040] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein a) independently upon each occurrence, the biomass is dehydrated, dehumidified, freeze-dried, and/or desiccated prior to extraction; b) independently upon each occurrence, the biomass is dried prior to extraction; c) independently upon each occurrence, the biomass is not dehydrated prior to extraction; d) independently upon each occurrence, the biomass is not dried prior to extraction; or e) independently upon each occurrence. The water content in the biomass is preferably about 20 wt % or less, about 15 wt % or less, about 10 wt % or less, about 8 wt % or less, about 5 wt % or less, about 2.5 wt % or less, or about 1 wt % of less, prior to extraction. Accordingly, the method of the ~~invention~~disclosure also includes ~~embodiment~~examples further comprising dehydrating, drying or desiccating the biomass prior to extraction.

[0041] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein a) prior to extraction, the biomass has an average particle size (d0.5) of less than about 1”, less than about 0.75”, less than about 0.5”, less than about 0.4”, less than about 0.3”, less than about 0.2”, less than about 0.1”, less than about 0.05”, or less than about 0.01”, said particle being with respect to ASTM sieve mesh opening size.

[0042] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein a) the content of oleandrin in said extract is about 0.01 wt % or higher, about 0.05 wt % or higher, about 0.1 wt % or higher, about 0.2 wt % or higher, about 0.5 wt % or higher, about 1 wt % or higher, about 2.5 wt % or higher, about 5 wt % or higher, about 10 wt % or higher, about 20 wt % or higher, about 30 wt % or higher, about 40 wt % or higher, about 50 wt % or higher, about 60 wt % or higher, about 70 wt % or higher, about 80 wt % or higher, or about 90 wt % or higher; b) the content of oleandrin in said extract is about 99 wt % or lower, about 95 wt % or lower, about 90 wt % or lower, about 80 wt % or lower, about 70 wt % or lower, about

60 wt % or lower, about 50 wt % or lower, about 40 wt % or lower, about 30 wt % or lower, about 20 wt % or lower, about 10 wt % or lower, about 5 wt % or lower, about 2.5 wt % or lower, about 1 wt % or lower, or about 0.5 wt % or lower; or c) a combination of any limit of item a) and any limit of item b).

[0043] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein the process further comprises one or more of the following: a) removing (or reducing the amount of) volatile organic compounds (VOC's) from said extract; b) removing (or reducing the amount of) semi-volatile organic compounds (SVOC's) from said extract; c) filtering said extract; d) said separating is conducted by filtration, centrifugation, decantation, or a combination thereof.

[0044] ~~Embodiment~~Examples of the ~~invention~~disclosure include those wherein said isolating is achieved by one or more of chromatography, liquid-liquid extraction, liquid-solid extraction, crystallization, precipitation, fractional precipitation, fractional crystallization, Soxhlet extraction, trituration, lyophilization (freeze-drying), or a combination thereof.

[0045] The individual steps of any of the methods of the ~~invention~~disclosure can be conducted at separate facilities or within the same facility.

[0046] Pharmaceutical compositions comprising oleandrin as the sole active ingredient are within the scope of the ~~invention~~disclosure. Another aspect of the disclosure provides a pharmaceutical composition comprising at least one pharmaceutical excipient and the combination composition of the disclosure. Compositions comprising oleandrin and plural active ingredients obtained from said biomass are within the scope of the ~~invention~~disclosure. Compositions comprising oleandrin and plural triterpenes as the active ingredients are within the scope of the ~~invention~~disclosure. In some ~~embodiment~~examples, said composition comprises oleandrin, oleanolic acid (free acid, salt, derivative or prodrug thereof), ursolic acid (free acid, salt, derivative or prodrug thereof), and betulinic acid (free acid, salt, derivative or prodrug thereof). The molar ratios of the compounds are as described herein. A triterpene is independently selected upon each occurrence from its free acid form, salt form, deuterated form and derivative form.

[0047] In addition to oleandrin, the extract may comprise one or more other compounds (which may or may not be pharmacologically active, therapeutically effective) extracted from the biomass during the extraction process. The one or more other compounds may or may not contribute to the therapeutic efficacy of oleandrin when administered to a subject receiving both. The one or more other compounds can be selected from the group consisting of cardiac glycoside, one or more glycone constituents of cardiac glycosides (such as glucoside, fructoside, and/or glucuronide), one or more aglycones, one or more glycoside

precursors (such as cardenolides, cardadienolides and cardatrienolides), one or more steroids, one or more triterpenes, one or more saccharides, one or more polysaccharides, one or more alkaloids, one or more proteins, fat, one or more other non-cardiac glycoside therapeutically effective agent, and any combination thereof. In some ~~embodiment~~examples, the composition comprises oleandrin and: a) two or three triterpenes; b) two or three triterpene derivatives; c) two or three triterpene salts; or d) a combination thereof. As used herein, the generic terms triterpene and cardiac glycoside also encompass free form, salt form and derivatives thereof, unless otherwise specified. In some ~~embodiment~~examples, the triterpene is selected from the group consisting of oleanolic acid, ursolic acid, betulinic acid and salts or derivatives thereof.

[0048] In some ~~embodiment~~examples, the organic extract comprises oleandrin and a) one or more volatile organic compounds; b) one or more semi-volatile organic compounds; c) one or more nonvolatile organic compounds; or d) any combination thereof.

[0049] In some ~~embodiment~~examples, the extract excludes a pharmacologically active polysaccharide (and/or saccharide) obtained during extraction. In some ~~embodiment~~examples, the extract comprises oleandrin and at least one pharmacologically active polysaccharide (and/or saccharide) obtained during extraction. The pharmacologically active polysaccharide may be an acidic homopolygalacturonan or arabinogalaturonan.

[0050] In some ~~embodiment~~examples, the extract comprises at least oleandrin, oleanolic acid, ursolic acid, betulinic acid, kanerocin, kanerodione, oleandrigenin, Nerium F, neritaloside, odoroside (A and H), adynerin, odoroside-G-acetate, and gitoxigenin. The combination composition may further comprise one or more of neriin, folinerin, gitoxigenin, digitoxigenin, nerigoside, rutin, ursonic acid, neridienone A, adynerigenin, deacetyloleandrin, odoroside G acetate, and/or quercetin.

[0051] The extract may further comprise one or more polyphenol(s), one or more carbohydrate(s), one or more flavonoid(s), one or more amino acid(s), one or more soluble protein(s), one or more cellulose(s), one or more starch(es), one or more alkaloid(s), one or more saponin(s), one or more tannin(s), and any combination thereof.

[0052] The amino acid can be selected from the group consisting of aspartic acid, glutamic acid, asparagine, serine, glutamine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine, tryptophan, and lysine. In some ~~embodiment~~examples, the amino is selected from the group

consisting of asparagine, arginine, threonine, alanine, proline, tyrosine, valine, isoleucine, leucine, phenylalanine, tryptophan, and lysine.

[0053] It should be understood that even though other components in the extract may be present in greater relative quantity than oleandrin, oleandrin may be the primary therapeutically effective compound with respect specific diseases, disorders or conditions.

[0054] Some ~~embodiment~~examples of the ~~invention~~disclosure include those wherein a pharmaceutical composition comprises at least one pharmaceutical excipient and the extract.

[0055] The oleandrin, oleandrin-containing extract, or oleandrin-containing composition of the ~~invention~~disclosure can be administered to a subject in need thereof to treat a condition, disease or disorder that is therapeutically responsive to treatment with cardiac glycoside (oleandrin, digoxin, digitoxin, ouabain, bufalin, cinobufatalin, cinobufagin, resibufogenin, lanatoside C, or other therapeutically effective cardiac glycoside), in particular to treatment with oleandrin, or which can be prevented by administration of cardiac glycoside, in particular administration of oleandrin.

[0056] An aspect of the ~~invention~~disclosure provides a method of treating or preventing a condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract), the method comprising administering to a subject in need thereof one or more doses of oleandrin (oleandrin-containing composition or oleandrin-containing extract), thereby treating or preventing said condition, disease or disorder. Target conditions, diseases and disorders are described herein.

[0057] Another aspect of the ~~invention~~disclosure provides a method of improving or boosting the immunological response of a subject, the method comprising administering to a subject in need thereof one or more doses of oleandrin (oleandrin-containing composition, oleandrin-containing combination composition, or oleandrin-containing extract) in an amount sufficient to boost the subject's immunological response. The one or more doses can be administered as a pharmaceutical composition, nutraceutical composition, and/or cosmeceutical composition.

[0058] Administration can be acute, chronic, or a combination thereof and can occur before and/or after initiation of said condition, disease or disorder. Administration can be to a subject that has or is at risk of getting said condition, disease or disorder.

[0059] The oleandrin, oleandrin-containing extract, oleandrin-containing composition, or oleandrin-containing combination composition of the ~~invention~~disclosure can be included in any

pharmaceutical composition, nutraceutical composition, cosmeceutical compositions, or dosage form as described herein.

[0060] The oleandrin, oleandrin-containing extract, or oleandrin-containing composition of the ~~invention~~disclosure can be administered locally, systemically or a combination thereof. Suitable modes of administration are described herein.

[0061] In some ~~embodiment~~examples, oleandrin is present as the primary therapeutic component, meaning the component primarily responsible for therapeutic activity, in a pharmaceutical composition.

[0061a] Another aspect of the disclosure provides use of the pharmaceutical composition of the disclosure in the manufacture of a medicament for the treatment or prevention of a condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract).

[0061b] Another aspect of the disclosure provides use of the combination composition of the disclosure in the manufacture of a medicament for the treatment or prevention of a condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract).

[0062] The ~~invention~~disclosure also provides use of oleandrin-containing extract in the manufacture of a medicament for the treatment, in a subject, of a condition, disease or disorder that is therapeutically responsive to cardiac glycoside. In some ~~embodiment~~examples, the manufacture of such a medicament comprises: providing oleandrin-containing extract; including a dose of oleandrin-containing extract in a pharmaceutical dosage form; and packaging the pharmaceutical dosage form. In some ~~embodiment~~examples, the manufacture can be conducted as described in PCT International Application No. PCT/US06/29061, the entire disclosure of which is hereby incorporated by reference. The manufacture can also include one or more additional steps such as: delivering the packaged dosage form to a vendor (retailer, wholesaler and/or distributor); selling or otherwise providing the packaged dosage form to a subject having a viral infection; including with the medicament a label and a package insert, which provides instructions on use, dosing regimen, administration, content and toxicology profile of the dosage form.

[0063] In some ~~embodiment~~examples, the treatment comprises: determining that a subject has a condition, disease or disorder that is therapeutically responsive to cardiac glycoside, e.g. oleandrin (oleandrin-containing composition or oleandrin-containing extract), digoxin, ouabain, bufalin, cinobufatalin, cinobufagin, resibufogenin, or other therapeutically effective cardiac glycoside; indicating administration of pharmaceutical dosage form to the subject according to a dosing regimen; administering to the subject one or more pharmaceutical dosage forms, wherein the one or more pharmaceutical dosage forms is administered according to the dosing regimen.

[0064] The pharmaceutical composition can further comprise a combination of at least one material selected from the group consisting of a water soluble (miscible) co-solvent, a water insoluble (immiscible) co-solvent, a surfactant, an antioxidant, a chelating agent, and an absorption enhancer.

[0065] The solubilizer is at least a single surfactant, but it can also be a combination of materials such as a combination of: a) surfactant and water miscible solvent; b) surfactant and water immiscible solvent; c) surfactant, antioxidant; d) surfactant, antioxidant, and water miscible solvent; e) surfactant, antioxidant, and water immiscible solvent; f) surfactant, water miscible solvent, and water immiscible solvent; or g) surfactant, antioxidant, water miscible solvent, and water immiscible solvent.

[0066] The composition(s) of the ~~invention~~disclosure optionally further comprises a) at least one liquid carrier; b) at least one emulsifying agent; c) at least one solubilizing agent; d) at least one dispersing agent; e) at least one other excipient; or f) a combination thereof.

[0067] In some ~~embodiment~~examples, the water miscible solvent is low molecular weight (less than 6000) PEG, glycol, or alcohol. In some ~~embodiment~~examples, the surfactant is a pegylated surfactant, meaning a surfactant comprising a poly(ethylene glycol) functional group.

[0068] The ~~invention~~disclosure also provides a buccal or sublingual dosage form comprising any extract(s) of the ~~invention~~disclosure as described herein, and at least one pharmaceutical excipient. Said dosage form is adapted to deliver components of the extract to the mucosa of the mouth. Suitable dosage forms include tablet, wafer, patch, spray, drop, liquid, solution, paste, gel, film, bioadhesive composition, gummy, or powder.

[0069] The combination composition can be included in any type of pharmaceutical dosage form. Suitable ones are selected from the group consisting of solid dosage form, liquid dosage form, parenteral dosage form, otic dosage form, ophthalmic dosage form, nasal dosage form, inhalable dosage form, buccal dosage form, sublingual dosage form, enteral dosage form, topical dosage form, oral dosage form, peroral dosage form, injectable dosage form, tablet, capsule, pill, caplet, troche, sache, solution, suspension, dispersion, vial, bag, bottle, injectable liquid, *i.v.* (intravenous), *i.m.* (intramuscular) or *i.p.* (intraperitoneal) administrable liquid.

[0070] The dosage form can be a rapid release, immediate release, controlled release, sustained release, prolonged release, extended release, burst release, continuous release, slow release, or pulsed release dosage form, or is a dosage form that exhibits two or more of those types of release. When placed in an aqueous environment or after administration to a subject, the dosage form may exhibit a drug release profile selected from the group consisting of zero order, pseudo-zero, first order, pseudo-first order or sigmoidal release profile.

[0071] In some ~~embodiment~~examples, the dosage form a) releases its charge of composition within a period of 0.5 to 1 hours or less; b) is enteric coated and releases its charge of composition downstream of the stomach, such as in the jejunum, ileum, small intestine, and/or large intestine; or c) is enteric coated and releases its charge of composition into the systemic circulation within 1-10 hr after oral administration

[0072] In some ~~embodiment~~examples, the inhalable, buccal or sublingual dosage form further comprises at least one bitter taste antagonist (also referred to as a bitter receptor blocker). The bitter taste antagonist inhibits the taste receptor activation caused by a bitter compound, such as a cardiac glycoside. In preferred ~~embodiment~~examples, the bitter taste antagonist inhibits activation of at least one of the receptors of the hTAS2R family of G protein-coupled receptors. As used herein, the bitter taste antagonist can include taste-masking agent(s). Suitable bitter taste antagonists include GIV3757 (4-(2,2,3-trimethylcyclopentyl)butanoic acid), gamma-aminobutyric acid (GABA), 6-methoxy-flavanone, 4′-fluoro-6-methoxyflavanone, 6,3′-dimethoxyflavanone, 6-methoxyflavanone, abscisic acid, (-)-hardwickiic acid (2-(fitran-3-yl)ethyl)-trimethyI-hexahydronaphthalene-carboxylic acid), fenchone, borneol, isoborneol, anethole, menthofuran, monosodium glycyrrhizinate, pullulan (α-1,4-; α-1,6-glucan), flavorant, or sweetener. Suitable flavorant(s) include mint, peppermint, menthol, eucalyptus, eucalyptol, chocolate, spearmint, tangerine, orange, grape, grapefruit, marshmallow, coffee, banana crème, caramel, bubble gum, cherry, lemon, lime, strawberry, maple, raspberry, and/or apple. Suitable sweetener(s) include magnasweet (110 or 135), sucralose, steviol glycoside(s), stevia, glycerin, acesulfame potassium, PCCA sweetness enhnancer, PCCA bitter stop, di-d-fructofuranose 1,2′: 2,3′-di-anhydride, aspartame, neohesperidine dihydrochalcone and hesperidine dihydrochalcone 4′-β-d glucoside, d-sorbitol, sodium saccharin, sodium gluconate, and/or monosodium glutamate. The inhalable, buccal or sublingual dosage form can also include an oil-based excipient such as a fixed oil (e.g. from almond, peanut, sesame, olive, avocado, or corn), or hydrogenated oil. The inhalable, buccal or sublingual dosage form may also be taste-masked with taste masking by polymer coating; taste masking by conventional granulation; taste masking with ion-exchange resins; taste masking by spray congealing with lipids; taste masking by formation of inclusion complexes with cyclodextrins; taste masking by the freeze-drying process; taste masking by making multiple emulsions; and taste masking with gelatin, gelatinized starch, liposomes, lecithins or lecithin-like substances, surfactants, salts, or polymeric membranes.

[0073] In some ~~embodiment~~examples, the inhalable, buccal or sublingual dosage form further comprises one or more permeation enhancers that improve mucosal permeability and absorption of compounds, such as cardiac glycoside(s). Suitable permeation enhancer(s) include surfactants, bile salts, fatty acids, cyclodextrins, and chelators.

[0074] The ~~invention~~disclosure includes all combinations of the aspects, ~~embodiment~~examples and sub-~~embodiment~~examples of the ~~invention~~disclosure disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

[0075] The following figures form part of the present description. The skilled artisan will, in light of these figures and the description herein, be able to practice the invention without undue experimentation.

[0076] FIG. 1 depicts a phase diagram for carbon dioxide indicating the solid, liquid, gas and supercritical phases as well as the triple point and critical point temperatures and pressures. The solid and liquid phases are separated by the solid-liquid phase line. The liquid and gas phases are separated by the liquid-gas phase line. The supercritical fluid and liquid phases are separated by the supercritical fluid-liquid phase line. The liquid region is thus bounded by the liquid-gas phase line, solid-liquid phase line, supercritical fluid-liquid phase line, triple point (temperature and pressure) and critical point (temperature and pressure).

[0077] FIG. 2 depicts a phase diagram for carbon dioxide indicating the supercritical region and two of the subcritical regions: a subcritical liquid region and a subcritical gas region.

[0078] FIG. 3 depicts chromatograms from GC-mass spectrometry analysis, with total ion current (TIC) detection, of the ethanolic extract (top), the subcritical CO_2 extract (middle), and the prior art supercritical fluid extract PBI-05204 (bottom) determined according to Example 18. The data can be used to identify volatile and semi-volatile components of the extract.

[0079] FIG. 4 depicts the HPLC chromatogram of PBI-05204 (prior art) whereby oleandrin is resolved from oleanolic acid, ursolic acid, and betulinic acid.

DETAILED DESCRIPTION OF THE INVENTION

[0080] The invention provides an oleandrin-containing subcritical extract, an oleandrin-containing organic solvent extract, and a combination composition comprising said subcritical extract and said organic solvent extract. The invention also provides

CLAIMS

1) An improved oleandrin-containing combination composition comprising a portion of oleandrin-containing organic solvent extract and a portion of oleandrin-containing subcritical liquid (SbCL) extract, wherein the organic solvent extract comprises at least oleandrin and other components extracted from the biomass, and the compositional profile of extracted volatile and semi-volatile components in said organic solvent extract is different than the compositional profile of said components in said subcritical liquid extract.

2) The combination composition of claim ~~3~~1, wherein substantially equal portions of the two extracts are present in the combination composition.

3) The combination composition of claim ~~3~~1 or 2, wherein a) ~~the organic solvent extract comprises at least oleandrin and other components extracted from the biomass; b)~~ in the organic solvent extract, the molar ratio of oleandrin to other components extracted from the biomass is greater than said molar ratio in the subcritical liquid extract; ~~c~~b) the SbCL extract comprises at least oleandrin and other components extracted from the biomass; ~~d~~c) the content of oleandrin in the organic solvent extract is greater than the content of oleandrin in the subcritical liquid extract; ~~e~~d) the content of extracted non-oleandrin components in the organic solvent extract is greater than the content of said components in said subcritical liquid extract; ~~f~~e) ~~the compositional profile of extracted volatile and semi-volatile components in said organic solvent extract is different than the compositional profile of said components in said subcritical liquid extract;~~ and/or ~~g~~f) relative to oleandrin content, the subcritical liquid extract comprises a greater proportion of extracted volatile and semi-volatile components than does the organic solvent extract.

4) The combination composition of any one of claims1-3, wherein a) the content of oleandrin in said combination composition is about 0.01 wt % or higher, about 0.05 wt % or higher, about 0.1 wt % or higher, about 0.2 wt % or higher, about 0.5 wt % or higher, about 1 wt % or higher, about 2.5 wt % or higher, about 5 wt % or higher, about 10 wt % or higher, about 20 wt % or higher, about 30 wt % or higher, about 40 wt % or higher, about 50 wt % or higher, about 60 wt % or higher, about 70 wt % or higher, about 80 wt % or higher, or about 90 wt % or higher; b) the content of oleandrin in said extract is about 99 wt % or lower, about 95 wt % or lower, about 90 wt % or lower, about 80 wt % or lower, about 70 wt % or lower, about 60 wt % or lower, about 50 wt % or lower, about 40 wt % or lower, about 30 wt % or lower, about 20 wt % or lower, about 10 wt % or lower, about 5 wt % or

lower, about 2.5 wt % or lower, about 1 wt % or lower, or about 0.5 wt % or lower; or c) a combination of any limit of item a) and any limit of item b).

5) The combination composition of any one of claims 1-3, wherein said combination composition comprises oleandrin and one or more compounds selected from the group consisting of one or more cardiac glycosides, one or more glycone constituents of cardiac glycosides (such as glucoside, fructoside, and/or glucuronide), one or more aglycones, one or more glycoside precursors (such as cardenolides, cardadienolides and cardatrienolides), one or more steroids, one or more triterpenes, one or more saccharides, one or more polysaccharides, one or more alkaloids, one or more proteins, fat, one or more other non-cardiac glycoside therapeutically effective agent, and any combination thereof.

6) The combination composition of claim ~~7~~5, wherein said combination composition comprises oleandrin and one or more compounds selected from the group consisting of neritaloside, odoroside, oleanolic acid, ursolic acid, betulinic acid, oleandrigenin, oleaside A, betulin (urs-12-ene-3β,28-diol), 28-norurs-12-en-3β-ol, urs-12-en-3β-ol, 3β,3β-hydroxy-12-oleanen-28-oic acid, 3β,20α-dihydroxyurs-21-en-28-oic acid, 3β,27-dihydroxy-12-ursen-28-oic acid, 3β,13β-dihydroxyurs-11-en-28-oic acid, 3β,12α-dihydroxyoleanan-28,13β-olide, 3β,27-dihydroxy-12-oleanan-28-oic acid, homopolygalacturonan, arabinogalaturonan, chlorogenic acid, caffeic acid, L-quinic acid, 4-coumaroyl-CoA, 3-O-caffeoylquinic acid, 5- O-caffeoylquinic acid, cardenolide B-1, cardenolide B-2, oleagenin, neridiginoside, nerizoside, odoroside-H, 3-beta-O-(D-diginosyl)-5-beta, 14 beta-dihydroxy-card-20(22)-enolide pectic polysaccharide composed of galacturonic acid, rhamnose, arabinose, xylose, and galactose, polysaccharide with MW in the range of 17000-120000 D, or MW about 35000 D, about 3000 D, about 5500 D, or about 12000 D, cardenolide monoglycoside, cardenolide N-1, cardenolide N-2, cardenolide N-3, cardenolide N-4, pregnane, 4,6-diene- 3,12,20-trione, 20R-hydroxypregna-4,6-diene-3,12-dione, 16beta,17beta-epoxy-12beta-hydroxypregna-4,6-diene-3,20-dione, 12beta-hydroxypregna-4,6,16-triene-3,20-dione (neridienone A), 20S,21-dihydroxypregna-4,6-diene-3,12-dione (neridienone B), neriucoumaric acid, isoneriucoumaric acid, oleanderoic acid, oleanderen, 8alpha-methoxylabdan-18-oic acid, 12-ursene, kaneroside, neriumoside, 3β-*O*-(D-diginosyl)-2α- hydroxy-8,14β-epoxy-5β-carda-16:17, 20: 22- dienolide, 3β-*O*-(D-diginosyl)-2α,14β- dihydroxy-5β- carda-16:17,20:22-dienolide, 3β,27-dihydroxy-urs-18-en-13,28-olide, 3β,22α,28-trihydroxy-25-nor-lup-1(10),20(29)-dien-2-one, *cis*-karenin (3β-hydroxy-28-Z-p-coumaroyloxy-urs-12-en-27-oic acid), *trans*-karenin (3-β-hydroxy-28-E-p-coumaroyloxy-urs-12-en-27-oic acid), 3beta-hydroxy-5alpha-carda-14(15),20(22)-

dienolide (beta- anhydroepidigitoxigenin), 3 beta-O-(D-digitalosyl)-21-hydroxy-5beta-carda-8,14,16,20(22)-tetraenolide (neriumogenin-A-3beta-D-digitaloside), proceragenin, neridienone A, 3beta,27-dihydroxy-12-ursen-28-oic acid, 3beta,13beta-dihydroxyurs-11-en-28-oic acid, 3beta-hydroxyurs-12-en-28-aldehyde, 28- orurs-12-en-3beta-ol, urs-12-en-3beta-ol, urs-12-ene-3beta,28-diol, 3beta,27-dihydroxy-12-oleanen-28-oic acid, (20S, 24R)-epoxydammarane-3beta,25-diol, 20beta,28-epoxy-28alpha-methoxytaraxasteran-3beta-ol, 20beta,28-epoxytaraxaster-21-en-3beta-ol, 28-nor-urs-12-ene-3beta,17 beta-diol, 3beta-hydroxyurs-12-en-28-aldehyde, alpha-neriursate, beta-neriursate, 3alpha-acetophenoxy-urs-12-en-28-oic acid, 3beta-acetophenoxy-urs-12-en-28-oic acid, oleanderolic acid, kanerodione, 3β-*p*-hydroxyphenoxy-11α-methoxy-12α-hydroxy-20-ursen-28-oic acid, 28-hydroxy-20(29)-lupen-3,7-dione, kanerocin (3α-hydroxy-urs-18,20-dien-28-oic acid), 3alpha-hydroxy-urs-18,20-dien-28-oic acid, D-sarmentose, D-diginose, neridiginoside, nerizoside, isoricinoleic acid, gentiobiosylnerigoside, gentiobiosylbeaumontoside, gentiobiosyloleandrin, folinerin, 12β-hydroxy-5β-carda-8,14,16,20(22)-tetraenolide, 8β-hydroxy-digitoxigenin, Δ16-8β- hydroxy-digitoxigenin, Δ16-neriagenin, uvaol, ursolic aldehyde, 27(p-coumaroyloxy)ursolic acid, oleanderol, 16-anhydro-deacteyl-nerigoside, 9-D-hydroxy-cis-12-octadecanoic acid, adigoside, adynerin, alpha-amyrin, beta-sitosterol, campestrol, caoutchouc, capric acid, caprylic acid, choline, cornerin, cortenerin, deacetyloleandrin, diacetyl-nerigoside, foliandrin, pseudocuramine, quercetin, quercetin-3-rhamnoglucoside, quercitrin, rosaginin, rutin, stearic acid, stigmasterol, strospeside, urehitoxin, and uzarigenin.

7) A pharmaceutical composition comprising at least one pharmaceutical excipient and the combination composition of any one of claims 1-~~3~~6.

8) Use of the pharmaceutical composition of claim 7 in the manufacture of a medicament for the treatment or prevention of ~~A method of treating or preventing~~ a condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract)~~, the method comprising administering to a subject in need thereof one or more doses of said pharmaceutical composition of claim 9, thereby treating or preventing said condition, disease or disorder~~.

9) Use of the combination composition of any one of claims 1-6 in the manufacture of a medicament for the treatment or prevention of ~~A method of treating or preventing~~ a condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract)~~, the method comprising administering to a subject in need thereof one or more doses of said combination~~

~~composition of any one of claims 1-3, thereby treating or preventing said condition, disease or disorder~~.

10) The ~~method~~ use of claim 8 or 9, wherein said medicament is to be administered via a) acute, chronic, or a combination thereof; and/or b) local, systemic, or a combination thereof.

~~11) The method of claim 8, wherein said administering is locally administering, systemically administering, or a combination thereof.~~

~~12)~~11) The ~~method~~ use of claim 8 or 9, wherein said condition, disease or disorder that is therapeutically responsive to oleandrin (oleandrin-containing composition or oleandrin-containing extract) is selected from the group consisting of cancer, tumor, carcinoma, sarcoma, glioblastoma, adenocarcinoma, lymphoma, melanoma, brain tumor, glioma, astrocytoma (which include low-grade astrocytomas (Grade I pilocytic astrocytoma, and Grade II diffuse astrocytoma), anaplastic astrocytoma (Grade III), glioblastoma (Grade IV, GBM, also known as glioblastoma multiforme)), ependymoma, oligodendroglioma, excessive cell proliferation, malignant neoplastic disease, noncancerous inflammatory skin diseases, actinic keratosis, psoriasis, eczema, neurological disease, neurological disorders, neurodegenerative disease, neurodegenerative disorder, neurological disease or disorder having an etiology associated with altered Na,K-ATPase activity, Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, dementia, amyotrophic lateral sclerosis, bovine spongiform encephalopathy, multiple sclerosis, diabetic neuropathy, autism and juvenile neuronal ceroid lipofuscinosis, tauopathy, Down's syndrome, Pick's disease, corticobasal degeneration, some variants of prions disease, progressive supranuclear palsy, frontotemporal dementia, hemorrhoids, ulcer, leprosy, snake bite, and viral infection.

~~13)~~12) The ~~method~~ use of claim ~~12~~11, wherein said viral infection is caused by a virus of the Arenaviridae family, Arterviridae family, Bunyaviridae family, Filoviridae family, Flaviviridae family (Flavivirus genus), Orthomyxoviridae family, Paramyxoviridae family, Rhabdoviridae family, Retroviridae family (Deltaretrovirus genus), Coronaviridae family, (+)-ss-envRNAV (positive sense single stranded enveloped RNA virus), (-)-ss-envRNAV (negative sense single stranded enveloped RNA virus), or Togaviridae family.

~~14)~~13) The ~~method~~ use of claim 12, wherein said viral infection is selected from the group consisting of flavivirus, Yellow Fever virus, Dengue Fever virus, Japanese Enchephalitis virus, West Nile virus, Zikavirus, Tick-borne Encephalitis virus, Kyasanur Forest Disease virus, Alkhurma Disease virus, Omsk Hemorrhagic Fever virus, Powassan virus, arborvirus, eastern equine encephalomyelitis virus (EEEV), western equine encephalomyelitis virus

(WEEV), Venezuelan equine encephalomyelitis virus (VEEV), Chikungunya virus (CHIKV), O'nyong'nvirus (ONNV), Pogosta disease virus, Sindbis virus, Ross River fever virus (RRV), Semliki Forest virus, Lassa virus, aseptic mengitis, Guanarito virus, Junin virus, Lujo virus, Machupo virus, Sabia virus, Whitewater Arroyo virus, Hantavirus, Crimean-Congo hemorrhagic fever orthonairovirus, Mumps virus, Nipah virus, Hendra virus, respiratory syncytial virus (RSV), human parainfluenza virus (HPIV), NDV, influenza virus (A through C), Isavirus, Thogotovirus, Quaranjavirus, H1N1, H2N2, H3N2, H1N2, Spanish flu, Asian flu, Hong Kong Flu, Russian flu, rabies virus, vesiculovirus, Lyssavirus, Cytorhabdovirus, SARS-CoV, MERS-CoV, COVID-19 (SARS-CoV-2), CoV 229E, CoV NL63, CoV OC43, CoV HKU1, and CoV HKU20.

~~15) The combination composition of any one of claims 1-3, wherein the oleandrin-containing SbCL extract is produced by a method comprising~~

~~subjecting oleandrin-containing biomass to subcritical liquid extraction (SbCLE) employing subcritical liquid (SbCL), comprising carbon dioxide and optionally one or more modifiers, for a period of time sufficient to extract the oleandrin and form extraction milieu;~~

~~separating said biomass from said extraction milieu to provide oleandrin-containing SbCL; and~~

~~removing SbCL from said oleandrin-containing SbCL to provide said oleandrin-containing SbCL extract (OCE).~~

~~16) The combination composition of claim 15, wherein said isolating is achieved by one or more of chromatography, liquid-liquid extraction, liquid-solid extraction, crystallization, precipitation, fractional precipitation, fractional crystallization, Soxhlet extraction, trituration, or a combination of any thereof.~~

~~17) The combination composition of claim 15, wherein the method further comprises one or more of the following steps: a) separating said biomass from the extraction milieu by filtering the extraction milieu; b) adding modifier to the carbon dioxide or extraction milieu; c) heating and pressurizing the interior of a vessel containing the extraction milieu to form subcritical liquid phase extraction milieu; d) removing said SbCL from said oleandrin-containing SbCL by volatilizing said SbCL to form raw SbCL extract; e) diluting the raw SbCL extract with organic solvent or aqueous organic solvent; f) filtering the diluted raw or crude SbCL extract one or more times; g) treating the diluted raw or crude SbCL extract with activated carbon; h) removing most of the organic solvent from the diluted raw extract to form; and/or i) sterile filtering the raw or crude extract to obtain the SbCL extract.~~

18) The combination composition of claim 15, wherein the SbCL comprises carbon dioxide and one or more modifiers.

19) The combination composition of claim 18, wherein the one or more modifiers is selected from the group consisting of alcohol and ethanol.

20) The combination composition of claim 15, wherein the carbon dioxide (along with any modifier, if any is present) is in the subcritical liquid phase during about 50% or more of the extraction time period.

21) The combination composition of claim 20, wherein the extraction milieu, comprising extraction fluid (carbon dioxide along with any modifier, if any is present) in the subcritical liquid phase and biomass, will remain in liquid form for at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% of the extraction period, or the extraction milieu remains in liquid form throughout the extraction period.

22) The combination composition of claim 15, wherein, with respect to the phase diagram of CO_2, the SbCLE is conducted a) at or below the critical pressure (p_c), below the critical temperature (t_c), and above the liquid-gas phase line; b) at or below the t_c and below the p_c, and above the liquid-gas phase line; c) at or above the p_c, below the t_c, above the liquid-gas phase line, and below the solid-liquid phase line; or d) at a temperature and pressure falling within the liquid region bounded by the solid-liquid phase line, the liquid-gas phase line, the liquid-supercritical phase line, the triple point (solid-liquid-gas) temperature (t_{tp}), the triple point pressure (p_{tp}), the critical point (liquid-gas-supercritical) temperature (t_{cp}), and the critical point pressure (p_{cp}).

23) The combination composition of claim 15, wherein the SbCLE is conducted at a) a temperature of about temperature of about 0°C up to about 31°C and a pressure of about 5.2 bar to about 3000 bar; b) at a temperature of about 0°C up to about 31°C and a pressure of about 5.2 bar to about 1000 bar; c) a temperature of about 2°C to about 16°C and a pressure of about 115 bar to about 135 bar; d) a temperature of about 5-10 °C and a pressure of about 120-130 bar; or e) a temperature in the range of about 5°C to about 15°C and a pressure in the range of about 50 bar to 70 bar.

24) The combination composition of claim 15, wherein the weight ratio of extraction liquid (comprising carbon dioxide and optionally further comprising modifier) to biomass is in the range of about 20:1 to about 100:1, about 30:1 to about 75:1, about 50:1, about 45:1 to 60:1, or about 40:1 to about 45:1, based on weight of both the solvent and the raw material.

~~25) The combination composition of claim 15, wherein the modifier is present and the weight ratio of carbon dioxide to modifier is in the range of about 100: about 0.01-20, about 100: about 0.01-15, about 100: about 0.01-10, or about 100: about 0.01-5.~~

~~26) The combination composition of claim 15, wherein a) independently upon each occurrence, the biomass is dehydrated, dehumidified, freeze-dried, and/or desiccated prior to extraction; b) independently upon each occurrence, the biomass is dried prior to extraction; c) independently upon each occurrence, the biomass is not dehydrated prior to extraction; d) independently upon each occurrence, the biomass is not dried prior to extraction; or e) independently upon each occurrence,.~~

~~27)~~14) A method of producing the ~~The~~ combination composition of any one of claims 1-~~36~~, ~~wherein the combination composition is produced by a method~~ the method comprising

subjecting oleandrin-containing first biomass to organic solvent extraction to provide an oleandrin-containing organic solvent extract;

subjecting oleandrin-containing second biomass to subcritical liquid (SbCL) extraction (SbCLE) to provide an oleandrin-containing SbCL extract, wherein the SbCLE is performed with SbCL comprising carbon dioxide and optionally one or more modifiers; and

combining said oleandrin-containing organic solvent extract and said oleandrin-containing SbCL extract to provide said combination composition.

~~28)~~15) The ~~combination composition~~ method of claim ~~27~~14, wherein a) said organic solvent extraction is conducted with organic solvent or aqueous organic solvent; b) independently upon each occurrence, said organic solvent or aqueous organic solvent comprises alcohol; c) independently upon each occurrence, said organic solvent is selected from the group consisting of methanol, ethanol, propanol, butanol, chloroform, methylene chloride, ethyl acetate, and a combination thereof; d) said organic solvent extraction comprises extracting said oleandrin-containing biomass with organic solvent or aqueous organic solvent one or more times; e) independently upon each occurrence, said aqueous organic solvent comprises up to about 20 % w/w, about 5% w/w to about 15% w/w, or about 10% w/w of water; f) said organic solvent extract is conducted at a temperature of from about 0°C to about 75°C, about 5°C to about 70°C, about 10°C to about 60°C, about 10°C to about 50°C, about 10°C to about 45°C, or about 20°C to about 40°C; g) the v/w ratio of organic solvent (or aqueous organic solvent) to biomass is in the range of about 50-1: about 1, about 40-1: about 1, about 30-1: about 1, about 20-1: about 1, about 20-5: about 1, or about 10: about 1.

~~29)~~16) The ~~combination composition~~ method of claim ~~27~~14, wherein the organic solvent extraction further comprises one or more of the following: a) separating said biomass from the extraction milieu by filtering the extraction milieu to form crude extract; b) adding aqueous liquid to the organic solvent or extraction milieu; c) reducing the content of organic solvent from said crude extract; d) treating the crude extract with activated carbon; e) filtering the crude extract one or more times; f) adding additional organic solvent to the crude extract; g) sterile filtering the organic solvent extract; and/or h) adding water to the crude extract and extracting the aqueous crude extract with organic solvent.

~~30) The combination composition of claim 15, wherein prior to extraction the water content in the biomass is about 20 wt % or less, about 15 wt % or less, about 10 wt % or less, about 8 wt % or less, about 5 wt % or less, about 2.5 wt % or less, or about 1 wt % of less.~~

~~31) The combination composition of claim 15, wherein a) prior to extraction, the biomass has a particle size (d0.5) of less than about 1", less than about 0.75", less than about 0.5", less than about 0.4", less than about 0.3", less than about 0.2", less than about 0.1", less than about 0.05", or less than about 0.01", said particle being with respect to ASTM sieve mesh opening size.~~

~~32)~~17) The ~~combination composition~~ method of any one of claims ~~15~~14-16, wherein the oleandrin-containing biomass is *Nerium* sp., *Nerium oleander*, *Nerium oleander* L (Apocynaceae), *Nerium odourum*, white oleander, pink oleander, *Agrobacterium tumefaciens* transformed and elicited in *Nerium* sp., cell culture, or a combination thereof.

~~33) The combination composition of claim 15, wherein said combination composition excludes a pharmacologically active polysaccharide (and/or saccharide) obtained during the SbCLE.~~

~~34)~~18) A nutraceutical composition comprising the combination composition of any one of claims 1-~~3~~ 6 and one or more dietary ingredients and/or or food additives.

~~35)~~19) A cosmetic composition comprising the combination composition of any one of claims 1-~~3~~ 6 and one or more cosmetically acceptable ingredients.

~~36)~~20) The ~~combination composition~~ method of claim ~~27~~14, wherein prior to extraction the water content in the biomass is about 20 wt % or less, about 15 wt % or less, about 10 wt % or less, about 8 wt % or less, about 5 wt % or less, about 2.5 wt % or less, or about 1 wt % of less.

~~37) The combination composition of claim 27, wherein a) prior to extraction, the biomass has a particle size (d0.5) of less than about 1", less than about 0.75", less than about~~

0.5", less than about 0.4", less than about 0.3", less than about 0.2", less than about 0.1", less than about 0.05", or less than about 0.01", said particle being with respect to ASTM sieve mesh opening size.

38) The combination composition of claim 15, wherein the oleandrin-containing biomass is *Nerium* sp., *Nerium oleander*, *Nerium oleander* L (Apocynaceae), *Nerium odourum*, white oleander, pink oleander, *Agrobacterium tumefaciens* transformed and elicited in *Nerium* sp., cell culture, or a combination thereof.

39) The combination composition of claim 27, wherein said combination composition excludes a pharmacologically active polysaccharide (and/or saccharide) obtained during the SbCLE.

40) A nutraceutical composition comprising the combination composition of claim 15 and one or more dietary ingredients and/or or food additives.

41) A nutraceutical composition comprising the combination composition of claim 27 and one or more dietary ingredients and/or or food additives.

42) A cosmetic composition comprising the combination composition of claim 15 and one or more cosmetically acceptable ingredients.

43) A cosmetic composition comprising the combination composition of claim 27 and one or more cosmetically acceptable ingredients..