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 example of the 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 of the 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 composition of the disclosure, the method comprising:

subjecting oleandrin-containing biomass to Subcritical liquid extraction (SbCLE) employing SbCL,

comprising carbon dioxide for a period of time sufficient to extract the oleandrin and extraction milieu; separating said biomass from said extraction to milieu to provide oleandrin-containing SbCL extract to provide said composition of oleandrin-containing extract (OCE).

[0020b] In another aspect of the disclosure, there is provided a method of producing an oleandrin-containing composition, the method comprising:

subjecting oleandrin-containing first biomass to organic solvent extraction by forming an extraction milieu and thereby 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 oleandrin-containing composition.

[0020c] In another aspect of the disclosure, there is provided an oleandrin-containing composition produced according to the method as described herein.

[0021] In some 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 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 of the 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.

[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 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 oleandrin-containing 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 was prepared by extraction with alcohol or alcohol and water, and the SbCL extract was prepared by extraction with carbon dioxide or carbon dioxide and alcohol. In some 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.

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] Examples of the 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] Examples of the 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 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 disclosure. Another aspect of the disclosure provides a pharmaceutical composition comprising at least one pharmaceutical excipient and the composition of the disclosure. Compositions comprising oleandrin and plural active ingredients obtained from said biomass are within the scope of the disclosure. Compositions comprising oleandrin and plural triterpenes as the active ingredients are within the scope of the disclosure. In some 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

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 examples of the 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 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] The 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] The 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 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 disclosure can be administered locally, systemically or a combination thereof. Suitable modes of administration are described herein.

[0061] In some examples, oleandrin is present as the primary therapeutic component, meaning the component primarily responsible for therapeutic activity, in a pharmaceutical composition. Another aspect of the disclosure provides an oleandrin-containing composition as described herein or the pharmaceutical composition as described herein for use in therapy.

[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, wherein the condition, disease, or disorder is COVID-19 (SARS-CoV-2).

[0061b] Another aspect of the disclosure provides use of the 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, wherein the condition, disease, or disorder is COVID-19 (SARS-CoV-2).

[0062] The 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 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 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.

CLAIMS

1) An oleandrin-containing 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 was prepared by extraction with alcohol or alcohol and water, and the SbCL extract was prepared by extraction with carbon dioxide or carbon dioxide and alcohol.

2) The composition of claim 1, wherein a) 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 b) 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.

3) The composition of claim 1 or 2, wherein said 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, one or more aglycones, one or more glycoside precursors, 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.

4) The composition of claim 1, wherein the composition comprises at least oleandrin, oleanolic acid, ursolic acid, betulinic acid, kanerocin, kanerodione, oleandrigenin, Nerium F, neritaloside, odoroside A, and odoroside H, adynerin, odoroside-G-acetate, and gitoxigenin.

5) The composition of claim 4, wherein a) in the organic solvent extract, the relative contents of compounds is kanerocin>kanerodione>oleanolic acid>ursolic acid~oleandrin~betulinic acid>oleandrigenin~Nerium F, odoroside A, odoroside H~adynerin~odoroside-G-acetate>gitoxigenin; and b) in the SbCL extract, the relative contents of compounds is oleanolic acid>ursolic acid~oleandrin~betulinic acid>oleandrigenin~Nerium F>odoroside A, odoroside H~adynerin~odoroside-G-acetate>kanerocin.

6) The composition of claim 4 further comprising one or more of neriin, folinerin, gitoxigenin, digitoxigenin, nerigoside, rutin, ursonic acid, neridienone A, adynerigenin, deacetyloleandrin, odoroside G acetate, and/or quercetin.

7) The composition of claim 4 further comprising 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), or any combination thereof.

8) The composition of claim 4, wherein a) in the organic solvent extract made by ethanolic extraction, the relative content of components is total free amino acids>total sugars>total cellulose>total alkaloids>total polyphenols~total flavonoids>total starch>total crude protein; b) in the organic solvent extract made by aqueous ethanolic extraction, the relative content of components is total free amino acids>total cellulose>total alkaloids>total sugars>total polyphenols~total flavonoids>total starch>total crude protein; or c) in the SbCL extract, the relative content of components is total free amino acids>total starch>total alkaloids>total cellulose>total flavonoids>total sugars>total polyphenols>total crude protein.

9) The composition of claim 1, wherein the composition is included in a pharmaceutical composition, nutraceutical composition, cosmeceutical composition, or dosage form.

10) A pharmaceutical composition comprising at least one pharmaceutical excipient and the composition of any one of claims 1-9.

11) Use of the pharmaceutical composition of claim 10 in the manufacture of a medicament for the treatment or prevention of a condition, disease or disorder that is therapeutically responsive to oleandrin, wherein the condition, disease, or disorder is COVID-19 (SARS-CoV-2).

12) Use of the composition of any one of claims 1-9 in the manufacture of a medicament for the treatment or prevention of a condition, disease or disorder that is therapeutically responsive to oleandrin, wherein the condition, disease, or disorder is COVID-19 (SARS-CoV-2).

13) A method of producing the composition of any one of claims 1-9, the method comprising:

subjecting oleandrin-containing biomass to Subcritical liquid extraction (SbCLE) employing SbCL, comprising carbon dioxide for a period of time sufficient to extract the oleandrin and extraction milieu; separating said biomass from said extraction to milieu to provide oleandrin-containing SbCL extract to provide said composition of oleandrin-containing extract (OCE).

14) The method of claim 13, 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; b) adding modifier to the carbon dioxide or extraction milieu; c) heating and pressuring the interior vessel containing the extraction milieu to form subcritical liquid phase extraction milieu; d) removing said SbCL from said oleandrin-containing SbCL by volatizing 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.

15) The method of claim 13, wherein the SbCL comprises carbon dioxide and one or more modifiers.

16) The method of claim 15, wherein the one or more modifiers is alcohol.

17) The method of claim 13, wherein the carbon dioxide, along with any modifier, if any is present, is in the subcritical liquid phase during 50% or more of the extraction time period.

18) The method of claim 17, wherein the extraction milieu, comprising extraction fluid, carbon dioxide along with any modifier, if any is present, in the subcritical liquid phase and biomass, remains in liquid form for at least 60% of the extraction period.

19) The method of claim 13, 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}).

20) The method of claim 13, wherein the SbCLE is conducted at a temperature of 0° C up 31° C and a pressure of 5.2 bar to 3000 bar.

21) The method of claim 13, wherein the weight ratio of extraction liquid, comprising carbon dioxide and optionally further comprising modifier, to biomass is in the range of 20:1 to 100:1, based on weight of both the solvent and the raw material.

22) The method of claim 17, wherein the weight ratio of carbon dioxide to modifier is in the range of 100: 0.01-20.

23) The method of claim 13, 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; or d) independently upon each occurrence, the biomass is not dried prior to extraction.

24) A method of producing an oleandrin-containing composition, the method comprising:

subjecting oleandrin-containing first biomass to organic solvent extraction by forming an extraction milieu and thereby 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 oleandrin-containing composition.

25) The method of claim 24, 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 20% w/w, 5% w/w to 15% w/w, or 10% w/w of water; f) said organic solvent extract is conducted at a temperature of from 0° C to 75° C, 5° C to 70° C, 10° C to 60° C, 10° C to 50° C, 10° C to 45° C, or 20° C to 40° C; g) the v/w ratio of organic solvent to biomass or of aqueous organic solvent to biomass is in the range of 50-1: 1, 40-1: 1, 30-1: 1, 20-1: 1, 20-5: 1, or 10: 1.

26) The method of claim 24, 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 the extract; b) adding aqueous liquid to the organic solvent or extraction milieu; c) reducing the content of organic solvent from said extract; d) treating the extract with activated carbon; e) filtering the extract one or more times; f) adding additional organic solvent to the extract; g) sterile filtering the organic solvent extract; and/or h) adding water to the extract to form aqueous extract and extracting the aqueous extract with organic solvent.

27) The method of claim 13 or 21, wherein a) prior to extraction the water content in the biomass is 20 wt % or less; b) prior to extraction the biomass has a particle size d0.5 of less than 1″, less than 0.75″, less than 0.5″, less than 0.4″, less than 0.3″, less than 0.2″, less

than 0.1″, less than 0.05″, or less than 0.01″, said particle being with respect to ASTM sieve mesh opening size; and/or c) the oleandrin-containing biomass is *Nerium* sp., *Nerium oleander*, *Nerium oleander* L, *Nerium odourum*, white oleander, pink oleander, *Thevetia* sp., *Thevetia peruviana*, yellow oleander, *Thevetia nerifolia*, *Agrobacterium tumefaciens* transformed and elicited in *Nerium* sp., cell culture, or a combination thereof.

28) An oleandrin-containing composition produced according to the method of claim 24.

29) An oleandrin-containing composition according to claims 1 to 9 or the pharmaceutical composition of claim 10 for use in therapy.