

CLAIMS

- 1) An oleandrin-containing extract produced by extraction of oleandrin-containing biomass with subcritical liquid carbon dioxide.
- 2) The oleandrin-containing extract of claim 1, wherein the subcritical liquid comprises subcritical liquid carbon dioxide and one or more modifiers.
- 3) An improved oleandrin-containing combination composition comprising a portion of oleandrin-containing organic solvent extract and a portion of oleandrin-containing SbCL extract.
- 4) The combination composition of claim 3, wherein substantially equal portions of the two extracts are present in the combination composition.
- 5) The combination composition of claim 3, 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.
- 6) The invention of any one of claims 1-5, 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).

7) The invention of any one of claims 1-5, wherein said extract 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.

8) The invention of any one of claims 7, wherein said extract 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-3 β ,28-diol), 28-norurs-12-en-3 β -ol, urs-12-en-3 β -ol, 3 β ,3 β -hydroxy-12-oleanen-28-oic acid, 3 β ,20 α -dihydroxyurs-21-en-28-oic acid, 3 β ,27-dihydroxy-12-ursen-28-oic acid, 3 β ,13 β -dihydroxyurs-11-en-28-oic acid, 3 β ,12 α -dihydroxyoleanan-28,13 β -olide, 3 β ,27-dihydroxy-12-oleanan-28-oic acid, homopolygalacturonan, arabinogalacturonan, 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, 3 β -O-(D-diginosyl)-2 α -hydroxy-8,14 β -epoxy-5 β -carda-16:17, 20: 22- dienolide, 3 β -O-(D-diginosyl)-2 α ,14 β -dihydroxy-5 β - carda-16:17,20:22-dienolide, 3 β ,27-dihydroxy-urs-18-en-13,28-olide, 3 β ,22 α ,28-trihydroxy-25-nor-lup-1(10),20(29)-dien-2-one, *cis*-karenin (3 β -hydroxy-28-Z-

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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, 3 β -*p*-hydroxyphenoxy-11 α -methoxy-12 α -hydroxy-20-ursen-28-oic acid, 28-hydroxy-20(29)-lupen-3,7-dione, kanerocin (3 α -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, 12 β -hydroxy-5 β -carda-8,14,16,20(22)-tetraenolide, 8 β -hydroxy-digitoxigenin, Δ 16-8 β - hydroxy-digitoxigenin, Δ 16-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, campesterol, caoutchouc, capric acid, caprylic acid, choline, cornerin, cortenerin, deacetyloleandrin, diacetyl-nerigoside, foliandrin, pseudocuramine, quercetin, quercetin-3-rhamnoglucoside, quercitrin, rosaginin, rutin, stearic acid, stigmasterol, strospeptide, urehitoxin, and uzarigenin.

9) A pharmaceutical composition comprising at least one pharmaceutical excipient and the invention of any one of claims 1-5.

10) 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.

11) 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 invention of any one of claims 1-5, thereby treating or preventing said condition, disease or disorder.

12) The method of claim 10 or 11, wherein said administering is acute, chronic, or a combination thereof.

13) The method of claim 10, 11, or 12, wherein said administering is locally administering, systemically administering, or a combination thereof.

14) The method of claim any one of claims 10, 11, 12, or 13, 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.

15) The method of claim 14, wherein said viral infection is caused by a virus of the Arenaviridae family, Arteriviridae 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.

16) The method of claim 14, wherein said viral infection is selected from the group consisting of flavivirus, Yellow Fever virus, Dengue Fever virus, Japanese Encephalitis virus, West Nile virus, Zikavirus, Tick-borne Encephalitis virus, Kyasanur Forest Disease virus, Alkhurma Disease virus, Omsk Hemorrhagic Fever virus, Powassan virus, arbovirus,

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eastern equine encephalomyelitis virus (EEEV), western equine encephalomyelitis virus (WEEV), Venezuelan equine encephalomyelitis virus (VEEV), Chikungunya virus (CHIKV), O'nyong'nyong virus (ONNV), Pogosta disease virus, Sindbis virus, Ross River fever virus (RRV), Semliki Forest virus, Lassa virus, aseptic meningitis, Guanarito virus, Junin virus, Lujo virus, Machupo virus, Sabia virus, Whitewater Arroyo virus, Hantavirus, Crimean-Congo hemorrhagic fever orthonaviruses, Mumps virus, Nipah virus, Hendra virus, respiratory syncytial virus (RSV), human parainfluenza virus (HPIV), NDV, influenza virus (A through C), Isavirus, Thogotovirus, Quarantavirus, 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.

17) A method of producing an oleandrin-containing extract, the method comprising subjecting oleandrin-containing biomass to subcritical liquid extraction (SbCLE) employing subcritical liquid (SbCL), comprising carbon dioxide, 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 extract (OCE).

18) A method of producing oleandrin comprising subjecting oleandrin-containing biomass to subcritical liquid extraction (SbCLE) employing subcritical liquid (SbCL), comprising carbon dioxide, 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.

19) The method of claim 18, 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.

20) The method of any one of claims 17-19 further comprising one or more of the following steps: a) separating said biomass from the extraction milieu by filtering the

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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.

21) The method of any one of claims 17-20, wherein the SbCL comprises carbon dioxide and one or more modifiers.

22) The method of claim 21, wherein the one or more modifiers is selected from the group consisting of alcohol and ethanol.

23) The method of any one of claims 17-22, 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.

24) The method of claim 23, 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.

25) The method of any one of claims 17-24, wherein, with respect to the phase diagram of CO₂, 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}).

26) The method of any one of claims 17-25, 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.

27) The method of any one of claims 17-26, 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.

28) The method of any one of claims 17-27, wherein the weight ratio of carbon dioxide to modifier is in the range of about 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.

29) The method of any one of claims 17-28, 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,.

30) 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.

31) The method of claim 30, 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.

32) The method of claim 30 or 31, 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.

33) The method of any one of claims 17-32, 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 % or less.

34) The method of any one of claims 17-33, wherein a) prior to extraction, the biomass has a particle size (d_{0.5}) 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.

35) The method of any one of claims 17-34, wherein the oleandrin-containing biomass is *Nerium* sp., *Nerium oleander*, *Nerium oleander* L (Apocynaceae), *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.

36) An oleandrin-containing extract produced by the method of any one of claims 17-35.

37) The invention of any one of the above claims, wherein said extract excludes a pharmacologically active polysaccharide (and/or saccharide) obtained during the SbCLE.

38) A nutraceutical composition comprising oleandrin and one or more dietary ingredients and/or food additives.

39) A cosmetic composition comprising oleandrin and one or more cosmetically acceptable ingredients.

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- 40) A SbCL extract as described herein.
- 41) A method of producing a SbCL extract as described herein.
- 42) A pharmaceutical composition as described herein.
- 43) A dosage form as described herein.
- 44) An oleandrin-containing composition as described herein.
- 45) A combination composition as described herein.
- 46) A dual extract combination composition as described herein.
- 47) A method of treatment as described herein.
- 48) A nutraceutical composition as described herein.
- 49) A cosmetic composition as described herein.