PLANT EXTRACT COMPOSITIONS AND USES THEREOF FOR TREATING CENTRAL NERVOUS SYSTEM DISEASES

FIELD OF THE INVENTION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/566,741 filed March 18th, 2024, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to *Wikstroemia indica (Linn.) C. A. Mey* extracts, compounds thereof, compositions containing the foregoing, and uses thereof for treating central nervous system diseases.

BACKGROUND OF THE INVENTION

[0003] *Wikstroemia indica* (Linn.) C. A. Mey was originally recorded in "Lingnan Medicine Collection" as a plant of the genus Wikstroemia. Wikstroemia is also known as *Wikstroemia indica* (Taxonomy of Chinese Trees). Wikstroemia its fruits, roots, stems and leaves can be used as medicine. According to traditional Chinese medicine, *Wikstroemia indica (Linn.) C. A. Mey* is suggested to have effects that include heat-clearing, detoxification, resolving masses, dispersing stasis, reducing swelling, and relieving pain.

[0004] It has not been appreciated until now that extracts of *Wikstroemia indica* (Linn.) C. A. Mey can be used to treat a wider variety of diseases and conditions. Accordingly, there is a need in the art for improved treatments derived from or based on compounds present in *Wikstroemia indica* (Linn.) C. A. Mey.

SUMMARY OF THE INVENTION

[0005] Provided herein is a pharmaceutical composition. The pharmaceutical composition may comprise a plant extract, which may comprise one or more compounds. The pharmaceutical composition may comprise one or more compounds of the plant extract. The pharmaceutical composition may further comprise at least one pharmaceutically acceptable excipient. The plant extract may be obtained from *Wikstroemia indica*, which may be *Wikstroemia indica* (Linn.) C. A. Mey.

[0006] The one or more compounds may comprise one or more of a coumarin, a flavonoid, and a lignan. The one or more compounds may be one or more of a daphnetin; a dicoumarin; a coumarin; a tricoumarin; a naringin; a daphnol; a genkwal B; a genkwal C; a genkwarin; a daphnetin B; a daphnone; a genkwanin; a daphnetin D1; a naphthol; and a daphnetin; and a

derivative, stereoisomer, hydrate, ester, solvate, eutectic, cocrystal, metabolite, pharmaceutically acceptable salt, glycoside, and prodrug thereof. The one or more compounds may be one or more of daphnoretin, 6'-hydroxy,7-O-7'-dicoumarin, daphnogitin, umbelliferone, wikstrocoumarin, wikstrosin, triumbelletin, 5-hydroxy-7,4'-dimethoxy flavone, thevetiaflavone, 5,6,7-trihydroxy-4'-methoxy-dihydroflavonol, stellaranol, genkwanol B, genkwanol C, primev-ersyl genkwanine, daphnodorin B, daphnolone, genkwanine, daphnodorin D1, wikstromol, and daphnoretin-O-β-D-glucoside. The pharmaceutical composition may comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 of the one or more compounds. The pharmaceutical composition may comprise daphnoretin, 6'-hydroxy,7-O-7'-dicoumarin, daphnogitin, umbelliferone, wikstrocoumarin, wikstrosin, triumbelletin, 5-hydroxy-7,4'-dimethoxy flavone, thevetiaflavone, 5,6,7-trihydroxy-4'-methoxy-dihydroflavonol, stellaranol, genkwanol B, genkwanol C, primev-ersyl genkwanine, daphnodorin B, daphnolone, genkwanine, daphnodorin D1, wikstromol, and daphnoretin-O-β-D-glucoside. The pharmaceutical composition may be suitable for oral administration, intravenous administration, topical administration, or injection.

[0007] Provided herein is a method of treating a central nervous system (CNS) disease or condition in a subject in need thereof, which may comprise administering to the subject the pharmaceutical composition. Further provided herein are use of the pharmaceutical composition in the manufacture of a medicament for treating the CNS disease or condition, and the pharmaceutical composition for treating the CNS disease or condition. The CNS disease or condition may be Alzheimer's disease, Parkinson's disease, Parkinsonism, dementia, vascular dementia, depression, or anxiety. The depression may be a major depressive disorder. The depression may be associated with menopause, and may be perimenopausal depression. The treatment may reduce one or more of emotional depression, stress, and anxiety.

[0008] The present invention also relates to a method for treating a central nervous system (CNS) disease or condition said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica (Linn.) C. A. Mey*.

[0009] The present invention also relates to a method for treating a central nervous system (CNS) disease or condition said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica (Linn.) C. A. Mey* and an excipient.

[0010] The present invention also relates to a method for treating or preventing disease or conditions associated with a central nervous system (CNS) disease or condition said method

comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica (Linn.) C. A. Mey.*

[0011] The present invention also relates to a method for treating or preventing disease or conditions associated with a central nervous system (CNS) disease or condition said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica (Linn.) C. A. Mey.* and an excipient.

[0012] The present invention further relates to a process for preparing a plant extract obtained from *Wikstroemia indica (Linn.) C. A. Mey.*

[0013] The amount of the plant extract or the one or more compounds of the plant extract administered to the subject, in the pharmaceutical composition, or in the medicament may be about 0.01-250 g. The amount may be 10-50 g. The amount may be 20-25 g. The pharmaceutical composition may be administered orally.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1A-E show the morphology and activity in a mouse model of depression (ovariectomized (OVX) combined with chronic unpredictable mild stimulation (CUMS)) from a model (untreated) group (FIG. 1A), E2-treated group (FIG. 1B), Sham operation (no OVX) group (FIG. 1C), low-dose *Wikstroemia indica* extract group (FIG. 1D), and high-dose *Wikstroemia indica* extract group (FIG. 1E).

[0015] FIG. 2A-E show fecal observations of OVX mice in each of the following treatment group: model (FIG. 2A), E2-treated (FIG. 2B), Sham (FIG. 2C), low-dose *Wikstroemia indica* extract (FIG. 2D), and high-dose *Wikstroemia indica* extract (FIG. 2E).

[0016] FIG. 3 shows weight measurement of treatment and control groups in OVX mice.

[0017] FIG. 4A-D show results of vaginal epithelium keratosis test of OVX mice at the following stages: pre-estrus (FIG. 4A); estrus (FIG. 4B); late estrus (FIG. 4C); and estrus interval (FIG. 4D).

[0018] FIG. 5 shows the effects of low-dose and high-dose *Wikstroemia indica* extract on immobility time of OVX mice, as compared to controls.

[0019] FIG. 6 shows the effects of low-dose and high-dose *Wikstroemia indica* extract on standing times of OVX mice, as compared to controls.

[0020] FIG. 7 shows the activity track of mice in each group in the open-field test.

[0021] FIG. 8 shows the effects of low-dose and high-dose *Wikstroemia indica* extract on preference value in OVX mice, as compared to controls.

[0022] FIG. 9 shows hematoxylin and eosin (H&E) staining in the hippocampal CA1 region of mice in *Wikstroemia indica* extract treatment groups as compared to controls (H&E, ×200).

[0023] FIG. 10 shows Nissl staining results of the hippocampal CA3 region of mice in *Wikstroemia indica* extract treatment groups as compared to controls (x200).

[0024] FIGS. 11A-C show the general state of mice in the control (FIG. 11A), model (FIG. 11B), and *Wikstroemia indica* extract groups in 4-Chloro-DL-phenylalanine model mouse experiments.

[0025] FIGS. 12A-D show the effects of a *Wikstroemia indica* extract on social interaction tests in chronic social defeated stress (CSDS) model mice. Compared with the control group, #P < 0.05; compared with the model group, *P < 0.05.

[0026] FIGS. 13A-D show 2 the effects of a *Wikstroemia indica* extract in a three-chamber social approach test in CSDS mice. Compared with the control group, ##P < 0.01; compared with the model group, **P < 0.01.

[0027] FIG. 14 shows the effects of a *Wikstroemia indica* extract on sucrose preference in CSDS mice. Compared with the control group, ##P < 0.01; compared with the model group, **P < 0.01.

[0028] FIG. 15A-D shows the results of FST, SIT, and TCT analyses for each group of mice (mean ± S, n = 8). Note: compared with control group, ** P<0.01; compared with Model group, # P<0.05, ## P<0.01.

[0029] FIG. 16 shows the morphology of neurons in various regions of the hippocampus of mice in each group (H&E, ×400). Note: White arrows are normal nerve cells, black arrows are damaged nerve cells.

[0030] FIG. 17 shows the morphology of Nissl vesicles in various regions of the hippocampus in various groups of mice (Nissl, ×400). Note: Black arrows are more complete Nissl vesicles.

[0031] FIG. 18A-F show the effects of Phyto-N on serum CRH (FIG. 18A), ACTH (FIG. 18B), CORT (FIG. 18C), IL-1β (FIG. 18D), IL-6 (FIG. 18E) and TNF-α (FIG. 18F) expression in CSDS model mice (± S,n=3). Note: Compared with Control group, ** P<0.01, *** P<0.001; Compared with Model group, ## P< 0.01, ### P< 0.001.

[0032] FIG. 19A-I show the effects of Phyto-N on the expression of Bcl-2 (FIG. 19F), Caspase-1 (FIG. 19D), NLRP3 (FIG. 19C), Caspase-3 (FIG. 19B), Caspase-9 (FIG. 19E) and Iba1 (FIG. 19A) proteins in the hippocampus of CSDS model mice (± S,n=3). Note: Compared with Control group, *P<0.05, **P<0.01, ***P<0.001; Compared with Model group, #P< 0.05. FIG. 19G shows Western blots for Caspase-1 vs. β-actin in various treatment groups, while FIG. 19H shows the blots for Iba1, Caspase-3, and NLRP3, compared to β-actin and FIG. 19I shows the blots for Bcl-2, and Caspase-9 compared to β-actin.

[0033] FIG. 20 shows measurement results of pole climbing experiment in each group of mice ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0034] FIG. 21 shows localization navigation latency of each group of mice ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0035] FIG. 22 shows space exploration ability of mice in each group ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0036] FIG. 23 shows the results of open field experiments on mice in each group ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0037] FIG. 24 shows gait experiment results of mice in each group ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0038] FIG. 25 shows effects of Phyto-N on neurons in the substantia nigra of mouse brain. (H&E, x400).

[0039] FIG. 26 shows the effects of Phyto-N on the apoptosis rate of dopaminergic neurons in the substantia nigra of mice brains (Tunel, x400) ($\bar{x}$±S, n=10). Note: **P<0.01, *P<0.05.

[0040] FIG. 27 shows the effects of Phyto-N on TH expression and number of neurons in substantia nigra of mice (IHC, x400).

[0041] FIG. 28 shows trends in body mass of mice in each group ($\bar{x}$ ± S, n=10). Compared to the Control group, ## P<0. 01； Compared to the Model group, *P<0. 05.

[0042] FIG. 29 shows new object recognition index in mice in each group ($\bar{x}$ ± S, n=10). Note: Compared with Control group, ## P < 0. 01; compared with Model group, **P < 0. 01.

[0043] FIG. 30 shows the spontaneous alternation rate in mice in each group ($\bar{x}$ ± S, n=10). Compared with Control group, ## P<0. 01; compared with Model group, *P<0. 05, **P<0. 01.

[0044] FIG. 31 shows latency of localized navigation in each group of mice ($\bar{x} \pm S$, n=10). Note: Compared with Control group, # P<0. 05, ## P<0. 01; compared with Model group, *P<0.05, **P<0. 01.

[0045] FIG. 32 shows spatial exploration ability of mice in each group ($\bar{x} \pm S$, n=10). Note: Compared with Control group, #P<0.05, ## P<0. 01; compared with Model group, *P<0.05, **P<0.

[0046] FIG. 33 shows swimming trajectories of mice in each group.

[0047] FIG. 34 shows Pathological morphology of mouse hippocampal neurons. Note: Arrows indicate nuclear consolidation, necrotic neuronal cells.

[0048] FIG. 35 shows Nissl body expression in hippocampus of each group.

[0049] FIG. 36 shows the number of positive cells stained by ni-staining in different hippocampal subdivisions in each group of mice ($\bar{x} \pm S$, n=10).

[0050] Fig. 37: Effects of Phyto-N on 5XFAD in NOR. Representative heatmaps during testing session of NOR for a) control, b) 5XFAD, and c) 5XFAD+ Phyto-N groups. d) Exploration time for left vs. right object during the familiarization session. Control: n = 16, 5XFAD: n = 9, and 5XFAD+ Phyto-N: n = 17. e) Exploration time for familiar vs. novel object in the test session. Control: n = 18, 5XFAD: n = 11, and 5XFAD+ Phyto-N: n = 13.Two-way ANOVA followed by Bonferroni's *post-hoc* tests. f) Discrimination ratio (DR) calculated for the familiarization and testing sessions. Control: n = 15, 5XFAD: n = 6, and 5XFAD+ Phyto-N: n = 12.Two-tailed paired t-test. Individual data points are shown in addition to mean ± SEM. Control: n = 20, 5XFAD: n = 12, and 5XFAD+ Phyto-N: n = 17. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

[0051] Fig. 38: Quantification of locomotor activity and anxiety-like behavior in OFT. Representative track plot images from a) control, b) 5XFAD, and c) 5XFAD+Phyto-N groups. d) Time in the center zone. e) Entries to the center zone. f) Total distance traveled. Individual data points are shown in addition to mean ± SEM. One-way ANOVA followed by Bonferroni's multiple comparison test. Control: n = 20, 5XFAD: n = 13, and 5XFAD+ Phyto-N: n = 9. *p < 0.05, **p < 0.01, ***p < 0.001.

[0052] Fig. 39: Effects of Phyto-N on chronic CORT treated mice. a) Experimental timeline of chronic CORT exposure in drinking water for 20 days, followed by voluntary oral administration (p.o.) of either vehicle (Nutra-Gel) or Phyto-N in Nutra-Gel for 14 days, then behavioral tests including open field test (OFT), sucrose splash test (SS) and tail suspension test (TST). OFT: b)

Time spent in the center zone, c) Entries to the center zone, d) Distance traveled. SS: e) grooming time, f) Number of grooming episodes, g) Latency to groom. TST: h) Immobility time, i) Number of immobility bouts, j) Latency before the first immobility bout. One-way ANOVA with Bonferroni's multiple comparison post-hoc tests. Individual data points are shown in addition to mean ± SEM. Control: n = 20, CORT: n = 20, CORT+ Phyto-N: n =9. *p < 0.05, **p < 0.01.

DETAILED DESCRIPTION

[0053] The inventors have discovered that, surprisingly, a plant extract of *Wikstroemia indica* (Linn.) C. A. Mey and compounds thereof have therapeutic effects beyond any currently known. The therapeutic effects include the ability to alleviate symptoms of central nervous system diseases.

[0054] Definitions

[0055] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. It is noted that the claims can be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only," and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0056] For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

[0057] As used herein, the term "about" is used to provide flexibility to a numerical range endpoint by providing that a given value may be "a little above" or "a little below" the endpoint. For example, the endpoint may be within 10%, 8%, 5%, 3%, 2%, or 1% of the listed value. Further, for the sake of convenience and brevity, a numerical range of "about 50 mg/mL to about 80 mg/mL" should also be understood to provide support for the range of "50 mg/mL to 80 mg/mL." The endpoint may also be based on the variability allowed by an appropriate regulatory body, such as the FDA, USP, etc.

[0058] As used herein, "comprises," "comprising," "containing," and "having" and the like may have the meaning ascribed to them in U.S. Patent Law and may mean "includes," "including," and the like, and are generally interpreted to be open ended terms. The terms "consisting of" or "consists of" are closed terms, and include only the components, structures, steps, or the like specifically listed in conjunction with such terms, as well as that which is in accordance with U.S. Patent law. "Consisting essentially of" or "consists essentially of" have the meaning generally ascribed to them by U.S. Patent law. In particular, such terms are generally closed terms, with the exception of allowing inclusion of additional items, materials, components, steps, or elements, that do not materially affect the basic and novel characteristics or function of the item(s) used in connection therewith. For example, trace elements present in a composition, but not affecting the composition's nature or characteristics would be permissible if present under the "consisting essentially of" language, even though not expressly recited in a list of items following such terminology. In this specification when using an open-ended term, like "comprising" or "including," it is understood that direct support should be afforded also to "consisting essentially of" language as well as "consisting of" language as if stated explicitly and vice versa.

[0059] As used herein, "pharmaceutically acceptable" may denote an attribute of a material which is useful in preparing a pharmaceutical composition or pharmaceutical formulation that is generally safe, non-toxic, and neither biologically nor otherwise undesirable and is acceptable for veterinary as well as human pharmaceutical use. "Pharmaceutically acceptable" can refer to a material, such as a carrier, or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, e.g., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0060] As used herein, "pharmaceutically acceptable excipient" may refer to any pharmaceutically acceptable ingredient in a pharmaceutical composition or pharmaceutical formulation having no therapeutic activity and being non-toxic to the subject administered, such as disintegrators, binders, fillers, solvents, buffers, tonicity agents, stabilizers, antioxidants, surfactants, carriers, diluents, excipients, preservatives or lubricants used in formulating pharmaceutical products.

[0061] As used herein, the terms "subject," "individual," and "patient" are used interchangeably. None of the terms are to be interpreted as requiring the supervision of a medical professional (e.g., a doctor, nurse, physician's assistant, orderly, hospice worker). As used herein, the subject may be any animal, including a mammal (e.g., a human or non-human animal) or a non-mammal. In one embodiment, the subject is a human.

[0062] As used herein, the terms "treat," "treating", or "treatment," and other grammatical equivalents, include ameliorating the underlying causes of one or more symptoms of a disease or condition; alleviating, abating, or ameliorating one or more symptoms of a disease or condition; ameliorating or reducing the appearance, severity, or frequency of one or more symptoms of a disease or condition; inhibiting the disease or condition, such as, for example, arresting the development of the disease or condition, relieving the disease or condition, causing regression of the disease or condition, relieving a condition caused by the disease or condition, or inhibiting the symptoms of the disease or condition either prophylactically and/or therapeutically. Methods of treatment as disclosed herein include disclosures of the use of the compounds, pharmaceutical compositions, or pharmaceutical formulations provided herein for the treatment of any indication described herein, and include disclosures of the compounds, pharmaceutical compositions, or pharmaceutical formulations provided herein for the use in treating any indication described herein.

[0063] As used herein, "*Wikstroemia indica* (Linn.) C. A. Mey" may be identified by other terms, which include Phyto-N, *Wikstroemia indica, Wikstroemia indica* (L.) C. A. Mey, Indian Stringbush, Indian Wikstroemia, Liao Gē Wáng, Le Gē Wáng, Nan Ling Rao Hua, Di Pi Mian, Shan Mian Pi, Cui Shui Rao Hua, Yin Du Rao Hua, Pu Lun, Ye Mian Hua Nan Ling Wan Hua, Jiu Xin Cai Jiu Xin Yao, Jiu Xin Cao, Pu Yin Cao, Gou Xin Cao, Gou Xin Qiang, Gou Xin Bao, Gou Xin Cang, Gou Jing Shu, Gou Xin Yao, Du Gou Zai, Shan Dou Liao, Shan Huang Pi, Shan Ma Pi, Shan Luo Ma, Shan Liu Ma, Shan Yan Pi, Shan Shi Liu, Ji Zi Ma, Ji Zi Ma, Ji Mang Tou, Niao Zi Ma, Wu Zi Ma, Ji Er Ku wan, Ji Er Ku, Ji Duan Chang, Que Er Ma, Que Zi Ma, Que Ji Ma, Que Zi Ma, Ye Fa Ma, Liao Ge Ma, Zhi Pi Ma, Shan Ma, Di Gu Ma, Di Ba Ma Di Mian Ma, Ye Ma Pu, Po Yin Ma, Di Mian, Ma Shu, Di Jin Gen Shu, Di Mian Gen, Huo Suo Mu, Da Huang Tou Shu, Gou Jing Shu, Ling Dai Guan Mu, Ruan Mu Cai, Jia Huang Pi, Shi Mian Pi, Shi Gu Pi, Tong Pi Zi, Shan Mian Pi, Shan Ma Pi, Shan Yan Pi, Shan Huang Pi, Di Mian Gen Pi, Di Mian, Guang Pi Zi, Ye Cao Zhi Pi, Bo Zi, Tong Zi Pi, Bai Zhi Pi, Jin Yao Dai, Zei Ku Dai,

Xiao Ye Jin Yao Dai, Ge Chun Guang, Da Jiu Jia, Du Chu Gen, Di Gu Gen, Bie Nan Gen, Qi Ma Gen, Du Shu Gen, Pu Yin, Ding Yuan Gen, Di Jin Shu Gen, Di Mian Gen, Yu Dan Gen, Du Shu Gen, Du Yu Teng, Bao Ya Lang, Pu Yi Cao, Tou Gu Cao, Xie Dai Guan, Tie Gu Shan, Tie Wu San, Tie Gu San, Xie Ye, Shan Pu Lun, Shan Pu Yu, Shan Pu Cang, Pu Yin, Pu Yu, Hong Deng Long, Qian Nian Ai, Bao Ya Lang, Shu Shu, Shu Zhu, Shan Zhi Yi, Bai Mian Er, Shan Shi Liu, Hong Chi Qi, Dui Kou Jan, and Gang Bu Qu.

[0064] Plant extract

[0065] Provided herein are a plant extract and a composition comprising the plant extract. The plant extract may be extracted from a plant material obtained from *Wikstroemia indica* (Linn.) C. A. Mey. The plant material may comprise a whole plant, or one or more of a fruit, a root, a stem, and a leaf of the plant. The plant material may comprise fresh or dried plant. The dried plant may have been dried by air drying or in an oven.

[0066] Extraction methods

[0067] Also provided herein is a method of extracting the plant extract from a plant material. The plant extract may be extracted from the plant from material using one or more of solvent extraction, water decoction, water vapor distillation, biological enzymatic hydrolysis, supercritical CO_2 extraction, supercritical enzymatic digestion combined extraction, ultrasonic-assisted eutectic solvent, macroporous adsorption resin, polyamide adsorption column chromatography, normal phase column chromatography, reversed phase column chromatography, Sephadex LH-20 gel column chromatography, semi-preparative high performance liquid chromatography, ion exchange resin, high-speed counter-current chromatography, and supercritical fluid chromatography. The extraction method may also comprise using a drying method such as concentration drying, freeze drying, decompression drying, vacuum drying, vacuum freeze drying, vacuum decompression drying, spray drying, atmospheric drying, boiling drying, high-pressure steaming, steam distillation, alcohol extraction, spirit distillation, hot air drying, natural shade drying, infrared drying, and microwave drying. The plant extract may be extracted using a method comprising one or more of macerating, heating refluxing, percolating, ultrasonicating, dual-frequency ultrasonic extraction, and microwaving.

[0068] The plant extract may be extracted by contacting the plant material with a solvent. The solvent may comprise one or more of water, dichloromethane, petroleum ether, acetone, ethyl

acetate, n-hexane, petroleum ether, ethyl acetate, and other lower fatty alcohols such as methanol, ethanol, n-propanol, isopropanol, propyleneglycol, n-butanol, isobutanol and n-pentanol. Any two or more of these solvents may be used in any ratio. The one or more solvents may comprise water or a lower fatty alcohol such as methanol, ethanol, n-propanol, isopropanol, propyleneglycol, n-butanol, isobutanol and n-pentanol in combination with at least one other solvent. The plant material may be suspended in water and extracted directly with one or more of ethyl acetate, n-butanol, aqueous ethanol acetate, and aqueous n-butanol. Alternatively, after degreasing the plant material with a low-polar solvent such as petroleum ether, pentane hexane, heptane, octane, benzene, toluene, dichloromethane, chloroform, carbon tetrachloride, tera-butyl alcohol, octane, ether, acetone, and ethylacetate, the plant extract may be extracted using one or more of ethyl acetate, n-butanol, aqueous ethyl acetate, and aqueous n-butanol. One or more such extraction steps may be performed.

[0069] The plant extract may be extracted using a water decocting method, which may comprise soaking the plant material in a volume of water that is 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times the volume of the plant material, for 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more hours. The plant extract may be decocted one or more times, and each decoction step may be for 0.5, 1, 2, 3, 4, or more hours.

[0070] The plant extract may be obtained by a hydrodistillation method. The method may comprise preparing a solution of ethyl acetate-water in a ratio 1:1 to 1:20, and adding sodium chloride to about 1-2% of the whole volume of the solution. A crude powder of the plant material and the solvent may be added to a distillation flask in a ratio of about 1:1 to 1:30 of the plant material-to-liquid ratio, and the extraction may be carried out by hydrodistillation.

[0071] The plant extract may be obtained by using a biological enzymatic method. The method may comprise crushing the plant material into a coarse powder and adding an enzyme or enzyme complex in a phosphate buffer solution at pH 2.5 to 6.5. The enzyme or enzyme complex may comprise one or more of cellulase, pectinase, galacturonase, and papain. In one example, the enzyme complex comprises cellulase and pectinase or cellulase and papain. The method may further comprise stirring crushed plant material in solution while adding the phosphate buffer solution with enzyme to form a mixture. The above mixture may be placed into an ultrasonic extractor and enzymatically digested while being exposed to ultrasonic waves. The mixture of material may be filtered and the filtrate may be passed through one or more of a microfiltration

membrane and a nanofiltration membrane, which may produce a clear filtrate comprising the plant extract.

[0072] The plant extract may be extracted by using a supercritical CO_2 extraction method. The method may comprise passing super critical CO_2 through the plant material at a flow rate of 10-50 L/h or greater and an extraction temperature of about 30-65°C for about 30-120 min. The plant extract may then be separated at a temperature of about 20-80°C.

[0073] The plant extract may be extracted by using supercritical enzymatic compound extraction. The method may comprise contacting the plant material with an enzymatic solution comprising a biological enzyme and water, to form a mixture. The biological enzyme may comprise one or more of amylase, hemicellulase, cellulase, ligninase, and pectin lyase. The mixture may be incubated at a temperature of about 25-75°C and an enzymatic pressure of about 8-15 MPa for about 5-20 h. The biological enzyme may be inactivated by adjusting the temperature to about 35-85°C.

[0074] The plant extract may be extracted by using an ultrasonic-assisted low eutectic solvent extraction technique. The method may comprise contacting the plant material with an aqueous solution of a low eutectic solvent selected from table A.

[0075] Table A: Low eutectic solvents

Low eutectic solvent	ratio
Choline chloride: urea	1:2
Choline chloride: glycerol	1:1
Choline chloride: lactic acid	1:2
Betaine: propylene glycol	1:3
ChCl:oxalic acid,	1:2
BeHCl:sorbitol	1:3
BeHCl:glycerol	1:3
ChCl:L-(+)-tartaric acid:water	1:1:2
Sucrose:lactic acid:water	1:5:7
Fructose:lactic acid:water	1:5:5
ChCl:tartaric acid	1:1
ChCl:oxalic acid	1:1

ChCl:citric acid	1:1
Acetyl ChCl:lactic acid	2:1
ChCl:glycerol	1:1
ChCl:citric acid:glucose	1:1:1
ChCl:citric acid	1:1
ChCl:maltose	1:3
ChCl:phenol	1:4
Lactic acid:glucose	5:1
Choline chloride:1,3-butanediol	1:2
Proline:malic acid	1:1
Choline chloride:1,3-butanediol	1:6
Citric acid:glucose	1:1
Choline chloride:lactic acid	1:1
Sucrose:lactic acid:water	1:5:7
L-proline:oxalic acid	1:1
Choline chloride:lactic acid	3:1
Choline chloride:Malicacid	1:2
Betaine:Glycerol	1:2
Betaine:Sucrose	1:3
Betaine: Lacticacid	1:2
Betaine: Urea	1:2
ChCl:HFIP	1:2
Choline chloride:propylene glycol	1:1
Choline chloride:urea:water	1:2:4
Choline chloride:sorbitol	1:1
Choline chloride:xylose	2:1
ChCl:glucose	5:2
Acetyl chloride:D, L-Malic acid	1:1
Acetyl chloride:Citric acid	1:1
Betaine:Citric acid	1:1

Proline:Choline chloride	1:1
Tartaric acid:DMU	1:1
Choline Chloride:Glycerol	1:1
Choline Chloride:Oxalic acid	1:1
Proline:Glutamic acid	2:1
Proline:xylitol	5:1
ChCl:D-(+)-glucose:H_2O	5:2:5
ChCl:acetic acid	1:4.35
H_2O:ChCl:fructose	5:5:2
Glycine:lactic acid	1:1
Lactic acid:glucose/menthol:lauric acid (biphasic system)	5:1/2:1
Lactic acid:glucose:water	5:1:9.3
Citric acid:glycine:water	2:1:1
Magnesium chloride hexahydrate [$MgCl_2 \cdot 6H_2O$]: urea	1:1/2:1

[0076] The solvent may comprise one or more of a hydrogen bond acceptor, a hydrogen bond donor, and water. The hydrogen bond donor may comprise one or more of lactic acid, glucose, propanetriol, butylene glycol, and ethylene glycol.

[0077] The plant extract may be extracted by contacting the plant material with an adsorbent resin. The adsorbent resin may comprise one or more of AB-8, D101, D201, DA201, D4006, HPD-BJQH, HPD-100, HPD950, DM301, and DM130. The method may comprise contacting the resin and plant material with an elution medium comprising an eluent, wherein the eluent comprises one or more of methanol, ethanol, acetone, aqueous methanol, aqueous ethanol, and aqueous acetone. The method may comprise eluting the plant extract from the resin, which may comprise contacting the plant material and the resin more than once with the elution medium, wherein in the series the eluent is in equal concentrations or a concentration gradient.

[0078] The plant extract may also be extracted using a polyamide adsorption column chromatography method comprising contacting the plant material with a polyamide adsorption column. The plant extract may be eluted from the column using an elution medium comprising an eluent, wherein the eluent comprises methanol or aqueous acetone. The method may comprise eluting the plant extract from the column, which may comprise serially contacting the plant

extract more than once with the elution medium, wherein in the series the eluent is in equal concentrations or a concentration gradient.

[0079] The plant extract may be extracted by contacting the plant material with a normal phase chromatography column. The method may be performed at atmospheric pressure or higher. In one example, the method comprises pressurized silica gel column chromatography. The column may comprise a packing material comprising silica gel suitable for column chromatography or silica gel suitable for thin layer chromatography. The plant extract may be eluted from the column by contacting the column with an elution medium comprising an eluent, wherein the eluent comprises one or more of benzene, acetone, dichloromethane, and acetone; trichloromethane and acetone; dichloromethane and methanol; trichloromethane and methanol; ethylacetate and methanol; benzene, acetone, and water; di-chloromethane, acetone, and water; ethyl acetate, methanol, and water; dichloromethane, methanol, and water; trichloromethane, methanol, and water; and trichloromethane, acetone, and water. The method may comprise eluting the plant extract from the column, which may comprise serially contacting the plant extract with the elution medium, wherein in the series the eluent is at equal concentrations or a concentration gradient.

[0080] The plant extract may be extracted by contacting the plant material with a reverse-phase chromatography column. The method may be performed at atmospheric pressure or higher. In one example, the method comprises pressurized reversed-phase column chromatography. The column may comprise a packing material comprising one or more of an octadecyl bonded phase (ODS) and an octaalkyl bonded phase. The plant extract may be eluted from the column by contacting the column with an elution medium comprising an eluent, wherein the eluent comprises aqueous methanol or aqueous acetonitrile. The method may comprise eluting the plant extract from the column, which may comprise serially contacting the plant extract with the elution medium, wherein in the series the eluent is at equal concentrations or a concentration gradient.

[0081] The plant extract may be extracted by contacting the plant material with a Sephadex LH-20 gel chromatography column. The column may comprise a mobile phase comprising an organic solvent, which may comprise methanol, or chloroform and methanol (which may comprise chloroform and methanol in a ratio of 1:1 or another ratio).

[0082] The plant extract may be extracted by subjecting the plant material to semi-preparative high performance liquid chromatography. The method may comprise contacting the plant material with a C18 column. The method may comprise eluting the plant extract from the column by contacting the column with aqueous methanol.

[0083] The plant extract may be extracted by using an ion exchange resin. The method may comprise contacting the plant material with an anion exchange resin or hydrogen type cation exchange resin. The hydrogen type cation exchange resin may comprise a 732 type cation exchange resin or a 001×7 type cation exchange resin.

[0084] The plant extract may be extracted by subjecting the plant material to high-speed counter-current chromatography. The plant material may be contacted with a solvent comprising one or more of hexane, ethyl acetate, methanol, and water, which may be present in any ratio.

[0085] The plant extract may be extracted by subjecting the plant material to supercritical fluid chromatography. The method may comprise dissolving the plant extract in a low-polar solvent to obtain an extract solution. The extract solution may be subjected to supercritical fluid chromatography separation. The plant extract may be subsequently purified from the extract solution.

[0086] The plant extract may be extracted by subjecting the plant material to supercritical fluid chromatography. The plant material may be contacted with a column, which may be a modified silica gel column or other type. The modified silica gel column may be packed with silica gel comprising one or more of a surface bonded pyridine, amino, phenyl, cyano, diol group, C18C, C18P, and C18H. The column may comprise a mobile phase comprising supercritical carbon dioxide and a lower alcohol.

[0087] The plant extract may be extracted by contacting the plant material with a volume fraction of an aqueous ethanol solution, wherein the ethanol concentration is 1-100%. The extraction may be performed at a temperature of 0 to 99°C, and may be performed with stirring.

[0088] The plant extract may be extracted by subjecting the plant material to percolation. The plant material may be contacted with a percolation solution, which may comprise one or more of hydrochloric acid, methanol, ethanol, and ammonia. The percolation may be complete when the color of percolating drops from the percolation solution is extremely light or the volume of the percolating solution is equal to 10 times the weight of the plant material or a larger volume.

[0089] The plant extract may be extracted by subjecting the plant material to microwave extraction in a solvent selected from the group consisting of water, methanol, ethanol, acetone, isopropanol, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, and dimethyl sulfoxide. The extraction temperature may be about 40-60°C. The microwave power may be about 300-600w.

[0090] The plant extract may be extracted by subjecting the plant material to dual-frequency ultrasonic extraction in a solvent selected from the group consisting of water, methanol, ethanol, acetone, isopropanol, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, and dimethyl sulfoxide. The extraction may be performed at a first frequency and a second frequency. The first frequency may be about 15-40kHz and the second frequency may be about 41-60kHz.

[0091] The plant extract may be extracted by subjecting the plant material to ultrasonic extraction. The resulting product may be added to a dual aqueous phase system. The dual aqueous phase system may comprise one or more of ethanol-ammonium sulfate, ethanol-dipotassium hydrogen phosphate, ethanol-sodium citrate, and methanol-ammonium sulfate. In one example, the dual aqueous phase system comprises methanol-ammonium sulfate.

[0092] Pharmaceutical compositions

[0093] Provided herein is a pharmaceutical composition comprising the plant extract or one or more compounds of the plant extract, and a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may not exist in nature in combination with the plant extract or one or more compounds of the plant extract. The pharmaceutically acceptable excipient may be other than water, although in some embodiments combinations of pharmaceutically acceptable excipients described herein include water. In one example, the pharmaceutical composition comprises the one or more active ingredients. The pharmaceutical composition may be suitable for oral administration, injection, intravenous administration, or topical administration. The oral formulation may comprise a powder, bulk, tablet, pill, punch, granule, capsule, gel, suspension, emulsion, aromatic aqueous solution, solution, syrup, glycerin, gum paste, tincture, elixir, emulsion, micro-ecological formulation, immediate-release, extended-release, controlled-acceleration, microcapsule, microsphere, nano-agent, nanocapsule, liposome, or reservoir. The topical pharmaceutical formulation may comprise an aerosol, tincture, patch, fire wool, applicator, coating, ointment, or suppository. The injectable pharmaceutical

formulation may comprise an infusion, an emulsion, or a sterile powder or lyophilized powder for reconstitution prior to injection.

[0094] The pharmaceutically acceptable excipient may comprise one or more of a solvent, preservative, antioxidant, stabilizer, buffer, filler, diluent, binder, wetting agent, lubricant, disintegrant, flow aid, suspension aid, emulsifier, osmolarity regulator, pH modifier, permeation enhancer, flavor modifier, colorant, compression aid, plasticizer, encapsulant, liposomal material, and microsphere material. The pharmaceutical composition may comprise water, which in some embodiments is purified water, sterile water, or sterile water for injection.

[0095] The solvent may comprise one or more of glycerol, Tween 80, propylene glycol, phenoxyethanol, polyethylene glycol 400, ethanol, acetone, and ethyl acetate.

[0096] The preservative may comprise one or more of benzoic acid with sodium benzoate, methyl paraben, sorbic acid, ethanol, cresol, chlorocresol, neocresol, duprenorphine, glycerin, and chlorhexidine acetate. The antioxidant may comprise one or more of an antioxidant, sodium sulfite, sodium bisulfite, sodium metabisulfite, sodium thiosulfate, ascorbic acid, tert-butyl p-hydroxyanisole (BHA), 2,6-di-tert-butylated hydroxytoluene (BHT), vitamin E, tea polyphenol, phytic acid, ascorbyl palmitate, inert gas, and metal complex.

[0097] The stabilizer may comprise one or more of an antioxidant, metal ion chelator, polyethylene oxide, polyethylene oxide derivatives, polysorbate, sodium deoxycholate, sodium docusate, polysorbate, poloxamer (e.g., poloxamer 188, poloxamer 124, and/or poloxamer 407), polyethoxylated vegetable oil, polyethoxylated castor oil, dehydrated sorbitan palmitate, lecithin, polyvinyl alcohol, human serum albumin, polyvinylpyrrolidone, povidone, polyethylene glycol, sodium chloride, calcium chloride, dextrose, propanetriol, mannitol and cross-linked polymers, dibutylphenol, and butylhydroxytoluene. The buffering agent may comprise one or more of phosphate, phosphate, citrate, sodium citrate, hydrochloric acid, sodium hydroxide, and trimethylolaminomethane.

[0098] The filler may comprise one or more of microcrystalline cellulose, lactose, dextrin, sucrose, mannitol, dicalcium phosphate dihydrate, starch, pregelatinized starch, and inorganic salt. The diluent may comprise one or more of starch, sucrose, dextrin, lactose, inorganic salt, and microcrystalline cellulose. The binder may comprise one or more of ethanol, polyethylene glycol, glycerol, rice flour, rice paste, batter, starch syrup, molasses, condensed honey, caramel, liquid glucose, cellulose derivative (e.g., hydroxypropyl methylcellulose, methyl cellulose,

hydroxypropyl cellulose, sodium/calcium hydroxymethylcellulose, and/or ethyl cellulose), carbomer, gum Arabic powder, and gelatin. The wetting agent may comprise one or more of ethanol, povidone (e.g., povidone 188, povidone 124, and/or povidone 407), sodium docusate, sodium deoxycholate, and tween.

[0099] The lubricant may comprise one or more of glycerol sorbate, sodium stearyl fumarate, magnesium stearate, calcium stearate, stearic acid, stearate, talc, liquid paraffin, propylene glycol (PG), PEG 6000, and magnesium or sodium lauryl sulfate. The disintegrant may comprise one or more of polyvinylpyrrolidone, sodium starch hydroxyethylate, starch or carboxymethyl cellulose, dry starch, sodium hydroxymethyl starch, low-substituted hydroxypropyl cellulose, cross-linked sodium hydroxymethyl cellulose, cross-linked povidone, and effervescent disintegrant. The flow aid may comprise one or more of talc, and silica derivative (e.g., colloidal silica such as Cab-O-Sil or Aerosil). The suspension aid may comprise one or more of sodium carboxymethylcellulose, polyethylene glycol and povidone. The povidone may comprise one or more of povidone K12, povidone K17, PLASDONETM C-12 povidone, PLASDONETM C-17 povidone, and PLASDONETM C-30 povidone.

[00100] The emulsifier may comprise one or more of a surfactant, natural emulsifier (gum Arabic, yarrow gum, gelatin, lecithin, apricot gum), solid powder emulsifiers (magnesium hydroxide, aluminum hydroxide, zinc hydroxide, calcium hydroxide, silicon dioxide, soap clay, magnesium stearate, etc). The surfactant may comprise one or more of an anionic surfactant, which may be one or more of sodium stearate, potassium stearate, sodium dodecyl sulfate, and cetyl sulfated castor oil; a nonionic surfactant, which may be a water-in-oil type nonionic emulsifier, which may be one or more of sorbic acid ester (e.g., Span, Span), sorbic acid monolaurate (e.g., Span 20), sorbic acid monopalmitate (e.g., Span 40), sorbic acid monostearate (e.g., Span 60), sorbic acid monooleate (e.g., Span 80), and sorbic acid trioleate (e.g., Span 85); and a non-ionic surfactant, which may be an oil-in-water (O/W) type nonionic emulsifier, which may be one or more of polysorbates (e.g., Tween, Tween), polysorbate 20 (e.g., Tween 20), polysorbate 40 (e.g., Tween 40), polysorbate 60 (e.g., Tween 60), polysorbate 80 (e.g., Tween 80), a polyoxyethylene fatty acid ester (e.g., Myrij, sellzer, O/W type), polyoxyethylene fatty alcohol ether (e.g., Brij, benzylzer, O/W type) and poloxamer.

[00101] The osmolarity regulator may comprise one or more of sodium chloride, mannitol, and sucrose. The pH modifier may comprise one or more of phosphoric acid,

phosphate, citrate, sodium citrate, hydrochloric acid, and sodium hydroxide. The permeation enhance may comprise one or more of capric acid, lauric acid, laurazepin, lecithin, and bile acid salt. The flavor modifier may comprise one or more of a sweetener (e.g., sucrose, stevioside, sodium saccharin, and/or aspartame), aromatizer (e.g., a natural flavor such as lemon volatile oil, cherry volatile oil, anise volatile oil, and/or peppermint volatile oil; and/or a synthetic flavor such as an alcohol, aldehyde, ketone, acid, ester, amine, ether, phenol, lactone, terpene, and/or an acetal such as apple flavor, orange flavor, and/or banana flavor, a gum paste agent such as sodium carboxymethyl cellulose, methyl cellulose, starch, sodium alginate, gum Arabic, gum yarrow, agar, and/or gelatin), effervescent agent (e.g., bicarbonate and/or an organic acid such as citric acid and/or tartaric acid).

[00102] The colorant may comprise one or more of a plant-based pigment (e.g., chypre pigment, red kale pigment, red currant, perilla extract, glucose red, luteolin, comfrey red, curcumin, carotene, copper chlorophyllate sodium salt, and/or caramel), synthetic pigment (e.g., amaranth red, lemon yellow, carmine, and/or carmine blue), and mineral-based pigment. The compression aid may comprise one or more calcium hydrogen phosphate dihydrate compound. The plasticizer may comprise triethyl citrate. The encapsulant may comprise a cyclodextrin. The liposomal material may comprise one or more of natural brain phospholipid, lecithin, soy phospholipid, and synthetic phospholipid. The microsphere material may comprise chitosan.

[00103] Method of treatment

[00104] Provided herein is a method of treating a central nervous system (CNS) disease or condition in a subject in need thereof, comprising administering the plant extract, the composition comprising one or more active ingredients thereof, or a pharmaceutical composition comprising the foregoing to the subject. Also provided are the plant extract, the composition comprising one or more active ingredients thereof, or the pharmaceutical composition comprising the foregoing for use in treating the disease or condition; and use of the plant extract, the composition comprising one or more active ingredients thereof, or the pharmaceutical composition comprising the foregoing in the manufacture of a medicament for treating the CNS disease or condition. The treatment, composition, or medicament may reduce one or more symptoms of the CNS disease or condition.

[00105] The CNS disease or disorder may be Parkinson's disease; parkinsonism; Alzheimer's disease; depression; anxiety; dementia; or vascular dementia. In one example, the

CNS disease or condition is depression. The depression may be major depressive disorder. The depression may be associated with menopause, and may be perimenopausal depression. The disease or condition may also be a symptom of depression, which may be one or more of emotional depression, anxiety, and stress. Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest. Depression includes major depressive disorder and clinical depression. Symptoms of depression include feelings of sadness, tearfulness, emptiness or hopelessness; angry outbursts, irritability or frustration, even over small matters; loss of interest or pleasure in most or all normal activities, such as sex, hobbies or sports; sleep disturbances, including insomnia or sleeping too much; tiredness and lack of energy, so even small tasks take extra effort; reduced appetite and weight loss or increased cravings for food and weight gain; anxiety, agitation or restlessness; slowed thinking, speaking or body movements; feelings of worthlessness or guilt, fixating on past failures or self-blame; trouble thinking, concentrating, making decisions and remembering things; frequent or recurrent thoughts of death, suicidal thoughts, suicide attempts or suicide; and unexplained physical problems, such as back pain or headaches. For many people with depression, symptoms may be severe enough to cause noticeable problems in day-to-day activities, such as work, school, social activities or relationships with others. Symptoms of depression also include high blood pressure (also called hypertension), diabetes, arthritis, multiple sclerosis (MS), stress, anxiety, and physical changes within body that disrupt its normal functions, including higher levels of inflammation and/or stress hormones and changes in heart rate, circulation, and metabolism. The plant extract may improve Hypothalamic-Pituitary-Adrenal axis hyperactivity, reduce the level of corticosterone in the brain, reduce the excessive activation of microglia, reduce the expression of inflammatory factors in the brain, and protect neurons.

[00106] Alzheimer's disease (AD) is a progressive neurodegenerative disease with insidious onset. Clinically, it is characterized by comprehensive dementia manifestations such as memory impairment, aphasia, apraxia, agnosia, impairment of visuospatial skills, executive dysfunction, and personality and behavioral changes.

[00107] Alzheimer's disease is the most common type of dementia. It is a progressive disease beginning with mild memory loss and possibly leading to loss of the ability to carry on a conversation and respond to the environment. Symptoms of Alzheimer's disease include memory loss; repeating statements and questions over and over; forgetting conversations, appointments or

events; forgetting the names of family members and everyday objects; misplacing items; getting lost; changes in personality and behavior and moods; depression; loss of interest in activities; social withdrawal; mood swings; distrust in others; anger or aggression; changes in sleeping habits; wandering; loss of inhibitions; delusions, such as believing something has been stolen; confusion with time or place; and trouble understanding visual images and spatial relationships. The plant extract may one or more of enhance the ability to explore new things, working memory, or spatial exploration; improve the structural morphology of neurons in the hippocampus; increase the number of neurons in the hippocampus; or reduce the number of Nystagmus.

[00108] Parkinson's disease (PD) is a chronic neurodegenerative disease that affects the central nervous system, mainly affecting the motor nervous system. Symptoms usually appear slowly over time. The main motor symptoms caused by Parkinson's disease are collectively called parkinsonism or Parkinson's Syndrome. Symptoms of Parkinson's disease include tremor; bradykinesia (slowness of movement); rigidity; limb stiffness; decreased motor function and abnormal gait or imbalance including speech and writing change; cognitive and behavioral problems or dementia and depressive disorder and anxiety disorders; problems with perception, sleep, neurasthenia and mood; pain, sensory impairment. decreased arm swing when walking; difficulty turning over in bed; difficulty getting out of bed, a deep chair, or a car seat; dysarthria (slurring of speech); hypophonia (soft; sometimes breathy and hoarse; voice); dyskinesia; involuntary, erratic writhing movements of the face, arms, legs or trunk; dystonia, sustained or repetitive muscle cramping, twisting or tightening; facial masking (hypomimia), a serious or mad facial "masking" appearance, and decreased blink rate; freezing, masked face (hypomimia) resulting from the combination of bradykinesia and rigidity; micrographia (small, crowded handwriting due to bradykinesia); shuffling gait, which may be accompanied by short steps and often a stooped posture; and stooped posture, often accompanied by short steps (shuffling gait) and trouble walking. The plant extract may reduce the loss of dopaminergic (DA) neurons in the substantia nigra or reduce DA neuron apoptosis.

[00109] The plant extract or the one or more compounds of the plant extract may be administered at a dose of about 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, or 250 g, or a range thereof. The dose may be a daily dose. The dose

may be split into multiple administrations during a day, which may be 1, 2, 3, 4, or 5, or a range thereof. In one example, the dose is 20 g per day. In another example, the dose is 25 g per day. The plant extract or the one or more compounds of the plant extract may be administered over a period of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, or 52 weeks, or a range thereof. The plant extract or the one or more compounds of the plant extract may be administered over a period of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, or 52 weeks.

[00110] Examples

[00111] Example 1: *Wikstroemia indica (Linn.) C. A. Mey* extract treats depression in an ovariectomized mouse model

[00112] This example demonstrates that a *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein can be used to treat depression, as indicated by its effects on the depressive behaviors of bilateral ovariectomized (OVX) mice induced by chronic stress. Fifty female Kunming (KM) mice were randomly divided into sham operation (Sham), model (negative control), positive control (E2), low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (3.8 g/kg) group, and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (6.5 g/kg) groups. The mouse model of depression was established by OVX combined with chronic unpredictable mild stimulation (CUMS). On the 8th day after bilateral ovariectomy, CUMS and control or extract administration were started for mice in each group. Tail suspension, open-field, and sucrose preference tests were used to evaluate the depressive behaviors of mice in each group. Hematoxylin and eosin (H&E) staining and Nissl staining were used to observe the histological changes of the hippocampus in mice. Compared with the Sham group, the immobility time in the tail suspension test of model group mice was significantly increased, standing times in the open-field test were significantly reduced, the value in the sucrose preference test was significantly reduced, and the morphology of the hippocampus was destroyed. Compared with the model group, the immobility time in the tail suspension test of mice in the E2 group decreased, standing times in the open-field test increased, and the value in the sucrose preference test increased. In the low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract group, the immobility time in the tail suspension test decreased, standing times in the open-field test increased, and the value in the sucrose preference test increased. In the high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract group, the immobility time in the tail suspension test decreased, standing times in the open-field

test increased, and the value in the sucrose preference test increased, but not significantly. Hippocampus morphology in the above three groups of mice improved. The results of behavioral tests and brain histopathology experiments show that the *Wikstroemia indica (Linn.) C. A. Mey* extract has antidepressant effects on OVX + CUMS mice.

[00113] Depression is mainly manifested as a mood disorder and affective disorder, with significant and lasting depression as the main clinical feature, and it is a psychiatric disease closely related to stress. The prevalence of major depressive disorder (MDD) is nearly twice as high in women as compared to men. The menopause transition is the midlife time when women experience an increased risk for the onset of depression, as well as relapse for women with a history of depression. The main symptoms in this transition include emotional depression, anxiety, and stress, accompanied by endocrine dysfunction, especially hypogonadism, and senescence. Robust correlation between depressive symptoms and dramatic fluctuation in gonadal hormones, especially the withdrawal in estrogen indicates that the "perimenopausal depression (PMD)" is a unique subtype of depression under perimenopausal state.

[00114] During perimenopause, women experience many symptoms caused by erratic fluctuations in ovarian hormones, which are driven by the hypothalamic-pituitary-gonadal (HPG) axis and result in the physiological and behavioral changes. Risk of menopause-related hormones contributed to the onset of PMD include low level of estradiol (E2) as well as high levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Estrogen withdrawal is well accepted as the main factor to trigger depressive symptoms in menopause transition. Hence, estrogen/hormone replacement therapy (ERT/HRT) has been considered the first-line clinic therapy for PMD treatment. However, long-term ERT might increase the risk of breast and ovarian cancer, stroke, and cardiovascular disease, which has limited the clinical applications of ERT.

[00115] Patients with depression often also show strong aggressiveness, impulsiveness, helplessness, and lack of pleasure, which are also often seen in animal models of depression induced by stress or inflammation. The animal model of bilateral ovariectomy (OVX) induced by chronic unpredictable mild stress (CUMS) is a good evaluation model for studying the etiology of depression at this stage. The modeling method is similar to the pathogenesis of clinical depression patients. The OVX-CUMS model effectively mimics the pathogenesis of PMD. In particular, the onset of PMD is characterized by depression. The OVX+ CUMS animal model is

of great significance for the pathogenesis, prevention, and treatment of PMD. OVX+ CUMS, as a commonly used animal model of depression, with estrogen withdrawal as the main feature, has been widely used in the study of neurodegenerative diseases such as PMD. OVX+CUMS is a relatively mature animal model that can successfully simulate depression caused by estrogen deficiency through bilateral ovariectomy and different stimulation for 21 days. The model reflects the pathological characteristics of PMD and is an ideal animal model of PMD.

[00116] Methods

[00117] Fifty female KM mice (30-35g) were purchased from Sibeifu Biotechnology Co., Ltd. (Beijing, China) [SCXK (Beijing) 2019-0010]. Mice were raised in specific pathogen free-grade animal facilities, with a temperature of 20–24℃ and relative humidity of 50–60%. The light source was natural, and the light and dark alternated for 12 hours (12L:12D), with *ad libitum* access to water and food. To make the mice adapt to the environment, they were adaptively fed for 1 week before the experiment. The operations were carried out under general anesthesia to reduce pain. The study was approved by the Ethical Committee of the Heilongjiang University of Chinese Medicine and was conducted according to accepted animal care practices.

[00118] *Wikstroemia indica (Linn.) C. A. Mey* extract was prepared by a method described herein. Estradiol valerate tablets (PROGYNOVA, E2) were purchased from Bayer Healthcare Co., Ltd. (Guangzhou, China). Dye for hematoxylin-eosin (H&E) staining was purchased from Beyotime Biotechnology (Shanghai, China).

[00119] Bilateral OVX combined with chronic unpredictable mild stimulation (CUMS) was used to construct the depression model. For OVX, the mice were fixed on a hard plate in the supine position after anesthesia. Then, an incision was made in the abdomen, and the ovaries on both sides were tied with medical sutures and then removed. Successful ovariectomy was identified using a vaginal epithelium keratosis test in which 4-7 days after OVX, no estrous cycle was observed upon continuous monitoring of the rat vagina. In the Sham group, a fat mass of the same size was removed to replace the ovary after ovary exposure. For CUMS, all mice were housed in a single cage after the surgery, and after 7 days of recovery from surgery, they were treated with CUMS for 21 days. The following seven stimuli were employed: fasting for 24 hours, water deprivation for 24 hours, damp pad stimulation for 24 hours, tilting cage (45° angle tilt), shaking cage (30 min/cage/time), black and white inversion (7 :00-19:00 black, 19:00-7:00 white), and ultrasonic stimulation (20 min) for 3 consecutive weeks.

[00120] Fifty mice were randomly divided into 5 groups: Sham group, Model group, E2 group (0.13 mg/kg), low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (3.8g/kg) group, and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (6.5g/kg) group. After 7 days of recovery from surgery, distilled water, E2, low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (twice a day), or high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract (twice a day) was administered according to the group classification for 21 days, with an oral administration at the dosage of 10 mL/kg.

[00121] Tail Suspension Test

[00122] Mice both acoustically and visually isolated were suspended 20 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during 2 to 6 minute periods. Mice were considered immobile only when they hung passively and completely motionless.

[00123] Open-Field Test

[00124] Animals were individually placed in a box (50 × 50 × 40 cm). The mice were placed in the center and their behavior was noted immediately and continued for 5 minutes. The apparatus was cleaned between test intervals. At the end of the experiment, the vertical activities (the standing times) of each mouse in the open field were analyzed.

[00125] Sucrose Preference Test

[00126] Following 2 hours pre-exposure to 2.5% sucrose to overcome neophobia, the baseline preference of the mice for 1% sucrose or water was measured for 12 hours. Bottles were weighed before and after use by researchers blind to the mouse's group allocation. Sucrose and water bottles were placed on randomly assigned sides of the cage and were switched after 6 hours to eliminate side preference artifacts. During the ensuing five weeks, water intake and 1% sucrose were measured once a week, on the day without exposure to stress, and not immediately following exposure to food deprivation. Preference for sucrose was calculated as the percentage of total liquid intake attributed to 1% sucrose solution, according to the equation: preference value (%) = sucrose intake / (sucrose intake + water intake)×100%.

[00127] H&E Staining

[00128] After the behavioral tests, the anesthetized mouse heart was exposed. A needle was inserted from the apex of the heart to the left ventricle, and normal saline was slowly injected until the blood was washed away. The normal saline was replaced with 4% paraformaldehyde

solution, and the mouse was stopped when its limbs became stiff. Their brains were removed and fixed with 4% paraformaldehyde for 48 hours, and stained pathological sections were prepared. Brain tissue was fixed in 4% paraformaldehyde, and then dehydrated and embedded by conventional pathological methods to make wax blocks. A rotary slicer was used to cut the wax block into brain sections (5.0 μm thick). The sections were dewaxed in xylene and dehydrated with 95% ethanol. Then, the sections were stained with H&E solution and dehydrated with graded alcohol. Sections were made transparent with xylene and the sections sealed with neutral glue. H&E staining was performed to observe the morphology of the hippocampus.

[00129] Nissl Staining

[00130] The same steps were performed as for the H&E staining method to perfusion. Whole brain was placed in 4% paraformaldehyde solution for storage. After 12 hours, the brain was removed, flushed, and the hippocampus was quickly peeled off. Wax blocks of the hippocampus were sliced, dewaxed with xylene until completely transparent, dyed with toluidine blue dye for 5 minutes, soaked with distilled water, dehydrated step by step with gradient ethanol, treated twice with xylene, and sealed with neutral gum. The morphology of hippocampal CA3 neurons was observed under a light microscope.

[00131] Morphology and Activity Observation

[00132] The morphology and activity of mice were observed before modeling, and there was no significant difference. In the process of modeling, except the Sham group, the morphology and activity of mice changed, showing dark fur, reduced activity, and death in severe cases. At the end of modeling, the appearance of mice in the E2 group was relatively healthy and their activity was slightly better, followed by mice in the low-dose and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract groups (Figs. 1A-E).

[00133] Fecal Observation

[00134] Compared with the Sham group, mice in the model group had fewer feces, smaller particles, lighter colors, and were drier. Compared with the mice in the model group, the mice in the E2 group, and the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* groups had more feces, which were darker, black or brown, and wetter. According to the observation and analysis of mouse feces, it is believed that the unpredictability of the stress mode may cause anorexia in the model group mice during the stress process, thus affecting gastrointestinal function disorder.

Low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract improved the gastrointestinal function of OVX mice (FIG. 2).

[00135] Weight Measurement

[00136] Body weight is an indicator of the development of bones and muscles and the degree of obesity. A change in body weight can reflect the nutrition and muscle development, which is of great significance for measuring the growth and health of the body. The experimental results showed that the weight growth of the model group mice was slow, and even negative. Compared with the model group, the weight of mice in the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract groups increased rapidly. The weight loss may have occurred because depression reduced appetite. In a negative emotional state, irregular diet or gastrointestinal dysfunction causes unhealthy weight development, resulting in weight loss. In this experiment, due to the high indoor temperature and humidity caused by the power failure in the animal room, the weight of mice in the Sham and the E2 group was significantly reduced on the fourth day (FIG. 3).

[00137] Vaginal Epithelium Keratosis Test

[00138] The types and characteristics of vaginal cells in normal mice showed periodic changes with changes in sexual cycle, including the pre-estrus, estrus, late estrus, and estrus intervals. The results of vaginal epithelium keratosis test showed that the vaginal cells of OVX mice disappeared periodically and remained in the estrus interval, characterized by a large number of white blood cells accompanied by a small number of epithelial cells, indicating the success of the OVX operation. The vaginal cells in the Sham group showed typical periodic changes, as shown in FIG. 4: A: Complete epithelial cells; B: a large number of keratinocytes densely distributed in patches; C: Keratinocytes and leukocytes; D: White blood cells accompanied by a few nucleated epithelial cells.

[00139] Tail Suspension Test

[00140] Compared with the Sham group, the immobility time of the tail suspension test in the model group was significantly prolonged. Compared with the model group, the immobility time of the E2 group mice was significantly shortened; the immobility time of mice in the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* groups was significantly shortened, and the depressive behavior of mice was improved (Table 1 and FIG. 5).

[00141] Table 1: Effects of low-dose and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract on immobility time of OVX mice

Group	Immobility time in seconds（$\bar{x}$±s）
Sham	42.46±9.02
Model	114.69±18.84$^{###}$
E_2	57.98±17.92***
Low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	45.32±21.63***
High-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	48.96±26.43***
Compared with the control group, ###P < 0.001; compared with the model group, ***P < 0.001.	

[00142] Open Field Test

[00143] Compared with the Sham group, the standing times in the open-field test of the model group mice were significantly reduced, indicating that the mice had depressive behavior. Compared with the model group, the standing times of mice in the E2 group increased. The standing times in the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract groups increased, although there was no statistical difference. They also showed that the *Wikstroemia indica (Linn.) C. A. Mey* extract had a certain effect on antidepressant behavior (Table 2 and FIG. 6-7).

[00144] Table 2: Effects of low-dose and high-dose *Wikistroemia indica* on standing times of OVX mice

Group	Times of standing in seconds（$\bar{x}$±s）
Sham	29.60±8.19
Model	20.3±11.78
E_2	26.1±9.66
Low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	30.6±9.25
High-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	34.3±14.93

[00145] Sucrose Preference Test

[00146] Compared with the Sham group, the preference value in the sucrose preference test in the model group was significantly reduced. Compared with the model group, the preference value in the high-dose *Wikstroemia indica (Linn.) C. A. Mey* group increased. The preference value in the E2 group and low-dose *Wikstroemia indica (Linn.) C. A. Mey* group increased significantly, and the depressive behavior of mice was improved (Table 3and FIG. 8).

[00147] Table 3: Effects of low-dose and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract on sucrose preference value in OVX mice

Group	Preference value%（$\bar{x}$±s）
Sham	74.16±3.99
Model	34.65±3.64$^{###}$
E_2	54.55±5.14**
Low-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	54.47±20.63**
High-dose *Wikstroemia indica (Linn.) C. A. Mey* extract	39.94±9.88
Compared with the control group, ###P < 0.001; compared with the model group, **P < 0.05.	

[00148] H&E Staining

[00149] The hippocampal neurons in the Sham group were round, with clear and complete structure and good arrangement. In the model group, the nucleus of hippocampal neurons was pyknotic, the staining was deepened, and the arrangement of neurons was loose. Compared with the model group, most of the neurons in the E2 group were intact, and there was little nuclear shrinkage. In the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract groups, some neurons were arranged in a more ordered fashion and some morphology recovered (FIG. 9).

[00150] Nissl staining

[00151] In the Sham group, the neurons in the hippocampal CA3 area of mice were arranged orderly, with normal morphology and no obvious necrosis. In the model group, neurons in the hippocampal CA3 area were atrophied, disordered, and sparse. Compared with the model group, the morphology of neurons in the CA3 area of the hippocampus in the E2 group was more regular, and there were fewer necrotic neurons. The morphology of neurons in the hippocampal CA3 area of mice in the low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract-treated groups were more regular, and there were fewer necrotic neurons (FIG. 10).

[00152] Conclusion

[00153] Depression belongs to the category of "depression" in traditional Chinese medicine, which is often caused by the failure of the liver to convey and disperse, and stagnation of liver qi. In the whole life cycle of women, perimenopause is a critical period for the development of depression, with a high risk of onset.

[00154] Some studies have shown that, in the open-field test, standing times of mice decrease when they have depressive behaviors. The sucrose preference test mainly evaluates whether rodents have depressive behaviors or whether their depressive behaviors have been improved in terms of sugar preference percentage from the aspect of pleasure loss. In this study, three behavioral experimental methods – a tail suspension test, open-field test, and sucrose preference test – were used to jointly confirm that under the same chronic stress induction conditions, the depression-like behavior of model mice was significantly worse than that of the Sham group. This study shows that compared with the Sham group, the immobility time in tail suspension test of the model group mice was prolonged, standing times in the open field were reduced, and the value in the sucrose preference test was significantly reduced. Compared with the model group, the immobility time in the tail suspension test of mice in the E2 group, and low- and high-dose *Wikstroemia indica (Linn.) C. A. Mey* extract-treated groups decreased, standing times in the

open-field test increased, and the value in the sucrose preference test increased significantly. The behavioral results showed that *Wikstroemia indica (Linn.) C. A. Mey* extract has significant antidepressant effects on OVX+CUMS mice.

[00155] Example 2

[00156] *Wikstroemia indica (Linn.) C. A. Mey* extract treats depression as indicated in a 4-Chloro-DL-phenylalanine-treated mouse model

[00157] This example demonstrates that a *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein can be used to treat depression-like behaviors, as determined in 4-Chloro-DL-phenylalanine (PCPA)-treated model mice. The experiment was divided into a control group, a model group, and a *Wikstroemia indica (Linn.) C. A. Mey* extract group. The effect of *Wikstroemia indica (Linn.) C. A. Mey* extract on depression-like behavior in PCPA model mice was tested by behavioral methods of open field, tail suspension, and forced swimming. The open field experiment showed that the central activity distance and time of the model group were decreased compared with the control group. Compared with the model group, the two indices of central activity distance and time were improved in the *Wikstroemia indica (Linn.) C. A. Mey* extract-treated group. The results of the tail suspension experiment showed that compared with the control group, the immobility time of the model group was significantly increased. Compared with the model group, the immobility time of mice in the treatment group was significantly reduced. The results of forced swimming experiment showed that compared with the control group, the immobility time of the model group was significantly increased. Compared with the model group, the immobility time of mice in the *Wikstroemia indica (Linn.) C. A. Mey* extract group was significantly reduced. The results show that *Wikstroemia indica (Linn.) C. A. Mey* extract improves depression-like behavior in PCPA mice.

[00158] Introduction

[00159] Depression is a common mental illness and a major cause of sub-health and disability worldwide, affecting nearly 300 million people. It is characterized by persistent symptoms, including low mood, decreased pleasure, suicidal thoughts, psychomotor retardation or agitation, changes in appetite and sleep patterns, decreased energy or increased fatigue, inattention, difficulty in decision making, irritability or physical restlessness, and slower speech or movement. The existence and severity of symptoms vary from individual to individual. Patients with depression have a higher rate of other physical diseases, decreased social function and

increased mortality. The complexity of the disorder is compounded by the fact that it often co-occurs with other mental illnesses. Therefore, it is of great medical and social significance to study the etiology, pathogenesis and treatment of depression and improve the living standard of patients. At present, the pathogenesis of depression is mainly based on the monoamine neurotransmitter hypothesis. The monoamine neurotransmitters and their metabolites in the brain mainly include norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT), and their content changes are closely related to the pathogenesis of depression. 5-HT plays a key role in antidepressant regulation and can regulate various physiological responses in the central nervous system and peripheral nervous system. 4-Chloro-DL-phenylalanine is a 5-HT synthesis inhibitor, which blocks the synthesis of 5-HT by inhibiting tryptophan hydroxylase. Depressive symptoms occur when 5-HT is depleted in the brain.

[00160] In this study, a PCPA-treated mouse model for depression was used to study the antidepressant effect of a *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein. Open field, tail suspension, and forced swimming experiments were used to verify the effects of *Wikstroemia indica (Linn.) C. A. Mey* extract on depression-like behavior in PCPA model mice. This study provides experimental basis for the clinical treatment of depression with *Wikstroemia indica (Linn.) C. A. Mey* extract.

[00161] Materials and methods

[00162] Experimental animals

[00163] Thirty Kunming strain female mice, aged 16 weeks, weighing 30±5 g, were provided by Sibeifu (Beijing) Biotechnology Co., LTD., License number: SCXK (Beijing) 2019-0010. The mice were handled at feeding temperature 22-26℃ and humidity 40%-70%.

[00164] Drug

[00165] *Wikstroemia indica (Linn.) C. A. Mey* extract prepared as described herein.

[00166] Main reagents and instruments

[00167] Para-Chlorophenylalanine (PCPA) (purity > 98%, lot No. D831376) was purchased from Shanghai MacLean Biochemical Technology Co., LTD. Tail hanging and forced swimming are self-made equipment. The open field was provided by Anhui Zhenghua Biological Equipment Co., LTD.

[00168] Animal model copy, grouping and methods

[00169] Thirty mice were randomly divided into a control group, a model group and a *Wikstroemia indica (Linn.) C. A. Mey* extract group, with 10 mice in each group. After 7 days of adaptive feeding, except for control group, mice in each group were intraperitoneally injected with 100 mg/kg PCPA, once a day for 4 consecutive days. After modeling, the administration group was given gavage (6.5 g/kg) for 7 days.

[00170] General Status Observation

[00171] Before the behavioral test, we observed the general state of each group of mice, including activity, reaction sensitivity, hair gloss, and other aspects.

[00172] Ethology

[00173] Open field experiment

[00174] The outer dimension of the open field test chamber was 105 cm × 105 cm, and the bottom wall and the surrounding side wall were made of black plastic. The experiment was carried out in a quiet environment. The mice were placed in the center of the bottom of the mouse open field box, and cameras and timing were performed at the same time. Mice were observed for 5 minutes and the camera was stopped. At the end of the experiment, the inner wall and bottom surface of the open field box were cleaned with alcohol to prevent it from affecting the test results of the next animal. At the end of the experiment, the central activity time (s) and central activity distance (cm) of each mouse were analyzed.

[00175] Forced swimming experiment

[00176] Each group of mice was placed in a cylindrical container with a diameter of 10 cm and a water surface height of about 12 cm, and the water temperature was 20-25°C. For each mouse, the water surface height was based on the mouse's hind paws not touching the ground and the head just above the water surface. The forced swimming experiment was performed for 6 minutes, and the immobility time of the mice within 4 minutes after the recording was recorded.

[00177] Tail suspension experiment

[00178] The self-made tail test suspension equipment was placed on a 45 cm high iron frame and fixed on a bracket in a box with elastic rope at the end of the tail, about 1 cm away from the end, keeping the head of the mouse about 20 cm away from the ground. The excrement of each mouse was cleaned up after the test, and the environment was kept quiet throughout the test. The mice were kept in a suspended state for 6 minutes and the time of immobility within 4 minutes after the analysis was recorded.

[00179] Statistical analysis

[00180] After data collection, SPSS 21.0 software was used for analysis, and data were expressed as ($\bar{x} \pm S$). Data consistent with normal distribution and overall homogeneity of variance were analyzed by one-way ANOVA, and $P<0.05$ indicated significant differences.

[00181] Results

[00182] General status observation

[00183] Compared with the control group (FIG. 11A), the activity of mice in the model group (FIG. 11B) was reduced, the response sensitivity was poor, and hair gloss was poor. Compared with the model group, the activity of mice in the *Wikstroemia indica (Linn.) C. A. Mey* extract group (FIG. 11C) was increased, the response sensitivity was better, and hair gloss was better.

[00184] Open field test

[00185] The open field experiment results showed that, as shown in Table 4, compared with the control group, the central activity distance and central activity time of the model group were reduced. Compared with the model group, the central activity distance and time of the *Wikstroemia indica (Linn.) C. A. Mey* extract group were increased.

[00186] Table 4: Effect of *Wikstroemia indica (Linn.) C. A. Mey* extract on central activity distance (cm) and central activity time (s) in open field tests in PCPA mice

Group	**Central activity distance (cm)**	**Central activity time (seconds)**
Control	253.21±43.03	11.60±3.01
Model	139.54±37.85	6.77±1.98
Wikstroemia indica (Linn.) C. A. Mey extract	206.06±43.14	9.81±4.26

[00187] Tail Suspension test

[00188] The results are shown in Table 5. Compared with the control group, the immobility time of the model group was increased. Compared with the model group, the immobility time of mice in the *Wikstroemia indica (Linn.) C. A. Mey* extract group was reduced.

[00189] Table 5: Effects of *Wikstroemia indica (Linn.) C. A. Mey* extract on tail suspension test immobility time in PCPA mice

Group	the immobility time (seconds)
Control	44.39±7.70
Model	117.36±19.05
Wikstroemia indica (Linn.) C. A. Mey extract	92.80±19.46

[00190] Forced swimming experiment

[00191] The results showed that, as shown in Table 6, compared with the control group, the immobility time of the model group was increased; Compared with the model group, the immobility time of mice in the *Wikstroemia indica (Linn.) C. A. Mey* extract group was reduced.

[00192] Table 6: Effects of *Wikstroemia indica (Linn.) C. A. Mey* on forced swimming test immobility time in PCPA mice

Group	immobility time (seconds)
Control	33.07±11.61
Model	50.87±17.54
Wikstroemia indica (Linn.) C. A. Mey extract	43.78±18.29

[00193] Conclusion

[00194] Depression is a common clinical mood disorder, with fatigue, low emotion, slow thinking, and decline in activity as the main symptoms. 5-hydroxytryptamine (5-HT) and its receptor play an important role in the development of depression. Most patients with depression have abnormal metabolism of 5-HT and related products, resulting in the destruction of the balance between some neurotransmitters and endocrine networks.

[00195] PCPA is a selective and irreversible inhibitor of the initiation enzyme and the rate-limiting enzyme tryptophan hydroxylase in 5-HT biosynthesis, which can block the synthesis of 5-HT by inhibiting tryptophan hydroxylation and significantly reducing the content of 5-HT in the brain and peripheral blood. 5-HT plays a key role in the regulation of depression, and the reduction of 5-HT content causes depression. In this study, a depression model was established by intraperitoneal injection of PCPA, and an open field test, tail suspension test, and forced swimming test were used to evaluate the depression of mice. The results showed that the number of autonomous activities of mice in the PCPA model group significantly decreased, the

immobility time of tail suspension and forced swimming significantly increased, and depression was aggravated. After administering a *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein, the autonomous activity of mice significantly increased, the tail hanging and forced swimming immobile time significantly decreased, and depression was significantly alleviated. The results indicate that *Wikstroemia indica (Linn.) C. A. Mey* extract has alleviating effects on the autonomous activity ability and depression of depressed mice, which provides a basis for the clinical treatment of depression.

[00196] Example 3

[00197] *Wikstroemia indica (Linn.) C. A. Mey* extract treats depression in chronic social defeated stress mouse model – study 1

[00198] Chronic social defeated stress (CSDS) are a common method used to model depression in animals. Depression is among the most prevalent mental illnesses, with 3.6% of the global population suffering from mood-related disorders, and depression is currently the leading worldwide cause of disability. Various animal models have been established to study the pathogenesis of depression, such as maternal deprivation, learned helplessness, chronic mild/unpredictable stress, isolated stress, early weaning, and sleep deprivation. However, these models cannot imitate psychiatric symptoms occurring in response to stress in humans in a representative manner. Social defeat stress, a process of losing a confrontation between individuals of the same species, is one of the most major risk factors for neuropsychiatric disorders. In humans, social defeat can be defined as feelings of being put in a secondary or repellent position. In animals, social defeat is caused by introducing a male into the home cage of an older and aggressive male. This defeat experience, seemingly a simple physical stress, actually induces psychological stress and behavioral changes through a chronic process.

[00199] Appropriate animal models are essential for the study of the pathogenesis of human diseases. In contrast to other experimental animal models of depression that are based on simple external "stress" or drug-induced depression, the CSDS model is derived from social affiliation, which gives experimental animals stress from both physical and psychological aspects. It can better simulate the typical depression states such as depression and anhedonia caused by excessive social pressure and frequent frustration in human daily social activities, and can reflect whether there is social avoidance behavior in the social process. It can better simulate the disease incentives of human depression from both physiological and psychological aspects. Therefore, it

has a high consistency with human diseases and is an ideal animal model for the study of human mental and psychological diseases.

[00200] This example demonstrates the effects of an *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein (also referred to as "Plant Drug") on depression-like behavior in CSDS model mice. The experiment was divided into control group, model group and *Wikstroemia indica (Linn.) C. A. Mey* extract group. The effect of *Wikstroemia indica (Linn.) C. A. Mey* extract on depression-like behavior in CSDS model mice was tested by behavioral methods of social interaction test, three-chamber social approach test and sucrose preference test. The results of the social interaction test showed that, in the presence of social targets, the model group spent less time in social contact and more time in the corner area. Compared with the model group, both measures of social contact time and time spent in corner areas were improved in the *Wikstroemia indica (Linn.) C. A. Mey* extract group. The results of three-chamber social approach test showed that compared with the control group, the model group had no significant increase in social strength and social preference, and compared with the model group, the *Wikstroemia indica (Linn.) C. A. Mey* extract group had significant increases in social strength and social preference. The sucrose preference results showed that the sucrose consumption of the model group was significantly lower than that of the control group. Compared with the model group, the sucrose consumption of the plant extract group was significantly increased. The results demonstrate that *Wikstroemia indica (Linn.) C. A. Mey* extract can improve the depression-like behavior of CSDS mice.

[00201] Materials and Methods

[00202] Experimental animals

[00203] Male C57BL6/J mice (7-8 weeks of age) were obtained from Hangzhou Qizhen Experimental Animal Technology Co., LTD (Hangzhou, China) [SCK(Zhe)2022-0005], and retired CD-1 mice breeders (5-6 months of age) were obtained from Hangzhou Ziyuan Experimental Animal Technology Co., LTD (Hangzhou, China) [SCK(Zhe)2019-0004]. Mice were housed in a regulated environment (23 ± 1°C, 60 ± 5% humidity) with a 12 h light/12 h dark cycle (lights on at 07:00). Animals were allowed to habituate for 1 week before the start of experiments. All studies were conducted in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation.

[00204] Drug

[00205] *Wikstroemia indica (Linn.) C. A. Mey* extract prepared as described herein.

[00206] Main reagents and instruments

[00207] Social interaction, three-chamber social approach and sucrose preference are self-made equipment.

[00208] Animal model copy, grouping and methods

[00209] Thirty mice were randomly divided into control group, model group and *Wikstroemia indica (Linn.) C. A. Mey* extract group, with 10 mice in each group. The CSDS paradigm was conducted as described previously. Retired male CD-1 mice were screened for 3 consecutive days and the aggressors were selected according to the following criteria: (i) CD1 mice attacked the C57BL/6J mice for 2 consecutive days and (ii) the latency to the initial attack on C57BL/6J mice was under 60 s. For 10 consecutive days, mice were placed in the home cage of CD1 and were defeated for 5 min. After physical defeat, a perforated divider separated them for 24 h until the next physical defeat. Each day, intruder mice always faced a different resident. After 10 days of CSDS, retired male CD-1 mice and experimental mice were singly housed and performed 24 h later for behavioral tests. After the model was established, the mice in the drug group were given intragastric administration (2.6 g·kg-1) for 21 consecutive days, and the behavioral test was performed 24 hours later.

[00210] Ethology

[00211] Social interaction test (SIT)

[00212] The SIT is used to assess social avoidance behavior. This is a two-step test. In the first 2.5 minute session, a c57BL/6 mouse is allowed to freely explore an open field arena (40 cm length × 40 cm width × 40 cm height; Figure 12A shows the schematic). Along one side of the arena is a circular (7 cm diameter) wire cage that remains empty during the first trial (target absent). The experimental c57BL/6 mouse is then removed from the open field arena and a novel CD1 male mouse is placed into the wire cage. In the second 2.5 min trial (target present), the experimental c57BL/6 mouse is reintroduced into this arena now containing a social target (unfamiliar CD1 mouse) within the wire cage. In this investigation, time (s) spent in the interaction zone (8 cm wide corridor surrounding the wire cage) and the corner zones (10 × 10 cm) were the dependent variables. Additionally, we recorded the distance traveled (cm) during the first 2.5 minutes of the social interaction test to examine whether basal locomotor activity could be influenced by social defeat stress.

[00213] Three-chamber social approach test

[00214] The test was performed as previously described. The day before experiments, novel mice were trained in cages located in two side chambers. On the testing day, subject mice were first allowed to freely explore the three-chamber apparatus for 10 minutes to habituate. In stage I sociability test, subject mice were initially placed in the center chamber and then allowed to visit an empty cage and a novel mouse (stranger 1) in two opposite side chambers for 10 min. In stage II social novelty preference test, the empty cage was replaced by another novel mouse (stranger 2), and the subject mice freely moved in the three-chamber apparatus for 10 minutes. The behaviors were recorded and analyzed using Any-maze.

[00215] Sucrose preference test (SPT)

[00216] Before the experiment, mice were habituated with two identical water bottles for 3 days. Each mouse was water and food-deprived for 24 hours and then provided with 1% water and sucrose solution. After 12 hours, the bottles were weighted and the sucrose preference was calculated as [sucrose water intake/(sucrose water intake + pure water intake)] × 100%.

[00217] Statistical analysis

[00218] After data collection, SPSS 26.0 software was used for analysis, and data were expressed as ($\bar{x}$± S). Independent sample t test was used to compare data between two groups if normal distribution was satisfied. For comparison between multiple groups, one-way analysis of variance was used for those satisfying normal distribution, and rank sum test was used for those with uneven variance, and $P<0.05$ indicated significant differences.

[00219] Results

[00220] Social interaction test results

[00221] After CSDS modeling, the mice in the model group showed obvious social avoidance behavior. Control group mice showed significantly higher levels of social interaction when the target was present. In contrast, social interaction levels were significantly reduced in mice exposed to social defeat in the presence of a social target. The level of social interaction in the model group improved significantly after receiving the *Wikstroemia indica (Linn.) C. A. Mey* extract (Figure 12B). The time spent in the corner zones (Figure 12C), another measure of social avoidance. Specifically, socially defeated C57BL/6 mice spent significantly more time in the corner zones in the presence of the social target, when compared to controls. Importantly, no

differences in total distance traveled were observed between each group during the target absent (2.5 min) condition of the social interaction test (Figure 12D).

[00222] Three-chamber social approach test results

[00223] In the first phase of the social preference test, there was no significant change in the social preference and intensity of the mice in each group. Each group of mice spent more time with the mouse (stranger 1) than with the object (Figure 13A-B). In phase two of the social preference test, the control groups and *Wikstroemia indica (Linn.) C. A. Mey* extract groups spent twice as much time with the new mice (stranger 2) as they did with the familiar mice (stranger 1). In contrast, mice in model group spent almost the same amount of time with familiar mice (stranger 1) and new mice (stranger 2) (Figure 13C). To assess the strength of social preferences, the preference index was evaluated (Figure 13D). The results showed that the *Wikstroemia indica (Linn.) C. A. Mey* extract could significantly improve the social deficits of mice in model group. The above experimental results showed that the loss of preference in the mice in model group was reversed by the *Wikstroemia indica (Linn.) C. A. Mey* extract to some extent, which was similar to that in the control group.

[00224] Sucrose preference test results

[00225] In sucrose preference tests (SPT), sucrose consumption was significantly reduced in the model group compared to the control group, reflecting anhedonia. Nevertheless, *Wikstroemia indica (Linn.) C. A. Mey* extract intervention greatly reversed CSDS-induced reduction in sucrose preference (Figure 14).

[00226] Conclusion

[00227] Depression has become a serious social issue that cannot be ignored because of the serious impact of depression on the health and quality of life of the patients. Research shows that drug treatment is an effective treatment for depression. Therefore, it is very urgent for us to search for novel and effective agents for treatment of depression.

[00228] CSDS is an animal model with both face and predictive validity. Analogous to a human population subjected to daily life stress. In this experiment, to investigate the effects of a *Wikstroemia indica (Linn.) C. A. Mey* extract on CSDS-induced depressive-like behaviors, SIT, TCT and SPT were performed after administering the extract.

[00229] In the SIT, the mice in the model group showed an avoidance behavioral response, spending significantly less time in the interaction zone and more time in the corner zone

compared to the control group. This stress-induced avoidance behavior mirrors the exposure of C57BL/6 male mice to the stress of social failure and can be reversed with *Wikstroemia indica (Linn.) C. A. Mey* extract. There are several advantages to the TCT, which improve data reliability for examination of sociability, social affiliation, social memory and preference, using similar procedures. This prevents direct physical contact, eliminating fighting and/or aggressive behavior but allows sensory interactions (smell, sight, sound, taste). Under these conditions, the subject mouse is solely in control of actively seeking and investigating the social stimulus. The three-chamber test thus provides an elegant but simple design with high experimental control and offers easy objective scoring, as compared to a social interaction test involving two freely moving animals. In the first phase of the social preference test, each group of mice spent significantly more time in the chamber with stranger 1 than in the chamber with the empty cage. In n phase two of the social preference test, control group and *Wikstroemia indica (Linn.) C. A. Mey* extract group mice spent significantly more time in the chamber with the unfamiliar mouse (stranger 2) than in the chamber with the now familiar mouse (stranger 1). However, mice with social defeat showed no significant social preference, which was reversed after administration of *Wikstroemia indica (Linn.) C. A. Mey* extract.This study also evaluated whether social failure stress affected sucrose preference with SPT as a complementary measure of depression-like behavior. Specifically, we chose this additional measure because anhedonia, the reduced ability to experience pleasure, is one of the core symptoms of clinical depression. The results showed that the defeated mice had a reduced preference for the sucrose solution compared to the control group.

[00230] Collectively, the data from the social interaction, social preference, and sucrose preference tests suggest that exposure to social defeat stress during adolescence results in the expression of core and common endophenotypes of major depressive disorder, which include avoidance and anhedonia, with significant ameliorative effects of *Wikstroemia indica (Linn.) C. A. Mey* extract, providing a rationale for clinical treatment of depression.

[00231] Example 4

[00232] A *Wikstroemia indica (Linn.) C. A. Mey* extract effectively treats depression-like behaviors in chronic social defeated stress model mice – study 2

[00233] This example demonstrates the effects of a *Wikstroemia indica (Linn.) C. A. Mey* plant extract described herein on depression-like behavior in CSDS model mice.

[00234] Methods: The experiment was divided into blank, model, fluoxetine and *Wikstroemia indica (Linn.) C. A. Mey* extract (also referred to as Phyto-N) groups. Depression-like behaviors of mice in each group were assessed using forced swimming experiment, social interaction experiment and three-box social contact experiment. Histological changes in the hippocampal region of mice were observed by hematoxylin and eosin (H&E) staining and Nichols staining. The serum levels of CRH, ACTH, CORT, IL-1β, IL-6 and TNF-α were detected by ELISA. The protein expressions of Bcl-2, Caspase-1, NLRP3, Caspase-3, Caspase-9 and Iba1 in hippocampus were detected by Western blotting.

[00235] Results: Behavioral results showed that, compared with the blank group, mice in the model group had a significantly longer immobility time in the forced swimming experiment; a significantly lower rate of social contact in the social interaction experiment; and no significant social preference in the three-box social contact experiment. Compared with the model group, the immobility time in the forced swimming experiment was significantly shorter in the fluoxetine group and the Phyto-N group; the social contact rate was significantly increased in the social interaction experiment; and there was a significant social preference in the three-box social contact experiments. H&E staining results showed that, compared with the blank group, the neurons in various regions of the hippocampus of the mice in the model group were similarly lost to varying degrees; compared with the model group, the fluoxetine group, Compared with the model group, the fluoxetine group and the Phyto-N group showed a more regular arrangement of neurons in each area of the hippocampus and a normal neuronal morphology. The results of Niehl's staining showed that compared with the blank group, a decrease or even disappearance of Niehl's vesicles and partial atrophy and deformation of cells in the hippocampus of mice in the model group were clearly observed; compared with the model group, the number of Niehl's vesicles in the hippocampus of mice in the fluoxetine group and the Phyto-N group was increased, and Niehl's vesicles were larger, bluish-purple in colour and with a normal morphology of the cells after staining. ELISA results showed that compared with the blank group, the serum levels of CRH, ACTH, CORT, IL-1β, IL-6 and TNF-α in the model group were significantly increased; Compared with the model group, the expression of all indicators in the fluoxetine and Phyto-N groups showed a significant correction trend. Western blotting results showed that compared with the blank group, the expression of Iba1, Caspase-1, Caspase-3 and NLRP3 protein in hippocampal neurons of the model group was significantly up-

regulated, and the expression of Bc-l 2 protein was significantly down-regulated, but Caspase-9 did not change significantly. Compared with the model group, the expression of each protein in the fluoxetine group and Phyto-N group showed a significant downward trend.

[00236] Conclusion: The results of behavioral test, histopathology, ELISA and Western blotting showed that Phyto-N could significantly improve the depression-like behavior of CSDS mice.

[00237] Depression is a chronic, recurring and potentially life-threatening illness that affects up to 20% of the population across the world. The cardinal symptoms of MDD include depressed mood (reduced motivation or hopelessness), anhedonia (diminished ability to experience pleasurable activity such as food, sex and social interactions), anergia, irritability, difficulty in concentrating, disrupted sleep, appetite and cognition and tendency to suicide. Chronic social defeat stress (CSDS)—an ethologically valid animal model of depression—induces a depression-like state in mice that is similar to depressive states in humans, with similarities in brain neurochemical changes and in symptoms, etiology, and sensitivity to antidepressants.

[00238] In this study, the antidepressant effect of Phyto-N was investigated using the CSDS depression model. The effects of Phyto-N on social dysfunction and despairing behaviour of CSDS model mice were verified by forced swimming experiment, social interaction experiment and three-box social contact experiment, and the protective effects on neurons in hippocampus were observed by H&E and Nichols' staining. This study provides an experimental basis for Phyto-N clinical treatment of depression.

[00239] Materials and Methods

[00240] Experimental animals

[00241] Male C57BL6/J mice (7-8 weeks of age) were obtained from Hangzhou Qizhen Experimental Animal Technology Co., LTD (Hangzhou, China) [SCK(Zhe)2022-0005], and retired CD-1 mice breeders (4-6 months of age) were obtained from Hangzhou Ziyuan Experimental Animal Technology Co., LTD (Hangzhou, China) [SCK(Zhe)2019-0004]. Mice were housed in a regulated environment (23 ± 1°C, 60 ± 5% humidity) with a 12 hours light/12 hours dark cycle (lights on at 07:00). Animals were allowed to habituate for 1 week before the start of experiments. All studies were conducted in accordance with the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation.

[00242] Drug

[00243] Phyto-N was prepared as described herein.

[00244] Main instruments

[00245] Social interaction, three-chamber social approach and sucrose preference are self-made equipment. -80°C ultra-low temperature refrigerator (Thermo Fisher), STP 120 automatic centrifugal tissue dehydrator (Myr&MICROM, Spain), HistoStar tissue embedding machine (Thermo Fisher), HM355S pathology sectioning machine (Thermo Fisher), GEMINI AS Automatic Staining Machine (Fisher Scientific), BX53 Microscope (OLYMPUS, Japan), SpectraMAX i3 Enzyme Labeler (Molecular Devices, China), EPS300 Gel Electrophoresis Instrument (Tennent Technology, China), 5200 Multi Gel System Imaging Analysis Software (Tennent Technology, China). Tennant Technology Corporation).

[00246] Main reagents

[00247] Fluoxetine hydrochloride capsules (J20130010, Fatheon France), sucrose (BS058-1KG, Biosharp), 4% paraformaldehyde (BL539A, Biosharp), paraffin wax (39601095, Leica), Hematoxylin-Eosin (H&E) Staining Kit (G1120, Beijing Solepol Science and Technology Co.), Nysted staining solution (C0117, Biyun Tian), PBS buffer (10×) (E-BC-R187, Elabscience), Iba1 Rabbit Polyclonal Antibody (10904-1-AP, proteintech), Anti-Caspase-3 CASP3 Rabbit Monoclonal Antibody (M00334-5, Boster Bio), Anti-Caspase-9/CASP9 Antibody PICOBAND™ (PB9332, Boster Bio), Anti-Bcl-2 Rabbit Monoclonal Antibody (M00040-1, Boster Bio), NLRP3 Polyclonal antibody (27458-1-AP, proteintech), Caspase 1 Recombinant antibody (81482-1-RR, proteintech). SDS-PAGE protein supersampling buffer (P0015L, biosharp), RIPA lysate (strong) (P0013B, biosharp), Colour Prestained Protein Marker (10-180kDa) (BL712A, biosharp) Mouse IL-1β (Interleukin 1 Beta) ELISA Kit (E- EL-M0037c, Elabscience), Mouse TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit (E-EL-M3063, Elabscience), Mouse IL-6 (Interleukin 6) ELISA Kit (E-EL- M0044c, Elabscience), CRH Kit (E02C0372, Shanghai Bluebase Biotechnology Co., Ltd.), ACTH Kit (E02A0005, Shanghai Bluebase Biotechnology Co., Ltd.), CORT Kit (E02C0008, Shanghai Bluebase Biotechnology Co., Ltd.)

[00248] Animal model copy, grouping and methods

[00249] Thirty mice were randomly divided into control group, model group and Phyto-N group, with 10 mice in each group. The CSDS paradigm was conducted as described previously. Retired male CD-1 mice were screened for 3 consecutive days and the aggressors were selected according to the following criteria: (i) CD1 mice attacked the C57BL/6J mice for 2 consecutive

days and (ii) the latency to the initial attack on C57BL/6J mice was under 60 seconds. For 10 consecutive days, mice were placed in the home cage of CD1 and were defeated for 5 minutes. After physical defeat, a perforated divider separated them for 24 hours until the next physical defeat. Each day, intruder mice always faced a different resident. After 10 days of CSDS, retired male CD-1 mice and experimental mice were singly housed and performed 24 h later for behavioral tests. After the model was established, the mice in the drug group were given intragastric administration (2.6 $g \cdot kg^{-1}$) for 21 consecutive days, and the behavioral test was performed 24 hours later.

[00250] Ethology

[00251] Social interaction test (SIT)

[00252] The SIT is used to assess social avoidance behavior. This is a two-step test. In the first 2.5 minute session, a c57BL/6 mouse is allowed to freely explore an open field arena (40 cm length × 40 cm width × 40 cm height). Along one side of the arena is a circular (7 cm diameter) wire cage that remains empty during the first trial (target absent). The experimental C57BL/6 mouse is then removed from the open field arena and a novel CD1 male mouse is placed into the wire cage. In the second 2.5 minute trial (target present), the experimental c57BL/6 mouse is reintroduced into this arena now containing a social target (unfamiliar CD1 mouse) within the wire cage. Record and analyse the time mice spend in the social zone around the wire cage (8 cm). For the SIT the SI ratio was calculated.SI ratio = time spent in the social zone of CD1 mice/time spent in the social zone in the absence of CD1 mice.

[00253] Forced swim test (FST)

[00254] The day before the experiment, C57BL/6 mice were placed in a transparent experimental tank with 25°C water for 15 minutes for swim acclimatisation, and then dried or air-dried and placed in their original cages. At the beginning of the experiment, the mice were placed in a transparent cylindrical container (beaker) filled with clean water at a depth of 15-20 cm and a temperature of 22±1°C. The mice were forced to swim, and the immobility of the mice for the last 4 minutes within the 6-minute period was recorded with a video camera system. After the experiment, the mice were dried and returned to their original cages. The water tank was rinsed and the water in the swimming tank was replaced before the next mouse was tested at the end of each mouse experiment to avoid the influence of mouse odor, such as excreta, on the behavioral science of the mice in this experiment.

[00255] Three-chamber social approach test (TCT)

[00256] The experiment was performed according to known methods. On the day before the experiment, new mice were trained in cages located on either side. On the day of testing, experimental mice were first allowed to freely explore the three-chamber device for 10 minutes to acclimatize. In the first phase of the social competence test, the subject mice were first placed in the center chamber and then allowed to visit an empty cage and a familiar C57BL/6J mouse in the two chambers on the opposite side for 10 minutes. in the second phase of the social novelty preference test, the unfamiliar C57BL/6J mice replaced the empty cages and were free to move around the three-chamber setup for 10 min. Any-maze software was used to record and analyze their behavior.

[00257] Histopathology

[00258] H&E staining

[00259] Fresh mouse brain tissues were dissected and removed and placed in fixative at room temperature for 24 hours. At the end of fixation, the tissues were rinsed with running water overnight to remove paraformaldehyde adequately. The fixed tissues were sequentially immersed in low-high gradient ethanol for 1 h each for tissue dehydration. Then, the tissues were immersed in n-butanol solution for 1 hour and xylene for 40 minutes for transparency treatment; the tissues were immersed in wax cylinders containing waxes with melting point of 56-58°C for 1 hours each, embedded and sliced into 6 μm, and dried at 37°C for 12 hours. The waxes were first dewaxed, and the wax slices were immersed in xylene solution for 20 minutes to remove the wax around the tissues; the tissues were immersed in high-low-gradient ethanol solution for 5 minutes each to dehydrate them. Sections were then rinsed under running tap water. Sections were stained in hematoxylin for 7-10 min, counter-blue in ethanol hydrochloride for 3 seconds, and immersed in eosin stain for 30 seconds. Each staining was rinsed under running water. After staining, the sections were dehydrated and transparent, i.e., the sections were sequentially immersed in low-high gradient ethanol solution and xylene solution for 5 seconds each for dehydration and transparency, and the sections were sealed with neutral gum. Microscopic observation and image acquisition and analysis showed the neuronal morphology with blue nuclei and red cytoplasm.

[00260] Nissl staining

[00261] The morphology of nidus vesicles in various regions of the hippocampus was observed using Niehl's staining. Sections were deparaffinised to water at the place described above for H&E staining method; Niehl's staining solution was added for 10-20 min, rinsed in running water, dehydrated, transparent and sealed. When viewed under the microscope, the Nichols' vesicles appeared blue in color

[00262] ELISA was used to measure the levels of CRH, ACTH, CORT, IL-1β, IL-6 and TNF-α

[00263] Detect the levels of CRH, ACTH, CORT, IL-1β, IL-6, TNF-α in mouse serum according to the instructions of ELISA kit.

[00264] Western blotting was used to detect the protein expression of Bcl-2, Caspase-1, NLRP3, Caspase-3, Caspase-9 and Iba1 in hippocampus

[00265] RIPA lysate containing protease inhibitors and phosphatase inhibitors was added to the hippocampus of mice in each group. The total protein was extracted, and the protein concentration was quantified by BCA method and normalized. Protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membranes and blocked in 5% skim milk for 2 h at room temperature. Primary antibodies Bcl-2, Caspase-1 (1:2500), NLRP3 (1:1000), Caspase-3 (1:1000), Caspase-9 (1:1000), Iba1 (1:1000) and β-actin (1:4000) were added and incubated overnight at 4 ° C. After rinsing three times with PBST, secondary antibodies (1:500) were added and incubated for 2 hours at room temperature with shaking. After PBST rinsing three times, ECL chromography was performed and images were obtained using an automatic gel imaging system. The gray value of the bands was calculated using Image J software.

[00266] Statistical analysis

[00267] After data collection, SPSS 26.0 software was used for analysis, and data were expressed as ($\bar{x} \pm$ S). Independent sample t test was used to compare data between two groups if normal distribution was satisfied. For comparison between multiple groups, one-way analysis of variance was used for those satisfying normal distribution, and rank sum test was used for those with uneven variance, and $P<0.05$ indicated significant differences.

[00268] Results

[00269] Social interaction test results

[00270] Compared with the blank group, the social contact rate of mice in the model group was significantly reduced (Figure 15A, $P<0.01$), with obvious social avoidance behavior; compared

with the model group, the social contact rate of mice in the fluoxetine group and the Phyto-N group increased significantly (Figure 15A, $P<0.01$). The results indicated that Phyto-N had a significant improvement effect on the social avoidance behavior of model mice. 2.2 Forced swim test results.

[00271] Compared with the blank group, the immobility time of the mice in the model group during the forced swimming experiment was significantly prolonged (Figure 15B, $P<0.01$), and the mice in the model group had an obvious state of behavioural despair; the immobility time of the mice in the fluoxetine and Phyto-N groups was significantly reduced compared with the model group (Figure 15B, $P<0.01$). The results indicated that Phyto-N had a significant improvement effect on the despair state of the model mice.

[00272] Three-chamber social approach test results

[00273] In the first stage of the social preference test, there was no significant change in the social preference of mice in all groups (Figure 15C, $P>0.05$). In the second stage of the social preference test, compared with the blank group, the social preference rate of mice in the model group was significantly reduced (Figure 15D, $P<0.01$); compared with the model group, the social preference rate of mice in the fluoxetine group and the Phyto-N group was significantly increased (Figure 15D, $P<0.01$; $P<0.05$). The results indicated that Phyto-N could significantly improve the social deficits of mice in the model group, and reversed the lack of social preference in the model mice to a certain extent.

[00274] H&E Staining results

[00275] In order to observe the effect of social eye on the neurons in the hippocampus of mice, H&E staining was performed on the brain tissue of mice. The results of H&E staining showed that the hippocampal neurons of mice in the blank group had a large number of neurons, the cells were arranged tightly, the cellular morphology was complete, the cytosolic nucleus and cytoplasmic demarcation were obvious, the nuclear membrane was clear, and the cytoplasmic staining was homogeneous. In the model group, the hippocampal neurons were severely lost, the cells were loosely arranged and irregular, the structure was blurred, the nuclei and cytosol were solidified, the blank area was visible in the cells, and the cytoplasm was deeply stained; the neuronal cells of CA1, CA2, CA3 and DG areas were lost to different degrees, the cell structure was blurred, the nuclei of vacuoles were solidified inside, and the cytoplasm was deeply stained. Compared with the model group, the morphology of neurons in the hippocampus of mice in the

fluoxetine group and Phyto-N group was similar to that of the blank group, with neurons arranged in a more regular manner, in larger numbers, in normal morphology, with clearer nuclei and no obvious nuclear condensation. The morphology of neurons in each area of the hippocampus of mice in each group is shown in Figure 16.

[00276] Nissl staining results

[00277] The brain tissue structure of blank group mice was normal, neuronal cells in all regions of the hippocampus had normal morphology, were closely arranged with more cytosolic protrusions, and the Nysted's body granules were thick and dense, dark blue in colour, and widely distributed in the cytoplasm. The model group mice hippocampus CA1, CA2, CA3 and DG area can be clearly observed that the Nystagmus granules are swollen and broken, the number is reduced, fuzzy or even disappeared, the cells are atrophied and deformed, and the cytosolic body is fuzzy and slush-like. Compared with the model group, the fluoxetine group and Phyto-N group showed an increase in the number of Nitrosomes in the hippocampal region of the mice in each group, and the Nitrosomes were larger, bluish-purple in colour after staining, and the cell morphology was more normal, with a small spacing between the cells and a dense arrangement of the cells. The morphology of Nitrosomes in the hippocampal regions of mice in each group is shown in Figure 17.

[00278] ELISA was used to detect the contents of CRH, ACTH, CORT, IL-1β, IL-6 and TNF-α

[00279] Compared with the blank group, the serum levels of CRH, ACTH and CORT in the model group were significantly increased (P< 0.001); Compared with the model group, the expression of CRH, ACTH and CORT in the fluoxetine and Phyto-N groups showed a downward trend (P< 0.01, P< 0.001; P< 0.05, P< 0.01). Compared with the blank group, the serum levels of IL-1β, IL-6 and TNF-α in the model group were significantly increased (P< 0.01); Compared with the model group, the expressions of IL-1β, IL-6 and TNF-α in the fluoxetine and Phyto-N groups showed a downward trend (P< 0.01, P< 0.001). The results are shown in Figure 18.

[00280] Western blotting was used to detect the protein expression of Bcl-2, Caspase-1, NLRP3, Caspase-3, Caspase-9 and Iba1 in hippocampus

[00281] Compared with the blank group, the expression of Iba1, Caspase-1, Caspase-3 and NLRP3 protein in hippocampal neurons of the model group was significantly up-regulated (P< 0.05, P< 0.01, P< 0.001), and the expression of Bc-l 2 protein was significantly down-

regulated (P< 0.05), but Caspase-9 did not change significantly. Compared with the model group, the expression of each protein in the fluoxetine group and Phyto-N group showed a significant downward trend (P< 0.05, P< 0.01). The results are shown in Figure 19.

[00282] Conclusion

[00283] Depression has become a serious social issue that cannot be ignored because of the serious impact of depression on the health and quality of life of the patients. Research shows that drug treatment is an effective treatment for depression. Therefore, it is very urgent for us to search for novel and effective agents for treatment of depression.

[00284] CSDS is an animal model with both face and predictive validity. Analogous to a human population subjected to daily life stress. In this experiment, to investigate the effects of Phyto-N on CSDS-induced depressive-like behaviors, SIT, TCT and SPT were performed after Phyto-N.

[00285] In the SIT test, mice in the model group exhibited avoidance behavioural responses, spending significantly less time in the interaction zone and more time in the corner zone compared to the blank group. This stress-induced avoidance behavior reflects the fact that C57BL/6 male mice are exposed to the stress of social failure and can be reversed by Phyto-N. The present study also assessed whether social failure stress affects despairing behaviour due to the state of despair, one of the core symptoms of clinical depression. The results showed significant despairing behaviour in the defeated mice compared to the control group. The TCT has several advantages and using a similar procedure could improve the reliability of data from tests of sociability, social relationships, social memory and preferences. This prevents direct physical contact and eliminates fighting and/or aggressive behaviour, but allows sensory interaction (smell, sight, sound, taste). Under these conditions, the subject rats were in full control of actively seeking and investigating social stimuli. Thus, compared to social interaction tests involving two free-ranging animals, the three-chamber test offers an elegant and simple design with a high degree of experimental control and provides easy objective scoring. In phase 1 of the social preference test, each group of mice spent significantly more time in the social area of familiar C57BL/6J mice than in the social area of empty cages. In phase 2 of the social preference test, mice in the blank, fluoxetine, and Phyto-N groups spent significantly more time in the social area of the unfamiliar C57BL/6J mice than in the familiar mice. However, the socially failed mice did not show a significant social preference, which was reversed after Phyto-N administration. In the histopathological results, neuronal cells in various regions of the

hippocampus of mice in the model group were lost to varying degrees, with blurred cellular structures, vacuolated nuclei within them that were solidified, deeply stained cytoplasm, and swollen and fragmented Nysted's vesicles that were reduced in number, blurred, or even disappeared, whereas all of the above was reversed after Phyto-N administration.

[00286] Corticosterone is the main glucocorticoid hormone in animals, which is secreted by the adrenal gland of HPA axis. When the body is under stress, accompanied by endocrine disorders and HPA axis hyperactivity, the level of corticosterone is significantly higher than that under normal conditions. High concentrations of corticosterone can activate glucocorticoid receptors in the hippocampus and other parts of the brain and induce neuronal apoptosis. In brain diseases such as depression and schizophrenia, corticosterone content is significantly increased. Microglia (MG) are the main immune cells in the central nervous system, which is the key to mediate neuroinflammatory response. Some scholars have confirmed that the treatment of mouse-derived MG with corticosterone can induce the secretion of pro-inflammatory factors, suggesting that the activation of MG by GC may play an important role in the neuroinflammatory response caused by stress. In recent years, studies have confirmed that GC is involved in the neuroinflammatory response caused by stress, and stress neuroinflammatory response has become one of the main pathogenesis of depression, post-traumatic stress disorder and other diseases. In this study, the results of ELISA showed that there were HPA axis hyperfunction and significantly increased levels of inflammatory factors in CSDS model mice, which further suggested that HPA axis activity was closely related to neuroinflammation. Western blotting results showed that the expression of Iba1, Caspase-1 and NLRP3 protein in hippocampal neurons of the model group was significantly increased, suggesting that increased corticosterone level may induce excessive activation of microglia and lead to increased expression of inflammatory factors. Caspases are a group of proteases in the cytoplasm, which are called cysteinyl aspartate specific proteinases (caspases). When activated, they cleave at the specific aspartate residues of target proteins. It plays an important role in programmed cell death (including apoptosis, pyroptosis and necroptosis) and inflammation. The results of Western blotting showed that the expression of apoptotic protein caspase-3 was significantly up-regulated, the expression of anti-apoptotic protein Bc-l 2 was significantly down-regulated, and the expression of caspase 9 was not significantly changed. Caspase-9, as the most important promoter and key protease of the apoptosis pathway, plays an important role in the endogenous apoptosis pathway. BCL2 family

of pro-apoptotic factors binds to apoptotic protease activator 1 (Apaf-1) to form apoptotic bodies that approach caspase 9 monomer, resulting in its dimerization and proteolytic signaling. In turn, caspase 3 and 7 are activated to induce apoptosis. In the extrinsic apoptotic pathway, extracellular death ligands (such as tumor necrosis factor-α or TNF-related apoptosis-inducing ligand) bind to various transmembrane death receptors to form DISC, which in turn activates caspase 3 and 7 to initiate apoptosis. These results further suggest that corticosterone can cause excessive activation of microglia, resulting in increased expression of inflammatory factors, leading to neuronal apoptosis, and promoting the occurrence and development of depression. Phyto-N can significantly improve HPA hyperactivity, reduce the level of corticosterone in the brain, reduce the excessive activation of microglia, and reduce the expression of inflammatory factors in the brain. It plays a role in protecting neurons.

[00287] Taken together, the data from the social interaction, social preference, and sucrose preference tests suggest that exposure to social defeat stress results in the expression of core and common endophenotypes of major depressive disorder, including avoidance and anhedonia. The results of histopathology showed that Phyto-N had obvious neuroprotective effect. Western blotting results showed that Phyto-N significantly inhibited the activation of microglia and decreased the expression of apoptotic proteins. These results indicate that a *Wikstroemia indica (Linn.) C. A. Mey* extract disclosed herein can significantly improve the depression-like behavior of CSDS model mice, which provides a theoretical basis for the clinical treatment of depression.

[00288] Example 5

[00289] A *Wikstroemia indica (Linn.) C. A. Mey* extract effectively treats Parkinson's disease

[00290] This example demonstrates that a *Wikstroemia indica (Linn.) C. A. Mey* extract (also referred to as Phyto-N) disclosed herein has neuroprotective effects in PD model mice treated with neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

[00291] Experimental Animals

[00292] Sixty male Kunming mice, 3 months old, SPF grade, weighing 25 ± 5 g, were purchased from Liaoning Changsheng Biotechnology Co., Ltd., Certificate No.: SCXK (Liao) 2023-0001. Feeding conditions: temperature is 25 ± 1°C, relative humidity is 60 ± 10%, day and night natural light, water, feed freedom, animal experiment process are in line with the experimental animal management and protection regulations.

[00293] Methods

[00294] Experimental grouping and administration

[00295] Fifty 3-month-old male Kunming mice were randomly divided into blank group (Control), model group (Model), low dose group (PhytoN-L), medium dose group (PhytoN-M) and high dose group (PhytoN-H). After one week of adaptability, the blank group and the model group were given distilled water by gavage on the 8th to 56th day. The low-dose group, the middle-dose group and the high-dose group were given low, medium and high PhytoN solution, respectively, on the 56th to 79th days, once every two days. On the 22nd day, the model was established. The blank group was subcutaneously injected with normal saline, and the other groups were continuously subcutaneously injected with MPTP. The model was completed on the 70th day.

[00296] MPTP solution:normal saline as solvent, MPTP was prepared into a concentration of $10mg \cdot mL^{-1}$. According to the method of human and animal body surface area, the dosage of MPTP (dosage = daily adult dosage × 70 kg × 0.0026 / mouse body weight g) was $100mg \cdot kg^{-1} \cdot d^{-1}$. The blank group was subcutaneously injected with 0.2 mL normal saline, and the other groups were subcutaneously injected with 0.2 mL MPTP solution.

[00297] PhytoN solution to 9, 18, or 36 g PhytoN were weighed and placed in a 50 mL volumetric flask, dissolved in 75% ethanol to a constant volume to the scale, and shaken well to yield concentrations of 0.52g/mL, 0.36g/mL, 0.18g/mL (high, medium and low, respectively) of test solution.

[00298] Behavioral experiments

[00299] Before modeling, after modeling and after treatment, the following behaviors of mice in each group were tested. Each mouse was trained 3 times a day for 3 consecutive days before the test.

[00300] Pole Test

[00301] A smooth stainless-steel rod with a diameter of 1 cm and a length of 0.6 m was made. A marking line was made in the center of the rod, and the rod was vertically fixed on a horizontal plane. The head of the mouse was placed on the top of the rod to make it climb down naturally along the rod. The sliding behavior of the mouse was scored. The score of the climbing rod experiment was 0 points, and the limbs were used to climb down from the rod smoothly at one time. 0.5 points, climbed down from the pole at one time, but the hind limbs appeared to slide; 1 point, pause several times after the pole and climb down; 1.5 points, sliding down after sliding,

with slight tremor; 2 points, tremors, limbs stiff, cannot grasp the pole, direct drop; 2.5 points, continuous tremor, limb paralysis, inability to move or die.

[00302] Morris water maze test

[00303] Morris water maze experiment includes positioning navigation experiment and space exploration experiment. Positioning navigation experiment. The circular platform is placed 2cm underwater in the fourth quadrant, and the position of the platform is kept unchanged. The mice were put into the water facing the wall of the pool at the water entry point specified in the 1, 2, and 3 quadrants. Within 90 seconds, the mice that found the target platform stayed on the platform for 30 seconds, and the mice that did not find the platform were artificially placed on the platform for 30 seconds to enhance memory. The time for mice to find the platform was the escape latency. The experimental period was four days, and the escape latency was recorded. Space exploration experiment: The platform was removed, and the mice were placed in the water maze at the water entry points in the 1st, 2nd, and 3rd quadrants, and their residence time in the target quadrant (fourth quadrant) was recorded within 90 seconds. The platform position and the number of times that the effective area (2 times the diameter of the platform area) passes through are recorded.

[00304] Open field test

[00305] The open field analysis box is a 100cm × 100cm × 40cm (length × width × height) wooden open field box. The bottom of the box is divided into 25 squares (20 cm × 20 cm) on average. The inner side wall is blackened. The grids along the side wall are called the peripheral area, and the rest are the central area. The camera was placed in the center above the open field box and connected to the computer. The mice were placed in the central compartment, and the activities of the mice within 5 minutes were observed. The residence time (latency), the number of uprights and the total distance of movement of the mice in the central compartment were recorded.

[00306] Gait Analysis Experiment

[00307] A simple gait analysis device was made according to the method reported in the literature. The front and rear claws of the mice were smeared with red and blue ink respectively, and then the mice were placed on one end of the runway (90 cm × 4.5 cm × 15 cm) covered with white paper to induce them to form a series of continuous footprints when climbing to the other end of the runway. Three clear and continuous footprints were selected to measure the following

parameters: the stride width of the front and rear claws (mm), the percentage of swing time of the front and rear claws (%), and the average footprint area of the front and rear claws (mm^2).

[00308] Animal sampling process and application

[00309] After the behavioral observation, the mice were anesthetized with 2% pentobarbital sodium at a dose of 1.5 mL / kg by intraperitoneal injection. After the peripheral blood was taken, the mice were sacrificed by cervical dislocation. The brain tissue containing the substantia nigra was taken and fixed in 4% paraformaldehyde. Part of the substantia nigra was stored at -80 °C.

[00310] Observation of organizational morphology

[00311] Observation of hippocampal tissue morphology using H&E staining light microscopy

[00312] The whole brain was fixed with 4% paraformaldehyde for 24 hours, embedded in paraffin, and sliced with a slicing knife. The slices were dewaxed with xylene, washed with 95% ethanol, 90% ethanol and 85% ethanol, and then placed in distilled water for dyeing. Hematoxylin staining was performed for 5 min, and the excess dye was washed with distilled water and differentiated in acidic alcohol for 5-10 seconds. Distilled water was used to wash the sections, and if the staining in the nucleus did not reach the standard, re-staining could be performed ; immerse in 1% eosin aqueous solution for 5 minutes and then wash off the excess eosin ; gradient alcohol dehydration, sealing. The pathological changes of hippocampus in mice were observed under light microscope.

[00313] Detection of apoptosis levels of DA neurons in the substantia nigra of the brain using Tunel staining

[00314] The fixed brain tissue was dehydrated with gradient ethanol, embedded in paraffin, sliced at 4 μm, dewaxed to water, added with 20 μg/mL of protease K, incubated at 37 °C for 20 minutes, washed with PBS, and blocked with 1% goat serum for 30 minutes. The TdT enzyme reaction solution was covered on the slide, incubated at 37 °C for 20 minutes, incubated with horseradish peroxidase (HRP), washed with PBS, stained with DAB for 1 minute, rinsed with running water, re-stained with hematoxylin, dehydrated and transparent with xylene, and sealed with central gum. The images were observed and collected under a 400-fold microscope. The nucleus was brown indicating positive cells. The average positive rate was calculated in 6 fields of view.

[00315] Immunohistochemical staining

[00316] Frozen sections were taken and washed with PBS three times, 5 minutes each time. Dropped with 0.3% hydrogen peroxide solution, incubated at room temperature for 10 min ; washed with PBS 3 times, 3 minutes each time; 5% BSA solution and incubated at 37 °C for 10 minutes. Add TH rabbit polyclonal antibody, 4 °C refrigerator overnight; hRP-labeled goat anti-rabbit IgG antibody was added and incubated at 37 °C for 1 hour. Washed with PBS 3 times, 5 minutes each time; dAB staining for 6 minutes, hematoxylin staining for 5 minutes, washing, fishing, dehydration, transparent, neutral resin sealing, and microscopic observation.

[00317] Data analysis

[00318] The data were analyzed by GraphPad Prism9.5.1 statistical software. The data were expressed as mean ± standard deviation ($\bar{x} \pm S$). One-way analysis of variance was used for comparison between multiple groups, and LSD test was used for comparison between two samples. $P < 0.05$ indicates statistical significance.

[00319] Results

[00320] The effect of PhytoN on the behavior of MPTP model mice

[00321] Pole Test

[00322] The results of climbing pole test showed that there was no significant difference in the climbing pole test scores of mice in each group before modeling ($P > 0.05$). After modeling, compared with the blank group, the climbing pole test scores of the model group, low-dose group, medium-dose group and high-dose group were significantly increased ($P < 0.01$). After treatment, compared with the model group, the climbing pole test scores of mice in the low-dose group, the middle-dose group and the high-dose group were significantly decreased ($P < 0.01$, $P < 0.05$). Compared with the low-dose group and the middle-dose group, the climbing pole test scores of mice in the high-dose group were significantly decreased ($P < 0.05$). The results are shown in FIG. 20.

[00323] Morris water maze test

[00324] Positioning navigation latency

[00325] The results of the water maze test showed that compared with the blank group, there was no significant change in the positioning latency of the model group on the first day, but the positioning latency was relatively long. The positioning latency increased significantly on the second day and the third day ($P < 0.05$), and the positioning latency increased significantly on the fourth day ($P < 0.01$). Compared with the model group, there was no significant change in the

positioning latency of the mice in the low-dose group on the first, second and third days, but the positioning latency was relatively short, and the positioning latency on the fourth day was significantly reduced ($P < 0.05$). There was no significant change in the positioning latency of the mice in the middle-dose group and the high-dose group on the first day, and the positioning latency on the third and fourth days was significantly reduced ($P < 0.05$), and the positioning latency on the fourth day of the high-dose group was significantly reduced ($P < 0.01$). The results are shown in FIG. 21.

[00326] Number of times crossing platforms

[00327] Compared with the blank group, the number of mice crossing the platform in the model group was significantly reduced ($P < 0.01$). Compared with the model group, the number of crossing the platform in the low-dose group and the middle-dose group increased significantly ($P < 0.05$), and the number of crossing the platform in the high-dose group increased significantly ($P < 0.01$). The results are shown in FIG. 22.

[00328] Open field experiment

[00329] The results of open field experiment showed that compared with the blank group, the total distance of horizontal movement and the total distance of central movement in the model group decreased significantly ($P < 0.01$), and the resting time increased significantly ($P < 0.01$). Compared with the model group, there was no significant difference in the total distance of horizontal movement, the total distance of central movement and the resting time in the low dose group. The total distance of horizontal movement in the middle dose group increased significantly ($P < 0.05$), and the resting time decreased significantly ($P < 0.05$). The total distance of horizontal movement in the high dose group increased significantly ($P < 0.01$), the resting time decreased significantly ($P < 0.01$), and the total distance of central movement increased significantly ($P < 0.05$). The results are shown in FIG. 23.

[00330] Gait Analysis Experiment

[00331] The results of gait analysis showed that compared with the blank group, the step width and the percentage of standing time in the model group were significantly increased ($P < 0.01$), while the step length, the percentage of swing time and the average print area were significantly decreased ($P < 0.01$). Compared with the model group, the step width and the percentage of standing time in the low-dose group were significantly increased ($P < 0.05$), while the step length, the percentage of swing time and the average footprint area of the hind paw were

significantly decreased ($P < 0.05$). The percentage of standing time in the middle dose group did not increase significantly, while other indicators were significantly different from those in the model group ($P < 0.01$). The high dose group was significantly different from the model group ($P < 0.01$). The results are shown in FIG. 24.

[00332] The effect of PhytoN on the morphology of whole brain tissue in MPTP model mice.

[00333] H&E staining light microscopy observation results

[00334] The results of H&E staining showed that compared with the blank group, a small amount of glial cell infiltration and aggregation could be observed in the substantia nigra region of the model group, and the aggregation of glial cells was more obvious. At the same time, neuronal swelling and karyopyknosis appeared in the substantia nigra region of the brain. Compared with the model group, the number of neurons in the low-dose group, middle-dose group and high-dose group was relatively large, the degree of neuronal damage was lighter, and there was no obvious karyopyknosis. The results are shown in FIG. 25.

[00335] Results of Tunel staining for detecting apoptosis levels of DA neurons in the substantia nigra of the brain

[00336] Tunel staining results showed that compared with the blank group, the apoptosis rate of dopaminergic neurons in the substantia nigra of the model group was increased, and the difference was statistically significant ($P < 0.01$). Compared with the model group, the apoptosis rate of dopaminergic neurons in the substantia nigra of the mice in the low-dose group decreased, and the difference was statistically significant ($P < 0.05$). The apoptosis rate of dopaminergic neurons in the substantia nigra of the mice in the middle-dose group and the high-dose group decreased significantly ($P < 0.01$). The results are shown in FIG. 26.

[00337] Immunohistochemical staining results

[00338] The results of immunohistochemistry showed that compared with the blank group, the number of neurons in the substantia nigra of the model group decreased, the positive expression of Tyrosine Hydroxylase (TH) decreased significantly, the number of neurons decreased significantly, and the cell body was small and irregular in shape. Compared with the model group, the number of neurons in the right substantia nigra of the low-dose group, the middle-dose group and the high-dose group was more, the nucleus was large and round, the cytoplasm was rich, and the morphology was complete. The results are shown in FIG. 27.

[00339] Conclusion

[00340] In summary, these experiments show that that *Wikstroemia indica (Linn.) C. A. Mey* extract exerts a neuroprotective mechanism on MPTP model mice by reducing the loss of DA neurons in the substantia nigra and striatum and improving the apoptosis of DA neurons, which provides experimental and theoretical basis for the clinical application of *Wikstroemia indica (Linn.) C. A. Mey* extract in the prevention and treatment of PD and the development of new anti-PD plant extract medicine.

[00341] Example 6

[00342] A *Wikstroemia indica (Linn.) C. A. Mey* extract effectively treats Alzheimer's disease

[00343] This example demonstrates the protective effects of a *Wikstroemia indica (Linn.) C. A. Mey* extract (also referred to as Phyto-N) disclosed herein against Alzheimer's disease. The general state of mice in each group was observed by macroscopic characterization and body weight. New Object Recognition (NOR) was used to detect the ability of mice in each group to explore new things, Y Maze (YM) was used to detect the working memory ability of mice in each group, and Morris Water Maze (MWM) was used to detect the ability of spatial exploration and memory in each group. The number of neurons in the hippocampus and the structural morphology of the hippocampal region of each group were observed by H&E staining. The number of Nitinol vesicles in the hippocampal region of each group was observed by Nitinol staining. The number of neurons and structural morphology in the hippocampus of each group was observed by H&E staining. The number of Nystrom's vesicles in the hippocampus of each group was observed by Nystrom's staining. And the neuroprotective effect of Phyto-N on the LPS model mice was investigated.

[00344] Experimental materials

[00345] Experimental animal

[00346] Sixty male C57BL/6 mice, 3 months old, SPF grade, weighing 18-22 g, were purchased from Spectrum (Beijing) Biotechnology Co., Ltd, Certificate of Conformity No.: SCXK (Beijing) 2019 - 0010. The rearing conditions were: temperature of 23 ± 3°C, relative humidity of 50 ± 10%, day and night natural illumination, and free access to drinking water and feed, and all the feeding and animal experimental processes were approved by the All the feeding and animal experiments were approved by the Animal Ethics Committee of Yunnan Branch, Institute of Medicinal Plants, Chinese Academy of Medical Sciences (2022006).

[00347] Experimental Instruments

[00348] Instruments used are shown in the following table.

[00349] Table 7

Instrument name	Manufacturer
New Object Recognition	Shifter Information Technology Co,Shanghai
Morris water maze	Shifter Information Technology Co,Shanghai
Y Maze	Shifter Information Technology Co,Shanghai
-80℃ Ultra-low temperature refrigerator	Thermo Fisher Company, USA
STP 120 Automatic Centrifugal Tissue Dehydrator	Myr & MICROM Company, Spain
HistoStar Tissue Embedder	Thermo Fisher Company, USA
HM355S Pathology Slicer	Thermo Fisher Company, USA
NX50 Frozen Slicer	Thermo Fisher Company, USA
GEMINI AS automatic dyeing machine	Fisher Scientific Company, USA
BX53 Microscope	OLYMPUS Company, Japan

[00350] Experimental materials

[00351] Materials used are shown in the following table.

[00352] Table 8

Instrument Name	Lot No.	Manufacturer
H&E Staining Kit	G1120	Beijing Soleberg Technology Co.
Nysted's stain	C0117	Shanghai Biyuntian Biotechnology Co.

[00353] Experimental Methods

[00354] Modelling, dosing and grouping of animals

[00355] SPF grade male C57BL/6 mice were randomly divided into Control, Model, Positive (piracetam) and Phyto-N groups. After one week of acclimatization feeding, pure water was given by gavage to Control and Model groups on days 1-14; piracetam (208 $mg \cdot kg^{-1} \cdot d^{-1}$) and Phyto-N (2.6 $g \cdot kg^{-1} \cdot d^{-1}$) were given once a day to Positive and Phyto-N groups, respectively (0.3g/ml aqueous suspension by oral gavage). Modelling was started on day 7 with subcutaneous injection of saline in the blank group and continuous intraperitoneal injection of LPS (250 $\mu g \cdot kg^{-1} \cdot 2d^{-1}$) in the other groups, modelling was done once in two days. Behavioral assessment was started on day 14.

[00356] General indicators

[00357] Macro-epidemiology

[00358] The general condition of the mice was monitored, such as mental status, coat colour and gloss, stress response and other systemic conditions.

[00359] Body mass

[00360] The body mass of rats in each group was weighed and recorded weekly after the start of the experiment to monitor the changes in body mass in each group.

[00361] Behavioral experiments

[00362] New Object Recognition (NOR)

[00363] After 3 minutes of acclimatization in the empty box environment, two identical objects (odor removed and fixed objects) were placed in the empty box, with the objects positioned approximately 10 cm from the two side walls of the device. Training phase: mice were placed in the device with their backs facing the objects at equal distances and allowed to explore freely for 3 minutes before being wiped with alcohol to remove the odor. Test phase: After an interval of 1 hour, one of the old objects was replaced by an unfamiliar object. Again, the mice were placed in the device with their backs facing the wall for 5 minutes of free exploration. The number of times the mice explored the old and new objects and the time spent exploring the objects were recorded. RI = time spent exploring the new object/ (time spent exploring the new object + time spent exploring the old object).

[00364] Y Maze (YM)

[00365] Mice were placed into the triangular starting area with their backs facing the wall of the device and allowed to explore freely for 8 minutes, and the trajectories of the mice were recorded. Between each trial, alcohol wipes were used to remove odor. The total number of arm entries and the total number of alternations were recorded for each mouse (3 consecutive entries of mice into 3 different arms of the Y-maze were recorded as 1 alternation). Autonomous alternation rate = total number of alternations/ (total number of arm entries - 2) × 100%.

[00366] Morris Water Maze (MWM)

[00367] The diameter of the maze equipment was 120 cm, the water tank was divided into four quadrants of the same size, the platform was reasonably placed in one quadrant, the water surface was higher than the platform by 1.5 cm, a milky solution of water and milk powder was added to the water tank to prevent the mice from seeing the platform, and the temperature of the

water was adjusted to 20-22°C. Positioning navigation measurement phase: A total of 4 days of training, every day the mice were placed into the water from the four quadrants with their heads facing the wall of the pool and were given 120 seconds to find the platform. After the 120 seconds were over, no matter whether the mice had found the hidden platform or not, they were required to take a rest on the platform for 15 seconds, and the mice that failed to find the platform were manually guided to the platform. The escape latency was recorded as the time the mice found the platform hidden under the water surface. Spatial exploration measurement phase: On day 5, when the hidden platform was removed, the mice were placed into the water from the quadrant opposite to the original platform and were free to explore for 120 seconds. The time spent in the target quadrant, the number of times the platform was crossed, and the swimming trajectory of the mice were recorded.

[00368] Animal sampling process and application

[00369] At the end of the behavioral observations, the mice were anaesthetized with 2% isoflurane by inhalation, and the eyeballs were removed for blood sampling for each group of mice separately. Among them, three mice from each group were perfused with 5 mL of 4% paraformaldehyde, and the whole brain was taken immediately after the end of perfusion for H&E and Nichols staining. The remaining mice in each group were quickly stripped of the hippocampus after blood sampling and stored in liquid nitrogen for subsequent experiments.

[00370] Histomorphological observations

[00371] H&E staining to observe hippocampal tissue morphology

[00372] Neurons in the hippocampal region of mice were observed by H&E staining. Fresh brain tissue was dissected and taken out, fixed at room temperature for 24 hours. Tissues were dehydrated and processed for transparency, i.e., 70%, 80%, 90% ethanol for 1 hour, 95% I, 95% II ethanol for 50 minutes, 100% ethanol for 40 minutes, 100% ethanol for 30 minutes; anhydrous ethanol: xylene = 1:1 for 30 min, xylene, xylene were transparent for 20 minutes each; and the wax cylinders I, wax cylinder II each immersed in wax for 1.5 hours, followed by tissue embedding. Tissue sections (4 μm in thickness) were made at 37°C overnight in an oven. Sections were dewaxed with xylene, washed with 100% ethanol, 95% ethanol, 85% ethanol, 70% ethanol, and subsequently placed in distilled water to be stained; hematoxylin stained for 10 minutes, the excess dye was washed away with distilled water, and it was put into acid alcohol for 30 s. Sections were washed in distilled water; immersed in eosin solution for 4 minutes, and

then washed to remove the excess eosin; gradient of 95% ethanol, 100% ethanol Alcohol dehydration, xylene transparency, and sealing the slices. Microscopic observation, image acquisition and analysis, the neuron morphology can be seen, the nucleus is blue, the cytoplasm is red.

[00373] Morphology of hippocampal tissue by Nissl staining of brain tissue.

[00374] Nissl staining was used to observe the morphology of Nissl vesicles in the hippocampus. Dewatering, embedding, sectioning, dewaxing and washing were as described in 2.4.1 H&E staining method; Nissl staining solution was immersed for 25 minutes, rinsed with running water for 1 minute, dehydrated, transparent and sealed. It was placed under the microscope for observation, and the images were captured and analyzed, and the Nysted vesicles were blue in color.

[00375] Data processing

[00376] Data results were analyzed and processed with SPSS 21.0 statistical software, data were expressed as mean ± standard deviation ($x \pm S$), comparisons between multiple groups were processed using one-way analysis of variance (ANOVA), and comparisons between two samples were made using the LSD test, with $P<0.05$ indicating statistical significance.

[00377] Results

[00378] General status evaluation

[00379] Macro-epidemiology

[00380] Before the start of modelling, the mice in each group were in good mental state, did not like to pile up, had black moist and glossy fur, active mobility, strong resistance to gavage, and the stools were in the shape of rice grains and moderately dry and sparse. One week after administration, mice in Control, Model, Positive and Phyto-N groups were in good mental state, with neat and glossy fur, active activity, strong resistance to gavage, and stools of normal shape and moderate texture. After one week of modelling, mice in the Model, Positive and Phyto-N groups were sometimes depressed, had messy but glossy fur, slightly weakened mobility, still resisted gavage violently, and had normal stools with a thinner texture. The stools were more normal in shape and thinner in texture, which were slightly different from those of Control group mice. Two weeks after modelling, mice in the Model group showed weakened mental resistance, unshaped stools and thinner texture. mice in the Positive and Phyto-N groups fared better than those in the Model group, but differed from mice in the Control group. Comparison of the

macroscopic characterization of the mice in each group at different time periods is shown in the following table.

[00381] Table 9

Groups		mental state	hair color	Activities	gavage resistance	stool
	0 week	Well	neatly	enlivened	strongly	moderate
Control	1 weeks	Well	neatly	enlivened	strongly	moderate
	2 weeks	Well	neatly	enlivened	strongly	moderate
	0 week	Well	neatly	enlivened	strongly	moderate
Model	1 weeks	dispirited	slight mess	sluggish	fall off	dilute
	2 weeks	dispirited	disarrayed	sluggish	fall off	dilute
	0 week	Well	neatly	enlivened	strongly	moderate
Positive	1 weeks	dispirited	slight mess	a little slow	strongly	moderate
	2 weeks	Well	slight mess	a little slow	strongly	moderate
	0 week	Well	neatly	enlivened	strongly	moderate
Phyto-N	1 weeks	dispirited	slight mess	a little slow	strongly	dilute
	2 weeks	Well	slight mess	a little slow	strongly	dilute

[00382] Weight of body

[00383] Weight of body

[00384] The results are shown in the following table and FIG. 28, randomly grouped, there was no statistically significant difference in the body mass of mice in each group ($P>0.05$). After 1 week of modelling, the body mass of mice in each group gradually increased, with no statistically significant difference ($P>0.05$). After 2 weeks of modelling, the body mass of mice in the Control group tended to be stable, and there was a decreasing trend in body mass of mice in the Model ($P<0.01$), Positive ($P<0.01$) and Phyto-N groups, but it was lower than that of the Model group but not significantly different from the Positive group ($P<0.01$), Positive group ($P<0.01$) and Phyto-N group showed a decreasing trend in body mass, but compared with the Model group, the Phyto-N group was lower than the Model group but there was no significant difference, which may be due to the diarrhea-causing effect of Phyto-N based on the results of macroscopic characterization.

[00385] Table 10: Changes in body mass of mice (grams) in each group (mean ± S, n=10)

Groups	Day 1	Day 7	Day 14
Control	21.64 ± 0.93	22.79 ± 0.78	22.81 ± 1.10
Model	21.58 ± 0.93	22.48 ± 1.18	20.74 ± 1.15##
Positive	21.68 ± 0.50	22.64 ± 0.84	21.81 ± 0.88*
Phyto-N	21.61 ± 0.83	21.85 ± 1.34	20.58 ± 0.98

Compared to the Control group # P < 0. 05,## P < 0. 01; compared with Model group,, ** P<0.01

[00386] Animal Behavoir

[00387] New Object Recognition (NOR)

[00388] The experimental results are shown in the following table and Fig. 29. Compared with the Control group, the new object recognition index of the mice in the Model group was significantly reduced (P<0.01). Compared with the Model group, the new object recognition index of the mice in the Positive group and the Phyto-N group was significantly increased (P<0.01) , and the ability of exploring the new things was significantly enhanced.

[00389] Table 11: New objects for each group of mice (mean ± S, n=10)

Groups	Recognition Index (RI)
Control	0.61 ± 0.02
Model	0.33 ± 0.02##
Positive	0.57 ± 0.03**
Phyto-N	0.48 ± 0.04**

Compared with Control group, ## P < 0. 01; compared with Model group, **P < 0. 01.

[00390] Y Maze (YM)

[00391] The experimental results are shown in the following table and FIG. 30. Compared with the Control group, the spontaneous alternation rate of the mice in the Model group was significantly reduced (P<0.01). Compared with the Model group, the spontaneous alternation rate

of the mice in the Positive group and Phyto-N group was significantly increased (P<0.01) , and the working memory capacity was significantly enhanced.

[00392] Table 12: (mean ± S, n=10)

Groups	Spontaneous alternation rate
Control	0.72 ± 0.05
Model	0.47 ± 0.03$^{\#\#}$
Positive	0.70 ± 0.10*
Phyto-N	0.74 ± 0.12**

Note: Compared with Control group, ## P<0. 01; compared with Model group, *P<0. 05, **P<0. 01

[00393] Morris Water Maze (MWM)

[00394] Positioning Navigational Latency

[00395] The experimental results are shown in the following table and FIG. 31. Compared with the Control group, the localization latency of mice in the Model group was significantly increased (P<0.05) , and compared with the Model group, the localization latency of mice in the Positive group and Phyto-N group was significantly reduced (P<0.05) , and the length of time of searching for a platform latency was gradually reduced in each group during the training process.

[00396] Table 13: Latency of localized navigation (seconds in each group of mice (mean ± S, n=10)

Groups	Day 1	Day 2	Day 3	Day 4
Control	60.50 ± 6.52	40.40 ± 10.18	29.90 ± 5.48	24.30 ± 1.47
Model	102.80 ± 6.82$^{\#\#}$	76.43 ± 10.72$^{\#}$	82.10 ± 7.98$^{\#\#}$	64.17 ± 10.05$^{\#\#}$
Positive	79.83 ± 8.99*	44.73 ± 8.10*	40.10 ± 6.14**	34.70 ± 6.04*
Phyto-N	80.10 ± 5.47*	42.23 ± 10.91*	42.43 ± 9.09**	45.80 ± 3.32*

Note: Compared with Control group, # P<0. 05, ## P<0. 01; compared with Model group, *P<0.05, **P<0. 01.

[00397] Number of space exploration traverses

[00398] The experimental results are shown in the following table and FIG. 32 and FIG. 33. Compared with the Control group, the number of times the mice in the Model group traversed the platform and the residence time in the target quadrant were significantly reduced (P<0.05) ;

compared with the Model group, the number of times the mice in the Positive group and Phyto-N group traversed the platform and the residence time in the target quadrant were significantly increased (P<0.05) , and the swimming trajectories appeared in the target quadrant probability was significantly higher, concentrated in the second quadrant where the platform was located.

[00399] Table 14: Space exploration ability of each group of mice (mean ± S，n=10)

Groups	Number of platform crossings	Target image dwell time (s)
Control	3.40 ± 0.58	40.68 ± 3.28
Model	1.20 ± 0.33##	17.64 ± 1.96##
Positive	2.80 ± 0.47*	31.44 ± 2.09**
Phyto-N	3.00 ± 0.33**	38.39 ± 1.94**

Note: Compared with Control group, ##P<0.01; compared with Model group, *P<0.05, **P<0. 01.

[00400] Histopathology

[00401] H&E staining observations

[00402] \As shown in Figure 34, the Control group mice had a regular arrangement of hippocampal cells, a large number of neurons, homogeneous staining, clear nuclei and no nuclear condensation; the Model group mice had a disorganized arrangement of hippocampal cells, some neurons were lost, cells were shrunken, the nuclei were not obvious and there was obvious nuclear condensation; the Positive group was similar to the Phyto-N group; the arrangement of the neurons was more regular, the number of neurons was large, the morphology was normal, the nuclei were clearer and there was no obvious nuclear condensation. The Positive group was similar to the Phyto-N group; the neurons in the hippocampus were arranged in a more regular manner, the number of neurons was larger, the morphology was normal, the nuclei were clearer, and there was no obvious nuclear condensation.

[00403] Nichols staining observations

[00404] The results are shown in FIG. 35 and FIG. 36 and the following table. In Control group, the hippocampal neurons were closely arranged with intact morphology, and the nuclei and nidus were clear and obvious; compared with Control group, in Model group, except for the dentate gyrus area of the hippocampus where there was no obvious damage to the neurons, the neurons in the other areas of the hippocampus were loosely arranged, with an increase in the necrotic neurons, and a significant decrease in the number of positive cells (P<0.01) and a significant

decrease in the number of nuclei and nidus. Compared with the Model group, the cell morphology of hippocampal regions in the Positive and Phyto-N groups was significantly improved, with neurons arranged in a regular manner, with clear and complete boundaries, and the number of positive cells was significantly increased (P<0.01), as well as the number of nidus bodies.

[00405] Table 15: The number of positive cells in each group was determined by Nissl staining (mean ± S, n=3)

groups	CA1	CA2	CA3	DG
Control	87.33 ± 4.16	101.00 ± 18.00	77.33 ± 9.87	239.00 ± 17.35
Model	56.33 ± 9.61$^{\#\#}$	69.67 ± 4.93$^{\#}$	44.33 ± 15.50$^{\#}$	238.33 ± 14.22
Positive	92.00 ± 16.00**	126.00 ± 25.06**	82.33 ± 12.42**	204.33 ± 67.10
Phyto-N	77.67 ± 7.37*	115.33 ± 8.51**	75.33 ± 14.84*	265.33 ± 34.48

Note: Compared with Control group, #P<0.05, ## P<0. 01; compared with Model group, *P<0.05, **P<0. 01

[00406] Conclusion

[00407] Alzheimer's disease is an age-related neurodegenerative disease.AD has a slow or insidious onset, slow disease progression, and complex pathogenesis, and patients will exhibit symptoms such as progressive memory impairment and cognitive dysfunction, which have a huge impact on social life, seriously affecting the health and quality of life of the elderly, and it is a major disease and a social problem threatening the health of the population of our country in today's society. Our results indicate that *Wikstroemia indica (Linn.) C. A. Mey* extract can significantly enhance the ability to explore new things, working memory and spatial exploration, improve the structural morphology of neurons in the hippocampus, increase the number of neurons in the hippocampus and the number of Nystagmus vesicles in the LPS model mice, and thus exert a neuroprotective effect on the LPS model mice.

[00408] Example 7

[00409] Use of Phyto-N to improve memory and depressive-like behaviors in mouse model of AD..

[00410] In this study, we tested the effects of Phyto-N on cognitive function and anxiety-like behaviors in a commonly used mutant AD mouse model, the 5XFAD mouse. We found that Phyto-N improved the performance of 5XFAD in the novel object recognition test (NOR) and

the open field test (OFT). In addition, to directly assess the effect of Phyto-N on anxiety-like and depressive-like behaviors, we also performed behavioral tests in a chronic corticosterone (CORT) administration mouse model[12]. CORT treatment or Phyto-N treatment did not have any effects on anxiety-like behaviors measured in OFT. Phyto-N did not increase grooming in sucrose splash test (SS) and but increased time to immobility in tail suspension test (TST). In summary, our results show that Phyto-N may have positive effects on cognitive function, as well as improving aspects of anxiety-like and depressive-like behaviors in mice.

[00411] To test the memory-enhancing effects of Phyto-N, we used 7- and 11-month-old male 5XFAD transgenic mice (Jackson Laboratories catalog #JAX034840). Age-matched C57BL6/J wild-type (WT) mice were used as controls. 8-week-old C57BL6/J wild-type (WT) mice were used in chronic CORT experiments. All mice were provided with food and water *ad libitum* and group-housed in a room maintained on a 12-hour light/dark cycle (7 am to 7 pm) at 72 °F with 45% humidity.

[00412] Phyto-N administration: The 5XFAD mice were randomly assigned to two groups: 5XFAD and 5XFAD+ Phyto-N. Similarly, the chronic CORT mice were randomly divided into two groups: CORT and CORT+ Phyto-N. Mice in the 5XFAD+ Phyto-N and CORT+ Phyto-N groups were administered Phyto-N (3.3 g/kg body weight/day) through voluntary oral consumption of a nutritionally complete diet gel (Nutra-Gel, Bio-Serv, Frenchtown, NJ). The dosage was calculated based on the average body weight of all 5XFAD or CORT mice at the start of the experiment. While it is typical for mice to consume approximately 10-12 grams of Nutra-Gel per day, all animals were also provided standard food and water *ad libitum* in addition to Nutra-Gel. Consequently, a slightly reduced amount of 8 grams of Nutra-Gel per animal per day was provided. The calculated amount of Phyto-N was thoroughly mixed into the appropriate amount of Nutra-Gel (8 grams per animal × number of animals in the cage) using a food processor. This mixture was then placed in a weighing boat on the bedding for the mice to consume. Mice in the 5XFAD and CORT groups received only 8 grams per animal of vehicle (Nutra-Gel). The 7-month-old and 11-month-old mice were included in NOR experiments, and only 11-month-old mice were used for OFT. Phyto-N diet was provided for 6 months until behavior testing. The CORT mice were administered Phyto-N for 14 days following chronic corticosterone exposure. Open-field test, Sucrose splash test, and tail suspension test were performed in the same mice on consecutive days following Phyto-N treatment.

[00413] Behavioral assessment: Open-field test (OFT): OFT measures general locomotor activity, anxiety-like, and exploratory behavior. Individual mice were placed in the center of an enclosed platform (18 × 18 × 14 inches) made of white acrylic and recorded for 10 min in experiments with 5XFAD and corresponding control mice, or 5 minutes in experiments with chronic CORT and corresponding control mice. The time in the center zone (9 $inches^2$), the number of entries to the center zone, and the total distance traveled were automatically scored with ANY-maze (ANY-maze, USA) software.

[00414] Novel object recognition (NOR) test: The NOR test consisted of three sessions: habituation, familiarization, and testing. The open field was cleaned with 70% (vol/vol) ethanol between each session. On Day 1, mice were individually habituated to the open field for 5 minutes. After a brief cleaning and setup period (~2 minutes), the familiarization session began, during which two identical objects were placed 1 inch away from the walls on opposite sides of the arena. Mice were placed in the center of the arena, facing a wall without an object in front of it, and allowed to explore for 5 minutes. Time spent investigating both objects was recorded using ANY-maze. Exploratory activity was defined as an object-oriented gaze while actively sniffing and/or pawing within 15 mm of the object. The orientation angle was set to 60° on ANY-maze. Due to reduced locomotor activity observed in the 5XFAD groups (Fig. 37), the training trial for the 5XFAD and 5XFAD+ Phyto-N groups was extended to 10 minutes to ensure that the majority of the animals in each group had adequate exploration time of at least 20 seconds for both objects. The testing session was conducted 24 hours after familiarization (Day 2), in which one of the familiar objects was replaced with a novel object of similar size. The placement of the novel object was counterbalanced across subjects. Mice were reintroduced to the arena, and the time spent investigating the familiar (F) and novel (N) objects was recorded. Mice that failed to interact with either objects for more than 10 seconds were excluded from analysis. The discrimination ratio (DR) was calculated for mice that had interacted with both objects for more than 10 seconds in both familiarization and testing sessions, by subtracting the time spent investigating the familiar object ($T_{familiar}$) from the time spent investigating the novel object (T_{novel}) and dividing by the total time spent investigating; $DR = (T_{novel} + T_{familiar}) / T_{total}$. DR for familiarization session was calculated as $DR = (T_{left} + T_{right}) / T_{total}$.

[00415] Chronic corticosterone (CORT) administration: Animals were self-administered corticosterone (Chronic CORT (25 μg/ml; 4-pregnen-11β 21-DIOL-3 20-DIONE 21-

hemisuccinate; Steraloids Inc., Newport, RI) orally in drinking water for 20 days. Following CORT treatment, vehicle or Phyto-N was administered orally for 14 days during the washout period.

[00416] Sucrose splash test (SS): Mice were sprayed with a 10% sucrose solution onto their dorsal coat, which induced grooming due to its high viscosity. Each animal was individually placed in a clean cage without bedding to prevent the bedding from adhering to the animal's coat. Grooming behavior was video-recorded for 5 minutes and scored by an observer blinded to the treatment groups.

[00417] Tail suspension test (TST): Each mouse was individually suspended by its tail from a hook using a 17 cm-long piece of adhesive tape, with 2 cm being used to attach the tape to the tail. The test was conducted in a small, three-sided chamber to prevent visual and social interactions between animals. The duration of immobility was recorded over a 6-minutes test period by an observer blinded to the treatment groups.

[00418] Statistical analyses: Statistical analyses were performed and graphs were generated using Graphpad Prism 10 software. For most of the behavioral tasks, one-way ANOVA was conducted to determine significant differences between treatment groups. To compare object exploration time of left/right or familiar/novel objects between treatment groups in NOR, two-way ANOVA was used. All ANOVA were followed by Bonferroni's *post-hoc* tests. The difference between discrimination ratios in familiarization vs. test was analyzed using two-tail paired t-test for each group. Sample size, statistical tests, p-values and significance for each experiment are indicated in the legends. Significance was determined at 95% confidence level ($p < 0.05$). All statistical tests were two-sided. Data were presented as mean ± SEM.

[00419] Results: NOR is among the most commonly used behavioral assays to assess learning and memory in mice, utilizing their innate preference for novelty. A mouse remembering a familiar object will spend more time exploring a novel object[15]. On day 1, interaction time with two identical objects was measured during the familiarization session. Control, 5XFAD and 5XFAD+ Phyto-N spent similar time exploring left versus right object (Fig. 37a-d). On day 2 during the testing session, one object was replaced with a novel object, and time spent investigating familiar (F) versus novel (N) object was measured. Control mice spent significantly more time exploring the novel object. 5XFAD spent less time exploring the novel object, and the time they spent exploring the two objects was not significantly different, indicating deficits in

learning and memory. 5XFAD+ Phyto-N mice preferred exploring the novel object similar to control (Fig. 37e). When DR was calculated, DR during the testing session was significantly higher than during familiarization session in control and 5XFAD+ Phyto-N groups, but not in 5XFAD group (Fig. 37f). These results indicate that Phyto-N improved the performance of 5XFAD mice in NOR test, indicating that it improves learning and memory in this AD model.

[00420] To assess anxiety-like behaviors and locomotor activity level, we performed OFT in 11-month-old mice (Fig. 38). While time spent in center was not significantly different between control, 5XFAD, and 5XFAD+ Phyto-N groups, entries into center zone was significantly reduced in 5XFAD (Fig. 38a-e). Number of entries in 5XFAD+ Phyto-N was no longer significantly different from control levels but also not significantly higher than 5XFAD, suggesting it may result in partial improvement in exploratory behaviors (Fig. 38e). 5XFAD mice had significant less distance traveled, indicating impaired locomotor activity at this age, consistent with previous reports[9–11] (Fig. 38f). 5XFAD+ Phyto-N showed slightly higher average distance traveled compared to 5XFAD but remained significantly lower than control (Fig. 38f). These results indicate that 5XFAD mice show decreased locomotor activity, and Phyto-N moderately improves locomotion.

[00421] We used a chronic CORT administration mouse model to assess the effect of Phyto-N on anxiety-like and depressive-like behaviors[12] . CORT (25 μg/ml) was added to drinking water for 20 days, followed by 14 days of washout period with no CORT, during which time Phyto-N was added. Following the washout period, we performed OFT, SS and TST on consecutive days (Fig. 39a). Chronic CORT or Phyto-N treatment did not have any effect on time in center, center entries or distance traveled measured in OFT (Fig. 39b-d). Grooming behavior serves as an index of self-care and motivational behavior in rodents. In SS, longer and more frequent grooming episodes, along with a shorter latency to groom, are typically associated with reduced depressive-like, apathy-like behavior[18]. Chronic CORT treatment led to lower grooming time and significantly lower number of grooming episodes, and Phyto-N did not improve grooming in CORT-treated mice (Fig. 39e-g). In TST, a behavioral paradigm that measures despair-like depression-like behavior and learned helplessness, chronic CORT treatment did not result in increased immobility time, number of immobility bouts, or latency to immobility (Fig. 39h-j). Interestingly, CORT+ Phyto-N showed significantly increased latency to immobility time compared to CORT and control, suggesting that Phyto-N may specifically alleviate despair-like

behaviors (Fig. 39j). Together, these results suggest that chronic CORT did not induce anxiety-like or despair-like behaviors, but resulted in significantly higher apathy-like behaviors. Phyto-N reduced despair-like behavior in mice measured by latency to immobility in TST.

[00422] In this study, we tested the efficacy of Phyto-N in the 5XFAD AD mouse model and in the chronic CORT mouse model in improving memory, alleviating anxiety-like and depressive-like behaviors. Phyto-N improved the performance of 5XFAD mice in NOR, and showed moderate enhancement in locomotor activity in OFT. These results support that Phyto-N is a new promising therapeutic for alleviating or delaying cognitive decline and motor deficits in AD.

[00423] We used a chronic CORT mouse model to induce anxiety-like and depressive-like behaviors. Phyto-N for 14 days following chronic CORT significantly increased the time to immobility in TST, indicating that it improves select aspects of depressive-like behaviors.

CLAIMS

What is claimed is:

1. A pharmaceutical composition consisting of an extract of the plant Wikstroemia indica (Linn.) C. A. Mey.

2. The pharmaceutical composition according to claim 1, further comprising at least one excipient.

3. A method of treating a central nervous system disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 1 to said subject.

4. The method of claim 3 wherein the central nervous system disease is selected from the group consisting of wherein the CNS disease is selected from the group consisting of Parkinson's disease, parkinsonism, Alzheimer's disease, dementia, vascular dementia, depression, major depressive disorder, perimenopausal depression, stress, and anxiety.

5. A method of treating a central nervous system disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica (Linn.) C. A. Mey wherein said extract is produced by a method comprising the steps of: contacting extracting a water suspension of dried Wikstroemia indica (Linn.) C. A. Mey with a solvent selected from the group consisting of water, methanol, ethanol, dichloromethane, petroleum ether, acetone, ethyl acetate, n-butanol, n-hexane, methanol, petroleum ether, and ethyl acetate, or a mixture of up to three of said solvents, then removing said solvent and adding at least one pharmaceutically acceptable excipient.

6. The method of claim 5 wherein said solvent is heated while it is in contact with the dried Wikstroemia indica (Linn.) C. A. Mey.

7. The method of claim 5 wherein said solvent is heated with a microwave while it is in contact with the dried Wikstroemia indica (Linn.) C. A. Mey.

8. A method of treating a central nervous system disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica (Linn.) C. A. Mey wherein said extract is produced by a method comprising the steps of: contacting dried *Wikstroemia indica (Linn.) C. A. Mey* in a water suspension with an enzyme selected from the group consisting of cellulase, pectinase, galacturonase, and papain amylase, hemicellulase, and ligninase, then filtering said aqueous suspension, removing said water and adding at least one pharmaceutically acceptable excipient.

9. A method of treating a central nervous system disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica (Linn.) C. A. Mey wherein said extract is produced by a method comprising the steps of: contacting dried Wikstroemia indica (Linn.) C. A. Mey with an absorbent resin selected from the groups consisting of AB-8, D101, D201, DA201, D4006, HPD-BJQH, HPD-100, HPD950, DM301, and DM130 and then eluting the absorbent resin with a solvent selected from the group consisting of methanol, ethanol, acetone, aqueous methanol, aqueous ethanol, aqueous acetone, and a mixture of up to three of said solvents, then removing said solvent and adding at least one pharmaceutically acceptable excipient.

10. A method of treating a central nervous system disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica (Linn.) C. A. Mey wherein said extract is produced by a method comprising the steps of: contacting dried Wikstroemia indica (Linn.) C. A. Mey with a chromatography column, then eluting said column with a solvent, then removing said solvent, and adding at least one pharmaceutically acceptable excipient.

11. The method of claim 9 wherein the chromatography column is a polyamide adsorption column chromatography and the solvent is selected from the group consisting of aqueous methanol and aqueous acetone.

12. The method of claim 9 wherein the chromatography column is a normal phase chromatography column and the solvent is selected from the group consisting of benzene, dichloromethane, acetone, trichloromethane, methanol, ethyl acetate, water, and a mixture of up to three of said solvents.

13. The method of claim 9 wherein the chromatography column is a reverse-phase chromatography column, and the solvent is selected from the group consisting of aqueous methanol and aqueous acetonitrile.