PLANT EXTRACT COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. US 63/566,747 filed March 18th, 2024, U.S. Provisional Application No. US 63/566,749 filed March 18th, 2024, and U.S. Provisional Application No. US 63/631,786 filed April 9th, 2024, which are herein incorporated by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (6701-0010WO01.xml; Size: 10 kb; and Date of Creation: March 13, 2025) is herein incorporated by reference in its entirety.

FIELD of the Invention

[0003] The present invention relates to *Wikstroemia indica* (Linn.) C. A. Mey extracts, compounds thereof, compositions containing the foregoing, and uses thereof for treating autoimmune diseases, preventing or treating viral infections, and metabolic diseases.

BACKGROUND OF THE INVENTION

[0004] *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 indica* (Linn.) C. A. Mey, 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.

[0005] 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

[0006] 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.

[0007] Provided herein is a method of treating an autoimmune disease or condition in a subject in need thereof, which may comprise administering to the subject a composition, which may be a pharmaceutical composition. Further provided herein are use of the composition in the manufacture of a medicament for treating the autoimmune disease or condition, and the composition for treating the autoimmune disease or condition. The autoimmune disease or disorder may be ulcerative colitis, Crohn's disease, inflammatory bowel disease (IBD), atopic dermatitis, eczema, psoriasis, or rheumatoid arthritis. The psoriasis may be chronic plaque psoriasis. The subject with ulcerative colitis, Crohn's disease, IBD, or psoriasis, which may be chronic plaque psoriasis, may be 18 years of age or younger.

[0008] Also provided herein is a method of treating a metabolic disease or condition in a subject in need thereof, which may comprise administering to the subject a composition, which may be a pharmaceutical composition. Further provided herein are use of the composition in the manufacture of a medicament for treating the metabolic disease or condition, and the composition for treating the metabolic disease or condition. The metabolic disease or condition may comprise gout or one or more complications or conditions thereof, which may comprise one or more of inflammation, redness, swelling, pain, and excess uric acid; hyperuricemia; acute or chronic gouty arthritis; gouty nephritis; diabetes or a complication or condition thereof; fatty liver disease; non-alcoholic fatty liver; obesity or a related condition; hyperlipidemia; hypertriglyceridemia; hypercholesterolemia; dyslipidemia; or a lipid metabolism disorder or condition. The diabetes or complication or condition thereof may comprise type 2 diabetes; insulin resistance; diabetic nephropathy; diabetic peripheral neuropathy; diabetic retinopathy; cataracts; diabetic foot or hand; diabetic neuropathy; diabetic hypertension; diabetic vascular occlusion; diabetic blood vessel lesions; diabetic numbness of hands or feet; diabetic Alzheimer's disease; non-healing oral ulcers; or skin ulcers. Treating the metabolic disease may comprise one or more of lowering total cholesterol, lowering low density lipoprotein cholesterol, and lowering triglycerides.

[0009] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, which may comprise administering to the subject a composition, which may be a pharmaceutical composition. Further provided herein are use of the composition in the manufacture of a medicament for treating or preventing the viral infection, and the composition for treating or preventing the viral infection. The viral infection may be severe-acute-respiratory-

syndrome-related coronavirus-2 (SARS-CoV-2). The subject may be suffering from coronavirus disease (COVID-19) or the flu, or one or more symptoms thereof. In one example, the method, medicament, or composition is for treating the viral infection.

[0010] Also provided herein is a method of treating acne in a subject in need thereof, which may comprise administering to the subject a composition, which may be a pharmaceutical composition. Further provided herein are use of the composition in the manufacture of a medicament for treating acne, and the composition for treating acne.

[0011] The composition may comprise a plant extract. The composition may comprise Daphnoretin (I). The composition may comprise Daphnogitin (II). The 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.

[0012] 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.

[0013] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0014] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica* and an excipient.

[0015] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0016] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*. and an excipient.

[0017] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0018] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica* and an excipient.

[0019] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0020] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*. and an excipient.

[0021] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0022] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica* and an excipient.

[0023] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*.

[0024] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of a plant extract obtained from *Wikstroemia indica*. and an excipient.

[0025] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0026] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of Daphnoretin (I) and an excipient.

[0027] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0028] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of Daphnoretin (I). and an excipient.

[0029] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0030] The present invention also relates to a method for treating an autoimmune disease said method comprising administering to a subject an effective amount of Daphnogitin (II) and an excipient.

[0031] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0032] The present invention also relates to a method for treating or preventing disease or conditions associated with an autoimmune disease said method comprising administering to a subject an effective amount of Daphnogitin (II). and an excipient.

[0033] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0034] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of Daphnoretin (I) and an excipient.

[0035] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0036] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of Daphnoretin (I). and an excipient.

[0037] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0038] The present invention also relates to a method for treating a viral infection said method comprising administering to a subject an effective amount of Daphnogitin (II) and an excipient.

[0039] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0040] The present invention also relates to a method for treating or preventing disease or conditions associated with a viral infection said method comprising administering to a subject an effective amount of Daphnogitin (II). and an excipient.

[0041] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0042] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of Daphnoretin (I) and an excipient.

[0043] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of Daphnoretin (I).

[0044] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of Daphnoretin (I). and an excipient.

[0045] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0046] The present invention also relates to a method for treating a metabolic disease said method comprising administering to a subject an effective amount of Daphnogitin (II) and an excipient.

[0047] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of Daphnogitin (II).

[0048] The present invention also relates to a method for treating or preventing disease or conditions associated with a metabolic disease said method comprising administering to a subject an effective amount of Daphnogitin (II). and an excipient

[0049] The present invention further relates to a process for preparing a plant extract obtained from *Wikstroemia indica*.

[0050] The composition may be suitable for oral administration, intravenous administration, topical administration, or injection. The amount of the plant extract or the one or more compounds of the plant extract administered to the subject, in the 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 composition may be administered orally.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIG. 1 shows the effects of a *Wikstromia indica* extract (referred to as Phyto-N) on dextran sulfate sodium (DSS)-induced colonic length in mice. A: Control; B: DSS; C: Positive control; D: Phyto-N. Compared to Control group, ** $P<0.01$; compared to DSS group, ##$P<0.01$.

[0052] FIG. 2 shows gross observations of colon morphology in each group of mice.

[0053] FIG. 3 shows a line graph of body weight changes in mice.

[0054] FIG. 4 shows a line graph of disease activity index (DAI) scores in mice.

[0055] FIG. 5 shows observations of H&E stained sections of mouse colon tissue (200x).

[0056] FIG. 6 shows immunohistochemical findings of mouse colon tissue (400x).

[0057] FIG. 7A-D show statistics of positive area of IL-6 (FIG. 7A), TNF-α (FIG. 7B), IL-1β (FIG. 7C) and IL-18 (FIG. 7D) in colonic tissues of mice.

[0058] FIG. 8 shows mouse skin condition in the blank group, model group, positive drug group (Y), and Phyto-N (N) group.

[0059] FIG. 9A-E show changes in the scores of erythema (FIG. 9A), infiltration (FIG. 9B), scales (FIG. 9C), and edema (FIG. 9D), as well as total scores (FIG. 9E) in the blank group, model group, positive drug group, and Phyto-N group (mean ± S, n = 10).

[0060] FIG. 10 shows mouse back skin tissue H&E staining for the blank group, model group, positive drug group (Y), and Phyto-N group (N) (200x).

[0061] FIG. 11 shows mouse epidermal thickness for the Blank group (KB), model group (MX), positive drug group (Y), and Phyto-N group (GY) (x ± S, n = 10). Compared with the blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0062] FIG. 12 shows expression of TNF-α in skin tissue of mice in blank group (KB), model group (MX), positive drug group (Y) and Phyto-N group (GY) (x±S, n = 10). Compared with blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0063] FIG. 13 shows skin condition of mice in the blank group (control), model group (model), Phyto-N group (Y) and positive drug group (N).

[0064] FIG. 14A-D show changes in the total score of skin erythema (FIG. 14A), infiltration (FIG. 14B), and skin lesions (FIG. 14C), as well as total score (FIG. 14D) in the blank group (KB), model group (MX), Phyto-N (Y) group and positive drug group (N) (mean ± S, n = 10).

[0065] FIG. 15 shows H&E staining (100x, 200x) of back skin tissue of mice in the blank group, model group, Phyto-N group (Y) and positive drug group (N).

[0066] FIG. 16 shows epidermal thickness of mice in the blank group (KB), model group (MX), Phyto-N group (Y) and positive drug group (GY) (mean±S, n=10). Compared with the blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0067] FIG. 17 shows back skin tissue toluidine blue staining of mice for the blank group, model group, Phyto-N group (Y), and positive drug group (N) (200x).

[0068] FIG. 18 shows expression of TNF-α in skin tissue of mice in blank group (KB), model group (MX) Phyto-N (Y) and positive drug group (N) (mean ± S, n = 10). Compared with blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0069] FIG. 19 shows back skin of BALB/c mice in blank group, model group (MX) and Phyto-N group (GY) on the day of sampling.

[0070] FIG. 20A-D show changes in the total scores of skin erythema (FIG. 20A), infiltration (FIG. 20B), scales (FIG. 20C) and skin lesion (FIG. 20D) in BALB/c mice in the model group and the administration group (n = 10).

[0071] FIG. 21 shows spleen index of mice in blank group (KB), model group (MX) and Phyto-N group (N) (mean ± S, n = 10). Compared with blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0072] FIG. 22 shows H&E staining (100x, 200x) of back skin tissue of mice in blank group, model group and Phyto-N group (N).

[0073] FIG. 23 shows epidermal thickness of mice in the blank group, model group and Phyto-N group (N) (mean ± S, n = 10). Compared with blank group, *** was $P < 0.05$ and **** was $P < 0.01$. Compared with the model group, ### was $P < 0.05$ and #### was $P < 0.01$.

[0074] FIG. 24A-C show swelling of ankle joints in rats (FIG. 24A), body weight of rats (FIG. 24B), and arthritis rating scale of rats (FIG. 24C)(mean ± X, n=7).

[0075] FIG. 25A-B show rat ankle foot thickness (FIG. 25A) and rat ankle foot volume (FIG. 25B) (mean ± S, n=7).

[0076] FIG. 26 shows pathological staining of rat ankle joints (histopathological morphology, 100x).

[0077] FIG. 27A-B show levels of TNF-α (FIG. 27A) and IL-1β (FIG. 27B) inflammatory factors in synovial tissues of rats in each group (Note: Compared with blank group,**$P< 0.01$; compared with model group, ##$P< 0.01$) (mean ± S, n=3).

[0078]

[0079] FIG. 28shows body weight of rats in each group. Compared with the control group, ** is $P < 0.01$; compared with the model group, # is $P < 0.05$.

[0080] FIG. 29 shows fasting blood glucose concentration of rats in each group. Compared with the control group, ** is $P<0.01$; compared with the model group, ## is $P< 0.01$; compared with the metformin group, △△ is $P < 0.01$.

[0081] FIG. 30 shows HbA1c concentration in rats in each group. Compared with the control group, * is $P< 0.05$; compared with the model group, # is $P< 0.05$.

[0082] FIG. 31 shows insulin concentrations of rats in each group. Compared with the control group, **is $P < 0.01$.

[0083] FIG. 32A-B show an oral glucose tolerance test (OGTT) curve (FIG. 32A) and its area under the curve (AUC) (FIG. 32B) for rats in each group. Compared with the control group, **is $P < 0.01$, compared with the model group, # is $P < 0.05$, ## is $P < 0.01$; compared with the metformin group, △ is $P < 0.05$.

[0084] FIG. 33A-B show an insulin tolerance test curve (FIG. 33A) and its AUC (FIG. 33B) for rats in each group. Compared with the control group, **is $P < 0.01$; compared with the model group, # is $P < 0.05$; compared with the metformin group, △ is $P < 0.05$, △△ is $P < 0.05$.

[0085] FIG. 34A-B show the HOMA-IS (FIG. 34A) and HOMA-IR (FIG. 34B) for rats in each group. Compared with the control group, **is $P < 0.01$; compared with the model group, # is $P < 0.05$, ## is $P < 0.01$.

[0086] FIG. 35A-D show triglycerides (TG) (FIG. 35A), total cholesterol (CHOL) (FIG. 35B), high density lipoprotein cholesterol (HDLC) (FIG. 35C) and low density lipoprotein cholesterol (LDLC) (FIG. 8D) concentration of rats in each group. Compared with the control group, * is $P < 0.05$, ** is $P < 0.01$; compared with the model group, # is $P < 0.05$, ## is $P < 0.01$; compared with the metformin group, △ is $P < 0.05$, △△ is $P < 0.01$.

[0087] FIG. 36 shows photomicrographs of the histology of pancreatic tissue (H&E, 400×). The arrows indicate pancreatic islets.

[0088] FIG. 37A-B show serum alanine transaminase (ALT) (FIG. 37A) and aspartate transaminase (AST) (FIG. 37B) concentration of rats in each group. Compared with the control group, ** is $P < 0.01$; compared with the model group, # is $P < 0.05$; compared with the metformin group, $\triangle P < 0.05$.

[0089] FIG. 38A-B show photomicrographs of the histology of liver tissue (H&E, 400×). FIG. 38A shows the non-portal vein area. FIG. 38B shows the portal vein area. Black arrows indicate fat droplets and grey arrows indicate inflammatory cell infiltration.

[0090] FIG. 39A-B show urea (FIG. 39A) and creatinine (FIG. 39B) concentration in rats in each group. Compared with the control group, ** is $P < 0.01$; compared with the model group, # is $P < 0.05$, compared with the metformin group, $\triangle P < 0.05$.

[0091] FIG. 40A-B show photomicrographs of the histology of kidney tissue (H&E, 400×). FIG. 40A shows the cortical area and FIG. 40B shows the medulla area. Black arrows indicate adipose

deposit, dark grey arrows indicate renal tubule basal membrane thickening, and lighter grey arrows indicate renal tubule atrophy.

[0092] FIG. 41 shows the results of the analysis of the number of twisting times in rats (n=8). Note: Compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; Compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[0093] FIG. 42A-B show the effects of Phyto-N on food intake (FIG. 42A) and body weight (FIG. 42B) in rats with gouty arthritis (n=8).

[0094] FIG. 43 shows the activity time of rats in each group (mean±SD, n=8). Note: Compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[0095] FIG. 44A-G show ankle swelling index of rats 2 h (FIG. 44A), 4 h (FIG. 44B), 6 h (FIG. 44C), 8 h (FIG. 44D), 10 h (FIG. 44E), 12 h (FIG. 44F) and 24 h (FIG. 44G) (mean±S, n=8), and the change in circumference over time for each group (FIG. 17H). Note: Compared with the blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[0096] FIG. 45A-E show results of serum biochemical indices in rats (mean±SD, n=8). Note: compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[0097] FIG. 46A-B show trend analysis of body weight (FIG. 46A) and food intake (FIG. 46B) changes in mice.

[0098] FIG. 47A-B show kidney weight (FIG. 47A) and renal coefficient (index) (FIG. 47B). Note: compared with control group, #P<0.05, ## P<0.01; compared with model group,*P<0.05, **P<0.01.

[0099] FIG. 48A-D show observation results of H&E stained sections of mouse kidney tissue (×200) in the control group (FIG. 48A), model group (FIG. 48B), positive control group (FIG. 48C), and Phyto-N group (FIG. 48D).

[00100] FIG. 49A-D show observations of Mosson stained sections of mouse kidney tissue (×200) in the control group (FIG. 49A), model group (FIG. 49B), positive control group (FIG. 49C), and Phyto-N group (FIG. 49D).

[00101] FIG. 50A-C show results of uric acid (UA) (FIG. 50A), creatinine (Cr) (FIG. 50B), and serum urea nitrogen (BUN) (FIG. 50C) analysis in mice. Comparison with control group, #P<0.05, ## P<0.01; comparison with model group, *P<0.05, **P<0.01.

[00102] FIG. 51A-B show analysis results of uric acid (FIG. 51A) and albumin (FIG. 51B) content in mouse urine. Comparison with control group, #P<0.05, ## P<0.01; comparison with model group,*P<0.05, **P<0.01.

[00103] FIG. 52A-C show differential analysis of the results of kidney xanthine dismutase (XOD) (FIG. 52A), adenosine deaminase (ADA) (FIG. 52B) and super oxide dismutase (SOD) (FIG. 52C) analysis in mice. Comparison with control group, #P<0.05, ## P<0.01; comparison with model group,*P<0.05, **P<0.01.

[00104] FIG. 53A-B show difference analysis of the results of renal inflammatory factor analysis in mice for TNF-α (FIG. 53A) and Caspase-1 (FIG. 53B). Comparison with control group, #P<0.05, ## P<0.01; comparison with model group,*P<0.05, **P<0.01.

[00105] FIG. 54 shows expression of IL-6, IL-18, IL-1β, NLRP3 in mouse kidney tissues (immunohistochemistry, ×200), where each protein is shown in each row for the control group (column 1), model group (column 2), positive control group (column 3), and Phyto-N group (column 4).

[00106] FIG. 55A-D show analysis results of the expression of IL-6 (FIG. 55A), IL-18 (FIG. 55B), IL-1β (FIG. 55C) and NLRP3 (FIG. 55D) in mouse kidney tissues. Comparison with control group, #P<0.05, ## P<0.01; comparison with model group,*P<0.05, **P<0.01.

[00107] FIG. 56A-C show analysis of the trend of body weight and blood glucose in mice. FIG. 56A shows body weight; FIG. 56B shows blood glucose, FIG. 56C shows body weight before sampling, and FIG. 29D shows blood glucose before sampling. Compared with control group, # P<0.05, ## P<0.01; compared with model group,* P<0.05, ** P<0.01.

[00108] FIG. 57A-C show liver appearance (FIG. 57A; upper left: control group; upper right: model group; lower left: positive control group; lower left: Phyto-N group); liver weight (FIG. 30B; and liver index (FIG. 57C). Compared with control group,# P<0.05, ## P<0.01; compared with model group,* P<0.05, ** P<0.01.

[00109] FIG. 58A-D show observation results of H&E stained sections of mouse liver tissue (×200) for the control group (FIG. 58A), model group (FIG. 58B), positive control group

(FIG. 58C), and Phyto-N group (FIG. 58D). The blue arrow shows fatty vacuoles. The orange arrows indicate balloon-like lesions.

[00110] FIG. 59A-D show observation results of oil red O stained sections of mouse liver tissue (×200) for the control group (FIG. 59A), model group (FIG. 59B), positive control group (FIG. 59C), and Phyto-N group (FIG. 59D).

[00111] FIG. 60A-D show difference analysis of serum AST (FIG. 60A), ALT (FIG. 60B), alkaline phosphatase (ALP) (FIG. 60C), total cholesterol (TC) (FIG. 60D), and triglycerides (TG) (FIG. 60E) in mice. Compared with control group, # $P<0.05$, ## $P<0.01$; compared with model group,*$P<0.05$,** $P<0.01$.

[00112] FIG. 61A-D show analysis of the trend of body weight and blood glucose in mice, where FIG. 61A shows body weight, FIG. 61B shows blood glucose, FIG. 61C shows body weight before sampling, and FIG. 61D shows blood glucose before sampling. Compared with Control group,# $P<0.05$, ## $P<0.01$; Compared with Model group,* $P<0.05$, ** $P<0.01$.

[00113] FIG. 62A-D show analysis of OGTT and ITT in mice, where FIG. 62A shows a OGTT line chart, FIG. 62B shows a ITT line chart, FIG. 62C shows AUC for the OGTT curve, and FIG. 62D shows AUC for the ITT Curve. Compared with Control group,# $P<0.05$, ## $P<0.01$; Compared with Model group,* $P<0.05$, ** $P<0.01$.

[00114] FIG. 63A-C show liver appearance (FIG. 63A; upper left panel: control group; upper right panel: model group; lower left panel: positive group; lower right panel: Phyto-N group); liver weight (FIG. 63B) and liver index (FIG. 63C) of mice. Compared with Control group,# $P<0.05$, ## $P<0.01$; Compared with Model group,* $P<0.05$, ** $P<0.01$.

[00115] FIG. 64A-D show observation results of H&E stained sections of mouse liver tissue (×200). FIG. 64A shows the Control group, FIG. 64B shows the Model group, FIG. 64C shows the Positive control group, and FIG. 64D shows the Phyto-N group. The blue arrow shows fatty vacuoles; The orange arrows indicate balloon-like lesions.

[00116] FIG. 65A-D show observation results of oil red O stained sections of mouse liver tissue (×200). FIG. 65A shows the Control group, FIG. 65B shows the Model group, FIG. 65C shows the Positive control group and FIG. 65D shows the Phyto-N group.

[00117] FIG. 66A-E show difference analysis of serum AST (FIG. 66A), ALT (FIG. 66B), ALP (FIG. 66C), TG (FIG. 66D) and TC (FIG. 66E) in mice. Compared with the Control group,# $P<0.05$, ## $P<0.01$; Compared with Model group,*$P<0.05$,** $P<0.01$.

[00118] FIG. 67A-B show difference analysis of LDL-C (FIG. 67A) and HDL-C (FIG. 67B) analysis results in mouse liver. Compared with Control group,# P<0.05, ## P<0.01; Compared with Model group,* P<0.05,** P<0.01.

[00119] FIG. 68A-B show difference analysis of TC (FIG. 68A) and TG (FIG. 68B) analysis results in mouse liver. Compared with the Control group,# P<0.05, ## P<0.01; Compared with Model group,* P<0.05,** P<0.01.

[00120] FIG. 69 shows analysis of differences in the results of liver inflammatory factors analysis in mice. Compared with Control group, # P<0.05, ## P<0.01; Compared with the Model group,* P<0.05,** P<0.01.

[00121]

[00122] FIG. 70 shows a schematic of a first study design.

[00123] FIG. 71 shows a schematic of a second study design.

[00124] FIG. 72 shows body weight loss in treated (Phyto-N) versus control (UT) hamsters infected with SARS-CoV-2. Left panel represents animals treated pre-infection and right panel represents animals treated post-infection.

[00125] FIG. 73 shows changes in body weight in treated (Phyto-N) versus control (UT) hamsters uninfected or infected with SARS-CoV-2.

[00126] FIG. 74 shows viral titer in lungs in treated (Phyto-N) versus control (UT) hamsters infected with SARS-CoV-2. Left panels represent animals treated post-infection and right panels represent animals treated pre-infection.

[00127] FIG. 75 shows viral titer in liver and kidney in treated (Phyto-N) versus control (UT) hamsters infected with SARS-CoV-2. Left panels represent animals treated post-infection and right panels represent animals treated pre-infection. Top panels show liver and bottom panels show kidney.

[00128] FIG. 76 shows viral titer in spleen, heart, and brain in treated (Phyto-N) versus control (UT) hamsters infected with SARS-CoV-2. Top panels represent animals treated post-infection and bottom panels represent animals treated pre-infection. Left panels show spleen, middle panels show heart, and right panels show brain.

[00129] FIG. 77 shows lung histology in treated versus control hamsters infected with SARS-CoV-2.

[00130] FIG. 78 shows liver histology in treated versus control hamsters infected with SARS-CoV-2.

[00131] FIG. 79 shows spleen histology in treated versus control hamsters infected with SARS-CoV-2.

[00132] FIG. 80 shows kidney histology in treated versus control hamsters infected with SARS-CoV-2.

[00133] FIG. 81 shows brain histology in treated versus control hamsters infected with SARS-CoV-2.

[00134] FIG. 82 shows immunohistochemical analyses of lungs sections from treated versus control hamsters infected with SARS-CoV-2.

[00135] FIG. 83 shows lung infiltration levels of CD3-positive T cells, IBA1-positive macrophages, and cytokine levels in treated versus control hamsters infected with SARS-CoV-2.

[00136] FIG. 84 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster lungs in treated versus control hamsters infected with SARS-CoV-2.

[00137] FIG. 85 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster spleens in treated versus control hamsters infected with SARS-CoV-2.

[00138] FIG. 86 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster kidneys in treated versus control hamsters infected with SARS-CoV-2.

[00139] FIG. 87 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster livers in treated versus control hamsters infected with SARS-CoV-2.

[00140] FIG. 88 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster brains in treated versus control hamsters infected with SARS-CoV-2.

[00141] FIG. 89 shows cytokine and immune marker expression in SARS-CoV-2 infected hamster hearts in treated versus control hamsters infected with SARS-CoV-2.

[00142] Fig. 90: Animals from atopic dermatitis model: Blank group, model group, Phyto-N, Group Daphnoretin (I) and Group Daphnogitin (II) mouse skin condition

[00143] Fig. 91: Change plot of skin erythema, infiltration, scales, edema, and skin lesions in mice (Model group (Mod) Phyto-N (N) , Daphnoretin (I) (A) and Daphnogitin (II) (B)) ($x \pm S$, $n=7$).

[00144] Fig. 92: Spleen index of mice in blank group (Con), model group (Mod), Phyto-N group (N), Daphnoretin (I) group (A) and Daphnogitin (II) group (B) ($x \pm S$, $n = 7$) HE staining

of back skin tissue of mice in blank group (Con) model group (Mod), Phyto-N group, Daphnoretin (I) group and Daphnogitin (II) group (100x).

[00145] Fig. 93: HE staining of back skin tissue of mice in blank group (Con) model group (Mod), Phyto-N group, Daphnoretin (I) group and Daphnogitin (II) group (100x).

[00146] Fig. 94: Epidermal thickness of blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B) ($x \pm S$, n=7). Compared with the blank control group,$^{\#}P<0.05$,$^{\#\#}P<0.01$;compared with the model group, $^{*}P<0.05$,$^{**}P<0.01$.

[00147] Fig. 95: Number of white blood cells ($X \pm S$, N = 7 blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B) $^{**}P<0.01$.

[00148] Fig. 96: Number of neutrophils ($X \pm S$, N = 7 blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B), $^{*}P<0.05$,$^{**}P<0.01$.

[00149] Fig. 97: Number of lymphocytes ($X \pm S$, N = 7 blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B), $^{*}P<0.05$.

[00150] Fig. 98: Number of platelets ($X \pm S$, N = 7 blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B). $P<0.05$.

[00151] Fig. 99: Number of monocytes ($X \pm S$, N = 7 blank (Con), model (Mod), and treated group (Phyto-N (N), Daphnoretin (I) (A) and Daphnogitin (II) (B), ,$^{***}P<0.01$.

[00152] Fig. 100: Figure 100: Acute colitis experimental flow.

[00153] Fig. 101: Line graph of percent body weight change in mice.

[00154] Fig. 102: Line graph of DAI scores in mice.

[00155] Fig. 103: Line graph of occult blood and stool dilution scores in mice.

[00156] Fig. 104: Histogram of mouse spleen index Daphnoretin (I) and Daphnogitin (II).

[00157] Fig. 105: H&E staining of colon of mice in each group of mice in acute colitis model (100x).

DETAILED DESCRIPTION

[00158] 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 autoimmune diseases. The therapeutic effects also include the ability to alleviate symptoms of or prevent viral infections.

[00159] Definitions.

[00160] 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.

[00161] 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.

[00162] 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.

[00163] 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.

[00164] 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.

[00165] 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.

[00166] 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.

[00167] 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.

[00168] As used herein, "*Wikstroemia indica* (Linn.) C.A. Mey" may be identified by other terms, which include *Wikstroemia indica* (L.) C. A. Mey, *Wikstroemia indica,* 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.

[00169] Plant extract

[00170] Provided herein are a plant extract and a composition comprising the plant extract. The plant extract may comprise one or more compounds. The plant extract may be extracted from a plant material obtained from *Wikstroemia indica* (Linn.) C. A. Mey. The plant extract may be referred to herein as "Phyto-N." 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.

[00171] Extraction methods

[00172] 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.

[00173] The plant extract may be extracted by contacting the plant material with a solvent. The solvent may comprise one or more of water, methanol, ethanol, dichloromethane, petroleum ether, acetone, ethyl acetate, n-butanol, n-hexane, methanol, petroleum ether, ethyl acetate, and other lower fatty alcohols. 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 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 (which may be petroleum ether), 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.

[00174] 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.

[00175] 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.

[00176] 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.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.

[00177] 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.

[00178] 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. 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.

[00179] 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.

[00180] 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.

[00181] 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.

[00182] 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.

[00183] 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).

[00184] 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.

[00185] 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.

[00186] 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.

[00187] 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.

[00188] 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.

[00189] 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.

[00190] 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.

[00191] The plant extract may be extracted by subjecting the plant material to microwave extraction. The extraction temperature may be about 40-60°C. The microwave power may be about 300-600w.

[00192] The plant extract may be extracted by subjecting the plant material to dual-frequency ultrasonic extraction. 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.

[00193] 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.

[00194] Also provided herein are compounds isolated from the plant extract, Daphnoretin (I) and Daphnogitin (II). that may be used for the treatment of autoimmune diseases, metabolic disease, and viral infection.

[00195] Compositions

[00196] Provided herein is a composition comprising the plant extract. Also provided herein is a composition comprising Daphnoretin (I). Also provided herein is a composition comprising Daphnogitin (II). The composition may be a pharmaceutical composition further comprising 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.

[00197] 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.

[00198] The solvent may comprise one or more of glycerol, Tween 80, propylene glycol, phenoxyethanol, polyethylene glycol 400, ethanol, acetone, and ethyl acetate.

[00199] 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.

[00200] 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.

[00201] 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.

[00202] 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.

[00203] 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.

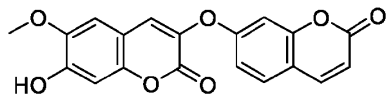
[00204] The osmolarity regulator may comprise one or more of sodium chloride, mannitol, and sucrose. The pH modifier may comprise one or more of phosphoric acid, phosphate, citrate, sodium citrate, hydrochloric acid, and sodium hydroxide. The permeation enhance may comprise one or more of capric acid, 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).

[00205] 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.

[00206] Method of treatment

[00207] Provided herein is a method of treating an autoimmune disease or condition in a subject in need thereof, comprising administering the plant extract, the composition comprising one or more active ingredients thereof, or the 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 autoimmune 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 autoimmune disease or condition. The treatment, composition, or medicament may reduce one or more symptoms of the autoimmune disease or condition.

[00208] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.



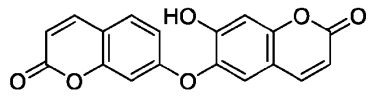
[00209] **Daphnoretin (I)**

[00210] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00211] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.

[00212] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00213] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering Daphnogitin (**II**) to the subject.



[00214] **Daphnogitin (II)**

[00215] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00216] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering Daphnogitin (**II**) to the subject.

[00217] Also provided herein is a method of treating or preventing an autoimmune disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00218] The autoimmune disease or condition may be ulcerative colitis, Crohn's disease, inflammatory bowel disease (IBD), atopic dermatitis, eczema, psoriasis, or rheumatoid arthritis. The psoriasis may be chronic plaque psoriasis. The subject with ulcerative colitis, Crohn's disease, IBD, or chronic plaque psoriasis, which may be chronic plaque psoriasis, may be 18 years of age or younger.

[00219] The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may one or more of decrease inflammatory cell infiltration, increase the number of structurally intact cup cells in crypts, decrease expression one or more of IL-6, TNF-α, IL-1β, and IL-18 in the colonic tissues, and decrease levels of one or more of IL-6, TNF-α, IL-1β, and IL-18 in serum. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may reduce pathological damage of the colon, alleviate inflammatory infiltration, or promote mucosal healing.

[00220] Atopic dermatitis (AD) is a chronic, recurrent, inflammatory skin disease characterized by severe itching and eczematous skin lesions. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may one or more of improve the skin lesions of atopic dermatitis, reduce the epidermal thickness and spleen index, improve the pathological structure of skin lesions, and reduce the content of inflammatory factor TNF-α in skin tissues,

[00221] Eczema is a common inflammatory skin disease of the epidermis and superficial dermis caused by a variety of internal and external factors. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may one or more of improve skin lesions associated with eczema, reduce epidermal thickness and spleen index, improve the pathological structure of skin lesions, and reduce the content of inflammatory factor TNF-α in skin tissues.

[00222] Psoriasis is a skin disease stimulated by environmental factors. It is a multigenic disease and immune-mediated. Psoriasis typically manifests as scaly erythema or plaque. The

plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may one or more of improve the skin lesions associated with psoriasis, reduce epidermal thickness and spleen index, and improve the pathological structure of skin lesions.

[00223] Rheumatoid arthritis is a systemic, refractory autoimmune disease that causes non-infectious inflammation of small joints and periarticular tissues throughout the body. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may one or more of have a joint protective effect on collagen-induced rheumatoid arthritis; reduce the incidence of arthritis, foot swelling, and arthritis score; reduce synovial inflammation; increase cartilage area; reduce the inflammatory reaction; and reduce expression of one or more of IL-1β and TNF-α. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may have anti-inflammatory and anti-rheumatoid effects.

[00224] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering the plant extract or the pharmaceutical composition comprising the plant extract to the subject.

[00225] Also provided are the plant extract or the pharmaceutical composition comprising the plant extract for use in treating or preventing the viral infection; and use of the plant extract or the pharmaceutical composition comprising the plant extract in the manufacture of a medicament for treating or preventing the viral infection. The treatment, composition, or medicament may reduce one or more symptoms of the viral infection. In one example, the plant extract is administered prior to the viral infection, and the plant extract may reduce one or more symptoms of the viral infection as compared to a population of individuals who did not receive the plant extract.

[00226] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.

[00227] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00228] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.

[00229] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00230] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering Daphnogitin (**II**) to the subject.

[00231] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00232] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering Daphnogitin (**II**) to the subject.

[00233] Also provided herein is a method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00234] The viral infection may be SARS-CoV-2. The viral infection may be selected from the groups consisting of SARS-CoV-2, H1N1, influenza A, influenza B, influenza C, influenza D, and influenza E. The subject may be suffering from COVID-19 or one or more symptoms thereof. The subject may be suffering from a flu or one or more flu-like symptoms such as fever, chills. cough. sore throat, sinusitis, muscle aches, headaches, fatigue, vomiting and diarrhea.

[00235] Also provided herein is a method of treating a metabolic disease or condition in a subject in need thereof, comprising administering the plant extract or the pharmaceutical composition comprising the foregoing plant extract to the subject. Also provided are the plant extract or the pharmaceutical composition comprising the plant extract for use in treating the metabolic disease or condition; and use of the plant extract or the pharmaceutical composition comprising the plant extract in the manufacture of a medicament for treating the metabolic disease or condition. The treatment, composition, or medicament may reduce one or more symptoms of the metabolic disease or condition.

[00236] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.

[00237] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00238] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering Daphnoretin (**I**) to the subject.

[00239] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (**I**) to the subject.

[00240] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering Daphnogitin (**II**)to the subject.

[00241] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00242] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering Daphnogitin (**II**) to the subject.

[00243] Also provided herein is a method of treating or preventing a metabolic disease or condition in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (**II**) to the subject.

[00244] The metabolic disease or condition may be gout or one or more complications or conditions thereof, which may include one or more of inflammation, redness, swelling, pain, and excess uric acid; hyperuricemia; acute or chronic gouty arthritis; gouty nephritis; diabetes or a complication or condition thereof; fatty liver disease; non-alcoholic fatty liver; obesity or a related condition; hyperlipidemia; hypertriglyceridemia; hypercholesterolemia; dyslipidemia; or a lipid metabolism disorder or condition. Diabetes and complications and conditions thereof may include one or more of type 2 diabetes; insulin resistance; diabetic nephropathy; diabetic peripheral neuropathy; diabetic retinopathy; cataracts; diabetic foot or hand; diabetic neuropathy; diabetic hypertension; diabetic vascular occlusion; diabetic blood vessel lesions; diabetic numbness of hands or feet; diabetic Alzheimer's disease; non-healing oral ulcers; and skin ulcers. Treating the metabolic disease or condition may comprise reducing one or more of total cholesterol, low density lipoprotein cholesterol (LDL-C), and triglycerides. The total cholesterol, LDL-C, or triglycerides may be reduced to normal levels. Normal levels may be defined by the American College of Cardiology (ACC), as described in Lloyd-Jones, D, et al., "2022 ACC Expert Consensus Decision Pathway on the Role of Nonstatin Therapies for LDL-Cholesterol Lowering in the Management of Atherosclerotic Cardiovascular Disease Risk: A Report of the American College of Cardiology Solution Set Oversight Committee," J Am Coll Cardiol., 80 (14) 1366–1418 (2022), the contents of which are incorporated herein by reference. The subject

may be in need of reducing one or more of total cholesterol, LDL-C, and triglycerides according to relevant medical guidelines such as those of the ACC. The plant extract may also prevent non-alcoholic fatty liver.

[00245] The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may treat gout, or complications or conditions thereof, including by reducing one or more of inflammation, redness, swelling, and pain. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may reduce one or more of uric acid (UA), creatinine (Cr), and urea nitrogen (BUN) in serum. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may reduce one or more of xanthine oxidase (XOD) and adenosine deaminase (ADA) in liver. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may reduce one or more of renal fibrosis, glomerular atrophy, and tubular dilatation induced by potassium oxonate and adenine. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**)may reduce levels of one or more inflammatory factors, which may be one or more of TNF-α, Caspase-1, IL-6, IL-18, IL-1β and NLRP3 in the kidneys.

[00246] In treating diabetes or complications thereof, the plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may decrease the blood glucose or lipids; improve insulin resistance; lower total cholesterol, LDL cholesterol, or triglycerides to normal levels; or protect the structure and function of the pancreas, liver, and kidneys.

[00247] In treating or preventing non-alcoholic fatty liver disease, the plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may reduce one or more of body weight, blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total cholesterol (TC). The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may improve a liver injury caused by a high-fat diet. The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may treat steatohepatitis or cirrhosis.

[00248] The plant extract, Daphnoretin (**I**), and Daphnogitin (**II**) may improve liver hypertrophy, histopathological changes and abnormal lipid deposition caused by high-fat diet. The pl;ant extract, Daphnoretin (**I**), and Daphnogitin (**II**) decreased the contents of IL-1β, IL-6 and TNF-α.

[00249] The plant extract, Daphnoretin (**I**), or Daphnogitin (**II**) 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, Daphnoretin (**I**), and Daphnogitin (**II**) 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.

[00250] Examples

[00251] Example 1: A *Wikstroemia indica* extract can treat ulcerative colitis

[00252] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) effectively treats ulcerative colitis. Ulcerative colitis (UC) is a chronic non-specific inflammatory disease whose etiology and pathogenesis are not well understood, and which is caused by the interaction of genetic, environmental, psychological, infectious, and immunologic balance factors. Recurrent episodes of abdominal pain, diarrhea, and mucus, pus, and blood are its main symptoms. In recent years, the incidence and prevalence of UC have continued to rise globally, with prolonged and complicated complications, resulting in a considerable socio-economic burden. Conventional Western medical treatments for UC, including hormones, 5-aminosalicylic acid (5-ASA), and biologics (tumor necrosis factor-alpha, TNF-alpha antagonists) are associated with a variety of adverse effects, with the problems of non-responsiveness and susceptibility to developing tolerance. Therefore, it is urgent to find a drug that is effective in the treatment of UC with few toxic side effects. In the present study, we used dextran sulfate sodium (DSS) to induce the establishment of UC mouse model, and recorded the general conditions (body weight, food and water consumption, fecal status and mortality), Disease activity index (DAI) scores, Hematoxylin-Eosin staining (H), and the general conditions of the mice in each group every day. The pathology of colon tissue was examined by Hematoxylin-Eosin staining (H&E), the levels of IL-6, TNF-α, IL-1β and IL-18 in serum were measured by Enzyme-linked immunosorbent assay (ELISA), and the levels of IL-6, TNF-α, IL-1β and IL-18 in colon tissue were examined by immunohistochemistry. -1β, IL-18, and mRNA expression of NLRP3, NF-κB, Caspase1, and GSDMD in colon tissues were detected by immunohistochemistry, and the protective effect of Phyto-N on UC was investigated based on the above experiments.

[00253] Experimental methods

[00254] Animal modeling, administration and grouping

[00255] Sixty C57BL/6 mice were randomly divided into four groups as follows: (1) Control group (n = 20); (2) DSS group (n = 20); (3) positive control (Positive) group (n = 20); (4) Phyto-N group (n = 20); The model and Phyto-N groups were acclimatized to feed for one week prior to modeling and weighed and values were recorded. In this experiment, DSS induction was used for modeling, and the blank group drank purified water; the model group, the positive drug group, and the Phyto-N group drank 2% DSS solution (2 g DSS dissolved in 100 ml purified water) for 5 days. After stopping drinking DSS and switching to purified water for 7 days, they switched to a 2% DSS solution for 5 days of free drinking. The positive drug group and the Phyto-N group were administered for 10 days starting from the 11th day of modeling. The positive control drug mesalazine was administered at a dose of 0.3 g/kg/d, and Phyto-N was administered at a dose of 3.3 g/kg/d, and mice in the blank group were given an equal amount of saline by gavage, and body weight measurements, fecal status, and hemorrhage were observed daily during the experiment.

[00256] Experimental sample collection

[00257] On the 21st day of the experiment, the mice were fasted for 12 hours. The mice were bloodied by removing the eyeballs, and the distal colon was quickly dissected from the anus to the cecum, and the length was straightened on weighing paper and photographed; the colon was cleaned of feces with saline, and then the colon was divided into two segments, one of which was preserved at -80°C, and the other was fixed with 4% paraformaldehyde for histopathological analysis.

[00258] General status evaluation vs. DAI evaluation

[00259] The general conditions of the mice were monitored prior to modeling, such as mental status, coat color and luster, stress response, and other systemic conditions. Body weight measurement, fecal status and bleeding were observed daily during the experiment. Based on the daily body weight of the mice after modeling, the following was calculated: body weight loss rate = (daily body weight - initial body weight)/initial body weight × 100%, and the scores of body weight loss, fecal status, and the degree of hemorrhage were recorded according to the scoring criteria in the table below, and the average of the three was taken as the DAI score.

[00260] Table 1: DAI scoring criteria for mice

Weight loss%	Fecal character	Occult blood level	Mark
0	Normalcy	Normalcy	0
1-5	Thin, but formed stools	Fecal Occult Blood Weakly Positive	1
5-10	Not rigorous	Bleeding (positive)	2
10-15	Meager stool	Bleeding (strong positive)	3
>15	Watery stool	Profuse bloody stools	4

[00261] Histopathologic observation of mouse colon

[00262] The H&E staining method was used to observe the mouse colon tissue. Fresh mouse colon tissues were dissected and removed and placed in fixative for 24 hours at room temperature. After fixation, the tissues were rinsed with running water 15 hours to remove the fixative 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 wax with a melting point of 56-58°C for 1 hour each, embedded and sectioned into 5 μm, and dried at 37°C for 12 hours. The tissues were then dried at 37°C for 12 hours. The tissues were then dipped into low-high gradient ethanol for 1 hour each for tissue dehydration. First, dewaxing was performed, and the wax slices were soaked in xylene solution for 20 min to remove the wax around the tissues; the tissues were dehydrated and processed by soaking in high - low gradient ethanol solution for 5 minutes each, soaked in distilled water for 5 min, and soaked and washed with phosphate buffer solution (PBS). Sections were medium-stained with hematoxylin for 7-10 minutes, incubated at room temperature for 10 min. 6.1% hydrochloric acid alcohol differentiation for 2-3 seconds, rinsed in tap water to return to the blue, washed again by immersion in PBS, with 1 drop of alcohol-soluble eosin staining solution, kept at room temperature for 3 seconds. After staining, the sections were dehydrated and made transparent, rinsed in anhydrous ethanol, and immersed in xylene for 2 times, 10 minutes each time, after which the sections were blocked by neutral tree resin, and optical microscope Observed under 200×, the images were captured and analyzed, and the morphological changes of the pathological colonic tissues could be seen.

[00263] Immunohistochemical assay to detect the area of IL-6, TNF-α, IL-18 and IL-1β positivity in colon tissue

[00264] An immunohistochemical (IHC) method was used to detect the positive area of IL-6, TNF-α, IL-18 and IL-1β in colon tissue. The colon tissue was taken after routine dehydration and embedded section of 5μm, and then processed for IHC according to Table 2, the primary antibody and secondary antibody solutions were prepared with appropriate concentration, and the antibody dilution concentration is shown in Table 3. The sections were routinely dehydrated, deparaffinized, and sealed with neutral tree glue. The slices were observed under a microscope, images were captured, and the area of the positive area was analyzed by ImageJ.

[00265] Table 2 IHC processing steps

Objective	**Liquid**	**Processing temperature and time**	**Cleaning time**
Repair of antigens	1×citric acid buffer	high to medium heat10 minutes	PBS：3minutes×3
Removal of endogenous peroxidase	3% H2O2 solution	25°C 10 minutes	PBS：3minutes×3
Serum seal	Goat serum solution	25°C 1hours	None
Incubation of primary antibody	Corresponding primary antibody solution	15 hours incubation at 4°C in the refrigerator	PBS：3minutes×3
Incubation of secondary antibodies	Secondary antibody solution	25°C 30min	PBS：3minutes×3
Labeled Antibodies	DAB color developing solution	25°C 1minute	Distilled water rinse
Labeling of nuclei	Hematoxylin solution	25°C 3minutes	Distilled water rinse

[00266] Table 3 Immunohistochemistry dilution concentration of each antibody

[00267]

Antibody Name	**Dilution**
IL-18 antibody	1：200
IL-6 antibody	1：200
IL-1β antibody	1：100
TNF-α	1：200
HRP Enzyme-labeled sheep anti-rabbit	1：500

[00268] Enzyme-linked immunoassay (ELISA) for cytokines

[00269] The levels of cytokines (TNF-α, IL-6) in mouse serum were determined using an ELISA kit according to the instructions, and the absorbance A was detected at 450 nm, and the content was calculated from the standard curve.

[00270] Expression of NLRP3, Caspase-1, GSDMD-N, NF-κB, and β-actin in rat colon tissues by Real-time PCR

[00271] Take 50 mg of colon tissue and add TRIzo1 600 μL, trichloromethane 120 μL, and an equal volume of isopropanol and centrifuge, from which RNA was extracted and reverse transcribed to obtain cDNA, and use this cDNA as a template, and use the primers synthesized with the target gene design to perform Real-time PCR, and based on the results of the amplification, the ratio of the target gene in the tissue and the internal reference gene, β-actin, which is the relative expression amount. The primer sequences were synthesized by Bioengineering (Shanghai) Co.

[00272] Table 4 Primer sequences

Primer name	Upstream primer sequences	SEQ ID NO:	Downstream primer sequences	SEQ ID NO:
NLRP3	TGGCATCGTGAAGTGGTTGT	1	GCCAAATGCTTACCAGAAAGT	6
Caspase-1	TGCCCAGAGCACAAGACTTC	2	TCCTTGTTTCTCTCCACGGC	7
GSDMD-N	TAAACTGGGCGGAGGGATGAA	3	GAAGGGGATTTCTGGTGGTGT	8
NF-κB	CGACGTATTGCTGTGCCTTC	4	TAGGATCTGCCCAGGTGGTAA	9
β-actin	ACCTTCTACAATGAGCTGCG	5	CTGGATGGCTACGTACATGG	10

[00273] Experimental results

[00274] Observations on colon length and colon gross morphology in each group of mice

[00275] The mice in the DSS group were affected by inflammation and showed a significant decrease in colon length compared with the blank group ($P<0.01$) (FIG. 1). The Phyto-N group showed a significant increase in colon length compared with the DSS group. In addition, as shown in FIG. 2, the intestinal contents of the colon of mice in the blank group were in good shape and there was no hemorrhage in the intestinal wall, whereas the mice in the DSS group and the Phyto-N group had severe intestinal hemorrhage in the colon and the intestinal contents were in watery form, which was more severe in the DSS group.

[00276] Improvement in general condition and DAI score in DSS-induced mice

[00277] During the experiment, mice in the blank group had smooth and soft fur, normal diet and defecation, formed feces, and maintained a stable body weight. Over the course of the modeling time, mice in the DSS group, the positive drug group, and the Phyto-N group had a significant decrease in body weight, diarrhea and blood in the stools, and a significant increase in the DAI scores. After the administration of the positive drug and the Phyto-N, the mice had a significant alleviation of the symptoms of UC, and the decrease in body weight and the elevation of the DAI scores were differently reversed. After administration of the positive control and Phyto-N, UC symptoms were significantly alleviated, and weight loss and elevated DAI scores were reversed to different degrees. The results are shown in FIG. 3 and 4.

[00278] Analysis of H&E staining results of colon tissue

[00279] Mice in the blank group had intact mucosa and crypt structure of colon tissue and no inflammatory infiltration; mice in the DSS group had severe damage to the mucosa and crypt structure of colon tissue, a large number of inflammatory cells infiltration, a significant decrease in the number of cup cells, and the cells had detachment and necrosis. Mice in the positive drug group and the Phyto-N group still had a small portion of inflammatory cells infiltration, but the intact structure of the crypts could be seen, and the arrangement of glands was basically intact with an increase in the number of cup cells. It indicated that the Phyto-N group had milder mucosal structural damage and inflammatory reaction compared with the DSS group. The results are shown in FIG. 5.

[00280] Immunohistochemical results

[00281] Protein expression of IL-6, TNF-α, IL-1β, and IL-18 was elevated in the colon tissues of mice in the DSS group compared to the blank group ($P<0.01$), and decreased in the colon tissues of mice in the positive drug group and the Phyto-N group compared to the DSS group ($P<0.01$). The results are shown in FIG. 6 and 7.

[00282] 2.5 ELISA results

[00283] Compared with the blank group, serum IL-6, TNF-α, IL-1β, and IL-18 levels were elevated in mice in the DSS group ($P<0.01$); and serum IL-6, TNF-α, IL-1β, and IL-18 levels were reduced in mice in the positive and Phyto-N groups compared with mice in the DSS group ($P<0.01$).

[00284] Table 5 Comparison of serum IL-6 and TNF-α levels in mice of each group (ng/L, **mean ±S, n=3)**

Groups	IL-6 (pg/mL)	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-18 (pg/mL)
Control	113.071±7.1780	135.260±3.0105	100.66±12.5117	122.78±5.734
DSS	193.643±4.5550**	253.408±3.6916**	136.5±13.8512**	168.32±3.486**
Positive	137.405±6.3678##	164.616±3.3802##	109.39±7.0269##	134.52±7.845##
Phyto-N	118.404±4.8911##	161.489±1.8284##	112.17±11.3707#	132.86±7.9345##

Note: compared with Control group ** P<0.01; compared with DSS group ## P<0.01.

[00285] Results of PCR experiments

[00286] The results of RT-qPCR assay are shown in the table below. Compared with the control group, the expression of NLRP3, NF-κB, Caspase1, and GSDMD in mice in the DSS group was significantly higher (P<0.01). Compared with DSS group, the expression of NLRP3, NF-κB, Caspase1, and GSDMD in the colonic tissues of mice in the Positive drug group and the Phyto-N group were significantly decreased (P<0.01).

[00287] Table 6 NLRP3, NF-κB, Caspase1, and GSDMD mRNA expression levels in mice of each group ($\bar{x}$±S, n=3)

Groups	(NLRP3/β-actin) $2^{-\Delta\Delta Ct}$	(NF-κB/β-actin) $2^{-\Delta\Delta Ct}$	(Caspase1/β-actin) $2^{-\Delta\Delta Ct}$	(GSDMD/β-actin) $2^{-\Delta\Delta Ct}$
Control	1.000±0.125	1.000±0.204	1.000±0.053	1.000±0.034
DSS	1.998±0.215**	1.920±0.604**	1.421±0.161**	1.886±0.412**
Positive	1.181±0.240##	1.359±0.281##	1.358±0.125##	1.762±0.613##
Phyto-N	1.182±0.126##	1.673±0.506##	1.377±0.393#	1.550±0.172##

Note: compared with Control group ** P<0.01; compared with DSS group ## P<0.01.

[00288] Experimental Summary

[00289] In this experiment, the body weight of mice in the DSS group were all significantly decreased compared with mice in the blank group, the length of the colon was significantly shortened, and the DAI score was significantly increased. Pathological results showed that mice in the DSS group showed structural destruction of the colonic mucosa, a decrease in the number of cup cells, accompanied by a large number of inflammatory cell infiltration, and elevated expression of the proteins of IL-6, TNF-α, IL-1β, and IL-18 in the colon tissues, and serum IL-6, TNF-α, and IL-18, IL-1β, and IL-18 levels were elevated. Mice in the positive drug group and

the *Wikstroemia indica* extract group had elevated body weights compared with the DSS group, regressed colon length, decreased DAI scores, and pathologic findings showed decreased inflammatory cell infiltration, increased number of structurally intact cup cells in the crypts, and decreased protein expression of IL-6, TNF-α, IL-1β, and IL-18 in the colonic tissues, and decreased levels of IL-6, TNF-α, IL-1β, and IL-18 in serum. The results demonstrate that pathological damage of the colon was reduced, inflammatory infiltration was alleviated, and mucosal healing was promoted in mice by the *Wikstroemia indica* extract.

[00290] Example 2: A *Wikstroemia indica* extract can treat atopic dermatitis

[00291] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) demonstrates protective effects against atopic dermatitis by observing the skin lesions and severity scores, histopathological structure of skin lesions, epidermal thickness, spleen index and TNF-α content in skin tissues of mice in each group.

[00292] Experimental methods

[00293] Animal modeling, administration and grouping

[00294] Sixty specific pathogen free (SPF) male BALB/c mice were selected and adaptively fed for 7 days (room temperature 22 °C -24 °C, humidity 50 % -60 %, normal feeding, free feeding). They were randomly divided into blank group, model group, phyto-N (3.3g/kg, equivalent to 20g per day for adults) positive drug (dexamethasone) group. Adaptive feeding began to model.

[00295] On the 1st to 3rd day of the experiment, except for the blank group, the mice in the other groups were coated with 100 μL 1 % Dinitrochlorobenzene (DNCB) on the back hair removal area of the mice. From the 5th day, 0.5 % DNCB was applied once every other day. The mice in the blank group were smeared with the same amount of vaseline.

[00296] On the 8th day of modeling, Phyto-N (3.3 g/kg) was administered by gavage for 21 days. The blank group was given the same amount of normal saline, and the positive drug group was given dexamethasone according to the clinical dosage.

[00297] Lesion performance and severity score

[00298] The area of skin lesions was measured with reference to relevant studies, and the severity of skin lesions was scored. According to the severity of erythema, papules, exudation, crusting and dryness in the skin lesions, 0 was scored as no skin lesions, 1 was scored as mild, 2

was scored as moderate, and 3 was scored as severe. The higher the score, the more serious the AD lesions.

[00299] Histopathological observation and epidermal thickness measurement of skin lesions in mice.

[00300] After the mice were sacrificed, half of the skin lesions were cut off with clean scissors, fixed in 4 % paraformaldehyde, and then dehydrated, embedded in paraffin, sectioned, and stained with H&E stain. Three sections of each specimen were selected under the microscope for observation and photographing, and the pathological changes of mice in each group were analyzed.

[00301] Determination of inflammatory factor TNF-α content in skin lesions of mice

[00302] The remaining skin lesion tissue of mice was placed in a test tube and stored in a refrigerator at-20 °C. Before the experiment, the thaw was taken out, weighed and cut into pieces. Normal saline was added at 1:9, and steel balls were added to the test tube. The tissue homogenate machine was used for crushing, and the supernatant was taken after centrifugation. Enzyme-linked immunosorbent assay (ELISA) was used to detect the content of inflammatory factor TNF-α.

[00303] Experimental results

[00304] Skin lesion performance and severity score

[00305] The skin lesions were scored on the 4th, 8th, 12th, 16th, 20th, 24th and 28th days of the experiment. From the 5th day to the 8th day, except for the blank group, the severity of skin lesions in each group gradually increased. After treatment, the skin lesion scores of the positive drug group and the Phyto-N group were lower than those of the model group. The results showed that Phyto-N could improve the erythema, infiltration, scales and edema of atopic dermatitis model mice, and reduce the skin lesion score. Results are shown in FIG. 8 and 9.

[00306] Histopathological observation and epidermal thickness measurement of skin lesions in mice.

[00307] In the blank group, there was no obvious abnormality in the structure of the epidermis and dermis of the skin tissue of the mice in the blank group, the cell morphology was normal, and there was no obvious inflammatory reaction in the superficial dermis. Compared with the blank group, the skin lesions of the model group showed obvious inflammatory proliferative changes, including hyperkeratosis, acanthosis, sponge edema, dermal vasodilation and

congestion, and inflammatory cell infiltration such as a large number of lymphocytes and eosinophils in the dermis. The pathological manifestations of mice in the positive drug group and Phyto-N group were slight hyperkeratosis, the spinous layer cells were thinner than those in the model group, and the infiltration of dermal inflammatory cells was less than that in the model group (see FIG. 10 and 11).

[00308] Determination of inflammatory factor TNF-α in mouse skin lesions

[00309] The level of TNF-α in skin lesions of mice in the model group was higher than that in the blank group ($P < 0.05$). The levels of TNF-α in the skin lesions of mice in the Phyto-N group and the positive drug group were lower than those in the model group ($P < 0.01$). The results showed that Phyto-N could effectively reduce the content of inflammatory factor TNF-α in the skin tissue of atopic dermatitis mice, and had a good improvement effect on immune function. (see FIG. 12).

[00310] Experimental summary

[00311] Atopic dermatitis is a chronic, recurrent, inflammatory skin disease characterized by severe itching and eczematous skin lesions, which seriously affects the physical and mental health and quality of life of patients. Our results show that a *Wikstroemia indica* extract can significantly improve the skin lesions of atopic dermatitis model mice, reduce the epidermal thickness and spleen index of model mice, improve the pathological structure of skin lesions, and reduce the content of inflammatory factor TNF-α in skin tissues, which has good development potential.

[00312] Example 3

[00313] A *Wikstroemia indica* extract can treat eczema

[00314] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) has protective effects against eczema by observing the skin lesions and severity scores, pathological structure of skin lesions, epidermal thickness, spleen index of mice, and inflammatory factor TNF-α content in skin tissue of each group of mice.

[00315] Experimental methods

[00316] Animal modeling, administration and grouping

[00317] Sixty SPF male BALB/c mice were selected and adaptively fed for 7 days (room temperature 22 °C -24 °C, humidity 50 % -60 %, normal feeding, free feeding). They were

randomly divided into blank group, model group, phyto-N (3.3g/kg, equivalent to 20g per day for adults) positive drug (dexamethasone) group. Adaptive feeding began to model.

[00318] The mice in each group were treated with electric depilation at 2cm × 3cm on the back, and then treated with depilation ointment to expose the skin of the mouse model. On the first and second days of the experiment, except for the blank group, the mice in the other groups were applied with 100 μL 5 % DNCB to the hair removal area on the back of the mice. From the fourth day, 1 % DNCB was applied once every other day. The mice in the blank group were smeared with the same amount of vaseline.

[00319] On the second day of modeling, Phyto-N (3.3 g/kg) was administered by gavage for 14 days. The blank group was given the same amount of normal saline, and the positive drug group was given dexamethasone according to the clinical dosage.

[00320] Eczema area and severity index scoring method.

[00321] The eczema area and severity index (EASI) scoring method is widely used in the verification of new drugs for eczema, that is, four clinical manifestations of erythema (E), scleredema (edema)/papule (I), scaly (S), lichenification (L) score. The severity of the four manifestations was scored from 0 to 3 points. The greater the score, the more serious the symptoms.

[00322] Histopathological observation and epidermal thickness measurement of skin lesions in mice.

[00323] After the mice were sacrificed, half of the skin lesions were cut off with clean scissors, fixed in 4 % paraformaldehyde, and then dehydrated, embedded in paraffin, sectioned, and stained with H&E stain. Under the microscope, 3 sections of each specimen were selected for observation and photographing, and the pathological changes of each group were analyzed.

[00324] Toluidine blue staining of mouse back skin tissue

[00325] After the mice were sacrificed, half of the skin lesions were cut off with clean scissors, fixed in 4 % paraformaldehyde, and then dehydrated, embedded in paraffin, sliced, and stained with toluidine blue. Three sections of each specimen were selected under the microscope for observation and photographing, and the changes of mast cells in each group were analyzed.

[00326] Determination of inflammatory factor TNF-α content in skin lesions of mice

[00327] The remaining skin lesions of mice were placed in a test tube and stored in a refrigerator at-20 °C. Before the experiment, the thaw was taken out, weighed and cut into

pieces. Normal saline was added at 1:9, and steel balls were added to the test tube. The tissue homogenate machine was used for crushing, and the supernatant was taken after centrifugation. Enzyme-linked immunosorbent assay (ELISA) was used to detect the content of inflammatory factor TNF-α.

[00328] Experimental results

[00329] Skin lesion performance and severity score

[00330] There was no skin lesion on the back of the mice in the blank group, and the overall state was good. In the model group, edema, exfoliation and erosion exudate appeared on the back of the mice, and some moss-like changes were seen, which was consistent with the typical manifestations of AD. The score of skin lesion inflammation was higher than that of the blank group, indicating that DNCB could effectively induce AD and the model was successful. After treatment, compared with the model group, the skin lesions of the other groups were improved to varying degrees. On the 14th day, the scores of skin lesion inflammation in the Phyto-N group and the positive drug group were lower than those in the model group. The score of skin inflammation in Phyto-N group was lower than that in control group. The results showed that Phyto-N could improve the erythema, infiltration and edema of eczema model mice and reduce the EASI score. The results are shown in FIG. 13 and 14.

[00331] Histopathological observation and epidermal thickness measurement of skin lesions in mice

[00332] In the blank group, the skin epidermis of the mice was thinner, the boundary between the epidermis and the dermis was clear, and there was no edema and lymphocyte infiltration. The epidermis of the mice in the model group was thicker, the stratum corneum was hyperkeratotic or incomplete, the stratum spinosum was hypertrophic and edematous, the dermis was thicker and accompanied by a large number of inflammatory cell infiltration, suggesting that the model was successful. The epidermis and dermis of Phyto-N group and positive drug group were slightly thickened, and there was a small amount of inflammatory cell infiltration. (see FIGs. 15 and 16).

[00333] Toluidine blue staining of mouse back skin tissue

[00334] Sporadic mast cells were seen in the dermis of the skin of mice in the blank group. The number of mast cells in the dermal layer of the skin lesions of the model group was higher than that of the blank group, and some of them were degranulated. The infiltration degree of mast

cells in Phyto-N group and positive drug group was lower than that in model group, and the number of mast cells was lower than that in model group (see FIG. 17).

[00335] Determination of inflammatory factor TNF-α in mouse skin lesions

[00336] The level of TNF-α in skin lesions of mice in the model group was higher than that in the blank group ($P < 0.05$). The levels of TNF-α in the skin lesions of mice in the Phyto-N group and the positive drug group were lower than those in the model group ($P < 0.01$). The results showed that Phyto-N could effectively reduce the content of inflammatory factor TNF-α in the skin tissue of eczema mice, and had a good improvement effect on immune function (see FIG. 18).

[00337] Experimental summary

[00338] Eczema is a common inflammatory skin disease of the epidermis and superficial dermis caused by a variety of internal and external factors. It occurs in the head and face, limb flexion and perineum. Some patients directly manifested as chronic eczema. It is characterized by severe itching, polymorphic skin lesions, symmetrical distribution, tendency to exudate, easy to relapse. Our results show that a *Wikstroemia indica* extract significantly improves the skin lesions of eczema model mice, reduces the epidermal thickness and spleen index of model mice, improves the pathological structure of skin lesions, and reduces the content of inflammatory factor TNF-α in skin tissues.

[00339] Example 4: A *Wikstroemia indica* extract can treat psoriasis

[00340] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) has protective effects against psoriasis by observing the skin lesions and severity scores, histopathological structure of skin lesions, epidermal thickness and spleen index of mice in each group.

[00341] Experimental methods

[00342] Animal modeling, administration and grouping

[00343] Thirty SPF male BALB/c mice were selected and fed adaptively for 7 days (room temperature 22 °C -24 °C, humidity 50 % -60 %, normal feeding, free feeding). They were randomly divided into blank group, model group and Phyto-N (3.3g/kg, equivalent to 20g per day for adults). Adaptive feeding began to model. 62.5 mg 5 % imiquimod ointment was applied on the back of mice for 8 consecutive days. The next day, the drug was administered, and the blank group and the model group were given the same amount of normal saline.

[00344] Skin lesion performance and severity score

[00345] The changes of skin lesions on the back of mice were observed daily, and the back skin of mice in each group was photographed on the 9th day before sampling. The erythema (E), desquamation (D), degree of infiltration (I) and total score of skin lesions in mice were recorded. The severity of skin lesions in mice was evaluated according to the psoriasis area and severity index (PASI) score. The criteria are shown in the table below.

[00346] Table 7: PASI score standard of mouse back

Score Index	Scoring Method
Erythema	0 points : no erythema 1 point : the erythema is pale red 2 points : red spot is bright red 3 points : the erythema is dark red 4 points : red spot red deep
Desquamation	0 points : no scales on the surface 1 point : some skin lesions were covered with slight scales 2 points : Most skin lesions were covered with flake scales. 3 points : almost all the skin lesions were covered with layered thick scales. 4 points : All the skin lesions were covered with layered thick scales.
Degree of infiltration	0 points : no thickening of skin lesions 1 point : slight thickening of skin lesions compared with normal skin. 2 : The lesion was moderately elevated compared with normal skin, and the edge was round or sloped. 3 points : skin lesions thickening, more obvious than the normal skin uplift. 4 points : skin lesions are highly thickened, and the uplift is more obvious than that of normal skin.

[00347] Determination of organ index in mice

[00348] The mice were sacrificed by cervical dislocation after blood collection. The spleen was weighed and its weight was recorded. The spleen was weighed and its weight was recorded. The organ index was calculated according to the following formula: organ index (mg/g) = organ weight (mg)/body weight (g).

[00349] Histopathological observation and epidermal thickness measurement of skin lesions in mice

[00350] After the mice were sacrificed, half of the skin lesions were cut off with clean scissors, fixed in 4 % paraformaldehyde, and then dehydrated, embedded in paraffin, sectioned, and stained with H&E stain. Three sections of each specimen were selected under the microscope for observation and photographing, and the pathological changes of mice in each group were analyzed.

[00351] Experimental results

[00352] Skin lesion performance and severity score

[00353] The pictures of the back of mice on the 8th day showed that compared with healthy mice, the skin erythema and scales on the back of mice in the model group increased, and Phyto-N could reduce skin erythema and scales. The results of PASI score showed that Phyto-N could reduce the PASI score of psoriasis in mice. The results are shown in FIG. 19 and 20.

[00354] Determination of organ index in mice

[00355] The results of spleen index showed that the spleen index of model group was significantly higher than that of healthy mice ($p < 0.01$), and Phyto-N could significantly reduce the spleen index of mice ($p < 0.01$). It shows that Phyto-N has a good effect on improving immune function. The results are shown in FIG. 21.

[00356] Histopathological observation and epidermal thickness measurement of skin lesions in mice

[00357] The skin structure of the skin lesions of the mice was evaluated by H&E staining. The results showed that the epidermis of the skin of the model group was thickened, and the epidermal process was prolonged and increased. After Phyto-N treatment, the thickness of skin epidermis decreased and the infiltration of inflammatory cells was significantly improved, which

further confirmed the potential of Phyto-N in the treatment of psoriasis. The results are shown in FIG. 22 and 23.

[00358] Experimental summary

[00359] Psoriasis is a skin disease stimulated by environmental factors, genetically controlled by multiple genes, and immune-mediated. It is typically manifested as scaly erythema or plaque, which is limited to one site or widely distributed throughout the body. Psoriasis seriously affects the quality of life of patients. Although the current treatment is still unable to avoid recurrence, active treatment can significantly reduce skin lesions or promote recovery, avoid serious complications, and significantly improve the quality of life of patients. Our results show that a *Wikstroemia indica* extract significantly improves skin lesions of psoriasis model mice, reduces the epidermal thickness and spleen index of model mice, and improves the pathological structure of skin lesions.

[00360] Example 5: A *Wikstroemia indica* extract can treat rheumatoid arthritis

[00361] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) can be used to treat rheumatoid arthritis. In this experiment, a rat model of arthritis induced by bovine type II collagen was used to verify the therapeutic effect of Phyto-N on rheumatoid arthritis. The effects of Phyto-N on arthritic rats were observed by morphological, pathological, and enzyme-linked immunosorbent methods to preliminarily elucidate the joint-protective effects of Phyto-N on rheumatoid arthritis rats.

[00362] Experimental methods

[00363] Animal modeling, drug administration and grouping

[00364] Twenty-eight SPF-grade female Sprague-Dawley (SD) rats, weighing 180-200 g, were selected and acclimatized for seven days, and then randomly divided into a blank group, a model group, a positive (methotrexate 1.8 mg/kg/w) drug group, and a Phyto-N (2.7 g/kg/d) group. Bovine type II collagen (CII) emulsion with a final concentration of 1 mg/mL was prepared by thoroughly mixing and emulsifying bovine type II collagen (CII) with an equal volume of Freund's incomplete adjuvant on an ice bath using an electric stir bar. The emulsion was injected subcutaneously at a dose of 0.2 ml each into the tail root of each rat except the blank group for the initial immunization, and 0.2 ml of the same emulsion as the first was injected into the tail root of the rats on day 8 of the initial immunization at a site different from the site of the initial immunization, and the success of the modelling was assessed on day 21 after the initial

immunization. Dosing was started seven days after the second immunization and continued for 4 weeks.

[00365] Weight monitoring

[00366] The first weighing was carried out on the day of the first exemption, and then every 3 days thereafter, to observe the behavioral and dietary status of the rats in each group, so as to evaluate the growth of the rats in different groups.

[00367] Arthritis scoring

[00368] Arthritis scoring was performed after the second exemption and every 3 days thereafter. The scoring was based on the degree and extent of redness and swelling of the joints, as well as joint enlargement and deformity. The arthritis index was assessed using the following scale: 0 = no sign of arthritis, 1 = mild swelling and erythema of toes or ankles, 2 = moderate swelling and erythema of toes or ankles, 3 = severe swelling and severe erythema of toes or ankles, and 4 = deformation or stiffness of toes and ankles. The total score for each rat was the sum of the two hind paw scores, with a maximum value of 8 points.

[00369] Assessment of the extent of hindfoot swelling

[00370] Measurement of foot swelling was performed as follows. The first measurement was performed on the day of second exemption, and every 3 days thereafter, and the foot thickness of each group of rats was measured synchronously with a digital vernier caliper. Press and hold the Off/On key to activate the digital vernier caliper, push the frame to make the two sides fit closely with the paw, and press and hold the Zero key to zero the vernier caliper. Adjust the distance between the two measuring claws to slightly greater than the rat foot thickness, stuck in the middle of the rat foot paw position, and then gently push the ruler frame so that the two sides of the measuring claw and the rat foot paw fit, readings can be taken. Each measurement was carried out by the same person, and each rat was measured 3 times in parallel, and then the average value was taken. The final value of foot thickness for each rat was expressed as the average of the left and right foot thickness. This was used to determine the degree of foot swelling.

[00371] Foot Volume Measurement was performed as follows. Measure the left and right hindfoot volume of the rat with the foot-plantar swelling measuring instrument, firstly, calibrate the instrument, after calibration, carry out accurate measurement, press the “Zero” button, immerse the animal’s paw into the pool, mark the place where the paw is first immersed with a

marker pen, which can reduce the error when it is immersed for the second time, step on the pedal switch, after freezing the data. Record the data.

[00372] hematoxylin-eosin staining

[00373] After the experiment was completed, the ankle joints of rats were taken and fixed with 4% paraformaldehyde for 48 hours. The ankle joints were put into a decalcification bucket, and EDTA (10%) decalcification solution was added, the decalcification solution was changed every three days and the degree of decalcification was observed, and the ankle joint tissues were dissected longitudinally when the samples could be pricked with a needle to accelerate the progress of softening. Afterwards, the samples were gradient dehydrated with different concentrations of ethanol solution, transparent with xylene, embedded in paraffin, sectioned, stained with hematoxylin and eosin sequentially, and then sealed for microscopic observation and comparison of synovial hyperplasia and inflammatory cell infiltration of ankle joints in each group.

[00374] Enzyme-linked immunoassay (ELISA) for cytokines

[00375] The levels of TNF-α, IL-1β inflammatory factors were detected separately after taking homogenates of rat synovial tissue according to the kit instructions.

[00376] Experimental results

[00377] Effect on body weight and arthritis score of rats

[00378] The results, as shown in Figure 24 showed that after type II collagen modelling, the rats' body mass was reduced, and the toes successively appeared red and swollen, and the joints were enlarged, leading to difficult walking, indicating that the CIA rat model was successfully established. The body mass and arthritis scores of the rats were monitored during continuous administration of the drug, and the results showed that with the prolongation of the administration time, the positive drug group and the Phyto-N group improved the body mass of the arthritic rats and significantly reduced the arthritis scores.

[00379] The effect on rat foot and degree of paw swelling

[00380] The results are shown in Fig. 25. All groups of rats showed an increase in foot thickness and foot volume after modelling, except for the blank group of rats. But after the intervention of the positive drug and Phyto-N, both groups showed a significant decrease in foot thickness and foot volume.

[00381] Histopathological morphology of the ankle joint

[00382] The results are shown in Figure 26, the structure of the ankle joint of rats in the blank group was clear and intact, the surface of the articular cartilage was smooth, and there was no obvious infiltration of inflammatory cells in the tissues; compared with the blank group, there was obvious destruction of the cartilage and bone tissues of the ankle joint of the rats in the model group, and a large number of inflammatory cells appeared in the tissues, and the treatment with the positive drug and Phyto-N led to the subsidence of the swelling, the significant reduction of inflammatory cell infiltration, and the marked decrease in the destruction of the cartilage and bone.

[00383] Effect of pro-inflammatory cytokine levels

[00384] The results are shown in Figure 27. Compared with the blank group, the levels of IL-1β and TNF-α in the synovial tissue of rats in the model group were significantly higher ($P<0.01$); compared with the rats in the model group, the levels of IL-1β and TNF-α in the synovial tissue of the rats in the positive drug group and the Phyto-N drug group were significantly lower ($P<0.01$).

[00385] Experimental summary

[00386] Rheumatoid arthritis is a systemic, refractory autoimmune disease with non-infectious inflammation of small joints and periarticular tissues throughout the body. Joint swelling and pain and activity limitation are seen in the early stage, followed by joint dysfunction in the middle and late stages, followed by stiffness and deformity, and even disability and loss of labor. Synovial hyperplasia leads to bone damage and cartilage degradation, resulting in impaired joint function is one of the most important pathological manifestations of rheumatoid arthritis. Our experimental results showed that *Wikstroemia indica* extract had a better joint protective effect on collagen-induced rheumatoid arthritis rats, which could significantly reduce the incidence of arthritis, foot swelling and arthritis scores, meanwhile, the pathological results showed that the synovial inflammation was reduced, the cartilage area was increased, the inflammatory reaction of the paw was reduced, and the expression of pro-inflammatory cytokines, such as IL-1β, TNF-α, was significantly reduced, indicating that *Wikstroemia indica* extract is an anti-inflammatory and anti-rheumatoid arthritis treatment.

[00387] Example 6: *Wikstroemia indica* extract can treat type 2 diabetes mellitus

[00388] This example demonstrates the efficacy and pharmacodynamics of anti-diabetes mellitus *Wikstroemia indica* extract (also referred to as Phyto-N) in streptozotocin-induced

(STZ) combined with high-sugar- and high-fat diet-induced diabetic SD rats by biochemical analysis and histomorphological observation.

[00389] Materials and methods

[00390] Modeling, grouping and administration of diabetes mellitus animal model

[00391] The study employed 8-week-old male SD rats weighing around 180 g - 200 g (n=120). Animals were exposed to standard conditions of 12-hours light/dark cycle, 50% - 60% relative humidity, 22 °C - 24 °C room temperature and provided with standard food pellets and water ad libitum allowing proper acclimatization to the laboratory conditions for 1 week.

[00392] Rats were randomly divided into two groups: control (n=30) and diabetic model (n=90), they were respectively fed with common diet and high-fat and high-sugar diet (10% lard, 20% sucrose, 2.5% cholesterol, 0.5% sodium cholate, 67% conventional feed) for 4 weeks. Rats in diabetic model group were induced by single dose of 20 mg/kg streptozotocin (STZ, made by 0.1 mol/L icy citrate buffer preparation with pH4.2, the concentration is 1%. The solution should be freshly prepared just before use, keep away from light and injection within 20 min) intraperitoneal injection after 15 hours fasting for 12 hours. After 2 hours of STZ injection, rats were supplemented with diet and glucose solution to prevent hypoglycemia. Control group was intraperitoneal injection citrate buffer under the same conditions. Injection of STZ was repeated for the next two days. Blood glucose after an 8-hour daytime fast were measured daily for several consecutive days since the 7th day after the last injection until diabetes was observed. Animals with fasting glucose above 11.1 mmol/L on 3 consecutive days will be considered diabetic. If the blood glucose was not up to the standard, repeated injection of STZ solution once according to the above conditions.

[00393] After successful modeling, the diabetic rats were randomly divided into three groups: model group, metformin group and phyto-N group, 30 rats in each group. The Metformin group and phyto-N group were provided intragastric administration with 0.2 g/kg/d Metformin and 1.8 g/kg/d phyto-N, respectively. The control group and model group were provided intragastric administration with saline of equal volume. Administration was performed once a day and each group was fed a common diet for 3 months. During this period, fasting blood glucose and body weight were measured weekly.

[00394] 1.2 Biochemical test

[00395] The blood was taken from the tail tip and the fasting plasma glucose (FPG) was measured by a blood glucose meter. Oral glucose tolerance (OGTT) and insulin tolerance (ITT) were measured at the end of the administration. The animals were sacrificed after indicators finished, blood samples were collected for biochemical analysis. Glycated hemoglobin (HbA1c) and fasting serum insulin (FSIN) were measured by ELISA and the insulin sensitivity index (ISI) and insulin resistance index (HOMA-IR) were calculated. Serum Alanine transaminase (ALT), aspartate transaminase (AST), triglyceride cholesterol (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), high density lipoprotein cholesterol (LDL-C), serum creatinine (CRE), uric acid (UA) and urea nitrogen (BUN) were measured by automatic biochemical analysis.

[00396] Histomorphological observation

[00397] Pancreases, livers and kidneys were collected after rats were sacrificed, rinsed in icy physiological saline, and weighed after sucking up solution. The organ and fixed in 4% formaldehyde buffer for more than 24 hours. After being embedded in paraffin, the tissue were cut into 5 μm thick sections, and stained with H&E stain for photomicrograph observation.

[00398] Results

[00399] Effects of Phyto-N on body weight of type 2 diabetic rats

[00400] The body weight of each diabetic group was significantly decreased as compared with the control group ($P<0.01$), but the metformin and treatment group were heavier than the model group, and the difference was significant in the treatment group ($P<0.05$). Results are shown in FIG. 28.

[00401] Effects of Phyto-N on fasting blood glucose of type 2 diabetic rats

[00402] The fasting blood glucose concentration of the model group was significantly increased as compared with the control group ($P<0.01$), and the indicator in treatment group was significantly decreased as compared with the model group ($P<0.01$) and the metformin group ($P<0.01$). Results are shown in FIG. 29.

[00403] Effects of Phyto-N on glycosylated hemoglobin (HbA1c) concentration of type 2 diabetic rats

[00404] The HbA1c concentration in the diabetic groups was significantly increased as compared with control group ($P<0.05$, $P<0.01$), while metformin and phyto-N could decrease the

Hba1c level of diabetic rats, and the change was significant in treatment group compared with the model group ($P<0.05$). Results are shown in FIG. 30.

[00405] Effects of Phyto-N on insulin concentration of type 2 diabetic rats

[00406] The fasting serum insulin concentration in the model group and metformin group were significantly increased as compared with control group ($P<0.05$), while there was no significant difference in the treatment group. Results are shown in FIG. 31.

[00407] Effects of Phyto-N on OGTT and ITT of type 2 diabetic rats

[00408] The peak of oral glucose tolerance test (OGTT) curve and the area under the curve (AUC) in all diabetic groups were significantly higher than those in control group ($P < 0.01$). Compared with the model group and the metformin group, the AUC of OGTT curve in the treatment group was significantly decreased ($P < 0.01$, $P < 0.05$), and the peak blood glucose level was significantly lower than that in the metformin drug group ($P < 0.05$). Results are shown in FIG. 32A-B.

[00409] After the injection of insulin, the blood glucose of the rats in the control group decreased slowly, and gradually recovered after 60 min of injection, during which the blood glucose remained at a normal level. The blood glucose level in model group and metformin group increased slowly in 30 min after insulin injection, and then decreased rapidly, and the blood glucose level and the area under insulin tolerance test (ITT) curve at each time point were significantly higher than those in control group ($P < 0.01$). Although the results in the treatment group was also significantly higher than that in the control group at all time points, the treatment group was decreased as compared with the model group and the metformin group, and the AUC of ITT curve was significantly lower than that in the above two groups ($P < 0.05$). Results are shown in FIG. 33A-B.

[00410] Effects of Phyto-N on HOMA-IR and HOMA-IS of type 2 diabetic rats

[00411] Compared with the control group, HOMA-IR was significantly increased ($P<0.01$) and HOMA-IS was significantly decreased ($P<0.01$) in the model group. Compared with model group, HOMA-IR was significantly decreased ($P<0.05$) and HOMA-IS was significantly increased ($P<0.01$) in the treatment group. Results are shown in FIG. 34A-B.

[00412] Effects of Phyto-N on blood lipids levels of type 2 diabetic rat

[00413] The levels of triglyceride (TG), cholesterol (CHOL), high density lipoprotein cholesterol (HDLC) and low density lipoprotein cholesterol (LDL-C) in the model group were

significantly increased when compared with the control group ($P<0.01$). These four indicators in treatment group were significantly decreased when compared with the model group. Results are shown in FIG. 35A-D.

[00414] The effects of Phyto-N on the pancreatic pathology of type 2 diabetic rats

[00415] In the control group, the pancreatic structure was intact, the islets were round or elliptical, and the cell profile was regular and neat. In the model group, the pancreatic tissue structure was disordered, the islet cells atrophied, the outline was not clear, and pancreatic acinar atrophied. Compared with the model group, the islet cells in the metformin group were clearer, and the islet cells in the treatment group recovered significantly. Results are shown in FIG. 36.

[00416] Effects of Phyto-N on liver function and structure of type 2 diabetic rat

[00417] As show in FIG. 37A-B, HFD feeding combined with STZ injection increased levels of AST ($P<0.01$) and ALT ($P<0.01$), which are indicators of liver functional injuries. Metformin and Phyto-N could improve liver functions by decreasing levels of AST and ALT ($P<0.05$), and the results of phyto-N were better than positive drug.

[00418] FIG. 38A shows the non-portal vein area. In the control group, the liver tissue cells were arranged neatly with clear nuclei. In the model group, large area of vacuolar fat droplets appeared in liver cells; in the treatment group, only a small amount of intracellular fat droplets existed, showing a significant improvement effect. FIG. 38B shows the portal vein area. Except for the model group, other groups showed different degrees of inflammatory cell infiltration.

[00419] Effects of Phyto-N on renal function and structure of type 2 diabetic rat

[00420] The levels of UREA and CREA in the model group increased significantly as compared with the control group ($P < 0.01$). The UREA and CREA concentration of treatment group were decreased significantly as compared with the model group ($P < 0.01$), and the result of CREA was better than the metformin group ($P < 0.01$). Results are shown in FIG. 39A-B.

[00421] FIG.40A-B shows the cortical area (FIG. 40A) and medulla area (FIG. 40B) of the kidney, respectively. In the control group, the structure of glomeruli and renal tubules was intact with normal volume and no basal membrane thickening. In the model group, large adipose deposit was observed, and the basal membrane of renal tubules was thickened and necrotic. In the treatment group, intracellular adipose deposit was significantly reduced, and the basal membrane thickening was improved, and the metformin group was slightly improved compared with the model group.

[00422] Conclusion

[00423] The results obtained from this study support the antidiabetic use of a *Wikstroemia indica* extract disclosed herein and show that it worked better than metformin. The extract could be used to decrease the blood glucose and lipids, improve insulin resistance, and protect the structure and function of the pancreas, liver, and kidneys. The extract could also be used as an adjunct in the management of diabetes mellitus.

[00424] Example 7: *Wikstroemia indica* extract can be used to treat gouty arthritis

[00425] This example demonstrates the protective effects of *Wikstroemia indica* extract (also referred to as Phyto-N) on gouty arthritis by observing the number of twists to acetic acid-induced pain, absentee activity time, ankle joint bound circumference, and biochemical indexes of gouty arthritis in each group of rats.

[00426] Experimental methods

[00427] Animal modeling, drug administration and grouping

[00428] 32 male SD rats of SPF grade were selected, and after 3 days of adaptive feeding (room temperature 22°C-24°C, humidity 50%-60%, normal feed, free-feeding), they were randomly divided into blank group, model group, positive drug group (colchicine 0.3 mg/kg), and Phyto-N (1.8 g/kg, equivalent to 20 g per day for adults), and were administered for 7 consecutive days, and on the seventh day, they were administered for 1 After 1 hour, the rats were injected intraperitoneally with 0.7% acetate saline solution according to their body weight, and the number of twisting of the rats within 15 minutes was observed and recorded to compare the differences between the groups.

[00429] After the rats in each group had recovered for one week, the drug was administered again for 7 days, and the body weight of the rats in each group was measured every day. One hour after the administration of the drug on the 6th day, the lower part of the right calf of the rats was shaved and sterilized with alcohol, and a sterile needle was used to aspirate 0.1 ml of 50 mg/ml sodium urate suspension and injected it into the ankle joints of the other three groups of rats, except the blank group, for the modeling of gouty arthritis.

[00430] Determination of joint swelling index in rats

[00431] Before modeling, a horizontal line was marked above the ankle joint of rats with an indelible marker, and the subsequent measurements were based on the horizontal line, and the circumference of the ankle joint was measured by the bounding line method before modeling and

2, 4, 6, 8, 10, 12, and 24 hours after modeling, respectively. Joint swelling index = (ankle circumference at measurement time - ankle circumference before modeling) / ankle circumference before modeling *100%.

[00432] The open field test experiment

[00433] The open field test was performed 24 hours after the gouty arthritis modeling, and the trajectory of the distance walked by the rats within 5 minutes was recorded to reflect the joint mobility. At the beginning of the test, the rats were placed in the central area of the experimental box, and the surrounding environment was kept quiet and dark, and the 5-minute activities of the rats were recorded by the animal movement tracking system in the open-field experimental box, and the tests were conducted between 9:00 a.m. and 5:00 p.m. The rats were placed in the central area of the experimental box and the surrounding environment was kept quiet and dark. At the end of each test, the rats were put back into the cage, the bottom and side walls of the chamber were disinfected, and the chamber was dried and odorless before the next test was conducted.

[00434] Biochemical indicators

[00435] Uric acid (Uric), creatinine (Crea), urea nitrogen (Urea), alanine aminotransferase (ALT) and azelaic aminotransferase (AST) were detected by biochemical kit to evaluate the protective effect of Phyto-N on liver and kidney, and the higher the value, the more serious the damage to liver and kidney.

[00436] Experimental results

[00437] Number of acetic acid-induced writhings

[00438] The analgesic effect of Phyto-N was expressed in terms of the number of twists after the rats were injected with acetic acid solution, and the pain index was proportional to the number of twists. The results are shown in the table below and Figure 41. Compared with the blank group, the number of twists in the model group was significantly increased ($P<0.01$). Compared with the model group, the number of twists in the positive drug group and Phyto-N group was significantly decreased ($P<0.01$), which indicated that Phyto-N had a better analgesic effect.

[00439] Table 8: Effects of Phyto-N on pain caused by rats (me±SD ; n=8)

Group	Writhing Times
Control	39.50± 3.34
Model	52.38 ± 6.39###
Positive	39.88 ± 6.19***
Phyto-N	40.00 ± 3.16***

Note: Compared with blank group, #*P*< 0.05, ##*P*< 0.01, ###*P*< 0.001; Compared with model group, **P*< 0.05, ***P*< 0.01, ****P*< 0.001.

[00440] Body weight and food intake

[00441] The body weight and intake of rats in each group for 7 days of drug administration are shown in FIG. 42A-B. After urate injection into the ankle joints of rats, the intake and body weight of rats in the model group, the positive drug group and the Phyto-N group decreased, and the intake and body weight of rats in the model group regressed on the next day, with the model group of rats regressing at a slower rate.

[00442] Results in the open field test

[00443] The efficacy of Phyto-N on urate-induced joint pain was reflected by the activity time of rats in the open field, and the voluntary activity of rats increased after the pain was relieved, i.e., the activity time of rats was proportional to the efficacy of Phyto-N. The results of the field experiment are shown in the table below and FIG. 43. Compared with the blank group, the activity time of the model group was significantly lower ($P < 0.001$); compared with the model group, the activity time of the positive drug group and Phyto-N group were significantly higher ($P < 0.001$), which indicated that Phyto-N could effectively treat the joint pain caused by uric acid.

[00444] Table 9: Activity time of rats in each group (mean±SD, n=8)

Group	Activity time (seconds)
Control	242.63± 13.73
Model	61.75± 13.18###
Positive	160.50 ± 17.48***
Phyto-N	156.75 ± 13.98***

Note: Compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; Compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[00445] Joint swelling index

[00446] The anti-inflammatory effect of Phyto-N on uric acid-induced arthritis was evaluated by measuring the circumference of the right ankle joint of rats in each group at 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 24 hours after urate modeling, and the anti-inflammatory effect of Phyto-N was inversely proportional to the circumference of the joint. The results of the detection of the degree of joint swelling in each group of rats are shown in the table below and FIG. 44A-H. Compared with the blank group, the joint swelling index of rats in the model group increased at all time periods ($P < 0.001$); compared with the model group, the degree of joint swelling in rats in the positive drug group was reduced at 4 hours, 6 hours, 10 hours, 12 hours, and 24 hours ($P < 0.05$ and $P < 0.01$); in the Phyto-N group The degree of joint swelling was reduced at all stages ($P < 0.05$, $P < 0.01$, $P < 0.001$), and the anti-inflammatory effect of Phyto-N was more obvious than that of colchicine.

[00447] Table 10: Swelling index of the ankle joint in all groups of rats each time period (mean±SD, n=8)

Group	2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	24 hours
Control	0.0578± 0.0136	0.0934± 0.0199	0.0576± 0.0206	0.0377± 0.0168	0.0189± 0.0165	0.0021± 0.0254	0.0053± 0.0179
Model	0.2161± 0.0323###	0.3105± 0.0364###	0.3671± 0.0376###	0.3823± 0.0568###	0.4022± 0.0552###	0.3586± 0.0631###	0.1384± 0.0243###
Positive	0.2075± 0.0232	0.2615± 0.0299**	0.3108± 0.0410**	0.3279± 0.0524	0.3086± 0.0488*	0.2774± 0.0525**	0.1132± 0.0237*
Phyto-N	0.1483± 0.0206***	0.2358± 0.0258***	0.2938± 0.0365***	0.3025± 0.0442*	0.2680± 0.0455**	0.2314± 0.0406***	0.0394± 0.0191***

Note: Compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; Compared with model group, *P< 0.05, **P< 0.01

[00448] Results of serum biochemical indexes in rats

[00449] Finally, uric acide, creatinine, urea, ALT and AST were detected in the serum of rats in each group to evaluate the toxic effects of Phyto-N on liver and kidney. The results are shown in the table below and FIG. 45A-E. Compared with the blank group, all five indexes in rats of the

model group increased ($P < 0.01$, $P < 0.001$). Compared with the model group, the above five indexes in rats of the positive drug group and the Phyto-N group decreased ($P < 0.05$, $P < 0.01$, $P < 0.001$).

[00450] Table 11: Results of serum biochemical indices in rats (mean±SD, n=8)

Group	Uric (μmol/L)	Urea (μmol/L)	Creatinine (μmol/L)	ALT (μmol/L)	AST (μmol/L)
Control	156.25±20.77	3.84±0.87	20.86±2.53	45.63±4.47	71.00± 6.44
Model	248.13±39.36$^{\#\#\#}$	5.68± 0.48$^{\#\#}$	27.88±3.31$^{\#\#\#}$	57.00±5.55$^{\#\#}$	86.63±8.40$^{\#\#}$
Positive	169.63±28.67***	4.86±0.45*	18.63±2.70***	44.00±4.21**	73.00±8.49**
Phyto-N	165.25±23.60***	5.01±0.24*	14.88±1.73***	42.25±7.76***	66.88±12.26***

Note: Compared with blank group, #P< 0.05, ##P< 0.01, ###P< 0.001; Compared with model group, *P< 0.05, **P< 0.01, ***P< 0.001.

[00451] Experimental summary

[00452] Due to the impaired purine metabolism in the human body, blood uric acid production is excessive or excretion is reduced, which in turn is saturated in the body for a long time and precipitates crystalline urate deposited in the joints, triggering gouty arthritis and causing disturbances in patients' behaviors and lives. The results show that a *Wikstroemia indica* extract can significantly increase the activity time, reduce the number of writhing and joint swelling during acetic acid analgesia in gouty arthritis model rats, which reflects a better analgesic and anti-inflammatory effect.

[00453] Example 8: *Wikstroemia indica* extract can be used to treat gouty nephritis

[00454] This example demonstrates the protective effects of a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) against gouty nephritis. Gouty nephritis is a metabolic disease caused by abnormal elevation of blood uric acid concentration due to purine metabolism disorder. Uric acid produced by the catabolism of nucleic acids in the organism is endogenous uric acid, and exogenous uric acid is mainly from the intake of purine-rich foods. Various enzymes such as adenosine deaminase (ADA) and xanthine oxidase (XOD) are produced in the liver to catalyze the final formation of uric acid from purines. The kidneys play a dominant role in the excretion of uric acid. When blood uric acid concentration is high, urate will precipitate crystals and be deposited in the interstitial parts of the renal tubules, which impairs

tubular function. The content of xanthine oxidase (XOD) and adenosine deaminase (ADA) in the liver and the content of SOD in the kidney were determined using kits; the content of uric acid (UA), creatinine (Cr) and urea nitrogen (BUN) in the serum of each group of mice was determined by a 24-item blood biochemistry reagent panel; the structural morphology of glomerulus and tubule and the degree of kidney fibrosis were observed using H&E staining of kidney and Mosson staining, respectively. The degree of renal fibrosis, and uric acid and albumin content in urine were measured using ELISA, and the inflammatory factors (IL-6, IL-18, IL-1β, NLRP3) in kidney were measured by immunohistochemistry to investigate the ameliorative effect of Phyto-N on renal inflammation.

[00455] Experimental drugs and materials

[00456] Table 12

Name	**Product number**	**Factory**
Potassium oxonate	HY-17511	MedChemExpress
Adenine	HY-B0152	MedChemExpress
Febuxostat	H20130058	Jiangsu Wanbang Biochemical
Blood Biochemistry Reagent Tray	9231404	Smuts
H&E staining kit	G1120	Solarbio
Mosson staining kit	G1340	Solarbio
XOD kit	A001-3-2	Nanjing Jiancheng Bioengineering Institute
ADA kit	A048-2-1	Nanjing Jiancheng Bioengineering Institute
SOD kit	A001-3-2	Nanjing Jiancheng Bioengineering Institute
TNF-α kit	MB-2868A	Jiangsu Meibiao Biotechnology
caspase-1 kit	MB-6205A	Jiangsu Meibiao Biotechnology
Sodium Citrate Antigen Retrieval Solution	E-BC-R115	Elabscience
PBS Buffer（10×）	E-BC-R187	Elabscience
IL-18 Polyclonal antibody	10663-1-AP	Proteintech
IL-1 Beta Polyclonal antibody	16806-1-AP	Proteintech
IL-6 Polyclonal antibody	21865-1-AP	Proteintech

NLRP3 Polyclonal antibody	19771-1-AP	Proteintech
Endogenous Peroxidase Blocking Buffer	E-IR-R115	Elabscience
Normal Goat Blocking Buffer	E-IR-R110	Elabscience
Antibody Dilution Buffer	E-IR-R106	Elabscience
DAB development kit	AR1027-3	Boster

[00457] Experimental Methods

[00458] Animal modeling, drug administration and grouping:

[00459] Sixty male KM mice of SPF grade were taken, and after 3 days of adaptive feeding (room temperature 20°C-25°C, humidity 60%-70%, free feeding), they were randomly divided into 4 groups: blank group, model group, positive drug group (febuxostat) 5.2 mg/kg, and Phyto-N 2.6 g/kg. Except for the blank group, each group was gavaged with adenine+potassium oxonate diluted with 5% CMC-Na (adenine 100 mg/kg+potassium oxonate 500 mg/kg) suspension every day for 3 consecutive weeks. suspension (adenine 100 mg/kg+potassium oxonate 500 mg/kg) diluted in 5% CMC-Na for 3 consecutive weeks, and the drug was administered while modeling. At the end of drug administration, urine was collected from all mice in metabolic cages.

[00460] General indicators

[00461] Closely observe the mental condition and food intake of the mice in each group every day. Weigh and record the body weight every 3 days.

[00462] Measurement of serum biochemical indexes

[00463] After the end of drug administration, fast for 12hours, perform orbital blood sampling, leave it for 2 hours, centrifuge it at 3000 r/min for 15 minutes, collect the upper layer of serum, and use biochemical reagent disk to determine the content of uric acid (UA), creatinine (Cr) and urea nitrogen (BUN) in the serum of mice.

[00464] Determination of renal index (RI) in mice

[00465] After blood sampling, mice were quickly placed on an ice table to separate their bilateral kidneys with sterilized scissors and forceps, filter paper was blotted dry and then the bilateral kidneys were weighed with an electronic analytical balance to calculate the renal index. The formula for calculating the organ index:RI = kidney weight/body weight (mg/g).

[00466] Detection of liver and kidney indexes

[00467] Xanthine oxidase (XOD) and adenosine deaminase (ADA) kit method was used to detect the content of xanthine oxidase and adenosine deaminase in the liver; superoxide dismutase (SOD) kit method was used to detect the content of oxidative dismutase in the kidneys, and inflammatory factor indexes (TNF-α, caspase-1) in the kidneys were determined using ELISA.

[00468] Renal pathologic observation

[00469] The left kidney was quickly placed in 4% paraformaldehyde solution for fixation, and then subjected to routine paraffin embedding and sectioning 24 hours later. H&E staining was used to observe the pathological changes of renal structure in each group of mice; Mosson staining was used to observe renal fibrosis; immunohistochemistry was used to determine the indexes of inflammatory factors (IL-6, IL-18, IL-1β, NLRP3) in the kidney.

[00470] Experimental results

[00471] Mouse body weight

[00472] Changes in mouse body weight are shown in FIG. 46A, before sampling, the body weight of the Model group was lower than that of the Control group, and the body weight of Phyto-N was higher than that of the Model group, which was comparable to that of the Positive group. The food intake of mice was shown in FIG. 46B, the food intake of the Model group was lower than the Control group, and Phyto-N food intake was higher than the Model group, which was comparable to the Positive group.

[00473] Results of double kidney weight and kidney index analysis

[00474] Comparison of bilateral kidney weights and renal indices of mice are shown in the table below and FIG. 47A-B. Compared with the Control group, the kidney weights ($P<0.01$) and indices of the Model group were significantly decreased ($P<0.05$). The bilateral kidney weights and renal indices of the Positive and Phyto-N groups were significantly higher than those of the Model group ($P<0.05$).

[00475] Table 13: Results of kidney weight and kidney index analysis in mice ($\bar{x}\pm S$)

Group	Kidney Weight (g)	Kidney index (%)
Control	0.50±0.04	11.99±1.03
Model	$0.36\pm0.11^{\#\#}$	$9.94\pm3.03^{\#\#}$
Positive	$0.43\pm0.10^{*}$	$11.97\pm0.91^{*}$

Phyto-N	0.42±0.11*	12.13±1.34*

Comparison with Control group, #P<0.05, ## P<0.01; Comparison with Model group,*P<0.05, **P<0.01

[00476] H&E staining of mouse kidney

[00477] As shown in Figure 48A-D, the glomeruli and tubules of mice in Control group had normal morphology, clear structure, neat cell arrangement and no inflammatory cell infiltration; the glomeruli of mice in Model group were wrinkled, the tubular lumen of renal tubules was enlarged, and the renal interstitium could be seen infiltrated by inflammatory cells. Compared with the Model group, the renal tubular epithelium of the Phyto-N group and the mice of the Positive group could be seen to be full of renal tubular epithelial cells. The distribution of inflammatory cells was reduced in the Phyto-N and Positive groups. The results showed that the Phyto-N group had a certain degree of protective effect on the kidney.

[00478] Mosson staining of mouse kidney

[00479] As shown in FIG. 49A-D, the glomeruli and renal interstitium of Control group mice did not show obvious changes, and no collagen fiber deposition was observed. The renal tubular epithelial cells of Model group mice could be seen to see the formation of vacuoles, and the tubular lumen was dilated to varying degrees, with sclerotic and atrophic glomeruli, and some of the glomeruli could be observed to have focal fibrotic changes, and the thylakoid zone and renal interstitium were blurred, and significant collagen fiber deposition could be observed, and the fibrosis was Focal fibrosis in mice in Phyto-N and Positive groups was significantly reduced compared with the model group, and collagen fiber deposition was improved to different degrees compared with the model group.

[00480] Results of serum index analysis

[00481] As shown in FIG. 50A-C, uric acid (UA), creatinine (Cr), and urea nitrogen (BUN) are important indicators of whether kidney function is impaired. The results of the study showed that the serum levels of UA, Cr, and BUN were significantly higher in the Model group compared with the Control group ($P < 0.01$). Compared with the Model group, the serum levels of UA and Cr in the Positive and Phyto-N groups were significantly lower than those in the Model group ($P < 0.01$), and the level of BUN was significantly lower than that in the Model group ($P < 0.05$), suggesting that the Phyto-N group had a uric acid-lowering effect.

[00482] Results of urine index analysis

[00483] As shown in Figure 51A-B, compared with the Control group, the content of uric acid and albumin in the urine of the Model group was significantly higher ($P < 0.01$). Compared with the Model group, the content of uric acid and albumin in the urine of the Positive and Phyto-N groups was significantly lower than that of the Model group ($P < 0.01$).

[00484] Results of analyzing liver XOD, ADA and kidney SOD activities in mice

[00485] As shown in FIG. 52A-B, XOD and ADA ($P<0.01$) were significantly increased in the liver of the Model group compared with the Control group. After Phyto-N intervention, the XOD and ADA content in the liver were significantly decreased ($P<0.01$), which was comparable to that of the Positive group.

[00486] As shown in Figure 52C, SOD activity ($P<0.01$) in the kidney was significantly decreased in the Model group compared with the Control group. After Phyto-N intervention, SOD activity in the kidney was significantly increased ($P<0.01$) and was comparable to the Positive group.

[00487] Results of analysis of inflammatory factors in mouse kidney

[00488] As shown in FIG. 53A-B, the renal inflammatory factors TNF-α and Caspase-1 ($P<0.01$) were significantly increased in mice in the Model group compared to the Control group; TNF-α and Caspase-1 ($P<0.01$) were significantly decreased in the Phyto-N group compared to the Model group, which was comparable to the Positive group.

[00489] Results of renal inflammatory factors analysis in mice

[00490] As shown in FIG. 54, FIG. 55A-D, and the table below, compared with the Control group, the renal inflammatory factors IL-6, IL-18, IL-1β and NLRP3 ($P<0.01$) were significantly increased in the Model group of mice; compared with the Model group, IL-6, IL-18, IL-1β and NLRP3 ($P<0.01$) in the Phyto-N and Positive groups were significantly decreased. This indicates that Phyto-N group could reduce the inflammatory response in the kidney.

[00491] Table 14: Results of analysis of average optical density of mouse kidney tissue ($\bar{x}\pm S$)

Group	IL-6	IL-18	IL-1β	NLRP3
Control	0.06±0.010	0.07±0.007	0.13±0.010	0.14±0.004
Model	0.13±0.005##	0.13±0.104##	0.21±0.026##	0.26±0.031##
Positive	0.08±0.007**	0.09±0.008**	0.16±0.025**	0.18±0.017**
Phyto-N	0.07±0.007**	0.09±0.006**	0.15±0.035**	0.19±0.010**

Comparison with Control group, #P<0.05, ## P<0.01; Comparison with Model group,*P<0.05, **P<0.01.

[00492] Summary

[00493] Gouty nephritis is mainly characterized by hyperuricemia accompanied by renal injury. Due to the disorder of purine metabolism within the human body, excessive production of blood uric acid or decreased excretion of blood uric acid, thus blood uric acid is saturated for a long period of time in the body, and subsequently, crystalline urates precipitated from uric acid are deposited in the kidneys, which causes damage to the kidneys and triggers gouty nephritis. Uric acid (UA), creatinine (Cr), and urea nitrogen (BUN) are important indicators for evaluating renal function, xanthine oxidase (XOD) and adenosine deaminase (ADA) are key enzymes in the process of uric acid production, and TNF-α, Caspase-1, IL-6, IL-18, IL-1β, and NLRP3 are important inflammatory factors in the body.

[00494] The results herein illustrate that a *Wikstroemia indica* extract disclosed herein can reduce UA ($P < 0.01$), Cr ($P < 0.01$), BUN ($P < 0.05$) in serum and XOD ($P < 0.01$), ADA ($P < 0.01$) in liver of mice, and can ameliorate the renal fibrosis, glomerular atrophy and tubular dilatation induced by potassium oxonate and adenine. Meanwhile, the extract resulted in the elevation of SOD activity and significant reduction of the levels of inflammatory factors TNF-α, Caspase-1, IL-6, IL-18, IL-1β and NLRP3 in the kidneys.

[00495] Example 9: Wikstroemia indica extract can be used to treat NAFLD

[00496] This example demonstrates the therapeutic effect of a *Wikstroemia indica* extract disclosed herein (also referred to herein as Phyto-N) on nonalcoholic fatty liver disease (NAFLD). The contents of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglyceride (TG) and total cholesterol (TC) in serum of mice in each group were determined with 24 blood biochemical reagents to observe the improvement effect of Phyto-N on NAFLD induced by high fat diet. Liver H&E staining and oil red O staining were used to observe the structure and morphology of liver cells and liver lobules, as well as the quantity and size of lipid droplets.

[00497] Experimental Materials

[00498] Experimental Drugs

[00499] Metformin (Lot No. ACG0153), produced by Bristol Myers Squibb.

Name	Lot number	Manufacturer
Gubra-Amylin NASH	D09100301	Readydietech
Blood glucose test strip	07124287	Roche
Blood glucose meter	06993788001	Roche
Blood biochemical reagent tray	9231404	Seamaty Technology
H&E staining kit	G1120	Solarbio
Improved oil red O staining kit	G1261	Solarbio

[00500] Experimental Methods

[00501] Experimental Animals

[00502] Forty male SPF C57BL/6 male mice, weighing 20±2g, were purchased from Beijing Huafukang Biotechnology Co., LTD., experimental animal production license No. SCXK (Beijing) 2019-0003. This animal experiment was approved by the Experimental Animal Ethics Committee of the Yunnan Branch of the Institute of Materia Medica, Chinese Academy of Medical Sciences, and the Animal Ethics approval number is 20230315008.

[00503] Animal Grouping

[00504] Forty male SPF grade C57BL/6 male mice (Ethics number) were fed adaptively for 7 days and randomly divided into 4 groups (10 mice per group) : Control group, Model group, Positive group (metformin) and Phyto-N group. The Control group was given ordinary diet, and the other groups were given Gubra-Amylin NASH(GAN) diet for continuous 22 weeks, drug intervention was given starting from the 22th week and GAN was fed a control diet. The dose of Positive group was 0.221 g/kg/d. The dose in the Phyto-N group was 3.9g/kg/d (equivalent to 30 g per day for adults).

[00505] General Indicators

[00506] The mental state and food intake of each group of mice were observed closely every day. Weight was weighed and recorded every 7 days, and fasting blood glucose was measured every 14 days.

[00507] Determination of serum biochemical indexes

[00508] After the end of administration, fasting for 12 h, orbital blood collection was performed for 2 hours, centrifugation at 3000 r/min for 15 minutes, upper serum was collected, and the contents of total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT) and

aspartate aminotransferase (AST) in serum of mice were determined by biochemical reagent tray. The contents of LDL-C and HDL-C in serum were determined by low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) kits.

[00509] Liver pathological observation

[00510] The same leaf of liver tissue was taken and divided into two parts. One portion is quickly fixed in 4% paraformaldehyde solution. Conventional paraffin embedding and section were performed 24 hours later. H&E staining was used to observe the liver lobular structure and the pathological changes of liver cell morphology in each group. One portion was frozen at -80°C, then coated with optimal cutting temperature compound OCT embedding agent and sliced by frozen microtome, and then oil red O staining was used to observe the changes of liver fat.

[00511] Experimental Results

[00512] Analysis of trends of body weight and blood glucose in mice

[00513] As shown in FIG. 56A, the weight of mice in the Model group increased more rapidly than Control group after the mice were fed high-fat diet. After the administration of metformin and Phyto-N, weight loss began in the Phyto-N and Positive groups. Before sampling, the body weight of Model group was significantly higher than Control group ($P<0.01$), and the body weight of Phyto-N group was significantly lower than the Model group ($P<0.05$) (FIG. 56C).

[00514] The blood glucose changes of mice are shown in FIG. 56. One week before sampling, the blood glucose of Model group was significantly higher than Control group ($P<0.01$), and Phyto-N group was significantly lower than Model group ($P<0.01$) (FIG. 56D), which was similar to Positive group.

[00515] Analysis results of mouse liver appearance, liver weight and liver index

[00516] As shown in FIG. 57A, the liver in the Control group was dark red in color and normal in volume. In Model group, the liver became lighter in red, white and reflective in some areas, and the liver showed a sense of smoothness. After Phyto-N intervention, the liver color returned to deep red and the greasy sensation disappeared. Liver weight and liver index pairs of mice were shown in the table below and FIG. 57B-C. Compared with Control group, liver weight of Model group was significantly increased ($P<0.05$). The Positive and Phyto-N groups showed a downward trend. Compared with Control group, liver coefficient in Model group was significantly increased ($P<0.01$), but decreased in the Positive control ($P<0.05$) and Phyto-N ($P<0.05$) groups.

[00517] Table 15: Analysis results of liver weight and liver index in mice ($\bar{x}\pm S$)

	Liver Weight (g)	Liver index (%)
Control	0.90±0.094	3.22±0.36
Model	1.66±0.37#	4.14±0.61##
Positive	1.20±0.14	3.54±0.25*
Phyto-N	1.31±0.12	3.60±0.27*

Comparison with Control group, #P<0.05, ## P<0.01; Comparison with Model group,*P<0.05, **P<0.01.

[00518] Mouse liver H&E staining

[00519] H&E staining results are shown in FIG. 58. In the Control group, the outline of liver cells was clearly visible, the cells were evenly distributed and neat, the nuclei were clearly colored, and no adipose vacuoles and inflammatory cell aggregation were observed. The hepatocytes of Model group mice were filled with a large number of white vacuoles, and the volume of liver cells was also significantly enlarged, with a few hepatocyte balloon-like changes. After the intervention of metformin and Phyto-N, the number of white vacuoles in mouse hepatocytes became significantly smaller, indicating that hepatic steatosis was inhibited, and the volume of hepatocytes was reduced, and occasionally vesicular fat vacuoles were observed without inflammation.

[00520] Mouse liver oil red O staining

[00521] The results of oil red staining in frozen sections of mouse liver tissue are shown in FIG. 59. The number of red fat droplets in liver cells of mice in the Control group was small, the volume was small, and the nucleus was obviously visible. The amount of red fat in Model group mice was significantly increased, the volume of fat droplets was enlarged, the color of oil red was bright and occasionally the nucleus was seen, indicating that the lipid deposition in liver cells was increased. After Phyto-N intervention, metformin reduced the number and volume of red lipid droplets and reduce the accumulation of fat in liver cells.

[00522] Results of serum index analysis

[00523] AST, ALT, ALP, TG and TC are important indicators to measure hepatic steatosis, and the results are shown in the table below and FIG. 60. Compared with Control group, serum AST (P<0.01), ALT (P<0.01) and TC (P<0.01) in Model group were significantly increased. Compared with the Model group, serum AST (P<0.05), ALT (P<0.01) and TC (P<0.05) in

Phyto-N group were significantly decreased, and were better than Positive group. Compared with the Control group, serum ALP and TG in the Model group had an upward trend, while serum ALP and TG in the Phyto-N group had a downward trend compared with the Model group, which was similar to Positive group.

[00524] Table 16: Analysis results of serum AST, ALT, ALP, TG and TC in mice ($\bar{x}\pm S$)

	AST (U/L)	ALT (U/L)	ALP (U/L)	TG (mmol/L)	TC (mmol/L)
Control	107.75±25.01	44.00±9.70	105.50±6.07	1.07±0.069	2.95±0.22
Model	165.56±37.62$^{\#\#}$	73.78±32.20$^{\#\#}$	117.44±20.40	1.18±0.17	5.68±0.56$^{\#\#}$
Positive	125.25±14.54**	48.88±20.02*	122.00±12.49	1.06±0.25	5.07±0.86
Phyto-N	132.56±22.50*	42.67±8.89**	111.33±15.84	1.03±0.16	4.85±0.66*

Comparison with Control group, #P<0.05, ## P<0.01; Comparison with Model group,*P<0.05, **P<0.01.

[00525] Summaries

[00526] The results show that:

[00527] (1) *Wikstroemia indica* extract reduced body weight and blood glucose in NAFLD mice.

[00528] (2) *Wikstroemia indica* extract reduced liver weight and hepatobody ratio, but neither was significant.

[00529] (3) *Wikstroemia indica* extract significantly reduced AST, ALT and TC, and improved the liver injury of high-fat diet-induced NAFLD mice. However, the effect of TG was not significant. It is postulated that the mouse feed was changed into the control feed of GAN.

[00530] In summary, *Wikstroemia indica* extract, on the basis of its preventive and therapeutic effect on GAN diet-induced NAFLD, combined with light diet, can treat NAFLD.

[00531] Example 10: A *Wikstroemia indica* extract can prevent non-alcoholic fatter liver disease

[00532] This example demonstrates the preventive effects of a *Wikstroemia indica* extract disclosed herein (also referred to as Phyto-N) on non-alcoholic fatty liver disease (NAFLD). Insulin resistance is an important factor in the occurrence and development of NAFLD. After insulin resistance occurs in the body, glucose in the blood will be converted into triglycerides and stored in the liver, which will then induce liver damage, which may lead to inflammation. Therefore, glucose tolerance and insulin resistance were evaluated by OGTT and ITT. The

contents of TC and TG in liver were determined by the kit, and the contents of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglyceride (TG) and total cholesterol (TC) in serum of each group were determined by 24 blood biochemical reagents and the contents of low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined by the kit to observe the effect of Phyto-N on the improvement of NAFLD induced by GAN diet. Liver H&E staining and oil red O staining were used to observe the structure and morphology of liver cells and liver lobules, as well as the quantity and size of lipid droplets. Inflammatory factors (IL-6, IL-β and TNF-α) in liver were determined by ELISA to observe the ameliorating effect of Phyto-N on liver inflammation.

[00533] Table 17 Experimental Drugs

Name	**Lot number**	**Manufacturer**
Metformin	ACG0153	Bristol Myers Squibb
Insulin	D545815C	Lilly France
Glucose	230427080	——

[00534] Table 18: Experimental Materials

Name	**Lot number**	**Manufacturer**
Gubra-Amylin NASH	D09100301	Readydietech
Blood glucose test strip	07124287	Roche
Blood glucose meter	06993788001	Roche
Blood biochemical reagent tray	9231404	Seamaty Technology
H&E staining kit	G1120	Solarbio
Improved oil red O staining kit	G1261	Solarbio
HDL-C kit	ADS-W-D011	AIDISHENG
LDL-C kit	ADS-W-D012	AIDISHENG
TC kit	ADS-W-ZF014	AIDISHENG
TG kit	ADS-W-ZF013	AIDISHENG
IL-1β kit	MB-2776A	Jiangsu Meibiao Biotechnology
TNF-α kit	MB-2868A	Jiangsu Meibiao Biotechnology
IL-6 kit	MB-2899A	Jiangsu Meibiao Biotechnology

[00535] Experimental Methods

[00536] Experimental Animals

[00537] Forty male SPF C57BL/6 male mice, weighing 20±2g, were purchased from Beijing Huafukang Biotechnology Co., LTD., experimental animal production license No.: SCXK (Beijing) 2019-0003. This animal experiment was approved by the Experimental Animal Ethics Committee of the Yunnan Branch of the Institute of Materia Medica Chinese Academy of Medical Sciences, and the Animal Ethics approval number is 20230315008.

[00538] Animal Grouping

[00539] Forty male SPF grade C57BL/6J male mice were fed adaptively for 7 days and randomly divided into 4 groups (10 mice per group Control group, Model group, Positive control group (metformin) and Phyto-N group. The Control group was given ordinary diet, and the other groups were given Gubra-Amylin NASH(GAN) diet for continuous 22 weeks, and drug intervention was given starting from the 4th week. The dose of Positive group was 0.221g/kg/d. The dose in the Phyto-N group was 3.9g/kg/d (equivalent to 30g per day for adults).

[00540] General Indicators

[00541] The mental state and food intake of each group of mice were observed closely every day. Weight was weighed and recorded every 7 days, and fasting blood glucose was measured every 14 days.

[00542] Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

[00543] In the final treatment week, OGTT and ITT were performed respectively, as follows: (1) OGTT: After fasting 15 hours, blood glucose levels were measured and defined as 0min time point blood glucose. All mice were given 2g/kg of glucose orally, and blood glucose concentrations were measured at 30, 60, and 120 minute time points. (2) ITT: blood glucose detected after fasting for 6 hours, defined as blood glucose at 0min time point. All mice were then given intraperitoneal injections of 0.5U/kg insulin, and blood glucose concentrations were measured at 15, 30, 60, and 120 minute time points after administration.

[00544] Determination of serum biochemical indexes

[00545] After the end of administration, fasting for 12 hours, orbital blood collection was performed for 2 hours, centrifugation at 3000r/min for 15 minutes, upper serum was collected, and the contents of total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT) and

aspartate aminotransferase (AST) in serum of mice were determined by biochemical reagent tray. The contents of LDL-C and HDL-C in serum were determined by low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) kits.

[00546] Liver index detection

[00547] Total cholesterol (TC) and total triglyceride (TG) kit were used to detect the contents of cholesterol and triglyceride in liver. Inflammatory cytokines (IL-6, IL-1, TNF-α) in the liver were determined by ELISA.

[00548] Liver pathological observation

[00549] The same leaf of liver tissue was taken and divided into two parts. One portion is quickly fixed in 4% paraformaldehyde solution. Conventional paraffin embedding and section were performed 24 hours later. H&E staining was used to observe the liver lobular structure and the pathological changes of liver cell morphology in each group. One portion was frozen at -80°C, then coated with OTC embedding agent and sliced by frozen microtome, and then oil red O staining was used to observe the changes of liver fat.

[00550] Experimental Results

[00551] Analysis of trends of body weight and blood glucose in mice

[00552] As shown in Figure 61A, the weight of mice in the Model group increased more rapidly than that in the Control group after the mice were fed high-fat diet. After administration of metformin and Phyto-N, the Phyto-N and Positive group gained weight at a much lower rate than the Model group (Figure 61A). Before sampling, the body weight of Model group was significantly higher than Control group ($P<0.01$), and the body weight of Phyto-N was significantly lower than that of Model group ($P<0.01$), which was similar to the Positive control group (FIG. 61C).

[00553] The changes of blood sugar in mice were shown in Figure 61B. Starting from the eighth week, the blood sugar in the Model group increased significantly faster than that in the other three groups (Figure 61B). Two weeks before sampling, blood glucose in Model group was significantly higher than Control group ($P<0.01$), and that Phyto-N group was significantly lower than Model group ($P<0.01$), which was better than Positive group (FIG. 61D).

[00554] Analysis results of glucose tolerance and insulin sensitivity of mice in each group

[00555] In OGTT, the blood glucose of the Model group was higher than that of the other three groups at 0min, which was the fasting blood glucose value. After gavage of glucose, the blood

glucose of mice in each group increased rapidly and reached the maximum value at 30min. After 30min, the blood glucose of the four groups of mice gradually decreased, and the blood glucose of the Phyto-N group of mice was close to that Control group (Figure 62A). By calculating the area under OGTT curve (AUC), the AUC of Model group was significantly higher than Control group ($P<0.01$), indicating that the glucose tolerance of model mice was decreased. Compared with the Model group, AUC in Phyto-N group was significantly decreased ($P<0.01$), indicating that Phyto-N improved the glucose tolerance of mice fed a high-fat diet (table below, Figure 62C), which was better than the Positive group.

[00556] In ITT, the fasting blood glucose of mice in each group was very similar. After intraperitoneal injection of insulin, the blood glucose of mice in the four groups began to decrease, the blood glucose dropped to the lowest level at 60min, and the blood glucose began to recover after 60min (Figure 62B). ITT AUC in the Model group was higher than Control group ($P<0.05$), indicating that the insulin sensitivity of model mice was decreased. Compared with the Model group, AUC in the Phyto-N group was significantly decreased ($P<0.05$), which was similar to Positive group, indicating that Phyto-N improved insulin sensitivity in GAN diet-induced NAFLD mice (table below, Figure 62D).

[00557] Table 19: Area under curve (AUC) analysis results of OGTT and ITT in mice ($\bar{x}\pm S$)

	OGTT (mmol/L*min)	ITT (mmol/L*min)
Control	1286.75±124.95	567.50±122.34
Model	1983.75±429.08##	704.74±61.70#
Positive	1466.75±290.46	546.30±99.64*
Phyto-N	1283.25±83.01**	461.03±119.87*

[00558] Analysis results of mouse liver appearance, liver weight and liver index

[00559] As shown in Figure 63A, the liver in the Control group was dark red in color and normal in volume. In the Model group, the liver became lighter in red, white and reflective in some areas, and the liver showed a sense of smoothness. After Phyto-N intervention, the liver color returned to deep red and the greasy sensation disappeared. As shown in the table below and Figure 63B-C, liver weight ($P<0.05$) and liver index ($P<0.05$) in Model group were significantly increased compared with Control group. Liver weight in the Positive control group was

significantly lower than the Model group ($P<0.05$). Liver weight ($P<0.05$) and liver index ($P<0.05$) in Phyto-N group were significantly lower than the Model group.

[00560] Table 20: Analysis results of liver weight and liver index in mice ($\bar{x}\pm S$)

	Liver Weight (g)	Liver index (%)
Control	0.94±0.066	3.14±0.18
Model	1.75±0.43$^{\#}$	4.54±0.73$^{\#}$
Positive	1.10±0.17*	3.54±0.23
Phyto-N	1.08±0.15*	3.49±0.30*

[00561] Mouse liver H&E staining

[00562] H&E staining results were shown in Figure 64. In the Control group, the outline of liver cells was clearly visible, the cells were evenly distributed and neat, the nuclei were clearly colored, and no adipose vacuoles and inflammatory cell aggregation were observed. The hepatocytes of Model group mice were filled with a large number of white vacuoles, and the volume of liver cells was also significantly enlarged, with a few hepatocyte balloon-like changes. After the intervention of metformin and Phyto-N, the number of white vacuoles in mouse hepatocytes became significantly smaller, indicating that hepatic steatosis was inhibited, and the volume of hepatocytes was reduced, and occasionally vesicular fat vacuoles were observed without inflammation.

[00563] Mouse liver oil red O staining

[00564] The results of oil red staining in frozen sections of mouse liver tissue were shown in Figure 65. The number of red fat droplets in liver cells of mice in the Control group was small, the volume was small, and the nucleus was obviously visible. The amount of red fat in Model group mice was significantly increased, the volume of fat droplets was enlarged, the color of oil red was bright and occasionally the nucleus was seen, indicating that the lipid deposition in liver cells was increased. After Phyto-N and metformin intervention, can reduce the number and volume of red lipid droplets and reduce the accumulation of fat in liver cells.

[00565] Results of serum index analysis

[00566] AST, ALT and ALP are important indexes to measure hepatic steatosis. TC and TG are important indicators to measure blood lipid levels, and the results are shown in the table below and Figure 66. Compared with the Control group, serum AST ($P<0.05$), ALT ($P<0.01$),

TC ($P<0.01$) and TG ($P<0.05$) in the Model group were significantly increased. Compared with the Model group, serum AST ($P<0.05$), ALT ($P<0.05$), TC ($P<0.05$) and TG ($P<0.01$) in the Phyto-N group were significantly decreased, and were better than the Positive control group. Compared with Control group, the serum ALP in the Model group had an upward trend, and the serum ALP in the Phyto-N group was significantly decreased compared with Model group ($P<0.01$), which was better than the Positive control group. It can be seen that the lipid level of mice in the Model group was abnormal and the liver function was damaged. The administration of Phyto-N improved the lipid level and liver function of mice.

[00567] Table 21: Analysis results of serum AST, ALT, ALP, TG and TC in mice ($\bar{x}\pm S$)

	AST (U/L)	ALT (U/L)	ALP (U/L)	TG (mmol/L)	TC(mmol/L)
Control	107.80±27.79	42.80±6.21	99.00±17.71	1.34±0.20	3.06±0.21
Model	240.90±106.76$^{\#}$	185.80±90.50$^{\#\#}$	107.20±33.36	1.65±0.18$^{\#}$	4.42±0.38$^{\#\#}$
Positive	153.85±28.87	72.00±34.39*	95.15±9.14	1.23±0.32**	4.14±0.44
Phyto-N	134.50±55.63*	64.43±27.76*	81.00±26.94**	1.05±0.18**	4.05±0.46*

[00568] Analysis results of LDL-C and HDL-C in mouse liver

[00569] The contents of LDL-C and HDL-C in mouse liver tissue were measured as shown in Figure 67. Compared with the Control group, LDL-C in liver of the Model group was significantly increased ($P<0.01$). After Phyto-N intervention, LDL-C content in liver was significantly decreased ($P<0.01$), which was better than Positive group. (table below, Figure 67A). Compared with the Control group, HDL-C in liver of the Model group was significantly increased ($P<0.05$). After Phyto-N intervention, the HDL-C content in liver showed a decreasing trend, which was similar to Positive group. (table below, Figure 67).

[00570] Table 22: Analysis results of LDL-C and HDL-C in mouse liver ($\bar{x}\pm S$)

	LDL-C (mmol/L)	HDL-C (mmol/L)
Control	2.40±2.21	5.29±0.51
Model	8.35±1.17$^{\#\#}$	6.71±0.17$^{\#}$
Positive	5.35±2.85	5.47±1.03
Phyto-N	2.27±2.08**	5.58±0.78

[00571] Analysis results of TC and TG in mouse liver

[00572] TG in the Model group increased significantly compared with the Control group ($P<0.01$), while TC in model group increased significantly compared with the Control group. This suggests that excessive accumulation of liver TG is the main feature of GAN diet. Compared with Model group, TG content in liver in Phyto-N group was significantly decreased ($P<0.01$), which was similar to that in Positive group. The results are shown in Figure 68 and the table below.

[00573] Table 23: Analysis results of TC and TG in mouse liver ($\bar{x}\pm S$)

	TC (μg/g)	TG (μg/g)
Control	153.78±17.72	4664.94±421.77
Model	182.85±42.65	6136.76±1013.53$^{\#\#}$
Positive	169.32±39.26	4850.37±925.46**
Phyto-N	181.60±38.46	4297.75±694.42**

[00574] Analysis results of liver inflammatory factors in mice of each group

[00575] The inflammatory factors were shown in the table below and Figure 69. Compared with the Control group, the liver inflammatory factors IL-1β ($P<0.05$), IL-6 ($P<0.01$) and TNF-α ($P<0.01$) in the Model group were significantly increased. Compared with the Model group, IL-1β ($P<0.05$), IL-6 ($P<0.01$) and TNF-α ($P<0.05$) in Phyto-N group were significantly decreased, which was similar to the Positive control group.

[00576] Table 24: Analysis results of liver inflammatory factors in mice ($\bar{x}\pm S$)T

	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
control	79.89±11.83	112.61±17.25	446.48±53.52
model	100.80±17.87$^{\#}$	151.32±12.16$^{\#\#}$	581.83±28.99$^{\#\#}$
Positive	71.83±17.10*	121.68±20.44**	515.49±36.56**
Phyto-N	83.17±12.84*	123.68±16.51**	529.47±38.56*

[00577] Summaries

[00578] NAFLD refers to a pathological syndrome characterized by excessive lipid deposition in liver cells, which is caused by alcohol and other specific liver damaging factors (mainly including drugs, viral infections, autoimmunity, etc.). The pathogenesis is complex, the most

classic is the "second blow theory", obesity and insulin resistance as the "first blow" induced the accumulation of fat in liver cells, leading to the development of a healthy liver into simple fatty liver, at this time the liver only lipid accumulation, no inflammation. If the accumulated fat causes the liver to become overburdened, it can cause a host of problems including inflammation, endoplasmic reticulum stress, oxidative stress and mitochondrial dysfunction, and deliver a "second blow" to the liver, leading to steatohepatitis and cirrhosis.

[00579] Weight is an important index to measure obesity. OGTT and ITT evaluated whether the mice developed insulin resistance. ALT and AST are important indicators of liver function, and the former is mainly distributed in liver cytoplasm, reflecting the function of liver cell membrane. The latter is mainly distributed in hepatocyte plasma and hepatocyte mitochondria, reflecting the physiological function of liver organelles, and the elevation of both indicates liver injury. TG, TC, LDL-C and HDL-C are the gold standard of blood lipids, and the increase of TG, TC and LDL-C and the decrease of HDL-C are the important manifestations of hyperlipidemia. IL-1β, IL-6 and TNF-α are important pro-inflammatory factors.

[00580] Our experiments showed that a *Wikstroemia indica* extract improved body weight, hepato-body ratio and insulin resistance in GAN diet mice. The contents of ALT ($P<0.05$), AST ($P<0.05$), TG ($P< 0.01$), TC ($P<0.05$) and LDL-C ($P<0.01$) in serum and TG ($P< 0.01$) in liver of NAFLD mice were decreased. It can improve liver hypertrophy, histopathological changes and abnormal lipid deposition caused by GAN. Meanwhile, the extrac\ decreased the contents of IL-1β ($P < 0.05$), IL-6 ($P < 0.01$) and TNF-α ($P < 0.05$) in liver of GAN diet-induced NAFLD mice, effectively preventing the deterioration of NAFLD. These results were similar to those reported in the current NAFLD literature, but TC levels in blood and liver did not change significantly before and after treatment with the *Wikstroemia indica* extract and positive metformin, which was consistent with the data of HDL-C experiment, which did not change significantly before and after treatment with *Wikstroemia indica* extract and positive metformin. This is because LDL-C is mainly responsible for loading lipids and transporting them from the liver to the periphery, while HDL-C mainly loads cholesterol from the periphery to the liver for metabolism and then excreted from the intestine, so there is no significant change in TC before and after administration. However, increased expression of LDL-C will lead to increased TG in the blood, and the LDL-C content is significantly reduced after administration, thus improving the TG deposition in the blood and liver of NAFLD mice induced by high fat diet. Compared with the

previous parallel experiment, liver H&E staining in this experiment showed weaker treatment effect of a *Wikstroemia indica* extract disclosed herein, which may be due to too long modeling time. In conclusion, *Wikstroemia indica* extract has a significant preventive effect on GAN diet-induced NAFLD.

[00581] Example 11: *Wikstroemia indica* extract treats symptoms of COVID-19

[00582] This example demonstrates that a *Wikstroemia indica* extract disclosed herein (also referred to as "Phyto-N") is useful for treating symptoms of COVID-19. The severity of COVID-19 is a strong predictor of mortality. Therefore, preventing the progression of disease from mild to severe to critical is key to reducing morbidity and mortality. Vaccination is effective in reducing severity but the efficacy of vaccines against the new SARS-CoV-2 variant is diminished. Therefore, therapeutic and immunomodulatory drugs are being tested currently by several clinical studies, including the SOLIDARITY trial by the World Health Organization (WHO). Immune boosting through administration of non-antimicrobial small molecule may augment better treatment outcome for COVID-19 cases. As disclosed in this Example, the therapeutic effects of *Wikstroemia indica* extract on pneumonia and other disease pathologic sequelae caused by SARS-CoV-2 infection in a hamster model were evaluated by viral load assays and histopathology of infected animal tissues.

[00583] Materials

[00584] Virus propagation and titration and PFU calculation

[00585] Severe Acute Respiratory Syndrome- Corona Virus-2 (SARS-CoV-2; strain USA-WA1/2020) was obtained from BEI Resources (BEI Resources, Manassas, VA, USA). Virus propagation, virus titration and infectivity assays were performed using Vero E6 cells (ATCC, Manassas, MA, USA) as described previously (Ramasamy et al., 2022). SARS-CoV-2 was propagated by infecting the Vero E6 cell monolayer at a multiplicity of infection (MOI) of 0.2 in DMEM media (supplemented with 2% FBS). The culture supernatants after 48 hrs of viral infection were collected, centrifuged and filtered and stored at -80 °C. For virus titration and infectivity assay, Vero E6 cells were seeded onto 6-well plates at $5x10^5$ cells per well for 18-24 hours. The next day, an aliquot of virus supernatant was thawed at 37 °C and made 10-fold dilutions from 10^{-2} to 10^{-6}. The spent media from 6-well plates were aspirated and added with each virus dilutions at 400µL/well. The cells were incubated at 37°C for 1 hour. Unattached viruses were then removed by media aspiration, and the infected monolayers were overlaid with

a mixture containing equal amounts of 2X MEM with 8% FBS and 1.6% low-melting agarose. The plaques were visualized by staining with 0.2% crystal violet on the third day. All the experiments involving infectious SARS-CoV-2 were conducted in Biosafety level 3 facilities at Rutgers University as per approved standard operating procedures. Unless specified, all chemicals and reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

[00586] Golden Syrian hamster infection and Phyto-N treatment

[00587] Male Golden Syrian hamsters (*Mesocricetus auratus*) of 6-8 weeks of age were purchased from Envigo (Envigo Corporation, Denver, PA, USA). Hamsters were housed as two animals/cage and standard food, and water were given ad libitum throughout the experiment. Animals were acclimatized for seven days in the BSL3 facilities before infection. Bodyweight, food and water intake were monitored daily for each animal throughout the experiment. To investigate the effect of Phyto-N treatment during SARS-CoV2 infection, the experiment was performed in two different ways: 1) Hamsters were treated with Phyto-N (260 mg/ animal) by oral gavage (n=10) for one week before infection; or, 2) hamsters were infected with SARS-CoV-2 first and then treated with Phyto-N (260 mg/ animal) simultaneously for the duration of the study or hamster kept as no-drug/placebo (FIG. 70 and 71). All hamsters were infected with SARS-CoV-2 (10^3 PFU) via intranasal inoculation with 50µL inoculum prepared in sterile 1X PBS as described earlier (Ramasamy et al., 2022). Five hamsters from each group were euthanized at 4 and 7-days post-infection. The lungs, liver, spleen, kidney, heart and brain were harvested and weighed aseptically. A portion of all organs were transferred in 10% buffered formalin for histopathology analysis.

[00588] All animal procedures were performed in bio-safety level 3 (BSL3) facilities following procedures approved by the Rutgers University Institutional Animal Care and Use Committee, which is consistent with the policies of the American Veterinary Medical Association (AVMA), the Center for Disease Control (CDC) and the United States Department of Agriculture (USDA).

[00589] Quantification of infectious viral load in the lungs

[00590] Approximately 40% of lungs, liver, spleen, kidney, heart, and brain homogenized in DMEM containing penicillin-streptomycin with help of Mini Bead Mill Homogenizer (VWR, PA, USA) by five cycles of 20 seconds each. The lung homogenates were centrifuged, and the supernatant was filtered through a 0.45 µ filter. For plaque-forming unit (PFU) assay, the filtrate

was diluted in serum-free DMEM, and 400 µL od diluted sample was used to infect the Vero E6 cell monolayers in the six-well plates as described previously (Ramasamy et al., 2022). Briefly, the spent media from 6-well plates were aspirated and the cells were incubated with each virus dilutions at 400µL/well. The cells were incubated at 37°C for 1 hour. Unattached viruses were then removed, and the infected monolayers were overlaid with a mixture containing equal amounts of 2X MEM with 8% FBS and 1.6% low-melting agarose. The infectious virus in the samples were enumerated as PFUs by visualizing plaques as described above.

[00591] Histopathology analysis of internal organs

[00592] Portions of hamster lungs, liver, spleen, kidney, heart and brain were treated with 10% buffered formalin for 3-5 days for histopathology analysis. The formalin-fixed tissues were embedded into paraffin blocks, cut into 5-micron sections and stained with hematoxylin and eosin (H&E) described previously (Ramasamy et al., 2022). For histopathological examination, the H&E-stained slides were analyzed in a Nikon Microphot-FX microscope equipped with NIS elements software for image capture and analysis. Histopathology images were organized and labeled in Adobe Photoshop v22.1.1.

[00593] Immunohistochemistry (IHC) and mRNA-Fluorescent in situ hybridization (mRNA-FISH) analysis of lungs

[00594] The FFPE tissue sections on glass slides were deparaffinized by treating with xylene for 10 minutes (2 times), followed by washing for 10 minutes each in graded ethanol solution (absolute ethanol, 95% and 70% ethanol). For antigen retrieval, tissue sections were treated with citrate buffer at 90°C for 40 minutes, followed by thorough washing with deionized water. Immunostaining was performed on tissue sections by blocking them with 5% bovine serum albumin (BSA) in 1x PBS, followed by addition of antibodies against ACE2 (viral entry receptor), IBA1 (macrophages) or CD3 (T-cells), prepared in 5% BSA at 1:1000 dilution. For mRNA-FISH technique, fluorescently labelled mRNA probes for SARS-CoV-2, or IL-6, IL-1β and TNF-α were used for hybridization. The IHC and mRNA-FISH were performed as per previously published standard protocol (Ramasamy et.al, 2023). For IHC, the tissue sections were washed with 1x PBS and Alexa-488 tagged secondary antibody (Abcam, MA, USA) prepared at 1:2000 dilution in 5% BSA, was added and incubated at room temperature for 1h. The slides were further processed for sm-FISH by treating with wash buffer containing 10% formamide in 2X SSC (ThermoFisher Scientific, Waltham,MA, USA), followed by hybridization

buffer, containing 10% dextran sulphate (Sigma Aldrich, St Louis, MO, USA), 1mg/ml E. coli tRNA (Sigma Aldrich, St Louis, MO, USA), 2mM ribonucleoside vanadyl complexes (New England Biolab, Ipswich, MA, USA), 0.02% ribonuclease-free BSA (ThermoFisher Scientific, Waltham, MA, USA), 10% formamide and 2X SSC for 30 minutes at room temperature. Labelled FISH probes (Biosearch Technologies, Dexter, MI, USA) were added and the sections were incubated 15 hours at 37°C in a moist chamber. The probes were removed, and the slides were treated with wash buffer for 30 minutes. Finally, TrueBlack Lipofuschin autofluorescence quencher (Biotium Inc, Fremont, CA, USA) was applied to tissue sections and mounted with cover slip for microscopic analysis.

[00595] Image acquisition and analysis

[00596] An Axiovert 200M inverted fluorescence microscope (Zeiss, Oberkochen, Germany) with 20X objective or 63X oil-immersion objective and a Prime sCMOS camera (Photometrics, Tucson, A2) controlled by Metamorph image acquisition software (Molecular Devices, San Jose, CA) was used for capturing images. ImageJ (NIH, Bethesda, MD, USA) software was used for analyses. The number of cells positive for a marker was normalized to the total number of cells in each field (3-5 fields, each containing at least 500 cells, were analyzed per sample). Further, GraphPad Prism-8 (GraphPad Software, San Diego, CA) was used for the statistical analysis of data. P values <0.05 were considered statistically significant.

[00597] Determination of cytokine and immune marker expression in the lungs by quantitative real-time PCR (qPCR)

[00598] Lung total RNA from hamsters with or without Phyto-N treatment was isolated using Trizol reagent and purified by RNeasy mini columns (Qiagen, USA), and the eluted RNAs was checked for concentration on a Nanodrop (Nanodrop, USA). For complementary DNA (cDNA) synthesis, total lung RNA was added with reverse transcriptase, dNTP and buffer from the High-Capacity cDNA Reverse Transcription Kit as per the recommended protocol (Applied Biosystems, USA). qPCR was performed using gene-specific forward and reverse primers for and Power SYBR green PCR Master Mix as per the manufacturer's instructions (Applied Biosystems, CA, USA). The expression level of the target gene was normalized to housekeeping beta-actin (ACTNB) level and fold change was calculated using values from the untreated and Phyto-N-treated.

[00599] Statistical analysis

[00600] Statistical analysis was performed using GraphPad Prism-9 (GraphPad Software, La Jolla, CA) and the mean ± standard error (SE) values were plotted as graphs. Unpaired Student's t-test with Welch correction was used to analyze the data between two groups and One-way Anova with Tukey's correction was used for multiple group comparison. For all the experimental data, $p \leq 0.05$ was considered statistically significant.

[00601] Results

[00602] In the present study, we carried out a preclinical study to understand the beneficial role of *Wikstroemia indica* extract (Phyto-N) supplementation during SARS-CoV-2 infection. Hamsters were treated with Phyto-N for one week before infection (260 mg/ animal) or infection and treatment (25mg/Kg) started together or kept as untreated controls. All the hamsters were infected with SARS-CoV-2 intranasally. Body weight was monitored daily from day-0 of treatment and infection and continued till the last time point of the study. Body weight was increased in untreated as well as Phyto-N treated group before SARS-CoV-2 infection (FIG. 72 and 4). All hamster from pre-infection treatment group, post-infection treatment group and untreated groups showed a reduction in body weight from the day of infection until seven days post-infection (FIG. 72 and 73). Interestingly, hamsters treated with Phyto-N before infection (pretreated) showed a less body weight loss from day-1 to day-7 post infection as compared to hamsters received Phyto-N treatment after infection (post-treated) as well as untreated group. The mean body weight loss was ~ 2% in pre-treated group vs ~5% in post treated group at 2-4 dots per inch (dpi) (FIG. 72 and 73).

[00603] Phyto-N pre-treatment to infection decreased virus load in extrapulmonary organs

[00604] To study the effect of Phyto-N treatment on the replicative viral load in the lungs, liver, spleen, kidney, heart and brain, the hamsters treated before or after viral infection were euthanized at 4 and 7 days of post infection. About 40% of these organs were homogenized and the infectious SARS-CoV-2 in the homogenates were determined by plaque assay. The infectious viruses were found in the lungs of all study groups at 4 and 7 dpi (FIG. 74). In the Phyto-N treated post-infection group, the virus titer was decreased in the lung compared to no treatment group at 4 and 7 dpi. However, the difference was not statistically significant (FIG. 74, left panels). In contrast, in hamsters treated with Phyto-N before infection, a significant reduction in viral load was noted in the lungs at both 4 and 7 dpi as compared to untreated groups ($P<0.05$; FIG. 574, right panels).

[00605] We determined the infectious virus load in extrapulmonary organs such as was liver, spleen, kidney, heart, and brain (FIG. 75 and 76). Replicating virus were found in all these organs of hamsters treated with Phyto-N before or after SARS-CoV-2 infection or untreated controls at 4-dpi. However, the virus load in the liver, kidney, heart, and brain of hamsters treated with Phyto-N after viral infection was not statistically significant as compared to the untreated group. (FIG. 75, left panel and FIG. 76, top panel). In contrast, the replicating viral load was significantly decreased in the liver ($P<0.005$), kidney ($P<0.05$), spleen ($P<0.005$), heart ($P<0.05$) and brain ($P<0.005$) of hamsters treated with Phyto-N before to viral infection, as compared to the untreated groups at 4dpi (FIG. 75, right panel and FIG. 76, bottom panel). We did not find infectious virus in the extrapulmonary organs in any of the study groups at 7-dpi. This result suggests that pre-treatment with Phyto-N before infection may have a beneficial role on the host to control the SARS-CoV-2 dissemination and to curb the viral load in the lungs as well as the extra pulmonary organs, particularly during early phase of infection (e.g., 4dpi).

[00606] Phyto-N pre-treatment alters the histopathology of organs

[00607] H&E-stained sections of the lungs, liver, spleen, kidney, and brain from untreated and Phyto-N treated hamsters at 4 and 7 dpi were used for histopathology analysis. A moderate level of inflammation, inflammatory cells infiltration and bronchiolitis was noted in the untreated-SARS-CoV-2 infected hamster lungs by 4 dpi (FIG. 877). This pattern was prominent with a moderate to severe infiltration of inflammatory cells, congestion of capillaries in the alveolar wall, edema, and alveolar collapse at 7 dpi. In contrast, mild-to-moderate levels of inflammation, inflammatory cell infiltration, and bronchiolar epithelial hyperplasia were noted in the lungs of Phyto-N-treated hamsters at 4dpi. At 7 dpi, a similar disease presentation was observed in the treated-infected hamsters (FIG. 77). Thus, it appears that Phyto-N treatment may dampen the inflammation, perhaps through reducing inflammatory immune cell recruitment to the site of infection.

[00608] The liver histology showed inflammation, hepatocytic degeneration, particularly perivascular area, and thrombotic lesions in the SARS-Cov-2 infected untreated hamsters at 4 and 7 dpi, with slightly severe pathology at the later time point (FIG. 78). These anomalies, particularly the thrombotic lesions and degeneration of tissue were reduced in the liver of Phyto-N treated hamsters (FIG. 78).

[00609] The spleen of SARS-CoV-2 infected, untreated hamsters showed reduction in overall size and the area of white pulp region at 4 and 7 dpi (FIG. 79). A similar level of disease pathology was noted in the spleen of Phyto-N treated hamsters, and no remarkable differences was noted between 4 and 7 dpi (FIG. 79).

[00610] The histology section of kidney showed moderate-to-severe hypertrophy mainly due to immune cell infiltration, thickening of the vascular smooth muscle layer and thrombotic lesions in SARS-CoV-2 infected untreated hamsters at 4 and 7 dpi (FIG. 80). The extent and severity of these pathologic manifestations in the kidney was mild to moderate levels in hamsters treated with Phyto-N prior to SARS-CoV-2 infection.

[00611] In the brain of SARS-CoV-2 infected untreated hamsters, mild inflammatory cellular accumulation in the meninges, accompanied by vascular congestion and hemorrhage. These brain pathologic manifestations showed a progressive severity between 4 and 7dpi in the untreated animals (FIG. 81). However, in Phyto-N pre-treated hamster infected with SARS-CoV-2, a slightly reduced hemorrhagic response was noted at 4dpi, although the immune cell infiltration was unremarkable, compared to the untreated hamsters at this time point. At 7dpi, the brain disease pathology was comparable between the untreated and Phyto-N treated hamsters infected with SARS-CoV-2 (FIG. 81).

[00612] Phyto-N pre-treatment increased infiltration of T cells in lungs, while decreased that of macrophages

[00613] Immunohistochemical analyses of lungs sections from Phyto-N treated hamsters and infected with SARS-Cov-2 viruses showed increased infiltration of CD3 positive T cells compared to untreated but infected hamsters (FIG. 82 and 83). However, we did not observe any significant difference in the expression of IL1B cytokine expressed by these cells. Contrary to CD3 positive cells, we observed lower infiltration of activated macrophages (IBA1 positive cells) upon Phyto-N treatment in infected hamsters. There was no significant difference in expression of TNFA and IL6 cytokines expressions between the groups (FIG. 82 and 83).

[00614] Phyto-N pre-treatment enhanced protective immune response in pulmonary and extrapulmonary organs

[00615] To study the effect of Phyto-N treatment during SARS-CoV-2 infection, the expression of markers associated with viral entry, inflammatory response and protective T cells responses was quantified in lung and extrapulmonary organs such as spleen, liver, kidney, heart

and brain. The expression of IFN-γ and IL-1β, TNF-α, CCL2 (both in 4 and 7-dpi), IL-10 (7-dpi) and IL-4 (both in 4 and 7-dpi) was upregulated, whereas the expression of IL-6 and CRP (7-dpi) was decreased in lung of Phyto-N treated hamsters as compared to untreated hamsters (FIG. 84). The expression of IFN-γ, IL-10, IL-4, ACE2, IP-10 and GMCSF were upregulated in the spleen of Phyto-N treated hamsters (FIG. 85). Similarly, the expression of IFN-γ, IL-1β, IL-10, IL-4 and CCL5 were enhanced in the liver and kidney of Phyto-N treated hamsters (FIG. 86 and 87). The expression of IL-1β and CCL5 in the brain, and IFN-γ in the heart were upregulated Phyto-N treated hamsters (FIG. 88 and 89). In addition, a decreased expression of CRP in the spleen, liver and brain (FIG. 85, 87, and 88), and IL-6 and TMPRSS in liver and brain (FIG. 87 and 88) were observed in Phyto-N treated hamsters as compared untreated hamsters. In contrast to other organs, the expression of TNF-α and IL-1β were decreased in the heart of Phyto-N treated hamsters (FIG. 20). The study results suggest that pretreatment of Phyto-N enhanced the immune response and help to control the inflammatory response during SARS-CoV2 infection in pulmonary and extrapulmonary organs.

[00616] Summary and Conclusion

[00617] Based on the findings of this study in a hamster model of pulmonary SARS-CoV-2 infection, it appears that treatment with Phyto-N prior to infection, but not after infection, has host beneficial effects in protecting against disease progression. In particular, pre-treatment with Phyto-N led to reduced replicative viral burden at the site of infection (lungs) and viral dissemination, as noted by reduction in viral load in extrapulmonary organs, such as liver, kidney, spleen, heart and brain. The improved viral load clearance in Phyto-N pre-treated hamster was remarkable during early stages (i.e, 4dpi) of SARS-CoV-2 infection. Therefore, it can be hypothesized that administration of Phyto-N acts as an immune-booster and prepares the host to defend the viral replication in the internal organs. The reduced viral load in organs might also have a positive association with the improvement in body weight loss in the Phyto-N pretreated hamsters, compared to untreated animals. The histopathologic analysis of internal organs of SARS-CoV-2 infected hamsters also indicated signs of immune modulation, marked by a mild to moderate level of reduction in disease severity in the Phyto-N pretreated animals, compared to the untreated animals. Consistent with these histologic changes and reduction in replicating viral load, immune cell distribution, particularly distribution of T cells was increased while macrophages were reduced in the lungs of Phyto-N pretreated animals, compared to the

untreated animals. These changes in immune cell environment are also supported by the immune activation gene expression analysis in various organs.

[00618] Taken together, these data suggest that *Wikstroemia indica* extract is a potent immune-modulatory agent that can be harnessed for effective management of COVID cases.

[00619] Example 12: Evaluation of the impact of Phyto-N, Daphnoretin (I), and Daphnogitin (II) on animal models of atopic dermatitis

[00620] The purpose of this experiment was to investigate the therapeutic effect of drugs Phyto-N, Daphnoretin (I), and Daphnogitin (II) on atopic dermatitis by observing the performance and severity score of skin lesions, the histopathological structure of skin lesions, epidermis thickness and spleen index of mice.

[00621] Experimental methods

[00622] Animal modeling, drug administration, and group grouping

[00623] Experimental groups: 35 SPF male BALB / c mice were selected after 7 days of adaptive feeding (room temperature 22°C -24°C, humidity 50% -60%, normal feeding, ad libitum feeding) and randomly divided into group Control (n=7), Model (n=7), Group Phyto-N (n=7), Group Daphnoretin (I) (n=7), and Group Daphnogitin (II) (n=7).

[00624] Doose: drug Phyto-N (3g / k g/d), Daphnoretin (I) (0.03g / k g/d), Daphnogitin (II) (0.03g / k g/d), equal amount of distilled water to blank group and model group, from the 12th day of the experiment, each group received gavage twice a day.

[00625] The back skin of all mice was shaved one day before each mold, with a range of about 2cm 4cm. On Day 1 to day 3, 200 μL of 0.5% DNCB solution was applied to the back skin (matrix fluid acetone: olive oil =4:1). From Day 14, 200 μL of 1% DNCB solution was applied to the back skin for the same dose of matrix solution. Mice were killed at 2 hours after the last challenge dose on day 36.

[00626] Skin injury degree findings and severity score

[00627] The severity of the skin lesions was scored. According to the severity of erythema, infiltration, scales, and edema in the skin lesions, they were scored: 0: no skin lesions, 1 mild, 2 moderate, and 3 severe. A higher score indicates more severe AD skin lesions.

[00628] Determination of spleen index in mice

[00629] The mice were sacrificed by cervical dislocation after blood collection, and then the spleen was removed. The spleen was weighed, and its weight was recorded. The spleen index

was calculated according to the following formula: spleen index (mg / g) = spleen weight (mg)/body weight (g).

[00630] Histopathological observation of mouse skin lesions and measurement of epidermal thickness

[00631] After the mice were sacrificed, half of the lesional tissue was cut with clean scissors and fixed in 4% paraformaldehyde, then dehydrated, paraffin-embedded, sectioned, and HE stained. Three slides of each specimen were selected under the microscope for observation and photographed to analyze the pathological changes of each group.

[00632] Blood cell analysis

[00633] Peripheral blood samples were collected from each group and collected into centrifuge tubes with EDTA anticoagulant and were thoroughly mixed. Then 200 μL of white blood cells (WBC), RBC cells (RBC), neutrophils (Neu), lymphocytes (Lym), and platelets (PLT) were analyzed.

[00634] Experimental results

[00635] Skin injury degree findings and severity score

[00636] Skin lesion condition was scored on days 1, 3, 12, 15, 18, 21, 24, 27, 30, 33, and 36 of the experiment. In the Model group, mice showed obvious edema and peeling in the second day of formal molding, and erosion and exudation began to appear in about 15 days. The back skin began to gradually recover in about 24 days. By the end, the skin lesions gradually became moss-like changes, and the surface was covered with thick scab skin, which was consistent with AD symptoms. Compared with the Model group, group Phyto-N, Group Daphnoretin (I) and Group Daphnogitin (II) all decreased these adverse symptoms, reduced the thickening of scab skin and the increase of erythema, and also improved the degree of dryness and shedding of the epidermis (Fig. 90). The results showed that Phyto-N, Daphnoretin (I) and Daphnogitin (II) improved the erythema, infiltration, scales and decreased PASI score in this mouse model of atopic dermatitis (Fig. 91).

[00637] Splenic index measurement in mice

[00638] The spleen index results showed that compared with healthy mice, the model group was significantly increased ($P < 0.01$), and Phyto-N, Daphnoretin (I) and Daphnogitin (II) could significantly inhibit the increase of spleen index in mice. It shows that Phyto-N, Daphnoretin (I) and Daphnogitin (II) good effect in improving immune function (Fig. 92).

[00639] Histopathological observation of mouse skin lesions and measurement of epidermal thickness

[00640] In the blank group of mice, the epidermis and dermis showed no obvious inflammatory reaction, and the cell morphology was normal. Compared with the blank group, the model group showed obvious inflammatory hyperplasia changes, with visible spinous layer thickening, dermal vascular expansion and congestion, significant increase in epidermal thickness, and large inflammatory cell infiltration of lymphocytes and eosinophils in the dermis. Mice in Phyto-N, group, Daphnoretin (I) group and Daphnogitin (II) group showed slight hyperkeratosis, thinning of spinous cells compared with the model group, less infiltration of true dermatitis cells, (Fig. 93) and reduced epidermal thickening compared with the model group (Fig. 94).

[00641] Blood cell analysis

[00642] Compared with the blank group, the number of leukocytes, neutrophils, monocytes and platelets were significantly increased, but lymphocytes were significantly decreased; compared with the model group, the number of leukocytes, neutrophils, monocytes, platelets and lymphocytes were suppressed. The results show that drugs Phyto-N, Daphnoretin (I) and Daphnogitin (II) can effectively reduce the number of inflammation-related cells in mice with atopic dermatitis and improve the immune function (Table 25 and Fig 95-99).

[00643] Table 25: Blood cell count

Variable	Control group	Model group	Phyto-N	Daphnoretin (I)	Daphnogitin (II)
White blood cell count(10^9/L)	10.75	14.03	6.14	13.86	8.04
Neutrophil count(10^9/L)	5.70	16.62	4.76	13.14	5.45
Lymphocyte count(10^9/L)	4.28	2.39	3.24	5.74	1.87
Platelet count(10^9/L)	1115.25	667	1267	1206	1305.75
Mononuclear	0.77	1.01	0.8	1.65	0.73

cell count(10^9/L)					

[00644] Experimental summary

[00645] Atopic dermatitis is a common pruritic inflammatory skin disease, and its main clinical manifestations are red skin.Plaque, plaque. Over the past few decades, the incidence of atopic dermatitis has increased year by year, and it has become a global health problem. Our results indicate that by assessing epidermal thickness, splenic index and inflammation, Phyto-N, Daphnoretin (I) and Daphnogitin (II) can improve the pathological structure of skin lesions, reduce PASI score, reduce the increase of splenic index, and improve inflammatory markers in blood cell counts. Phyto-N, Daphnoretin (I) and Daphnogitin (II) have significant therapeutic potential for atopic dermatitis.

[00646] Example 13: Evaluation of the impact of Phyto-N, Daphnoretin (I), and Daphnogitin (II) on animal models of acute colitis

[00647] The objective of this study was to evaluate the efficacy of two compounds Daphnoretin (**I**) and Daphnogitin (**II**) against acute colitis.Materials: SPF grade female C57BL/6 mice, 6-8 weeks old, weighing 16-18 g, were purchased from Changsheng (Liaoning) Biotechnology Co.METHODS: In this study, dextran sodium sulfate (DSS) induction method was used to establish a mouse model of acute colorectal inflammation. On the first day of the experiment, the Model and drug-dosing groups were free to drink 2.4% DSS (induction period) for 7 d + 0.5% DSS (maintenance period) for 4 d, and the Control group was free to drink purified water, and the administration of the drug was initiated on the 0th day of the experiment, with modelling accompanied by the administration of the drug.Body weight, faecal condition and DAI score were recorded for each group of mice, which were sampled after day 12 and tissue specimens of blood and colorectal tissue were retained. H&E staining method was used to stain and observe the histopathological changes of mouse colon under microscope.

[00648] Materials and methods

[00649] Laboratory animals

[00650] SPF grade female C57BL/6 mice, 6-8 weeks old, body mass 16-18g, provided by Liaoning Changsheng Biotechnology Co Ltd, Certificate of Conformity No. SCXK (Liao) 2020-0001.The animals were kept in an aseptic environment at a temperature of 21±2°C, relative

humidity of 60±5% and free access to food and water. The animal experiments were approved by the Experimental Animal Ethics Committee of Heilongjiang University of Traditional Chinese Medicine.

[00651] Experimental herbs and reagents

[00652] The reagents are shown in Table 25.

[00653] Table 25: Reagents

Reagent name	product number	factory owners
75% alcohol	——	Dezhou Chengze Disinfection Technology Co., Ltd.
Rodent maintenance feed	24041911	Liaoning Changsheng Biotechnology Co., Ltd
Dextran Sulfate Sodium Salt	S14049	Shanghai yuanye Bio-Technology Co., Ltd
Urine Fecal Occult Blood Test Kit	60403ES60	Yeasen Biotechnology (Shanghai) Co.

[00654] Experimental apparatus

[00655] The experimental apparatus is shown in Table 26.

[00656] Table 26: Experimental Apparatus

Instrument name	model number	factory owners
Electronic Analytical Balance	JA5203	Shanghai Fangrui Instrument Co.
electronic balance	A6-001	Yuyao Jiming Weighing and Calibration Equipment Co., Ltd.

[00657] Modelling, grouping and dosing of animals:

[00658] All mice were acclimatized and fed for 7 days. After excluding mice that were not in good condition during the period, female C57BL/6 mice were randomly grouped into Control (n=12), Model (n=12), Daphnoretin (**I**) (n=9), Daphnogitin (**II**) (n=9).

[00659] In this study, Dextran Sulfate Sodium Salt (DSS) induction method was used, and on the 1st day of the experiment, an acute colitis mouse model was established, and the Control group drank purified water. The Model group and each of the administered groups consumed 2.4% DSS solution (2.4 g DSS dissolved in 100 ml of purified water) ad libitum for 7 days to induce acute colitis, which was discontinued and then switched to 0.5% DSS ad libitum for 4 days to maintain acute colitis.

[00660] Each dosing group was administered by gavage twice in the morning and evening from day 0 of the experiment, 60 mg/Kg/d for Daphnoretin (**I**) and Daphnogitin (**II**). Equal amounts of purified water were administered by gavage in the Control and Model groups. The experimental flow is shown in Fig. 100. The general condition of the mice in each group (body weight, fecal condition and mortality) was recorded during the experiment. Disease activity index (DAI) score was determined. The mice in each group were executed after 12 days and blood and colorectal tissue specimens were retained. H&E Staining was used for staining and microscopic observation of histopathological changes in the mouse colon.

[00661] Evaluation indicators

[00662] General status evaluation: The general conditions of the mice were monitored daily, such as mental status, coat colour and gloss, stress response and other systemic conditions. The weight of the mice was weighed and recorded daily, and the survival of the mice was checked.

[00663] DAI score: Weight measurements, fecal status and bleeding were observed daily during the experiment. The rate of weight loss was calculated based on the daily body weight of the mice after modelling: rate of weight loss = (daily body weight - initial body weight)/initial body weight × 100%, and the scores of weight loss, fecal status and degree of haemorrhage were recorded according to the scoring criteria in Table 3, and the sum of the three was taken as the DAI score.

[00664] **Table 27: DAI scoring criteria in mice**

Weight loss%	Fecal character	Occult blood level	Mark
0	Normalcy	Normalcy	0
1-5	Thin, but formed stools	Fecal Occult Blood Weakly Positive	1
5-10	Not rigorous	Bleeding (positive)	2
10-15	Meager stool	Bleeding (strong positive)	3
>15	Watery stool	Profuse bloody stools	4

[00665] Results

[00666] Effect of each administration group on DSS-induced body weight in mice:

[00667] The mice in each group showed a decreasing trend in body weight after drinking DSS. There was a significant decrease in per cent body weight in the Model group as compared to the Control group with the administered group. There was a trend of increasing body weight in the administered groups compared to the Model group, but none of them were significantly different (fig. 101).

[00668] Effect of each administration group on DSS-induced DAI and occult blood stool dilution in mice

[00669] The DAI scores showed an increasing trend in all groups of mice after drinking DSS.DAI scores in the Model group were significantly higher from Day6 compared to the Control group. DAI scores were significantly lower in Groups Daphnoretin (**I**) and Daphnogitin (**II**) compared to Model group (fig. 102).

[00670] Occult blood and stool dilution scores showed an increasing trend in all groups of mice after drinking DSS.DAI scores in the Model group were significantly higher from Day6 compared to the Control group.Occult blood and stool dilution scores were significantly lower in Groups Daphnoretin (**I**) and Daphnogitin (**II**) compared to the Model group (Fig. 103).

[00671] The effect of each dosing group on the splenic index of mice

[00672] The splenic index was significantly higher in the Model group compared to the Control group.Splenic index was significantly lower in Group Daphnoretin (**I**) and Daphnogitin (**II**) compared to Model group (Fig. 104).

[00673] Analysis of HE staining results of colonic tissues in each group

[00674] Compared with the Control group, mice in the Model group had severe damage to the mucosa and crypt structure of the colon tissue, with a large number of inflammatory cells infiltrating, a significant decrease in cup cells, and cells with detached necrosis; compared with the Model group, mice in groups Daphnoretin (**I**) and Daphnogitin (**II**) still had a small portion of inflammatory cells infiltrating, but intact crypts could be seen. structure, the arrangement of glands was basically complete, and the number of cup cells increased (Fig. 105).

[00675] Experimental summary

[00676] The aim of this experiment was to evaluate the efficacy of Daphnoretin (**I**) and Daphnogitin (**II**) against acute colitis. Compared with the Control group, the Model group showed significant weight loss, significant increase in DAI score, significant shortening of colon length, severe damage to the mucosa and crypt structure of the colon tissue, massive infiltration of inflammatory cells, significant reduction of cup cells, and shedding necrosis of cells, suggesting that the modelling was successful.

[00677] Administration of Daphnoretin (**I**) and Daphnogitin (**II**) improved the condition of mice with thinning of occult blood stools, lowered DAI score, lowered splenic index, and significantly reduced the mucosal structural damage and inflammatory response as compared to the Model group.

CLAIMS

What is claimed is:

1. A pharmaceutical composition consisting of an extract of the plant Wikstroemia indica.

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

3. A method of treating an autoimmune disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 2 to said subject.

4. The method of claim 3, wherein the autoimmune disease is selected from the group consisting of ulcerative colitis, Crohn's disease, inflammatory bowel disease, atopic dermatitis, eczema, psoriasis, chronic plaque psoriasis, and rheumatoid arthritis.

5. A method of treating a metabolic disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 2 to said subject.

6. The method of claim 5, wherein the metabolic disease is selected from the group consisting of gout, acute gout arthritis, chronic gouty arthritis, hyperuricemia, gouty nephritis, diabetes, fatty liver disease, non-alcoholic fatty liver, obesity, diabetes, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, dyslipidemia and lipid metabolism disorder.

7. A method of treating a condition associated with metabolic disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 2 to said subject.

8. The method of claim 7, wherein the wherein said condition associated with metabolic disease is selected from the group consisting of insulin resistance, diabetic nephropathy, diabetic peripheral neuropathy, diabetic retinopathy, cataracts, diabetic foot, diabetic hand, diabetic neuropathy, diabetic hypertension, diabetic vascular occlusion, diabetic blood vessel lesions, diabetic numbness of hands, diabetic numbness of feet, Alzheimer's disease, non-healing oral ulcers, and skin ulcers.

9. A method of treating or preventing a viral infection in a subject in need thereof, comprising administering the pharmaceutical composition of claim 2 to said subject.

10. The method of claim 9, wherein the viral infection is selected from the group consisting of severe-acute-respiratory-syndrome-related coronavirus-2 (SARS-CoV-2), H1N1, influenza A, influenza B, influenza C, influenza D, and influenza E.

11. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprsing: contacting extracting a water suspension of dried Wikstroemia indica 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, ethyl acetate, and a mixture of up to three of said solvents, then removing said solvent and adding at least one pharmaceutically acceptable excipient.

12. The method of claim 5 wherein said solvent is heated while it is in contact with the dried Wikstroemia indica.

13. The method of claim 5 wherein said solvent is heated with a microwave while it is in contact with the dried Wikstroemia indica.

14. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced a method comprising: contacting dried *Wikstroemia indica* 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.

15. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried Wikstroemia indica 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.

16. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried

Wikstroemia indica with a chromatography column, then eluting said column with a solvent, then said solvent and adding at least one pharmaceutically acceptable excipient.

17. 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.

18. 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,

19. 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.

20. A method of treating a metabolic disease in a subject in need thereof, comprising administering the pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting extracting a water suspension of dried Wikstroemia indica 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, ethyl acetate and a mixture of up to three of said solvents, then removing said solvent and adding at least one pharmaceutically acceptable excipient.

21. The method of claim 20 wherein said solvent is heated while it is in contact with the dried Wikstroemia indica.

22. The method of claim 20 wherein said solvent is heated with a microwave while it is in contact with the dried Wikstroemia indica.

23. A method of treating a metabolic disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried *Wikstroemia indica* 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.

24. A method of treating a metabolic disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried Wikstroemia indica 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.

25. A method of treating a metabolic disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried Wikstroemia indica with a chromatography column, then eluting said column with a solvent, then said solvent and adding at least one pharmaceutically acceptable excipient.

26. The method of claim 25 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.

27. The method of claim 25 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,

28. The method of claim 25 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.

29. A method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting extracting a water suspension of dried Wikstroemia indica 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, ethyl acetate and a mixture of up to three of said

solvents, then removing said solvent and adding at least one pharmaceutically acceptable excipient.

30. The method of claim 29 wherein said solvent is heated while it is in contact with the dried Wikstroemia indica.

31. The method of claim 29 wherein said solvent is heated with a microwave while it is in contact with the dried Wikstroemia indica.

32. A method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried *Wikstroemia indica* 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.

33. A method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried Wikstroemia indica 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.

34. A method of treating or preventing a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising an extract from the plant Wikstroemia indica wherein said extract is produced by a method comprising: contacting dried Wikstroemia indica with a chromatography column, then eluting said column with a solvent, then said solvent and adding at least one pharmaceutically acceptable excipient.

35. The method of claim 34 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.

36. The method of claim 34 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,

37. The method of claim 34 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.

38. A method of treating an autoimmune disease in a subject in need thereof, comprising administering Daphnoretin (I) to said subject.

39. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (I) and an excipient to said subject.

40. The method of claim 39, wherein the autoimmune disease is selected from the group consisting of ulcerative colitis, Crohn's disease, inflammatory bowel disease, atopic dermatitis, eczema, psoriasis, chronic plaque psoriasis, and rheumatoid arthritis.

41. The method of claim 38, wherein the autoimmune disease is selected from the group consisting of ulcerative colitis, Crohn's disease, inflammatory bowel disease, atopic dermatitis, eczema, psoriasis, chronic plaque psoriasis, and rheumatoid arthritis.

42. A method of treating an autoimmune disease in a subject in need thereof, comprising administering Daphnogitin (II) to said subject.

43. A method of treating an autoimmune disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (II) and an excipient to said subject.

44. The method of claim 42, wherein the autoimmune disease is selected from the group consisting of ulcerative colitis, Crohn's disease, inflammatory bowel disease, atopic dermatitis, eczema, psoriasis, chronic plaque psoriasis, and rheumatoid arthritis.

45. The method of claim 43, wherein the autoimmune disease is selected from the group consisting of ulcerative colitis, Crohn's disease, inflammatory bowel disease, atopic dermatitis, eczema, psoriasis, chronic plaque psoriasis, and rheumatoid arthritis.

46. A method of treating a metabolic disease in a subject in need thereof, comprising administering Daphnoretin (I) to said subject.

47. A method of treating a metabolic disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (I) and an excipient to said subject.

48. The method of claim 46, wherein the metabolic disease is selected from the group consisting of insulin resistance, diabetic nephropathy, diabetic peripheral neuropathy, diabetic retinopathy, cataracts, diabetic foot, diabetic hand, diabetic neuropathy, diabetic hypertension, diabetic vascular occlusion, diabetic blood vessel lesions, diabetic numbness of hands, diabetic numbness of feet, Alzheimer's disease, non-healing oral ulcers, and skin ulcers.

49. The method of claim 47, wherein the metabolic disease is selected from the group consisting of insulin resistance, diabetic nephropathy, diabetic peripheral neuropathy, diabetic retinopathy, cataracts, diabetic foot, diabetic hand, diabetic neuropathy, diabetic hypertension, diabetic vascular occlusion, diabetic blood vessel lesions, diabetic numbness of hands, diabetic numbness of feet, Alzheimer's disease, non-healing oral ulcers, and skin ulcers.

50. A method of treating an metabolic disease in a subject in need thereof, comprising administering Daphnogitin (II) to said subject.

51. A method of treating an metabolic disease in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (II) and an excipient to said subject.

52. The method of claim 50, wherein the metabolic disease is selected from the group consisting of insulin resistance, diabetic nephropathy, diabetic peripheral neuropathy, diabetic retinopathy, cataracts, diabetic foot, diabetic hand, diabetic neuropathy, diabetic hypertension, diabetic vascular occlusion, diabetic blood vessel lesions, diabetic numbness of hands, diabetic numbness of feet, Alzheimer's disease, non-healing oral ulcers, and skin ulcers.

53. The method of claim 51, wherein the metabolic disease is selected from the group consisting of insulin resistance, diabetic nephropathy, diabetic peripheral neuropathy, diabetic retinopathy, cataracts, diabetic foot, diabetic hand, diabetic neuropathy, diabetic hypertension, diabetic vascular occlusion, diabetic blood vessel lesions, diabetic numbness of hands, diabetic numbness of feet, Alzheimer's disease, non-healing oral ulcers, and skin ulcers.

54. A method of treating a viral infection in a subject in need thereof, comprising administering Daphnoretin (I) to said subject.

55. A method of treating a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnoretin (I) and an excipient to said subject.

56. The method of claim 54, wherein the viral infection is selected from the group consisting of severe-acute-respiratory-syndrome-related coronavirus-2 (SARS-CoV-2), H1N1 influenza A, influenza B, influenza C, influenza D, and influenza E.

57. The method of claim 55, wherein the viral infection is selected from the group consisting of severe-acute-respiratory-syndrome-related coronavirus-2 (SARS-CoV-2), H1N1 influenza A, influenza B, influenza C, influenza D, and influenza E.

58. A method of treating a viral infection in a subject in need thereof, comprising administering Daphnogitin (II) to said subject.

59. A method of treating a viral infection in a subject in need thereof, comprising administering a pharmaceutical composition comprising Daphnogitin (II) and an excipient to said subject.

60. The method of claim 58, wherein the viral infection is selected from the group consisting of severe-acute-respiratory-syndrome-related coronavirus-2 (SARS-CoV-2), H1N1, influenza A, influenza B, influenza C, influenza D, and influenza E.

61. The method of claim 59, wherein the viral infection is selected from the group consisting of severe-acute-respiratory-syndrome-related coronavirus-2 (SARS-CoV-2), H1N1, influenza A, influenza B, influenza C, influenza D, and influenza E.