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(54) Title: A METHOD OF OBTAINING EXTRACTS OF SPATHOLOBUS SUBERECTUS DUNN (SSD), FRACTIONS AND COMPOSTIONS THEREOF AND USING AGAINST VIRAL DISEASES

(57) Abstract: Disclosed is a method of obtaining preparing an extract of Spatholobus suberectus Dunn, which contains total flavonoid and proanthocyanidins. The disclosure relates to a method for extracting an active fraction from Spatholobus suberectus. Also provided is the SSP, the active fraction, and a composition thereof. Further provided is a method of treating viral diseases such as COVID-19 disease caused by SARS-CoV-2, and use of the SSP and the active fraction in the manufacture of preparations for prevention and/or treatment of a viral disease.

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Description

Title of Invention: A METHOD OF OBTAINING EXTRACTS OF SPATHOLOBUS SUBERECTUS DUNN (SSD) , FRACTIONS AND COMPOSTIONS THEREO F AND USING AGAINST VIRAL DISEASES

FIELD OF THE INVENTION

[0001] Disclosed is a method of preparing a natural product of antiviral extract from Spatho lobus suberectus Dunn (SSD) , which contains total flavonoid and pro anthocyanidins. The disclosure relates to a method for discovering an active proantho cyanidins of Spatholobus suberectus (SSP) as viral entry inhibitor. Also provided is a method of preventing and treating viral diseases such as coronavirus disease 2019 (COVID-19) .

BACKGROUND OF THE INVENTION

[0002] Recent epidemic outbreaks caused by emerging and re-emerging viruses in the advent of globalization and rapid urbanization have underscored their preventi on and treatment as a critical threat to public health. Examples include the recent o utbreak of COVID-19 caused by severe acute respiratory syndrome viru s type 2 (SARS-CoV-2) . Despite the progress made in immunization and drug development, many viruses lack preventive vaccines and efficient antiviral thera pies. Thus, identifying novel agents especially natural products that are highly effi cacious and cost-effective for the management and control of viral infections is of c ritical importance. Broad-spectrum antivirals will be particularly beneficial to combat new variants of related viruses.

[0003] Herbal medicines and purified natural products provide a rich resour ce for novel antiviral drug development. Identification of the antiviral mechanisms f rom these natural agents has shed light on where they interact with the viral life cy cle, such as viral entry, replication, assembly, and release. Traditional Chinese Med icine (TCM) is particularly appreciated. With the existence of between 250,000 to 300 ,000 plant species in the world, Chinese herbal medicine provides a fast track and im portant source for drug discovery [6-10]. Artemisia annua is the representative herb. The d iscovery of Qinghaosu (artemisinin) , an antimalarial drug from Artemisia annual, and subsequent development of several therapies to inhibit the malaria parasite has save d millions of lives.

[0004] The plant SSD. Slightly sweet, warm in nature, belongs to Chinese me dicine liver and kidney. "Compendium of Materia Medica" claims that it can nourish th

e stomach and dry stomach; "Compendium of Compendium of Materia Medi ca" claims that it can promote blood circulation and warm the waist and knees. There is a large medical demand for the treatment of patients with closedness, rheumatism, numbness, paralysis, blood deficiency and chlorosis. In recent years, there have been some reports on the common use of Centipede to treat diseases caused by viruses, but it has not been reported in Covid-19. Till now, no publication has indicated SSP as a viral entry inhibitor.

[0005] The chemical constituents of SSD mainly include flavonoids, terpenes , sterols, lignin, anthraquinones, polyphenols, proanthocyanidins and some trace elem ents, among which the content of flavonoids is the most. The research on the extracti on method of content mostly uses the total flavonoid extraction rate as an indicator and does not consider the effect of the extraction method on the drug efficacy of the extract.

SUMMARY OF THE INVENTION

[0006] Provided herein is a method of obtaining an extract of SSD, said met hod comprising: (i) cutting SSD into pieces; (ii) immersing and treating SSD pieces i n a solvent at room temperature to obtain an extract; (iii) concentrating the extract ; and (iv) drying the concentrated extract to obtain the powder of the extract of SSD (SSP) .

[0007] Provided herein is a method of obtaining an active fraction from SSD, the active frac tion including proanthocyanidins, said method comprising: (i) cutting SSD into pieces ; (ii) immersing and treating SSD pieces in a solvent at room temperature to obtain a n extract; (iii) concentrating the extract; (iv) drying the concentrated extract to o btain the powder of the extract of SSD (SSP) ; (v) dispersing SSP in water to obtain an aqueous solution; (vi) extracting the SSP aqueous solution with a series of solven t with different polarities at equal volume to obtain parts of SSP corresponding to e ach solvent, the series of solvent comprising n-butanol; and (vii) separating the n- butanol part (SSP-n-BuOH) with eluents to obtain the active fraction.

[0008] In one embodiment, the treating process in (ii) comprises percolation.

[0009] Provided herein are an extract of SSD and an active fraction from SS D obtained by the above methods, as well as a composition comprising the extract or t he active fraction.

[0010] Provided herein is a method of preventing and/or treating a viral disease comprising administering an effective amount of an extract of SSD or an active fraction from SSD obtained by the above methods to a subject in need thereof.

[0011] In one embodiment, the viral disease is caused by SARS-CoV-2 including Omicron variants, Ebola virus (EBOV) , HIV-1 with either CCR5 or CXCR4 tropism, SARS-CoV, H5N1.

[0012] In one embodiment, the effective dose is 0.5 μg/ml to 5000 μg/ml.

[0013] Provided herein is use of an extract of SSD or an active fraction from SSD obtained by the above methods in the manufacture of preparations for prevention and/or treatment of a viral disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1. Flow chart of the study. Preparation and quality-controlled of SSP which is used to determine the broad-spectrum antiviral activity and its underlying mechanism. The powder SSP was further extracted to obtain the effective active parts.

[0015] Figure 2. Representative pictures of SSP's quality control. (a) HPLC chromatogram of different batches of SSP after ethyl acetate processing. (b) TLC chromatogram of different batches of SSP and positive control of SS.

[0016] Figure 3. Antiviral activity of SSP against SARS-CoV-2. Serially diluted SSP was added to HEK293T-ACE2 cells infected with SARS-CoV-2 (a) and VSV (b) respectively. The luciferase level was measured 2 or 3 days post-infection. To test SSP cytotoxicity, cells viability (c) was measured. (d) Pre-treatment of SARS-CoV-2 and target cells inhibited viral infection. (e) Binding of RBD to ACE2 expressing 293T cells, but not 293T control cells. (f) SSP inhibited RBD binding to target cells. SSP pre-treated HEK293T-ACE2 cells were incubated with RBD-PD1 for 30 mins on ice, followed by antibody staining of ACE2 (upper panel) and RBD (lower panel) and flow cytometry analysis. The data represent the mean ± SEM of triplicate experiments.

[0017] Figure 4. Antiviral activity of SSP against SARS-CoV-1, H5N1, and HIV, and EBOV viruses. Serially diluted SSP was added to HEK293T-ACE2, MDCK, GHOST-CCR5, and GHOST-CXCR4 infected with SARS-CoV-1 (a) H5N1 (b) HIV_{ADA} (c) , and HIV_{HXB2} (d) respectively. The luciferase level was measured 2 or 3 days post-infection. Cells viability (e) was measured to test SSP cytotoxicity. (f) Serially diluted SSP was added to 293T cells infected with EBOV. The luciferase level was measured 2 days post infection. Cells viability was measured to test SSP cytotoxicity against the 293T cells. The data represent the mean ± SEM of triplicate experiments.

[0018] Figure 5. Mechanisms of SSP mediated virus entry inhibition. (a-b) . Pre-treatment of SARS-CoV-1 (a) , H5N1 (b) and target cells inhibited viral infection. SARS-CoV-1 or H5N1 pseudovirus and HEK293T-ACE2 (a) or MDCK

(b) cells pre-treated with serial diluted SSP were recovered and subsequently subj ected to infect target cells, or incubated with untreated SARS-CoV-1 pseudovirus for 48 hours, respectively. The luciferase level was measured 2 or 3 days post-infection. (c-d) . Post-entry assay. GHOST-CD4-CCR5 or CXCR4 cells were co-incubated with pseudovirus for 2 hrs, washed, and then treated with the presence of 5 0 mg/ml SSP and 1 mM AZT as a positive control for 48 hr. SSP does n ot inhibit either HIV-1_{ADA} or HIV_{HxB2} virus gene replication after the viral entry is ac hieved. (e-f) . SSP-virus interaction assay. HIV-1_{ADA} and HIV_{HxB2} pseudovirus pre-treated with 50 mg/ml SSP and entry inhibitor enfuvirtide (T-20) as a positive control. SSP pre-treatment inhibited both HIV_{ADA} and HIV_{HxB2} pseudovirus infe ction to a similar degree as T-20. (g-h) SSP-cell binding assay. GHOST cells we re pretreated with SSP and the CCR5 antagonist maraviroc and the CXC R4 antagonist JM2987 as positive controls for 1 hr at 37℃ prior to being infected. SS P pretreatment with the cells had no antiviral effect, whereas maraviroc and JM2987 p re-treatment showed strong inhibition, as expected. Dimethyl sulfoxide (DMSO) was used as the negative control. The data represent the mean ± SD of triplica te experiments.

[0019] Figure 6. Isolation of SSP and UPLC-MS analysis. (a) UPLC-DAD spectra of SS P; (b) UPLC spectra of Fr. B (Part I) ; (c) UPLC spectra of Fr. G (Part II) ; (d) Ext racted ion chromatogram (EIC) of monomer and dimer of the proanthocy anidins in SSP; (e) Extracted ion chromatogram (EIC) of polymers wit h mDP between 3 and 7 of the proanthocyanidins in SSP; (f) Extracted ion chromatogram (EIC) of polymers with mDP between 8 and 10 of the proanthocyanidins in SSP.

[0020] Figure 7. Antiviral activity of SSP and their extracted parts against SARS-CoVs, and VSV. As shown in a～d, serially diluted SSP, SSP-n-BuOH, Fr. B, F, a nd G were added to HEK293T-ACE2 cells infected with SARS-CoV-2 (a) , SARS-CoV (b) , and VSV (c) . The luciferase level was measured 2 or 3 days post-infection. To test their cytotoxicity, cells viability (d) was also measured. (e-h) Pre-treatment of SARS-CoV-2 and target cells inhibited viral infection. SARS-CoV-2 pseudovirus and HEK293T-ACE2 pre-treated with serial diluted SSP-n-BuOH (e) , Fr. B (f) , F (g) , and G (h) were recovered and subsequently subjected to infect HEK293T-ACE2, or incubated with untreated SARS-CoV-2 pseudovirus for 48 hr, respectively. As controls, HEK293T-ACE2 cells were infected with SARS -CoV-2 pseudovirus and treated with gradient diluted SSP simultaneously. The data represent the mean ± SEM of triplicate experiments.

[0021] Figure 8. SSP did not show significant cytotoxicity in multiple cell lines, or long-term in vivo toxicity in rats. (a～ d) To test SSP cytotoxicity, cells viability was m

easured in HEK293T-ACE2 (a) , MDCK (b) , GHOST (3) -CD4-CXCR4 (c) /CCR5 (d) . (e) Effects of SSP administration for 4 weeks on the bodyweight of SD rats. (f) Effects of SSP administration for 4 weeks on food intake of SD rats. (g) Effect of SS P administration for 4 weeks on the organ coefficient of SD rats.

[0022] Figure 9. SSP shows higher potency and broader spectrum against SARS-CoV-2 variants entry in comparison with GSE and TP. (A) After keeping SS P solution for 6 and 13 days, the anti-viral activity and cyto-toxicity were tes ted. (B) SSP had a fairly strong inhibitory effect against major SARS-CoV-2 variants [including Alpha (B. 1.1.7) , Beta (B. 1.351) , Gamma (P1) , Delta (B. 1.6 17.2) , and Omicron (BA. 1, BA. 2, BA2.1.2.1 and BA. 4/5)] . (C～D) The anti-viral efficacy of grape seed extract (GSE) and tea polyphenol (TP) against SARS-CoV-2 and Omicron variants, as well as their cyto-toxicity.

[0023] Figure 10. SSP shows efficacy in protection against nasal challenge of SARS_CoV-2 omicron in mice. (A) Flowchart of the in vivo experiments. (B) Omi cron BA. 2 virus-infected cells (green) and inflammatory cells (red) were significant ly reduced in the lung tissues of mice treated with SSP compared with the saline grou p. (C) In the lung tissue of mice treated with SSP, the expression level of RNA-dependent RNA polymerase and the virus titer were significantly lowe r than those of mice treated with saline. *$P < 0.05$.

DETAILED DESCRIPTION

[0024] The present invention is related to a method of obtaining an extract of SSD, said met hod comprising: (i) cutting SSD into pieces; (ii) immersing and treating SSD pieces i n a solvent at room temperature to obtain an extract; (iii) concentrating the extract ; and (iv) drying the concentrated extract to obtain the powder of the extract of SSD , the extract of SSD including proanthocyanidins, named SSP.

[0025] As used herein, the term “SSD” refers to Spatholobus suberectus Dunn . As used herein, the term “SSP” refers to the extract of SSD including proanthocyani dins. Herein, SSP can be obtained by the above method comprising steps (i) to (iv) .

[0026] In step (ii) , suitable treating process can comprise percolation, ultrasonic, heatin g and refluxing, decoction, and/or solvent extraction. Suitably, the treating process in (ii) is percolation.

[0027] In step (ii) , the solvent used can comprise water, and/or an organic solvent. In one embodiment, the solvent in (ii) comprises water and ethanol, such as 60%ethanol/wate r (v/v) .

[0028] For example, step (ii) comprises treating SSD pieces via a percolation process with a 60%ethanol (in water, v/v) at room temperature. The percolation process can be perfo rmed for 12 hours.

[0029] In step (iii) , the concentrating process can comprise reduced pressure rotary evaporation, centrifugation, and/or nitrogen blowing. In one embodiment, the concentrating process in (iii) is reduced pressure rotary evaporation. The concentrating process can be performed at a temperature under 50°C.

[0030] In step (iv) , the drying process can comprise freeze drying, spray drying, microwave drying, and/or infrared heating drying. In one embodiment, the drying process in (iv) is freeze drying.

[0031] The invention also relates to the SSP obtained by the above method. SSP exhibited significant inhibitory ability against SARS-CoV-2 IC_{50} values of 3.574 μg/mL and 3.648 μg/mL. Innovatively, SSP uniquely inhibited the entry of SARS-CoV-2, HIV-1, Ebola virus (EBOV) , SARS-CoV-1 and influeunza H5N1 infections but failed to block vesicular stomatitis virus (VSV) . Furthermore, we showed that SSP had no effects on post-entry events of replication. It blocked SARS-CoV-2, HIV-1, H5N1, EBOV and SARS-CoV-1 entry by acting on viral envelope directly. Our findings suggested SSP is a novel entry inhibitor, which has a potential for preventing and treating COVID-19, also can fight EBOV, HIV-1, H5N1 and SARS-CoV infection, and stockpiling for possible use against future pandemic caused by related virus.

[0032] Our findings provide scientific evidence for the potential use of SSP for preventing &treating COVID-19 (SARS-CoV-2) infection, and stockpiling for possible use against future pandemic caused by related virus, as well as for the search of ingredient small molecular compounds as entry inhibitor.

[0033] Further, the invention is related to a method of obtaining an active fraction from SSD, the active fractions including proanthocyanidins, said method comprising: (i) cutting SSD into pieces; (ii) immersing and treating SSD pieces in a solvent at room temperature to obtain an extract; (iii) concentrating the extract; (iv) drying the concentrated extract to obtain the powder of the extract of SSD (SSP) ; (v) dispersing SSP in water to obtain an aqueous solution; (vi) extracting the SSP aqueous solution with a series of solvent with different polarities at equal volume to obtain parts of SSP corresponding to each solvent, the series of solvent comprising n-butanol; and (vii) separating the n-butanol part (SSP-n-BuOH) with eluents to obtain the active fraction.

[0034] As used herein, the term “active fraction” refers to the fraction extracted from SSD which is active as entry inhibitor and/or has anti-virus activity. Herein, one or more active fractions can be obtained by the above method. The active fraction (s) can include concentrated proanthocyanidins than SSP. Thus, the active fraction (s) exhibits entry inhibitory, anti-virus, immunoregulation and/or anti-inflammatory effects as SSP.

[0035] The active fraction (s) can have different mean degrees of polymeriz ation (mDP) . The active fraction (s) can have higher mDP than SSP. For example, one active fraction can have a mDP of 7～10.

[0036] Each of the steps (i) - (iv) can have any of the features as described abo ve in the method of obtaining an extract of SSD.

[0037] In step (vi) , the series of solvent can further comprise water, ethanol, petroleum e ther (PE) , and/or ethyl acetate. In one embodiment, the series solvent is petroleum ether (PE) , ethyl acetate and n-butanol. In this case, the parts of SSP correspondi ng to each solvent comprise petroleum ether part (SSP-PE) , ethyl acetate part (SSP-EA) , ethyl acetate insoluble part (SSP-EAin) , and n-butanol part (SSP-n-BuOH) .

[0038] In step (vii) , suitable separating process can comprise macroporous resin columns se paration, fractional precipitation, crystallization, normal phase chromatography, rev erse phase chromatography, and/or ion exchange resin columns separat ion. In one embodiment, the separating process is macroporous resin columns separatio n.

[0039] In step (vii) , suitable eluents can comprise water, and/or an organic solvent. In on e embodiment, the eluents in (vii) are gradient ethanol-water from 10%to 95%.

[0040] The method of obtaining an active fraction from SSD can further comp rise: (viii) concentrating the active fraction; and (ix) drying the concentrated acti ve fraction. The concentrating process in step (viii) can have any of the features of the corresponding process in the above step (iii) . The drying process in step (ix) can have any of the features of the corresponding process in the above step (iv) .

[0041] The invention also relates to the active fraction obtained by the above method.

[0042] Other than the above SSP extract, the above active fraction from SSD, related nature products from SSD may be useful in this disclosure. Meanwhile, the prescript or formu la similar to SSP can also have anti-viral function against COVID-19.

[0043] The invention further relates to a composition comprising the SSP or the active fract ion obtained by the above methods. The composition can further compr ise auxiliary materials. The auxiliary materials can include, but are not limited to fillers, such as starch, pregelatinized starch, lactose, mannitol, chitin, microcryst alline cellulose, sucrose, etc. ; disintegration agents, such as, starch, pregelatini zed starch, microcrystalline cellulose, sodium carboxymethyl starch, cross-linked polyvinylpyrrolidone, low-substituted hydroxypropyl cellulose, cross-linked polymethyl cellulose sodium, etc. ; additives, such as, stearic acid magnesium , sodium lauryl sulfate, talc, and/or silica suspending agents, binders, etc. ; suspe nding agents, such as, polyvinylpyrrolidone, microcrystalline cellulose, sucrose, aga r, hydroxypropyl methylcellulose, etc. ; binders, such as, starch Pulp, polyvinylpyrr olidone, etc. The composition can further comprise herbs other than SSD.

[0044] Disclosed herein is that SSP is an entry inhibitor for SARS-CoV-2 via direct binding to viral envelope and downregulating viral receptor ACE2 on target cell surface. The invention relates to a method of preventing and/or treating a viral disease comprising administering an effective amount of the SSP or the active fraction obtained by the above methods to a subject in need thereof. Suitably, the effect dose of SSP can be ranging from 0.5 μg/mL to 5000 μg/mL.

[0045] The invention also relates to use of SSP or the active fraction obtained by the above methods in the manufacture of preparations for prevention and/or treatment of a viral disease. The preparations can be in the form of injections, tablets, granules, emulsions, gels, sustained-release preparations, nasal wash/spray, oral liquids, throat spray, like-tea pills/candy, nano preparations, “throat lozenges” , “throat sprays” , “nasal washes” , and/or “nasal sprays” .

[0046] The viral disease include that caused by coronaviruses, e.g., SARS-CoV-2 and SARS-CoV-1 and their variants, Ebola virus (EBOV) , HIV-1, H5N1 and other enveloped viruses except for VSV.

[0047] The following Examples further illustrate the findings of the present disclosure.

[0048] EXAMPLES

[0049] The following examples are intended to illustrate the present invention, but are not intended to limit the scope of the present invention.

[0050] Materials and methods

[0051] 1. SSP Extract of SSD

[0052] Dried SS stems were purchased from KangMei Pharmaceutical Company Ltd (Guang Xi Province, China) and the plants were authenticated by inspectors in the School of Chinese Medicine, the University of Hong Kong, Hong Kong. Briefly, SS was cut into pieces and extracted using a percolating device with 10 times volume (v/w) of 60%ethanol with 12 hours at room temperature. The extract was then concentrated by reduced pressure under 50℃ and freeze-dried by vacuum freeze dryer to obtain the percolation powder, named SSP (Figure 1) . SSP can be dissolved in dimethyl sulfoxide (DMSO) to reach a concentration of 40 mg/mL for future use.

[0053] 2. Active fractions extraction

[0054] About 200 g SSP extract was weighed and dispersed evenly in an appropriate amount of water to obtain the aqueous solution, followed by extraction with equal volumes of petroleum ether (PE) , ethyl acetate (EA) , and n-butanol (n-BuOH) to obtain the following parts: 2 g petroleum ether part (SSP-PE) , 21 g ethyl acetate part (SSP-EA) , 8.2 g EA insoluble part (SSP-EAin) , 62 g n-butanol part (SSP-n-BuOH) and 113 g water layer residue (SSP-W) . Then the n-butanol part (SSP-

n-BuOH) was subjected to macroporous resin columns for gradient elution ethanol-water to obtain fraction A～J (Figure 1) .

[0055] 3. Quality control

[0056] The quality control methods for SSP extract contains: 1) the contents of proanthocyanidins by vanillin-hydrochloric acid method; 2) content determination of references compounds by UPLC; 3) TLC identification.

[0057] 1) The contents of PACs (proanthocyanidins) in SSP were determined by the vanillin-hydrochloric acid method with minor modification (Y. CHENG et al., 2011) . 2) UHPLC of SSP was performed by Ultimate 3000 system (Thermo Fisher Scientific Inc., Waltham, Massachusetts, U.S.) with a diode array detector (DAD) . An ACE Excel 2 C_{18} column (100 mm × 2.1 mm id, Scotland, UK) was used for the chromatographic separation, and the mobile phase was composed of acetonitrile (solvent A) and Milli-Q water with 0.1%formic acid (solvent B) , using a gradient elution of 0 min 95%B, 3 min 92%B, 20 min 85%B, 26 min 70%B, 30 min, 40%B, 35 min 5%B and keep at 5%B for five more minutes. The solvent flow rate was set as 0.4 mL/min, and the wavelength for analysis was conducted at 280 nm (Figure 2a) . This method was validated. Flavonoids, and proanthocyanidins were present in the SSP extract UPLC spectrum.
3) Identification of SSP by thin-layer chromatography (TLC) according to the Chinese Pharmacopoeia (version of the Year 2015) . Based on the color spot on the chromatogram, the compounds of SSP were identified according to the corresponding position of the control (Figure 2b) .

[0058] 4. Cell Culture

[0059] Highly sensitive and transferable cell lines, 293T, TZM-bl, and MDCK, which were obtained from ATCC company (American Type Culture Collection) and passage numbers were within 10 times, were cultivated in Dulbecco's modified Eagle medium (DMEM, Gibco, Grand Island, NY, USA) with 10%inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) , 1%mixture of penicillin and streptomycin (P/S, Sigma-Aldrich, St. Louis, MO, USA) . HEK 293FT-ACE2 was cultivated in DMEM with 10%FBS, 1%P/S, and 1 μg/mL puromycin (Sigma-Aldrich, St. Louis, MO, USA) . GHOST (3) -CD4-CCR5/CXCR4 cell lines were cultivated in DMEM with 10%FBS, 1%P/S, and 100 μg/mL hygromycin B, 500 μg/mL G418, and 1 μg/mL puromycin (Sigma-Aldrich, St.Louis, MO, USA) .

[0060] 5. Pseudovirus generation

[0061] We inserted an optimized full-length S gene of SARS-CoV-2 (QHR63250) into the pVAX-1 vector, namely pVax-1-S-COVID19. The construction was confirmed by sequence analysis. Single-cycle luciferase SARS-CoV-2, SARS-CoV-1, HIV

$_{ADA}$, HIV_{HXB2}, H5N1, and Vesicular stomatitis Indiana virus (VSV) pseudoviruses were constructed as previously described (L. Liu et al., 2007; Yi, Ba, Zhang, Ho, &Chen, 2005) . Briefly, pseudoviruses were generated by co-transfection of 293T cells with HIV-1 NL4-3 ΔEnv Vpr Luciferase (pNL4-3. Luc. R-E-) or HIV-1 NL4-3 ΔEnv EGFP Reporter Vector (NIH AIDS Reagent Program, Cat #3418 and 11100) and envelope protein from different strains of viruses (polyethyleneimine [PEI] ; Polysciences Inc., Warrington, PA) . Cell-free supernatant was collected 48h post-transfection and frozen at -80 ℃. To determine the viral titer, around 10,000 HEK293T-ACE2 cells per well in 96-well plates were seeded in 10%FBS-containing media. Cells were infected with serially diluted SARS-CoV-2 pseudovirus in a final volume of 200 μL. After 48 hours, infected cells were lysed to measure luciferase activity using a commercial kit (Promega, E1500, Madison, WI) . The 50%tissue culture infective dose (TCID50) was calculated using the "TCID" macro as described previously (Richards &Clapham, 2006) .

[0062] 6. Cytotoxicity assays

[0063] Cells were incubated in the presence or absence of serially diluted SSP at 37 ℃ in 5%CO_2 for 48 hours. The viability of the cells was then measured using theCellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, Wisconsin, USA) . The cytotoxicity was generated according to the sample concentrations and luminescent values. The result was analyzed based on the formula:

$$\%\ \text{cell viability} = 100\ \text{X}\left(1 - \frac{\text{Luminescent value of the sample}}{\text{Luminescent value of cells alone}}\right)$$

[0064] 7. Antiviral assays

[0065] The inhibitory activities of the SSP against viruses are evaluated as previously described (L. Liu et al., 2007; X. Lu et al., 2012) . Briefly, serially diluted SSP was tested against 100TCID50 viral infection. HEK 293FT-ACE2 were used for SARS-CoV-1/2; MDCK was used for H5N1; GHOST (3) -CD4-CCR5/CXCR4 were used for HIV_{ADA} and HIV_{HXB2} infection. On day three, the viral infection was determined by measuring the reporter luciferase activity in target cells post-infection using commercially available kits (Promega, Wisconsin, USA) . Antiviral data are reported as the concentration of drugs required to inhibit viral replication by 50% (EC_{50}) . Similarly, the different SARA-CoV-2 variants including Alpha (B. 1.1.7) , Beta (B. 1.351) , Gamma (P1) , Delta (B.1.617.2) , and Omicron (BA. 1, BA. 2, BA2.1.2.1 and BA. 4/5) were also used to test the inhibition rate of SSP. The anti-viral activity of grape seed extract (GSE) and tea polyphenol (TP, CAS: 84650-60-2) (Shanghai Yuanye Company) were also tested by the same method.

[0066] 8. Safety evaluation

[0067] Model: Acute Toxicity-Mice and rats; Long-term Toxicity-Rats; Nasal spray irritation test-Rabbits

[0068] Acute toxicity experiment: observe and study the toxicity of SD rats and NIH mice given single or multiple times by intragastric administration of SSP within 24 hours to determine the acute toxic dose and the maximum tolerated dose and possible toxicity for target organs for its clinical safety. Method: Several SD rats and NIH mice were selected, half male and female, and randomly divided into vehicle control group and SSP group. The rats were fasted for 12h-16h before administration and weighed. The SSP group was given SSP powder 0.25g / mL was given to rats by 20mL /kg, and the vehicle control group was given an equal volume of purified water, orally or twice a day (each dosing interval 8h), continuous observation for 4 hours after each administration , D1 ～ D14 were observed once a day in the morning and afternoon; D1, D4, D7, D14 weighed the rats and their situations were also recorded. After animals sacrifice and visual observation, any changes in volume, color, and texture of any tissues and organs were recorded and histopathological checked.

[0069] Long-term toxicity: Twenty-four male and female Sprague-Dawley rats (150-180 g) were used to determine long time adverse effect of SSP. All experiments were approved by the Institutional guidelines of Laboratory Animal Care and Committee on the Use of Live Animals in Teaching and Research, South China Agricultural University, Guangzhou, China. The animals were randomly divided into four groups, with three males and three females per group. SSP was dissolved in water and administered via gavage once a day at doses of 2, 4, 6 g/kg, or water. Their body weights, general behavior, signs of toxicity, food intake, and mortality were recorded at an interval of every two-four days. After 25 days, the animals were sacrificed by excessive anesthesia. Gross necropsy, calculation of organ index (such as brain, heart, lung, liver, kidney, etc.) , pathological examinations of the organs, blood collection, and analysis were performed.

[0070] Nasal spray irritation test-Rabbits: This experiment was completed in the CFDA-certified GLP-compliant Drug Safety Evaluation Research Center (Shandong Xinbo Drug Research Co., Ltd.) , and the code of this experiment was KY22233.

[0071] 9. Stability

[0072] The stability of SSP powder is determined by vanillin-hydrochloric acid method as mentioned in 3-1) to detect the content of proanthocyanidins, and the detection samples are the SSP aqueous solution prepared immediately, and the SSP aqueous solution after keeping at room temperature for over 1 month.

[0073] Besides, it was found that the anti-viral activity of the SSP aqueous solution after being placed for 6 and 13 days.

[0074] 10. In vivo anti-viral experiments

[0075] Efficacy of SSP against live intranasal SARS-CoV-2 Omicron BA. 2 challenge were tested in K18-hACE2 mice. The units of 2*10^4 moi of viruses were administered to the nasal cavity of the mice. As shown in Figure 10a, one dose of SSP intervention (40 mg/ml *20 μL, 800 μg) was given at the time of 30 minutes before virus infection, and the control group was saline group (n = 5 per group) . After 3 days of virus infection, the lung tissues of the mice were collected.

[0076] Results

[0077] 11. SSP inhibits SARS-CoV-2 entry

[0078] To determine the antiviral activity of SSP, SSP was weighed and dissolved in 40 mg/mL as described. As previously described, the SARS-CoV-2 pseudoviruses were generated (L. L. Liu et al., 2019; X. ;) . To examine the antiviral activity of SSP against SARS-CoV-2, HE293T-ACE2 cells were then infected with 100 TCID50 SARS-CoV-2 pseudoviruses in the presence of serially diluted SSP. Virus pseudotyped with a second, unrelated viral envelope glycoprotein of VSV-G was included as a control to reduce the false positives. As shown in Figure 3, SSP displayed an EC_{50} of 3.5 μg/mL in the inhibition of SARS-CoV-2 pseudovirus infection (Figure 3a) and was devoid of overt cytotoxicity (Figure 3c) . No inhibition was observed when SSP was tested against VSV pseudovirus (Figure 3b) . Because SARS-CoV-2 and VSV pseudoviruses share the common genetic HIV backbone expressing protease (PR) , reverse transcriptase (RT) , and integrase (IN) and differ only in their glycoproteins on the surface of the viruses. This result suggested that SSP antagonizes SARS-CoV-2 pseudovirus entry rather than post-entry events (e.g., reverse transcription) . Moreover, the lack of antiviral activity against VSV also ruled out the possibility that SSP simply inactivates the SARS-CoV-2 virus by acting on viral lipids as a disinfectant.

[0079] 12. SSP acts by blocking the attachment of SARS-CoV spike envelope protein to entry receptor ACE2

[0080] As known, SARS-CoV-2 enter cells in the host through a virus surface-anchored S-protein. The S protein mediates viral entry through the RBD in the S1 subunit that specifically recognizes ACE2 as its receptor and then fuses the viral into host membranes through the S2 subunit. Entry inhibitors usually target protein-protein interactions within the viral envelope proteins or between viral envelope proteins and host cells or inhibit protein-lipid interactions. To determine whether SSP act by targeting SARS-CoV-2 S protein or host cells, we pre-treated pseudovirus and HEK-293T-ACE2 cells with serially diluted SSP for 2 hours at 37 ℃. The viruses and HEK-

293T-ACE2 cells were then recovered and subsequently subjected to infect HEK293T-ACE2 or incubated with untreated SARS-CoV-2 pseudovirus (Ra pista et al., 2011) . As controls, HEK293T-ACE2 cells were infected with SARS-CoV-2 pseudovirus and then treated with gradient diluted SSP immediately or 2-hours post-infection (hpi) . As shown in Figure 3d, SSP treatment at 2-hours post-infection showed limited anti-SARS-CoV-2 activity compared with the virus and SSP being added simultaneously. This result confirmed the antiviral entry activi ty of SSP against SARS-CoV-2. Moreover, pre-treatment of pseudovirus with SSP resulted in increased inhibition activities with the EC_{50} value of 2.3 μg/mL, suggesting that SSP targets SARS-CoV-2 viral envelop S protein to inhib it SARS-CoV-2 entry. Notably, pre-treatment of target cells with SSP halted SARS-CoV-2 infection with the EC_{50} value of 2.1 μg/mL, suggesting that SSP also targe ts cellular components to inhibit SARS-CoV-2 entry.

[0081] To determine whether SSP targets ACE2 receptor to block virus entry, RBD binding assay was performed. HEK-293T-ACE2 cells were treated with seria lly diluted SSP for 2 hours at 37°C, washed, and were then incubated with supernatant collected from RBD-PD1 expressing plasmid transfected HEK293T cells or a goat polyclonal antibody against ACE2 for 30 min on ice. Cells we re then washed and stained with a fluorescent-labeled secondary antibody against goat-IgG or PD-1, respectively. As shown in Figure 3e, untreated HEK-293T-ACE2 cells were positive for both ACE2 and RBD-PD1 staining but negative for the staining of antibodies against goat-IgG and PD-1, which suggested that HEK-293T-ACE2 cells were having a high expression level of ACE2 and can bind to RBD of SARS-CoV-2 S protein. SSP-treated cells showed significantly reduced geometric mean signal for ACE2 staining but not the percentage of $ACE2^+$ cells, suggesting the likelihood that SSP partially blocked ACE2 specific antibody b inding rather than downregulating ACE2 expression. In contrast, SSP treatment deducted both geometric mean signal and percentage of posi tive cells of RBD staining when compared to untreated cells, suggesting that SSP trea tment partially blocked binding of RBD to ACE2 protein (Figure 3f) .

[0082] 13. SSP shows broad-spectrum antiviral activities against SARS-CoV, H5N1, EBOV and HIV-1

[0083] To determine whether SSP possesses broad-spectrum antiviral activities, a p anel of pseudoviruses was generated in our previous studies, including SARS-CoV (L. Liu et al., 2019) , $H5N1_{Turkey}$ (Xiao et al., 2013) , CCR5-tropic HIV-1_{ADA}, and CXCR4-tropic HIV-1_{HXB2} (Liang et al., 2013) , were utilized to test the i nhibition rate of SSP. As shown in Figure 4a-d, SSP inhibited their infection with EC

$_{50}$ around 3.64, 5.13, 3.61, and 8.15 μg/mL, respectively, and was devoid of overt cytot oxicity (Figure 4e) . This result suggested that SSP has a broad-spectrum antivira l activity against entry of SARS-CoV, H5N1, and HIV-1. Additionally, recent re-emergence of Ebola virus disease in West Africa has led us to exam whether S SP also inhibit EBOV infection. EBOV pseudovirus was produced in 293T cells as described previously. We tested pseudoviral EBOV and VSV as contr ol. 293 T cells were infected with EBOV and VSV, respectively, in the presence of ser ial diluted SSP. We found that SSP inhibited EBOV with EC_{50} value around 4 μg/ ml without cell toxicity observed (Figure 4f) . Consistently, SSP did not inhibit V SV infection, indicating that SSP blocked EBOV entry to the target cells.

[0084] SSP blocks SARS-CoV-1 and H5N1 entry by binding to viral envelope and i ts target cells. To determine whether SSP inhibits viral entry by binding to the SARS -COV-1 envelope or its receptor on target cells, SSP-virus binding and SSP-cell binding assays were performed. In the SSP-virus binding assay, pseudovirus w as initially pre-treated with 50 μg/mL of SSP. The viruses were then recovered by ult racentrifugation and subjected to infect target cells. We found that SSP inhibited SA RS-CoV-1 pseudovirus infection at a similar degree as when the virus and SS P were added into cells simultaneously (Figure 5) . Meantime, the SSP-cell binding assays were performed by pre-treatment of the target cells with SSP for 1 hour at 37℃. After washing by PBS, cells were incubated with SARS-CoV-1 at 37 ℃ for 48 hrs (Rapista et al., 2011) . We found that pre-treatment of SSP on the target cells inhibited virus infection (Figure 5) . The same approach was also used to test the inhibitory effect of SSP against H5N1 with a single high dose of 50 μg/mL. Similar to SARS-CoV-1, SSP treatment with virus particles or their targ et cells efficiently blocked H5N1 virus entry (Figure 5b) .

[0085] SSP blocks HIV-1 entry by binding to the viral envelope. We tested HIV-1 with a similar but slightly modified experimental protocol by including four anti-HIV drugs: AZT, a potent NRTI, HIV-1 fusion blocker T20, CCR5 antagon ist Marvaroc (MVC) , and the CXCR4 antagonist JM2987 as positive con trols. As shown in Figure 5, AZT, but not SSP, significantly inhibited HIV-1 infection when the treatment was initiated at 2 hours post-infection. In con trast, pre-treatment of the virus with 50 μg/mL of SSP significantly inhibited HIV-1 infection at a similar degree as T-20, which blocks HIV-1 fusion (Figure 5c～ 5d) . Subsequently, the SSP-cell binding assays were performed by treating the target GHOST cells with SSP or with control compounds the CCR5 antag onist Marvaroc (MVC) or the CXCR4 antagonist JM2987 for 2 hours at 3 7℃. After washing by PBS, cells were subjected to HIV-1_{ADA} or HIV-1_{HXB2} infection and cultivated at 37 ℃ for 48 hrs (Rapista et al., 2011) . We found that pre-

treating the target cells with SSP had virtually no antiviral effect, while MVC and J M2987 showed potent viral inhibition against the respective ADA and HXB2 pseudoviruses at 1 μM as expected (Figures 5e～5f) . This eviden ce demonstrated that SSP inhibited HIV-1 infection by actions on the vira l envelope glycoprotein gp160, which mediates the viral entry into the host target ce lls.

[0086] 14. Antiviral activity of SSP and its effective constituents

[0087] To interpret the materials containing SS, a feasible UPLC method was used to separate SSP, in which SSP was divided into three parts, namely Part I, II, and III (Figure 6a) . UPLC-ESI-MS/MSn experiment was also perform ed under the same method (Figure 6d～6f) , and found that: (1) Monome r and dimer were mainly enriched in the 3～9 min (Part I) ; (2) 8～10m er were mainly enriched in the 23～33 min (Part II) ; (3) 3～7mer were both enriched in the two parts. Nevertheless, polymers with a degree of polymerizati on (DP) above 10 were undetected due to the limited range of molecul ar weight. Based on this result, different polar solvents were used for the extractio n, and the n-butanol part (SSP-n-BuOH) was selected for further separation by using the macroporous resin columns. Through UPLC's detection, Fr. B and F r. G were separated into two parts, Part I and Part II, respectively, as mentioned ab ove (Figure 6b～6c) . In this study, we extracted and greatly enriched proanthocyanidi ns in SSP, reaching about 60%concentration. The thiolysis experiments also proved tha t the mean degrees of polymerization (mDP) of SSP, SSP-n-BuOH, Fr. B, and Fr. G were 3.49, 4.57, 2.95, and 7.52 respectively, which meant macroporous resin col umns could effectively separate and obtain parts with different mDPs. Using the metho d as previously described, we found that SSP, SSP-n-BuOH, and Fr. G e xhibited lower EC_{50} in the inhibition of SARS-CoV and SARS-CoV-2 pseudovirus inf ection than Fr. B (Figure 7a～7b) . No significant inhibitory effects on VSV pseudovir us (Figure 7c) or cytotoxicity were observed (Figure 7d) . Moreover, pre-treatment of pseudovirus or cells with SSP-n-BuOH, Fr. F, or Fr. G inhibited vi ral entry with lower EC_{50} value than adding the virus and fragments simultan eously, suggesting that those fragments target both SARS-CoV-2 viral envelop S protein and target cells to inhibit SARS-CoV-2 entry. More importantly, Fr. G e xhibited more outstanding bioactivity when compared to Fr. B, suggesting that Part II of SSP contains more effective antiviral activities than Part I. These results revea led that Fr. G contains an intermediate polymer of proanthocyanidins, which may have better antiviral effects.

[0088] 15. Safety evaluation result

[0089] To evaluate the safety of SSP, the cytotoxicity in multiple cell lines was determined by cell toxicity assay (IC_{50} = 181.2～339.8 μg/mL as shown in Figure 8a～8d) .

[0090] To test the acute toxicity in vivo, NIH mice and SD rats were employed to obtain the maximum tolerated dose. The maximum tolerated dose for one-time administrati on is 67 g crude drug /kg in mice (Table 1) . The maximum tolerated dose for one-time administration is 107 g crude drug /kg in rats (Table 2) .

[0091] To further examine the long-term toxicity in vivo, 24 male and female SD rats w ere divided into four groups: water (blank control) , 2 g/kg, 4 g/kg, and 6 g/ kg SSP. Drug or water was administered via oral gavage once a day for 25 da ys. No mortality was observed in any group of rats during the monitoring period (Figu re 8) . The body weight and food intake were shown in Figures 8e-8f. Administratio n of SSP at the doses did not manifest any signs of toxicity to rats except the loss of body weight in the high dose group. No significant differences in organ indexes (r atio of organ weight to body weight) were observed compared with the control mice (Figure 8g) . No abnormality was observed in levels of blood biochemica l parameters were not harmed, indicating that SSP has no apparent toxicity to the exp erimental rats (Table 3) .

[0092] Mice result:

[0093] Table 1 acute toxicity of SSP in mice (NIH)

Dosage	Index	♂			♀		
		1	2	3	4	5	6
20g/kg	dose	0.5g/ml (Maximum concentration) × 40ml/kg=20g/kg (133 g crude herb/kg)					
	Dead	3.5h	5.5h	5.5h	4.5h	4h	4h
10g/kg	Dose	0.25g/ml × 40ml/kg=10g/kg (67 g crude herb /kg)					
	Time of death	22h	/	3h	3h	4h	/
	FBG(6h)	3.2	2.3	dead	dead	dead	2.3
	PBG(24h)	dead	3.1				8.4
	PBG(48h)		2.8				8.8
5g/kg	dose	0.125g/ml × 40ml/kg=5g/kg (33 g crude herb/kg)					
	Dead	/	/	/	/	/	/
	FBG(6h)	5.2	3.7	2.2	5.1	2.7	3.8
	PBG(24h)	7.4	5.9	3.4	3.2	7.3	7.4
	PBG(48h)	8.7	7.0	3.8	5.5	9.2	9.5

[0094] Rat result:

[0095] Table 2 acute toxicity of SSP in rat (SD)

Administration			Oral taken (No. TT2-2018515)		
Dose			0.4g/ml (Maximum concentration)× 20ml/kg × 2=16 g/kg (107 g crude herb /kg)		
FBG(mmol/L)			FBG(1h)	PBG(6h)	PBG(24h)
SSP	♂	236.5g	7.9	9.8	1.7(Dead)
		247.8g	7.2	8.3	8.4
		272.5g	7.2	7.2	5.7
		215.9g	4.8	6.8	6.6
	♀	223.0g	9.3	11.2	1.2
		190.6g	5.9	9.6	(Dead)
		173.5g	5.8	7.2	(Dead)
		192.4g	7.9	8.2	(Dead)

[0096] Table 3 Blood biochemical index testing items in male and female rats

Indicators	Control ♂ (n=3)		SSP-II ♂ (n=3)		Control ♀ (n=3)		SSP-II ♀ (n=3)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ALP (U/L)	211.2	22.44	235.5	85.27	105.97	21.77	119.5	6.59
ALT (U/L)	42	13.45	28.67	10.97	27.67	4.62	20.67	1.53
AST (U/L)	91.37	25.26	91.97	17.09	89	16.05	78.53	9.01
LDH (U/L)	356.33	49.86	682	205.47	612.67	208.08	578.67	192.76
UREA (mmol/L)	5.38	0.35	3.89	0.53	6.98	0.68	5.68	0.51
CRE (μmol/L)	29	4	27.33	2.89	36.33	6.66	32.33	3.06
TP (g/L)	55.8	3.72	55.83	1.1	55.43	4.68	55.27	3.52
ALB (g/L)	36.77	1.14	32.93	6.75	39.3	1.14	39.53	3.2
GLB (g/L)	19.03	4.86	22.9	7.61	16.13	3.73	15.73	0.42
A/G	2.02	0.52	1.64	0.94	2.51	0.48	2.51	0.16
GLU (mmol/L)	15.92	2.34	13.58	3.98	11.48	3.56	9.23	1.99
CHO (mmol/L)	1.35	0.14	1.46	0.15	1.24	0.2	1.14	0.13
TG (mmol/L)	0.97	0.34	0.64	0.21	0.94	0.36	0.53	0.36
K (mmol/L)	5.98	1.47	5.73	0.39	4.77	0.17	4.28	0.24
Na (mmol/L)	135.33	2.31	138.67	1.53	133.33	6.35	134	10.44
Cl (mmol/L)	97.47	3.65	98.47	1.08	96.67	6.34	95.8	8.43
CK (U/L)	1336.33	753.96	818.67	551.85	906.33	700.2	478.33	122.35
TBIL (μmol/L)	0.67	0.06	1.03	0.12	0.57	0.31	0.9	0.36
u/c	186.9	16.06	144.96	36.21	194.14	16.62	176.21	13.77

[0097] 16. Stability results

[0098] According to the same method, the proanthocyanidins contained in the immediately prepared SSP aqueous solution were 794.794 ± 13.619 mg/

g, while the content in the SSP aqueous solution after being placed for 1 month was 7 78.964 ± 63.589mg/g, which was within the quality standard range of 5%, so the SSP aqueous solution is stable in terms of proanthocyanidin content.

[0099] Besides, it was found that the anti-viral activity of the SSP aqueous solution after be ing placed for 6 and 13 days were no significant reduction, and cyto-toxicity was also no changes (Figure 9a) .

[0100] 17. In vitro anti-viral experiments against SARS-CoV-2 virus variants

[0101] As shown in Figure 9a-9b, the inhibitory activity of SSP against SARS-CoV-2 virus variants was also quite potent, meanwhile, the cell toxicity was minor. Compa red with SSP, the GSE (Figure 9c) and TP (Figure 9d) , which also co ntained PACs, exhibited much weaker inhibitory effect and not all variants were inhib ited. SSP shows higher potency and broader spectrum against SARS-CoV-2 variants entry, which is different from GSE and TP. To sum up, SSP is the unique wi th higher potency and broader spectrum against virus, which deserves deeper explorati on.

[0102] 18. In vivo anti-viral experiments

[0103] As shown in Figure 10b, Omicron BA. 2 virus-infected cells (green) and inflamm atory cells (red) were significantly reduced in the lung tissue of mice treated with SSP compared with the saline group. Also, in the lung tissue of mice treated with SSP , the expression level of RNA-dependent RNA polymerase and the virus titer were s ignificantly lower than those of mice treated with saline (Figure 10c) . Overall, SSP showed efficacy in protection against nasal challenge of SARS-CoV-2 omicron variant virus in mice.

[0104] 19. Nasal spray irritation test

[0105] This experiment was completed in the CFDA-certified GLP-compliant Drug Sa fety Evaluation Research Center (Shandong Xinbo Drug Research Co., L td.) , and the code of this experiment was KY22233. During the test period, the anim als were generally in good condition, and no death occurred; no obvious abnormalities were observed immediately after each administration. 24 hours after the last adminis tration and 14 days after recovery, the necropsy was performed to observe the local m ucous membrane tissue (oral cavity, nasal cavity, larynx, trachea, and bronchi) with the naked eye. No obvious abnormalities were found in the negative control group and the test product group. The results of histopathological examination also showed that 24 days after the last administration and the end of the recovery period, no drug-related histopathological changes were found in all animal parts of the negative cont rol group and the test product group.

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[0120] The foregoing description of the specific embodiments will so fully reveal the genera l nature of the disclosure that others can, by applying knowledge within the skill of the relevant art (s) (including the contents of the documents cited and incorporated by reference herein) , readily modify and/or adapt for various applications such spe cific embodiments, without undue experimentation, without departing from the general concept of the present disclosure. Such adaptations and modification s are therefore intended to be within the meaning and range of equivalents of the dis closed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of descripti on and not of limitation, such that the terminology or phraseology of the present spe cification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one skilled in the re levant art (s) .

[0121] While various embodiments of the present disclosure have been descri bed above, it should be understood that they have been presented by way of examples, and not limitation. It would be apparent to one skilled in the relevant art (s) that various changes in form and detail could be made therein without departing from the s pirit and scope of the disclosure. Thus, the present disclosure should not be limited by any of the above-described exemplary embodiments but should be defin ed only in accordance with the following claims and their equivalents.

[0122] All references cited herein are incorporated herein by reference in their entirety an d for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Claims

[Claim 1] A method of obtaining an extract of Spatholobus sub erectus Dunn (SSD) , said method comprising: (i) cutting SSD into pi eces; (ii) immersing and treating SSD pieces in a solvent at room te mperature to obtain an extract; (iii) concentrating the extract; and (iv) drying the concentrated extract to obtain the powder of the ex tract of SSD, the extract of SSD including proanthocyanidins (SSP) .

[Claim 2] A method of obtaining an active fraction from Spatholobus suberectus Dunn (SSD) , the active fraction including proanthocyanidins, said method comprising: (i) cutting SSD into pieces; (ii) immersing and t reating SSD pieces in a solvent at room temperature to obtain an ext ract; (iii) concentrating the extract; (iv) drying the concentrated extract to obtain the powder of the extract of SSD (SSP) ; (v) dispe rsing SSP in water to obtain an aqueous solution; (vi) extracting th e SSP aqueous solution with a series of solvent with different polar ities at equal volume to obtain parts of SSP corresponding to each s olvent, the series of solvent comprising n-butanol; and (vii) separating the n-butanol part (SSP-n-BuOH) with eluent s to obtain the active fraction.

[Claim 3] The method of claim 1 or 2, wherein the treating process in (ii) com prises percolation, ultrasonic, heating and refluxing, decoction, and/ or solvent extraction.

[Claim 4] The method of claim 3, wherein the treating process in (ii) comprise s percolation.

[Claim 5] The method of claim 1 or 2, wherein the solvent in (ii) comprises wa ter, and/or an organic solvent.

[Claim 6] The method of claim 5, wherein the solvent in (ii) comprises water a nd ethanol.

[Claim 7] The method of claim 6, wherein the solvent in (ii) is 60%ethanol/wat er (v/v) .

[Claim 8] The method of claim 1 or 2, wherein the concentrating process in (ii i) comprises reduced pressure rotary evaporation, centrifugation, and/ or nitrogen blowing.

[Claim 9] The method of claim 8, wherein the concentrating process in (iii) co mprises reduced pressure rotary evaporation.

[Claim 10] The method of claim 1 or 2, wherein the concentrating process is per formed at a temperature under 50℃.

[Claim 11] The method of claim 1 or 2, wherein the drying process in (iv) comprises freeze drying, spray drying, microwave drying, and/or infrared heating drying.

[Claim 12] The method of claim 11, wherein the drying process in (iv) comprises freeze drying.

[Claim 13] The method of claim 1 or 2, wherein the series of solvent in (vi) further comprises water, ethanol, petroleum ether (PE) , and/or ethyl acetate.

[Claim 14] The method of claim 1 or 2, wherein the separating process in (vii) comprises macroporous resin columns separation, fractional precipitation, crystallization, normal phase chromatography, reverse phase chromatography, and/or ion exchange resin columns separation.

[Claim 15] The method of claim 14, wherein the separating process in (vii) comprises macroporous resin columns separation.

[Claim 16] The method of claim 1 or 2, wherein the eluents in (vii) comprises water, and/or an organic solvent.

[Claim 17] The method of claim 16, wherein the eluents in (vii) are gradient ethanol-water from 10%to 95%.

[Claim 18] The method of claim 2, further comprising: (viii) concentrating the active fractions.

[Claim 19] The method of claim 18, further comprising: (ix) drying the concentrated active fractions.

[Claim 20] An extract of SSD obtained by the method of any one of claims 1 and 3-17.

[Claim 21] An active fraction from SSD obtained by the method of any one of claims 2-19.

[Claim 22] A composition comprising an extract of SSD obtained by the method of any one of claims 1 and 3-17, or an active fraction from SSD obtained by the method of any one of claims 2-19.

[Claim 23] A method of preventing and/or treating a viral disease comprising administering an effective amount of an extract of SSD obtained by the method of any one of claims 1 and 3-17 to a subject in need thereof.

[Claim 24] A method of preventing and/or treating a viral disease comprising administering an effective amount of an active fraction from SSD obtained by the method of any one of claims 2-19 to a subject in need thereof.

[Claim 25] The method of claim 23 and 24, wherein the effective dose is ranging from 0.5 μg/mL to 5000 μg/mL.

[Claim 26] Use of an extract of SSD obtained by the method of any one of claims 1 and 3-17 in the manufacture of preparations for prevention and/or treatment of a viral disease.

[Claim 27] Use of an active fraction from SSD obtained by the method of any one of claims 2-19 in the manufacture of preparations for prevention and/or treatment of a viral disease.

[Claim 28] The method/use of any one of claims 23-26, wherein the viral disease is caused by coronaviruses including SARS-CoV-2 and SARS-CoV-1 and their variants, Ebola virus (EBOV) , HIV-1, H5N1, and other enveloped viruses except for VSV.

[Claim 29] The use of claim 26 or 27, wherein the preparations are in the form of injections, tablets, granules, emulsions, gels, sustained-release preparations, nasal wash/spray, oral liquids, throat spray, like-tea pills/candy, nano preparations, “throat lozenges” , “throat sprays” , “nasal washes” , and/or “nasal sprays” .

[Claim 30] The use of claim 26 or 27, where in the preparations further comprise fillers, disintegration agents, additives, suspending agents, and/or binders.

[Claim 31] The use of claim 30, wherein the fillers comprise starch, pregelatinized starch, lactose, mannitol, chitin, microcrystalline cellulose, and/or sucrose.

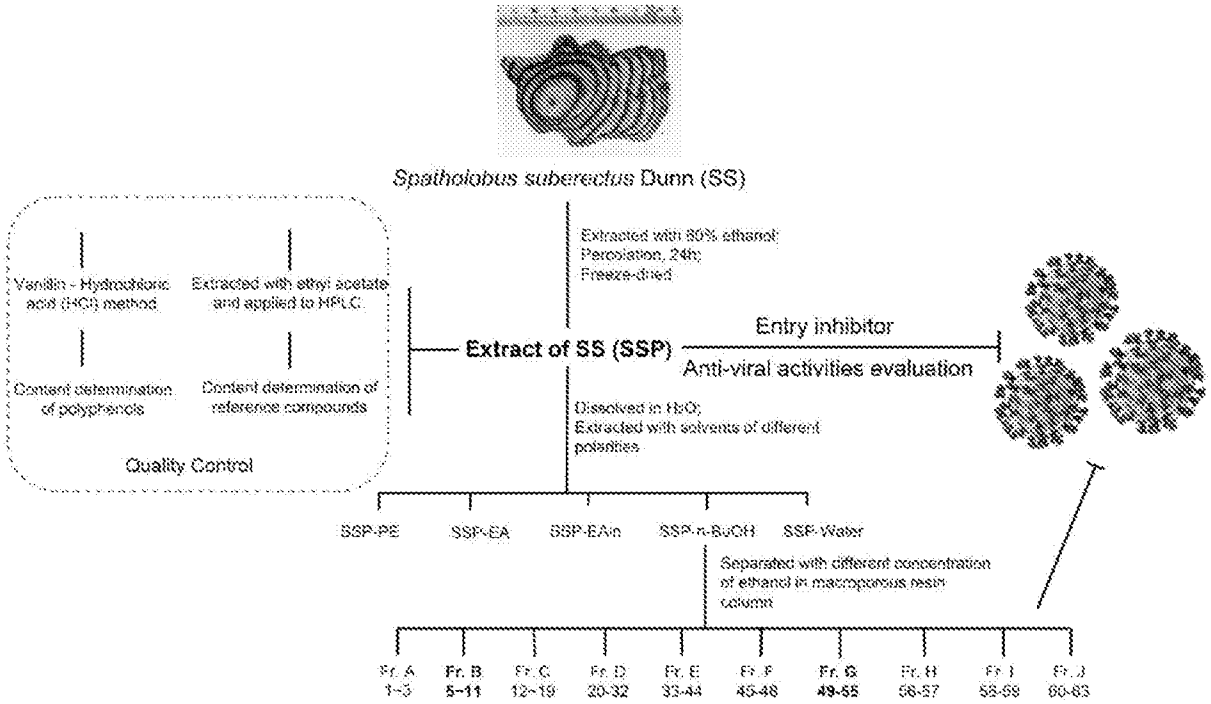
[Claim 32] The use of claim 30, wherein the disintegration agents comprise starch, pregelatinized starch, microcrystalline cellulose, sodium carboxymethyl starch, cross-linked polyvinylpyrrolidone, low-substituted hydroxypropyl cellulose, and/or cross-linked polymethyl cellulose sodium.

[Claim 33] The use of claim 30, wherein the additives comprise stearic acid magnesium, sodium lauryl sulfate, talc, and/or silica.

[Claim 34] The use of claim 30, wherein the suspending agents comprise polyvinylpyrrolidone, microcrystalline cellulose, sucrose, agar, and/or hydroxypropyl methylcellulose.

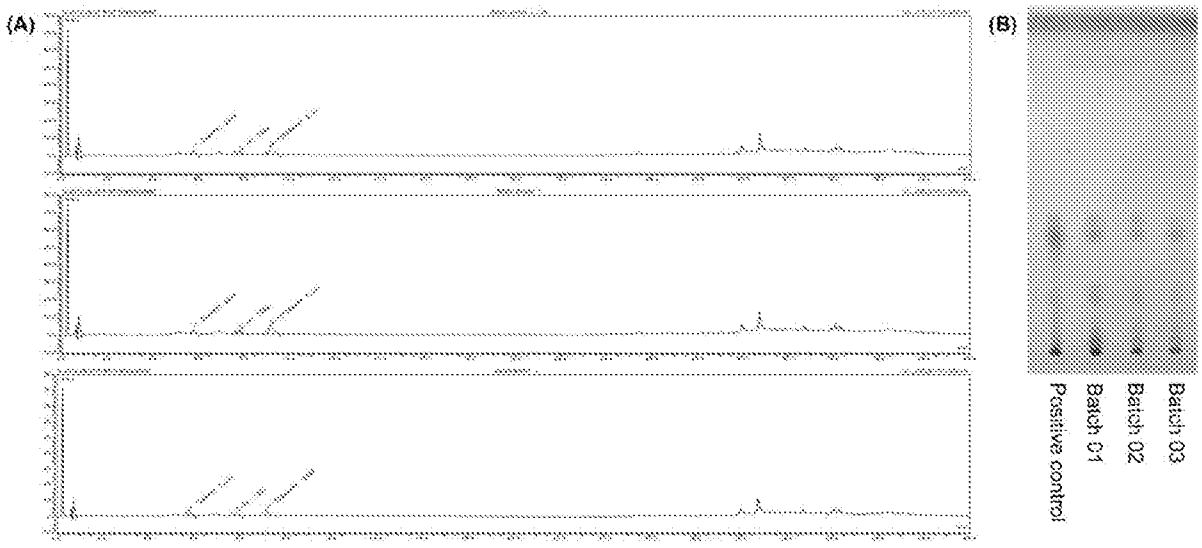
[Claim 35] The use of claim 30, wherein the binders comprise starch Pulp, and/or polyvinylpyrrolidone.

[Fig. 1]



Rule 26, 12.09.2024

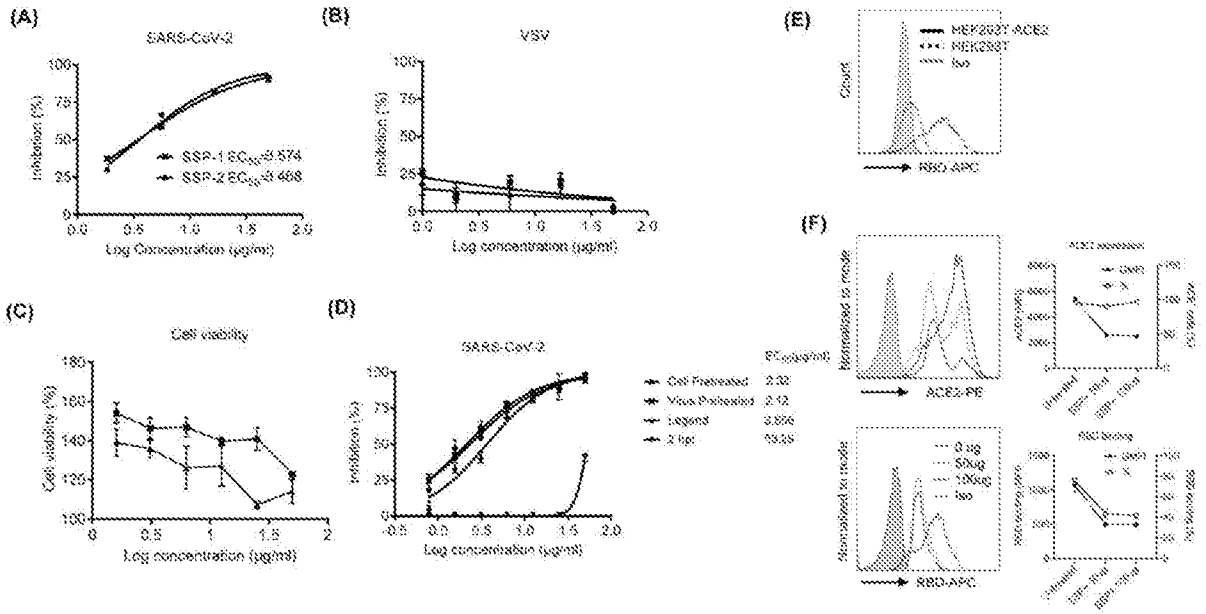
[Fig. 2]



Rule 26, 12.09.2024

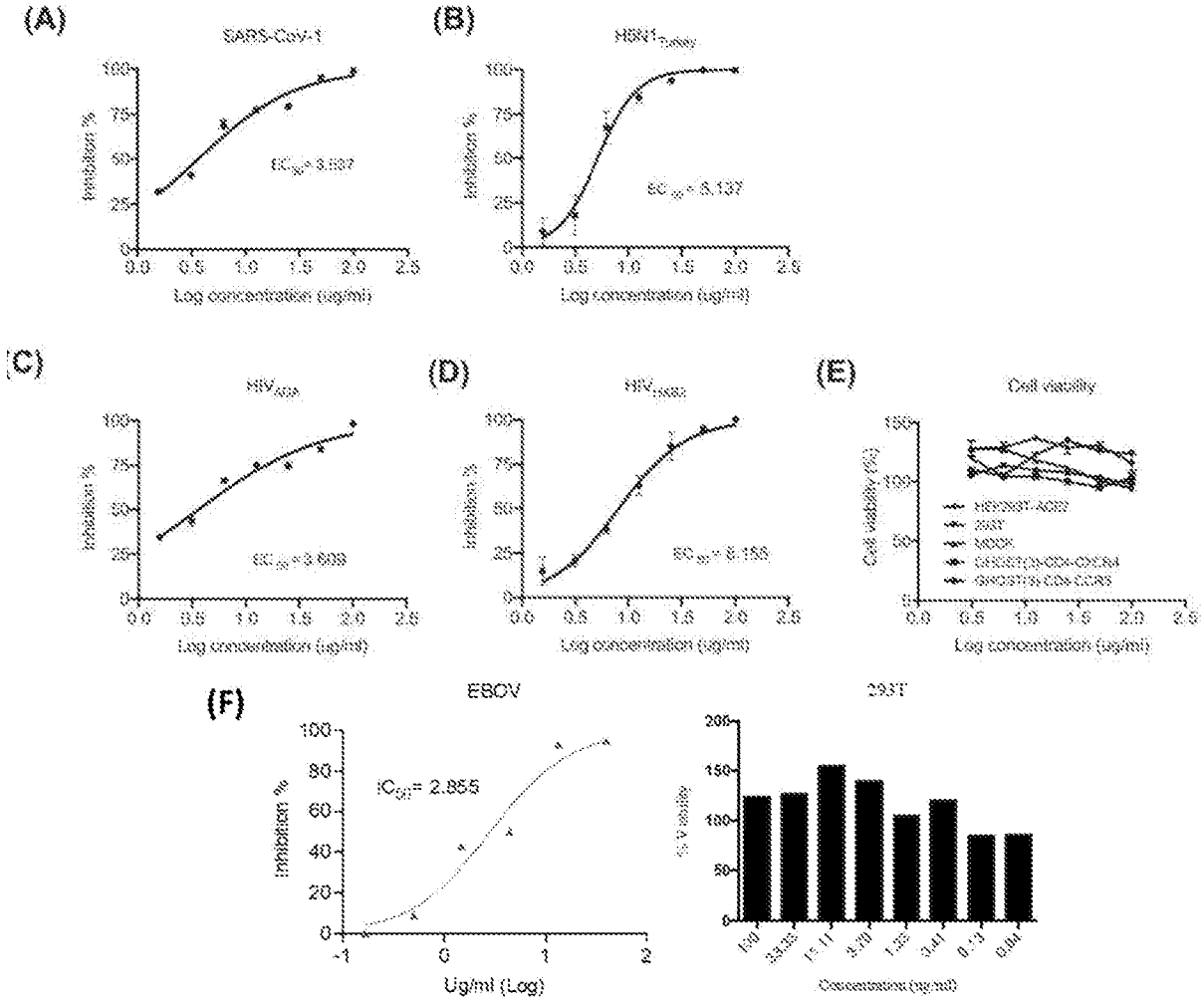
[Fig. 3]

Rule 26, 12.09.2024

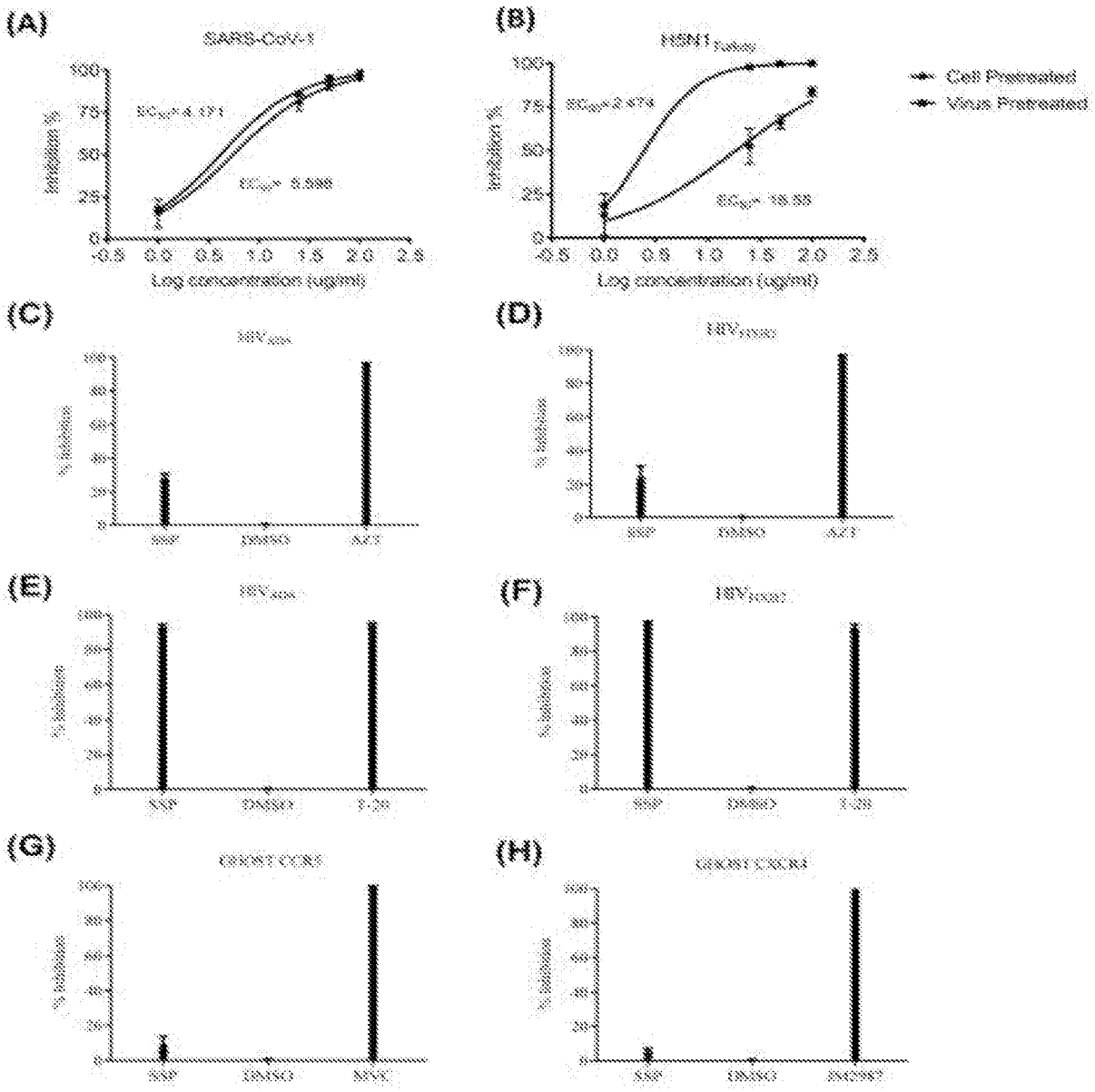


[Fig. 4]

Rule 26, 12.09.2024



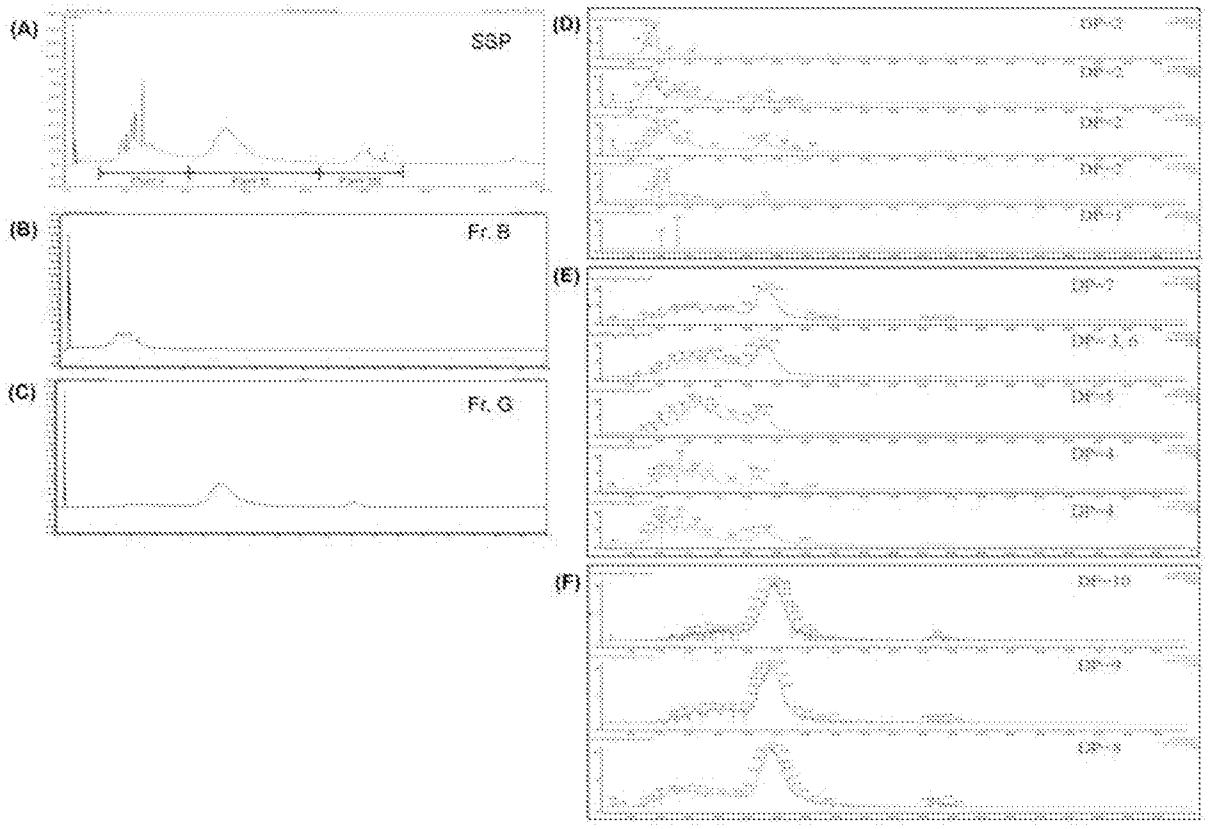
[Fig. 5]



Rule 26, 12.09.2024

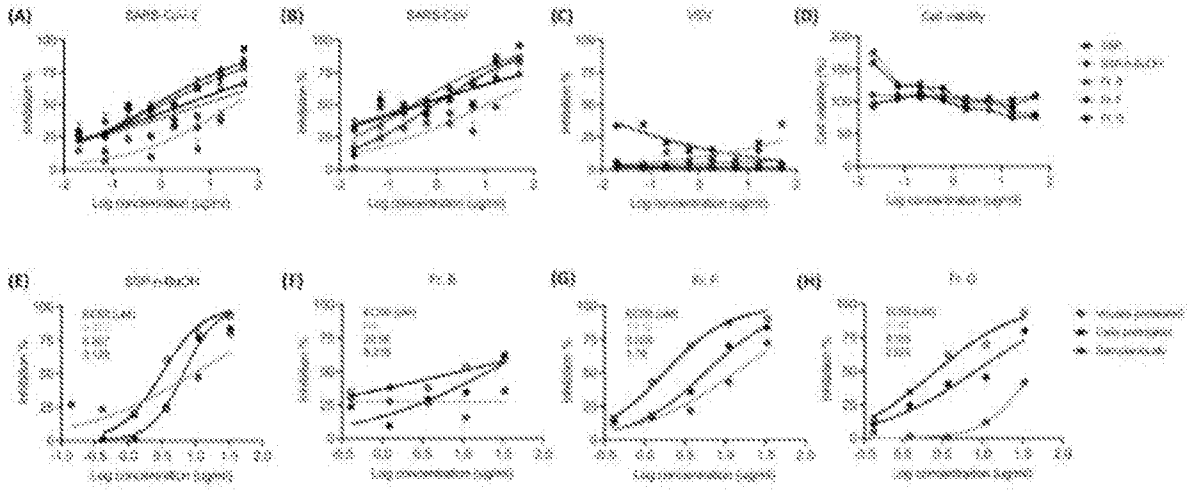
[Fig. 6]

Rule 26, 12.09.2024

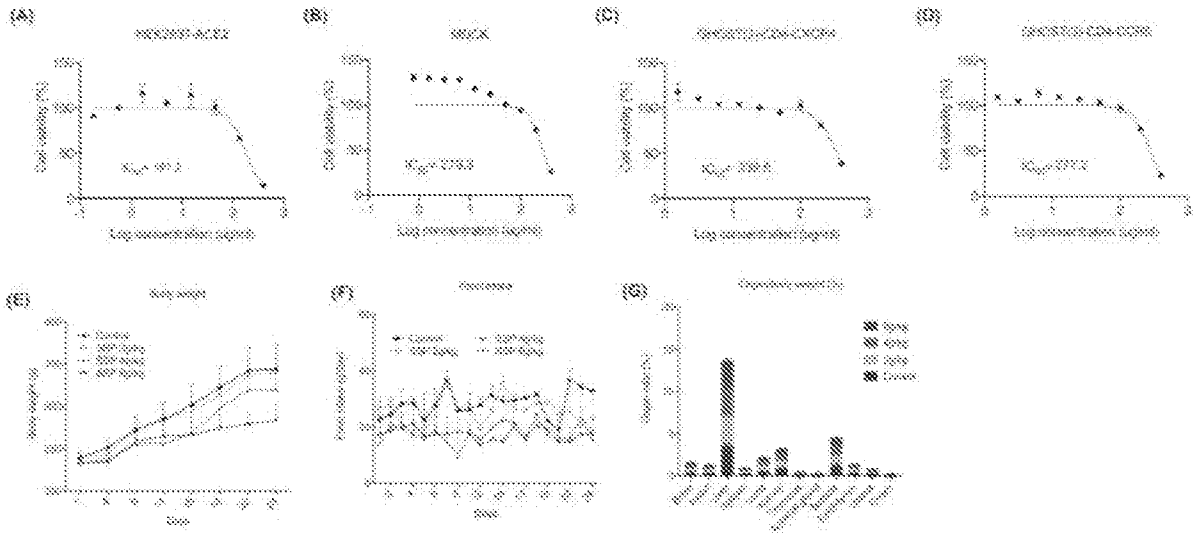


[Fig. 7]

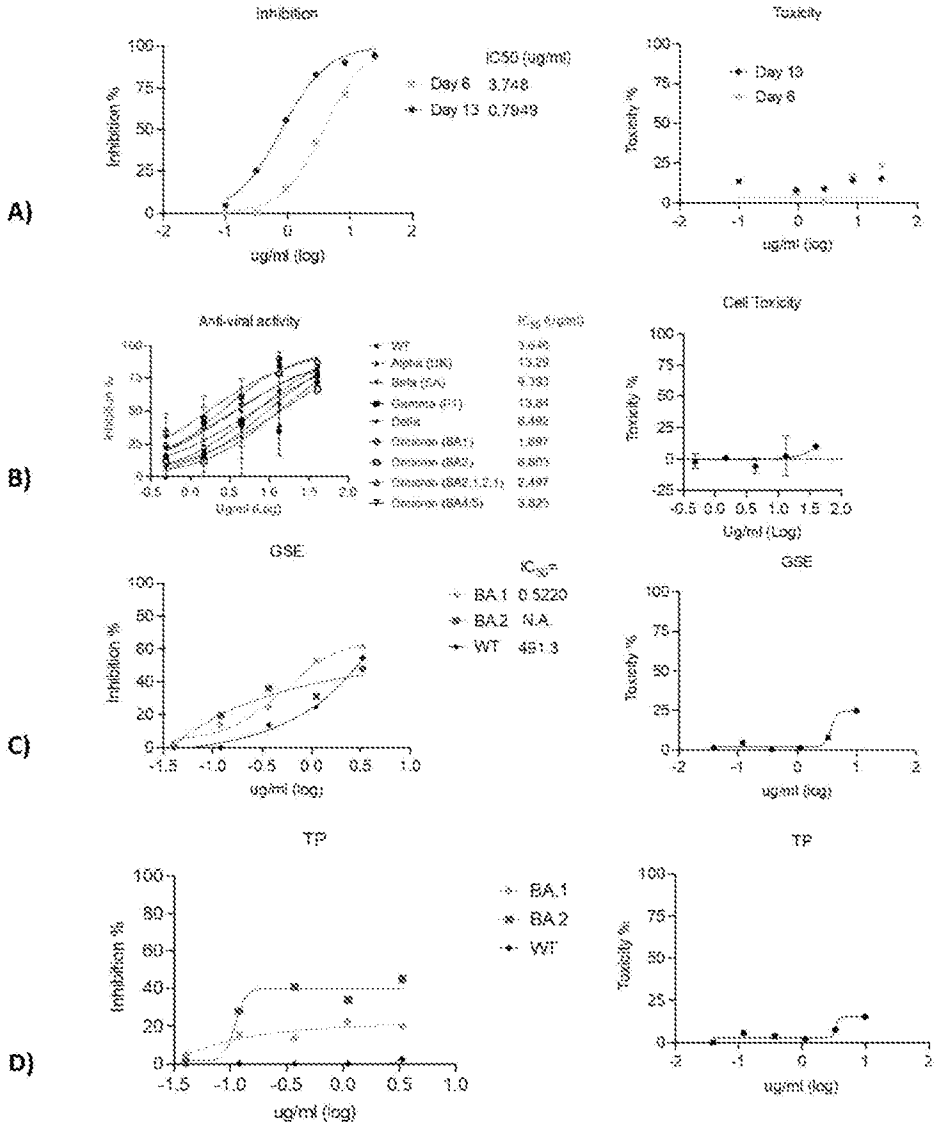
Rule 26, 12.09.2024



[Fig. 8]



[Fig. 9]



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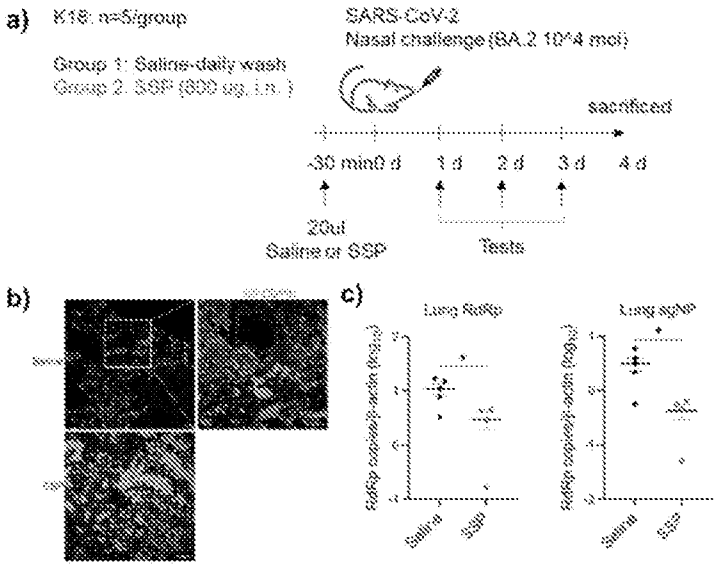
[Fig. 10]



Rule 26,
12.09.2024

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/081058

A. CLASSIFICATION OF SUBJECT MATTER

A61K36/486(2006.01)i; A61K31/352(2006.01)i; A61P31/12(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K,A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS,CNTXT,DWPI,ENTXT,ENTXTC,VEN,WPABS,WPABSC,CNABS,CNTXT,CNMED,CJFD,CNKI,MEDNPL, ZYNPL,Elsevier Science,ISI Web of Science,STN:Spatholobus suberectus,proanthocyanidin#,butanol,BuOH,viruses,virus,HIV, H5N1,EBOV,SRAS,coronavirus,MERS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Qingqing Liu et al. "Broad-spectrum antiviral activity of Spatholobus suberectus Dunn against SARS-CoV-2, SARS-CoV-1, H5N1, and other enveloped viruses" *Phytotherapy Research,* Vol. 36, No. 8, 09 August 2022 (2022-08-09), pages 3232-3247 Pages 3236-3243	1-35
X	CN 113925890 A (THE UNIVERSITY OF HONG KONG) 14 January 2022 (2022-01-14) Claims 1-7,page 4 of the description	1,3-12,20-22
Y	CN 113925890 A (THE UNIVERSITY OF HONG KONG) 14 January 2022 (2022-01-14) Claims 1-7,page 4 of the description	2,13-19,23-35
Y	ZENG, Fanli et al. "Study on in vitro Antiviral Activities of Different Extracts from Caulis Spatholobi" *Traditional Chinese Drug Research&Clinical Pharmacology,* Vol. 22, No. 1, 31 January 2011 (2011-01-31), Pages 16-20 Abstract and page 20	2,13-19,23-35

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 June 2023	**25 June 2023**
Name and mailing address of the ISA/CN **CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	Authorized officer **CHENG,Cheng** Telephone No. **(+86) 010-62411848**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FU,Ying et al. "Advances in studies on chemical constituents in Spatholobi Caulis and their pharmacological activities" *Chinese Traditional and Herbal Drugs,* Vol. 42, No. 06, 12 June 2011 (2011-06-12), Pages 1229-1233 Page 1232	28
A	CN 101474247 A (INST HYGIENE & ENVIRONMENTAL MEDICINE MI) 08 July 2009 (2009-07-08) Claim 1	23-28
A	JP 2005314316 A (KIKKOMAN CORP) 10 November 2005 (2005-11-10) Abstract	1-35
A	DONG,Pan et al. "Extraction and antioxidant activity of Proanthocyanidins from Spatholobus suberectus Dunn" *Acta Scientiarum Naturalium Universitatis Sunyatseni,* Vol. 56, No. 1, 15 January 2017 (2017-01-15), Pages 8-13 Abstract	1-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/081058

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **23-25,28(part)**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 23-25, and 28(part) relates to a treatment method of the human or animal body, and therefore, according to the criteria set out in Rule 39.1(iv), relates to subject matter for which an international search is not required. But the search report has been carried out on the basis of the claims 23-25, and 28(part) which are revised as follows: "use of an active fraction from SSD for manufacture of a medicament for treating or preventing a viral disease".

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/081058

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 113925890 A	14 January 2022	None	
CN 101474247 A	08 July 2009	None	
JP 2005314316 A	10 November 2005	None	