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Herbs vs AIDS

Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis
Calcium-Spirulan
Reverse HIV Naturally with Spirulina Filipina
ABAMAV vs HIV

Anti-HIV Activity of Medicinal Herbs: Usage and Potential Development
Preparation of a novel molecularly imprinted polymer for the highly selective extraction of baicalin
Different extraction pretreatments significantly change the flavonoid contents of Scutellaria baicalensis
Patents : Extraction of Baicalin
Black Seed Extract 'Cures' HIV Patient Naturally
Nigella Sativa Concoction Induced Sustained Seroreversion in HIV Patient
Olive Leaf Extract (20% oleuropein), Oregano Oil, Turmeric Curcumin
Traditional Chinese Herbal Medicines for Treating HIV Infections and AIDS
Randomized double-blinded and controlled clinical trial on treatment of HIV/AIDS by Zhongyan-4
Randomized, placebo-controlled trial of Chinese herb therapy for HIV-1- infected individuals
Effects of Jingyuankang capsules on leukocyte level in AIDS patients
YouTube : HIV/AIDS Herbal Remedy / HIV/AIDS Herbal Remedy - Real testimonies (Part 2) / Dr. Mitch interviews Dr. Paul Chepkwony
Dr. Paul Chepkwony : US7556830 -- Medicinal herbal composition for treating infection
US5837257 : Use of plant extracts for treatment of HIV, HCV and HBV infections
US6696094 : Herbal pharmaceutical composition for treatment of HIV/AIDS patients
US5178865 : CHINESE HERBAL EXTRACTS IN THE TREATMENT OF HIV RELATED DISEASE IN VITRO
US6455078 : Medicinal herbal composition for treating liver diseases and HIV
See also : FARHADI, Mohammed, *et al* : Urtica vs AIDS ~ Extract of Nettle treated with pulsed high frequency EMF (45 W, 150 Tesla, 750 KHz) being tested by Iran.

Spirulina

http://journals.lww.com/jaids/Abstract/1998/05010/Inhibition_of_HIV_1_Replication_by_an_Aqueous.2.aspx
http://www.ncbi.nlm.nih.gov/pubmed/9593452
J Acquir Immune Defic Syndr Hum Retrovirol. 1998 May 1;18(1):7-12.

Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis).

Ayehunie S, Belay A, Baba TW, Ruprecht RM.

Abstract

An aqueous extract of the blue-green filamentous algae Arthrospira platensis (previously called Spirulina platensis) inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells (LC). Extract concentrations ranging between 0.3 and 1.2 microg/ml reduced viral production by approximately 50% (50% effective concentration [EC50]) in PBMCs. The 50% inhibitory concentration (IC50) of extract for PBMC growth ranged between 0.8 and 3.1 mg/ml. Depending on the cell type used, therapeutic indices ranged between 200 and 6000. The extract inactivated HIV-1 infectivity directly when preincubated with virus before addition to human T-cell lines. Fractionation of the extract revealed antiviral activity in the polysaccharide fraction and also in a fraction depleted of polysaccharides and

tannins. We conclude that aqueous A platensis extracts contain antiretroviral activity that may be of potential clinical interest.

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http://www.lightparty.com/Health/Spirulina.html

...Another group of medical scientists has published new studies regarding a purified water extract unique to Spirulina named Calcium-Spirulan. It inhibits replication of HIV-1, herpes simples, human cytomegalovirus, influenza A virus, mumps virus and measles virus in-vitro, yet is very safe for human cells. It protects human and monkey cells from viral infection in cell culture. According to peer reviewed scientific journal reports this extract, "...holds great promise for treatment of ...HIV-1, HSV-1, and HCM infections, which is particularly advantageous for AIDS patients who are prone to these life-threatening infections."

Calcium-Spirulan is a polymerized sugar molecule unique to Spirulina, containing both sulfur and calcium. Hamsters treated with this water soluble extract had better recovery rates when infected with an otherwise lethal herpes virus. How does it work? When attacking a cell, a virus first attaches itself to the cell membrane. However, because of spirulina extract, the virus cannot penetrate the cell membrane to infect the cell. The virus is stuck, unable to replicate. It is eventually eliminated by the body's natural defenses. Spirulina extracts may become useful therapeutics that could help AIDS patients lead longer, more normal lives.

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https://www.youtube.com/watch?v=ywwap1HNyxw

Reverse HIV Naturally with Spirulina Filipina

THE SAN FRANCISCO MEDICAL RESEARCH FOUNDATION
The Study of Spirulina

Effects on the AIDS Virus, Cancer and the Immune System

Spirulina is gaining more attention from medical scientists as a nutraceutical and source of potential pharmaceuticals. There are several new peer reviewed scientific studies about Spirulina's ability to inhibit viral replication, strengthen both the cellular and humoral arms of the immune system and cause regression and inhibition of cancers. While these studies are preliminary and more research is needed, the results so far are exciting.

In April 1996, scientists from the Laboratory of Viral Pathogenesis, Dana-Farber Cancer Institute and Harvard Medical School and Earthrise Farms, Calipatria, California, announced on-going research, saying "Water extract of Spirulina platensis inhibits HIV-1 replication in human derived T-cell lines and in human peripheral blood mononuclear cells. A concentration of 5-10 mg/ml was found to reduce viral production." HIV-1 is the AIDS virus. Small amounts of Spirulina extract reduced viral replication while higher concentrations totally stopped its reproduction. Importantly, with the therapeutic index of 100, Spirulina extract was non-toxic to human cells at concentrations stopping viral replication.

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http://www.naturalpharmainternational.com/1/abamav_vs_hiv_1671420.html
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http://www.worldscientific.com/doi/abs/10.1142/S0192415X01000083
Am. J. Chin. Med. 29, 69 (2001).
DOI: 10.1142/S0192415X01000083

Anti-HIV Activity of Medicinal Herbs: Usage and Potential Development

Ji An Wu et al,

The acquired immunodeficiency syndrome (AIDS) is a result of human immunodeficiency virus (HIV) infection which subsequently leads to significant suppression of immune functions. AIDS is a significant threat to the health of mankind, and the search for effective therapies to treat AIDS is of paramount importance. Several chemical anti-HIV agents have been developed. However, besides the high cost, there are adverse effects and limitations associated with using chemotherapy for the treatment of HIV infection Thus, herbal medicines have frequently been used as an alternative medical therapy by HIV positive individuals and AIDS patients. The aim of this review is to summarize research findings for herbal medicines, which are endowed with the ability to inhibit HIV. In this article, we will emphasize a Chinese herbal medicine. **Scutellaria baicalensis Georgi** and its identified components (i.e. baicalein and baicalin) which have been shown to inhibit infectivity and replication of HIV. Potential development of anti-AIDS compounds using molecular modeling methods will also be discussed.

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http://onlinelibrary.wiley.com/doi/10.1002/jssc.201500865/abstract
DOI: 10.1002/jssc.201500865View

Preparation of a novel molecularly imprinted polymer for the highly selective extraction of baicalin

Xiao Liu, et al

Abstract

The selective extraction of baicalin is important to its quality control especially when the matrices are complicated. In this work, a novel molecularly imprinted polymer was prepared for the selective extraction of baicalin in herbs. The molecularly imprinted polymer was synthesized by the copolymerization of 4-vinyl pyridine and ethylene glycol dimethacrylate in the presence of baicalin by a precipitation polymerization method. After the optimization of parameters for molecularly imprinted polymer preparation, including the functional monomer, porogen, sampling solvent, and washing solvent, good selectivity was obtained, with an imprinting factor of about 4, which is much better than that achieved by the bulk-polymerization method. The performances of the prepared molecularly imprinted polymers were systematically investigated, including adsorption kinetics, isotherm experiment, and Scatchard analysis. On the basis of the good adsorptive capability of the prepared molecularly imprinted polymer, it was also applied for the pretreatment of baicalin in Scutellaria baicalensis Georgi. The result showed that most of the matrices were removed and baicalin was selectively enriched.

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http://www.tandfonline.com/doi/abs/10.3109/13880209.2013.784922?journalCode=iphb20#.VpMTe6TR1FU
Pharmaceutical Biology Volume 51, Issue 10, 2013
DOI: 10.3109/13880209.2013.784922

Different extraction pretreatments significantly change the flavonoid contents of

Scutellaria baicalensis

Chunhao Yuabc, et al.

Abstract

Context: Scutellaria baicalensis Georgi (Labiatae) is one of the most commonly used medicinal herbs, especially in traditional Chinese medicine. However, compared to many pharmacological studies of this botanical, much less attention has been paid to the quality control of the herb's pretreatment prior to extract preparation, an issue that may affect therapeutic outcomes.

Objective: The current study was designed to evaluate whether different pretreatment conditions change the contents of the four major flavonoids in the herb, i.e., two glycosides (baicalin and wogonoside) and two aglycones (baicalein and wogonin).

Materials and methods: A high-performance liquid chromatography assay was used to quantify the contents of these four flavonoids. The composition changes of four flavonoids by different pretreatment conditions, including solvent, treatment time, temperature, pH value and herb/solvent ratio were evaluated.

Results: After selection of the first order time-curve kinetics, our data showed that at 50 °C, 1:5 herb/water (in w/v) ratio and pH 6.67 yielded an optimal conversion rate from flavonoid glycosides to their aglycones. In this optimized condition, the contents of baicalin and wogonoside were decreased to 1/70 and 1/13, while baicalein and wogonin were increased 3.5- and 3.1-fold, respectively, compared to untreated herb.

Discussion and conclusion: The markedly variable conversion rates by different pretreatment conditions complicated the quality control of this herb, mainly due to the high amount of endogenous enzymes of S. baicalensis. Optimal pretreatment conditions observed in this study could be used obtain the highest level of desired constituents to achieve better pharmacological effects.

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http://worldwide.espacenet.com/advancedSearch?locale=en_EP

Patents : Extraction of Baicalin

CN104910225
Method for extracting baicalin from radix scutellariae
Inventor: CHEN XUESONG / DAI BAIAN

KR20150092954
COMPOSITION COMPRISING BAICALIN EXTRACTED FROM SCUTELLARIA BAICALENSIS FOR INHIBITING DENDRITIC CELL MATURATION
Inventor: LEE JUN SIK , et al.

CN104873573
Method for being suitable for industrial production and extracting crude baicalin from scutellaria baicalensis
Inventor: SUN GUOQIANG, et al.

CN104829666
Method for preparing high purity baicalin from radix scutellariae
Inventor: ZHOU YUZHI, et al.

CN104784254
Extraction method for producing baicalin with biological enzyme method
Inventor: WEI YOULIANG, et al.CN104784254

CN104650165
Preparation method of high-purity baicalin
Inventor: GAO QIANSHAN, et al.

CN104610401
Method for simultaneously extracting baicalin, baicalein and wogonin from scutellaria baicalensis
Inventor: YU BEIBEI, et al

CN104513285
Baicalin extracting technology process
Inventor: DU SHUQING

CN104356185
Method for quickly and efficiently extracting baicalin
Inventor: ZHANG LIWEI, et al.

Nigella sativa

http://www.greenmedinfo.com/blog/black-seed-completely-cures-hiv-case-study
December 7th 2013

Black Seed Extract 'Cures' HIV Patient Naturally

By

Sayer Ji

There are words you don't use in medicine today, such as "cure." But a remarkable case study in an HIV positive patient treated with black seed extract resulted in a sustained remission, indicating a safe, accessible and affordable alternative to highly toxic antiretroviral HIV drugs may already exist.

Nigella Sativa, also known as 'black seed,' has been studied for a wide rage of health benefits, but not until recently was it discovered to hold promise as a curative agent against potentially lethal viral infections, including Hepatitis C[i] and now HIV.

A remarkable case study published in August of this year in the African Journal of Traditional, Complementary, and Alternative Medicine described an HIV patient who after undergoing treatment with a black seed extract experienced a complete recovery, with no detectable HIV virus or antibodies against HIV in their blood serum, both during and long after the therapy ended.[ii]

This was a remarkable and unexpected observation, described by the researchers as follows:

"Nigella sativa had been documented to possess many therapeutic functions in medicine but the least expected is sero-reversion in HIV infection which is very rare despite extensive therapy with highly active anti-retroviral therapy (HAART). "…

At the outset of the study, the patient presented with classical symptoms of symptomatic HIV infection, "with [a] history of chronic fever, diarrhoea, weight loss and multiple papular pruritic lesions of 3 months duration." Examination identified moderate weight loss, with laboratory confirmed tests showing 'sero-positivity' to HIV infection with a "pre-treatment viral (HIV-RNA) load and CD4 count of 27,000 copies/ml and CD4 count of 250 cells/ mm(3) respectively."...

The patient was administered a black seed concoction of 10 mls twice daily for 6 months, resulting in a rapid improvement in symptoms, and significant reductions in viral load:

"Fever, diarrhoea and multiple pruritic lesions disappeared on 5th, 7th and 20th day respectively on Nigella sativa therapy. The CD4 count decreased to 160 cells/ mm3 despite significant reduction in viral load (≤1000 copies/ml) on 30th day on N. sativa."

By the 187th day on black seed therapy, testing indicating the blood was entirely cleared of signs of infection, a so-called 'sero-negative status'. The post-therapy CD4 counts increased from baseline to a normalized 650cells/ mm(3) with an undetectable viral (HIV-RNA) load.

"This case report reflects the fact that there are possible therapeutic agents in Nigella sativa that may effectively control HIV infection."...

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http://www.greenmedinfo.com/article/nigella-sativa-concoction-induced-sustained-seroreversion-hiv-patient

Afr J Tradit Complement Altern Med. 2013 Aug 12;10(5):332-5.

Nigella Sativa Concoction Induced Sustained Seroreversion in HIV Patient

Abdulfatah Adekunle Onifade, Andrew Paul Jewell, Waheed Adeola Adedeji.

Abstract:

Nigella sativa had been documented to possess many therapeutic functions in medicine but the least expected is sero-reversion in HIV infection which is very rare despite extensive therapy with highly active anti-retroviral therapy (HAART). This case presentation is to highlight the complete recovery and sero-reversion of adult HIV patient after treatment with Nigella sativa concoction for the period of six months. The patient presented to the herbal therapist with history of chronic fever, diarrhoea, weight loss and multiple papular pruritic lesions of 3 months duration. Examination revealed moderate weight loss, and the laboratory tests of ELISA (Genscreen) and western blot (new blot 1&2) confirmed sero-positivity to HIV infection with pre-treatment viral (HIV-RNA) load and CD4 count of 27,000 copies/ml and CD4 count of 250 cells/ mm(3) respectively. The patient was commenced on Nigella sativa concoction 10mls twice daily for 6 months.. He was contacted daily to monitor side-effects and drug efficacy. Fever, diarrhoea and multiple pruritic lesions disappeared on 5th, 7th and 20th day respectively on Nigella sativa therapy. The CD4 count decreased to 160 cells/ mm3 despite significant reduction in viral load (≤1000 copies/ml) on 30th day on N. sativa. Repeated EIA and Western blot tests on 187th day on Nigella sativa therapy was sero-negative. The post therapy CD4 count was 650cells/ mm(3) with undetectable viral (HIV-RNA) load. Several repeats of the HIV tests remained sero-negative, aviraemia and normal CD4 count since 24 months without herbal therapy. This case report reflects the fact that there are possible therapeutic agents in Nigella sativa that may effectively control HIV infection.

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http://www.destroydiseases.com/HIV.html

Olive Leaf Extract (20% oleuropein), Oregano Oil, Turmeric Curcumin

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http://dx.doi.org/10.1155/2012/950757
Evidence-Based Complementary and Alternative Medicine, Volume 2012 (2012), Article ID 950757

Traditional Chinese Herbal Medicines for Treating HIV Infections and AIDS

Wen Zou, Ying Liu, Jian Wang, Hongjuan Li, and Xing Liao

To assess the effects of TCHM on patients with HIV infection and AIDS, we reviewed eleven randomized placebo-controlled trials involving 998 patients. Due to the limited number of RCTs for included trials and the small sample size of each study, we are not able to draw firm conclusions concerning TCHM therapy in treating patients with HIV infection and AIDS. However, some high-quality clinical studies do exist. Studies of diarrhea and oral candidiasis, which are challenging symptoms of AIDS, were demonstrated to have positive effects. Study of peripheral leukocytes, which are a side effect of antiretroviral drugs, suggested that an integrated treatment approach may be of benefit. The overall methodological quality of the trials was adequate; however, randomization methods should be clearly described and fully reported in these trials according to the Consolidated Standards of Reporting Trials (CONSORT)…

3.3.1. IGM-1

A randomized trial tested a Chinese herbal formulation (IGM-1) composed of 31 Chinese herbs (Table 1) in 30 HIV-infected adults with symptoms and decreased CD4+ cell count (200–499/mm3) for treatment of HIV-related symptoms for duration of 12 weeks [13]. The study found a significant better effect in improvement of health-related QoL in terms of life satisfaction and symptoms than placebo. The number of symptoms was reduced in patients receiving herbs, but not in those receiving placebo. There were no statistically significant differences in overall health perception, symptom severity, CD4 counts, anxiety, or depression between groups. No adverse events were reported among participants. However, the above results need to be accounted for with care due to the small sample in the trial.

3.3.2. "35-Herb"

Interestingly, three years after the above trial was published, the same investigator who prescribed IGM-1 prescribed another Chinese herbal formulation that was tested in a trial in Switzerland [17]. The formulation was composed of 35 Chinese herbs containing most of the herbs listed in IGM-1 (Table 1). A trial tested the Chinese herbal formulation in 68 HIV-infected adults with decreased CD4+ cell count (less than 500/mm3) for a treatment period of six months [17]. The participants were randomized to receive "35-herb" ( ) or placebo ( ). Over 70% of the patients had received previous antiretroviral therapy, the two groups were comparable regarding sociodemographic characteristics, previous antiretroviral use, viral load, CD4+ cell counts, and other clinical laboratory tests at entry. A total of 53 (78%) patients completed treatment for 6 months, including 24 in the herb group and 29 in the placebo group. Analyses were based on complete data and on intention-to-treat principle in the trial report. After six months, there was no significant difference in CD4+ cell counts, viral load, new AIDS-defining events, number of reported symptoms, psychosocial measurements or QoL between two groups.

The total number of reported adverse events was 46 in the herb group and 20 in the placebo group, and included diarrhea, increased number of daily bowel movements, abdominal pain, constipation, flatulence, and nausea. Hematological or serum chemistry laboratory values showed no evidence of toxicity from the study herbs. Two patients in the herb group died during the study period and causes of death were believed to be due to severe immunodeficiency and pre-enrolment history of severe opportunistic complications, but not related to the study drugs.

3.3.3. Compound SH

Compound SH containing five herbs (Table 1) were combined with zidovudine and zalcitabine in the treatment of 60 HIV-infected Thai patients in a randomized trial [14]. The herbal formula was made from more than 1000 chinese herbs from 120 plant families by Kunming Institute of Botany of the Chinese Academy of Science. The trial found that adding SH herbs to the two nucleoside reverse transcriptase inhibitors has a greater antiviral activity than antiretrovirals only. However, the data analyses were based on participants, who had completed the trial, 22 subjects who lost followup or withdrawal due to adverse events were excluded, and the above benefits need to be accounted for with care.

3.3.4. Qiankunning

Qiankunning (Table 1) is a Chinese herb preparation extracted from 14 herbs. A randomized, double blind placebo controlled trial was conducted in 2003 in China [15], 36 adults with HIV infection or AIDS were randomized to receive Qiankunning ( ) or placebo ( ). Patients were comparable regarding age, body weight, average duration of drug abuse, and pre trial HIV RNA levels. No intention to treat analyses were applied, the data analyses were based on participants who had completed the trial. Significant decrease in HIV RNA levels was found in herb group than placebo after the end of treatment for 7 months. In this trial, the use of herbs was related to stomach discomfort and diarrhea. No adverse effects were reported from the placebo group. There were no serious adverse events observed.

3.3.5. Zhongyan-4

Chinese herbal medicine zhongyan-4 (ZY-4) (Table 1) is prepared by the Chinese Academy of Chinese Medical Sciences in Beijing, China. A randomized, double blind placebo controlled trial enrolled 72 patients with HIV infection or AIDS (36 with herbs and 36 with placebo) [16]. CD4+ cell counts in the ZY-4 group were increased by cells/mm3, while in the placebo group the CD4+ cell counts were decreased by cells/mm3 after treatment for 6 months ( ). A total of 15 out of 30 patients (6 dropped out) in the ZY-4 group had their CD4 count increased compared with 8 out of 33 patients (3 dropped out) in the placebo group ( ). The study concludes that ZY-4 is effective in enhancing immunity function based on CD4+ cell counts. However, this

study showed no significant difference in body weight or viral load after treatment between ZY-4 and placebo.

3.3.6. Aining Granule

The Chinese herbal medicine Aining Granule (AG) (Table 1) was tested in 100 patients compared with placebo in a double blind trial. Participants were randomized into two groups [18], AG group ( ) received AG+HAART (d4T+ddI+NVP) and Placebo group ( ) received placebo + HAART (d4T+ddI+NVP). CD4+ cell counts in the AG group were decreased by cells/mm3, while in the placebo group the CD4+ cell counts were decreased by cells/mm3 after treatment for 11 months ( ). Significant improvement of symptoms such as fatigue, anorexia, nausea, diarrhea, skin rash was found in AG group. The results showed that patients receiving Chinese herb AG had a lower risk for the decrease of CD4+ cell counts. However, this study showed no significant difference between two groups in viral load after treatment.

3.3.7. Xiaomi Granule

A randomized two arms positive-drug controlled open label trial was conducted in 2009 in china, in which 80 AIDS participants with oral candidiasis were included in the Xiaomi Granule (Table 1) plus Nystatin group ( ) and Nystatin group ( ) [19]. After treatment for 2 weeks, significant improvement of symptoms of oral candidiasis was found in herb group. No adverse event was found. Xiaomi Granule is a Chinese herb preparation developed from a prescription in classic Chinese medicine ancient book “jin kui yao lve”. There is no description of CD4+ cell counts and viral load in the paper available.

3.3.8. Jingyuankang Capsule

In a double-blind, double-analogue trial, 116 participants with HIV infection and peripheral leucopenia were randomized to receive Jingyuankang Capsule (JC) (Table 1) plus AZT, ddI, NVP, and analogue Leucogen Tablets ( ) or Leucogen Tablets plus AZT, ddI, NVP and analogue JC ( ) for 6 months [20]. The application of JC showed significant increase of peripheral leukocytes in herb group. CD4+ cell count outcome was not reported. There were no significant differences between the groups regarding adverse effect in the trial report.

3.3.9. Xielikang Capsule

A randomized, double-blind, double dummy and controlled clinical trial was conducted between 2009 and 2011 in china, in which 158 AIDS-related chronic diarrhea patients were randomized into Xielikang Capsule (XC) (Table 1) plus loperamide analogue group ( ) and loperamide capsule plus XC analogue group ( ) [21]. The primary efficacy parameters were stool weight, abnormal stool frequency and score of diarrhea questionnaire. All the patients have no recognized enteritis or intestinal canal identified from enteroscope or diarrhea resulted by protease inhibitors (PI) drugs. According to an analysis of the treatment effect over 7 and 14 days based on daily measurements, Patients who were treated with XC experienced a statistically significant reduction in stool weight (in 7 days and in 14 days) and in diarrhea questionnaire score (in 14 days). There were no significant differences between groups with respect to stool frequency. No serious adverse events were reported. There was no major difference between XC and placebo in the occurrence of adverse events or in laboratory abnormalities.

3.3.10. Aikang Capsule

A randomized placebo controlled trial enrolled 102 patients infected with HIV and AIDS with CD4+ cell counts between 250 and 600 cells/mm3 who were treated with Aikang Capsule (Table 1) or placebo for 6 months [22]. There was no significant difference in CD4+ cell counts between two groups.

3.3.11. Tangcao Tablets

In a China phase III clinical muti-center trial conducted between 2002 and 2003, 176 patients with CD4+ cell counts 200 cells/mm3 were randomized to receive a 6- month course of treatment with Chinese herbal medicine Tangcao Tablets (Table 1) ( ) or placebo ( ) [23]. Patients receiving antiretroviral drugs were excluded. Both intention to treat analysis and per-protocol analysis showed significant increase in CD4 counts, CD4/CD8 ratio and weight in herb group, significant increase of viral load in placebo group, improvement of symptoms in herb group.

The total number of reported adverse events was 21 in the herb group and 27 in the placebo group, and

included diarrhea, cold, abdominal pain, flatulence, and nausea. Hematological or serum chemistry laboratory values showed no evidence of toxicity from the study herbs. Two patients in the placebo group died during the study period and causes of death were believed to be due to severe immunodeficiency and pre-enrolment history of severe opportunistic complications, but not related to the placebo…

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http://www.scopus.com/record/display.uri?eid=2-s2.0-33645549859&origin=inward&txGid=0
Chinese Journal of Integrative Medicine, vol. 12, no. 1, pp. 6–11, 2006.

Randomized double-blinded and controlled clinical trial on treatment of HIV/AIDS by Zhongyan-4

J. Wang, F. Z. Yang, M. Zhao et al.

Objective: To assess the efficacy and safety of Zhongyan-4 ((Chinese characters), ZY-4, a Chinese herbal preparation worked out according to the therapeutic principle of supplementing qi, nourishing Yin, clearing heat and detoxication) in treating HIV/AIDS patients in the early or middle stage. Methods: Adopted was randomized double-blinded and placebo-parallel-controlled method, with 72 HIV/AIDS patients randomly divided into the ZY-4 group (36 patients) treated with ZY-4 and the control group (36 patients) treated with placebo. The treatment course was six months. The index of CD 4 +, CD 8 + counts, body weight, clinical symptom scoring were estimated at 4 time points (0, 1, 3 and 6 month in the course), and also the viral load before and after treatment. The whole course of observation was completed in 63 patients, 30 in the ZY-4 group and 33 in the control group. Results: CD 4 + count in the ZY-4 group got elevated by 7.70 ± 150.96/mm 3 on average, while that in the control group lowered by 27.33 ± 85.28/mm 3. Fifteen out of the 30 patients in the ZY-4 group had their CD 4 + count increased, which was evidently much higher than that in the control group (8/33, $P<0.05$), suggesting that the efficacy of ZY-4 is superior to that of placebo in elevating CD 4 + count. Moreover, ZY-4 showed actions in elevating CD 45RA + and CD 8 + count, reducing HIV virus load, improving clinical symptom/sign and increasing body weight of patients. No obvious adverse reaction was found in the clinical trial. Conclusion: ZY-4 has an immunity-protective and/or rebuilding function in HIV/AIDS patients in the early and middle stage, and also shows effects in lowering viral load, increasing body weight and improving symptoms and signs to a certain degree.

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http://www.scopus.com/record/display.uri?eid=2-s2.0-0344549402&origin=inward&txGid=0
Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, vol. 22, no. 1, pp. 56–64, 1999.

Randomized, placebo-controlled trial of Chinese herb therapy for HIV-1- infected individuals

R. Weber, L. Christen, M. Loy et al.,

Abstract

Context: Alternative medicine or complementary remedies that have not been scientifically tested are nonetheless widely used to treat chronic illnesses, particularly if curative options are limited. Objectives: To assess the effectiveness of Chinese medicinal herbs in reducing symptoms and improving the quality of life of HIV-infected persons. Design: Prospective, placebo-controlled double-blind study. Setting: University-based HIV outpatient clinic. Patients: 68 HIV-infected adults with CD4 cell counts <0.5 x 109/L. Intervention: Participants were randomized to receive four daily doses of seven pills containing a standardized preparation of 35 Chinese herbs or placebo for 6 months. Main Outcome Measures: Symptoms, HIV disease progression, HIV-1 RNA plasma vital loads, CD4 and CD8 cell counts, and scores on standard questionnaires for quality of life, depression, anxiety, and coping. Results: Intervention and placebo groups were equivalent at baseline regarding, respectively, previous antiretroviral therapy (74% versus 79%), median CD4 cell counts (0.20 x 109/L versus 0.25 x 109/L), and median HIV-1 plasma viral loads (35,612 copies/ml versus 52,027 copies/ml). At enrollment, none of the study subjects was seriously ill or depressed, and average coping and quality of life scores were in the normal range. In all, 53 (78%) participants completed the study. Patients taking Chinese herbs reported significantly more gastrointestinal disturbances (79% versus 38%; $p = .003$) than those receiving placebo. No therapy-related toxicities were observed. At completion of the study, no significant differences between the intervention and placebo groups were found regarding plasma viral loads,

CD4 cell counts, symptoms, and psychometric parameters. HIV-1 RNA level was unchanged at study end. Among participants who were not on concomitant antiretroviral therapy, median CD4 cell counts declined by 0.05 x 109/L in both the intervention and placebo groups. Conclusions: This standardized formulation of Chinese herbs for HIV-infected individuals did not improve quality of life, clinical manifestations, plasma virus loads, or CD4 cell counts. The data suggest that this formulation of Chinese herbs is not effective when administered in a Western medicine setting.

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http://www.scopus.com/record/display.uri?eid=2-s2.0-79956112856&origin=inward&txGid=0
Journal of Traditional Chinese Medicine, vol. 31, no. 1, pp. 32–35, 2011.

Effects of Jingyuankang capsules on leukocyte level in AIDS patients

S. Q. Jiang, H. X. Sun, Y. M. Xu, Y. L. Jiang, J. W. Pei, and H. L. Wang

Abstract

Objective: To observe the therapeutic effects of Jingyuankang capsules for leukopenia in AIDS patients. Methods: In this randomized double-blind trial, 58 patients orally took Jingyuankang capsule, analog Leucogen tablet and the HAART (highly active anti-retroviral therapy) drugs, and the other 58 patients took Leucogen tablet, analog Jingyuankang capsule and the HAART drugs all for 6 months, during which the peripheral hemogram was periodically examined to observe the therapeutic effects of Jingyuankang capsule for leukopenia of the AIDS patients. Results: With good therapeutic effect for leukopenia of the AIDS patients, Jingyuankang capsule can enhance leukocyte level as effective as Leucogen tablet in treating grade I and grade II leukopenia, and more effectively than Leucogen tablet in treating grade III leukopenia. No toxic side-effects and adverse reactions were found during the treatment and in the follow-up visit. Conclusion: Jingyuankang capsule can effectively treat leukopenia of the AIDS patients.

Dr. Paul Chepkwony

https://www.youtube.com/watch?v=BHz3wZvhuug
HIV/AIDS Herbal Remedy

https://www.youtube.com/watch?v=HzjZHlrkmXo
HIV/AIDS Herbal Remedy - Real testimonies (Part 2)

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Medicinal herbal composition for treating infection

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Abstract

Herbal compositions derived from Kenyan plants are provided for the treatment of HIV and other infectious diseases. The herbal compositions can include the extracts of up to 14 plants, including the root of **Dovyalis abyssinica and Clutia robust**a. Also provided are methods for extracting alkaloids and other compounds from the plants. Also provided are methods of treating a subject having an infectious disease, particularly HIV.

FIELD OF THE INVENTION

The present invention relates to combinations of extracts from plants that can be used in the treatment of infection.

BACKGROUND OF THE INVENTION

This application claims the benefit of provisional application Ser. No. 60/710,237, filed Aug. 22, 2005, incorporated herein by reference in its entirety. The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

Tens of millions of people world-wide are living with acquired immunodeficiency syndrome (AIDS), or are infected with the causative agent, human immunodeficiency virus (HIV). In some countries in sub-Saharan Africa, up to one in four adults has contracted the disease. Despite the costs and efforts spent attempting to identify new methods of treatment, a cure for the disease has remained elusive.

Ancient societies have traditionally turned to plants for their health needs. Documented use of herbs to treat illnesses dates back to as early as 2,000 B.C. Recently, individuals have resorted to nature as remedies and medicines for the treatment of modern illnesses have been derived from plants, such as for example, treatment of HIV and other infectious diseases.

For example, U.S. Pat. No. 5,178,865 discloses an experimental treatment with 56 herbs, and reports that 10 of the 56 herbs exhibit anti-HIV activity in in vitro experiments. The 10 herbs include: **Coptis chineusis, Ligusticum wallichii, Ilicium eanclolatum, Isatis tinctoria, Salvia miltiorrhiza, Erycibe obtusifolia, Acanthopanax graciliatylus, Bostaurus domesticus, Inula helenium and Lonicera japonica. Both Bostaurus domesticus and Lonicera japonica** are further described to be able to combine with Scutellaria baicaleusis to exhibit anti-HIV activity.

U.S. Pat. No. 5,837,257 discloses Chinese herbal medicines that exhibit in vitro antiviral activity against murine leukemia virus and HIV and for treatment of animals and humans infected with HIV. In one of the preferred embodiments, the Chinese herbal medicines contain **hedyotis, Scutellarial barbatae herba, Lonicera flos, Prunellae spica and Solani harba.**

U.S. Pat. No. 5,989,556 discloses various herbal compositions for treating viral infections which have shown in vitro antiviral activities against HIV. A first herbal composition contains **Aeginetiae herba, Blechni rhizoma, Lespedezae herba, Polygoni cuspidati rhizoma, Forsythiae fructus, and Ligustri fructus. A second herbal composition contains Cirsii rhizoma and radix, Breeae radix, Baphicacanthis rhizoma and radix, Phellodendri cortex, and Bletillae tuber. A third herbal composition disclosed in the patent includes Aeginetiae Herba, Lonicerae, Flos, Prunellae spica and Lespedezal herba.**

U.S. Pat. No. 6,696,094 discloses an herbal pharmaceutical composition for treating HIV/AIDS. The pharmaceutical composition contains 14 ingredients, including: **diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Asiatic moonseed rhizome, baical skullcap root, Bovine biliary powder, milkvetch root, barbary wolfberry fruit, sanqi, figwort root, Chinese magnoliavine fruit, turmeric root-tuber, hawthorn fruit and Chinese angelica.** Procedures are provided for the preparation of an "HIVCIDE condensate", which can be formulated as an injectible solution or as capsules. Results indicate that subjects injected with HIVCIDE solution showed no symptoms of acute or chronic toxicity. Further, the HIVCIDE injection solution was effective in inhibiting pathological changes in cells caused by HIV-1 in vitro. In a third experiment, the HIVCIDE injection solution was effective in reducing symptoms of HIV-infected subjects in a treatment regime together with administration of HIVCIDE capsules. HIV-positive subjects did not show adverse reactions to HIVCIDE injection solution. It was further reported three out of four subjects showed improvement in fatigue after treatment with HIVCIDE, and that HIV viral load studies indicated that all subjects demonstrated reduced HIV viral loads.

U.S. Pat. No. 6,455,078 discloses a medical herbal composition for treating liver diseases and HIV. The composition contains 15 ingredients, which includes **diffuse hedyotis, bistort rhizome giant knotweed rhizome, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, milkvetch root, barbary wolfberry fruit, sanqi, red gingseng, figwort root, Chinese magnoliavine fruit, turmeric root-tuber, hawthorn fruit and Chinese angelica. Among the 15 ingredients, diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit** are cited as being necessary to contribute to the efficacy of the pharmaceutical composition.

In U.S. Pat. No. 5,366,725, an **extract from the seeds of Aeginetia indica** was prepared which exhibited excellent carcinostatic effects and possesses interleukin-2 and interferon-gamma-inducing properties. The extract is believed to be a macromolecular polysaccharide, which may or may not contain Lipid A binding

with protein depending on whether the extraction is conducted using butanol or phenol. The extracted substance is soluble in water, insoluble in n-butanol, and has a molecular weight ranging from 100,000 to 200,000 Daltons.

U.S. Pat. No. 5,411,733 to Hozumi, et al., discloses a variety of plant extracts for use as anti-herpes viral, anti-polioviral, anti-varicella-zoster virus, anti-measles virus, anti-cytomegalovirus (CMV), and anti-DNA and anti-RNA virus agents.

U.S. Pat. No. 5,178,865 discloses the anti-HIV activity in vitro of a variety of herbs known in China to exhibit anti-viral activity. Water extractions of the mixtures, treatment with ethanol for precipitation and charcoal adsorption are disclosed for the preparation for the anti-HIV-active composition.

Two **lignans, phyllamycin B and retrojusticiden B**, have been reported to have an inhibitory effect on HIV-1 reverse transcriptase activity. The lignans are isolated from **Phyllanthus myrtifolius Moon,** a plant widely grown in Southern China. See, for example, Chang, et al., Antiviral Research, 27 (4), 367-374 (1995).

A mixture of **aqueous extracts of Lonicera japonica flower buds, Forsythia suspensa fruits, and Scutellaria baicalensis rootbark** have been shown to have antibacterial and antiviral properties. Subjects with severe respiratory disease treated with the mixture responded as well as a control group on standard antibiotic therapy. See Houghton, et al., Phytother. Res., 7(5), 384-386 (1993).

A water extract of Prunella vulgaris was reported to have anti-HIVB activity when administered in combination with zidovudine (AZT) and didanosine (dd1). Only a slight additive effect was observed for the administration of an extract of **Prunella vulgaris and zalcitabine (ddC)**. See John, et al., Abstr. Gen. Meet. Am. Sc. Microbiol., 94, 481 (1994).

Yamasaki et al. have reported the in vitro evaluation of 204 crude drugs commonly used in Japan for anti-HIV-1 activity and studies indicate that hot water extracts of **Lithospermum erythrorhizon (root) and Prunella vulgaris (spike)** showed strong in vitro anti HIV-1 activity with an IC100 of 16 μg/mL. See Yamasaki, et al., Yakugaku Zasshi, 113(11), 818-824 (1993).

Yao et al. have reported that water extracts of dried **Prunella vulgaris** (whole plant) were active in vitro for inhibiting HIV-1 replication, and showed relatively low cytotoxicity to MT-4 cells. The extract also demonstrated activity in the inhibition of reverse transcriptase. The active factor was purified and identified as anionic with a molecular weight of approximately 10,000 Daltons. This active component may be the same as the prunellin, as described by Tabba, et al., (1989). The purified extract inhibited HIV-1 replication in the lymphoid cell line MT-4, in the monocytoid cell line U937, and in peripheral blood mononuclear cells (PBMC) at effective concentrations of 6.3 and 12.5 μg/mL, respectively. Pretreatment of uninfected cells with the extract prior to viral exposure did not prevent HIV-1 infection upon subsequent exposure to the virus. Preincubation with the purified extract decreased HIV-1 infectiousness. The purified extract also blocked cell-to-cell transmission of HIV-1, prevented syncytium formation, and interfered with the ability of both HIV-1 and purified gp 120 to bind to CD4. PCR (polymerase chain reaction) analysis confirmed the absence of HIV-1 proviral DNA in cells exposed to virus in the presence of the extract, suggesting that the purified extract antagonized HIV-1 infection of susceptible cells by preventing viral attachment to the CD4 receptor. See Yao, et al., Virology, 187(1), 56-62 (1992).

Tabba, et al. isolated and partially characterized prunellin, a compound exhibiting anti-HIV properties, from **aqueous extracts of Prunella vulgaris, a Chinese herb. Prunellin** was identified as a carbohydrate (a partially sulfated polysaccharide) with an minimum inhibition concentration of 2.2 μg/mL against HIV-1 in vitro. It was identified as having a molecular weight of about 10,000 Dalton. See Tabba, et al., Antiviral Research, 11, 263-273 (1989).

Antiviral agents have been isolated from **Syzygium aromatica, Sapium sebiferum (Chinese tallow tree leaves), Scutellaria baicalensis, and Scutellaria rivularis. Eugeniin, (a tannin isolated from Syzygium aromatica), and methyl gallate, (isolated from Sapium sebiferum)**, exhibited anti-herpes simplex virus (HSV-2) activity in vitro. Plant flavonoids, such as 5,7,4-truhydroxyflavone, extracted from the whole herb Scutellaria rivularis, were reported to have anti-influenza virus activity. See Hozumi, et al., U.S. Pat. No. 5,411,733; Takechi, et al., Planta Medica, 42, 69-74 (1981); Kane, et al., Bioscience Report, 8, 85-94 (1988); and Nagai, et al., Chem. Pharm Bull. 38(5), 1329-1332 (1990).

Ethiopian medicinal plants known for treatment of a variety of ailments were screened for activity against

HIV-1 and HIV-2, as reported by Asres, et al. **Extracts from Bersama abyssinica root bark, Combretum paniculatum leaves, Dodonaea angustfolia leaves, and Ximenia Americana stem bark** each displayed anti-viral activity at concentrations that were non-toxic to MT-4 cells. Anti-viral activity of the extracts is noted to be more effective against HIV-1 than HIV-2. See Asres, et al., Phytother. Res., 15, 62-69 (2001).

Selected plants used in traditional Rwandan medicine for treatment of infections and/or rheumatoid diseases were investigated for antiviral activity in vitro against HIV-1. See Cos, et al., Phytomedicine 9, 62-68 (2002). Of 38 plant extracts tested, extracts from the leaves of **Aspilia pluriseta and Rumex bequaertii** had the highest antiviral activities.

SUMMARY OF THE INVENTION

The present invention is based upon the discovery of the unique antiviral properties of a herbal remedy composition prepared from a variety of plants native to Kenya. The herbal composition of the present invention can include plant material from between two and 14 different plants preferably including roots of abyssinica (representative seed of said line having been deposited under ATCC Accession No. PTA-6769) and Clutia robusta (representative seed of said line having been deposited under ATCC Accession No. PTA-6970). For treatment of infectious disease, the herbal composition of plant material may be extracted to produce a liquid herbal composition or further purified to obtain alkaloid compounds from the plant material. The liquid herbal composition prepared from aqueous extracts from the plants has demonstrated effectiveness in treating HIV-positive subjects, as subjects treated with the liquid herbal composition have experienced improvements in CD4+ cell counts, and in some cases, complete reversal of HIV positive status.

In one aspect, the invention provides a herbal composition for treating infectious diseases, such as for example, HIV. The composition containing plant material includes the roots of abyssinica and the roots of Clutia robusta. In other embodiments of the invention, the herbal pharmaceutical composition may also include plant material, as indicated, from one or more of the following: **stem bark of Prunus africanastem bark of Croton macrostachyus, stem bark of Acacia nilotica (representative seed of said line having been deposited under ATCC Accession No. PTA-7378), roots of Rhamnus prinoides, roots of Adenia gummifera, roots of Asparagus africanus, stem bark of Anthocleista grandiflora, whole plant of Plantago palmata (representative seed of said line having been deposited under ATCC Accession No. PTA-7377), roots of Clematis hirsuta, stem bark of Ekebergia capensis, stem bark of Bersama abyssinica, and roots of Periploca linearifolia.**

In another aspect, the invention provides a method for preparing a liquid extract of the solid herbal composition of the invention. The extraction of plant material can be done with hot water. In one embodiment, hot aqueous extraction is done under basic conditions, followed by hot aqueous extraction under acidic conditions. In further embodiments, desired alkaloid compounds purified from the liquid extracts are provided or produced from direct chemical synthesis.

The invention further provides aqueous extracts of the herbal compositions of the invention. Also provided are alkaloid compounds purified from aqueous extracts and the chemical synthesis of the herbal compositions of the invention.

In another aspect of the present invention a method for treating HIV-positive subjects is provided. Subjects are administered an effective amount of a herbal composition of the invention prepared from the **aqueous extracts of Dovyalis abyssinica and Clutia robusta, alone or in combination with one or more of the following: Prunus africana, Croton macrostachyus, Acacia nilotica, Rhamnus prinoides, Adenia gummifera, Asparagus africanus, Anthocleista grandiflora, Plantago palmata, Clematis hirsuta, Ekebergia capensis, Bersama abyssinica, and Periploca linearifolia**, in doses based on subjects' body weights. In other embodiments the herbal composition of the invention is prepared from purified alkaloid compounds obtained from the aqueous extracts. The herbal compositions are administered at least once a day. In other embodiments, the herbal composition is administered twice or three times daily, based upon the health of the subject. In other embodiments, the composition may be administered as a beverage, capsule, tablet, powder, candy, gel, nutritional product or pharmaceutical product.

In another aspect of the present invention provides an herbal composition for treating subjects having infection, such as for example, HIV or AIDS. The herbal composition consists essentially of extracts of abyssinica and Clutia robusta, and optionally one or more of the following: **Prunus africana, Croton macrostachyus, Acacia nilotica, Rhamnus prinoides, Adenia gummifera, Asparagus africanus, Anthocleista grandiflora, Plantago palmata, Clematis hirsuta, Ekebergia capensis, Bersama abyssinica,**

and Periploca linearifolia. In one embodiment, the herbal composition of the invention is prepared from purified alkaloid compounds obtained from aqueous extracts.

In another aspect of the present invention a method is provided for treating subjects having infection, such as for example, HIV or AIDS. Subjects are administered an effective amount of a herbal composition consisting essentially of extracts of **abyssinica and Clutia robusta, and optionally the extract of one or more of the following: Prunus africana, Croton macrostachyus, Acacia nilotica, Rhamnus prinoides, Adenia gummifera, Asparagus africanus, Anthocleista grandiflora, Plantago palmata, Clematis hirsuta, Ekebergia capensis, Bersama abyssinica, and Periploca linearifolia**, in doses based on subjects' body weights. In other embodiments the herbal composition of the invention is prepared from purified alkaloid compounds obtained from the aqueous extracts. The herbal compositions can be administered at least once a day. In other embodiments, the herbal composition can be administered twice or three times daily, based upon the health of the subject. In other embodiments, the composition may be administered as a beverage, capsule, tablet, powder, candy, gel, nutritional product or pharmaceutical product...

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 shows relationships between observed clinical symptomatology and CD4+ count results.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the discovery that combinations of extracts from plants native to Kenya can be used in the treatment of infection, such as for example, HIV and AIDS. Herbal compositions prepared from combinations of the extracts of the following: **the roots of abyssinica and Clutia robusta, Rhamnus prinoides, Adenia gummifera, Asparagus africanus, Clematis hirsuta, and Periploca linearifolia, the stem bark of Ekebergia capensis, Bersama abyssinica, Prunus africana, Croton macrostachyus, Acacia nilotica, and Anthocleista grandiflora, and the whole plant of Plantago palmata** have been shown to be particularly effective in improving the health of infected subjects. Specifically, herbal compositions of the present invention are particularly well suited for the treatment of infectious diseases including HIV.

Compositions of the invention can be prepared from plant material collected from the Mau Forest Complex in Western Kenya. Herbal compositions prepared from aqueous extractions and purified extracts of plants from this region of Kenya exhibit increased potency in the treatment of infectious diseases. The Mau Forest Complex is located at 0° 30′ South, 35° 20′ East and in the Rift Valley Province, and spans four Kenyan administrative districts: Narok, Nakuru, Bomet and Kericho. Mean annual rainfall varies from 1000 to 1500 mm with peaks in April and August. The rainfall pattern at the western flanks is governed by the moist monsoon winds from the Indian Ocean and dry winds from the Great Rift Valley. The western flanks of the Mau Forest Complex are influenced by the Lake Victoria macroclimatic region and are generally wetter with annual rainfall greater than 2000 mm and more evenly distributed. Mean annual temperatures for the Mau Forest Complex range from 12-16° C. The soil of the Mau Forest Complex is rich volcanic loam having a pH between 3.8-5.8.

The vegetational pattern follows an altitudinal gradient with local topographical ecolines. The closed canopy moist mountain forest at lower altitudes becomes increasingly intermixed with bamboo from 2200 m onwards. Between 2300 and 2500 m, pure bamboo (Arundinaria alpina) swards are found. Above 2500 m this gives way to mixed bamboo/tree stands, both associated with grass clearings that usually represent a sub-climax resulting from burning and cutting of bamboo. A marginal type of mountain sclerophyll forest, wherein the plants generally have hard leaves to prevent wilting during dry conditions, occupies the highest altitudes of the Mau complex.

Plants in the Western flank of the Mau Forest Complex have shown the highest potency for the herbal compositions. Plants growing in the Western flank, (which is generally a high rainfall, high altitude region), have fewer environmental stresses. It is therefore possible that plants of the Western flank have more biosynthetic pathways, which may in turn lead to the production of a greater number of diverse compounds, which may in turn explain the greater potency of plants from the Western flank (as compared to other regions of the Mau Forest Complex). Alternatively, the greater potency plant extracts from the Western flank plants may be a result of a greater variety and number of alkaloids and other compounds in the plant extracts, such that the combined effect is greater than the sum of their individual effects.

The East Mau Forest Complex has a drier vegetation of Cedar and Podo. Wherever these species have been extracted, colonizing species such as **Neuboutonia marcrocalyx and Macaranga capensis** can be found.

The compositions of the invention may be prepared using plants collected from three altitude ranges of the Mau Forest Complex: 2000 m (annual rainfall of 1000 mm), 2300 m (1500 mm), 2500 m (western Mau flank, annual rainfall greater than 2000 mm) above sea level. The Western flanks of the Mau Forest contain plants that are particularly preferred for preparing the herbal compositions of the invention. The plants grown in the drier. Eastern flank of the Mau Forest Complex also may be used.

Plant material for preparing compositions of the invention may also be obtained from plants grown in a greenhouse environment. The germination of the seeds of particular plants may be altitude or soil dependent. Seeds for greenhouse planting may require collection from the natural dispersal agents as they exist in the wild. Additionally, simulation of rainfall, sunlight (an average of 12 hours per day in the Mau Forest Complex), and soil conditions of the Mau Forest Complex (i.e., rich volcanic loam having a pH between 3.8-5.8) may be required to obtain plants of similar potency.

The seeds of abyssinica (representative seed of said line having been deposited under ATCC Accession No. PTA-6969) are contained in a fleshy fruit. There are about 4 seeds enclosed by the flesh. A ripe fleshy fruit can be soaked in water for about 4 days, to make it possible to squeeze with minimum force to release the small seeds, each being approximately the size of a tomato seed or slightly larger. The seeds are then washed, dried and stored, awaiting germination under Mau Forest-like environmental conditions. In the wild, the fruit flesh is soaked by rain water, which results in the release of the seeds. The seeds grow naturally under the environmental conditions of the Mau Forest Complex as described above.

The **Clutia robusta** (representative seed of said line having been deposited under ATCC Accession No. PTA-6970) seeds are much smaller and encased in berries having a nut-like outer covering which encases approximately 3 to 4 seeds the size of a grain of sand. When mature seeds are exposed direct sunlight, they disperse rapidly in a process called explosive dispersal. This is not a problem in the wild, but if one is interested in collecting the seeds, care and intelligence are required, or else all the seeds will fly away under the scattering effect of the hot sun.

To recover the **clutia robusta seeds**, the berries should be placed in a metallic container, and covered with a material that allows sunlight to enter, such as a transparent polyethylene film surrounding a container of appropriate wire mesh. Exposure to light will cause the shells to break open, releasing the seeds which can then be separated from the chaff.

The optimal time for planting the clutia and dovyallis seeds in their natural environment is during the long rains, typically around the month of April. However, in the wild, the plants will generally grow throughout the year, except during the dry season, as the plants require a considerable amount of water and light to grow.

Croton macrostachyus produces pale pea-sized capsules, on drooping spikes to 30 cm long, splitting open on the tree to release 3 shiny grey seeds, covered at one end by a soft, creamy aril, or envelope.

Prunus africana produces spherical fruit, about 10 mm in diameter and is pinkish brown in color.

The **Acacia nilotica** (representative seed of said line having been deposited under ATCC Accession No. PTA-7378) plant produces straight or curved pods measuring approximately 17×2 cm. When young, the pods are green and fleshy but get darker with age, and are usually velvety. Pods have a fruity odor and open on the ground to release seeds.

Ekebergia capensis produces rounded, thin skinned berries, up to 2.5 cm in diameter, on long stalks in heavy bunches, which are yellow to red in color when mature.

The berry-like fruits of **Rhamnus prinoides** are approximately the size of a pea (about 5 mm in diameter), roundish and clearly divided into three compartments. They are fleshy and green, turning red and then purple as they ripen.

The fruit of the **Asparagus africanus** is a round berry, approximately 0.5 cm in diameter, green aging to orange, found most of the year. It is spread mainly by birds carrying the seeds.

The **Anthocleista grandiflora** produces fruits that are oval in shape, measuring approximately 3 cm×2 cm, glossy, smooth and brown when mature. Multi-seeded, large fruits are found throughout the year.

The **Bersama abyssinica** produces a smooth, spherical capsule, measuring approximately 2.5 cm in diameter,

golden velvety at first, losing most of the hair and becoming brown by maturity; splitting into four valves to reveal attractive bright red seeds, about 10 mm long, enveloped for about their half length by a yellow, cup-shaped aril.

Adenia gummifera produces a fruit which is a stalked 3-valved capsule, leathery or fleshy, often red; seeds compressed with bony testa in a fleshy aril.

Plantago palmata (representative seed of said line having been deposited under ATCC Accession No. PTA-7377) produces a capsule-like fruit with two seeds per capsule.

Periploca linearifolia (representative seed of said line having been deposited under ATCC Accession No. PTA-7375) produces black seeds measuring approximately 10 mm long and 2 mm wide with white wool measuring around 3 cm attached to the tips of the seeds. The seeds are enclosed in pods measuring about 12 cm long. Upon maturity, the pods break open upon exposure to sunlight. This releases the seeds, which are borne aloft by the wool as they are dispersed by wind. Alternatively, these plants may be cultivated from stem cuttings, which when laid on or planted in the ground, grow roots and propagate new plants.

Clematis hirsuta (representative seed of said line having been deposited under ATCC Accession No. PTA-7383) produces yellowish seeds measuring approximately 3mm in length and 1 mm in breadth. The seeds are surrounded by yellowish-white wool which measures about 5 mm long. The wool carries the seeds upon the wind, which is the dispersal agent.

HIV Testing

As noted previously, for purposes of this application, a person is considered HIV-negative if the subject tested negative on a two-part HIV screening tests, consisting of an initial screening test and a confirmatory test.

An infected individual usually goes for testing for one or more of the following reasons: 1) the individual feels ill, 2) the individual's sexual partner is ill and has tested positive, 3) the individual's sexual partner died of AIDS; or 4) the individual suspects his/her sexual partner is sexually promiscuous.

The initial screening test is ELISA (Enzyme-Linked Immunosorbent Assay), an enzyme immunoassay (EIA) to determine the presence of HIV antibodies. The ELISA test uses artificial HIV proteins that capture antibodies to the virus and is more than 99 percent accurate. If antibodies to HIV are present (positive result), the test is typically repeated. However, other antibodies can cause a false-positive result.

Generally, HIV-1 antibodies are detectable approximately 25 days after acute infection, with nearly all infected subjects testing HIV positive 12 weeks after infection. The process of developing antibodies to a virus is termed seroconversion, and individuals who become antibody-positive are often called seroconverters.

Two types of HIV have been identified: HIV-1 and HIV-2, of which, HIV-1 is more common. HIV-1 and HIV-2 are similar in the modes of transmission (sexual contact, sharing needles, etc.) and infected individuals are generally subject to the same opportunistic infections. However, HIV-2 appears to weaken the immune system more slowly than HIV-1.

In Kenya, individuals are generally tested for antibodies to both HIV-1 and HIV-2. HIV-1 is generally more common in the Western world and HIV-2 is more common in Africa. In Kenya however, most HIV-positive individuals have the HIV-1 infection. It is believed that 90% of the HIV-positive cases in Kenya are HIV-1, with the remaining 10% of HIV-positive cases being the HIV-2. While rare, subjects occasionally are HIV antibody-positive to both types of HIV (i.e. HIV-1 and HIV-2).

The second part of the HIV screening test is called the confirmatory test. In the U.S., the most often used confirmatory test is the Western blot, wherein an electrical field is used to separate the various components by their molecular weight prior to evaluating antibody binding. This allows identification of antibodies to specific viral antigens, which show up as identifiable “bands” on a strip of test paper. The Western blot test is more difficult to perform and accurately interpret than the ELISA test, but it is less likely to give a false-positive result because it can distinguish HIV antibodies from other antibodies that may react to the ELISA. Other confirmatory tests may be used, including the indirect fluorescent antibody assay (IFA) and the radioimmunoprecipitation assay (RIPA).

One major drawback of antibody tests is the “window” period (i.e. the time it takes the body to produce antibodies after infection has begun). The screening tests do not correlate to the presence or absence of symptoms. The standard HIV tests do not detect the virus itself, but instead detect the antibodies that the body produces in response to the virus. During the period before the antibodies are produced, a person may be infected with HIV and can infect others, and still test negative on the HIV antibody test. It is therefore important to tell subjects who test negative to avoid engaging in high-risk behavior and to return for retesting at a later date.

The p24 antigen test can be used in diagnosing HIV early in the course of infection. It is primarily used to screen the blood supply but in some places it is used for testing for HIV. The p24 antigen is a protein that is part of the HIV. Early in the infection, it is produced in excess and can be detected in the blood serum by a commercial test. The p24 test can detect HIV infection before the HIV antibody test can and it is recommended 2-3 weeks after a risk exposure.

Individuals that test positive for HIV are regularly administered two tests to monitor HIV levels in the blood and to determine how the virus is affecting the immune system. These tests are: (1) a viral load measurement, and (2) CD4+ cell counts.

Viral load measurement (also called the HIV plasma RNA test) determines how many HIV viral particles are present in a given amount of a person's blood. Test results help determine the best treatment for the HIV infection as the viral load test shows how fast the virus is multiplying in the body. Because HIV reproduces by making copies of itself, the results are given as copies per milliliter (mL). Viral load testing can also reveal the presence HIV infection before antibodies can be detected and can also accurately determine whether a baby born to an infected mother has HIV.

CD4+ cell counts (T-lymphocyte measurements) provide an estimate of the immunologic status of an individual and help determine the immediate risk of opportunistic infection. The CD4+ count measures the number of a certain type of white blood cell that is most affected by HIV, and are measured every 3 to 4 months in individuals infected with HIV. On average, an individual infected with HIV loses approximately, 30-60 CD4+ cells per year, although in some subjects, CD4+ T-lymphocyte counts may remain stable for years followed by rapid decline.

CD4(T4) or CD4+ cells are a type of T cell involved in protecting against infections, such as for example, viral, fungal, and protozoal infections. Destruction of these cells is the major cause of immunodeficiency observed in AIDS, and decreasing CD4+ lymphocyte counts appear to be the best indicator for the potential development of opportunistic infections. In judging the severity of HIV/AIDS cases, the CD4+ lymphocyte count is more indicative of the severity of the disease than gross symptomalogy, although it is also true that certain symptoms may be associated with particular CD4+ lymphocyte levels. See, for example, FIG. 1. Average normal adult CD4+ cell counts typically ranges from 500 to 1,500/2,000 cells per cubic milliliter of blood.

As CD4+ cell counts decrease below the normal adult levels during primary HIV infection, CD8+ or cytotoxic T-lymphocytes also increase. However, most studies indicate that an increase in CD8 count is not a prognostic indicator of disease progression. Some clinicians in the U.S. use the CD4/CD8 ratio as an indicator of disease progression, however, this ratio varies not only with the severity of the disease, but with the ethnicity of the subject.

There are several systems for classifying and staging HIV infection. The most commonly-used system is the CDC (Centers for Disease Control) Scheme. The CDC scheme has three classifications based upon CD4 counts. The definitions of the three CD4+ T-lymphocyte categories I as follow: Category 1: >500 cells/mm<3 >(or CD4%>28%); Category 2: 200-499 cells/mm<3 >(or CD4% 14% -28%); and Category 3: <200 cells/mm<3 >(or CD4%<14%).

In addition to the CDC classification scheme, there are also 3 possible categories of clinical conditions, which are designated by the letters A, B and C. Therefore, a given individual can have the following CDC classification and clinical categorization designation: 1-A, or 1-B, or I-C, 2-A, 2-B, 2-C, 3-A, 3-B or 3-C.

An individual in category A is identified as an adolescent or adult (>13 years) with documented HIV infection having one or more of the following conditions (and lacking any of the conditions associated with categories B and C): asymptomatic HIV infection; persistent generalized lymphadenopathy; and acute (primary) HIV infection with accompanying illness or history of acute HIV infection.

An individual in category B is identified as an adolescent or adult (>13 years) with documented HIV infection having one or more of the following conditions (and lacking any of the conditions associated with category C) and that meet at least one of the following criteria: (a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or (b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples of conditions in clinical category B include but are not limited to: bacillary angiomatosis; candidiasis (oropharyngeal, i.e. thrush); candidiasis (vulvovaginal, persistent, frequent, or poorly responsive to therapy); cervical dysplasia (moderate or severe/cervical carcinoma in situ); constitutional symptoms, such as fever (body temperature of 38.5° C. or greater) or diarrhea lasting longer than 1 month; hairy leukoplakia (oral); herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome; idiopathic thrombocytopenic purpura; listeriosis; pelvic inflammatory disease (particularly if complicated by tubo-ovarian abscess); and (11) peripheral neuropathy. For classification purposes, Category B conditions take precedence over Category A conditions. For example, an individual previously treated for oral or persistent vaginal candidiasis (but not exhibiting a Category C disease or condition) who is now asymptomatic, should be classified in Category B.

An individual in category C is identified as an adolescent or adult (>13 years) with documented HIV infection having one or more of the following conditions Category C conditions include the following: candidiasis of bronchi, trachea, or lungs; candidiasis (esophageal); invasive cervical cancer; coccidioidomycosis (disseminated or extrapulmonary); cryptococcosis (extrapulmonary); cryptosporidiosis (chronic intestinal, greater than 1 month's duration); cytomegalovirus disease (other than liver, spleen, or nodes); cytomegalovirus retinitis (with loss of vision); encephalopathy (HIV-related); herpes simplex: chronic ulcer(s) (greater than 1 month's duration), or bronchitis, pneumonitis, or esophagitis; histoplasmosis (disseminated or extrapulmonary); isosporiasis (chronic intestinal, greater than 1 month's duration); Kaposi's sarcoma; lymphoma (Burkitt's, or equivalent term), lymphoma, (immunoblastic, or equivalent term); Lymphoma (primary, of brain); mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary; mycobacterium tuberculosis, (any site, pulmonary or extrapulmonary); mycobacterium, (other species or unidentified species, disseminated or extrapulmonary); pneumocystis carinii pneumonia; pneumonia (recurrent); progressive multifocal leukoencephalopathy; Salmonella septicemia (recurrent); toxoplasmosis of brain; and wasting syndrome due to HIV. For classification purposes, once a Category C condition has occurred, the individual will remain in Category C.

One method of treatment for HIV-positive individuals is the highly active antiretroviral therapy (HAART) regimen. HAART is a therapeutic treatment regime consisting of the combination of anti-HIV drugs, that is prescribed to HIV-positive individuals even before they develop symptoms of AIDS. The therapy usually includes one nucleoside analog, one protease inhibitor and either a second nucleoside analog or a non-nucleoside reverse transcription inhibitor (NNRTI). Frequently, the HAART regime is toxic to the individual, resulting in adverse side effects. For example, HAART can be toxic to blood because it almost always includes one or two nucleoside analogs, like AZT that are notorious for their toxicity to red and white blood cells and blood cell production. Various forms of anemia are very common and sometimes are irreversible. However, it is extremely rare for a subject on the HAART regimen reverse his/her HIV status in Kenya.

Examples of drugs administered for the HAART treatment regime include: azidovudine (AZT), didanosine (dideoxyinosine, ddI), zalcitabine (dideoxycytosine, ddC), lamivudine (epivir, 3TC), nevirapine (Viramune), abacavir (Ziagen), stavudine (Zerit, d4T), tenofovir (Viread), efavirenz (Sustiva), amprenavir (Agenerase), lopinavir (Kaletra), nefinavir (Viracept), saquinavir (Invirase), ritonavir (Norvir), indinavir (Crixivan), and delavirdine (Rescriptor).

Method for Extracting Alkaloid Compounds and Preparing Herbal Composition

The compositions of the invention are prepared using roots of abyssinica and Clutia robusta, and optionally one or more of the following: **the stem bark of Prunus africana, stem bark of Croton macrostachyus, stem bark of Acacia nilotica, roots of Rhamnus prinoides, roots of Adenia gummifera, roots of Asparagus africanus, stem bark of Anthocleista grandiflora, whole plant of Plantago palmata, roots of Clematis hirsuta, stem bark of Ekebergia capensis, stem bark of Bersama abyssinica, and roots of Periploca linearifolia**. Preferably, the ingredients collected are fresh, although dried samples may also be used. The ingredients are combined and chopped into small pieces and dried. Preferably, the dried ingredients are ground into a fine powder after drying. Alternatively, each ingredient may be processed individually and combined at a later stage. Preferably, if combined for the extraction process, the ingredients are combined in equal weight ratios. Optionally, Dovyalis abyssinica, Clutia robusta, Prunus africana, Croton macrostachyus, Acacia nilotica, Rhamnus prinoides, Adenia gummifera, Asparagus africanus, Anthocleista grandiflora,

Plantago palmata, Clematis hirsuta, Ekebergia capensis, Bersama abyssinica and Periploca linearifolia can be present in a weight ratio of 2:2:2:2:2:2:1:2:2:1:2:2:2:2.

The herbal plant material mixture may be extracted with a non-polar solvent to remove fats from the chopped herbal ingredients. Preferably, approximately 20% by volume non-polar solvent is added to the herbal ingredient mixture. Non-polar solvents are generally organic solvents having a dielectric constant less than 20. Non-polar solvents that may be used include, but are not limited to: alkanes, 1,4-dioxane, carbon tetrachloride, chloroform, methylene chloride, benzene, ethers, ethyl acetate, tetrahydrofuran, acetic acid, butanol, chlorobenzene, cycloalkanes, xylene, and the like. Preferred non-polar solvents are xylene and ether.

The non-polar solvent is decanted and discarded. The defatted herbal solids, are then allowed to dry. Sufficient base is added to the defatted herbal material to achieve a pH of approximately 8. The concentration of the base added can be adjusted to provide sufficient liquid volume to cover the defatted herbal solid mixture. Any suitable base may be used, with preferred bases including NaOH, KOH, Ca(OH)2, Mg(OH)2, NH4OH, and the like. The base extract is then heated for 2-4 hours. Preferably, the ingredients are slowly simmered under reflux conditions, although the same effect can be achieved by simmering the mixture in a covered pot.

Acid is added to the base extract to achieve a pH of approximately 3. Preferably the acid is HCl, although other acids, including but not limited to, HBr, HNO3, H2SO4, H3PO4, or any other acid suitable for achieving a pH of approximately 3 may be used as well. The concentration of the acid can be adjusted as necessary to provide sufficient volume to the mixture. The acidified solution is then boiled for approximately 2-4 hours under the same conditions employed for the heating of the basic solution. After heating, the mixture is cooled, and the aqueous layer is separated from the mixture, such as for example, by decanting the liquid from the remaining solids. Acid is then added to the remaining residue sufficient to achieve a pH of approximately 3, and the mixture is then reheated for approximately 2-4 hours under the same conditions previously employed. The aqueous layer is separated from the ingredients and the two acidified layers are combined. If necessary, additional acid extractions may be performed.

The acidic filtrate is extracted several times with a non-polar solvent until little or no emulsion forms. Preferable non-polar solvents are ether and xylene. Base is added to the aqueous layer to precipitate the alkaloid compounds. Preferably, base is added to achieve a pH of approximately 9. The precipitate is separated from the aqueous solution, neutralized and dried.

The precipitate is preferably collected in either crystalline or powder form, and may administered to an subject as a beverage, capsule, tablet, powder, candy, gel, nutritional product or pharmaceutical product.

The precipitate can be further purified as desired to isolate individual alkaloid compounds by any known chromatographic means.

It is understood that at any point during the process of extracting the alkaloid compounds from the herbal ingredients that the aqueous solution can be concentrated and stored for later use without the need for precipitation of the compounds from solution.

Alternatively, the alkaloid compounds for use in the present invention can be synthesized by known methods once the chemical structure has been determined. Isolated compounds can be analyzed by chemical analysis, mass spectroscopy, infrared spectroscopy, X-ray diffraction, NMR (including <1>H NMR, <13>C NMR, COSY, NOSEY, and the like), and other known analytical techniques to obtain the chemical structures. For example, chemical structures for four extracts obtained from abyssinica have been previously determined. (See, for example, http://www.dfuni.dk/uploads/media/Naturstofgruppen_BonnieRasmussen.pdf).

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Determination of Bioactivity of Plant Extracts

The efficacy of the individual plant extracts were tested against Escherichia coli and Staphylococcus aureus. Plant extracts were obtained as described above. Solutions containing 100 ppm (parts per million) of each plant extract were prepared for use in the anti-bacterial assay.

Preparation of bacterial culture of Escherichia coli and Staphylococcus aureus.

Standard cultures of E. coli (representing gram-negative strains of bacteria) and Staphylococcus aureus (representing gram-positive bacteria) were obtained from Moi University Teaching and Referral Hospital. Assays were conducted at the Moi University Department of Botany.

Bioassay procedure (Diffusion Method)

Nutrient agar was used as growth medium for both bacteria samples. The agar was sterilized in an autoclave at 120° C., cooled and poured into sterile Petri dishes and allowed to set. Sterile conditions were achieved and maintained by exposing the area to a UV lamp during sample preparation and the assay the procedure.

The cooled agar medium was streaked on the surface with each bacteria culture. Wells were dug in the middle of the medium, using a cork borer, where the prepared plant extract was deposited. A control experiment was also performed, using plain sterile water in place of the plant extracts.

Cultures were incubated for 12 hours, after which zones of inhibition of bacterial growth were determined and measured. Bacteria-growth inhibition was expressed in diameters (mm), and was determined by measuring the distance from edge of the well to area where the bacteria begin to show growth. Generally, the larger inhibition diameter indicates greater potency of the particular extract against the bacteria.

Of the 23 plants were screened in this assay, 14 of the plants had bacteria growth inhibition diameters greater than 8 mm, which was previously determined to be the minimum activity required for adoption of the extract for the herbal remedy. The anti-bacterial activities of the plants were compared with standard antibiotics. Of the 14 plants having inhibition diameters greater than 8 mm, abyssinica and Clutia robusta demonstrated the greatest anti-bacterial activity. Results for plant extracts exhibiting inhibition diameters greater than 8 mm are provided in the Table 1.

TABLE 1
Zones of Inhibition Expressed as Inhibition Diameter (mm)

Plant Name	E. coli	S. aureus
1. Dovyalis abyssinica	17.2	16.6
2. Clutia robusta	16.7	15.8
3. Prunus Africana	14.7	14.6
4. Croton macrostachyus	14.7	14.4
5. Acacia nilotica	13.6	13.2
6. Ekebergia capensis	12.8	13.0
7. Clematis hirsuta	11.9	12.8
8. Adenia gummifera	11.7	12.8
9. Asparagus africanus	11.3	11.2
10. Plantago palmata	11.0	11.0
11. Rhamnus prinoides	10.9	10.8
12. Periploca linearifolia	10.9	10.6
13. Bersama abyssinica	10.5	10.3
14. Anthocleista grandiflora	10.0	9.7

Administration of the Herbal Composition

The plant extract precipitates are preferably purified and collected in either crystalline or powder form. The precipitates can administered to a subject as a beverage, capsule, tablet, powder, candy, gel, nutritional product or pharmaceutical product. Preferably, between 0.1 and 25 grams of alkaloids are administered per day to an infected subject. The herbal composition is preferably administered as a beverage wherein approximately 1 tbsp of powdered extract is dissolved in approximately 250 mL of hot water, and drunk. Dosing is either twice daily at 12 hour intervals, or three times daily at eight hour intervals (depending on the level of infection of the test subject), and is preferably administered with a meal.

Subjects in the current trials were screened at the Walter Reed Hospital of the U.S. Army in Kericho, Kenya, the Moi University Hospital in Eldoret, and at various Voluntary Counseling and Testing (VCT) Centers scattered throughout the country.

Subjects' CD4 and CD8 counts were measured using a FACSCount™ system following procedures provided in the FACSCount White Paper (July 1994). HIV-1 and HIV-2 antibodies were detected using a bioMérieux Vironostika® HIV Uni-Form II Ag/Ab ELISA system.

All subjects administered the herbal composition were HIV-positive adults. Prior to administration of the herbal composition, an initial CD4 count for each subject was determined, followed by an assessment of the level of opportunistic infections. Those with fewer opportunistic infections were administered the herbal composition twice daily after meals, at twelve hour intervals. Those with more opportunistic infections were administered the herbal composition three times daily, at 8 hours intervals. Each subject was given one week's dosage during each visit to the clinic. This was done to make it possible to monitor compliance, and to avoid the possibility of subjects sharing the drug with others.

Example 1

Initial studies for the treatment of HIV positive subjects with herbal remedy were conducted by treating four HIV positive subjects with two different herbal remedies. Two subjects were administered a herbal composition which included the extract of abyssinica, while the other two subjects were administered a herbal remedy which included the extract of Clutia robusta. The subjects were each treated for a period of three months. The CD4 counts of both sets of subjects (i.e., those administered either abyssinica or Clutia robusta) increased by approximately 10 per month of treatment.

Example 2

In another study, three subjects were administered a herbal composition prepared with a 1:1 ratio by weight mixture of Doyalis abyssinica and Clutia robusta for a period of approximately three months. The CD4 counts of the subjects treated with the mixture increased by approximately 30 per month.

Example 3

In yet another experiment, 20 subjects were administered a herbal composition containing extracts of abyssinica, Clutia robusta, Prunus africana, Croton macrostachyus, Acacia nilotica, Ekebergia capensis, Clematis hirsuta and Adenia gummifera. The 8 plant extracts were selected from 23 total plant extracts which had been previously assayed against E. coli and S. aureus. As shown in Table 2, CD4 counts increased of subjects by up to 100 per month, but none of the subjects tested HIV negative within the three-month period.

TABLE 2
CD4/ul per month

Subject ID	Month 1	Month 2	Month 3	Month 4	Month 5
1b	118	150	399	420	—
2b	100	250	420	460	—
3b	04	93	190	320	—
4b	667	550	815	830	—
5b	160	120	480	620	—
6b	210	190	520	510	—
7b	420	500	780	780	—
8b	128	108	310	304	—
9b	110	150	380	348	—
10b	380	460	716	716	—
11b	300	410	390	560	—
12b	100	120	310	318	—
13b	250	180	340	420	—
14b	80	70	260	380	—
15b	140	110	300	420	—
16b	250	180	290	360	—
17b	300	380	460	580	—
18b	280	290	290	410	—
19b	118	190	170	320	—
20b	160	160	220	299	360

Example 4

In another experiment, 26 HIV-positive subjects were treated with a herbal composition consisting of the 14 herbal ingredients identified in Table 1. Subjects were administered a composition prepared by dissolving approximately 1 tbsp. (or 15 ml) of the powdered ingredients (a mixture prepared the 14 plants listed in Table 1) in approximately 8 ozs. (250 ml) of hot water. The supernatant liquid was then ingested by the subject.

The subjects were divided into two groups: the first group having 10 subjects (subject ID Nos. 1-10) and the second group having 16 subjects (Subject ID Nos 11-26). In the first group, each the 14 plants was present in the composition in equal weight ratios. In the second group, the concentrations of abyssinica and Clutia robusta were approximately half of the other 12 ingredients as disclosed.

As shown in Table 3, CD4 counts for each subject were measured on a monthly basis. The CD4 counts of the test subjects treated with the 14 ingredient herbal composition increased by up to 100 per month. Six subjects tested HIV-negative after four months of treatment. Two subjects tested HIV-negative after two months of treatment.

TABLE 3
Subject CD4/uL per month
ID Month 1 Month 2 Month 3 Month 4
1 420 450 570 HIV negative
2 320 390 480 520
3 100 115 250 —
4 80 150 310 —
5 340 370 480 560
6 120 180 299 —
7 118 350 360 HIV negative
8 125 105 225 —
9 300 200 400 HIV negative
10 280 399 410 HIV negative
11 400 500 520 HIV negative
12 250 250 310 —
13 250 460 600 —
14 400 520 780 —
15 250 330 480 HIV negative
16 667 550 815 830
17 150 250 380 —
18 620 640 660 —
19 310 400 480 —
20 243 245 280 —
21 180 216 434 —
22 280 390 — —
23 360 420 — —
24 190 280 — —
25 630 720; — —
HIV negative
26 N/A; N/A;

HIV positive HIV negative

By comparison with the results achieved with the present invention, in a study conducted on subjects on HAART in Moi University Teaching and Academic Model for Prevention and Treatment of HIV (AMPATH), the CD4 count increases were gradual, generally taking several years to reach above 500. The subjects were treated with conventional antiretroviral (ARV) therapy, consisting of twice daily dosing of Stavudine, Lamivudine and Nevirapine (d4T-3TC-NVP). Other ARV regimes include treatment with combinations consisting of ZDV-3TC-NVP, d4T-3TC-EFV and ZDV-3TC-EFV (wherein ZDV is Zidovudine and EFV is Efavirenz). Treatment guidelines are provided in the publication “Integrated Management of Adolescent and Adult Illness,” published in January 2004 by the World Health Organization. ARV therapy subjects rarely reverse their seroconversion status, and among those listed in Table 4, none did so.

TABLE 4
Comparative Results of CD4 Count Increases in Subjects Under Conventional ARV Therapy.

	6 Months	1 Year	1½ Years	2 Years	2½ Years	3 Years
1.	247	207	264	197	138	367
2.	315	327	150	260	—	—
3.	268	199	195	360	—	—
4.	99	163	—	—	—	—
5.	265	40	36	247	332	397
6.	138	311	584	578	—	—
7.	37	298	—	—	—	—
8.	201	261	—	—	—	—
9.	21	52	74	309	—	—
10.	2	156	—	—	—	—
11.	43	200	—	—	—	—
12.	169	295	—	—	—	—
13.	75	144	179	—	—	—

US5837257 / US5989556
Use of plant extracts for treatment of HIV, HCV and HBV infections

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Abstract

This invention relates to compositions derived from Chinese herbal medicines, medicinal plants and extracts thereof, and to their use for the treatment of animals infected with viruses, especially with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). More specifically, the compositions of the present invention are derived from various Chinese herbal medicines or medicinal plants which have a long history of human consumption. The compositions of the invention are obtained through specific techniques and have demonstrated outstanding efficacy for treating human HBV carriers and hepatitic C patients. Compositions according to the invention have also exhibited in vitro antiviral activities against murine leukemia virus (MuLV) and HIV. HIV is the virus known to cause acquired immunodeficiency syndrome (AIDS) in humans and AIDS presents special problems to the medical community which the present invention addresses.

RELATED APPLICATIONS

This application claims priority to a provisional application filed Jul 9, 1996, Ser. No. 60/016,100 entitled: ANTI-VIRAL AGENTS; and to a provisional application filed Jul. 10, 1996, Ser. No. 60/021,467 entitled: ANTI-VIRAL AGENTS FROM CHINESE MEDICINAL HERBS.

TECHNICAL FIELD

This invention relates to compositions derived from Chinese herbal medicines, medicinal plants and extracts thereof, and to their use for the treatment of animals infected with viruses, especially with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). More specifically, the compositions of the present invention are derived from various Chinese herbal medicines or medicinal plants which have a long history of human consumption. The compositions of the invention are obtained through specific techniques and have demonstrated outstanding efficacy for treating human HBV carriers and hepatitic C patients. Compositions according to the invention have also exhibited in vitro antiviral activities against murine leukemia virus (MuLV) and HIV. HIV is the virus known to cause acquired immunodeficiency syndrome (AIDS) in humans and AIDS presents special problems to the medical community which the present invention addresses.

BACKGROUND OF THE INVENTION

Modem medical science is constantly searching for new and more powerful agents to prevent, treat or retard bacterial and viral infections and cure the diseases they cause. Bacterial and viral infections of humans and domestic animals cost billions of dollars annually. Vast sums of money are spent each year by pharmaceutical companies to identify, characterize, and produce new antibiotics and antivirals to combat the emerging drug resistant strains which have become a serious problem. Reliable prophylactic treatments for disease

prevention are also of major interest. Yet, despite the costs and efforts to identify treatments for viral infections, such as hepatitis and AIDS, effective therapies remain elusive.

Hepatitis is a disease of the human liver. It is manifested with inflammation of the liver and is usually caused by viral infections and sometimes from toxic agents. Hepatitis may progress to liver cirrhosis, liver cancer, and eventually death. Several viruses such as hepatitis A, B, C, D, E and G are known to cause various types of viral hepatitis. Among them, HBV and HCV are the most serious. HBV is a DNA virus with a virion size of 42 nm. HCV is a RNA virus with a virion size of 30-60 nm. See D. S. Chen, J. Formos. Med. Assoc., 95(1), 6-12 (1996).

Hepatitis B is a major health problem worldwide, especially in Asia and Africa. Approximately 300 million people are chronically infected with HBV worldwide. More than one million carriers of HBV are found in the United States and HBV infection is currently the main cause of liver cirrhosis and cancer. HBV carriers not only become long-term reservoirs of the virus but also may develop chronic liver disease and have a greatly increased risk of developing liver cirrhosis and cancer. The progression from chronic hepatitis B to cirrhosis is frequently insidious and occurs without a noticeable change in symptoms. Once the symptoms of cirrhosis or cancer are manifested, therapies are of little value.

Current prevention of HBV infection is a hepatitis B vaccination which is safe and effective. However, vaccination is not effective in treating those already infected (i.e., carriers and patients). Many drugs have been used in treating chronic hepatitis B and none have been proven to be effective, except interferon. Treatment with interferon has limited success and has frequently associated adverse side effects such as fatigue, fever, chills, headache, myalgias, arthralgias, mild alopecia, psychiatric effects and associated disorders, autoimmune phenomena and associated disorders and thyroid dysfunction. Treatment with interferon for sixteen (16) weeks has been shown to be effective with a sustained loss of viral replication in approximately 40% of hepatitis B patients. The great majority of responders had normal serum aminotransferase levels and relapse rates appear to be low. See R. P. Perrillo, Digestive Diseases and Sciences, 38(4), 577-593 (1993). However, a higher long-term relapse rate (24%) was reported in Chinese patients with chronic hepatitis B who underwent interferon therapy. See A. S. F. Lok, H. T. Chung, V. W. S. Liu, & O. C. K. Ma, Gastroenterology, 105(6), 1833-1838 (1993).

Moreover, serum hepatitis B surface antigen (HBsAg) disappeared in 10-15% of patients treated with interferon. The loss of HBsAg coincided with the disappearance of HBV. Improvement in liver histology was sustained years later in HBsAg-negative patients. The lack of disease progression could thus conceivably result in the prevention of liver cancer when treatment is provided in the pre-cirrhotic stage of infection. See R. P. Perrillo, Digestive Diseases and Sciences, 38(4), 577-593 (1993).

Hepatitis C has been previously described as a non-A non-B hepatitis, which is caused by HCV. There are approximately 100 million HCV carriers worldwide. An estimated 3.5 million people have chronic hepatitis C in the United States. HCV infection will lead to liver cirrhosis and cancer with less clinical manifestation. Most hepatitis C patients do not have particular symptoms and can thus be easily overlooked until it is too late for therapy. This poses a potentially more serious problem than hepatitis B. HCV carriers also become long-term reservoirs of the virus and eventually develop chronic liver disease and have a greatly increased risk of developing liver cirrhosis and cancer. See D. S. Chen, Science, 262, 369-370 (1993).

No effective immunization is currently available, and hepatitis C can only be controlled by other preventive measures such as improvement in hygiene and sanitary conditions and interrupting the route of transmission. At present, the only acceptable treatment for chronic hepatitis C is interferon which requires at least six (6) months of treatment. Treatment with interferon has limited long term efficacy with a response rate about 25%. Initial treatment has a response rate of about 50% however, half of those which respond relapse after cessation of interferon treatment. Therefore, only about 25% of patients had a sustained response. See D. S. Chen, J. Formos. Med. Assoc., 95(1), 6-12 (1996) and N. Terrault & T. Wright, New Engl. J. Med., 332(22), 1509-1511 (1995).

Because the interferon therapy has limited efficacy and frequent adverse effects, a more effective regimen is needed. New antivirals and immune modulators are presently undergoing clinical trials.

AIDS is a deadly disease of an acquired immunodeficiency syndrome in humans caused by HIV. It has been plaguing the world since the first description of the disease in 1981 and the discovery of its causative agent, HIV, in 1983. About 13 million people were infected with HIV worldwide in 1993 and the number has increased to about 21 million in 1996. See B. Jasny, Science, 260(5112), 1219 (1993) and P. Piot, Science,

272(5270), 1855 (1996).

Several drugs have been approved for treatment of this devastating disease, including azidovudine (AZT), didanosine (dideoxyinosine, ddI), d4T, zalcitabine (dideoxycytosine, ddC), nevirapine, lamivudine (epivir, 3TC), saquinavir (Invirase), ritonavir (Norvir), indinavir (Crixivan), and delavirdine (Rescriptor). See M. I. Johnston & D. F. Hoth, Science, 260(5112), 1286-1293 (1993) and D. D. Richman, Science, 272(5270), 1886-1888 (1996).

All drugs currently approved for AIDS treatment utilize inhibition of viral proliferation and are viral reverse transcriptase inhibitors or viral protease inhibitors. More protease inhibitors, such as nelfinavir and improved saquinavir, are in development. An AIDS vaccine (Salk's vaccine) has been tested and several proteins which are chemokines from CD8 have been discovered to act as HIV suppressors.

In addition to the above synthetic nucleoside analogs, proteins, and antibodies, several plants and substances derived from plants have been found to have in vitro anti-HIV activity, such as Lonicera japonica and Prunella vulgaris, and glycyrrhizin from Glycyrrhiza radix. See R. S. Chang & H. W. Yeung, Antiviral Research, 9, 163-175 (1988) and M. Ito, et al., Antiviral Research, 7, 127-137 (1987).

Despite all of the available pharmaceuticals for the treatment of HIV, there is still no cure for the deadly disease. HIV viruses continue to mutate and become resistant to existing drugs such as the reverse transcriptase inhibitors and protease inhibitors. Recently, a therapy of using two (2) or three (3) anti-HIV drugs in combination has been found effective in significantly lowering the HIV loads in AIDS patients. The results have been promising, however the virus continues to develop resistance to the drugs and the long-term outcome (survival and cure rates) is still unknown. Thus, the medical communities throughout the world continue to search for drugs that can prevent HIV infections, treat HIV carriers to prevent them from progressing to full-blown deadly AIDS, and treat the AIDS patient.

The use of herbal drugs and folk medicines have been known for thousands of years in China. These herbal approaches to the treatment of numerous illnesses, from arthritis to viral infections, have been viewed by western modern medicine as ineffective and dangerous. Records of the use of herbs date from ancient China, Egypt and Biblical times. Early physicians used hundreds of herbs to treat a variety of ailments. The practice is still widespread, especially in Asia and Europe. During the 19th century, many home remedies containing herbs were patented and sold. Modern drugs have replaced those remedies, but many modern drugs contain ingredients derived from herbs.

In 1776, the English botanist and physician William Withering learned that an herbal tea made by an old farm woman was effective in treating dropsy, or excess water in the tissues, which is caused by the inability of the heart to pump strongly enough. He found that one ingredient of the tea, which was made with leaves of the foxglove plant, strengthened the heart's pumping ability. The drug made from the foxglove plant is now known as digitalis.

Folk medicine is a relatively modern term to the West and has come to mean the care and treatment of the sick through a variety of herbal medicines. In recent years, folk medicines have become of increasing interest to many people in the western scientific medical community.

PRIOR ART

A Chinese herbal medicine known as **AEGINETIAE HERBA (a.k.a. GOLDEN LOCK KEY or LOTUS HERBA**); has traditionally been used to treat illnesses such as swollen and sore throat, urinary tract infection, osteomyelitis, boils, tonsillitis, goiter, pharyngitis, thyroiditis, enteritis, liver disease, cancer, rheumatism, hematemesis, neurasthenia, eye redness, piles, menstruation irregularity, dropsy, jaundice, hernia, snake bite, and child developmental retardation. AEGINETIAE HERBA is prepared from the dried whole plant of Aeginetia indica which belongs to the family Orobanchaceae Dichondra micrantha, Striga lutea and Dichondra repens are also used to prepare this herbal medicine. Treatment dosage using the dried plant is typically from 4 to 150 g per day. It should be noted that the plant tastes bitter and is toxic.

Okubo et al. disclose that a phosphate buffered saline (PBS) extract (pH 7.2 at ambient to 4 DEG C.) from the seeds of Aeginetia indica exhibits excellent carcinostatic effect and possesses interleukin-2 and interferon-.gamma. inducing properties. The PBS was a 0.1M phosphate buffered physiological saline at pH 7.2, not containing calcium or magnesium ions. The extracted substance is taught to be a macromolecular polysaccharide which may or may not contain lipid A binding with protein depending on whether the

extraction is conducted using butanol or phenol. The extracted substance was soluble in water and insoluble in n-butanol. Its molecular weight was within the range of 100,000 to 200,000 Dalton. See S. Okubo, M. Sato, & K. Himeno, U.S. Pat. No. 5,366,725, issued on Nov. 22, 1994.

A Chinese herbal medicine known as **BAPHICACANTHIS RHIZOMA ET RADIX** has traditionally been used to treat illnesses such as fever, abscesses, erysipelas, swollen sore throat, hematemesis, epistaxis, typhus, typhoid, mumps, puerperal fever, flu, measles, beriberi, headache, jaundice, plague, leucorrhea, and syphilis. BAPHICACANTHIS RHIZOMA ET RADIX is prepared from the dried rhizoma and root of Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, or Polygonum tinctorium. It has been reported that this herbal medicine has exhibited inhibition of flu virus in vitro. Aqueous extracts from boiling the root of Isatis tinctoria have also exhibited antibacterial effect.

The dried leaf of Baphicacanthes cusia, Isatis tinctoria, Isatis indigotica, or Polygonum tinctorium have been used to prepare another herbal medicine known as BAPHICACANTHIS FOLIUM. BAPHICACANTHIS FOLIUM has traditionally been used to treat illnesses such as typhus, typhoid, measles, fever, erysipelas, sore throat, tonsillitis, dysentery, acute laryngitis, stomatitis, gum bleeding, and various infectious diseases with fever. It has also exhibited antibacterial effects and antipyretic effects. The leaf of Isatis tinctoria has been used as an antipyretic in the past.

The leaf of Baphicacanthes cusia, Isatis tinctoria, Isatis indigotica, or Polygonum tinctorium with additional processing has also been used to prepare a third related herbal medicine known as **INDIGO PULVERATA LEVIS. INDIGO PULVERATA LEVIS** has traditionally been used to treat illnesses such as epistaxis, rashes, sores, mumps, chronic skin boils, dermatitis, anemia, fever, swollen sores, stomatitis, acute laryngitis, tonsillitis, gingivitis, parasitic oral mucosa inflammation, snake or dog bites, malignant sores, and erysipelas. Ethanol extracts of INDIGO PULVERATA LEVIS have exhibited bacterial inhibition properties.

Baphicacanthes cusia and Strobilanthes cusia belong to the family of Acanthaceae. Isatis tinctoria and Isatis indigotica belong to the family of Cruciferae. Polygonum tinctorium belongs to the family of Polygonaceae. BAPHICACANTHIS RHIZOMA ET RADIX tastes bitter while BAPHICACANTHIS FOLIUM tastes bitter and salty, and is nontoxic. INDIGO PULVERATA LEVIS tastes salty and is also nontoxic. Treatment doses are typically 10 to 19 g per day for BAPHICACANTHIS RHIZOMA ET RADIX, 8 to 30 g per day for BAPHICACANTHIS FOLIUM, and 0.4-1.1 g per day for INDIGO PULVERATA LEVIS.

Ho et al. disclose the use of an extract from a mixture of herbs for the in vitro inhibition of HIV infection in human T lymphocyte cells and mononuclear phagocytic lineage cells. The activity was based on the test results of a water extract from a mixture of three herbs: **Isatis tinctoria (or Isatis indigotica), Lonicera japonica, and Polygonum bistorta**. See D. D. Ho & X. S. Li, U.S. Pat. No. 5,178,865, issued on Jan. 12, 1993.

The compound known as tryptanthrin has been identified as the principal antifungal agent in the leaf of Strobilanthes cusia and as the main antidermatophytic substance in the leaf of **Polygonum tinctorium and Isatis tinctoria**. See H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 758-759 (1985).

A Chinese herbal medicine known as **BLECHNI RHIZOMA or DRYOPTERIS CRASSIRHIZOMAE RHIZOMA** has traditionally been used to treat conditions such as cuts, swelling, fever, measles, hematemesis, menorrhagia, dysentery, stool with traces of blood, abdominal pain caused by parasites, wound bleeding, uterus bleeding, puerperal abdominal pain, and erysipelas. BLECHNI RHIZOMA is prepared from the dried root and stem of Blechnum orientate which belongs to the family of Polypodiaceae or Blechnaceae. DRYOPTERIS CRASSIRHIZOMAE RHIZOMA is prepared from the dried root and stem of Dryopteris crassirhizoma which belongs to the family of Aspidiaceae. Osmunda japonica(Osmundaceae family), Woodwardia orientalis and Woodwardia unigemmata (Blechnaceae family), Athyrium acrostichoides (Aspidiaceae or Athyriaceae family), Sphaeropteris lepifera (Cyatheaceae family), Cyrtomium falcatum, and Cyrtomium fortunei (Aspidiaceae family) have also been used for preparation of the herbal medicines. These herbal medicines taste bitter and astringent, and are slightly toxic. Treatment dosage is typically 4-11 g per day.

The sprout of Blechnum orientate has been used to treat swelling while the sprouts of **Sphaeropteris lepifera** (also known as (hereinafter "a.k.a.", Alsophila pustulosa) have been used to treat carbuncles. Blechnum orientate has also shown a strong inhibition effect against the influenza virus. Filmarone, filicin, aspidin, albaspidin, and filicic acid which are found in Dryopteris crassirhizoma have been characterized as having an

anthelmintic effect. See H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, R.O.C., 577-578 (1985); and H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 249-250 (1982).

Hozumi et al. disclosed that the **rhizome of Dryopteris crassirhizoma** was an antiherpesviral agent, antipolioviral agent, and anti-varicella-zoster virus agent. The rhizome of Cyrtomium fortunei and the rhizome of Woodwardia orientalis were also disclosed as antiherpesviral, antipolioviral, anti-measles virus, anti-varicella-zoster virus, anti-cytomegalovirus (CMV), and an anti-DNA and anti-RNA virus agents. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued May 2, 1995.

A Chinese herbal medicine known as **BLETILLAE TUBER** has traditionally been used to treat illnesses such as hemoptysis, epistaxis, hematemesis, abscesses, burns, dry and chapped skin, tuberculosis, gastric ulcers, and sores. BLETILLAE TUBER has astringent, antibacterial and antifungal properties. BLETILLAE TUBER is prepared from the dried tuber of Bletilla striata which belongs to the family of Orchidaceae. BLETILLAE TUBER tastes bittersweet, astringent and is nontoxic. Treatment dose is typically 2-11 g per day for an average human.

Bletilla-glucomannan is a mucilage in the tuber of Bletilla striata which has astringent properties (can be used to stop bleeding and decrease swelling). See H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, R.O.C., 381 (1985); and H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 114-115 (1982).

Chinese herbal medicines known as **CIRSII RHIZOMA ET RADIX and BREEAE RADIX** have traditionally been used to treat illnesses such as hematemesis, urine with traces of blood, stool with traces of blood, gonorrhea with traces of blood, menorrhagia, leucorrhoea, boils, acute infectious hepatitis, cuts, bleeding sores, and abscesses. CIRSII RHIZOMA ET RADIX is prepared from the dried rhizoma or root or the whole plant of plants such as Cirsium japonicum, Cirsium albescens, and Cirsium japonicum var. australe which are from the Compositae family. BREEAE RADIX is prepared from the dried root of Compositae family plants such as Breea segetum (a.k.a., Cephalanoplos segetum) and Breea setosum. Both herbal medicines taste sweet and slightly bitter, and are nontoxic. Treatment dose is typically 5 to 75 g per day for the average human.

A Chinese herbal medicine known as **FORSYTHIAE FRUCTUS** has traditionally been used to treat illnesses such as sores, abscesses, lymph node swelling, neck lymph node tuberculosis, erysipelas, gonorrhea, measles, ecchymosis, urethritis, and hypertension. It was also found to inhibit several bacteria and influenza viruses. FORSYTHIAE FRUCTUS is prepared from the dried mature fruit of Forsythia suspensa, Forsythia viridissima, or Forsythia koreana which belong to the family Oleaceae. The herbal medicine tastes bitter and is nontoxic. Treatment dosage is typically 3 to 11 g per day.

Hozumi et al. disclose that the fruit of **Forsythia suspensa** is an antipolioviral agent and an anti-measles virus agent useful in treating these viral infections. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, **U.S. Pat. No. 5,411,733,** issued May 2, 1995.

The compounds Forsythoside A (found in the leaf of Forsythia suspensa), forsythoside B (found in the stem of Forsythia koreana), forsythoside C and forsythoside D (found in the fruit of Forsythia suspensa) have been reported to exhibit antibacterial activity against Staphylococcus aureus at a concentration less than 2 mM. Suspensaside (found in the fruit of Forsythia suspensa, likely the same as forsythoside C) has also been reported to exhibit antibacterial activity against Staphylococcus aureus Terashima with a minimum inhibition concentration (MIC) of 2.6 mg/mL. See H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 53-55, 142-143 (1985).

A Chinese herbal medicine known as **HEDYOTIS (a.k.a., OLDENLANDIAE HERBA**) has traditionally been used to treat illnesses such as malignant swelling, urethra infection, pharyngitis, laryngitis, tonsillitis, toxic snake bites, subacute or chronic coccygodynia, prurigo, carbuncle, appendicitis, intestinal cancer, contusion injuries and eye diseases. It has also been found to have weak antibacterial activity in vitro. HEDYOTIS is prepared from the dried whole plant of Hedyotis diffusa (a.k.a., Oldenlandia diffusa) which belongs to the family Rubiaceae. The herbal medicine tastes sweet and is nontoxic. Treatment dosage is typically 19 to 300 g per day.

The Chinese herbal medicines known as **LESPEDEZAE HERBA and SENECINIS HERBA** have traditionally been used to treat illnesses such as urine incontinence, gonorrhea, leucorrhoea, asthma, stomach ache, general weakening and exhaustion, a children's disease characterized by swelling of the belly and limbs caused by malnutrition or parasitic worms, diarrhea, contusion injuries, eye diseases, visual impairment, eye redness, renal disease, breast abscess, acute inflammatory disease, cataracts, dysentery, enteritis, jaundice, flu, septicemia, abscesses, boils, ringworm, erysipelas, snake or dog bites, rheumatic pains, sores, swelling and a disease of the palm. LESPEDEZAE HERBA is prepared from the dried whole plant of Lespedeza cuneata which belongs to the family Leguminosae. SENECINIS HERBA is prepared from the dried whole plant of Senecio scandens which belongs to the family Compositae. The extracts of Lespedeza cuneata and Senecio scandens have been shown to have antibacterial effects. Both herbs taste sour, astringent and bitter. Treatment dose is typically 4 to 40 g per day.

A Chinese herbal medicine known as **LIGUSTRI FRUCTUS** has traditionally been used as a tonic and to treat illnesses such as debility, knee limpness, tinnitus and dizziness, palpitation, insomnia, constipation, early white hair, neck lymph node, tuberculosis, lung tuberculosis, intermittent fever and dropsy. LIGUSTRI FRUCTUS is prepared from the dried mature fruit of Ligustrum lucidum or Ligustrum japonicum which belongs to the family Oleaceae. The leaves of Ligustrum lucidum have been used as an antipyretics, analgesics and anti-inflammatory agents. The leaves of Ligustrum japonicum have also been used to treat illnesses such as ophthalmalgia, ulcerative stomatitis, mastitis, swelling, and burns. The fruit of Ligustrum lucidum taste bitter and are nontoxic. Typical treatment dosage of the dried fruit is typically 6 to 20 g per day. That of the dried leaves is typically 40 to 75 g per day.

A Chinese herbal medicine known as **LONICERAE FLOS** has traditionally been used to treat illnesses such as fever, febrile diseases, acute infectious diseases, measles, carbuncle, dysentery, malignant sores and swelling, abscesses, boils, gonorrhea, syphilis, poisoning, enteritis, swelling, ringworm and similar skin diseases. LONICERAE FLOS is prepared from the dried flower bud of Lonicera japonica or Lonicera confusa. Both plants belong to the family Caprifoliaceae. The flower of Lonicera japonica has diuretic, antipyretic, anti-inflammatory, anti-convulsive, antibacterial and antiviral properties. The flower bud has also been used as a diuretic. The herbal medicine tastes sweet and is nontoxic. Treatment dosage is typically 11 to 75 g per day for the typical human.

The dried vine, stem and leaf of Lonicera japonica is used for preparation of another herbal medicine called **LONICERAE CAULIS ET FOLIUM**, which has traditionally been used to treat illnesses such as paralysis and pain caused by rheumatism, rheumatism swelling, rheumatic pain, carbuncle swelling, arthritis, gonorrhea, enteritis, and various symptoms with pus, such as abscesses. Extracts have exhibited the ability to raise blood sugar levels in rabbits. The root of Lonicera japonica has also been used to treat illnesses such as venereal disease, syphilis, gonorrhea, lymph node tuberculosis, contusion injury, and skin disease. Treatment doses are typically 8 to 75 g per day for the stem or leaf and 110 to 150 g per day for the root.

Ho et al. disclose the anti-HIV activity in vitro of a mixture **Lonicera japonica, Isatis tinctoria (or Isatis indigotica) and Polygonum bistorta or a mixture of Lonicera japonica with Scutellaria baicalensis**. Water extractions of the mixtures, treatment with ethanol for precipitation and charcoal adsorption are disclosed for the preparation for the anti-HIV active composition. See D. D. Ho & X. S. Li, U.S. Pat. No. 5,178,865, issued on Jan. 12, 1993. Several tannins such as caffeoylquinates isolated from Lonicera japonica have been reported to have an inhibitory effect on HIV-1 reverse transcriptase activity. See C. W. Chang, M. T. Lin, S. S. Lee, K. C. S. C. Liu, F. L. Hsu, & J. Y. Lin, Antiviral Research, 27(4), 367-374 (1995).

A mixture of aqueous extracts of **Lonicera japonica flower buds and Forsythia suspensa** fruits with the crude flavonoids from Scutellaria baicalensis have been shown to have antibacterial and antiviral properties. A group of patients with severe respiratory disease were treated with the mixture and they responded as well as a control group on standard antibiotic therapy. See P. J. Houghton, Z. Boxu, & Z. Xisheng, Phytother. Res., 7(5), 384-386 (1993).

A Chinese herbal preparation which consisted of ten (10) herbs such as **Prunus armeniacae, Scutelaria baicalensis, Lonicera japonica**, etc. was shown to have strong inhibitory effects in vitro against Streptococcus hemolyticus, Staphylococcus aureus, Flexners Dysentery bacillus, Diplococcus pneumoniae and Pseudomonas aeruginosa. The preparation was shown to be as effective as penicillin and aminophylline in treating bronchopneumonia and acute bronchitis patients. See Y. Q. Li, W. Yuan, & S. L. Zhang, Chung Kuo Chung Hsi I Chieh Ho Tsa Chih, 12(12), 708, 719-721, 737 (1992).

Another Chinese herbal preparation which consisted of **Lonicera japonica, Ophiopogon japonicus, and**

Astragalus membranaceus was shown to be effective in treating viral myocarditis. The authors reported that the preparation could directly inactivate the virus of Coxsackie B3, protect heart cells in mice, prevent attack by Coxsackie B3, promote the production of interferon and increase the functionality of NK cells to regulate immunity in experimental mice. See H. J. Yan, Chung Hsi I Chieh Ho Tsa Chih, 11(8), 452, 468-470 (1991).

A Chinese herbal medicine known as **PHELLODENDRI CORTEX** has traditionally been used to treat illnesses such as dysentery, diarrhea, jaundice, stools with blood, piles, tinnitus, mouth and tongue boils, abscesses, sores, leucorrhea with blood, abdominal pain, indigestion, bacteroid enteritis, and tuberculoid diarrhea. The herbal medicine has also been used as an eye wash, for strengthening stomach and intestine, stimulate appetite, and as an astringent, anti-inflammatory, etc. It has antibacterial, anti-inflammatory, and wound healing properties. PHELLODENDRI CORTEX is prepared from the dried cortex of plants from the Rutaceae family such as Phellodendron amurense, Phellodendron chinense, Phellodendron amurense var. sachalinense, and Phellodendron wilsonii. PHELLODENDRI CORTEX tastes bitter and is nontoxic. Treatment dose is typically 1 to 11 g per day.

Hozumi et al. disclose the bark of **Phellodendron amurense** as an antiherpesviral, antipolioviral, anti-measles virus, anti-varicella-zoster virus, anti-CMV and anti-DNA virus and anti-RNA virus agents. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued on May 2, 1995.

A Chinese herbal medicine known as **POLYGONI CUSPIDATI RHIZOMA** has traditionally been used to treat illnesses such as dysentery, leucorrhea, fever, headache, menorrhagia, dysmenorrhea, breast abscesses, sores, boils, contusion injury, menstruation irregularity, puerperal ecchymotic abdominal distension and pain, dysuria, infantile growth and appendicitis. POLYGONI CUSPIDATI RHIZOMA is prepared from the dried rhizoma of Polygonum cuspidatum, Polygonum runcinatum, or Polygonum reynoutria (a.k.a. Reynoutria japonica) which belong to the family Polygonaceae. The tender leaf has also been used to treat contusion and cut injuries. Extracts of the herbal medicine have exhibited antibacterial and antiviral effects in vitro. Excessive use of the herbal medicine may cause a slight diarrhea. The herbal medicine tastes bitter and the treatment dose is typically 6 to 40 g per day.

Hozumi et al. disclose the root and rhizome of Polygonum cuspidatum as an antiherpesviral, antipolioviral, anti-varicella-zoster virus, and anti-CMV agent. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, **U.S. Pat. No. 5,411,733**, issued on May 2, 1995.

Resveratrol has also been reported as an antifungal and antibacterial component in the root of Polygonum cuspidatum. See H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 51 (1985).

A Chinese herbal medicine known as **PRUNELLAE SPICA** has traditionally been used to treat illnesses such as goiter, scrofula, neck lymph node tuberculosis, lymph node swelling, eye redness, pain, abscesses, sores, hemorrhoids, swollen eye, ophthalmalgia, leucorrhoea with traces of blood, gonorrhea, uterine disease, mastitis, breast abscesses, breast cancer, foot swelling, paralysis, chronic arthritis, conjunctivitis, and hypertension. PRUNELLAE SPICA is prepared from the dried spica or whole plant of Prunella vulgaris or Prunella vulgaris subsp. asiatica (a.k.a., Prunella vulgaris var. lilachina). Both plants belong to the family Labiatae. The whole plant can be used as a diuretic and also has antibacterial effects in vitro. The herbal medicine tastes bitter and is nontoxic. Treatment dosage is typically 4 to 110 g per day for the average human.

Hozumi et al. disclose that the spike of Prunella vulgaris as an antiherpesviral agent for treating herpes virus infection. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U**.S. Pat. No. 5,411,733**, issued May 2, 1995. The water extract of Prunella vulgaris (boiling 3 g in 100 mL water for 45 minutes) was also reported to have anti-HIV (strain H9/3B) activity. The extract also exhibited synergistic anti-HIV activity with zidovudine (AZT) and didanosine (ddl). Only a slight additive effect was observed for Prunella vulgaris and zalcitabine (ddC). See J. F. John, R. Kuk, & A. Rosenthal, Abstr. Gen. Meet. Am. Soc. Microbiol., 94, 481 (1994).

Yamasaki et al. evaluate in vitro, two hundred and four (204) crude drugs of common use in Japan for anti-HIV-1 activity and reported that the hot water extract of Prunella vulgaris (spike) showed a strong in vitro anti-HIV-1 activity with an IC100 of 16 .mu.g/mL. See K. Yamasaki, T. Otake, H. Mori, M. Morimoto, N. Ueba, Y. Kurokawa, K. Shiota, & T. Yuge, Yakugaku Zasshi, 113(11), 818-824 (1993).

Yao et al. report that the water extract of the dried entire plant of Prunella vulgaris was active in vitro in

inhibiting HIV-1 replication with relatively low cytotoxicity towards the MT-4 cells. The extract was also active in reverse transcriptase inhibition. The active factor was purified and identified as anionic with a molecular weight of approximately 10,000 Dalton. This active component may be the same as the prunellin, as described below by Tabba et al. The purified extract inhibited HIV-1 replication in the lymphoid cell line MT-4, in the monocytoid cell line U937, and in peripheral blood mononuclear cells (PBMC) at effective concentrations of 6, 30, and 12.5 .mu.g/mL, respectively. Pretreatment of uninfected cells with the extract prior to viral exposure did not prevent HIV-1 infection. Preincubation of HIV-1 with the purified extract dramatically decreased infectiousness. The purified extract was also able to block cell-to-cell transmission of HIV-1, prevented syncytium formation, and interfered with the ability of both HIV-1 and purified gp120 to bind to CD4. PCR (polymerase chain reaction) analysis confirmed the absence of HIV-1 proviral DNA in cells exposed to virus in the presence of the extract. The results suggested that the purified extract antagonized HIV-1 infection of susceptible cells by preventing viral attachment to the CD4 receptor. See X. J. Yao, M. A. Wainberg, & M. A. Pamiak, Virology, 187(1), 56-62 (1992).

Tabba et al. isolated and partially characterized an anti-HIV component, prunellin, from aqueous extracts of **Prunella vulgaris**. Prunellin is a carbohydrate with an MIC (minimum inhibition concentration) of 2.2 .mu.g/mL against HIV-1 in vitro. It was identified as a partially sulfated polysaccharide with a molecular weight of about 10,000 Dalton. See H. D. Tabba, R. S. Chang, & K. M. Smith, Antiviral Research, 11, 263-273 (1989).

Zheng evaluated four hundred seventy two (472) traditional medicinal herbs for antiviral effect on type 1 herpes simplex virus (HSV1). Prunella vulgaris was one of the ten herbs found to be highly effective in vitro. Clinically, 78 cases of herpetic keratitis due to HSV1 were treated with Prunella vulgaris and Pyrrosia lingua eye drops. Among them, 38 cases were effectively cured, 37 cases showed an improvement, and 3 cases showed no benefit. See M. Zheng, J. Tradit. Chin. Med., 8(3), 203-206 (1988).

Triterpene 1 and Triterpene 2 which have been isolated from Prunella vulgaris have shown antiviral activity against HSV1. Triterpene 1 was identified as betulinic acid and triterpene 2 was identified as 2.alpha.,3.alpha.-dihydroxyurs-12-en-28-oic acid. The EC50 was estimated to be 30 .mu.g/mL for triterpene 1 and 8 .mu.g/mL for triterpene 2 by plaque reduction assay. See S. Y. Ryu, C-K. Lee, C. O. Lee, H. S. Kim, & O. P. Zee, Arch. Pharmacal Res. (Seoul), 15(3), 242-245 (1992).

A Chinese herbal medicine known as **SCUTELLARIAE BARBATAE HERBA** has traditionally been used to treat illnesses such as hematemesis, gonorrhea with traces of blood, jaundice, sore throats, lung abscesses, boils, carbuncles, abscesses, neck lymph node swelling, sores, cancer, contusion or cut injuries, snake bite injuries, dysentery with traces of blood, convulsions, pneumonia, abdominal pains, congenital diseases, enteritis, coccygodynia, appendicitis, asthma, malaria, and rheumatism. It was also found to have antibacterial effect. SCUTELLARIAE BARBATAE HERBA is prepared from the dried whole plant of Scutellaria barbata, Scutellaria rivularis, or Scutellaria dependens which belong to the family Labiatae. The herbal medicine tastes bitter and should not be consumed by those who have anemia. Pregnant women should avoid taking this herb. Treatment dosage is typically 4 to 300 g per day.

Dried whole plants of **Scutellaria rivularis** have been used in folk medicine for the treatment of tumors, hepatitis, liver cirrhosis, and other diseases in China and Taiwan. See Y. L. Lin, Y. H. Kuo, G. H. Lee, and S. M. Peng, J. Chem. Research (S), 320-321.(1987).

Apigenin, isolated from the whole herb of Scutellaria rivularis, was found to have anti-influenza virus activity. See T. Nagai, et al., Chem. Pharm. Bull., 38(5), 1329-1332 (1990).

A Chinese herbal medicine known as **SOLANI HERB**A has traditionally been used to treat illnesses such as boils, abscesses, erysipelas, contusion or sprain injuries, chronic bronchitis, acute nephritis, cancer, swelling, hernia, ulcers, carbuncles with swelling and sores. SOLANI HERBA is prepared from the dried whole plant of Solanum nigrum which belongs to the family Solanaceae. Extracts of SOLANI HERBA have demonstrated anti-inflammatory properties. The fruit has also exhibited the effects of suppressing coughs and relieving bronchial inflammation. The herbal medicine tastes bitter and slightly sweet and is nontoxic. Treatment dosage is typically 11 to 60 g per day.

The root of Solanum nigrum was believed to have antipyretic activity and has been used for treating high fevers by some primitive tribes of western Ghats in India. A decoction prepared from **Solanum nigrum plants, Glycosmis Mauritania seeds and/or Santalum album wood chips** was believed to have expectorant activity and has been used for coughs and to treat hemoptysis. See P. Pushpangadan and C. K. Atal, J.

Ethnopharmacol., 11(1), 59-77 (1984).

The compound solasonine (found in the **whole herb, fruit, leaf, and fresh immature berries of Solanum nigrum**) has an anti-inflammatory effect similar to cortisone. Solasonine and solanine (also found in Solanum nigrum) possesses the ability of raising or lowering the blood sugar level in rats depending on the situation of the animals. Solasonine was also reported to have a stimulating effect on the heart, while solanine had a suppressive effect. When administered at small doses, solasonine enhances the stimulative process of the central nerve system in animals (i.e., rat and rabbit). On the other hand, it enhances the suppressive process when administered at large doses. Solasonine can also lower the blood coagulability. See (1) H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, R.O.C., 176-177 (1985); (2) H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 1400-1401, 1406 (1982); and (3) H. Y. Hsu, Y. P. Chen, & M. Hong, The Chemical Constituents Of Oriental Herbs, Vol. 2, Oriental Healing Arts Institute, Los Angeles, Calif., U.S.A., 742 (1985).

Additionally, Yamasaki et al. report that the hot water extract of **Lithospermum erythrorhizon (root)** showed a strong in vitro anti-HIV-1 activity with an IC100 of 16 .mu.g/mL. Yao et al. reported that the water extracts of the dried root of Arctium lappa and the dried aerial parts of Andrographis paniculata were anti-HIV-1 active in vitro and cytotoxic towards the MT-4 cells. Both extracts were also active in reverse transcriptase inhibition. See K. Yamasaki, T. Otake, H. Mori, M. Morimoto, N. Ueba, Y. Kurokawa, K. Shiota, & T. Yuge, Yakugaku Zasshi, 113(11), 818-824 (1993); and X. J. Yao, M. A. Wainberg, & M. A. Parniak, Virology, 187(1), 56-62 (1992).

Glycyrrhizin is reported to have an inhibitory effect on the in vitro infectivity and cytopathic activity of HIV. See M. Ito, et al, Antiviral Research, 7, 127-137 (1987). Glycyrrhizin is a saponin found in the herbal medicine **GLYCYRRHIZAE RADIX**. GLYCYRRHIZAE RADIX is prepared from the dried root of Glycyrrhiza uralensis, Glycyrrhiza glandulifera, Glycyrrhiza echinata, or Glycyrrhiza glabra all of which belong to the family Leguminosae.

Chang and Yeung screened the boiling water extracts of twenty seven (27) medicinal herbs for anti-HIV activity. They found eleven (11) of the extracts were active in inhibiting HIV in the H9 cells. **Lonicera japonica, Prunella vulgaris, Woodwardia unigemmata, and Senecio scandens** were among those active ones with moderate activities. **Forsythia suspensa, Isatis tinctoria, and Polygonum cuspidatum** were among those tested which did not display activity in the anti-HIV assay. The anti-HIV active extract of Viola yedoensis was further tested and found to be fairly specific. The extract did not inactivate HIV extracellularly and did not inhibit the growth of herpes simplex, polio, or vesicular stomatitis viruses in human fibroblast culture. See R. S. Chang & H. W. Yeung, Antiviral Research, 9, 163-175 (1988).

Antiviral agents have been isolated from **Syzygium aromaticum, Sapium sebiferum, Scutellaria baicalensis, and Scutellaria rivularis. Eugeniin** (a tannin) isolated from Syzygium aromaticum and methyl gallate isolated from Sapium sebiferum exhibited anti-herpes simplex virus activity in vitro. Plant flavonoids, such as 5,7,4'-trihydroxy-8-methoxyflavone from the root of Scutellaria baicalensis and apigenin (5,7,4'-trihydroxyflavone) from the whole herb Scutellaria rivularis, were also reported to have anti-influenza virus activity. See (1) T. Hozumi, et al., U.S. Pat. No. 5,411,733 (1995); (2) M. Takechi & Y. Tanaka, Planta Medica, 42, 69-74 (1981); (3) C. J. M. Kane, et al, Bioscience Reports, 8, 85-94 (1988); and (4) T. Nagai, et al., Chem. Pharm. Bull., 38(5), 1329-1332 (1990).

Hozumi et al. disclose ninety one (91) herbal medicines which demonstrated antiviral activity. More specifically, fifty two (52) of them had antiherpesviral activity, sixty four (64) had antipolioviral activity, thirty seven (37) had anti-measles virus activity, twenty seven (27) had anti-varicella-zoster virus activity, twenty three (23) had anti-CMV activity, and twenty eight (28) had anti-DNA virus and anti-RNA virus activity. See T. Hozumi, T. Matsumoto, H. Ooyama, T. Namba, K. Shiraki, M. Hattori, M. Kurokawa, & S. Kadota, U.S. Pat. No. 5,411,733, issued on May 2, 1995.

The anti-DNA virus and anti-RNA virus activity of the twenty eight (28) herbal medicines disclosed in the '733 patent solely based upon their antiherpesviral, antipolioviral, anti-measles virus, and/or anti-varicella-zoster virus and anti-CMV activities. However, the extrapolation to cover both anti-DNA virus and anti-RNA virus activities is unfounded from the work conducted.

The data of the present invention presented below evidenced little or no anti-HIV activity of the two herbal medicines at 2.5 and 0.5 mg/mL derived from the **rhizome of Cyrtomium fortunei and the bark of**

Phellodendron amurense. In contrast, the three (3) herbal medicines using the spike of Prunella vulgaris, the fruit of Forsythia suspensa, and the root and rhizome of Polygonum cuspidatum, will be shown to have a strong to moderate anti-HIV activity at 2.5 mg/mL.

Herbal medicines **LONICERAE FLOS, BAPHICACANTHIS RHIZOMA ET RADIX, and FORSYTHIAE FRUCTUS** have been used separately and/or in combination as antipyretic and detoxification agents along with other herbal medicines for treating acute hepatitis. The herbal medicines **BLECHNI RHIZOMA and POLYGONI CUSPIDATI RHIZOMA** have been used along with other herbal medicines in a formula for treating B hepatitis. The herbal medicines **SCUTELLARIAE BARBATAE HERBA and LIGUSTRI FRUCTUS** have occasionally been added to improve activity. Herbal medicine LIGUSTRI FRUCTUS was occasionally used along with other herbal medicines mainly as a tonic and HEDYOTIS was occasionally used along with other herbal medicines as a detoxification agent. The herbal medicine PRUNELLAE SPICA has also been used along with other herbal medicines to relief liver stress.

It is noted that in the practice of Chinese traditional medicine, herbal medicines were used to treat the symptoms of the patients, not the disease entity itself, and were therefore fairly nonspecific to a particular disease. Herbal medicines were used depending on the symptoms of the individual patient. The composition of herbal medicines would vary case by case and may even change for each individual patient during the course of the treatment according to each treatment result. It is therefore very difficult to have a universal herbal composition suitable for treating a specific disease within a population.

The present invention is directed to the discovery of antiviral herb compositions, extracts thereof and the active chemical constituents. The antiviral herb compositions of this invention are derived from individual herbs, herb mixtures and commercially available Chinese herbal medicines. These novel herb compositions and their extracts and/or active principles have demonstrated activities against viral diseases such as hepatitis B, hepatitis C, HBV and HCV carriers, HIV infection and AIDS.

SUMMARY OF THE INVENTION

As used herein and in the claims, the following nomenclatures will be used to identify the four (4) herb mixtures known as HHT888-4, HHT888-5, HHT888-45 and HHT888-54. HHT888-4 is a mixture of five single-herb Chinese herbal medicines at a preferred ratio of No.4(1): No.4(2): No.4(3): No.4(4): No.4(5) of about 3:3:3:3:4 (w/w). The weight ratio may vary up to 50% per component. By "variance of the weight ratio by 50%" means that each value of each component of the ratio may be increased or decreased by 50%. Thus, as an example, 1:1 can range from 1.5:0.5 to 0.5:1.5 (or 3:1 to 1:3).

HHT888-5 is a mixture of eleven (11) single-herb Chinese herbal medicines, No.5(1) to No.5(11) preferably at about equal proportions by weight. The weight ratio may vary up to 50% per component.

HHT888-45 is a mixture of four (4) to six (6) single-herb Chinese herbal medicines at a ratio of No.4(3): No.4(4): No.5(4): No.5(5): No.5(8): No.4(2) at a preferred ratio of about 1:1:1:1:0-1:0-1 (w/w). The weight ratio may vary up to 50% for each component.

HHT888-54 is a mixture No.5(5) and at least one single herb medicine selected from No. 4(2), No. 4(3), No. 4(4), No. 4(5), No. 5(1), No. 5(2), No. 5(4), No. 5(7), No. 5(8) and No. 5(11) wherein the weight ratio of No. 5(5) to each of the other single herb medicines is 1:1. Thus, HHT888-54 consists of No. 5(5) plus No. 4(3), No. 4(4) and No. 5(8); the most preferred weight ratio is 1:1:1:1.

More generally, the weight ratio of No. 5(5) to the sum of the other single herb medicines is from 1:10 to 10:1.

The single-herb components of HHT888-4 are:
No.4(1)=HEDYOTIS (a.k.a., OLDENLANDIAE HERBA) source: Hedyotis diffusa (a.k.a., Oldenlandia diffusa)
No.4(2)=SCUTELLARIAE BARBATAE HERBA source: Scutellaria barbata, Scutellaria rivularis, Scutellaria dependens
No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa
No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (a.k.a., Prunella vulgaris var. lilachina)
No.4(5)=SOLANI HERBA source: Solanum nigrum

The single-herb components of HHT888-5 are:
No.5(1)=HEDYOTIS (a.k.a., OLDENLANDIAE HERBA) source: Hedyotis diffusa (a.k.a., Oldenlandia diffusa)
No.5(2)=BLECHNI RHIZOMA or DRYOPTERIS CRASSIRHIZOMAE RHIZOMA, source: Blechnum orientale, Dryopteris crassirhizoma, Osmunda japonica, Woodwardia orientalis, Woodwardia unigemmata, Athyrium acrostichoides, Sphaeropteris lepifera, Cyrtomium falcatum, Cyrtomium fortunei
No.5(3)=CIRSII RHIZOMA ET RADIX and BREEAE RADIX source: Cirsium japonicum, Cirsium albescens, Cirsium japonicum var. australe, Breea segetum (a.k.a., Cephalanoplos segetum), Breea setosum
No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens
No.5(5)=AEGINETIAE HERBA(a.k.a. GOLDEN LOCK KEY or LOTUS HERBA). source: Aeginetia indica, Dichondra micrantha, Striga lutea, Dichondra repens
No.5(6)=BAPHICACANTHIS RHIZOMA ET RADIX source: Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, Polygonum tinctorium
No.5(7)=POLYGONI CUSPIDATI RHIZOMA source: Polygonum cuspidatum, Polygonum runcinatum, Polygonum reynoutria (a.k.a., Reynoutria japonica)
No.5(8)=FORSYTHIAE FRUCTUS source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana
No.5(9)=PHELLODENDRI CORTEX source: Phellodendron amurense, Phellodendron chinense, Phellodendron amurense var. sachalinense, Phellodendron wilsonii
No. 5(10)=BLETILLAE TUBER source: Bletilla striata
No.5(11)=FLIGUSTRI FRUCTUS source: Ligustrum lucidum, Ligustrum japonicum

The single-herb components of HHT888-45 are:
No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa
No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (a.k.a., Prunella vulgaris var. lilachina)
No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens
No.5(5)=AEGINETIAE HERBA (a.k.a. GOLDEN LOCK KEY or LOTUS HERBA). source: Aeginetia indica in addition to No.5(5) are at least one selected from:
No.4(2)=SCUTELLARIAE BARBATAS HERBA (optional) source: Scutellaria barbata, Scutellaria rivularis, Scutelaria dependens
No.5(8)=FORSYTHIAE FRUCTUS (occasionally used) source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana

The single herb components of HHT888-54 in addition to No.5(5)are at least one selected from:
No.4(2)=SCUTELLARIAE BARBATAE HERBA source: Scutellaria barbata, Scutellaria rivularis, Scutellaria dependens
No.4(3)=LONICERAE FLOS source: Lonicera japonica, Lonicera confusa
No.4(4)=PRUNELLAE SPICA source: Prunella vulgaris, Prunella vulgaris subsp. asiatica (a.k.a., Prunella vulgaris var. lilachina)
No.4(5)=SOLANI HERBA source: Solanum nigrum
No.5(1)=HEDYOTIS (a.k.a., OLDENLANDIAE HERBA) source: Hedyotis diffusa (a.k.a., Oldenlandia diffusa)
No.5(2)=BLECHNI RHIZOMA or DRYOPTERIS CRASSIRHIZOMAE RHIZOMA, source: Blechnum orientale, Dryopteris crassirhizoma, Osmunda japonica, Woodwardia orientalis, Woodwardia unigemmata, Athyrium acrostichoides, Sphaeropteris lepifera, Cyrtomium falcatum, Cyrtomiumfortunei
No.5(4)=LESPEDEZAE HERBA or SENECINIS HERBA source: Lespedeza cuneata, Senecio scandens
No.5(7)=POLYGONI CUSPIDATI RHIZOMA source: Polygonum cuspidatum, Polygonum runcinatum, Polygonum reynoutria (a.k.a., Reynoutria japonica)
No.5(8)=FORSYTHIAE FRUCTUS source: Forsythia suspensa, Forsythia viridissima, Forsythia koreana
No.5(11)=LIGUSTRI FRUCTUS source: Ligustrum lucidum, Ligustrum japonicum

The names of the Chinese herbal medicines for the single-herb components are shown in capital letters, followed by their plant sources listed in italics.

As used herein and in the claims, the term HHT888-4, HHT888-5, HHT888-45 and the like include the actual herbal blends, aqueous extracts thereof and the individual active components or principles of the extract. In similar fashion, the use of the terms No.5(5), No. 5(8) and the like include the actual herb, extracts thereof and the isolated active molecular agents.

As also used in the specification and in the claims, No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(3), No.5(4), No.5(5), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11) are the single-herb

components described above, including their respective source plants. It should be noted that No.4(1) is the same as No.5(1) (HEDYOTIS).

Specific details and descriptions of the above recited Chinese herbal medicines and medicinal herbs can be found in the following references: (1) H. C. Chang, Medicinal Herbs I, Holiday Publishing Co., Taipei, Taiwan, R.O.C., 15, 36, 100, 113, 127, 147 (1990); (2) H. C. Chang, Medicinal Herbs II, Holiday Publishing Co., Taipei, Taiwan, R.O.C., 15, 131, 135, 155 (1991); (3) W. S. Kan, Pharmaceutical Botany, National Research Institute Of Chinese Medicine, Taipei, Taiwan, R.O.C., 113, 124-130, 200-201, 206-207, 289-290, 353-354, 442-444, 485, 487-488, 497, 505, 513-514, 522, 527-529, 558, 562-563, 648-649 (1971); (4) M. S. Lee, Frequently Used Chinese Crude Drugs And Folk Medicines Handbook, 12th Ed., Sheng-Chang Medicinal Record Magazine Publishing Co., Taipei, Taiwan, R.O.C., 4-6, 17, 21, 29, 36, 38, 40, 48, 64, 71, 79, 85 (1992); and (5) H. Y. Hsu, Y. P. Chen, S. G. Hsu, J. S. Hsu, C. J. Chen, & H. C. Chang, Concise Pharmacognosy, New Medicine Publishing Co., Taipei, Taiwan, R.O.C., 90, 97, 105-106, 117-118, 126-127, 130-131, 133, 144-145, 152-153, 156-157, 161-162, 174, 176-177, 357-358, 381-382, 384-385, 456-457, 577-578 (1985).

The present invention in its broadest aspect relates to the use of the described herbal medicines and various mixtures thereof to prevent and treat viral infections. More specifically, the viral infections are those caused by HBV, HCV and HIV. The antiviral mixtures according to the invention have been described above as HHT888-4, HHT888-5, HHT888-45 and HHT888-54. In addition, the single herb agents designated No. 4(2), No. 4(5), No. 5(5), No. 5(7), No. 5(8) and No. 5(11) have been shown to have antiviral activity. These single herb agents have not been shown by the prior art to have antiviral activity.

A more specific aspect of the present invention resides in the discovery that HHT888-5 is efficacious in reducing hepatitis B viruses in HBV carriers. An additional aspect of the invention resides in the discovery that HHT888-45 is efficacious in treating hepatitis C patients and returning their liver function to normal.

The herb mixtures HHT888-4 and HHT888-5 and their aqueous extracts have both been shown by the inventors herein to also have antiretroviral activities against MuLV and HIV in vitro. In addition, eleven (11) of the fifteen (15) single-herb components of HHT888-4 and HHT888-5, i.e., No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(4), No.5(5), No.5(7), No.5(8), and No.5(11) have shown anti-HIV activities by effectively suppressing viral proliferation in HIV infected human peripheral blood lymphocytes (PBLs).

There is further disclosed as a composition of matters, the herb mixtures HHT888-4, HHT888-5, HHT888-45 and HHT888-54. As described above, HHT888-54 is No.5(5) or its extract or active principle and at least one single-herb herbal medicine or its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.4(5), No.5(1), No.5(2), No.5(4), No.5(7), No.5(8), and No.5(11). These compositions of matter have not been described before and are unobvious.

There is further disclosed a method of treating viral infections in a mammal, said method comprising administering to said mammal from 0.4 to 120 g per day of at least one composition selected from the group consisting of HHT888-4, HHT888-5, HHT888-45, HHT888-54, No. 4(2), No. 4(5), No. 5(1), No. 5(2), No. 5(4), No. 5(5), No. 5(7), No. 5(8), No. 5(11) and their respective extracts or active principles.

More specifically, there is disclosed a method for reducing the viral load of humans infected with hepatitis B virus, said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-5.

There is also disclosed a method for reducing the viral load of humans infected with hepatitis C virus, said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-45.

There is also disclosed a method of reducing the viral load of a human carrier of the hepatitis B virus and a method of preventing hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount of a composition comprising No.5(5) and at least one selected from the group consisting of No.5(1), No.5(2), No.5(3), No.5(4), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11). There is further disclosed a method of treating a hepatitis C virus carrier and a method of treating or preventing hepatitis C in a human, said method comprising administering to said human a therapeutically effective amount of a composition comprising the mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.5(4), No.5(8), and No.5(11).

Also disclosed is a method of treating hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount of at least one composition selected from HHT888-45 and HHT888-5.

There is disclosed a method of treating hepatitis B in a human, said method comprising administering to said human a therapeutically effective amount of at least one composition selected from: 1) a mixture of the single herb medicine No. 5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.5(4), No.5(8), and No.5(11); and 2) a mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.5(1), No.5(2), No.5(3), No.5(4), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11).

There is further disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-4.

There is disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-5.

There is disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-45.

There is disclosed a method for treating humans infected with HIV, HBV and HCV said method comprising administering to said human a therapeutically effective amount of a composition comprising HHT888-54.

There is also disclosed a method for treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(5), No.5(1), No.5(2), No.5(4), No.5(5), No.5(7), No.5(8), and No.5(11).

There is also disclosed a method of treating humans infected with HIV, said method comprising administering to said human a therapeutically effective amount of a composition comprising the mixture of the single-herb herbal medicine No.5(5), its extract or active principle and at least one single-herb herbal medicine, its extract or active principle selected from the group consisting of No.4(2), No.4(3), No.4(4), No.4(5), No.5(l), No.5(2), No.5(4), No.5(7), No.5(8), and No.5(11).

The dosage of the compositions of the invention can range from 0.4 to 120 g per day for the mammal in need of therapy. One skilled in the art will appreciate that depending upon the weight of the individual and the progression of the viral infection, that higher doses of the compositions will be required. As the compositions according to the invention have demonstrated virtually no side effects, high doses may be initiated with reduction of dosage upon manifestation (i.e., reduction of viral load) of therapeutic effect. One skilled in the art can tailor each dosage rate for a given individual without undue experimentation. More specifically, the dosages for a given composition can range from 0.4 to 25 g per day. Preferably, the compositions are administered at least three (3) times per day however, bolus administration will be effective. More specifically, oral dosages of 5.5 g three (3) times a day (total 16.5 g per day) of the herb mixture HHT888-5 have been found to be effective to reduce HBV load in carriers. Oral dosages of 2.7-5.7 g three times a day (total 8-17 g per day) of the herb mixture HHT888-45 have been found to be effective to return normal liver function to hepatitis C patients. Dosages as high as 121 g per day for HHT888-5 and 63 g per day for HHT888-45 have not evidenced serious side effects. It will be appreciated that the dosages recited herein are for the herbal medicine (extract deposited on ground plant) in dry form. Further, extracts of the inventive compositions will increase the concentration of the actives and therefore reductions in the dosage levels will be realized. Dosages as low as 10% of those recited herein for the inventive compositions are contemplated.

The preferred dosage for No. 5(5) to treat HCV infection is from 0.4 to 17 g per day.

The compositions of the invention are preferably administered enterally, however, intravenous (i.v.) and/or intramuscular (i.m.) administration is also contemplated herein. Those skilled in the art will understand how i.v. and i.m. formulations can be prepared and how the effective dosages can be obtained.

In the method according to this invention a mammal may be a human or animal. The human may be an adult, child or infant. Thus, for infants, an infant formula containing the hereinafter described plant extracts or active principles will be effective in treating the infants infected with HBV, HCV, or HIV. For children and

adults, a medical food or nutritional product, such as milks and yogurts, containing the plant extracts or active principles described herein will also be effective in treating humans infected with HBV, HCV, or HIV.

The herbs used as starting materials for this invention may be obtained from commercial sources as single-herb herbal medicines which may be mixed, or extracted and concentrated, and placed in compositions for the administration to a human. The plant extracts, once isolated from the plant material, may be concentrated and then placed in compositions for the administration to a human. The active principles, once isolated from the plant material or herbal medicine, may be concentrated and then placed in compositions for the administration to a human. The compositions of this invention may take a variety of forms such as capsules, tablets, powder, candies, gels, beverages, teas, nutritional products, and the like.

Also disclosed is a medicinal product produced by the process comprising the steps of: (a) contacting comminuted plant material selected from the group consisting of No.5(1) to No.5(11), No.4(2) to No.4(5), and mixtures thereof, with water to form an aqueous dispersion; (b) heating the aqueous dispersion to about 100 DEG C. and holding at that temperature for about 0.5 to about 3 hours; (c) separating the insoluble plant material from the aqueous phase; and (d) concentrating the solute contained in the aqueous phase. The concentrated solute may be obtained through freeze drying, spray drying, evaporation and ultrafiltration.

As described in more detail in the following examples, the herbal compositions of the invention contain components that are active against viruses in vitro and in vivo.

Most impressively, the clinical effects of HHT888-5 on hepatitis B virus carriers are shown in Table 1 while the clinical effects of HHT888-45 on type C hepatitis patients are shown in Table 6.

In a preferred embodiment, the herb mixtures, individual single-herb herbal medicines, their water extracts and/or active principles are incorporated into oral dosage forms such as capsules, tablets, teas, powders, candies, candy bars, beverages, nutritional products, and the like.

This application sets forth the data available on the present discoveries and fully describes the compositions of matter, their preparation, and clinical applications. These and other aspects of the invention will become apparent to those skilled in the art as a result of the following examples which are intended as illustrative of the invention and not limitative.

BEST MODE FOR CARRYING OUT THE INVENTION

To acquaint persons skilled in the art with the principles of the invention, the following Examples are submitted.

EXAMPLE 1

Preparation of Herb Mixtures

In the preparation of the herbal compositions according to the invention, Chinese herbal medicines in single herb format were obtained from commercial sources in powder form. The individual single-herb herbal medicines were mixed in the appropriate proportions to prepare each herb mixture.

The herb mixture HHT888-4 was prepared by mixing five (5) single-herb herbal medicines No.4(1), No.4(2), No.4(3), No.4(4), and No.4(5) at a ratio of 3:3:3:3:4 by weight. The herb mixture HHT888-5 was prepared by mixing equal weights of eleven (11) single-herb herbal medicines No.5(1), No.5(2), No.5(3), No.5(4), No.5(5), No.5(6), No.5(7), No.5(8), No.5(9), No.5(10), and No.5(11).

The herb mixture HHT888-45 was prepared by mixing four (4) to six (6) single-herb herbal medicines No.4(3), No.4(4), No.5(4), No.5(5), No.5(8), and No.4(2) at a ratio of 1:1:1:1:0-1:0-1 by weight. The single-herb herbal medicine No.5(8) or No.4(2), or both, were not used in some cases in HHT888-45 for initial administrations. One of the two single-herb herbal medicines or both were added later when needed to enhance the therapy. The weight ratio of the single-herb herbal medicine No.4(2) in the herb mixture HHT888-45 also varied case-by-case between 0.5 and 1 when used.

It is noted that a mixture of decoctions prepared individually from the source plants of the single-herb herbal medicines or a decoction prepared from the pre-mixed source plants of the single-herb components of each herb mixture is well within the specification of the herb mixture.

EXAMPLE 2

Preparation of Single-herb Herbal Medicines

The single-herb herbal medicine used to prepare the herb mixtures has been described in the Prior Art section of this application. The plant source from which each single-herb herbal medicine is obtained was also listed in the Prior Art section. More than one species or genus of medicinal plant may be used to prepare the same herbal medicine as indicated in the plant source list of that herbal medicine. For example, the herbal medicine No.5(8) or FORSYTHIAE FRUCTUS may be prepared from either one of the three (3) species of Forsythia genus plants, i.e., Forsythia suspensa, Forsythia viridissima, Forsythia koreana or mixtures thereof. The herbal medicine No.5(6) or BAPHICACANTHIS RHIZOMA ET RADIX may be prepared from one of the five (5) plants of different genus and species, i.e., Baphicacanthes cusia, Strobilanthes cusia, Isatis tinctoria, Isatis indigotica, Polygonum tinctorium or mixtures thereof The herbal medicines were prepared from their respective plant sources as follows.

A suitable part or parts or the whole herb of a medicinal plant was obtained, washed with cold water, dried and comminuted. The plant materials were then extracted with boiling water on a basis of 1 part by weight of plant material to approximately 5 to 10 parts by weight of water. The amount of water used should at least cover the plant material in the extraction vessel. Samples were boiled for 0.5 to one hour, but not in excess of 3 hours, in order to allow effective extraction of the desired components. Shorter or longer heating would not substantially affect the extraction, except the yield and cost. The aqueous solution was separated from the plant material by filtration.

The aqueous solution may be freeze dried or spray dried, or reduced in volume by heating with or without an applied vacuum. The concentrate may then be spray dried or freeze dried or absorbed by powdered material of the same plant material or starch and thus the single-herb herbal medicine is prepared in powdered form.

It is noted that a decoction prepared from a source plant of the single-herb herbal medicine is well within the specification. A decoction is the aqueous solution of the plant material prepared by boiling the plant material in water as described above for about 0.5 to one hour. The decoction may be directly consumed after it is prepared and cooled to warm or ambient temperatures or preserved with proper sterilization for later consumption. Sterilization may be accomplished by microfiltration or heat.

EXAMPLE 3

Treatment of Hepatitis B Virus Carriers

Twenty-nine (29) HBV carriers with normal levels of serum liver enzymes, glutamine oxalacetate transferase (SGOT) and glutamine pyruvate transferase (SGPT), were treated with HHT888-5. Several HBV carriers who had elevated SGOT and SGPT levels were first treated with other remedies which returned their serum liver enzymes to normal levels (8-40 unit/mL for SGOT and 5-35 unit/mL for SGPT) but failed to reduce the HBV load. Treatment with HHT888-5 then began. HHT888-5 was prepared as described in Example 1 by mixing eleven (11) single-herb herbal medicines which were obtained from a commercial source and were manufactured following good manufacture practice (GMP) guidelines. Consent of the patients was obtained before their treatment began.

Patients were instructed to take the HHT888-5 three (3) times a day. Each dose was 5.5 g. Each 5.5 g packet of the herb mixture was mixed with warm water and consumed orally. Serum hepatitis B surface antigen (HBsAg) titers of each patient were determined at intervals as shown in Table 1 to monitor the progress of the treatment. Serum HBsAg titer was determined using a reverse-passive hemagglutination test as described herein: (1) Instruction of "Taifu" Serodia-HBs Test Reagent for HBsAg Detection, Taifu Pharmaceutical Co., Ltd., Taoyuan, Taiwan, R.O.C.; (2) D. S. Chen & J. L. Sung, J. Formosan Med. Assoc., 77, 263-270 (1978); and (3) T. Juji & T. Yokochi, Japan. J. Exp. Med., 39, 615-620 (1969).

Table 1 shows the treatment results of the twenty-nine (29) HBV carriers. Individual patients showed progressive improvement in their disease state over the course of treatment, as indicated by their HBsAg titer reductions and well being. Fourteen (14) carriers (48%) whose HBsAg titers ranged from 20 to 81,920 were significantly lowered (four (4) to 256-fold reductions, or from positive to negative) after 35 to 964 days of treatment. Four (4) carriers (14%) reduced their HBsAg titers from 20, 40, and 2,560 to negative (i.e., below 20 ng/mL detection level) after 56-153 days of treatment. Fourteen (14) carriers (48%) had no significant change (two-fold titer decrease or increase or no change) in HBsAg titers. That means these carriers had static

HBsAg titers during the course of the treatment (63-284 days). One carrier (3%) had a slightly four-fold titer increase.

The above HHT888-5 treatment results compare very favorably with the current interferon therapy. The response rates for interferon therapy and HHT888-5 treatment to lower the HBsAg titers in patients infected with HBV were comparable, approximately 40% vs. 48%. The serum HBsAg clearance rates were also comparable for both, 10-15% for interferon therapy and approximately 14% for HHT888-5 treatment. Furthermore, the interferon therapy is administered intramuscularly or intravenously and with frequent adverse effects. The HHT888-5 treatment is administered orally (like drinking a tea) and no apparent side effects were observed in all patients treated. Oral administration is a much more convenient and better way than intramuscular or intravenous administration. HHT888-5 can thus be safely and conveniently consumed even on a long-term basis to reduce or control HBV proliferation in HBV carriers and hepatitis B patients.

TABLE 1
Clinical Effects of HHT888-5 on Hepatitis B Virus Carriers

HBsAg Titer Duration
Patient Before After (days)
1 40 negative
56
2 2560 negative
72
3 20 negative
153
4 20 negative
88
5 2560 80 53
6 1280 320 101
7 2560 1280 32
1280 399
320 964
8 2560 1280 79
640 412
9 20480 5120 53
10 20480 5120 60
11 40960 10240 35
12 81920 40960 74
10240 461
13 81920 20480 6
14 5120 2560 170
2560 245
1280 556
1280 832
15 160 80 284
16 320 160 198
17 640 320 276
18 1280 640 120
19 2560 1280 69
20 5120 2560 263
21 20480 10240 77
22 40960 40960 120
20480 210
23 160 160 227
24 320 320 79
25 640 640 157
26 1280 1280 69
27 40960 40960 137
28 5120 10240 63
29 160 640 121

When the HBV viral load in an HBV carrier can be reduced or maintained at a sufficiently low level, the carrier is less likely to progress to hepatitis, liver cirrhosis, liver cancer, and death. Thus, HHT888-5 may be used to prevent and treat hepatitis B, or even prevent liver cirrhosis or liver cancer caused by HBV infection.

Since HHT888-5 was administered in the above treatments by mixing the powder in water first and then consumed orally, the water extract of HHT888-5 or a decoction from the herbal mixture comprising the single-herb components or plants of HHT888-5 is expected to be also effective and safe. Isolation of the active components of HHT888-5 and its administration to humans would also be efficacious in the treatment of HBV.

It is noted that HHT888-5 may be administered "as is" or in other solid dosage forms such as capsules, tablets, tea bags, candies, etc. The powdered herb mixture is typically mixed with warm or cold water and consumed orally. Its extracts may be administered as capsules, tablets, teas, candies, beverages, nutritional products, and the like.

Dosages range from 1 to 5 treatments per day at about 1 to 120 g per dosage depending upon the form and concentration of the herbal medicine. The effective minimum dose of a composition as a dried water extract of HHT888-5 is 1 g per day. The effective minimum dose of a composition comprising a more purified active component or components would be lower. The water extract of the tested HHT888-5 constituted 19% of the herb mixture by weight. Dosages of the herb mixture HHT888-5 as high as 120 g per day have been accomplished without serious side effects.

EXAMPLE 4

Antiretroviral Testing of Herb Mixtures and their Water Extracts

Two herb mixtures, HHT888-4 and HHT888-5, were tested for their antiretroviral activities and found to be active against EMuLV and HIV in the in vitro assay. Two in-vitro assays, anti-Ecotropic Murine Leukemia Virus (anti-EMuLV) and anti-HIV, were used to test the antiretroviral activities of the inventive compositions.

The anti-EMuLV assay uses a large, enveloped, RNA-containing retrovirus, EMuLV, which belongs to the same virus family as HIV and has many characteristics that are similar to HIV.

1. Anti-Ecotropic Murine Leukemia Virus Assay

The assay contained two parts, cytotoxicity test and virus suppression test. See QBI Protocol 39014 Final Report and QBI Protocol 39016 Final Report, Quality Biotech, Camden, N.J., USA, 1992. Each sample was initially tested for its cytotoxicity to the SC-1 indicator cells which were used for titration of infectious EMuLV in a XC plague assay. See QBI protocol C30015, Quality Biotech, Camden, N.J., USA. Each sample was dispersed in a virus resuspension buffer (50 mM Tris, pH 7.8, 10 mM KCL, 0.1 mM EDTA) without the virus. The solution was then subjected to the XC plague assay under the same conditions as those for the determination of EMuLV titer. A sample was considered cytotoxic if the indicator cells for the assay were less than 50% confluent. A noncytotoxic sample concentration was chosen for the virus suppression test.

In the virus suppression test, each sample was incubated with EMuLV (strain AKV623, titer 2.2-4.2.times.10@5 PFU/mL) in a virus resuspension buffer at 23-25 mg/mL (e.g., 100 mg/4.0 mL) for 12-32 minutes. The treated virus suspension was pH adjusted, if necessary, to within 6.8-7.2 and then tested for its titer in the XC plague assay.

An aliquot (1.5 mL) was diluted in the cell culture medium to the endpoint (10@0, 10@-1, 10@-2, 10@-3, 10@-4, 10@-5, 10@-6, 10@-7, and 10@-8 dilutions, or as appropriate). Each dilution was vortexed to resuspend any particulates if present and assayed in duplicate for infectious viral particles by the XC plaque assay. A positive control (virus suspension without treatment) and a negative control (cell culture medium, no virus) were also analyzed concurrently to validate the assay.

Anti-EMuLV activity of the sample was expressed in log10 reduction of the EMuLV titer when compared to the positive control. A sample with log10 to titer reduction greater than 0.5 is considered to be active.

HHT888-4 and HHT888-5 were initially tested "as is" and exhibited good antiviral activities (1.0 to 1.4 log10 reduction in viral titer) at 25 mg/mL and 12 minutes of incubation with the virus at room temperature. They were then tested again with a longer incubation time (32 minutes) with the virus at the same concentration.

Each sample was also tested for its soluble and insoluble fractions in the above virus resuspension buffer to see if any active component was water soluble. The soluble portion was separated from the insoluble one by centrifuge at room temperature and 10,000x g for 10 minutes. The soluble fraction was divided into two aliquots, one 0.45-.mu.m filtered and one unfiltered, and tested to see if residual particulates have any effect on the activity.

Table 2 summarizes the anti-EMuLV activity test results. The results confirmed that both HHT888-4 and HHT888-5 and their soluble and insoluble fractions have anti-EMuLV activities. The samples caused 1.0 to 2.6 log10 reduction in viral titer when they were incubated with the virus at 23-25 mg/mL for 32 minutes. Microfiltration did not significantly affect the activity of either soluble fraction.

2. Anti-Human Immunodeficiency Virus Assay

This assay also contained two parts, a toxicity test and a HIV suppression test. The sample was mixed in a cell culture medium, e.g., 50 mg in 1.00 mL. The mixture was vortexed and centrifuged to separate the soluble from the insoluble. The supernate was filtered through a 0.45-.mu.m filter and then diluted with cell culture medium to appropriate concentrations for the assay. The cell culture medium used in the assay was RPMI 1640 (pH 7.3.±.0.3) supplemented with 10% fetal calf serum, 2 mM glutamin, 50 U/mL penicillin and 50 .mu.g/mL streptomycin.

The sample was tested for its cytotoxicity and/or cytostatic activity towards the target cells, human peripheral blood lymphocytes (PBLs). A lymphocyte proliferation assay was used for the toxicity test, where a 100 .mu.L sample was incubated with 100 .mu.L of a cell suspension of uninfected PBLs (3.times.10@5 cells) under the same conditions as the HIV suppression test. Lymphocyte proliferation was measured by a colorimetric assay

TABLE 2
Anti-Ecotropic Murine Leukemia Virus Activity
Cytotoxicity* Anti-EMuLV Activity
Sample
Treatment
25 2.5
0.25 mg/mL
Log10 Titer Reduction**

HHT888-4
"as is" Yes No No 1.02 (90%)***
"as is" Yes No No 1.04 (91%)****
Soluble -- -- -- 1.74 (98%)****
Soluble, filtered
-- -- -- 1.59 (97%)****
Insoluble
-- -- -- 2.64 (99.8%)****
HHT888-5
"as is" Yes No No 1.35 (96%)***
"as is" Yes No No 2.10 (99.2%)****
Soluble -- -- -- 2.05 (99.1%)****
Soluble, filtered
-- -- -- 1.71 (98.1%)****
Insoluble
-- -- -- 1.72 (98.1%)****

*Sample was considered cytotoxic if the SC1 indicator cells for the assay were less than 50% confluent.
**As compared to a working virus suspension with a titer of 2.2-4.2.times. 10@5 PFU/mL, or Log10 (PFU/mL) = 5.34-5.62. The values in parentheses indicate percent reductions in viral titer from the workingvirus suspension.
***Incubation time 12 minutes, at 25 mg/mL test level. The activity may be caused by the sample, by microbial contaminant, or by a nonspecific
physical interaction between the particles of the sample and the virus, since the samples were not sterile filtered before assay.

****Incubation time 32 minutes, at 25 mg/mL test level for the "as is" unfractionated samples. For soluble, soluble & sterile filtered, and insoluble fractions, the test level was equivalent to 23 mg/mL of its unfractionated sample.

(MTT-Test). See T. Mosmann, J. Immunological Methods, 65, 55-63 (1983). A sample concentration which results in .gtoreq.70% of the control in Iymphocyte proliferation is considered to be acceptable for the HIV suppression test.

In the HIV suppression test, HIV-1 infected PBLs were cultivated in the presence of the sample for four (4) days as in the toxicity test. See H. Ruebsamen-Waigmann, et al., J. Med. Virology, 19, 335-344 (1986). The secreted viral core protein p24 and/or viral RNA were determined as indicators for virus proliferation status on day 3 and day 4 by an HIV-1 p24 capture ELISA technique and an HIV-RNA dot blot hybridization technique, respectively. The concentration of p24 synthesized by the HIV infected cells was determined by Sandwich ELISA. A standard preparation of recombinant p24 (MicroGeneSys, USA) was used for calibration of the ELISA. See Ch. Mueller, et al., Fresenius Z. Anal. Chem., 330, 352-353 (1988).

HIV-RNA synthesized in the infected cells was determined by a nucleic acid hybridization technique. Cellular RNA was prepared from the infected cells and analyzed by a dot blot hybridization technique. The hybridization solution contained the P@32 -labeled DNA probe which comprised a 5.5 kilobase DNA fragment of the HIV isolate D31. See H. v. Briesen, et al., J. Med. Virology, 23, 51-66 (1987). This fragment covering the gag/pol region of the virus is labeled with P@32 alpha-d CTP by oligonucleotide labeling. Plus-strand RNA transcripts derived from the gag/pol region of the viral isolate D31 were used as the external standard for the hybridization. These "run-off" transcripts were generated by means of the T7 polymerase reaction from negatively polarized HIV-DNA under T7-promotor control. The concentration of RNA transcripts was determined spectrophotometrically. The hybridized probe was detected by autoradiography and the processed autoradiograms were evaluated densitometrically.

A positive control, a negative control, and an AZT control were conducted concurrently to assure the validity of the HIV suppression test. All tests were performed in triplicates, and 96-well round bottom microtiter plates were used for all assays. A positive control was HIV-1 infected lymphocytes cultivated in the presence of the cell culture medium without the sample. A negative control was lymphocytes infected with a heat-inactivated virus inoculum incapable of replication. These "mockinfected" lymphocytes were cultivated and assayed in the same way as the infected cells. The amount of viral protein being present in the cultures solely due to the remaining inoculum was thus determined as the background level. The amount of viral protein p24 in the test sample and in the positive control due to viral replication was then determined by the respective p24 levels less the background level.

The amount of viral protein being present in the cultures containing the sample due to viral proliferation was compared with that in the positive control, i.e., the culture without the sample. The % suppression of HIV proliferation was determined by the difference in p24 levels between the positive control and the sample, divided by the p24 level of the positive control, and timed 100%.

The AZT control was conducted via HIV-1 infected lymphocytes that were cultivated in the presence of azidothymidine (AZT) at concentrations of 100, 10, 1 and 0.1 ng/mL, respectively. This provided an estimate of the sensitivity of the lymphocytes towards AZT, a known inhibitor of HIV-1 replication. The suppression of HIV-1 proliferation caused by AZT in a concentration of 10 ng/mL should be greater than 50% as compared to the untreated positive control.

TABLE 3
Anti-HIV Activities of HHT888-4 and HHT888-5

HIV Suppression
Test p24 RNA
Sample
Concentration
Cytotoxicity*
Day 3
Day 4
Day 3
Day 4

HHT888-4
2.5
mg/mL
>46% 100% 100% 100%
100%
50 .mu.g/mL
85% 1% 6% -- --
HHT888-5
5.0
mg/mL
75% 100% 97% 99%
100%
50 .mu.g/mL
86% 0% 12% -- --
AZT 100
ng/mL
-- 99-100%
100% -- --
10 ng/mL
-- 85-98%
77-96%
-- --
1 ng/mL
-- 20-39%
8-12%
-- --
0.1
ng/mL
-- 0% 0-3% -- --
*Percent proliferation of control. HHT8884 was 46% at 5.0 mg/mL. Both HHT8884 and HHT8885 were cytotoxic (<50% of control) at 25 mg/mL level.

Table 3 summarizes the cytotoxicity and the HIV suppression test results of HHT888-4 and HHT888-5, as well as the AZT controls. Both herb mixtures were active in suppressing HIV proliferation in infected human lymphocytes at 2.5-5.0 mg/mL, but not at 50 .mu.g/mL (50-100 times diluted). The AZT controls from all sets of anti-HIV assays herein and thereinafter exhibited the expected activities and thus assured the validity of the tests.

At 2.5-5.0 mg/mL of HHT888-4 and HHT888-5, HIV proliferation in infected human lymphocytes were essentially completely suppressed: 97-100% suppression based on viral protein p24 and 99-100% suppression based on viral RNA determined on both day 3 and day 4 after treatment. The anti-HIV activity at 50 .mu.g/mL was negligible, 0-12% suppression for both herb mixtures. The activities could not be attributed to insoluble particulates since they were filtered out by a 0.45-.mu.m filter before the assay. The activities were not due to cytotoxicity. Repeat tests on three lots of HHT888-4 showed 100% suppression at 2.5 mg/mL on both day 3 and day 4 with acceptable cytotoxicity (71-100% of control proliferation). Repeat tests on three lots of HHT888-5 at 2.5 mg/mL showed 93-98% suppression on day 3 and 89-99% suppression on day 4 with acceptable cytotoxicity (85-91% of control proliferation). Results of the repeat experiments are shown in Table 4.

It is noted that Lot 3 of HHT888-4 or HHT888-5 was prepared by mixing the respective single-herb components at equal proportion by weight. Lot 3 of HHT888-5 was composed of nine (9) single-herb components, excluding No.5(10) and No.5(11).

Water extracts of HHT888-4 and HHT888-5 from one to two lots were further tested to see whether the active components were extractable by water. Water extracts of HHT888-4 and 5 were prepared by extracting 5 g of the powder with 25 mL of MilliQ purified water twice. Each water suspension was vortexed for 1 minute, stood for 5 minutes, and vortexed again for 1 minute to facilitate the extraction. The extract was separated from the insoluble by centrifuge at 1,000-2,000 rpm for 20 minutes. The supernate was transferred into a clean preweighed 50-mL centrifuge tube, freeze dried, weighed, and tested for anti-HIV activity.

The percent weight of material extracted was 17.3% for the first 25 mL extract and 10.8% for the second 25

mL extract of HHT888-4 (Lot 2). That was 14.2% for the first 25 mL extract and 4.6% for the second 25 mL extract of HHT888-5 (Lot 2). The first (E1), the second (E2) and the combined (E) extracts of HHT888-4 (Lot 2) were tested for anti-HIV activity. All the other extracts were tested with the first and the second extracts combined. The results are summarized also in Table 4.

TABLE 4
Anti-HIV Activities of HHT888-4 and HHT888-5 and their Water Extracts

% Test HIV Suppression**
Sample Lot Weight
Concentration
Cytotoxicity*
Day 3
Day 4
HHT888-4
1 100% 2.5
mg/mL
>46% 100%
100%
2.5
mg/mL
98% 100%
100%
0.05
mg/mL
85% 1% 6%
2 100% 2.5
mg/mL
100% 100%
100%
3***
100% 2.5
mg/mL
71-79% 100%
100%
HHT888-4-E1
2 17% 1.0
mg/mL
98% 100%
96%
@ E2
2 11% 1.0
mg/mL
96% 100%
87%
@ E
2 28% 1.0
mg/mL
47% 100%
100%
0.5
mg/mL
78% 100%
100%
4 27 .±. 1% (2)
1.0
mg/mL
72% 100%
100%
1.0

mg/mL
100% 100%
93%
0.1
mg/mL
97% 34%
12%
0.02
mg/mL
82% 23%
2%
HHT888-5
1 100% 5.0
mg/mL
75% 100%
97%
2.5
mg/mL
89% 93%
91%
0.05
mg/mL
86% 0% 12%
2 100% 2.5
mg/mL
91% 94%
89%
3** 100% 2.5
mg/mL
44-85% 98%
99%
0.5
mg/mL
52-100%
0% 0%
HHT888-5-E
2 19% 1.0
mg/mL
91% 71%
26%

*Toxicity in percent of control proliferation.
**HIV suppression based on viral protein p24 levels.
***Composite of respective single herb components at equal proportions.

No. 5(10) and No. 5(11) were not included in Lot 3 of HHT8885.

All three Lots of each of the herb mixtures were very active, 100% suppression at 2.5 mg/mL for HHT888-4 and 89-100% suppression at 2.5-5.0 mg/mL for HHT888-5. The IC50 was between 0.05-2.5 mg/mL for HHT888-4 and between 0.5-2.5 mg/mL for HHT888-5. IC50 is the concentration of the test substance at which would cause 50% suppression of the viral proliferation.

The water extract of HHT888-4 showed very good activity: 93-100% suppression at 0.5-1.0 mg/mL. The first (E1) and the second water extract (E2) of Lot 2 exhibited comparable activities: 100% suppression on day 3 and 87-96% suppression on day 4 at 1.0 mg/mL. The IC50 of the water extract of HHT888-4 was between 0.1-0.5 mg/mL.

The water extract of HHT888-5 (lot 2) exhibited a substantially lower activity: 71% suppression on day 3 which dropped to 26% suppression on day 4 at 1.0 mg/mL. The main active component apparently stayed behind in the insoluble fraction and was not as easily extracted by water as that of HHT888-4 under the aforementioned conditions. It is noted that the water extract of HHT888-5 (Lot 2) constituted 19% by weight

of the herb mixture. The test concentration of the water extract of HHT888-5 (or HHT888-5-E) at 1.0 mg/mL is equivalent to 5.3 mg/mL of HHT888-5 itself. HHT888-5 was tested very active at both 2.5 mg/mL (93-98% suppression on day 3 and 89-99% on day 4) and 5.0 mg/mL (100% suppression on day 3 and 97% on day 4).

The above results clearly demonstrated that both HHT888-4 and HHT888-5 and their water extracts have in vitro antiretroviral activities, more specifically anti-EMuLV and anti-HIV activities. HHT888-5 has also been shown to be efficacious in treating hepatitis B virus carriers, while HHT888-4 has not been tested in vivo.

* EXAMPLE 5

Antiretroviral Testing of Individual Single-herb Herbal Medicines

The individual single-herb components of HHT888-4 and HHT888-5 were tested for anti-HIV activity. Table 5 shows the test results.

TABLE 5
Anti-HIV Activities of Single-herb

Components of HHT888-4 and HHT888-5
Test HIV Suppression**
Sample Lot Concentration
Cytotoxicity*
Day 3 Day 4
No. 4(1)**
1 2.5 mg/mL 98% 73% 50%
No. 4(2)
1 2.5 mg/mL 74-84% 92% 94%
No. 4(3)
1 2.5 mg/mL 75-78% 100% 100%
No. 4(4)
1 2.5 mg/mL 74-100% 100% 100%
No. 4(5)
1 2.5 mg/mL 41-79% 98% 92%
0.5 mg/mL 47-100% 0% 0%
No. 5(1)***
1 2.5 mg/mL 98% 73% 50%
No. 5(20
1 2.5 mg/mL 73-87% 18% 29%
No. 5(3)
1 2.5 mg/mL 89-100% 0% 0%
No. 5(4)
1 2.5 mg/mL 64% 100% 100%
1.0 mg/mL 69-91% 0% 0%
No. 5(5)
1 2.5 mg/mL 80-84% 93% 93%
No. 5(6)
1 2.5 mg/mL 94-100% 0% 0%
No. 5(7)
1 2.5 mg/mL 90-100% 50% 38%
No. 5(8)
1 2.5 mg/mL 32-59% 100% 100%
0.5 mg/mL 65-100% 0% 0%
No. 5(9)
1 0.5 mg/mL 24-78% 0% 0%
No. 5(10)
1 2.5 mg/mL 100% 65% 0%
No. 5(11)
1 2.5 mg/mL 100% 92% 74%
*Toxicity in percent of control proliferation.

**HIV suppression based on viral protein p24 levels.

No. 4(1) = No. 5(1)

All five (5) single-herb components of HHT888-4 exhibited anti-HIV activities with various degrees: 73-100% suppression on day 3 and 50-100% suppression on day 4 at 2.5 mg/mL. No. 4(3) and No. 4(4) exhibited the best activity: 100% suppression at 2.5 mg/mL on both day 3 and day 4. No. 4(2) and No. 4(5) were the next: 92-98% suppression on day 3 and 92-94% suppression on day 4 at 2.5 mg/mL. No.4(1) exhibited a moderate activity: 73% suppression on day 3 and 50% suppression on day 4 at 2.5 mg/mL. No.4(5) exhibited a slight cytotoxicity (41-79% of control proliferation) which was likely to contribute to the observed activity with an ID50 between 0.5 and 2.5 mg/mL.

Three (3) of the eleven (11) single-herb components of HHT888-5: No.5(4), No.5(5), and No.5(8) exhibited very good activities, 93-100% suppression of HIV proliferation on both day 3 and day 4 at 2.5 mg/mL. No.5(11) was the next: 92% suppression on day 3 and 74% suppression on day 4 at 2.5 mg/mL. Again, No.5(1), which was the same as No.4(1), had a moderate activity: 73% suppression on day 3 and 50% suppression on day 4 at 2.5 mg/mL. No.5(2) and No.5(7) exhibited only marginal activities: 18-50% suppression on day 3 and 29-38% suppression on day 4 at 2.5 mg/mL. No.5(10) exhibited a very slight activity: 65% suppression on day 3 which dropped to 0% on day 4 at 2.5 mg/mL. The remaining three (3) single-herb components, No.5(3), No.5(6), and No.5(9) exhibited no activity at 0.5-2.5 mg/mL. No.5(9) was not tested at 2.5 mg/mL level because of its cytotoxicity: already 24-78% of control proliferation at 0.5 mg/mL.

Although No.5(4) and No.5(8) appeared to be slightly more active than No.5(5) (100% vs. 93% suppression at 2.5 mg/mL), their activities might be partially due to cytotoxicity (32-64% of control proliferation at 2.5 mg/mL). This was supported by the loss of activity (0% suppression) when tested at lower levels, 0.5-1.0 mg/mL, where the cytotoxicity was lower and more acceptable to the assay.

EXAMPLE 6

Anti-HIV Testing of Medicinal Plant

The source plant of the single-herb herbal medicine No.5(5), Aeginetia indica, was obtained from a local herbal store in Taiwan and tested for its anti-HIV activity. This was to see whether the activity can be reproduced in the herbal medicine prepared directly from its source plant, instead of being obtained from the commercial source.

The whole plant was washed with cold water, dried, comminuted, and extracted with boiling water as described above in Example 2. The aqueous solution was separated from the plant material by filtration. The aqueous solution was then reduced in volume by heating. The concentrate was spray dried and absorbed onto powdered material of the same plant material and thus was prepared the herbal medicine in powder form, designated hereinafter as raw No.5(5).

The powdered herbal medicine prepared from Aeginetia indica, or raw No.5(5), was extracted with water at ambient temperature. Two (2) 5.00 g samples were each extracted twice with about 40 mL of water each time in a separate 50-mL plastic centrifuge tube by vortexing for one (1) minute, standing for ten (10) minutes, and vortexing again for one (1) minute. The tubes were centrifuged at 1500 rpm for twenty (20) minutes to separate the extracts from the insoluble residues. The extracts were filtered through a Whatman No.4 filter paper, freeze dried or nitrogen dried, and weighed.

The above extraction of the raw No.5(5) with water (pH.about.5.1) was repeated and the pH of the first extract was measured to be 5.7. The first and the second extracts were respectively separated from the residue, air dried, and weighed. The percent weight of the extractable was determined to be 18.7.±.2.8% (n=2).

The first water extract of the raw No.5(5) was tested for anti-HIV activity and found to be as active, 91% suppression on day 3 and 97% suppression on day 4 at 1.0 mg/mL. Cytotoxicity test showed that the extract was not cytotoxic at this level, 99% of control proliferation.

The above examples clearly demonstrate that both the herb mixtures HHT888-4 and HHT888-5 are very active against HIV proliferation. Complete (100%) or nearly complete (89-99%) suppressions of HIV

proliferation were achieved at 2.5 mg/mL. The water extract of HHT888-4 is also very active. Complete (100%) suppression of HIV proliferation was achieved at 0.5 mg/mL. The water extract of HHT888-5 is not as active as its original mixture. It only suppressed 26-71% of HIV proliferation at 1.0 mg/mL. Both HHT888-4 and HHT888-5 are not cytotoxic at 2.5 mg/mL. The water extracts of both HHT888-4 and HHT888-5 are also not cytotoxic at 1.0 mg/mL.

HHT888-5 has been demonstrated to be effective and safe in treating HBV infections in humans. That means, the active principle or principles of HHT888-5 must be bioavailable in humans through oral administration to cause the decrease of hepatitis B virus in those patients treated, as indicated by the decrease of their hepatitis B virus surface antigen (HBsAg) exhibited in Example 3. In addition, Hozumi et al. provide examples in U.S. Pat. No. 5,411,733 to support the belief that substances exhibiting antiviral activity in vitro also possess antiviral activity in vivo as described in the Prior Art section. It is therefore logical to believe that HHT888-4 or HHT888-5 and their water extracts or active principles should also be effective for treating HIV infections in humans.

To test the belief, six (6) of the most anti-HIV active single-herb components of HHT888-4 and HHT888-5 were selected to treat hepatitis C patients caused by hepatitis C virus infections. The logic is that both HCV and HIV are retroviruses. Viral hepatitis C tends to become a chronic disease and is therefore more suitable for the test of the treatment. If the treatment works for patients infected with HCV, it will also work for patients infected with HIV. Example 7 clearly demonstrates the validity of this belief.

EXAMPLE 7

Treatment of Hepatitis C Patients

Six (6) of the most anti-HIV active single-herb components of HHT888-4 and HHT888-5 were selected and mixed to treat hepatitis C patients caused by hepatitis C virus infections. The six (6) single-herb herbal medicines selected were No.4(2), No.4(3), No.4(4), No.5(4), No.5(5), and No.5(8). No.4(5) was not included although it exhibited a very good activity because it was learned that the herb might have a certain unconfirmed toxicity.

The six (6) single-herb herbal medicines were obtained from a commercial source and were manufactured following good manufacture practice (GMP) guidelines. They were mixed according to the desired ratio in various combinations and thus the herb mixture HHT888-45 was prepared as further described in Example 1. Patients' consents were obtained before the initiation of treatment.

Patients were instructed to take the herb mixture three (3) times a day, 2.7-5.7 g each time. Unit dosages of the herb mixture HHT888-45 were prepared in individual packets. Each unit dose packet (2.7-5.7 g) of the herb mixture was mixed with warm water and taken orally. All patients were treated with HHT888-45 containing No.4(3), No.4(4), No.5(4), and No.5(5). No.5(8) or No.4(2) or both were added in HHT888-45 for the treatment of some patients at the very beginning or during the course of the treatment to enhance the effectiveness of the treatment. During the course of the treatment, the daily dose of No.4(3), No.4(4), No.5(4), and No.5(5) varied from two (2) to three (3) g each. The daily dose of No.5(8) also varied from two (2) to three (3) g when used. The daily dose of No.4(2) varied from 1.5 to two g when used. The dose was varied according to the progress of the disease.

Seven (7) viral hepatitis C patients were treated. Their serum liver enzymes, SGOT and SGPT, were determined from time to time by a local clinical laboratory during the course of the treatment to monitor the progress of the disease. The SGOT and SGPT were determined using an enzyme assay. See (1) Instruction of Kyokuto TA-E Transaminase Assay Reagents, Permit No. (62AM)0885, Kyokuto Pharmaceutical Industry Co., Ltd., Tokyo, Japan, 1994; (2) Instruction of Yatrozyme TA-Lq Transaminase-assay Reagent Solution (Enzyme Assay), Commodity No. 817245 (RM163-K), Yatron Co., Ltd., Diayatron Co., Ltd., Tokyo, Japan; and (3) U. Lippi & G Guidi, Clin. Chim. Acta., 28, 431-437 (1970).

The levels of serum GOT and GPT closely correlate with the degree of cellular injury in the liver. These tests are widely used in the diagnosis of liver diseases and as an indicator of the liver function. The normal range for SGOT is 8-40 units/mL and that for SGPT is 5-35 units/mL. Elevated SGOT and SGPT levels usually indicate compromised liver functions.

The results of HHT888-45 treatment are shown in Table 6. All seven (7) patients treated had their serum liver enzymes returned from elevated levels (SGOT from 48 to 166 unit/mL and SGPT from 41 to 291 unit/mL) to

essentially normal range (SGOT from 8 to 40 unit/mL and SGPT from 5 to 35 unit/mL) after 17 to 178 days of treatment. Thus, the liver functions of the patients were returned to normal after consumption of the invention composition.

The results clearly demonstrate that the herb mixture HHT888-45 is effective in treating hepatitis C patients. To accomplish that, the causative hepatitis C virus needs to be eradicated or reduced to a tolerable level. Since HHT888-45 components have demonstrated very strong anti-HIV in vitro activity and several of the components have demonstrated efficacy in reducing HBV in carriers, the herb mixture will therefore be effective in treating patients infected with HIV and HBV.

It is therefore an aspect of this invention that the antiviral herbal medicines including the herb mixtures according to this invention and their single-herb components at various proportions and effective doses are effective in treating hepatitis C, hepatitis B, and other retroviral diseases, such as AIDS.

TABLE 6
Clinical Effect Of HHT888-45* On Type C Hepatitis Patients
SGOT**, unit/mL SGPT**, unit/mL
Duration
Patient
Before After Before After (days)

1 112 53 238 146 3
30 35 64
16 18 77
2 81 35 103 62 9
41 61 20
46 67 29
32 56 37
21 43 53
24 50 70
23 43 85
28 55 102
23 44 117
23 29 178
3 117 96 179 123 8
75 74 19
66 69 26
47 51 34
55 48 42
42 45 50
48 40 70
38 32 79
30 26 88
4 48 32 71 65 56
30 55 70
21 37 87
5 83 64 67 54 8
58 46 14
56 40 22
42 34 29
38 28 36
6 166 106 291 206 2
71 121 16
51 81 22
57 89 29
36 45 45
31 36 50
28 37 58
22 29 64
28 32 71

25 27 85
36 28 103
23 27 113
23 22 163
7 30 28 41 42 9
29 32 17

*Comprising mainly Nos. 4(3), 4(4), 5(4) and 5(5), and occasionally 4(2) and 5(8).
**SGOT = serum glutamine oxalacetate transferase; normal range = 8-40 unit/mL.

SGPT = serum glutamine pyruvate transferase; normal range = 5-35 unit/mL.

Since the precise chemical composition and pharmacological mechanism of the compositions of this invention have not yet been eludicated, it is possible that the antiviral activity may be due to a single herbal component, a combination of components or the biological metabolite or derivative thereof.

Industrial Applicability

The instant invention is directed in part, to the discovery that specific medicinal plants or herbal medicines or their mixtures possess surprising antiviral activities without causing damage to the host cells. Further, the invention is directed to methods of treating humans and mammals infected with viruses such as HBV, HCV, or HIV. The data presented in this application clearly demonstrate that the identified compositions possess antiviral activity without toxicity to the host cells.

It can be concluded from the foregoing experiments that the herb mixture designated HHT888-4 is effective in treating HBV carriers and thus can be used to treat humans infected with HBV. The reduction of viral load in HBV patients will thus result in the prevention of HBV disease in the human and will also be effective in the treatment of humans exhibiting HBV disease. The clinical experiments have also shown that the herb mixture HHT888-45 is effective in treating hepatitis C patients, and thus is expected to be effective in treating hepatitis B patients when administered alone or in combination with HHT888-5 or its antiviral single-herb components.

In addition, HHT888-5, HHT888-45, HHT888-54 and the individual anti-HIV active single-herb components have demonstrated efficacy in suppressing HIV proliferation in human cells. Furthermore, HHT888-5, HHT888-45 and HHT888-54 have shown efficacy in treating patients infected with HBV and HCV. HHT888-5, HHT888-45, HHT888-4 and HHT888-54 are also effective in treating humans infected with HIV, including HIV carriers and AIDS patients.

The therapeutic effects described herein may be accomplished through the administration of the herbal medicines "as is", or as teas, decoctions, beverages, candies or other confections, enteral liquid nutritional products such as infant formula and adult nutritional products, medical foods, nutritional supplements or neutraceuticals containing one or more of the herbal medicines or their extracts or active principles. For pharmaceutical preparations, one or more of the antiviral herbal medicines or their extracts or active principles described above may be administered in unit dosage forms such as capsules, packets or tablets, with or without controlled-release coating(s).

The medical community is constantly in search of methods and products that will effectively treat viral infections, especially methods and products for treating humans infected with HBV, HCV, and HIV. The herb mixtures HHT888-4, HHT888-5, HHT888-45, HHT888-54, the single-herb components, their extracts, active principles, and products containing these herbal compositions will be readily accepted by the medical community as an additional tool in the prevention and treatment of these devastating illnesses.

US6696094
Herbal pharmaceutical composition for treatment of HIV/AIDS patients

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The present invention provides a pharmaceutical composition for treating patients with HIV infection. The pharmaceutical composition is in the form of an intravenous injection solution and optionally capsules. The

pharmaceutical composition contains fourteen (14) ingredients, i.e., diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, milkvetch root, barbary wolfberry fruit, sanqi, figwort root, Chinese magnoliavine fruit, turmeric root-tuber, hawthorn fruit, and Chinese angelica.

CROSS-REFERNCE TO RELATED APPLICATION

The present application is a continuation-in-part (CIP) of U.S. patent application Ser. No. 09/906,791 filed on Jul. 18, 2001, now U.S. Pat. No. **6,455,078** which in turn claims the priority of U.S. Provisional Application No. 60/240,963 filed on Oct. 18, 2000. Both U.S. priority applications are herein incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to a novel herbal pharmaceutical composition and its use for treating patients with human immunodeficiency virus (HIV) infection. The pharmaceutical composition is in the form of an intravenous injection solution or capsules and contains aqueous extracts of fourteen (14) herbal ingredients, including diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Chinese magnoliavine fruit, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit, sanqi, barbary wolfberry fruit, figwort root, Chinese angelica, and milkvetch root. The intravenous injection solution can be administered alone or co-administered with the capsules. The present invention also includes the methods for making the pharmaceutical composition and for treating the patients with HIV infection.

DESCRIPTION OF THE RELATED ART

The present invention is a continuation-in-part (CIP) of the parent patent application, U.S. patent application Ser. No. 09/906,791, now U.S. Pat. No. 6,455,078, which is herein incorporated by reference. In the parent application, novel pharmaceutical compositions, which are particularly effective in treating patients with human immunodeficeincy virus (HIV), were described.

HIV infection causes acquired immunodeficiency syndromes (AIDS) in humans which presents special problems to the medical community. AIDS is a pandemic immunosuppressive disease which results in life-threatening opportunistic infections and malignancies. The retrovirus, HIV, has been isolated and identified as the etiologic agent of this disease. HIV has been detected in whole blood, plasma, lymphatic fluid, serum, semen, vagina fluid, breast milk, tears, saliva, and central nervous system tissue of infected patients. HIV can be transmitted through sexual contact with an infected person, by sharing needles or syringes (primarily for drug injection) with an infected person, or, less commonly, through transfusions of infected blood or blood clotting factors. Babies born to HIV-infected women may become infected before or during birth or through breast-feeding after birth. As of this time, complete cure for HIV infection/AIDS has been diligently pursued by scientists around the world, yet there has been no report of absolute success.

Pharmaceutical compositions made from natural herbs have been known and used for thousands of years to cure or ameliorate various diseases and injuries. Some of these herbal compositions have been disclosed for having medicinal properties for curing or ameliorating symptoms associated with HIV infection. For example, U.S. Pat. No. 5,178,865 discloses screening of fifty-six (56) individual herbs and have found that ten (10) out of 56 herbs exhibits anti-HIV activity in ex vivo experiments. The ten (10) individual herbs include Coptis chineusis, Ligusticum wallichii, Illicium lanceolatum, Isatis tinctoria, Salvia miltiorrhiza, Erycibe obtusifolia, Acanthopanax graciliatylus, Bostaurus domesticus, Inula helenium, and Lonicera japonica. Both Bostaurus domesticus and Lonicera japonica are further described to be able to combine with Scutellaria baicaleusis to exhibit anti-HIV activity.

U.S. Pat. No. 5,837,257 discloses Chinese herbal medicines that exhibit in vitro antiviral activities against murine leukemia virus and HIV and for treatment of animals and humans infected with HIV. In one of the preferred embodiments, the Chinese herbal medicines contain hedyotis, scutellariae barbatae herba, lonicerae flos, prunellae spica, and solani herba.

U.S. Pat. No. 5,989,556 discloses various herbal compositions for treating viral infection. One of the herbal compositions contains Aeginetiae Herba, Blechni Rhizoma, Lespedezae Herba, Polygoni Cuspidati Rhizoma, Forsythiae Fructus, and Ligustri Fructus. Another herbal composition contains Cirsii Rhizoma et Radix, Breeae Radix, Baphicacanthis Rhizoma et Radix, Phellodendri Cortex, and Bletillae Tuber. A third herbal composition disclosed in the patent has Aeginetiae Herba, Lonicerae Flos, Prunellae Spica, and Lespedezae Herba.

The present invention provides a novel pharmaceutical composition for treatment of HIV, both in vitro and in vivo, including treatment of AIDS patients, which are distinctively different from prior art disclosures. This novel pharmaceutical composition differs from the pharmaceutical compositions described in the parent application for not containing red ginseng (Radix ginseng rubra). The pharmaceutical composition of the present invention is a natural Chinese medicine with little or no side effects and has no toxicity.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical composition which is particularly effective in treating HIV-positive or AIDS patients. The pharmaceutical composition (in the name of "HIVCIDE") is in the form of an injection solution (for intravenous injection) or capsules. It contains aqueous extract of the following herbal ingredients: (1) an entire plant of Herba Hedyotidis diffusae (diffuse hedyotis); (2) a rhizome of Rhizoma Bistortae (bistort rhizome); (3) a rhizome of Rhizoma Polygoni Cuspidati (giant knotweed rhizome); (4) a ripe fruit of Fructus Schisandrae (Chinese magnoliavine fruit); (5) a rhizome of Rhizoma Menispermi (Asiatic moonseed rhizome); (6) a root of Radix Scutellariae (baical skullcap root); (7) bovine biliary powder; (8) a root tuber of Radix Curcumae (tumeric root-tuber); (9) a ripe fruit of Fructus Crataegi (hawthorn fruit); (10) a root of Radix Notoginseng (sanqi); (11) a ripe fruit of Fructus Lycii (barbary wolfberry fruit); (12) a root of Radix Scrophulariae (figwort root); (13) a root of Radix Angelicae sinensis (Chinese angelica); and (14) a root of Radix Astragali (milkvetch root). Examples of the aqueous solution for extracting the pharmaceutical ingredients from the herbs include, but are not limited to, water, ethanol, or a mixture thereof. The preferred aqueous solution is water.

The herbal ingredients of HIVCIDE differ from that in the parent application by not containing the root of Radix ginseng rubra (red ginseng). Also, the weight ratio of the pharmaceutical composition of the present invention is different from that of the pharmaceutical composition of the parent application by containing more quantity of diffuse hedyotis. The weight ratio of the 14 ingredients listed above is about 4:3:1:2:1:1:0.1:1:2:1:3:2:1:2.

The aqueous extract of the herbal ingredients is further filtered and condensed. The condensed aqueous extract is called "herbal condensate" or "HIVCIDE condensate."The volume of the herbal filtrate is about 1.4 fold of the herbal condensate.

The gram weight of the HIVCIDE for the purpose of determining the dosage amounts for patient treatment as described hereinafter is based on the weight of the "HIVCIDE condensate," not the weight of the original herbal ingredients.

The present invention also includes a method for preparing HIVCIDE which includes the steps of: (1) grinding and mixing diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Chinese magnoliavine fruit, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit, sanqi, barbary wolfberry fruit, figwort root, Chinese angelica, and milkvetch root to form a herbal mixture; (2) boiling the herbal mixture in water to form a water extract; (3) filtering the water extract to collect an herbal filtrate; and (4) concentrating the herbal filtrate to form an herbal condensate (also known as "HIVCIDE condensate"). The HIVCIDE condensate is then either dissolved in a suitable solution to form the intravenous injection solution or sprayed dried to form herbal powders. The herbal powders are further mixed with a binder, such as starch, and encapsulated.

The HIVCIDE injection solution contains about 0.1-1 g of the HIVCIDE condensate per ml of the injection solution. When administering to patients, the HIVCIDE is perferably diluted to about 1:5 to 1:10 by volume of 5% glucose solution.

The present invention further contains a method for treating patients with HIV infection which includes intravenous administration of an effective amount of HIVCIDE to patients with HIV infection. The preferred dosage is about 1 to 10 g of HIVCIDE condensate, most preferably, about 2-6 g of HIVCIDE condensate, per day.

Additionally, the present invention provides a method for treating patients with HIV infection which comprises: intravenous administering the HIVCIDE injection solution and orally administering the HIVCIDE capsules to patients. The preferred dosage of the HIVCIDE injection solution is about 1 to 10 g, most favorably, 2-6 g, of the HIVCIDE condensate per day, preferrably in one injection.

The preferred dosage for HIVCIDE capsules is about 0.1 to 2 g of HIVCIDE condensate per serving and for

about 2-4 times a day.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 shows the HIV numbers in the five HIV positive patients as described in EXAMPLE 5, infra. These five HIV-positive patients were treated with daily intravenous injection of HIVCIDE injection solution (Example 1, infra) for 3 months. Patient #1 (, solid line with solid arrow) had received AZT and DDI treatment for 3 months. Ten (10) days after the discontinuance of the AZT and DDI treatment, patient #1 started the HIVCIDE daily injection. Patient #5 (->, solid line with open arrow) received the HIVCIDE treatment concurrently with [alpha]-interferon. Results for Patents #2, #3 and #4 are indicated as (dash line with solid arrow), -- (solid line with solid circle) and - - - - (dash line with solid circle), respectively.

DETAILED DESCRIPTION OF THE INVENTION

Traditional Chinese medicine has been in existence for more than two thousand years. It has a proven record of success for curing many kinds of diseases. Traditional Chinese medicine utilizes a variety of herbs and natural substances. Each herb/natural substance has its unique characteristics. By combining and balancing the unique characteristics of herbs, a doctor can prescribe a formulation with enhanced medicinal activities and with less or no toxicity by synergizing the medicinal effects among various herbs, while in the meantime, cancelling out or neutralizing the toxic effects of the herbs. This, in Chinese herbal medicine, is regarded as to regulate between negative/hypoactive characteristics ("yin") and positive/hyperactive characteristics ("yang"),

Under the definitions set forth in the traditional Chinese medicine, "yin" is defined as drugs which cure cold syndrome (which itself has hot or warm property), and "yang" is defined as drugs which cure heat syndrome (which itself has cold or cool property).

The pharmaceutical combination of the present invention comprises fourteen (14) ingredients, which are particularly effective in treating patients with HIV. Out of the 14 ingredients, four (4) ingredients are the core ingredients which contribute to the primary efficacy and healing effect of the composition. They are: (1) diffuse hedyotis/spreading hedyotis (Pharmaceutical name: Herba Hedyotidis diffusae; Botanical name: Hedyotis diffusa Willd.); (2) bistort rhizome (Pharmaceutical name: Rhizoma Bistortae; Botanical name: Polygonum bistorta L.); (3) giant knotweed rhizome (Pharmaceutical name: Rhizoma Polygoni Cuspidati; Botanical name: Polygonum cuspidatum Sieb. et Zucc.), and (4) Chinese magnoliavine fruit (Pharmaceutical name: Fructus Schisandrae Chinensis; Botanical name: Schisandra chinensis (Turcz.) Baill., S. sphenanthera Rehd. et Wils.). The core ingredients are functioned in clearing heat and toxic substances while improving immune system and circulation, curing symptoms of jaundice, and having beneficial effect on internal organs.

There are six (6) additional ingredients that are used to improve and balance the pharmaceutical effects activities produced by the above named core ingredients. These six ingredients also have toning effect and can improve blood circulation in the liver. These six ingredients are: (1) Asiatic moonseed rhizome (Pharmaceutical name: Rhizoma Menispermi; Botanical name: Menisermum dauricum DC); (2) baical skullcap root (Pharmaceutical name: Radix Scutellariae; Botanical name: Scutellaria baicalensis Georgi); (3) bovine biliary powder (Zoological name: Vesica Fellea Bovus); (4) tumeric root-tuber (Pharmaceutical name: Radix Curcumae; Botanical name: Curcuma wenyujin Y. H. Lee et Cl Ling); (5) Hawthorn Fruit (Pharmaceutical name: Fructus Crataegi; Botanical name: Crataegus pinnatifida Bge.); and (6) sanqui (Pharmaceutical name: Radix Notoginseng; Botanical name: Panax notoginseng (Burk.)).

Finally, there are additional five (4) ingredients which are used to primarily provide nutrients and energy sources for patients so as to expedite the recovery process. These ingredients include: (1) barbary wolfberry fruit (Pharmaceutical name: Fructus Lycii; Botanical name: Lycium barbarum L.); (2) figwort root (Pharmaceutical name: Radix Scrophulariae; Botanical name: Scrophularia ningpoensis); (3) Chinese angelica (Pharmaceutical name: Radix Angelicae sinensis; Botanical name: Angelica sinensis (Oliv.) Diels); and (4) milkvetch root (Pharmaceutical name: Radix Astragali; Botanical name: Astragalus membranaceus (Fisch.) Bge.). Among these ingredients, milkvetch root (Radix Astragali) also has the capacity of improving immunological functions of the body to fense off diseases.

The pharmaceutical composition of the present invention differs from the composition described in the parent application by not containing red ginseng (Radix Ginseng Rubra). It was found that the presence of red ginseng appeared to cause relapse of the disease after termination of the use of the pharmaceutical composition. Also, the pharmaceutical composition of the present invention is particularly suitable for intravenous injection and less effective for oral administration, which is also slightly different from the

pharmaceutical composition of the parent application, where both capsules (in the form of HIVCIDE powder) and intravenous injection are effective.

The pharmaceutical names, botanical or zoological names, family names, common descriptions, and major ingredients of the herbs used in the present invention are shown in Table 1.

TABLE 1

Herbs of the Present Pharmaceutical Composition

Pharmaceutical Name	Botanical/ Zoological Name	Family	Common Description	Major Ingredients
Herba Hedyotidis Difffusae	Hedyotis diffusa (Willd.) Roxb., also known as Oldenlandia diffusa	Rubiaceae	heydyotis, oldenlandia	hentriacontane, stigmastatrienol, ursolic acid, oleanolic acid, [beta]-sitosterol, [rho]-coumaric, [beta]-sitosterol-D-glucoside
Radix et Rhizoma Polygoni Cuspidati	Polygonum cuspidatum Sieb. et Zucc.	Polygonaceae	Giant Knotweed root and Rhizome	emodin, chryso-phanol, rheic acid, emodin monomethyl ether, polygonim, and physcion-8-[beta]-D-glucoside
Rhizoma Bistortae	Polygonum bistorta L.	Polygonaceae	Bistort Rhizome	n/a
Rhizoma Menispermi	Menispermum dauricum DC.	Menispermaceae	Asiatic Moonseed Rhizome	n/a
Radix Scutellariae Baicalensis	Scutellaria Baicalensis Georgi	Labiatae	Baical Skullcap Root	baicalein, baicalin, wogonin, wogonoside, neobaicalein, oroxylin aglucuronide, camphesterol, [beta]-sitosterol, benzoic acid
Vesica Fellea Bovus	Bovine Biliary powder			n/a
Radix Astragali membranaceus	Astragalus membranaceus (Fisch.) Bge. var. mongholicus. (Bge.) Hsiao or Astragalus membranaceus (Fisch.) Bge.	Leguminosae	Milkvetch Root	D-[beta]-asparagine, 2',4'-dihydroxy-5,6-dimethoxyisoflavane, calycosin, formononetin, cycloastragenol, astragalosides, choline, betaine, kumatakenin, sucrose, glucoronic acid, [beta]-sitosterol
Fructus Lycii barbarum L.	Lycium	Solanaceae	Barbary Wolfberry Fruit	betaine, carotene, physalien, thiamine, riboflavin,

vitamin C, [beta]-
sitosterol,
linoleic acid

Radix Panax noto- Araliaceae San-chi, Arasaponin A,
Notoginseng ginseng (Burk.) notoginseng, arasaponin B,
F.H. chen, P. Tian qi, Shen san dencichine
pseudoginseng qi
Wall, P. sanchi
Hoo.

Radix Scrophularia Scrophulariaceae Figwort Root, 1-asparagine,
Scrophulariae ningpoensis Scrophularia oleic acid,
Ningpoensis Hemsl. orS. linoleic acid,
buergeriana stearic acid,
Miq. carotene

Fructus Schisandra Magnoliaceae Chinese sesquicarene, [beta]-
Schisandrae chinensis Magnoliavine bisabolene, [beta]-
Chinensis (Turcz.) Baill., Fruit, schisandra chamigrene, [alpha]-
S. sphenanthera fruit ylangene,
Rehd. et Wils. schizandrin,
pseudo-[gamma]-
schizandrin,
deoxyschizandrin,
schizandrol,
citral,
stigmasterol,
vitamin C,
vitamin E

Tuber Curcumae Curcuma Zingiberaceae Turmeric Root- d-camphene, d-
wenyujin Y. H. tuber, curcuma camphor, 1-[alpha]-
Lee et C. Ling., curcumene, 1-[beta]-
or Curcuma curcumene,
Longa L., or curcumin,
Curcuma demethoxycurcumin,
aromatica bisdemethoxy-
Salisb., or curcumin,
Curcuma turmerone, ar-
zedoaria Rosc., turmerone,
or Curcuma carvone, [rho]-
kwangsiensis tolylmethyl-
S. G. Lee et C. carbinoldiferuloyl-
F. Liang methane

Fructus Crataegi Crataegus Rosaceae Hawthorn Fruit crategolic acid,
pinnatifida Bge.; citric acid,
C. pinnatifida tartaric acid,
Bge. var. major flavone, sugars,
N.E. Br. or C. glycosides,
suneata Sieb. et vitamin C
Zucc.

Radix Angelicae Angelica Umbelliferae Chinese butylidene
Sinensis sinensis (Oliv.) Angelica root, phthalide, Diels tang-kuei ligustilide, n-butylidene-phthalide, sequiterpenes, carvacrol, dihydrophthalic anhydride, sucrose, vitamin B12, carotene, [beta]- sitosterol

Diffuse hedyotis or spreading hedyotis (Herba Hedyotidis Diffusae) belongs to the family of Rubiaceae. The entire plant is used as an herbal medicinal component. The herb has no toxicity. The herb is harvested in summer and autumn in mainland China and in late spring or early winter in Taiwan. In "Materia Medica" (Chinese Herbal medicine), compiled and translated by Dan Bensky & Andrew Gamble, diffuse hedyotidis clears heat and resolves dampness by promoting urination. It is particularly useful for relieving hot painful urinary dysfunction and damp-heat jaundice. Diffuse hedyotidis is the major ingredient in the present herbal pharmaceutical composition which contributes to the medicinal effect on liver diseases and HIV.

Bistort rhizome (Rhizoma Bistortae) is the dried rhizome of the plant Polygonum bistorta L. It belongs to the

family of Polygonaceae. Bistort rhizome has moderate cool property (meaning that bistor rhizome is an "yang" herb). It can be used to remove toxic heat, to promote the subsidence of swelling and to stop bleeding.

Giant knotweed rhizome (Radix et Rhizoma Polygoni Cuspidati) is the dried rhizome and root of polygonum cuspidatum Sieb. et Zucc. It belongs to the family of Polygonaceae. The plant is grown throughout China, especially Jiangsu, Zhejiang, Anhui, Guangdong, Guangxi, Sichuan, and Guizhou provinces. The plant is harvested in spring and autumn. Giant knotweed rhizome is normally used to dispel damp, to eliminate blood stasis and alleviate pain, to relieve cough, and to resolve phlegm.

Chinese magnoliavine fruit (Fructus Schisandrae) is the dried ripe fruit of Schisandra chinensis (Turcz.) Baill. or Schisandra sphenanthera Rehd. et Wils. It belongs to the family of Magnoliaceae. The former, the best of its kind, is produced in northern parts of China and is habitually called "Northern schisandra fruit"; the latter is commonly referred to as the "Southern schisandra fruit" as it is produced in the southern parts of China. Both kinds can be used for the pharmaceutical preparation of the present invention. The fruit is collected in autumn and dried under the sun after removing the fruit stalks. Chinese magnoliavine fruit is generally used to arrest disharges, replenish qi, promote fluid secretion, tonify the kidney, and induce sedation. Chinese magnoliavine fruit can also decrease the level of GPT (glutamate-pyruvate transaminase) in patients with hepatitis.

Asiatic moonseed rhizome (Rhizoma Menispermi) is the dried rhizome of Menispermum dauricum DC. It belongs to the family of Menispermaceae. Asiatic moonseed rhizome has cool property. It can be used to remove toxic heat and relieve rheumatic pains.

Baical skullcap root (Radix Scutellariae) is the dried root of Scutellaria baicalensis georgi. It belongs to the family of Labiatae. The plant is produced in the provinces of Hebei, Shanxi, Inner Mongolia, etc., and collected in spring or autumn. Baical skullcap root is used to remove damp-heat, counteract toxicity, arrest bleeding, and prevent abortion, in patients.

Bovine biliary powder is the gallbladder of the cow, Vesica Fellea Bovus. It can clear heat and alleviate spasms.

Turmeric root-tuber (Radix Curcumae) is the dried root tuber of Curcuma wenyujin Y. H. Lee et C. Ling., or Curcuma Longa L., or Curcuma aromatica Salisb., or Curcuma zedoaria Rosc., or Curcuma kwangsiensis S. G. Lee et C. F. Liang. The herb is mainly produced in Sichuan, Zhejiang, Guangdong, and Guangxi provinces in China, and harvested in winter or spring, washed clean after the removal of the hairy rootlets, boiled thoroughly, and dried in the sun. It belongs to the family of Zingiberaceae. Turmeric root-tuber tastes bitter and had cool property. It can be used to clear heat, alleviate spasms and chest pain, and resolve phlegm.

Hawthorn fruit (Fructus Crataegi) is the dried ripe fruit of Crataegus pinnatifida Bge. var major N. E. Br., or Crataegus pinnatifida Bge., or Crataegus cuneata Sieb. It is produced primarily in Henan, Jiangsu, and Shandong provinces of China. It is harvested in autumn, sliced, and dried in sunlight. It belongs to the family of Rosaceae. Hawthorn fruit is normally used to stimulate digestion and promote the functional activity of the stomach. It can also improve the normal blood flow and dissipate blood stasis.

Sanqi, or San-chi, (Radix Notoginseng) belong to the family of Araliaceae. Sanchi (Sanqi) is the dried root of Panax notoginseng (Burk.) F. H. Chen. The plant is also known as P. pseudoginseng Wall and P. sanchi Hoo. The plant grows in Yunnan, Guangxi, Sichuan, Guizhou, and Jiangxi provinces of China, and is harvested in the autumn or winter of the third or seventh year, either before the flowers bloom (better) or after the fruit is ripe. H. Gao et al., Pharmaceutical Research, (1996) 13(8): 1196-1200, disclose that polysaccharides from Panax notoginseng (San-Chi) have immuno-stimulating activities in vitro.

Barbary wolfberry fruit (Fructus Lycii) is the dried ripe fruit of Lycium barbarum L. It belongs to the family of Solanaceae. The plant is mainly produced in Ningxia, Gansu, and Qinghai provinces of China. It is harvested in summer and autumn. It nourishes and tonifies the liver and kidneys. It can also replenish vital essence and improve eyesight.

Figwort Root (Radix Scrophulariae) is the dried root of Scrophularia ningpoensis Hemsl. It belongs to the family of Scrophulariaceae. The herb is chiefly produced in Zhejiang and Sichuan provinces of China and harvested in winter when the part of the plant above-ground has withered. The roots are piled and dried in sunlight alternately until the inside becomes black and then sliced for use. Figwort root can reduce heat from blood. It als has nourishing capacity and can counteract toxicity.

Chinese angelica (Radix Angelicae Sinensis) is the dried root of Angelica sinensis (Oliv.) Diels. It belongs to the family of Umbelliferae. The herb is mainly produced in Gansu and Shanxi provinces of China. It is harvested in late autumn, smoked dry on slow fire after getting rid of the rootlets, sliced, or stir-baked with wine. Chinese angelica can enrich blood, promote blood circulation, regulate menstruation, relieve pain, and relax bowels.

Milkvetch root (Radix Astragali) is the dried root of Astragalus membranaceus (Fisch.) Bge. var. mongholicus. (Bge.) Hsiao or Astragalus membranaceus (Fisch.) Bge. It belongs to the family of Leguminosae. The herb is mainly produced in Shanxi, Gansu, Heilongjiang, and Inner Mongolia of China. The plant of four-year old or older is harvested in spring or autumn. Milkvetch root can promote discharge of pus and the growth of new tissue.

The pharmaceutical composition of the present invention is suitable for preparation in a scale typical for pharmaceutical industry as well as for smaller measure.

In the process for making the pharmaceutical composition of the present invention, the individual herbal components are pretreated according to the common procedures. The herbs are cut and put in a container with water to boil and simmer twice. The first time of simmering takes two hours, the solution is collected, and water is added for the second time of simmering for about 1.5 hour. The solutions from the simmering steps are collected and then filtered by a sieve/filter. The filtrate is then condensed from about 1.4 fold by volume to about 1.0 fold by volume. The resulting condensate is pasty-like. Optionally, the herbs can be simmered, filtered and condensed again. The second condensate is then mixed with the first condensate to form the "HIVCIDE condensate," which becomes the basic content of the HIVCIDE injection solution. When used, the HIVCIDE injection solution is further diluted 1:5 to 1:10 by volume with a suitable solution. The gram weight of the HIVCIDE injection solution described herein is referred to the weight of the "HIVCIDE condensate."

The following example is illustrative, but not limiting the scope of the present invention. Reasonable variations, such as those occur to reasonable artisan, can be made herein without departing from the scope of the present invention.

EXAMPLE 1
Pharmaceutical Preparation of HIVCIDE

The kinds and amounts of the herbal ingredients used in the process of making the pharmaceutical composition of the present invention are described in Table 1. The pharmaceutical composition is called "HIVCIDE," which is named after "HIV-killer" ("-cide" means "killer"). HIVCIDE was formulated as injection solution and capsules.

Table 2. Ingredients Used in Making HIVCIDE Injection Solution and Capsules

Component	Amount (kg)
Diffuse hedyotis	3.32
Bistort Rhizome	2.49
Giant Knotweed root and Rhizome	0.83
Asiatic Moonseed Rhizome	0.83
Baical Skullcap Root	0.83
Bovine Biliary powder	0.083
Milkvetch Root	1.66
Barbary Wolfberry Fruit	2.49
Sanchi	0.83
Figwort root	1.66
Chinese Magnoliavine Fruit	1.66
Turmeric Root-tuber	0.83
Hawthorn fruit	1.66
Chinese Angelica	0.83

(1) Quality Controls of Raw Materials

Quality control tests carried out for each individual raw material were according to conventional methods used in the herbal pharmaceutical field, which include, but are not limited to, physical appearance, loss on

drying, total ash, acid insoluble ash, alcohol extracts, water extracts, TLC, HPLC, heavy metals, microbial counts and residual pesticides. Bovine biliary powder was tested for appearance, TLC and general chemistry.

(2) Manufacturing Process

The individual herbal components were pretreated according to common procedures. The herbs were weighed according to Table 2. A flowchart of the manufacturing process for making HIVCIDE injection solution is provided in Table 3:

TABLE 3
Manufacturing Process For Making HIVCIDE
Manufacturing Process Quality Control Procedure
Raw Herbs Delivered Quality Control of Raw Herbs:
Physical Appearance

Preparation
(cutting, drying, etc.)

Quality Control of Raw Herbs: Physical Appearance Loss on Drying Total Ash Acid Insoluble Ash Alcohol Extracts Water Extracts TLC HPLC Heavy Metals Microbial Residues Pesticide Note: Bovine biliary powder (also known as Bovis Bezoar) is only tested for appearance, TLC and General Chemistry
1<st >Extraction
Parameter Set:
1. Dial Set Temperature at 95[deg.] C. with the Acceptable Range of 90[deg.] C.-100[deg.] C.
2. Dial Set Steam Pressure at 2 kg/cm<2>.
3. Dial Read Out the Lid Pressure at 0.2-0.4 kgf/cm<2 >Range.
4. Extract for 45 minutes.
In-Process Quality Control: (take 10-15 g as test sample) Concentration of Solid Content Concentration of Water Content

1<st >Concentration
Parameter Set:
1. Dial Read Out Vacuum at -60≈76 cmHg.
2. Dial Read Out Temperature at 40[deg.] C. ± 5[deg.] C.
3. Process for 40 minutes.

Raw Herbs Add
Water 350 L ± 10%
2<nd >Extraction
Parameter Set:
1. Dial Set Temperature at 95[deg.] C. with the Acceptable Range of 90[deg.] C.-100[deg.] C.
2. Dial Set Steam Pressure at 2 kg/cm<2>.
3. Dial Read Out the Lid Pressure at 0.2-0.4 kgf/cm<2 >Range.
4. Extract for 45 minutes

2<nd >Concentration
Parameter Set:
1. Dial Read Out Vacuum at -60-76 cmHg.
2. Dial Read Out Temperature at 40[deg.] C. ± 5[deg.] C.
3. Process for 40 minutes.

Combined Concentrated Extracts and
Pour into the Stainless Container
Final Yield: 6.1 kg ± 10%
Spraying Silo
Parameter Set:
1. Dial Set Temperature at 60[deg.] C.
2. Pre-heat for 15 minutes.

Fluid Bed Dryer
1. Add 3.9 kg of Starch (adj. Base on water content of the starch).
2. Dial Set In-Temperature at 60[deg.] C.; Out-Temperature at 50[deg.] C.
3. Pre-heat for 10 minutes.
4. Process for target of 2 hours ± 20 min. until LOD <5%.

Sievinig
Parameter Set:
1. Dial Read out Temperature at 23 ± 4[deg.] C.
2. Dial Read out RH at 50 ± 10%.
3. Sieve with #100 followed by #80.

HIVCIDE Powder Quality Control of Drug Substance:
Physical Appearance
Loss on Drying
Total Ash
Acid Insoluble Ash
Alcohol Extracts
Water Extracts
TLC
HPLC
Heavy Metals
Microbial
Residues Pesticide
Stability

The individual herb was cut into small pieces and thoroughly mixed together. The mixed herbs were placed in bags with sufficient space to spread out. The bags were placed in an extractor with 350 L (±10%) of water and soaked for about 60±10 min. The herbs were first extracted at 95±5[deg.] C. under steam pressure of 2 kg/cm<2 >and lid pressure of 0.2-0.4 kg/cm<2 >for 45 min. The water extract was collected into a concentrator and concentrated at 40±5[deg.] C. under vacuum of -60 to -76 cmHg for 40 min to form the first concentrate.

The herbs in the bags were recovered from the extractor, placed into another 350 L (±10%) of water and extracted again at 95±5[deg.] C. under steam pressure of 2 kg/cm<2 >and lid pressure of 0.2-0.4 kgf/cm<2 >for 45 min. The extract from the second extraction was collected into a concentrator and concentrated at 40±5[deg.] C. under vacuum of -60 to -76 cmHg for 40 min to form the second concentrate.

The first and second concentrates were combined and poured into a stainless container. The total weight of the combined concentrates was about 6.1 kg±10%. The combined 1<st >and 2<nd >concentrates were called the "HIVCIDE condensate."

For the HIVCIDE injection solution, about 0.1 to 1 g, preferably 0.4 g of the "HIVCIDE condensate" was dissolved in about 1 ml of the injection buffer. About 5 ml of the injection solution was poured into an ampoule.

For the HIVCIDE powders (which were packaged into HIVCIDE capsule), about 3.9 kg of starch (adjustable

based on the water content of the starch) were added to the HIVCIDE condensate and spray dried in a fluidized bed setting at in-temperature of 60[deg.] C. and out-temperature of 50[deg.] C. for approximately 120±20 min until LOD (limit of detection)<5%.

The resultant powders were passed through a 100-mesh sieve and then a 80-mesh sieve. The final yield of the HIVCIDE powders were about 9.5≈10.5 kg. The HIVCIDE powders were further packaged into capsules. Any conventional capsules, including, but not limited to, natural gelatin, pectin, casein, collagen, protein, modified starch, and polyvinyl pyrrolidone, were suitable for encapsulation.

There were two dosage forms of HIVCIDE capsules: A 500 mg of the HIVCIDE capsule, which contained about 305 mg of the "HIVCIDE condensate" and about 195 mg of starch; and a 220 mg of the HIVCIDE capsule, which contained about 134 mg of the "HIVCIDE condensate" and about 86 mg of starch.

(3) In-Process Quality Controls

After the extracts were concentrated, a 10-15 g sample was collected and the concentrations of the solid content and the water content were determined by methods described in US Pharmacopoeia, China Pharmacopoeia, and/or Japanese Pharmacopoeia.

EXAMPLE 2

Acute Toxicity Study of HIVCIDE in Animals

Purpose:

The following experiment was conducted at the Toxicology Laboratory of the Institute of Labor, Health, and Occupational Disease of Heilungkiang Province in China to examine acute toxicity of the HIVCIDE during intravenous injection in animals.

Methods:

Experimental animals were Japanese big-ear white rabbits obtained from the Animal Center of Haerbin Medical University in Haerbin, Heilungkiang Province, China. These rabbits were characterized by the obvious blood vessels on ears which facilitates the operation of injection during the experiments.

Ten (10) rabbits were obtained including six (6) males and four (4) females, each weighing between 1900 g to 3000 g.

The rabbits were randomly divided into two (2) groups, five rabbits in each group including two (2) females and three (3) males. The HIVCIDE injection solution was intravenously injected into the two groups of rabbits through the veins on their ears at dosages of 10 g and 15 g of HIVCIDE condensate per kg of rabbit body weight, respectively.

The HIVCIDE concentration was about 1 g/ml, so that the higher dosage group was about 15 ml/kg, which was roughly equal to a sixty (60) kg-weighted adult treated with 900 ml of the HIVCIDE injection solution.

The rabbits were observed for behaviour continuously for a period of two (2) weeks after intravenous injections. Observation was conducted hourly at day 1; during the following days, observation was conducted four to six (4-6) times per day.

At the end of the observation period, rabbits were sacrificed and dissected to examine the eyes, liver, lung, and spleen to determine adverse effects.

Results:

No abnormal behavior was found in the rabbits during the entire observation period. All animals exhibited normal body weight gains. No organ abnormality was observed. After sacrifice and dissection of the animals, inspection of the eyes, liver, lung, and spleen showed no extraordinary syndromes. The results indicate that, when comparing with the general acute toxicity index, the HIVCIDE injection solution showed no symptoms of acute or chronic toxicity.

Conclusion:

The intravenous administration of HIVCIDE injection solution up to 15 g/kg was well-tolerated, which was approximately equal to injection of 900 ml of HIVCIDE into a 60-kg of human. The HIVCIDE demonstrated no acute toxicity in rabbits.

EXAMPLE 3
Effects of the HIVCIDE on HIV in Cell Cultures

Purpose:

The following experiment was to determine the effectiveness of the HIVCIDE injection solution gainst HIV in cell cultures.

Methods:

MT4 cells were cultured in HIV-1 suspension liquid of 100 TCID50 in a 96-orifice culture plate. MT4 is a human T-cell lymphotropic virus type 1-transformed cell line. The culture condition was set at a temperature of 37[deg.] C. and under 5% CO2. The duration of the culture was seven (7) days.

The HIVCIDE injection solution of the present invention was added to the cultural wells at various concentrations. The morphology of the MT4 cells was observed under microscope by conventional methods.

Results:

No pathological changes of MT4 cells were observed in the cultural wells where the HIVCIDE injection solution was added to in adequate concentrations. The inhibition of the pathological changes of MT4 cells indicated that the HIVCIDE injection solution had inhibitory effect on pathological changes of the cultured cells caused by HIV.

The effective concentration of the HIVCIDE injection solution for inhibition of the pathological changes of MT4 cells was more than 12.5 mg/ml. To achieve a 50% of inhibition, the concentration of the HIVCIDE herbal composition was 25 mg/ml.

Conclusion:

The HIVCIDE injection solution was effective in inhibiting pathological changes in cells caused by HIV-1 in vitro.

EXAMPLE 4
A Case Study on an HIV-Patient Treated with HIVCIDE

Purpose:

The following clinical trial was conducted in the Infectious Disease Hospital in Shanghai, China to test the effectiveness of the herbal composition of the present invention in treating an HIV-infected patient.

Methods:

A fifty-year Chinese male patient diagnosed with HIV infection complicated by herpes zoster was treated with anti-virus regimens by the combination of western medicine and the herbal composition of the present invention during hospital stay.

Results:

The patient was confirmed of HIV-infection by Rapid Agglutinin Assay. At the time of the initial diagnosis, the patient showed no symptoms. Ten months after the initial diagnosis, the patient quickly developed an herpetiform rash over the front of the left side of the check extending over the nick, the shoulder, and the upper left arm. The patient was then admitted into the Hospital shortly thereafter.

At the hospital, the result of the physical examination was normal except the skin rash. The pathology tests confirmed normal renal function. The functional tests of the liver showed a slightly increased level of serum [gamma] glutamyl transpeptidase and acetyl glucuronidase. Hepatitis viral tests showed negative for Hepatitis B virus and Hepatitis C virus (HBV-DNA and HCV-RNA). However, Hepatitis G viral test showed positive

for HGV-RNA. The immunological studies showed that the [beta]-2 microglobulin level was 2.4-2.5 mg/ml.

During the hospital stay, haemoglobin and erythrocytes levels of the patient were slightly decreased, while the levels of the leukocyte and platelet were normal. Peripheral blood lymphocytes counts showed that T4 cells were decreased to 2.76*10/L (32.9%) and the ratio of T4/T8 cell was 1.16. Thus, the diagnosis is that the patient was with HIV infection complicated by herpes zoster.

During hospital stay, the patient had diarrhea and dry cough for a few days and was cured. The patient showed HIV antibody positive by ELISA, and his T4 cells further decreased to 25.4% and the ratio of T4/T8 cells was inverted to 0.94.

The patient's T4 cells and the ratio of T4/T8 gradually increased after intravenously injected with the HIVCIDE injection solution. In about three months of HIVCIDE treatment, his T4 cells were returned to 40.7%, and the ratio of T4/T8 was improved to 1.45. The skin rash gradually disappeared and completely recovered by the end of November.

Conclusion:

The HIVCIDE injection solution was effective in reducing symptoms of the HIV-infected patient in a treatment regime together with western medicine.

EXAMPLE 5

Clinical Study of Five HIV-Positive Patients Treated with HIVCIDE Injection Solution

Purpose:

The following clinical trial was conducted in De-Tang Hospital (National AIDS Therapy Center) in Beijing, China to test the effectiveness of HIVCIDE in HIV patients.

Methods:

Five (5) HIV-postive patients were intravenously injected with HIVCIDE daily. The HIV infection was confirmed by western blotting. The profile of the patients are shown in Table 4:

TABLE 4

Medical Profile of the HIV Patients Participated in the Clinical Study

Patients	Gender	Age	History	Diagnosis
1	Male	32	2 years	AIDS (Stage IV)
2	Male	25	0.5 year	AIDS (Stage II)
3	Female	32	1 year	AIDS (Stage IV)
4	Male	31	1 year	AIDS (Stage III)
5	Male	17	3 weeks	HIV acute infection

The patients were treated according to the following regimen:

The HIVCIDE injection solution was prepared according to EXAMPLE 1, supra. Prior to the injection, five (5) ml of HIVCIDE injection solution was diluted in 250 ml of 5% glucose solution. The diluted solution was injected intravenously once per day for three (3) consecutive days. If no adverse reactions were observed, the dosage was increased to 15 ml of HIVCIDE in 250 ml of 5% glucose solution, and the patients were injected intravenously once per day reactions for two (2)-three (3) months.

Additionally, patient #1 was treated with AZT and DDI therapy for 3 months. The AZT and DDI therapy was discontinued ten (10) days prior to the HIVCIDE treatment. Patients #2, #3, and #4 were given HIVCIDE daily injection alone. Patient # 5 was given combined treatment of [alpha]-interferon and HIVCIDE.

About 3 ml of blood sample were taken from the patients each time before, during, and after the treatment and further tested for HIV numbers. CD4 counts were determined in Patients #1, #2 and #4 before and after the respective treatment.

Results:

No adverse reactions to the HIVCIDE injection solution were observed. Three out of the four AIDS patients had different degrees of fatigue before the treatment of HIVCIDE. There was marked improvement in the fatigue after the treatment with HIVCIDE in the three AIDS patients.

The change in HIV viral load (copies/ml) in plasma before and after HIVCIDE treatment is shown in Table 5 and FIG. 1.

TABLE 5
Change In HIV Viral Load In Plasma of HIV-Positive Patients

Patients	before treatment	1st month during treatment	2nd month during treatment	3rd month, at the end of treatment
1	1.9 * 10<4>*	1.7 * 10<5>	6.3 * 10<3>	1.5 * 10<4>
2	1.5 * 10<4>	6.3 * 10<3>	3.8 * 10<2>	
3	7.3 * 10<3>	3.2 * 10<3>		
4	3.0 * 10<5>	3.7 * 10<3>***	1.1 * 10<6>	
5**	3.9 * 10<5>	2.6 * 10<3>	1.8 * 10<3>	

*The lower HIV numbers observed in Patient #1 before treatment might be contributed to the 3-month treatment of AZT and DDI prior to the start of HIVCIDE treatment.
**Patient #5 received concurrent treatment of HIVCIDE and [alpha]-interferon.
***The plasma HIV viral load of Patient #4 was detected 6 weeks after the HIVCIDE treatment.

As shown in Table 5 and FIG. 1, four of the five HIV-positive patients (i.e., patients #1, #3, #4, and #5) showed decrease in HIV viral load in two months. Two out of these four patients (i.e., patients #1 and #4) showed a rebound in viral load after the end of the third-month HIVCIDE treatment. The other two patients, (i.e., patients #3 and #5), showed significant clinical symptom relief, and did not provide plasma sample for analysis at the end of the third-month treatment. Patient #2, whose plasma sample was not taken at the end of the second month treatment, showed a 40-time reduction in HIV viral load at the end of the third-month HIVCIDE treatment. Patient #2 also demonstrated significant clinical symptom relief at the end of the third-month HIVCIDE treatment. However, because patient #5 received concurrent treatment with [alpha]-interferon, the results of this patient were difficult to assess.

The results of the HIV viral load study indicate that all of the patients demonstrated reduction in HIV viral load after HIVCIDE treatment. However, great fluctuation in the replication of HIV was observed in two of the five patients. For example, patient #4's HIV viral load decreased significantly after 6 weeks of treatment but rebounded after the end of the three-month HIVCIDE treatment. Patient #1 also experienced a rebound (although to a much lesser degree than that of patient #4) at the end of the third-month treatment.

In addition to HIV viral load, CD4 count (count/mm<3>) was measured in three patients (i.e., patients #1, #2, and #3) at the end of the three-month HIVCIDE treatment. For example, the CD4 count of patient #2 rose from 285 to 510/mm<3 >after 3 months of HIVCIDE treatment and continued to rise to 630/mm<3 >2 months after the discontinuance of the HIVCIDE treatment. The CD4 count of Patient 4 was 190/mm<3 >prior to HIVCIDE treatment, 40/mm<3 >after 3-month of HIVCIDE treatment, and 360/mm<3 >2 months after the discontinuance of the HIVCIDE treatment.

Conclusion:

The results indicated that HIVCIDE injection solution was effective in treating HIV/AIDS patients.

EXAMPLE 6
Clinical Study of HIV-Positive Patients with HIVCIDE and HIVCIDE Capsules Treatment in Russia

Purpose:

The following experiment was conducted in Hospital in Siberia, Russia to examine the effectiveness of the HIVCIDE injection solution and HIVCIDE capsules against HIV infection.

Methods:

Five (5) HIV-Positive patients were treated with the HIVCIDE injection solution and HIVCIDE capsules obtained from Gongming Pharmaceutical Co., Ltd, Heilongkiang Province, China. The HIVCIDE injection solution and HIVCIDE capsules were prepared according to EXAMPLE 1, supra.

The profile of the patients are shown in Table 6:

TABLE 6
Medical Profile of the HIV-Positive Patients
Participated in the Clinical Study in Russia

Patients	Gender	Age	History	Diagnosis
1	Female	23	2 years	AIDS (Grade A3), adenitis, hepatitis C, Syphilis, Citomegalo infection, Gonorrhea
2	Female	28	2 year	AIDS (Grade A3), adenitis, hepatitis B and C, Gerdeo and Citomegalo infection, Gonorrhea, drug abuse
3	Male	35	1 year	AIDS (Grade B2), adenitis
4	Male	22	1 year	AIDS (Grade B2), adenitis, hepatitis C, drug abuse
5	Male	34	several months	AIDS (Grade A3), adenitis, hepatitis B and C, 10% weight loss, drug abuse

The patients were concurrently treated with HIVCIDE injection solution and HIVCIDE capsules. For HIVCIDE injection solution, about 5 ml of HIVCIDE injection solution (containing approximately 0.4 g of HIVCIDE condensate per ml) were diluted in 250 ml of 5% glucose. Each patient was given an intravenous injection of the HIVCIDE injection solution once daily for 3 months. For HIVCIDE capsules, each patient was given 6 HIVCIDE capsules (220 mg of HIVCIDE capsules) per time (before meal), three times per day for 3 months.

Blood samples were taken from the patients before, during, and after the HIVCIDE treatment for tests of CD4 cells count.

Results:

All patients experienced pain in the stomach and right rib area during the first two weeks of the treatment period. Patient #5 also vomited every morning for a week in the third month of the treatment period.

The CD4 cells count of the patients is shown in Table 7:

TABLE 7

Change in CD4 Cells Count in Patients With HIV Infection

Patients	before treatment	2nd month during treatment	2 months after the completion of the 3-month treatment
1	477	641	849
2	740	1140	705
3	421	-	527
4	440	490	669
5	625	-	814

During the treatment period, all patients demonstrated positive response to the HIVCIDE treatment except the minor adverse reactions as mentioned above. The physical condition of all of the patients had clearly improved after one month of the HIVCIDE treatment. In particular, patients felt stronger, were happier and showed improved appetite. Anaphylaxis, melancholy, stomach pain and discomfort disappeared. For some of the patients who had constipation, the symptom was greatly improved. For some of the patients who had experienced insomnia, the sleepless problem was also greatly improved. Also, patients #4 and #5 had gained about 5 kg at the end of the three-month HIVCIDE treatment. And patients # 2 and #4 stopped taking narcotic analgesics for pain relief. The biochemical indicators, including HBV and HCV, in some of the patients were also clearly improved. The M antibody in Citomegalo virus, genorrhea antibody and HBC antibody also disappeared.

Also, as shown in the last column of Table 7, 2 months after the completion of the 3-month treatment period,

all of the patients CD4 cell counts were continued to increase, which indicated that no relapse had occurred after the termination of the HIVCIDE.

Patients #1 and #2 had received a second 3-month course of HIVCIDE treatment after the completion of the first 3-month HIVCIDE program. No adverse effect was observed at the end of the second course of HIVCIDE treatment.

Conclusion:

The combined use of HIVCIDE injection solution and HIVCIDE capsules was effective in reducing symptoms in HIV-positive patients.

US5178865

CHINESE HERBAL EXTRACTS IN THE TREATMENT OF HIV RELATED DISEASE IN VITRO

Inventor(s): HO DAVID D [US]; LI XILING, et al

The invention features herbal extracts from ten (10) Chinese Herbal Medicines demonstrating significant in vitro and ex vivo anti-HIV activity and their use for the diagnosis and treatment of HIV and HIV-related disease.

TECHNICAL FIELD

This invention is in the fields of medicine and pharmacology. In particular, the invention features ten (10) commercially available Chinese Herbal Extracts (CHEs) exhibiting in vitro and/or ex vivo activity against the etiologic agent of Acquired Immune Deficiency Syndrome (AIDS) and AIDS related complex (ARC).

BACKGROUND ART

Acquired Immune Deficiency Syndrome is a pandemic immunosuppressive disease which results in life threatening opportunistic infections and malignancies. A retrovirus, designated human immunodeficiency virus (HIV-1(HTLV-III LAV)), has been isolated and identified as the etiologic agent of this disease. This virus has been shown to be harbored by T helper lymphocytes and monocyte-macrophages, and it is detectable in whole blood, plasma, lymphatic fluid, serum, semen, saliva and central nervous system tissue. Ho et al., New England Journal of Medicine, 321:1621-1625 (1989). Although cells of the monocyte-macrophage lineage serve as important reservoirs of HIV infection, most of the cell-associated virus in the blood is contained within CD4+ T cells. Characteristically, then, AIDS is associated with a progressive depletion of T cells, especially the helper-inducer subset bearing the OKT4 surface marker.

Several agents have been reported to inhibit the growth of the human immunodeficiency virus in vitro. Among the agents exhibiting in vitro anti-HIV activity, some are now in clinical use, including ribavirin, zidovudine (AZT), the 2', 3'-dideoxynucleosides (DDI and DDC); ganciclovir alpha-interferon, interleukin-2, ampligen and isoprinosine. Anand et al., Lancet i,97-98 (1986); Balzarini et al., Int. J. Cancer 37:451-457 (1986); Ho et al., Lancet, i,602-604 (1985); McCormick et al., Lancet ii,1367-1369 (1984); Mitchell et al., Lancet i,890-892 (1987); Mitsuya et al., Proc. Natl. Acad. Sci. USA 83:1911-1915 and 82:7096-7100 (1985, 1986); Mitsuya et al., Science 226:172-174 (1984); Pert et al., Proc. Natl. Acad. Sci. USA 83:9254-9258 (1986); Pizzi et al., Human Biol. 22:151-190 (1950); Rozenbaum et al., Lancet i,450-451 (1985); Sandstrom et al., Lancet i,1480-1482 (1986); Veno and Kino, Lancet i,1379 (1987); Yamamoto et al., Interferon Res. 6:143-152 (1986), and Antiviral Research 7:127-137 (1987). However, no therapy to date is known to cure AIDS.

The majority of the compounds tested for use against HIV. including those referenced above, appear to be either too toxic for prolonged use or incapable of completely eliminating HIV infection from the human host. Blanche et al., Lancet i,863 (1986); De Clercq et al., J. Med. Chem. 29:1561-1569 (1986); Yarchoan et al., Lancet i,575-580 (1986); Wetterberg et al., Lancet i,159 (1987). In view of the severity of the AIDS situation and the toxicity and limited clinical efficacy of the compounds tested thus far, the scientists of the present invention have begun investigating the anti-HIV activity of extracts from Chinese medicinal herbs. Chang and Yeung, Antiviral Research 9:163-176 (1988); Chang et al., Antiviral Research 11:263-73 (1989). This interest in Chinese herbs was prompted by Chinese folklore, wherein a number of these herbs have been

reputed to have anti-infective activity and to be well tolerated by humans. A subset of these herbs now also appear to exhibit anti-HIV activity, and are disclosed herein.

However, Chinese folk medicine is based largely on anecdotal observations spanning the past several thousands of years. Hence, the effectiveness of the medicinal herbs used by folk medicine practitioners has, for the most part, not been substantiated by scientific methods. Despite this lack of scientific proof, it is quite possible that some herbal remedies may have specific therapeutic action, as was proven to be the case with the anti-malarial. qinghaosu, and perhaps even anti-HIV activity. Klayman, Science, 228:1049-1055 (1985). Consequently, with regard to the possible anti-HIV activity among Chinese herbal extracts, an urgent need exists for: 1) the identification of effective anti-HIV herbal extracts, 2) the substantive documentation, by modern scientific methods, of the effectiveness of these herbal extracts against HIV, and 3) the identification of effective anti-HIV Chinese herbal extracts that are less toxic than the currently available anti-HIV agents. The present invention satisfies this need and provides related advantages as well.

The papers cited throughout this application are incorporated herein by reference.

DISCLOSURE OF THE INVENTION

A total of fifty-six (56) herbal extracts. some of which are known to have anti-infective properties and to be non-toxic in clinical use in China, were screened for their anti-HIV activity using in vitro techniques. Of these fifty-six (56) herbal extracts, ten (10) were shown to have potent anti-HIV activity in in vitro experiments, and two (2) of these ten (10) also exhibited anti-HIV activity in ex vivo experiments.

These ten (10) include the extracts from: Sample #1--Coptis chineusis, which can be located in Western, Southern and Central China; Sample #8--Ligusticum wallichii, which can be found in Northern and Southwestern China, and Salvia miltiorrhiza, which can be located in most areas of China; Sample #21--Illicium lanceolatum, which can be located in Eastern and Southern China; Sample #30--Isatis tinctoria, which can be found in Central China, Lonicera japonica, which can be located in most areas of China, and Polygonum bistorta, which can be located in Northern, Eastern and Southwestern China; Sample #32--Salvia miltiorrhiza, which can be located in most areas of China; Sample #35--Erycibe obtusifolia, which can be found in Southern China, Taiwan, Japan, Indonesia and Northern Australia; Sample #39--Acanthopanax graciliatylus--which can be located in Central and Southwestern China and the Philippines; Sample #41--Bostaurus domesticus, which can be found in most areas of China and in Southern Africa, and Scutellaria baicaleusis, which can be located in Northern, Western and Central China and Southern Africa; Sample #44--Inula helenium, which can also be located in most areas of Northern China, and Salvia miltiorrhiza, which can be located in most areas of China; and Sample #49--Lonicera japonica, which can be located in most areas of China, and Scutellaria baicaleusis, which can be located in Northern, Western and Central China, as well as in Southern Africa. This information is reproduced in Table I below, which also provides alternative means for identifying the subject herbs.

TABLE I
SAMPLE
NAME OF HERB
CLASSIFICATION
MAJOR LOCATION
#1 Coptis chineusis
Ranunculaceae
Western, Southern and
Franch Central China
*#8 Ligusticum wallichii
Umbelliferae
Northern and
Franch and Southwestern China;
Salvia miltiorrhiza
Labiatae Most areas of China
Bunge
#21 Illicium lanceolatum
Illiciaceae
Eastern and Southern
A. C. Smith or China
Illicium henryi Diels

*#30 Isatis tinctoria L.
Cruciferae Central China
or Isatis indigotica
Fort.,
Lonicera japonica
Caprifoliaceae
Most areas of China
Thunb and
Polygonum bistorta L.
Polygonaceae
Northern, Eastern and
Southwestern China
#32 Salvia miltiorrhiza
Labiatae Most areas of China
Bunge
#35 Erycibe obtusifolia
Convolvulaceae
Southern China,
Benth Taiwan, Japan,
Indonesia and Northern
Australia
#39 Acanthopanax
Araliaceae Central and
graciliatylus Southwestern China,
W. W. Smith Philippines
*#41 Bostaurus domesticus
Bovine choleic
Most areas of China
Gmel. and
Scutellaria baicaleusis
Labiatae Northern, Western and
Georgi Central China, S.
Africa
*#44 Salvia miltiorrhiza
Labiatae Most areas of China
Bunge and
Inula helenium L.
Compositae Northern, Northeastern
and Northwestern China
*#49 Lonicera japonica
Caprifoliaceae
Most areas of China
Thunb and
Scutellaria baicaleusis
Labiatae Northern, Western and
Georgi Central China, S.
Africa

*A compound comprising more than one (1) herb.

In the context of the present specification, CHE is used to refer to any species of any of the herbs delineated above which, upon extraction, yields a fraction comprising a pharmacologically active agent, whether a component, a combination of components, a biological metabolite, a derivative thereof or a combination of the above, that exhibits in vitro and/or ex vivo anti-HIV activity. Since the precise chemical composition and pharmacologic mechanism of the CHEs has not yet been elucidated, it is possible that the anti-HIV activity may be due to a single CHE component, a combination of CHE components, or the biologic metabolite or derivative thereof.

By the terms "HIV," and "AIDS-related virus" is meant the commonly designated HIV series (human immunodeficiency virus) formerly called HTLV, LAV and ARV, and species thereof, as described in the incorporated references.

Similarly, the terms "HIV-related disease" and "AIDS-related disease" shall refer to any illness or syndrome, caused directly or indirectly by HIV or AIDS-related virus, including but not limited to infections whose source is fungal, viral and/or bacterial.

It is therefore an object of the present invention to employ the CHEs as therapeutic agents in hosts infected with HIV. In vitro studies, ex vivo studies, including the therapeutic indices (TI) calculated for each CHE, suggest that these CHEs will be useful in pharmacological preparations as in vivo anti-HIV agents. The pharmacological preparations may contain the pharmacological active ingredient alone or in admixture with an appropriate excipient or carrier, and administered to the HIV infected host by enteral, such as oral or rectal, and parenteral, such as intraperitoneal, intramuscular, intravenous or subcutaneous route. The pharmacological agent may also be administered in combination with a supplemental antiviral agent, an immune modulator, any other chemotherapeutic agent, an antibody or a combination thereof. In addition, the pharmacological preparations according to the invention may be, for example, in dosage unit form, such as tablets, capsules. suppositories or ampoules.

It is another object of the invention to use a CHE component or combination of CHE components, a biologic metabolite, a derivative thereof or a combination of the above, in a pharmacological preparation for the treatment of HIV-related illness in infected hosts.

It is a further object of the invention to use the CHE, its active component or combination of components, a biological metabolite, a derivative thereof, or a combination of the above, alone or conjugated to a label, in a diagnostic test for the diagnosis of HIV related illness. Such a test could be an immunofluorescent test, based upon a CHE's capacity to bind either the HIV infected T cells or the anti-idiotypic antibody derived from the CHE.

It is still a further object of the invention to use a CHE, a CHE component, a combination of CHE components, a biological metabolite, a derivative thereof, or a combination of the above to produce a vaccine. Once the CHE's "active site" has been determined, current immunologic techniques could be relied upon to produce such a vaccine.

These and other objects will become readily apparent to those skilled in the art from the following description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be described in connection with the accompanying drawings in which:

FIG. 1 is a composite graph depicting the neutralization assay results for each of the ten CHEs against the lab isolate IIIB.

FIG. 2 is a composite graph depicting the activity of the ten (10) CHEs against the laboratory isolate, HIV-1 AC.

FIG. 3 is a composite graph depicting the neutralization assay results for each of the ten CHEs against the lab isolate HIV-2.

FIG. 4 illustrates and compares the percent HIV neutralization of CHE #1 for each of seven separate clinical isolates of the virus.

FIG. 5 illustrates and compares the percent HIV neutralization of CHE #8 for each of seven separate clinical isolates of the virus.

FIG. 6 illustrates and compares the percent HIV neutralization of CHE #21 for each of seven separate clinical isolates of the virus.

FIG. 7 illustrates and compares the percent HIV neutralization of CHE #30 for each of seven separate clinical isolates of the virus.

FIG. 8 illustrates and compares the percent HIV neutralization of CHE #32 for each of seven separate clinical isolates of the virus.

FIG. 9 illustrates and compares the percent HIV neutralization of CHE #35 for each of seven separate clinical isolates of the virus.

FIG. 10 illustrates and compares the percent HIV neutralization of CHE #39 for each of seven separate clinical isolates of the virus.

FIG. 11 illustrates and compares the percent HIV neutralization of CHE #41 for each of seven separate clinical isolates of the virus.

FIG. 12 illustrates and compares the percent HIV neutralization of CHE #44 for each of seven separate clinical isolates of the virus.

FIG. 13 illustrates and compares the percent HIV neutralization of CHE #49 for each of seven separate clinical isolates of the virus.

FIG. 14 is a composite graph depicting the degree of HIV replication inhibition exhibited by CHE #32.

FIG. 15 illustrates and compares the anti-HIV activity of CHEs #21, #32 and #49 in chronically infected H9/IIIB cells.

FIG. 16 is a composite graph depicting each CHE's percent neutralization of HIV IIIB reverse transcriptase activity.

FIG. 17 illustrates and compares the ex vivo anti-HIV activity of CHE #32 in the peripheral blood mononuclear cells (PBMNs) and plasma of three (3) patients.

FIG. 18 illustrates and compares the ex vivo anti-HIV activity of CHE #49 in the PBMNs and plasma of three (3) patients.

DETAILED DESCRIPTION

The following detailed description and procedures are provided to illustrate the principles of the invention. They are not, however, intended to limit the invention, which extends to the full scope of the appended claims.

A. Preparation of Extracts

The fifty six (56) subject herbs were obtained from China in extract form, packaged in ampoules for parenteral use. However, the extracts of the present invention can be prepared from the subject herbs by utilizing the procedures set forth below, or any organic extraction procedure.

Cut into small pieces, one kilogram of dried herb. Soak the cut herb pieces in eight liters (8 L) of water at room temperature for six to eight hours, and then boil under reflux for one (1) hour. Decant the extract, filter it through a 0.45 .mu.m membrane filter, and concentrate to one liter (1 L).

To the concentrated extract, add three liters (3 L) of 100% ethanol, and maintain the mixture at room temperature for forty-eight (48) hours. Decant, filter and concentrate the extract to one liter (1 L) as above. Repeat this ethanol precipitation two (2) more times.

Add 0.3% charcoal into the concentrated extract and boil the charcoal-extract mixture for five (5) minutes. Filter the extract again.

To the filtered extract, add 10% sodium hydroxide until pH7. The final extract concentration obtained using these procedures should be 1 g herb/ml.

B. Determination Of The Subtoxic Concentrations Of Herbal Extracts

Before assessing the anti-HIV activity of the fifty-six (56) CHEs, toxicity studies were performed to ensure that the observed activity could not be attributed to the indiscriminate destruction of the host lymphocytes by the CHE. For these studies, the standard laboratory methods for T cell toxicity testing were followed. Chang et al., Antiviral Research 9:163-176 (1988); Merchant et al., Handbook of Cell and Organ Culture, Burgess

Publishing Co., Minneapolis, Minn. (1960).

Briefly, the CHE extract to be tested was diluted two-fold serially in medium. To 0.2 ml of the diluted extract, 0.8 ml of a freshly prepared H9 cell suspension was added. (The H9 cells had been obtained from the American Type Culture Collection (A.T.C.C.).) This was done in duplicate; and a medium control was included in every assay. This medium control consisted of 0.8 ml of the same H9 cell suspension added to 0.2 ml of medium; and the control was done in quadruplicate. After 4 days of incubation, the number of viable cells in each culture was counted with a hemacytometer by dye exclusion. When the viable count of extract-treated culture was 2 S.D. below the mean of the medium control, the extract-treated culture was considered to show evidence of cytotoxicity. The highest concentration of an extract which showed no evidence of cytotoxicity was taken as the subtoxic concentration, or maximum tolerated dose (MTD). The MTD's for the ten (10) CHEs exhibiting anti-HIV activity are disclosed in Table II which follows:

TABLE II
Maximum Tolerated Dose (MTD)
CHE MTD (.mu.l)
#1 5
#8 5
#21 40
#30 20
#32 20
#35 10
#39 80
#41 20
#44 40
#49 20

C. Neutralization Assay

Having determined their MTDs, the fifty-six (56) CHEs were then screened for their inhibitory activity against HIV-IIIB in H9 cells. (The HIV-IIIB had been obtained from Drs. Popovic and Gallo.) Employing a standard neutralization assay, which assay is described in the literature, HIV expression was detected by p24 production in the culture supernatant. Ho et al., Science 239:1021-1023 (1988); and Ho et al., J. Virol. 61:2024 (1987).

Specifically, the TCID50 (50% tissue culture infective doses) for the HIV-IIIB isolate was placed in contact with 1.times.10@6 human T lymphocytes, one hour after the CHE under investigation was added at varying doses. This culture was then followed for seven days and observed for signs of viral expression, as measured by the production of HIV core protein p24. A particular CHE was not deemed to have anti-HIV activity unless 90% of viral replication was blocked, as compared to control cultures.

An ID50 and ID90 (amount of CHE necessary to inhibit 50% and 90% of viral replication, respectively) was also calculated for each of the ten CHEs that exhibited anti-HIV activity. In addition, by dividing the MTD by the ID50, a therapeutic index was obtained. Generally, the therapeutic index (T.I.) is a measure of both drug efficacy and safety, and a high therapeutic index is desirable.

FIG. 1 demonstrates the anti-HIV activity of each of the ten (10) CHEs against the lab isolate IIIB. In brief, the ID50 for the ten (10) subject CHEs ranged from 0.15 .mu.l to 1.80 .mu.l, while the ID90 ranged from 0.38 .mu.l to 2.70 .mu.l. The T.I. for these same ten (10) ranged from 22 to 173. These values are all presented in Table III below.

TABLE III
Ten CHEs With Positive Activity Against
HTLV-IIIB Infection Of H9 Cells
Anti-HTLV-IIIB Activity
CHE MTD (.mu.l)
ID50 (.mu.l)
ID90 (.mu.l)
T.I.
#1 05 0.15 0.84 33.33
#8 05 0.14 0.38 35.71

#21 40 1.80 2.30 22.22
#30 20 0.26 1.70 76.92
#32 20 0.45 0.66 44.44
#35 10 0.41 1.70 24.39
#39 80 1.00 5.60 80.00
#41 20 0.34 2.70 58.82
#44 40 0.23 0.65 173.91
#49 20 0.24 0.53 83.33

Using a similar method, the CHEs were then tested against seven (7) clinical isolates (J, AP, L, B, P, C, F) and two (2) additional HIV laboratory isolates, (AC and HIV2), in normal stimulated PBMNs. (The clinical isolates, and the AC laboratory isolate, had been obtained from AIDS patients treated at Cedars-Sinai Medical Center, in Los Angeles, Calif. The HIV-2 (LAV-2ROD) had been obtained from Luc Montagnier, at the Institute Pasteur, in France.) The ten (10) CHEs were found to exhibit anti-HIV activity against most of the clinical isolates; but with varying efficacy. Similarly, in FIG. 2, all ten (10) CHEs exhibited activity against the AC laboratory isolates, whereas only two (2) of the ten (10) CHEs (#41 and #49), in FIG. 3, showed appreciable inhibitory activity against the HIV2 isolate. The results of this method are discussed more particularly as follows:

As illustrated in FIG. 4, CHE #1 exhibited greater than 90% inhibition for six (6) of the seven (7) primary HIV-1 isolates, with an ID90 ranging from 0.20 .mu.l to 3.5 .mu.l.

In FIG. 5, CHE #8 was found to have equal or greater than 90% inhibition for the seven (7) primary isolates, with an ID90 ranging between 0.35 .mu.l to 5.00 .mu.l.

As illustrated in FIG. 6, CHE #21 exhibited greater than 90% inhibition for all but one (1) of the seven (7) primary isolates, with an ID90 ranging from 1.74 .mu.l to 7.6 .mu.l.

In FIG. 7, CHE #30 exhibited greater than 90% activity against five (5) ofthe seven (7) primary isolates, with an ID90 of 0.52 .mu.l to 8.30 .mu.l.

FIG. 8 illustrates the neutralization activity for CHE #32. As the graph illustrates, CHE #32 exhibited ID90 activity against five (5) of the seven (7) primary isolates, with an ID90 ranging from 0.52 .mu.l to 7.00 .mu.l.

In FIG. 9, CHE #35 inhibited six (6) of the seven (7) primary isolates, with an ID90 ranging from 0.60 .mu.l to 8.2 .mu.l.

As illustrated in FIG. 10, only three (3) of the six (6) primary isolates were inhibited more than 90% by CHE #39, with an ID90 ranging from 2.2 .mu.l to 10 .mu.l.

In FIG. 11, all but one (1) primary isolate were inhibited greater than 90% by CHE #41, with an ID90 between 1.10 .mu.l to 5.00 .mu.l.

CHE #44 exhibited greater than 90% inhibition against three (3) of the six (6) primary isolates in FIG. 12, with an ID90 ranging from 1.00 .mu.l to 5.10 .mu.l.

In FIG. 13, CHE #49 inhibited all seven (7) primary isolates by greater than 90%, with an ID90 of 0.62 .mu.l to 2.05 .mu.l.

D. Syncytial Inhibition

Formation of syncytia, with progression to cell death, is a characteristic feature of in vitro cell cultures infected with HIV. Syncytia formation depends upon the interaction of HIV-expressing cells with neighboring cells bearing the CD4 differentiation antigen. Syncytial inhibition studies were therefore performed to determine whether a particular CHE had its primary effect upon the HIV envelope glycoproteins, or upon the uninfected target cells. Following a standard method, described in the literature, the two cell cultures Molt IIIB and HPBALL were employed as the sources of infected and uninfected cell specimens, respectively. See Lifson et al., Science 232:1123-7 (1986); Sodroski et al., Nature 322:470-4 (1986); and Lifson et al., Nature 323:725-8 (1986).

Varying amounts (0.3 .mu.l, 1.0 .mu.l, 3.0 .mu.l) of each of the ten (10) CHEs were preincubated separately

with either the infected Molt IIIB or the uninfected HPBALL cells for a standard time period. The cells were then washed several times and the two cell types were mixed in culture. The percent syncytial inhibition of both methods was then evaluated for all CHEs by light microscopy eighteen (18) hours after mixing. Table IV lists the preliminary results of syncytial inhibition activity exhibited by each CHE studied.

TABLE IV*

#1 #8 #21
#30
#32
#35
#39
#41
#44
#49
rsT4
A. CHE WAS PREINCUBATED WITH UNINFECTED CELLS (HPB-ALL)
3 .mu.l
96.2
98.1
3.7
70.9
93.6
98.1
67.9
77.4
50.9
86.1
32.1
(3 .mu.l)
1 .mu.l
62.3
26.4
1.9
62.3
74.7
83.0
15.1
58.5
35.8
62.3
35.8
(1 .mu.l)
0.3 .mu.l
28.3
15.1
0 28.3
37.7
58.5
0 16.9
15.5
50.9
0 (0.3 .mu.l)
B. CHE WAS PREINCUBATED WITH INFECTED CELLS (MoltIIIB)
3 .mu.l
0 20.8
7.6
47.2
73.6
26.4
39.6

50.6
92.5
73.6
92.5
(3 .mu.l)
1 .mu.l
0 26.4
13.2
39.6
37.2
20.8
16.9
50.9
88.7
47.2
62.3
(1 .mu.l)
0.3 .mu.l
0 9.4
0 16.9
33.9
0 18.3
43.4
77.4
32.1
35.8
(0.3 .mu.l)

*The results tabulated above were obtained from one (1) series of experiments. These experiments have not yet been repeated to verify the reproducability of the above results.

Briefly, as tabulated above in Section A of Table IV, four (4) of the ten (10) CHEs (#1, #8, #32 and #35) exhibited greater than 90% inhibition of syncytia formation when the CHE was preincubated with the uninfected cells. However, only one (1) (#44) exhibited greater than 90% inhibition of syncytia formation when the CHE was preincubated with the infected cells.

An additional experiment was thereafter performed to determine whether this anti-HIV activity produced by the CHEs occurred inside the cells. In this experiment, the cells were infected one (1) hour before adding the CHEs, using the reverse transcriptase inhibitor, AZT, as a control. In FIG. 14, 10 .mu.l of CHE #32 exhibited 100% inhibition of HIV replication in the preinfected cells with both doses of HTLV-IIIB (50 TCID50 and 100 TCID50). This result appears to indicate that the observed in vitro anti-HIV activity may actually occur within the cell, although the precise mechanism for this activity is still being investigated.

E. End-Point-Dilution Cultures

The end-point-dilution culture method, as described in the literature, was used to determine whether the CHEs exhibited an anti-HIV effect in chronically infected H9/IIIB cells. Ho et al., NEJM 321:1621-1625 (1989). As indicated in FIG. 15, 1 .mu.l of CHE #21 produced no viral titer change, although 10 .mu.l of CHE #21 produced a 10 fold decrease in viral titer. Moreover, 1 .mu.l of CHE #32 produced a 2 fold decrease in viral titer, while 10 .mu.l produced a 10 fold decrease; and 1 .mu.l of CHE #49 decreased the viral titer 2 fold, while 10 .mu.l resulted in a 100 fold decrease.

Hence, these results further strengthen the results obtained in the pre-infection studies in FIG. 14, (the cells were infected one hour before adding the CHEs), where it appeared that the anti-HIV activity of the CHEs may actually occur within the cell interior. However, as indicated above, the precise mechanism for the CHE activities is still under investigation.

F. Reverse Transcriptase Assay

The HIV III-B RT enzyme was isolated and mixed with varying amounts of each CHE, using a known reverse transcriptase (RT) biochemical assay. Ho et al., Science 226:451-453 (1984); Popovic et al., Proc.

Natl. Acad. Sci. U.S.A., 77:7415 (1980).

Specifically, virus particles were precipitated from cell-free supernatant as follows: 0.3 ml of 4M sodium chloride and 3.6 ml of 30% (weight volume) polyethylene glycol (Carbowax 6000) were added to 8 ml of harvested culture fluids and the suspension was placed on ice overnight. The suspension was centrifuged at 2000 rev/min at 30 minutes. The precipitate was resuspended in 300 .mu.l of 50% (by volume) glycerol (25 mM tris-HCl, pH 7.5, 5 mM dithiothreital, 150 mM potassium chloride and 0.025% Triton X-100). Virus particles were disrupted by addition of 100 .mu.l of 0.9% Triton X-100/1.5M potassium chloride solution.

The cell-free virus concentrate from a culture of H9/HIV III-B was layered on a 20 to 60% (by weight) sucrose gradient in 10 mM tris-HCl (pH 7.4) containing 0.1M sodium chloride and 1 mM EDTA and centrifuged overnight at 35,000 rev/min. Fractions of 0.7 ml were collected from the bottom of the gradient and 10 .mu.l portions, in a final volume of 100 .mu.l containing 40 mM tris-HCl (pH 7.8), 4 mM dithiothreital, 45 mM potassium chloride and 50 .mu.g of template--primer poly (A).dT12-18 and poly (C).dG12-18 per ml (with 10 mM Mg@2+) or 50 .mu.l of poly (dA).dT12-18 per ml (with 0.25 mM Mn@2+) were assayed for RT at 37 DEG C. for 1 hour. The mixture also contained 15 .mu.M of the appropriate labeled deoxyribonucleotide triphosphates, [@3 H]dTTP (16 Ci/mmole; 1 Ci-3.7.times.10@10 becquerels) or [@3 H]dGTP (12 Ci/mmole). The amount of each CHE necessary to inhibit 50% and 90% of the HIV III-B RT activity is reported in Table V below and FIG. 16. As illustrated, the results indicate that seven (7) of the ten (10) CHEs exhibited greater than 90% RT inhibition.

TABLE V
Anti-HTLV-IIIB RT Activity
CHE ID50 (.mu.l)
ID90 (.mu.l)
#1 0.53 >10.00
#8 2.40 9.00
#21 3.70 >10.00
#30 1.60 9.00
#32 3.00 9.00
#35 4.50 >10.00
#39 3.35 8.90
#41 1.20 9.20
#44 1.95 9.50
#49 1.12 6.80

G. Ex Vivo Experiments Utilizing CHEs #32 And #49, The Best Mode CHEs

Having determined their in vitro anti-HIV activity, ex vivo experiments were conducted in order to provide an experimental model that resembles as closely as possible, in vivo conditions for the CHEs. Ho et al., PNAS, 87:6574-6578 (1990). For these experiments, CHEs #32 and #49 were utilized, as they were considered the leading candidates among the ten (10) CHEs for anti-HIV activity. These two (2) particular (CHEs) were selected based upon the experimental data to date. However, this selection of CHEs #32 and #49 as the best mode CHEs is not meant to foreclose other possibilities, as future experiments may identify other more effective anti-HIV agents among the ten (10) CHEs.

Plasma and PBMNs were obtained from three (3) patients and denoted as follows: A, for a patient with AIDS; R for a patient with ARC; and H for a healthy patient. An end-point-dilution culture method as described above, was used for serial quantitation of HIV-1 in the PMBNs and plasma of the three (3) patients, and serum p24 core antigen levels were measured as a marker of viral burden. In FIG. 17, the HIV titers in PBMNs are illustrated on the top graph; and the HIV titers in plasma are illustrated on the bottom graph.

HIV-1 was recovered from the PBMNs of all three (3) patients, with titers ranging from 10 to 1,000 TCID/10@6 cells, and a mean titer of 370 TCID/10@6 cells. When 1 .mu.l of CHE #32 was added, however, HIV-1 was detected in titers ranging from 10 to 100 TCID/10@6 cells, a 10 fold decrease in viral titers. When 10 .mu.l of CHE #32 was added, viral titers in all patients were decreased to 1 TCID/10@6 cells. Meanwhile, the total HIV-1 titers in plasma before the addition of CHE ranged from less than 1 to 10 TCID/ml, and a mean value of 3.3 TCID/ml. However, the addition of 1 .mu.l or 10 .mu.l of CHE #32 decreased viral titers to less than 1 to 1 TCID/ml.

As illustrated in FIG. 18, in PBMNs treated with 0.1 .mu.l of CHE #49, HIV titers decreased from a mean titer of 370 TCID/10@6 to 10 TCID/10@6 cells. The addition of 1 .mu.l of CHE #49 further reduced the

viral titer to 0.67 TCID/10@6 cells. In plasma, however, 0.1 .mu.l of CHE #49 produced no change in HIV titer, although 1 .mu.l reduced the HIV titer 10 fold.

These experiments and the resulting data demonstrate that CHEs may be a rich source for potential in vivo anti-HIV therapy in an infected host. As illustrated, ten (10) of the fifty-six (56) CHEs tested were found to exhibit dose dependent anti-HIV activity in vitro. Five (5) of these CHEs (#1, #8, #32, #35 and #44) also demonstrated substantial syncytial inhibition activity, while seven (7) CHEs (#8, #30, #32, #39, #41, #44 and #49) exhibited inhibitory activity against reverse transcriptase. Finally, CHEs #32 and #49 even exhibited ex vivo dose dependent anti-HIV activity in patient plasma and PBMNs.

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Medicinal herbal composition for treating liver diseases and HIV

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The present invention provides a herbal pharmaceutical composition for treating patients with liver diseases and/or HIV. The composition contains fifteen (15) ingredients, which are diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, milkvetch root, barbary wolfberry fruit, sanqi, red ginseng, figwort root, Chinese magnoliavine fruit, turmeric root-tuber, hawthorn fruit, and Chinese angelica. Among the fifteen (15) ingredients, diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit are the required herbs which contribute to the efficacy of the pharmaceutical composition.

CROSS-REFERNCE TO RELATED APPLICATION

The present application claims the benefit of the filing date of U.S. Provisional Application No. 60/240,963, filed on Oct. 18, 2000, which is herein incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to a novel herbal pharmaceutical composition and its use for treating patients with liver diseases (e.g., viral hepatitis [such as Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, and Hepatitis E], alcoholic or fatty liver, liver cirrhosis, and liver cancer) and HIV. The major ingredients in the herbal composition are diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit. The composition further contains Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit, sanqi, barbary wolfberry fruit, red ginseng, figwort root, Chinese angelica, and milkvetch root. The present invention also relates to a method for making the medicinal herbal composition and methods for treating patients with the medicinal herbal composition.

DESCRIPTION OF THE RELATED ART

Liver diseases have great impact on human health. Hepatitis is a kind of liver diseases, which is caused by liver inflammation due to infection of a variety of pathogens, which include, but are not limited to, viruses, bacteria, fungi, and protozoa. Hepatitis can be categorized as acute, chronic, or fulminant.

Viral hepatitis is an enterically transmitted liver disease due to viral infection. The major transmission means for viral hepatitis is through ingestion. Viral hepatitis can also be transmitted through blood transfusion or similar means of hepatitis-virus-carrying blood or blood product such as blood plasma. Viral hepatitis is widespread around the world. For example, there are approximately thirty million (30,000,000) viral hepatitis patients in China including an estimated number of nine million (9,000,000) new patients each year, and about one hundred million (100,000,000) hepatitis B virus (HBV) carriers. It is estimated that 10% of the pregnant women in China are HBV carriers. About one hundred thousand (100,000) people in China die of liver cancer originated as liver diseases each year.

Depending on the major etiologic agent, viral hepatitis is categorized into Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, and Hepatitis E. Hepatitis A is caused by hepatitis A virus (HAV); Hepatitis A can affect anyone and occur in isolated cases as well as widespread epidemics. Hepatitis B is a serious disease caused by hepatitis B virus (HBV). HBV attacks the liver and can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death. Hepatitis C is caused by hepatitis C virus (HCV). Hepatitis D is

caused by the hepatitis D virus (HDV) which is a defective single-stranded RNA virus that requires the helper function of HBV to replicate and to synthesize envelope protein composed of HBsAg to encapsulate HDV's genome. Hepatitis E is caused by hepatitis E virus (HEV), which is an etiologic agent of enterically transmitted non-A, non-B hepatitis. HEV is a spherical, non-enveloped, single-stranded RNA virus of approximately 32 to 34 nm in diameter. HEV has been provisionally classified in the Caliciviridae family; however, the organization of the HEV genome is substantially different from that of other Caliciviruses, and HEV may eventually be classified in a separate family.

The most common types of viral hepatitis are Hepatitis A, Hepatitis B, Hepatitis C, and Hepatitis E, which have similar major symptoms including decreased appetite, nausea, unease upper abdomen, lack of strength, etc. Acute jaundice is also one of the common symptoms. Chronic hepatitis is very difficult to cure. Severe hepatitis often comes on quickly and results in high mortality.

Traditional Chinese herbal compositions have been developed and shown success for preventing and treating various liver diseases. The types of traditional Chinese herbal medicine for treating hepatitis include medications having single or multiple herbal components and medications made of active ingredients extracted from the herbs.

For example, Qianglining injection solution is made of glycyrrhizic acid extracted from licorice (Glycyrrhiza). Glycyrrhizic acid reacts with ammonia to form a water-soluble ammonium salt of glycyrrhizic acid, which then can compound with amino acids. The injection solution is useful for treating chronic viral hepatitis, liver cirrhosis, and hepatoma. The total effective rate of qianglining injection solution is about 87.5%, in which 64.1% is significant, according to clinical studies conducted on hepatitis patients provided by Shanghai Huashan Hospital, Shanghai, China.

Yanhuanglian injection solution is derived from ground herb Yanhuanglian grown in Guangxi Province in China. The solution is useful for treating various types of hepatitis, liver cirrhosis, and liver cancer, with a reported clinical efficacy rate of 81.47%. The solution has an effective rate of 93.88% in cases involving acute jaundice patients, 87.50% in non-jaundice type hepatitis patients, 87.09% in chronic active type hepatitis patients, 69.23% in prolonged type hepatitis patients, and 80.95% in chronic cirrhosis patients. However, only 17.91% of the patients show changes of HBV surface antigen from positive to negative.

Shandougen (Radix Sophorae Tonkinensis) injection solution is useful for both acute and chronic viral hepatitis, and especially effective for chronic active hepatitis. As studied by Guangxi Medical College in Guangxi province, China, the total effective rate is 91.79% for chronic active hepatitis patients, and the substantial effective rate is 54.23%. Also, 64.93% of the patients' glutamate-pyruvate transaminase (GPT) level returns to normal in two (2) months after the treatment. However, some patients show recurring symptoms of hepatitis after the treatment is discontinued.

Umbellate pore fungus (Polyporus umbellata) injection solution has functions of improving immune function, inhibiting tumor, lowering level of transaminase, and inhibiting replication of hepatitis virus. After treating patients with chronic viral hepatitis with umbellate pore fungus injection solution, 35.6% of the patients return to normal serum GPT (SGPT) level, 76.61% of the patients show some lowering effects on transaminase level, 38.6% of the patients show HBV E antigen turning negative, and 13.1% of the patients show surface antigen turning negative.

Qidun fruit acid tablet has a total effective rate of 94.4% in patients with acute jaundice-type hepatitis. The total recovery rate is 64.8%. Qidun fruit acid tablet also shows an effective rate of 69.8% in chronic active hepatitis, in which 43.7% of the patients show a significant effect. The rate for HBsAg positive turning negative is 16.8%.

Gandezhi (Liver-curing) capsule has Wuren alcohol, scutellarin, mulberry fruit-spike (Fructus Mori Albae), salvia root (Radix Salviae Miltiorrhizae), and licorice (Radix Glycyrrhizae Uralensis) and is useful for lowering transaminase level. It has an effective rate of 80.0% for treating prolonged hepatitis and chronic hepatitis, according to studies reported by Guangzhou Zhongshan Medical College Hospital in China. There has been no report which shows that Gandzhi has effect on HBV Antigen turning negative.

Danggui (Chinese angelica root) pill is made of Chinese angelica root (Radix Angelicae Sinensis) and licorice (Radix Glycyrrhizae Uralensis). In a study conducted by Beijing Medical College in China, Danggui pill is effective for treating prolonged hepatitis (with an effective rate of 84.4%), chronic hepatitis (with an effective rate of 79.1%), and cirrhosis resulted from hepatitis (with an effective rate of 73.6%).

Hugang (liver-protecting) tablet is made from schisandra fruit (Fructus Schisandrae Chinensis) alcohol extractant, liver-protecting extractant (including Junchen, Zihu, and woad root (isatis root, Radix Isatidis seu Baphicacanthi)), and biliary powder, etc. It has an effective rate of 95.08% for treating chronic hepatitis (70% with significant effect), and 82.5% for treating cirrhosis (63% with significant effect).

Jigu ("chicken bone") grass pill is made of Jigu grass, billiary powder, and bovine bezoar (Calculus Bovis). As studied by Beijing Children's Hospital in China, Jigu grass pill has a total effective rate of 100% in patients with acute viral hepatitis, 73.3% in patients with chronic active hepatitis, 70.4% in patients with chronic prolonged type hepatitis. However, Jigu grass pill does not appear to have any effect on other types of prolonged hepatitis.

Wuzi ("five ester") capsule is made from schisandra fruit (Fructus Schisandrae Chinensis) alcohol extractant. It shows function of lowering GPT level and is useful for treating chronic prolonged hepatitis. The total effective rate of wuzi capsule is 95.33%, in which 74.21% is significant.

Ganfuneng (liver-healing) formula contains astragalus (Radix astragali membranaceus), hawthorn fruit (Fructus crataegi), pueraria (Radix puerariae), Cornu Bubali powder, San-qi, etc. It has an effective rate of 88.7% for chronic hepatitis patients and 79.1% for GPT recovery.

Biyansha Hepatitis B-curing formulation is made from diffuse hedyotis (Hedyotis diffusa Willd.), rubia root (Radix Rubiae Cordifoliae), Indigo Pulverata Levis, glabrous greenbrier rhizome (Rhizoma Smilacis Glabrae), salvia root (Radix Salviae Miltiorrhizae), finger citron fruit (Fructus Citri Sarcodactylis), hawthorn fruit (Fructus Crataegi), Ganoderma Lucidum, Ophiopogon tuber (Tuber Ophiopogonis Japonici), and silkworm feces (Excrementum Bombycis Mori). The formulation has been used for treating infectious HBV, acute and chronic hepatitis, early-stage cirrhosis, swollen liver and spleen, etc. It has a total effective rate of 84.75% and an HBsAg turning negative rate of 41.35%, as shown in the study of 314 HBV patients at Xian Medical University Second Affiliated Hospital in China.

Ganpikang ("liver-spleen" health) capsule contains fourteen (14) herbal components including bupleurum (Radix Bupleuri), San-qi, and bear gallbladder (Vesica Fellea Ursi) powder. It has a curing rate of 53.33% and an effective rate of 40.0 for chronic active HBV, and a curing rate of 63.33% and an effective rate of 26.67 for chronic prolonged HBV.

Ruanjianhugan ("liver-protecting") tablet contains sophora root (Radix Sophorae Tonkinensis), prunella (Spica Prunellae Vulgaris), bushy knotweed root and rhizome (Radix et Rhizoma Polygoni Cuspidati), scutellaria (Radix Scutellariae Baicalensis), salvia root (Radix Salviae Miltiorrhizae), astragalus (Radix Astragali Membranaceus), ligustrum (Fructus Ligustri Lucidi), cardamon (Fructus Amomi), and hawthorn fruit (Fructus Crataegi). It shows that 78% of the patients having HBeAg turned negative, 28-57% of the patients having HBsAg turned negative.

However, despite the effectiveness of the above herbal medicinal compositions in treating hepatitis, none of these compositions demonstrates significant effects on HBV antigen turning negative.

The present invention provides a novel pharmaceutical composition for treating liver diseases, particularly for treating patients with viral hepatitis (e.g., HAV, HBV, HCV and HEV), alcoholic or fatty liver, and liver cancer. The compositions described in the present invention also demonstrates significant clinical effects on patients with HIV. This composition is a natural Chinese medicine with little or no side effects and has no toxicity.

BRIEF SUMMARY OF THE INVENTION

The novel medicinal composition of the present invention comprises herb extracts from diffuse hedyotis, giant knotweed rhizome, bistort rhizome, Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, milkvetch root, barbary wolfberry fruit, sanqi, red ginseng, figwort root, Chinese magnoliavine fruit, turmeric root-tuber, hawthorn fruit, and Chinese angelica. The composition is effective in treating patients with liver diseases, including, but not limited to viral hepatitis (e.g., HAV, HBV, and HCV, and HEV), alcoholic or fatty liver, liver cirrhosis and liver cancer. It is also effective for treating patients with HIV.

Among the herbs used in the composition, diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit are the necessary ingredients that provide for the efficacy of the composition. Asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit,

and sanqi are used mainly to improve or enhance the flavour, toning, and medicinal effects of, and to balance the excessive effects cause by diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit. In addition, barbary wolfberry fruit, red ginseng, figwort root, Chinese angelica and milkvetch root can be added to the composition to provide further nutrition to the liver during the recovery stage.

The weight ratio of diffuse hedyotis, bistort rhizome, giant knotweed rhizome, and Chinese magnoliavine fruit is preferred to be about 3:3:1:2. The weight ratio of diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Chinese magnoliavine fruit, asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit, and sanqi is preferred to be about 3:3:1:2:1:1:0.1:1:2:1. The weight ratio of diffuse hedyotis, bistort rhizome, giant knotweed rhizome, Chinese magnoliavine fruit, asiatic moonseed rhizome, baical skullcap root, bovine biliary powder, tumeric root-tuber, hawthorn fruit, sanqi, barbary wolfberry fruit, red ginseng, figwort root, Chinese angelica, and milkvetch root is preferred to be about 3:3:1:2:1:1: 0.1:1:2:1:3:1:2:1:3.

The present invention also provides a method for preparing the herbal pharmaceutical composition, which comprises the steps of: (1) grinding and mixing the entire plant of diffuse hedyotis, the dried rhizome of bistort rhizome, the dried rhizome of giant knotweed rhizome, and the dried ripe fruit of Chinese magnoliavine fruit to form a herbal mixture; (2) boiling the herbal mixture in water in two times (first by boiling the mixture in water for 2 hours, then, after the mixture has cooled down, boiling the mixture again for 1.5 hours); (3) filtering the boiled herbal mixture to separate the herbs from the herbal solution; (4) concentrating the herbal solution (preferrably concentrating from about 1.4 fold by volume to about 1 fold by volume); and (5) spray-drying and granulating the concentrated herbal solution into granules, which can be further encapsulated.

DETAILED DESCRIPTION OF THE INVENTION

Traditional Chinese medicine has been in existence for more than two thousand years. It has a proven record of success for curing many kinds of diseases. Traditional Chinese medicine utilizes a variety of herbs and natural substances. Each herb/natural substance has its unique characteristics. By combining and balancing the unique characteristics of herbs, a doctor can prescribe a formulation with enhanced medicinal activities and with less or no toxicity by synergizing the medicinal effects among various herbs, while in the meantime, cancelling out or neutralizing the toxic effects of the herbs. This, in Chinese herbal medicine, is regarded as to regulate between negative/hypoactive characteristics ("yin") and positive/hyperactive characteristics ("yang"),

Under the definitions set forth in the traditional Chinese medicine, "yin" is defined as drugs which cure cold syndrome (which itself has hot or warm property), and "yang" is defined as drugs which cure heat syndrome (which itself has cold or cool property).

The pharmaceutical combination of the present invention comprises fifteen (15) ingredients, in which four (4) ingredients are the core ingredients which contribute to the primary efficacy and healing effect of the composition. They are: (1) diffuse hedyotis/spreading hedyotis (Pharmaceutical name: Herba Hedyotidis diffusae; Botanical name: Hedyotis diffusa Willd.); (2) bistort rhizome (Pharmaceutical name: Rhizoma Bistortae; Botanical name: Polygonum bistorta L.); (3) giant knotweed rhizome (Pharmaceutical name: Rhizoma Polygoni Cuspidati; Botanical name: Polygonum cuspidatum Sieb. et Zucc.), and (4) Chinese magnoliavine fruit (Pharmaceutical name: Fructus Schisandrae Chinensis; Botanical name: Schisandra chinensis (Turcz.) Baill., S. sphenanthera Rehd. et Wils.). The core ingredients are functioned in clearing heat and toxic substances while improving immune system and circulation, curing symptoms of jaundice, and having beneficial effect on internal organs.

There are six (6) additional ingredients that are used to improve and balance the pharmaceutical effects activities produced by the above named core ingredients. These six ingredients also have toning effect and can improve blood circulation in the liver. These six ingredients are: (1) Asiatic moonseed rhizome (Pharmaceutical name: Rhizoma Menispermi; Botanical name: Menisermum dauricum DC); (2) baical skullcap root (Pharmaceutical name: Radix Scutellariae; Botanical name: Scutellaria baicalensis Georgi); (3) bovine biliary powder (Zoological name: Vesica Fellea Bovus); (4) tumeric root-tuber (Pharmaceutical name: Radix Curcumae; Botanical name: Curcuma wenyujin Y. H. Lee et Cl Ling); (5) Hawthorn Fruit (Pharmaceutical name: Fructus Crataegi; Botanical name: Crataegus pinnatifida Bge.); and (6) sanqui (Pharmaceutical name: Radix Notoginseng; Botanical name: Panax notoginseng (Burk.)).

Finally, there are additional five (5) ingredients which are used to primarily provide nutrients and energy

sources for patients so as to expedite the recovery process. These ingredients include: (1) barbary wolfberry fruit (Pharmaceutical name: Fructus Lycii; Botanical name: Lycium barbarum L.); (3) figwort root (Pharmaceutical name: Radix Scrophulariae; Botanical name: Scrophularia ningpoensis); (4) Chinese angelica (Pharmaceutical name: Radix Angelicae sinensis; Botanical name: Angelica sinensis (Oliv.) Diels); and (5) milkvetch root (Pharmaceutical name: Radix Astragali; Botanical name: Astragalus membranaceus (Fisch.) Bge.). Among these ingredients, red ginseng (Radix Ginseng Rubra) and milkvetch root (Radix Astragali) also have the capacity of improving immunological functions of the body to fense off diseases.

The pharmaceutical names, botanical or zoological names, family names, common descriptions, and major ingredients of the herbs used in the present invention is shown in Table 1.

TABLE 1

Herbs of the Present Pharmaceutical Composition

Pharmaceutical Name	Botanical/Zoological Name	Family	Common Description	Major Ingredients
Herba Hedyotidis Diffusae	Heydyotis diffusa (Willd.) Roxb., also known as Oldenlandia diffusa	Rubiaceae	heydyotis, olden-landia	hentriacontane, stigmastatrienol, ursolic acid, oleanolic acid, [beta]-sitosterol, [rho]-coumaric, [beta]-sitosterol-D-glucoside
Radix et Rhizoma Polygoni Cuspidati	Polygonum cuspidatum Sieb. et Zucc.	Poly-gonaceae	Giant Knotweed root and Rhizome	phanol, rheic acid, emodin monomethyl ether, polygonim, and physcion-8-[beta]-D-glucoside
Rhizoma Bistortae	Polygonum bistorta L.	Poly-gonaceae	Bistort Rhizome	n/a
Rhizoma Meni-spermi	Menispermum dauricum DC.	Meni-sperm-aceae	Asiatic Moonseed Rhizome	n/a
Radix Scutellariae Baicalensis	Scutellaria baicalensis Georgi	Labiatae	Baical Skullcap Root	baicalein, baicalin, wogonin, wogonoside, neobaicalein, oroxylin aglucuronide, camphesterol, [beta]-sitosterol, benzoic acid
Vesica Fellea Bovus	Bovine Biliary powder			n/a
Radix Astragali	Astragalus membranaceus (Fisch.) Bge. var. mongholicus. (Bge.) Hsiao or Astragalus membranaceus (Fisch.) Bge.	Leg-uminosae	Milkvetch Root	D-[beta]-asparagine, 2', 4'-dihydroxy-5,6-dimethoxyiso-flavane, caly-cosin, formono-netin,cyclo-astragenol,astra-galosides, choline, betaine, kumatakenin,

				sucrose, glucoronic acid, [beta]-sitosterol
Fructus Lycium Lycii barbarum L.	Sol- anaceae	Barbary Wolfberry Fruit	betaine, carotene, physalien, thiamine, riboflavin, vitamin C, [beta]-sitosterol, linoleic acid
Radix Panax noto- ginseng	Arali- aceae	San-chi, noto- ginseng (Burk.) F.H. chen, P. pseudoginseng Wall, P. sanchi Hoo.	Arasaponin A, arasaponin B, dencichine Tian qi, Shen san qi
Radix Panax Ginseng Ginseng Rubra	Arali- aceae	Red Ginseng C. A. Mey Other	Panaxatriol, Panaxadiol, Panoxisides, Panoquilon, Panaxin, Ginsenin, [alpha]-Panaxin, Protopanaxadiol, Protopanaxtriol, Panacene, Panaxynol, Panaenic Acid, Panose, Dammarane, Glucose, Fructose, Maltose, Sucrose, Nicrotinic Acid, Riboflavin, Thiamine
Radix Scroph- lariae ning- Ning- poensis	Scrophu- ularia lariaceae Scrophu- laria poensis Hemsl. or S. buer-geriana Miq.	Root,	oleic acid, linoleic acid, stearic acid, carotene
Fructus Schisandra Schis- andrae (Turcz.) Baill., Chinensis	Magno- liaceae vine S. sphenanthera Rehd. et Wils.	Chinese Magnolia- fruit schisandra	sesquicarene, [beta]-bisabolene, [beta]-chamigrene, [alpha]-ylangene, schizandrin, pseudo-[gamma]-schizandrin, deoxyschizandrin, schizandrol, citral, stigmasterol, vitamin C, vitamin E
Tuber Curcuma Curcumae wenyujin Y. H. Lee et C. Ling., or Curcuma	Zingi- beraceae tuber, curcuma	Turmeric Root-	d-camphene, d-camphor, 1-[alpha]-curcumene, 1-[beta]-curcumene,

Longa L., or Curcuma aromatica Salisb., or Curcuma zedoaria Rosc., or Curcuma kwangsiensis S. G.Lee et C. F. Liang		curcumin, demethoxycurcumin, bisdemethoxycurcumin, turmerone, turmerone, carvone, [rho]-tolylmethylcarbinoldiferuloylmethane
Fructus Crataegus Crataegi pinnatifida Bge.; C. pinnatifida Bge. var. major N.E. Br. or C. suneata Sieb. et Zucc.	Rosaceae	Hawthorn Fruit	crategolic acid, citric acid, tartaric acid, flavone, sugars, glycosides, vitamin C
Radix Angelicae Sinensis Diels	Umbelliferae	Angelica (Oliv.) root, tang-kuei	Chinese butylidene phthalide, ligustilide, n-butylidenephthalide, sequiterpenes, carvacrol, dihydrophthalic anhydride, sucrose, vitamin B12, carotene, [beta]-sitosterol

Diffuse hedyotis or spreading hedyotis (Herba Hedyotidis Diffusae) belongs to the family of Rubiaceae. The entire plant is used as an herbal medicinal component. The herb has no toxicity. The herb is harvested in summer and autumn in mainland China and in late spring or early winter in Taiwan. In "Materia Medica" (Chinese Herbal medicine), compiled and translated by Dan Bensky & Andrew Gamble, diffuse hedyotidis clears heat and resolves dampness by promoting urination. It is particularly useful for relieving hot painful urinary dysfunction and damp-heat jaundice. Diffuse hedyotidis is the major ingredient in the present herbal pharmaceutical composition which contributes to the medicinal effect on liver diseases and HIV.

Bistort rhizome (Rhizoma Bistortae) is the dried rhizome of the plant Polygonum bistorta L. It belongs to the family of Polygonaceae. Bistort rhizome has moderate cool property (meaning that bistor rhizome is an "yang" herb). It can be used to remove toxic heat, to promote the subsidence of swelling and to stop bleeding.

Giant knotweed rhizome (Radix et Rhizoma Polygoni Cuspidati) is the dried rhizome and root of polygonum cuspidatum Sieb. et Zucc. It belongs to the family of Polygonaceae. The plant is grown throughout China, especially Jiangsu, Zhejiang, Anhui, Guangdong, Guangxi, Sichuan, and Guizhou provinces. The plant is harvested in spring and autumn. Giant knotweed rhizome is normally used to dispel damp, to eliminate blood stasis and alleviate pain, to relieve cough, and to resolve phlegm.

Chinese magnoliavine fruit (Fructus Schisandrae) is the dried ripe fruit of Schisandra chinensis (Turcz.) Baill. or Schisandra sphenanthera Rehd. et Wils. It belongs to the family of Magnoliaceae. The former, the best of its kind, is produced in northern parts of China and is habitually called "Northern schisandra fruit"; the latter is commonly referred to as the "Southern schisandra fruit" as it is produced in the southern parts of China. Both kinds can be used for the pharmaceutical preparation of the present invention. The fruit is collected in autumn and dried under the sun after removing the fruit stalks. Chinese magnoliavine fruit is generally used to arrest disharges, replenish qi, promote fluid secretion, tonify the kidney, and induce sedation. Chinese magnoliavine fruit can also decrease the level of GPT (glutamate-pyruvate transaminase) in patients with hepatitis.

Asiatic moonseed rhizome (Rhizoma Menispermi) is the dried rhizome of Menispermum dauricum DC. It belongs to the family of Menispermaceae. Asiatic moonseed rhizome has cool property. It can be used to

remove toxic heat and relieve rheumatic pains.

Baical skullcap root (Radix Scutellariae) is the dried root of Scutellaria baicalensis georgi. It belongs to the family of Labiatae. The plant is produced in the provinces of Hebei, Shanxi, Inner Mongolia, etc., and collected in spring or autumn. Baical skullcap root is used to remove damp-heat, counteract toxicity, arrest bleeding, and prevent abortion, in patients.

Bovine biliary powder is the gallbladder of the cow, Vesica Fellea Bovus. It can clear heat and alleviate spasms.

Turmeric root-tuber (Radix Curcumae) is the dried root tuber of Curcuma wenyujin Y. H. Lee et C. Ling., or Curcuma Longa L., or Curcuma aromatica Salisb., or Curcuma zedoaria Rosc., or Curcuma kwangsiensis S. G. Lee et C. F. Liang. The herb is mainly produced in Sichuan, Zhejiang, Guangdong, and Guangxi provinces in China, and harvested in winter or spring, washed clean after the removal of the hairy rootlets, boiled thoroughly, and dried in the sun. It belongs to the family of Zingiberaceae. Turmeric root-tuber tastes bitter and had cool property. It can be used to clear heat, alleviate spasms and chest pain, and resolve phlegm.

Hawthorn fruit (Fructus Crataegi) is the dried ripe fruit of Crataegus pinnatifida Bge. var major N. E. Br., or Crataegus pinnatifida Bge., or Crataegus cuneata Sieb. It is produced primarily in Henan, Jiangsu, and Shandong provinces of China. It is harvested in autumn, sliced, and dried in sunlight. It belongs to the family of Rosaceae. Hawthorn fruit is normally used to stimulate digestion and promote the functional activity of the stomach. It can also improve the normal blood flow and dissipate blood stasis.

Sanqi, or San-chi, (Radix Notoginseng) belong to the family of Araliaceae. Sanchi (Sanqi) is the dried root of Panax notoginseng (Burk.) F. H. Chen. The plant is also known as P. pseudoginseng Wall and P. sanchi Hoo. The plant grows in Yunnan, Guangxi, Sichuan, Guizhou, and Jiangxi provinces of China, and is harvested in the autumn or winter of the third or seventh year, either before the flowers bloom (better) or after the fruit is ripe. H. Gao et al., Pharmaceutical Research, (1996) 13(8): 1196-1200, disclose that polysaccharides from Panax notoginseng (San-Chi) have immuno-stimulating activities in vitro.

Barbary wolfberry fruit (Fructus Lycii) is the dried ripe fruit of Lycium barbarum L. It belongs to the family of Solanaceae. The plant is mainly produced in Ningxia, Gansu, and Qinghai provinces of China. It is harvested in summer and autumn. It nourishes and tonifies the liver and kidneys. It can also replenish vital essence and improve eyesight.

Figwort Root (Radix Scrophulariae) is the dried root of Scrophularia ningpoensis Hemsl. It belongs to the family of Scrophulariaceae. The herb is chiefly produced in Zhejiang and Sichuan provinces of China and harvested in winter when the part of the plant above-ground has withered. The roots are piled and dried in sunlight alternately until the inside becomes black and then sliced for use. Figwort root can reduce heat from blood. It als has nourishing capacity and can counteract toxicity.

Red ginseng (Radix Ginseng Rubra) is the steamed and dried root of the cultivated form of Panax ginseng C. A. Mey (commonly known as "Yuanshen"). The herb turns red after being steamed and its properties become warmer in nature. It belongs to the family of Araliaceae. The pharmaceutical effects of ginseng is in its dried root. Ginseng has effects on central nervous system. It enhances both stimulatory and inhibitory processes in the central nervous system, thereby improving the adaptability of nervous responses. Ginseng can also lower serum glucose and cholesterol. It also shows therapeutic and preventive effect on peptic ulcer.

Chinese angelica (Radix Angelicae Sinensis) is the dried root of Angelica sinensis (Oliv.) Diels. It belongs to the family of Umbelliferae. The herb is mainly produced in Gansu and Shanxi provinces of China. It is harvested in late autumn, smoked dry on slow fire after getting rid of the rootlets, sliced, or stir-baked with wine. Chinese angelica can enrich blood, promote blood circulation, regulate menstruation, relieve pain, and relax bowels.

Milkvetch root (Radix Astragali) is the dried root of Astragalus membranaceus (Fisch.) Bge. var. mongholicus. (Bge.)Hsiao or Astragalus membranaceus (Fisch.) Bge. It belongs to the family of Leguminosae. The herb is mainly produced in Shanxi, Gansu, Heilongjiang, and Inner Mongolia of China. The plant of four-year old or older is harvested in spring or autumn. Milkvetch root can promote discharge of pus and the growth of new tissue.

The herbal composition of the present invention was suitable for preparation in a scale typical for

pharmaceutical industry as well as for smaller measure.

In the process for making the herbal composition of the present invention, the individual herbal components are pretreated according to the common procedures. The herbs are cut and put in a container with water to boil and simmer twice. The first time of simmering takes two hours, the solution is collected, and water is added for the second time of simmering for 1.5 hour. The solutions from the simmering steps are collected by passing through a sieve/filter. The filtrate is then condensed from about 1.4 fold by volume to 1.0 fold by volume. Subsequently, the liquid condensate is spray-dried and granulated to form particles. The particles are further packaged and preserved for use or for further analysis by the conventional means of the active ingredients to ensure their quality.

The composition of the present invention can further be processed and formulated in a form suitable for oral administration or intravenous injection.

The following example is illustrative, but not limiting the scope of the present invention. Reasonable variations, such as those occur to reasonable artisan, can be made herein without departing from the scope of the present invention.

EXAMPLE 1
Pharmaceutical Preparation

The kinds and amounts of herbal ingredients used in the process of making the pharmaceutical composition of the present invention are described in Table 2.

TABLE 2
Ingredients Used In Example 1.

Component	Amount (g)	Component	Amount (g)
Diffuse heydyotis	90	Sanchi	30
Bistort Rhizome	90	Red Ginseng	30
Giant Knotweed root and Rhizome	30	Figwort root	60
Asiatic Moonseed Rhizome	30	Chinese Magnoliavine Fruit	60
Baical Skullcap Root	30	Turmeric Root-tuber	30
Bovine Biliary powder	3	Hawthorn fruit	60
Milkvetch Root	60	Chinese Angelica	30
Barbary Wolfberry Fruit	90		

The individual herbal components are pretreated according to common procedures. The herbs are weighed according to Table 2. The herbs are cut into small pieces and put in a container with water to boil and simmer twice, the first time for two hours, and the second for 1.5 hour. After the first simmering, solution is poured out and water is added to the container for the second simmering. The solutions from the two simmering steps are collected to pass through a sieve/filter, and then, condensed at a ratio of 1:1.4. The liquid condensate is spray-dried and granulated to form particles. The particles were further packaged into about 1000 capsules. The capsules are called "Yigan Kang capsules", abbreviated "YGK" capsules. The liquid condensate can also be made for intravenous injection. The injection solution is called "YGK" herbal injection solution. The herbal composition of the present invention is called "YGK" herbal composition.

EXAMPLE 2
Efficacy of the YGK Herbal Composition on Treatment of Patients with Hepatitis B (HBV)

The clinical research was conducted in the Liberty Military Hospital 211 in China. The course of hepatitis B is determined by many factors, including immune response, host genetic factors, and HBV mutations. The chronic hepatitis distinguishes from the acute hepatitis. The acute hepatitis is the active and symptomatic infection of the liver. A patient with the acute hepatitis is contagious. Symptoms of acute HBV infection are non-specific, but may include malaise, anorexia or jaundice. A chronic hepatitis patient is asymptomatic. The HBV is present in the liver and blood, although there are usually no obvious physical symptoms. Specific blood tests will reveal the presence of the virus, and the patient is also contagious via blood, birth, sex,

needles, etc. Cirrhosis is the pathological dysfunctional state of the liver, the hardening of the liver as the result of chronic hepatitis, chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH).

A total of 948 patients with acute HBV, chronic HBV, and liver cirrhosis participated in a clinical comparative study. The patients were divided into two (2) groups. The study group had 642 patients and the comparative group has 306 patients. The data on patients who participated in this study are listed in Table 3.

TABLE 3
Patients Data in the Clinical Study

Group	Study Group	Comparative Group
Total Number of Patients	642	306
Sex Distribution of the Patients	Male: 482 Female: 160	Male: 229 Female: 77
Age Distribution of Patients	7 to 74 years old (average age: 32.5)	8 to 70 years old (average age: 30.5)
*Symptoms of Patients' Liver Disease	Acute Hepatitis B: 282 Chronic Hepatitis: 276 Cirrhosis: 84	Acute Hepatitis B: 109 Chronic Hepatitis B: 114 Cirrhosis: 83

*According to the diagnosis criteria of Hepatitis revised at the Shanghai Hepatitis Conference in 1980, Shanghai, China.)

The patients were treated according to the following regime:

(1) The patients in the study group were each orally administered eight (8) YCK herbal composition containing the herbal composition of the present invention per day.

(2) The patients in the comparative group were each orally administered four (4) Hugang ("liver protecting") tablets per day. A description of Hugang tablets has been provided in the "Background" section, supra.

The treatment lasts for ninety (90) days.

Table 4 shows the results of this clinical comparative study.

TABLE 4
Effects of YGK Capsule Treatment

Group	Number of Patients with Positive Effect* (%)
Study (642 patients)	456 (71.03%)
Comparative (306 patients)	104 (33.98%)
($p < 0.01$)	

*Positive effect means that the hepatitis B envelope antigen (HBeAg) and HBV DNA of the patients turn negative after taking the YGK herbal composition for 90 days.

As indicated in Table 4, approximately 71.03% of patients who took the YGK herbal composition for 90 days show positive responses to the herbal composition. This is contrary to the comparative group where the patients were given a popular "liver protecting" tablets which were available in the Chinese market. Patients who had taken the "liver protecting" tablets only have an effective rate of approximately 33.98% to show improvement in their liver diseases.

The Hepatitis B virus (HBV) consists of a surface and a core. The core contains a DNA polymerase and an e antigen. The DNA structure is double stranded and circular. HBV has four (4) genes encoding four (4) polypeptides: the S (surface), the C (core), the P (polymerase), and the X (transcriptional transactivating).

The S gene consists of three (3) regions, the pre-S 1 region, the pre-S2 region, and the region that encodes the surface protein (HBsAg). Very rarely a mutation occurs in the S gene which aborts the production of HBsAg so that a person maybe HBsAg negative but still has the virus present as determined by HBV DNA. In addition, the HBsAg particles are antigenically complex and the antigenic determinants have been identified as one single common determinant designated a, and four (4) major subdeterminants designated as d, y, w, and r. Thus, the four (4) major determinants are adw, adr, ayw, and ayr.

The C gene consists of two (2) regions, the pre-core region and the core region, which encodes for two different proteins, the core antigen (HBcAg), and the e antigen (HBeAg). A mutation in the pre-core region may stop the production of HBeAg, thus, a person maybe HBeAg negative, but HBsAg positive and HBV DNA positive. Another type of mutant in the core region is called HBV2. The patients that have HBV2 mutant are HBsAg positive but lack HBeAg and HBV DNA.

Because of the complexity and the antigenic differences among the virus, there are a number of tests available for HBV including:

(1) a test for HBsAg, which is an indicator of the presence of the HBV;

(2) a test for HBeAg, which correlates with the viral replication and infectivity, it indicates a high amount of the virus in the blood, thus, is an indicator of the activity and infectivity of the HBV; and

(3) a test for HBV DNA, which is an indication of the virus presence and activity.

Tables 5-7 indicated the change of Hepatitis B envelope Antigen ("HBeAg"), Hepatitis B surface antigen ("HBsAg"), heptomegaly, and splenomegaly in the patients after the treatment.

TABLE 5
Effect of Herbal Composition on HBeAg in Patients

Group	Study Group	Comparative Group	Acute Hepatitis Patients
Number of Patients with HbeAg(+)	260	78	
Number of Patients with HbeAg(+) After Treatment	48	59	
Percentage of Patients With HbeAg Turning Negative	81.5%	24.36%	
Chronic Hepatitis Patients			
Number of HbeAg(+) Patients	206	82	
Number of HbeAg(+) Patients After Treatment	74	64	
Percentage of Patients With HbeAg Turning Negative	64.0%	21.95%	
Cirrhosis Patients			
Number of HbeAg(+) Patients	24	26	
Number of HbeAg(+) Patients After Treatment	14	22	
Percentage of Patients With HbeAg Turning Negative	41.7%	15.38%	

As indicated in Table 5, the percentages of patients with HBeAg turning negative in all three (3) categories of patients (including acute hepatitis, chronic hepatitis, and cirrhosis) are 2.7-3.3 times higher than those of the comparative groups. This demonstrates that the YGK herbal composition had significant effect on HBeAg turning negative and inhibiting HBV activity and infectivity.

TABLE 6
Effect of Herbal Composition on HBsAg in Patients

Group	Study Group	Control Group
Acute Hepatitus Patients		
Number of	262	84
Patients with HBsAg(+)		
Number of	116	73
Patients with HBsAg(+) After Treatment		
Percentage of	55.7%	13.09%
Patients With HBsAg Turning Negative		
Chronic Hepatitis Patients		
Number of	216	87
HBsAg(+) Patients		
Number of	118	78
HBsAg(+) Patients After Treatment		
Percentage of	45.37%	10.30%
Patients With HBsAg Turning Negative		
Cirrhosis Patients		
Number of	64	43
HBsAg(+) Patients		
Number of	50	40
HBsAg(+) Patients After Treatrnent		
Percentage of	21.88%	6.98%
Patients With HBsAg Turning Negative		

As indicated in Table 6, the percentages of patients with HBsAg turning negative in all three (3) categories of patients including acute hepatitis, chronic hepatitis, and cirrhosis were 3.1-4.4 times of those of the comparative groups. This demonstrates that the YGK herbal composition had significant effect on HBsAg turning negative and inhibiting the HBV.

In addition to HBeAg and HBsAg turning negative, the YGK herbal composition also show greater effects on increased appetite and decreased various symptoms of liver diseases than the comparative group using Hugang "liver protecting" tablets.

TABLE 7
Effect on Hepato-Splenmegaly

Group	Reduced Hepatomegaly	Reduced Splenomegaly
Study Group	79.72%	58.54%
Comparative Group	30%	28.8%

Heptomegaly and splenomegaly are related to and possibly caused by viral infection. The reduced hepatomegaly and splenomegaly in patients was indicative to reduced symptoms of viral infection.

In summary, the YKG herbal composition demonstrates effect on treating patients with HBV, which including acute hepatitis B, chronic hepatitis B, and cirrhosis.

EXAMPLE 3

Effects of the YGK Herbal Composition on Treatement of Patients with Chronic Hepatitis B (HBV)

The clinical research was conducted in the Liberty Military 302 Hospital, Ninth Section, China. The research was conducted on treatment effects of the herbal composition of the present invention on chronic hepatitis B patients.

Chronic Hepatitis is an ongoing injury to the cells of the liver with inflammation which lasts for longer than six months. The causes of chronic hepatitis include: viruses, metabolic or immunologic abnormalities and medications. Symptoms resulted from the injury of hepatocytes, the inflammation or from the resulting scarring is called cirrhosis. Chronic hepatitis may follow acute hepatitis B or C or may develop quietly without an acute illness. Liver biopsy is helpful in that it confirms the diagnosis, aids in establishing the cause (etiology) and can demonstrate the presence of cirrhosis. It is less helpful in judging the response to treatment. Approximately 25% patients with chronic hepatitis B will develop cirrhosis, causing permanent and serious liver damage. Chronic carriers of HBV are far more likely to develop hepatocellular carcinoma than non-carriers.

It is believed that chronic infections develop as the result of a weak T helper (Th) cell response to the virus, in particular to the HBsAg. The T cell response is responsible for clearing the infected cells in the host's system. When the clearance is inefficient and the infected cells persist in the body, a chronic infection develops. As the HBsAg titer increases, the patient moves into acute, symptomatic disease. When the titer of anti-HBsAg rises, the symptoms of HBV begin to decline and patient reaches the immune state.

Chronic hepatitis has been divided into two categories based on histologic findings: chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH). Characteristically, specimens from liver biopsy identified as CPH show inflammation confined to the portal triad (does not penetrate the limiting plate). Specimens identified as CAH show inflammation that penetrates the limiting plate, extending to the surrounding individual hepatocyte and yielding piecemeal necrosis. Under this schema, CAH eventually reaches a point where lobular architecture is destroyed, and bands of necrosis (bridging necrosis) are replaced by scar tissue (bridging fibrosis), resulting in the characteristic features of cirrhosis.

Sixty (60) patients with chronic hepatitis B are divided into two (2) groups, one group for treatment with YKG herbal composition and the other with Hugang ("Liver protecting") tablets. The study was conducted and maintained for three (3) months. The patients information in the two (2) groups are shown in Table 8:

TABLE 8
Compositions of the Patients in the Clinical Study

Group	Study Group	Comparative Group
Total Number of Patients	30	30
Sex Distribution of the Patients	Male: 26 Female: 4	Male: 25 Female: 5
Age Average Patients	32.8	35.1
Duration of Illness	2 months to 11 years	2 months to 9 years
*Symptoms of Patients' Liver Disease	CPH: 13 CAH: 17	CPH: 10 CAH: 20

*According to the diagnosis criteria of hepatitis revised at the Shanghai Hepatitis Conference in 1980.

Table 9 shows the changes in HBsAg, HBeAg, and HBV-DNA in patients after treatment with the YGK herbal composition (the study group) or Hugang tablets (the comparative group).

As indicated above, HBsAg can be detected in patients with acute infection as well as patients who are chronic HBV carriers. In the serological test, decreased titer of HBsAg indicates that the symptoms of HBV are lessened and the patient is approaching the immune state.

TABLE 9
The Changes of HBsAg, HBeAg, and HBV-DNA in Patients

Group	HBsAg Sero-Negative (%)	HBsAg Decreased Titer (%)	HbeAg Sero-Negative (%)	HbeAg Decreased Titer (%)	HBV-DNA Sero-Negative	SGPT Recovery Rate (%)
Study Group	1/30 (3.33%)	6/30 (20.00%)	12/26 (46.15%)	6/26 (23.08%)	9/15 (60.00%)	73.33%
Comparative Group	0/30 (0%)	2/30 (6.67%)	5/27 (18.52%)	2/27 (7.41%)	4/18 (22.22%)	71.43%

$p<0.05$.

As indicated in Table 9, the YGK herbal composition has significant effects on chronic hepatitis patients. Patients treated with the YGK herbal composition have Serum Glutamic Pyruvic Transaminase (SGPT/ALT)

recovery rate of 73.33%, HBeAg turning negative rate of 46.15%, HBV-DNA turning negative rate of 60.00%, suggesting that the YGK herbal composition has significant effects on inhibition of HBV replication and presence and depletion of aminotransferase. In addition, there was no toxic adverse reaction on the patients treated with the YGK herbal composition, accodring to clinical obervation.

EXAMPLE 4
Case Studies on Effects of the YGK Herbal Composition on Patients with Hepatitis B

The clinical research was conducted in the Contagious Disease Department of People's Liberation Army Hospital Branch 113 in China. The research was conducted on treatment effects of the YGK herbal composition on hepatitis B patients.

Each patient was tested for various markers. Serum alanine aminotransferase (ALT) is an enzyme appears in liver cells, with lesser amounts in the kidneys, heart, and skeletal muscles. When such damage occurs, ALT is released from the liver cells into the bloodstream, often before jaundice appears, resulting in abnormally high serum level of ALT that last for days or weeks. ALT is a relatively specific indicator of acute liver cell damage. Serum bilirubin (BIL) is also tested as an indication of liver diseases.

Case #1 was a twenty-four years old male patient with chronic hepatitis B, with general weakness for more than one year. Table 10 shows the diagnoses of patient case #1 before and after treatment with the YGK herbal composition:

TABLE 10
Diagnoses of the Patient #1 Before and After the Treatment

	TBIL (nmol/L)	ALT (U/L)	HBSAg	HbsAb	HBeAg	HbeAb	HbcAg	HBcAb (IgM)	PCR HBV-DNA
Before Treatment	42	231	+	-	+	-	+	+	++ (1:64)
After Treatment	18.6	66	-	+	-	+	+	+	--

Table 10 indicates that the patient was in a state of immunity towards HBV and with alleviated infection as shown by the significant decrease of the viral DNA, and viral proteins, HBsAg, HBeAg, HBcAg, with increased amount of the antibodies against the viral protein in the serum.

Case #2 was a sixty-six years old male patient with recurrent abdominal fullness and general weakness for about ten (10) years with liver cirrhosis and splenomegaly. The following are the diagnoses of the patient before and after treatment with the YGK herbal composition (Table 11).

TABLE 11
Diagnoses of the Patient #2 Before and After Treatment with the YGK herbal composition

	TBIL (nmol/L)	ALT (U/L)	HBsAg	HbsAb	HBeAg	HbeAb	HbcAg	HBcAb (IgM)	PCR HBV-DNA
Before Treatment	44.8	382	+	-	+	-	+	+	+++ (1:64)
After Treatment	25.3	43.8	+	-	-	+	+	-	+ (1:32)

Table 11 shows that patient #2 was in a state of alleviated infection symptoms towards HBV as shown by the significant decrease of viral DNA, and viral proteins., The data also show an increase in immunity as evidenced by reduced amount of HBsAg, HBeAg, HBcAg, and an increased amount of the antibodies against the viral proteins in the serum.

Case #3 was a thirty-one years old male patient with general weakness for more than one (1) month, treated in local Chinese Medicine clinic and subsequently hospitalized as acute biliary hepatitis B patient. The following are the diagnoses of the patient before and after treatment with the YGK herbal composition (Table 12).

TABLE 12
Diagnoses of the Patient #3 Before and After

the Treatment With the YGK Herbal composition

	TBIL (nmol/L)	ALT (U/L)	HBcAb IgM	HBsAg	HbsAb	HBeAg	HbeAb	HBcAg	PCR HBV-DNA
Before Treatment	154	520	+	-	+	-	+	+	+++
After Treatment (1:64)	22.1	29.1	+	-	-	+	+	-	+
(1:32)									

Table 12 shows that the patient was in a state of alleviated infection symptoms towards HBV as shown by the significant decrease of viral DNA, and viral proteins. The data also show an increase in immunity as evidenced by reduced amount of HBsAg, HBeAg, HBcAg, and an increased amount of the antibodies against the viral proteins in the serum.

Case #4 was a forty-five years old male acute biliary hepatitis B patient with recurrent abdominal fullness, abdominal pain and general weakness for about one week. The following are the diagnoses of the patient before and after the treatment with the herbal composition of the present invention (Table 13).

TABLE 13
Diagnoses of the Patient #4 Before and After
the Treatment With the YGK Herbal composition

	TBIL (nmol/L)	ALT (U/L)	HBcAb IgM	HBsAg	HbsAb	HBeAg	HbeAb	HBcAg	PCR HBV-DNA
Before Treatment	143	966	+	+	+	-	+	+	++
After Treatment (1:64)	15.3	42.1	+	-	-	+	+	-	--
(1:32)									

Table 13 shows that the patient is in a state of alleviated infection symptoms towards HBV as shown by the significant decrease of viral DNA, and viral proteins. The data also show an increase in immunity as evidenced by reduced amount of HBsAg, HBeAg, HBcAg, and an increased amount of the antibodies against the viral proteins in the serum.

Case #5 was a thirty-one years old male acute biliary hepatitis B patient with abdominal fullness and general weakness for about five (5) days and then admitted. The following are the diagnoses of the patient before and after the treatment with the herbal composition of the present invention (Table 14).

TABLE 14
Diagnoses of the Patient #5 Before and After
Treatment With the YGK Herbal composition

	TBIL (nmol/L)	ALT (U/L)	HBcAb IgM	HBsAg	HbsAb	HBeAg	HbeAb	HBcAg	PCR HBV-DNA
Before Treatment	47.7	694	+	+	+	-	+	+	++
After Treatment (1:64)	19.8	138	+	+	+	-	+	-	-
(1:32)									

Table 14 shows that the patient is in a state of alleviated infection symptoms towards HBV as shown by the significant decrease of viral DNA, and viral proteins. The data also show an increase in immunity as evidenced by reduced amount of HBsAg, HBeAg, HBcAg, and an increased amount of the antibodies against the viral proteins in the serum.

Table 15 shows the percentage of patients with therapeutic effects in different markers.

TABLE 15
Therapeutic Effects on Patients After
Treatment with the YGK Herbal composition

Therapeutic Effects	Percentage of Patients*
Obvious therapeutic effects	80.9%
Improved therapeutic effects	19.10%
Hepatomegaly	75%
Splenomegaly	62.5%

Normalizationof liver function
ALT 93.7%
Bilirubin 91.1%
Seroconversion
HBsAg(+) to HbsAg(-) 33.3%
HBsAb(-) to HbsAb(+) 23.8%
HbeAg(+) to HbeAg(-) 68.6%
HbeAb(-) to HBeAb(+) 23.9%
HBcAb(+) to HbsAb(-) 43%
HBV-DNA(+) to HBV-DNA(-) 39.5%

*The study included a total number of 42 patients (male: 31; female: 11), who were aged between 16 and 63 (average age: 42). Before treatment, twenty six (26) of the patients were diagnosed with acute hepatitis B, eight (8) with chronic hepatitis B; and eight (8) with chronic active hepatitis B. Thirty eight (38) patients had abnormal serum ALT. Thirty four (34) patients had abnormal serum BIL. Forty two (42) patients had HBV Marker (positive+).

#Thirty eight (38) patients had HBV-DNA as tested by PCR (positive+). Thirty five (35) patients were HBeAg positive. Thirty two (32) patients were anti-HAV, anti-HCV, anti-HEV.

Results

The patients after being treated with the YGK herbal composition showed improvement of subjective symptoms, especially pain on liver area, fast normalization of liver function. Their ALT levels started to fall in about sixteen (16) days generally. Possible anti-viral activity was shown in the patients: the rate of HBeAg turning negative was commonly found in the YGK herbal composition treated patients (68.6%). No side-effects were noted in the treated patients.

EXAMPLE 5
Effects of the YGK Herbal Composition On Animals With Liver Diseases

The animal study was conducted at Korean Central Research Center.
Experiment 5.1
Analysis of Effect on Alcoholic or Fatty Liver in White Rats

Purpose

The experiment was conducted to investigate effects of the herbal composition on alcohol metabolism in white rats, especially, the influence on the ability to transform alcohol to triglyceride and cholesterol. The experimental dosage was 1 g/kg.

Method

The experimental animal used was male SD white rat with weight of 200 g. Blood sampling of the experimental animal was taken through orbital vein plexus. The animal was administered for the herbal composition of the present invention three (3) times a day for seven (7) days.

The experimental animals were divided into the control group and the study group. The control group animals were administered alcohol for one week. The study group animanls were administered alcohol and concomitantly with 1 g/kg of the YGK herbal composition for one week. The rats' livers were tested for triglyceride and cholesterol level, lipid hyperoxidation, and glutathione peptide.

Results

After one (1) week of alcohol administration, triglyceride and cholesterol levels in the rats' liver were increased; lipid hyperoxidation and diminished glutathione peptide occurred in the control group. In contrast, in the study group, the fatty metamorphosis of the liver was inhibited. Also, the processes of lipid hyperoxidation and diminished glutathione peptide were inhibited in the study group animals.

Conclusion

The YGH herbal composition prevents accumulation of triglyceride and cholesterol levels in the liver which follows alcohol consumption, thus providing beneficiary effects on the liver functions.

Experiment 5.2
Analysis of Effect on Liver Cirrhosis in White Rats

Purpose

The experiment was conducted to investigate the effect of the YGK herbal compositions on protein synthesis in white rats with liver cirrhosis.

Method

The experimental animal used was male SD white rat with weight of 200 g. Blood sampling of the experimental animal was taken through orbital vein plexus. The animal was administered for the herbal composition of the present invention three (3) times a day for seven (7) days.

1. Induction of Liver Cirrhosis in the Rats

The rats were injected subcutaneously on the back with 1 ml/200 g 50% chloroform (CCl_4) diluted in olive oil, for three (3) times a week for four (4) weeks. Liver biopsy was conducted through midline laparotomy. Most animals needed six (6) weeks of injection to induce liver cirrhosis. The injection dosage was adjusted each week in accordance to the weight of the rats.

Due to liver cirrhosis and partial liver resection, the serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) significantly increased in the rats.

2. Treating Rats with the YGK Herbal Composition

The rats in the study group were subdivided into three (3) groups which were respectively administered the YGK herbal composition of the present invention for 500 mg/kg, 1000 mg/kg, or 2000 mg/kg.

Results

1. ALT and AST Levels: after the treatment with the YGK herbal composition, the serum ALT and AST levels decreased in all three (3) different dosage treatment groups. The liver cirrhosis process was inhibited.

2. Hepatocyte Regeneration: after the administration of the herbal composition in three (3) different doses, the rates of liver regeneration in the rats were 19%, 30%, and 47%, respectively, higher than the rats with liver cirrhosis and partially resected livers which were not treated with the herbal composition, and the rates of liver regeneration in the treated rats were also 51%, 70%, and 92%, respectively, higher than the partially liver resected rats with normal liver functions.

Conclusion

The YGK herbal composition was effective in liver regeneration and had effectively inhibited the liver cirrhosis process.

EXAMPLE 6
Toxicity Study of the YGK Herbal Composition in Animals

Purpose

The following experiment was conducted at the Toxicology Laboratory of the Institute of Labor, Health, and Occupational Disease of Heilungkiang Province in China to examine acute toxicity of the YGK herbal composition during intravenous injection in animals.

Methods

Experimental animals were Japanese big-ear white rabbits obtained from the Animal Center of Haerbin Medical University in Haerbin, Heilungkiang Province, China. These rabbits were characterized by the

obvious blood vessels on ears which facilitates the operation of injection during the experiments.

Ten (10) rabbits were obtained including six (6) males and four (4) females, each weighing between 1900 g to 3000 g.

The rabbits were randomly divided into two (2) groups, five rabbits in each group including two (2) females and three (3) males. The YGK herbal composition was intravenously injected into the rabbits through the veins on their ears at dosages of 10 g/kg and 15 g/kg, respectively, for two groups.

The concentration of injection fluid containing the herbal composition was about 1 g/ml. So the higher dosage group at 15 g/kg has a concentration of about 15 ml/kg, which could be calibrated as a sixty (60) kg-weighted adult who was treated by 900 ml of the herbal composition at a time.

The rabbits were observed for behaviour continuously for a period of two (2) weeks after intravenous injections. observation was conducted hourly at day 1; during the following days, observation was conducted four-six (4-6) times per day.

At the end of the observation period, rabbits were sacrificed and dissected to examine the eyes, liver, lung, and spleen for adverse effects.

Results

No abnormal behavior was observed of the rabbits during the observation period. The rabbits showed normal body weight increase during the period. After the sacrifice and dissection, inspection of the eyes, liver, lung, and spleen showed no extraordinary syndromes. The results when compared to a general acute toxicity index were normal and no acute toxicity.

EXAMPLE 7
Effects of the YGK Herbal Composition on HIV in Cell Cultures

Purpose

The following experiment was conducted in the Military Medical Research Institute in China to examine the effectiveness of the YGK herbal composition of the present invention in the form of intravenous product against HIV.

Methods

MT4 cells were cultured in HIV-1 suspension liquid of 100 TCID50 in a 96-hole culture plate. The culture condition was set at a temperature of 37[deg.] C. and under 5% CO2. The culture time was seven (7) days.

The YGK herbal composition of the present invention were added into the wells at various concentrations. The morphology of the MT4 cells were observed by conventional methods.

Results

No pathological changes of MT4 cells were observed in wells where the YGK herbal composition was added to in adequate concentrations. The inhibition of the pathological changes of MT4 cells indicated that the YGK herbal composition had inhibitory effect on pathological changes of the cultured cells caused by HIV.

The effective concentration of the YGK herbal composition for inhibition of the pathological changes of MT4 cells was more than 12.5 mg/ml. To achieve a 50% of inhibition, the concentration of the YGK herbal composition was 25 mg/ml.

Conclusion

The YGK herbal composition was effective in inhibiting pathological changes in cells caused by HIV-1 in vitro.

EXAMPLE 8
A Case Study on an HIV-Patient Treated With the YGK herbal Composition

Purpose

The following clinical trial was conducted in the Infectious Disease Hospital in Shanghai, China to test the effectiveness of the herbal composition of the present invention in treating an HIV-infected patient.

Methods

A fifty-year Chinese male patient diagnosed with HIV infection complicated by herpes zoster was treated with anti-virus regimens by the combination of western medicine and the herbal composition of the present invention during hospital stay.

Results

The patient was confirmed of HIV-infection by Rapid Agglutinin Assay. At the time of the initial diagnosis in August 1996, the patient showed no symptoms. Starting Jun. 1, 1997, the patient quickly developed an herpetiform rash over the front of the left side of the check extending over the nick, the shoulder, and the upper left arm. The patient was then admitted into the Hospital in Jun. 24, 1997.

At the hospital, the result of the physical examination was normal except the skin rash. The pathology tests confirmed normal renal function. The functional tests of the liver showed a slightly increased levels of serum [gamma] glutamyl transpeptidase and acetyl glucuronidase. Hepatitis viral tests showed negative for Hepatitis B virus and Hepatitis C virus (HBV-DNA and HCV-RNA). However, Hepatitis G viral test showed positive for HGV-RNA. The immunological studies showed that the [beta]-2 microglobulin level was 2.4-2.5 mg/ml.

During the hospital stay, haemoglobin and erythrocytes levels of the patient were slightly decreased, while the levels of the leukocyte and platelet were normal. Peripheral blood lymphocytes counts showed that T4 cells were decreased to 2.76*10/L (32.9%) and the ratio of T4/T8 cell was 1.16. Thus, the diagnosis is that the patient was with HIV infection complicated by herpes zoster.

During hospital stay, the patient had diarrhea and dry cough for a few days and was cured. In September, 1997, the patient showed HIV antibody positive by ELISA, and his T4 cells further decreased to 25.4% and the ratio of T4/T8 cells was inverted to 0.94. Then, T4 cells and the ratio of T4/T8 gradually increased after treatment with the YGK herbal composition and as tested in November 1997, his T4 cells were 40.7%, and the ratio of T4/T8 1.45. The skin rash gradually disappeared and completely recovered by the end of November.

Conclusion

The YGK herbal composition was effective in reducing symptoms of the HIV-infected patient in a treatment regime together with western medicine.

EXAMPLE 9
Clinical Trial on HIV-Infected Patients Treated with the YGK Herbal Composition

Purpose

The following clinical trial was conducted in De-Tang Hospital (National AIDS Therapy Center) in Beijing, China to test the effectiveness of the herbal composition of the present invention in treating HIV-infected patients.

Methods

Five (5) HIV-infected patients were treated with the YGK herbal composition. The infection was confirmed by western blotting. The profile of the patients were as follows:

Patients	Sexuality	Age	History	Diagnosis
1	Male	32	2 years	AIDS (Stage IV)
2	Female	32	1 year	AIDS (Stage IV)
3	Male	31	1 year	AIDS

(Stage III)
4 Male 25 0.5 year AIDS
(Stage II)
5 Male 17 3 weeks HIV Infection

The patients were treated according to the following regimen:

Five (5) ml injection fluid herbal composition of the present invention was dissolved in 250 ml 5% glucose solution. The solution was injected intravenously once per day for three (3) days. Then, the dosage was increased to 15 ml injection fluid in 250 ml 5% glucose solution, and the patients were injected intravenously once per day without uncomfortable reactions for three (3) months.

Additionally, patient #1 was treated with AZT+DDI therapy for ten (10) days before being treated with the YGK herbal composition; patient #5 was treated with combination of interferon and the herbal composition.

Three (3) ml blood sample was taken from the patients each time before, during, and after the treatment and further tested for HIV.

Results

The HIV counts of the patients are as follows: 1st month 2nd month 3rd month,
before during during at the end
Patients treatment treatment treatment of treatment

1 1.9 * 10<4> 1.7 * 10<5> 6.3 * 10<3> 1.5 * 10<4>
2 1.5 * 10<4> 6.3 * 10<3> 3.8 * 10<2>
3 7.3 * 10<3> 3.2 * 10<3>
4 3.0 * 10<5> 1.9 * 10<4> 1.9 * 10<4>
5 3.9 * 10<5> 2.6 * 10<3> 1.8 * 10<3> *
Note: the control level of HIV is 3,000.

Based on the above table, all patients showed decreased HIV level and increased CD4 cells, except in patient #5 who was also treated with interferon. Especially, patient #2 had significant decrease of HIV; his CD4 counts also dropped from 285/mm<3 >to 510/mm<3>.

Conclusion

The herbal composition of the present invention is effective in reducing HIV in serum in HIV-infected patients.

EXAMPLE 10
Clinical Trial on HIV-Infected Patients Treated with the YGK Herbal Composition In Russia

Purpose

The following experiment was conducted in Hospital in Siberia, Russia to amine the effectiveness of the YGK herbal composition of the present invention against HIV.

Methods

Five (5) HIV-infected patients were treated with the YGK herbal composition. The profile of the patients were as follows:Patients Sexuality Age History Diagnosis

1 Female 23 2 years AIDS (Phase A3), adenitis,
hepatitis C, Syphilis,
Citomegalo infection, Gonorrhea

2 Female 28 2 year AIDS (Phase A3), adenitis,
hepatitis B and C, Gerpec and
Citomegalo infection, Gonorrhea,
drug abuse

3 Male 35 1 year AIDS (Phase B2), adenitis

4 Male 22 1 year AIDS (Phase B2), adenitis, hepatitis C, drug abuse

5 Male 34 several months AIDS (phase A3), adenitiis hepatitis B and C, 10% weight loss, drug abuse

2. The patients were treated with the herbal composition of the present invention.

Samples were taken from the patients each time before, during, and after the treatment and further tested for CD4 cells.

Results

The CD4 cells counts of the patients are as follows:

Patients before treatment / 2nd month treatment / 5th month at the end of treatment

1 477 641 849
2 740 1140 705
3 421 . . . 527
4 440 490 669
5 625 . . . 814

Note: the normal level of CD4 cell count is about 500.

During the treatment process, all patients had positive response except some minor side effects. The symptoms of the patients were improved after one month of treatment including alleviation of weakness, depression, and stegnosis. The abdominal region pain and uncomfortable feeling also disappeared. Patients #4 and #5 had 5 kg increase of body weight after three (3) months of treatment. Patients #2 and #4 were disintoxicated. The biological marker of the liver showed normal after all patients after the treatment.

Based on the above table, all patients showed increased CD4 cell counts except patient #2.

Conclusion

The herbal composition of the present invention is effective in reducing symptoms in AIDS patients.
