

HERBAL PHARMACEUTICAL COMPOSITIONS FOR TREATING IMMUNOLOGICAL DISORDERS

FIELD OF THE INVENTION

5 The present invention relates to pharmaceutical compositions, their use for treating patients with immunological disorders, and the methods for making the pharmaceutical compositions. Particularly, the pharmaceutical compositions contain herbs which are (1) Ophiopogon (*Tuber Ophiopogonis*), (2) pinellia (*Tuber Pinelliae*), (3) raw licorice (*Radix Glycyrrhizae*), and (4) one of the following herbs: lantern tridax (*Herba Tridaxis*
10 *procumbentis*), Taiwan adenostema (*Herba Adenostematis*), and heartleaf houttuynia (*Herba houttuyniae*). Optionally, a fifth herb, which is either tang-shen (*Radix Codonopsis*) or American ginseng (*Radix Panacis Quinquefolii*), can be added. The herbal pharmaceutical compositions are used for effective treatment of Immunoglobulin E (IgE) -mediated diseases, which include, but are not limited to, allergic rhinitis, allergic conjunctivitis,
15 allergic asthma, atopic eczema, atopic dermatitis, food allergy, hyper IgE syndrome, and rheumatoid arthritis.

BACKGROUND OF THE INVENTION

 Antigen-induced, particularly allergen-induced, immunological disorders afflict
20 more than 20% of the world population and cause serious health problems. One example of such immunological disorders is asthma. In recent years, the onset of allergy-related immunological disorders has shifted to the younger population, and more and more children and adolescents have developed symptoms of allergen-induced immunological disorders.

 For instance, in Taiwan, the prevalence of childhood asthma increased from 1.3% in
25 1974 to 5.07% in 1985, and to 5.8% in 1991. Also, the incidence of allergic rhinitis increased from 7.84% in 1985 to 20.67% in 1991. Further, the occurrence of atopic eczema was 1.43% in 1974 which rose to 1.23% in 1985 and 3.84% in 1991. The early onset of allergen-related immunological disorders may be attributable to environmental pollution.

 Respiratory allergies are IgE-mediated immune response. Brinker, J. Naturopathic
30 Medicine, 4: 64-68 (1993). There are two major respiratory allergic reactions: allergic rhinitis and allergic bronchial asthma. Allergic rhinitis, also known as hay fever, is brought on by the binding of antigen/Ig E to sensitized mast cells and basophils. The binding causes a decrease in cAMP, which in turn leads to the release of eosinophil chemotactic factor and

histamine. Histamine then binds to H1-receptors and causes the increase of vasodilation, capillary permeability, and smooth muscle contraction, as manifested by nasal congestion with watery discharge, sneezing, and itching eyes.

Allergic asthma is another IgE-mediated immune response in which mast cells
5 release histamine, bradykinin, and arachidonic acid metabolites including the leukotriene slow reacting substances of anaphylaxis and thromboxane/prostaglandin bronchoconstrictors. Platelet activating factor is another potent asthma mediator released from a number of leukocytes. Under this condition, histamine, which released after a decrease in cAMP, acts on H1-receptors to cause bronchospasm; histamine is also
10 responsible for broncho-constriction due to cholinergic reflex action and half of the prostaglandin generation in anaphylaxis.

As the percentage of the affected population and the severity of the diseases are rising, the treatment methods for allergic reaction still remain dependent primarily on empirical and serendipitous findings rather than scientific approach. Most allergy patients
15 are treated with drugs that aim at controlling symptoms resulted from the release of mediators by effector cells. Some drugs appear to be effective in short term release of the symptoms without adverse effect, however, drugs have not been found which have long-term effects for preventing disease progression and permanent destruction without any adverse effect. For example, long-term oral therapy for treating asthma, such as steroid
20 therapy, is now known to be associated with multiple debilitating effects such as growth delay, osteoporosis, and adrenal suppression.

Traditional Chinese herbs and medicinal combinations provide an alternative for treating immunological disorders. The traditional Chinese herbs and herbal combinations have been known for improving immune system function and treating various chronicle
25 immunological disorders. For example, Hamasaki et al., *J. Ethnopharmacology*, (1997) 56:123-131, disclose a Chinese herbal medicine, Shinpi-To, which inhibits IgE-mediated leukotriene synthesis in rat basophilic leukemia-2H3 cells. Shinpi-To is a freeze-dried granular Chinese herbal medicine prepared from extracts of seven medicinal herbs including
30 *Ephedrae herba* (*Ephedra sinica* Stapf), *Armeniacae semen* (*Prunus armeniaca* Linne), *Magnoliae cortex* (*Magnolia obovata* Thunberg), *Aurantii nobilis Pericarpium* (*Citrus unshiu* Markovich), *Glycyrrhizae radix* (*Glycyrrhiza uvalensis* Fischer), *Bupleuri radix*

(*Bupleurum falcatum* L.), and *Perillae herba* (*Perilla frutescens* Britton var. *acuta* Kudo).
Shinpi-To is useful for treating childhood asthma.

Toda et al., *J. Ethnopharmacology*, (1988) 24:303-309, discloses a Chinese herbal medicine, Saiboku-To, which shows inhibitory effects on histamine release from mouse
5 peritoneal mast cells. Saiboku-To is useful for treating asthma. Saiboku-To contains ten medicinal herbs including *Bupleurum falcatum* L., *Pinelliae ternata* Breitenbach, *Poria cocos* Wolf, *Scutellaria baicalensis* Georgi, *Zizyphus vulgaris* Lam., *Panax ginseng* C.A. Meyer, *Magnolia obovata* Thunberg, *Glycyrrhiza glabra* L., *Perillae frutescens* Britton var. *acuta* Kudo, and *Zingiber officinale* Roscoe.

10 Li et al., *Immunopharmacology*, (1999), 43:11-21, discloses a Chinese herbal medicine, Hochu-Ekki-To, which shows effects on restoring stress-induced immunosuppression. Hochu-Ekki-To consists of ten ingredients including *Ginseng Rx.*, *Atractylodis alba* Rz., *Astragali Rx.*, *Angelicae sinensis Rx.*, *Jujubae Fr.*, *Citri reticulatae Pc.*, *Bupleuri Rx.*, *Glycyrrhizae Rx. Preparata*, *Zingiberis recens* Rz., and *Cimicifugae* Rz.

15 Zou et al., *Chinese Traditional and Western Medical Magazine*, 529-532 (1996), discloses a Chinese herbal medicine, Wenyang Tongluo mixture, for treating cold-type asthma patients. It shows effects of improving pulmonary ventilation functions, regulating adrenergic β -receptors of peripheral blood lymphocytes, and decreasing the serum level of 5-hydroxytryptamine. Wenyang Tongluo mixture contains twelve medicinal herbs
20 including *Red Ginseng*, *Zhi Fu Pian*, *Yin Yang Huo*, *Dried Ginger* (*Zingiberis recens* Rz.), *Zhi Huang Zhi*, *Angelicae sinensis Rx.*, *Ephedra*, *Polygalae Rx.*, *Sang Baipi*, *Sheng Shi Gao*, *Schisandrae Fr.*, and *Licorice* (*Glycyrrhizae Rx. Preparata*).

A decoction for treating bronchitis and bronchial asthma has been disclosed on the internet (www.herb.com.tw). The decoction is made from a combination of six herbs
25 including *Ophiopogonis Rx.*, *Pinelliae Rz. Preparata*, *Oryzae Sm.*, *Jujubae Fr.*, *Ginseng Rx.*, and *Glycyrrhizae Rx.*

Quality control of the Chinese herbal medicine is critical in developing pharmaceutical composition from the herbs. The contents and active ingredients of the herbs may fluctuate with factors such as the growing condition and locality of growth,
30 harvesting season and technique, and manufacturing and processing of the harvested herbs. If any of the critical factors is not met, the effectiveness of the herbal medicine may be

compromised. Nevertheless, no scientific method for reliable monitoring of the quality of the Chinese herbal medicine has been reported or adopted.

The present invention provides novel and non-toxic pharmaceutical compositions derived from herbs which are primarily found in China and Taiwan. The combined use of these herbs has never been found in any literatures or reports. These pharmaceutical compositions are effective in treating patients with immunological disorders, especially IgE mediated diseases. The pharmaceutical compositions of the present invention have demonstrated special effects on down-regulation of the synthesis of interleukin 4 (IL-4) and suppression of IgE. In addition, to effectively control the quality of the herbs, the present invention fingerprints each herb using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) to ensure that each herb used in the composition contains consistent and reproducible ingredients.

SUMMARY OF THE INVENTION

The present invention provides pharmaceutical compositions which contain, as essential herbs, Ophiopogon (*Tuber Ophiopogonis*), Pinellia (*Tuber Pinelliae*), raw Licorice (*Radix Glycyrrhizae*), and an herb selected from the group consisting of Lantern Tridax (*Herba Tridacis procumbentis*), Taiwan Adenostema (*Herba Adenostematis*), and Heartleaf Houttuynia (*Herba Houttuyniae*). The pharmaceutical compositions of the present invention further contain an herb which is either tang-shen (*Radix Codonopsis*) or American ginseng (*Radix Panacis Quinquefolii*). The preferred weight ratio of the herbs, ophiopogon : pinellia : raw licorice : American ginseng/Tang-shen : lantern tridax/Taiwan adenostema/heartleaf houttuynia, is 3 : 2 : 1 : 1 : 1.

The most favorable pharmaceutical composition contains ophiopogon, pinellia, raw licorice, American ginseng, and lantern tridax. The effective ingredients of these herbs can be either extracted by water or organic solvent, or in powder form which is obtained from direct grinding of the herbs.

In one embodiment, the pharmaceutical composition contains ophiopogon in aqueous extract and the rest of the herbs in powder. The aqueous extract of ophiopogon is preferably prepared by extraction in water.

In another embodiment, the pharmaceutical composition contains aqueous extracts of ophiopogon, pinellia, raw licorice, and American ginseng, preferably by extraction in water;

and aqueous extract of lantern tridax, preferably extracted in alcohol (most favorably in 50% alcohol).

The present invention also provides methods for preparing the pharmaceutical compositions in which aqueous extract and/or powders of the herbs are prepared. In one embodiment, except for ophiopogon, all of the other herbs are cut into pieces, dehydrated, ground, and passed through a sieve to produce herbal powder. The ingredients of ophiopogon are extracted by boiling and condensing ophiopogon in water. The ophiopogon extract (filtered) is then obtained by passing the condensed ophiopogon-water mixture through a sieve. Second, the ophiopogon filtrate is mixed with the herbal power to form a herbal mixture. This herbal mixture is then placed into a fluidized granulation machine for the production of granules. The granules can optionally be sieved to produce granules with uniform sizes. The granules can be further encapsulated.

In yet another embodiment, the pharmaceutical composition is prepared as follows:

(a) Each of the aqueous extracts of ophiopogon, pinellia, raw licorice, American ginseng and lantern tridax is preferably separately filtered and condensed. (b) Except for ophiopogon which is not granulated, each of the condensates of pinellia, raw licorice, American ginseng, and lantern tridax are individually granulated from condensate to form individual granules. (c) Before granulation, an excipient, preferably corn starch, is added to the condensate to confer a suitable form of the granules. (d) The individual granules and the condensate of ophiopogon are then mixed together to form mixed granules, which are then encapsulated.

Preferably, the solid content of the condensate of ophiopogon constitutes about 20-30% by weight, preferably about 25% by weight, of the ophiopogon raw material. The solid content of the condensate is the condensate excluding the water. In the case of ophiopogon, the condensate contains substantial amount of water (i.e., about 58% of the condensate of ophiopogon is water). The solid contents of the rest of the herbs are about the same as the condensate (i.e., the water content was insignificant). The condensate of pinellia constitutes about 15-25% by weight, preferably about 20% by weight, of the pinellia raw material. The condensate of raw licorice constitutes about 15-25% by weight, preferably about 20% by weight, of the raw licorice raw material. The condensate of American ginseng constitutes about 19-29% by weight, preferably about 24.3% by weight, of American ginseng raw

material. The condensate of lantern tridax constitutes about 5-10% by weight, preferably about 7.8% by weight, of lantern tridax raw material.

The granules of pinellia constitute about 20-30% by weight, preferably about 25% by weight, of the pinellia raw material. The granules of raw licorice constitute about 16-26%
5 by weight, preferably about 21% by weight, of the raw licorice material. The granules of American ginseng constitute about 27-37% by weight, preferably about 32% by weight, of the American ginseng raw material. The granules of lantern tridax constitute about 37-47% by weight, preferably 42% by weight, of the lantern tridax raw material.

Also, the method requires that the extraction of ophiopogon, pinellia, raw licorice,
10 American ginseng and lantern tridax be conducted individually. In addition, ophiopogon, pinellia, raw licorice, and American ginseng are extracted in water by decocting. Lantern tridax is extracted in alcohol, preferably 50% alcohol, by refluxing. After the extraction, each individual extract is filtered to remove the remaining herb. The filtrate is then condensed by decompressed condensation at a temperature of about 55°C and a vacuum of
15 about 30 torr to form a condensate. An adequate amount of an excipient, preferably corn starch, is added to each of the condensate, except ophiopogon, to form an extraction paste, which is subject to granulation by spray-drying at an inlet temperature of about 105°C and an outlet temperature of about 76-77°C. The respectively granules from pinellia, raw licorice, American ginseng, and lantern tridax are mixed with the condensate of ophiopogon
20 to form the mixed granules, which can be encapsulated.

In addition, the ratio of aqueous extractant by volume to the herb by weight is about 20:1. For water extraction, the preferable decocting time is about 30 minutes. The preferred alcohol extraction time is also about 30 minutes.

The two pharmaceutical compositions of the present invention, one containing
25 aqueous extract of ophiopogon and powders of other herbs and the other containing aqueous extracts of all the herbs, are both effective in treating immunological disorders. However, the pharmaceutical composition containing aqueous extracts of all of the herbs is slightly favorably because it demonstrates better drug efficacy and efficiency than the one containing aqueous extract of ophiopogon and powders of other herbs. The difference in treatment
30 effects of these two methods may be due to the effectiveness of increase in the release of active ingredients during the extraction.

The pharmaceutical compositions of the present invention are effective in treating patients with immunological disorders, particularly IgE mediated diseases, which include, but are not limited to, allergic rhinitis, allergic conjunctivitis, allergic asthma, atopic dermatitis, food allergy, hyper IgE syndrome, atopic eczema, and rheumatoid arthritis.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an HPLC chromatogram of ophiopogon. Its entire chromatographic profile was detected at 214 nm wavelength using method 1 of the HPLC analysis (see *infra*). There were two discrete peaks, 6115-1 and 6115-2, which had a retention time of 11.5 min and 13 min, respectively, shown in the chromatogram. These two peaks could be used to identify ophiopogon.

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Figure 2 shows an HPLC chromatogram of pinellia. Two chemical markers, adenine and guanosine, were detected at 260 nm wavelength using method 2 of the HPLC analysis. Adenine has a retention time of 14.8 min. Guanosine has a retention time of 17 min.

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Figure 3 shows an HPLC chromatogram of raw Licorice. A chemical marker, glycyrrhizin (Gly), was detected at 250 nm wavelength using method 1 of the HPLC analysis. Gly has a retention time of 46.4 min.

Figure 4 shows an HPLC chromatogram of American Ginseng. A chemical marker, ginsenoside Rb₁ (Rb₁), was detected at 203 nm wavelength using Method 3 of the HPLC analysis. Rb₁ has a retention time of 37.5 min.

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Figure 5 shows an HPLC chromatogram of Lantern Tridax. There were four (4) distinctive peaks, FY-1 to FY-4, detected at 250.0 nm wavelength using method 2 of the HPLC analysis. FY-1 has a retention time of 28 min. FY-2 has a retention time of 31.6 min. FY-3 has a retention time of 33.5 min. FY-4 has a retention time of 18.3 min.

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Figure 6 shows the levels of Der p 5 (*Dermatophagoides pteronyssinus* allergen 5) specific IgG and IgE antibodies after Der p 5 inhalation challenge followed by treatment of 5 (A-F) decoction groups. The serum IgG and IgE levels were measured by ELISA. Values are Means $\pm$ SEM with bar indicating the SEM. A: White-Draining Powder Group; B: Green-Blue Dragon Decoction Group; C: Ophiopogon Decoction Group; D: Dryness-Clearing lung-Rescuing Decoction Group; E: Perilla Fruit Qi-Downbearing Decoction Group; and F: Placebo Group.

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Figure 7 shows the expression of IL-4 gene in splenocytes after Der p 5 inhalation challenge followed by treatment of 5 (A-F) decoction groups. The expression of IL-4 gene was determined by RT-PCR (reverse transcriptase - polymerase chain reaction). Values are Means $\pm$ SEM with bar indicating the SEM. A: White-Draining Powder Group; B: Green-Blue Dragon Decoction Group; C: Ophiopogon Decoction Group; D: Dryness-Clearing lung-Rescuing Decoction Group; E: Perilla Fruit Qi-Downbearing Decoction Group; and F: Placebo Group. * Indicates $p < 0.05$.

Figure 8 shows mice serum IgG levels after challenged by allergen Der p 5 (*Dermatophagoides pteronyssinus* allergen 5) and treated with individual herb. Lane 1 shows the effect of ophiopogon treatment. Lane 2 shows the effect of tang-shen treatment. Lane 3 shows the effect of pinellia treatment. Lane 4 shows the effect of raw licorice treatment. Lane 5 is the placebo treatment. The serum IgG levels were measured by ELISA. Values are Means $\pm$ SEM with bar indicating the SEM.

Figure 9 shows mice serum IgE levels after challenged by Der p 5 (*Dermatophagoides pteronyssinus* allergen 5) and treated with individual herb or the pharmaceutical composition (i.e., STA-36, *infra*). Lane 1 shows the effect of ophiopogon treatment. Lane 2 shows the effect of tang-shen treatment. Lane 3 shows the effect of pinellia treatment. Lane 4 shows the effect of raw licorice treatment. Lane 5 shows the effect of STA-36 treatment. Lane 6 is the placebo treatment. The serum IgG levels were measured by ELISA. Values are Means $\pm$ SEM with bar indicating the SEM.

Figure 10 shows the expression of IL-4 gene in mice splenocytes after challenged by Der p 5 (*Dermatophagoides pteronyssinus* allergen 5) and treated with individual herb or the pharmaceutical composition (i.e., STA-36, *infra*). The expression of IL-4 gene was determined by RT-PCR (reverse transcriptase -polymerase chain reaction). Lane 1 shows the effect of ophiopogon treatment. Lane 2 shows the effect of tang-shen treatment. Lane 3 shows the effect of pinellia treatment. Lane 4 shows the effect of raw licorice treatment. Lane 5 shows the effect of STA-36 treatment. Lane 6 is the placebo treatment.

Figure 11 compare the effects of American ginseng in the form of (1) powder, (2) aqueous extract in 95% alcohol, (3) aqueous extract in water, and (4) aqueous extract in 50% alcohol, on the level of IgG_{2a} and IgE. Values are means $\pm$ SEM. The figure indicates that American ginseng extracted by 50% alcohol produced slightly better effects in terms of decreasing IgE and increasing IgG, than water extract.

Figure 12 compares the effects of lantern tridax in the form of (1) powder, (2) aqueous extract in 95% alcohol, (3) aqueous extract in water, and (4) aqueous extract in 50% alcohol, on the level of IgE, IgG_{2a}, IL-4, and IFN- γ . Values are means $\pm$ SEM. * indicates significant difference from control. Results indicate that 50% ethanol extract of lantern tridax was most effective on inhibiting IgE and increasing IgG.

Figure 13 is a comparative study of the effects of STA-36, STA-4, and control (the placebo group) on IgG and IgE in serum of female BALB/c mice. * indicates significant difference ($p < 0.05$) between STA-4 and control. Values are means $\pm$ SEM. The dosage amount of STA-36 (20 mg/20 g mouse) was twice of the dosage amount (10 mg/20 g mouse) of STA-4 (i.e., STA-3 : STA-4 = 2: 1). The results show that in terms of IgE, although both STA-36 and STA-4 show inhibition on IgE, only STA-4 is statistically significant from control. As of IgG, both STA-36 and STA-4 show increase in IgG. However, although it appears that STA-36 shows greater increase in IgG level than STA-4, neither increase is significantly different from the control.

Figure 14 is a comparative study of the effects of STA-36 and STA-4 on Lung Functions. Values are means $\pm$ SEM. Both STA-36 and STA-4 show significant improvement in lung functions (as determined by measurement of acetylcholine) as compared to control ($P < 0.05$). Between the treatment of STA-36 and STA-4, although it appears that STA-4 had better effect than STA-36, the difference between STA-36 and STA-4 was nevertheless not statistically significant probably due to insufficient numbers of animals used in each group. The dosage amount of STA-36 (20 mg/20 g mouse) was twice of the dosage amount (10 mg/20 g mouse) of STA-4.

Figure 15 compares the effects STA-4 on IgE, IgG, IL-4, and IFN- γ with those of the conventional drugs (for treating asthma) including Ketotifen, Prednisolone, and Aminophline. Values are means $\pm$ SEM. * indicates significant difference from control. The results of this study indicate that STA-4 significantly lowered the level of IgE, but significantly increased the level of IgG_{2a}, IL-4 and IFN- γ .

DETAILED DESCRIPTION OF THE INVENTION.

Antigen or allergen-mediated asthma is a respiratory disease primarily due to chronic inflammatory reaction. The inflammatory reaction is mediated by the release of inflammatory factors from the cells such as platelet activation factor, histamine,

prostaglandin, and leukotrien. Inflammatory reaction stimulates antigenic response in T lymphocytes (T cells), particularly, CD4+ cells. There are two types of CD4+ cells, Th1 and Th2 CD4+ cells. Th1 type of CD4+ cells secretes cytokine (IFN- γ). Th2 type of CD4+ cells secretes interleukin-4 (IL-4). Most of the allergic asthma patients are Th2 type. The allergen stimulates Th2 cell to produce excess IL-4, which in turn induces the production and secretion of IgE from B lymphocytes.

The present invention provides seven pharmaceutical compositions: STA-31, STA-32, STA-33, STA-34, STA-35, STA-36, and STA-4. All of the compositions possess pharmaceutical effects on immunological disorders, particularly on suppression of IgE production and down regulation of the synthesis of interleukin-4 (IL-4) gene. The herbal contents of the herbal pharmaceutical compositions are shown in Table 1.

Table 1. Herbal Compositions in the Pharmaceutical Compositions

Herbal Composition	Herb #1 (prep form)	Herb #2 (prep form)	Herb #3 (prep form)	Herb #4 (prep form)	Herb #5 (prep form)
STA-31	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Tang-Shen (powder)	Adenostema (powder)
STA-32	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Tang-Shen (powder)	Houttuynia (powder)
STA-33	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Tang-Shen (powder)	Tridax (powder)
STA-34	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Ginseng (powder)	Adenostema (powder)
STA-35	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Ginseng (powder)	Houttuynia (powder)
STA-36	Ophiopogon (aq. ext.)	Pinellia (powder)	Raw Licorice (powder)	Ginseng (powder)	Tridax (powder)
STA-4	Ophiopogon (aq. ext.)	Pinellia (aq. ext.)	Raw Licorice (aq. ext.)	Ginseng (aq. ext.)	Tridax (50% alcohol extract)

aq. ext. = aqueous extract by water; Ophiopogon= *Tuber Ophiopogonis*; Pinellia= *Tuber Pinelliae*; Raw Licorice= *Radix Glycyrrhizae*; Tang-seng= *Radix Codonopsis*; Ginseng = *Radix Panacis Quinquefolii*; Adenostema= *Herba Adenostematis*; Houttuynia = *Herba Houttuyniae*; Tridax = *Herba Tridacis procumbentis*.

Among the seven compositions, STA-36 is preferred and STA-4 is the most preferred composition. STA-36 and STA-4 are particularly effective in treating immunological disorders including suppression of IgE production.

Each of the pharmaceutical compositions contains five herbs, three out of which are essential herbs. The essential herbs are ophiopogon (also known as black leek), pinellia, and raw licorice. The fourth herb can be either tang-shen or American ginseng. The fifth herb is

selected from the group consisting of Taiwan adenostema, Lantern Tridax, and Heartleaf houttuynia. Taiwan adenostema and Heartleaf houttuynia, according to available herbal literatures, can only be found in Taiwan. Lantern tridax can be found in Taiwan and South America. The pharmaceutical names, botanical names, family, and major ingredients of the compositions are shown in Table 2.

Table 2. *Pharmaceutical/Botanical Names and Major Ingredients of the Herbs in the STA Pharmaceutical Composition*

Herb	Pharmaceutical Name	Botanical /Zoological Name	Family	Major Ingredients
Ophiopogon (black leek)	<i>Tuber Ophiopogonis Japonici</i>	<i>Ophiopogon japonicus</i> Ker-Gawl.	Liliaceae	Ophiopogonin, Ruscogenin, β -Sitosterol, Stigmasterol
Tang-Shen	<i>Radix Codonopsis Pilosulae</i>	<i>Codonopsis pilosula</i> (Franch.) Nannf.	Campanulaceae	saponins, alkaloids, taraxerylacetate, friedelin, sucrose, glucose, inulin
American Ginseng	<i>Radix Panacis Quinquefolii</i>	<i>Panax quinquefolium</i> L.	Araliaceae	saponins, panaquilon
Pinellia	<i>Tuber Pinelliae, Rhizoma Pinellia Ternatae</i>	<i>Pinellia ternata</i> (Thunb.) Breitenbach	Araceae	coniine, protoanemonin, homogentisic acid, nicotine, aspartic acid, glutamic acid, arginine, β -sitosterol, cholesterol
Raw Licorice	<i>Radix Glycyrrhizae Uralensis</i>	<i>Glycyrrhizaeuralensis</i> Fisher or <i>Glycyrrhiza glabra</i> Linné.	Leguminosae	Azetidine-2-carboxylic acid, Aspartate, Homoserine, Diaminobutyric Acid, Digitalis Glycoside
Lantern Tridax	<i>Herba Tridacis procumbentis</i>	<i>Tridax procumbens</i> Linn.	Compositae	polysaccharide
Taiwan Adenostema	<i>Herba Adenostematis</i>	<i>Adenostema lavenia</i> (L.) Ktze.	Compositae	
Heartleaf Houttuynia	<i>Herba Houttuyniae</i>	<i>Houttuynia cordata</i> Thunberg	Saururaceae	decanoyl acetaldehyde, lauric aldehyde, methyl-n-nonylketone,

				myrcene, capric aldehyde, capric acid, cordarine, calcium sulfate, calcium chloride, isoquercitrin, reynoutrin, hyperin
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Ophiopogon (*Radix Ophiopogonis*) belongs to the family of *Liliaceae*. The tuber (root) of Ophiopogon has medicinal effects. The best harvesting season for ophiopogon is the summer. After harvested, the tuber of ophiopogon should be washed clean to get rid of unwanted dirt or rootlets, which was followed by drying under sun to preserve for later use. Ophiopogon tuber is the enlarged part of the root. Ophiopogon could be found in provinces such as Zhejiang, Sichuan, and Jiangsu, of China. The aqueous extract of ophiopogon was reported by Yu et al. (1991), China Journal of Chinese Materia Medica, 16:584-585, to enhance the clearance of iv charcoal particles in mice, and antagonize the leukopenia caused by clophosphamide. To be effectively used in the herbal composition, Tuber Ophiopogon can be prepared by aqueous extract by water or other organic solvents. It is preferred that the dosage preparation form for the herb is extraction by water.

Tang-shen (*Radix Codonopsis Pilosulae*) belongs to the family of *Campanulaceae*. The medicinal effects of Tang-Shen is in the root. Tang-shen can be either collected in the wild or cultivated. The typically harvest season for tang-shen is in autumn. After harvested, the root of tang-shen was dried under sun, and cut into segments for later use. Tang Shen could be found in Nantou Shien and Taichung Shien of Taiwan. To be effectively used in the herbal composition, the herb can be prepared by aqueous extract by water or other organic solvents or powder from grinding raw herb. The preferred dosage preparation for the herb is either aqueous extract through extraction by water or ground powder.

American ginseng (*Radix Panacis Quinquefolii*) belongs to the family of *Araliaceae*. The medicinal effects of American ginseng is in the root. American ginseng can be found in northern United States and Canada. It has also been widely cultivated in France and northern China. The best harvest season for American ginseng is in autumn. To be effectively used in the herbal composition, the herb can be prepared by aqueous extract by water or other organic solvents or in a form of ground powder. The preferred dosage

preparation for the herb is either aqueous extract through extraction by water or ground powder.

Pinellia (*Tuber Pinelliae*, *Rhizoma Pinellia Ternatae*) belongs to the family of *Araceae*. The tuber (after the cork layer has been removed) of pinellia has medicinal effects.

5 The plant grows in Sichuan, Hubei, Henan, Guizhou, and Anhui provinces of China. The normal harvest season of pinellia is between July and September. Pinellia can be found in Taichung Shien of Taiwan. To be effectively used in the herbal composition, the herb can be prepared by aqueous extract by water or other organic solvents or in a form of ground powder. The preferred dosage preparation for the herb is either aqueous extract by water or
10 ground powder.

Raw licorice (*Radix Glycyrrhizae Uralensis*) belongs to the family of *Leguminosae*. The root and stolon, with (unpeeled) or without (peeled) the periderm, of raw licorice have medicinal effects. Raw licorice contains not less than 2.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$), calculated on the basis of dried material, which can be used as chemical marker for raw
15 licorice. Raw licorice can be found in inner Mongolia, Gansu, Xinjiang, and northeastern China. The best harvest seasons for raw licorice are either in spring or autumn. In Taiwan, raw licorice can be found in Taichung Shien. Licorice root has anti-inflammatory properties and can be used alone to help release symptoms of asthma. See H.W. Morningstar, Sentient Times: Alternative for Personal and Community Transformation (1998), 6:16-17. Licorice
20 roots are also useful for treating bronchitis. See T. David, Miracle Medicines of the Rainforest: A Doctor's Revolutionary Work With Cancer And AIDS Patients (1997), pp. 66-79. To be effectively used in the herbal composition, the herb can be prepared by aqueous extract by water or other organic solvents or in a form of ground powder. The preferred dosage preparation for the herb is either aqueous extract by water or ground powder.

25 Lantern tridax (*Herba Tridacis procumbentis*) belongs to the family of *Compositae*. The medicinal effects of lantern tridax come from the dried whole plant. Lantern tridax can be found in Nantou Shien, Taichung Shien, and Yunlin Shien, and the city of Taichung of Taiwan. Lantern tridax has also been found in South America. Diwan et al., Indian Journal of Physiology & Pharmacology (1983), 27: 32-36, disclose that the juice extracted from the
30 leaves of lantern tridax has effects resembling dexamethasone on wound contraction and granulation, but significantly counteracts the effects of dexamethasone on tensile strength and epithelization. Gan et al., China Medical College Annual Bulletin (1977), 8: 526,

disclose that leaves of lantern tridax is useful for diminishing inflammation, releasing symptoms of fever, and curing pneumonia and cough.

To be effectively used in the herbal composition, lantern tridax can be prepared by aqueous extraction by water or other organic solvents or in a form of ground powder. The preferred dosage preparation for the herb is either ground powder or aqueous extract by alcohol, and preferably,, 50% alcohol for extraction.

Taiwan adenostema (Herba Adenostematis) belongs to the family of Compositae. Taiwan adenostema usually grows near water or in wetland. The medicinal effects of Taiwan adenostema come from the entire plant, either used freshly or in dried form. The best harvest seasons are in summer or early autumn. Taiwan adenostema can be found not only in Taiwan but also in Yunnan, Guangxi, Guizhou, Jiangxi, and Chekiang provinces of China. Taiwan adenostema contains 11-hydroxylated kauranic acid. To be effectively used in the herbal composition, the herb can be prepared in a form of ground powder. The preferred dosage preparation for the herb is ground powder.

Heartleaf houttuynia or Houttuynia Herb (Houttuyniae cum Radice Houttuyniae Cordatae) is the terrestrial part of *Houttuynia cordata* Thunberg. It belongs to the family of *Saururaceae*. Heartleaf houttuynia grows throughout the Yangtze river valley and south in China. The best harvest season is during the flowering season in late summer to early autumn. Hanhong Heartleaf houttuynia can be found in Taichung Shien of Taiwan. To be effectively used in the herbal composition, the herb can be prepared in a form of ground powder. The preferred dosage preparation for the herb is ground powder.

In general, the compositions are prepared as follows:

A. Preparation of STA-31, STA-32, STA-33, STA-34, STA-35, and STA-36:

(1) The roots of pinellia, raw licorice and American ginseng, and the entire plant of lantern tridax were cut, dehydrated, ground, and passed through a sieve (preferably 40-120 mesh) to produce herbal powders.

(2) The root of ophiopogon was cut into small pieces and immersed in water in a decocting container where the water volume was at least 10 cm above the top of ophiopogon. The submerged ophiopogon was heated at 97 to 103°C for about sixty (60) minutes twice to produce an ophiopogon extract solution. Then, the ophiopogon extract solution was passed through a sieve (approximately 100 mesh). The filtrate was collected. The ophiopogon filtrate was then condensed at a temperature of 50 to 60°C under vacuum

condition with pressure between 400 and 650 mm-Hg to obtain a liquid condensate with a desirable concentration. It was preferred that the liquid condensate was about 1/14 of the ophiopogon filtrate.

- (3) The liquid condensate of ophiopogon was mixed with the herbal powders of the rest of the herbs. The mixture was then transferred to the fluidized granulator and placed in a spraying tube made of filtered fabric. The granulator was set at 90 to 110°C. After pre-heating for about five (5) minutes, the mixture was sprayed at about 40 to 55 seconds per time for an appropriate amount of times and the spraying tube was patted at a frequency of once in every 10 seconds. After granulation, the granules were dried and then cooled off.
- 10 The granules were passed through sieves and packaged in PE bags. The preferred water content of the granules was less than 6%. The granules can be further encapsulated in any of the conventional capsules, including, but not limited to, natural gelatin, pectin, casein, collagen, protein, modified starch, and polyvinyl pyrrolidone.

B. Preparation of the STA-4 Herbal Composition:

- 15 The STA-4 herbal composition was prepared by the following method:

I. *For lantern tridax:*

- (1) The entire plant of lantern tridax was cut into small pieces.
- (2) About 20 times (by volume) of 50% alcohol (v/v) (*i.e.*, extractant) was added to and mixed with about 1 time (by weight) of the small pieces of lantern tridax.
- 20 (3) The herb, together with its extractant, was heated by refluxing heat for about 30 minutes. This process produced an alcohol extract of lantern tridax.
- (4) After the extract was cooled down, the alcohol extract of lantern tridax was filtered through a sieve (approximately 100 mesh). The filtrate was collected. The filtrate was then condensed by a decompressed condensation method at a temperature of 50 to 60°C
- 25 (preferably at 55°C) under vacuum condition (at 30 torr) to obtain a condensate. Preferably, the solid content of the condensate is about 7.8% by weight of the raw material. The solid content of the condensate is the condensate excluding 50% alcohol. In the case of lantern tridax, the solid content of the condensate is about the same as the condensate (because very little amount of water is in the condensate).
- 30 (5) An adequate amount of corn starch (as excipient) was added to the condensate to produce an herbal paste.

(6) The herbal paste was dried by spray-drying method to form the lantern tridax granules (preferably about 42% by weight of the raw material).

II. For pinellia, raw licorice and American ginseng:

5 (1) The tuber of pinellia and the roots of licorice of American ginseng were separately cut into small pieces.

(2) For each herb, about 20 times (by volume) of water was added to and mixed with about 1 time (by weight) of the small pieces of the individual herb, respectively.

10 (3) Each herb, which was submerged in water, was heated by decocting for about 30 minutes, resulting in producing water extracts of ophiopogon, pinellia, raw licorice, and American ginseng respectively.

(4) After the respective extract was cooled down, each extract was separately filtered through a sieve (approximately 100 mesh). Each of the filtrate was collected, and then condensed by a decompressed condensation method at a temperature of 50 to 60°C
15 (preferably at 55°C) under vacuum condition (at 30 torr) to obtain a liquid condensate for each of the herb. It is preferably that the solid content of the condensate of pinellia is about 20% of the pinellia raw material; the solid content of the condensate of raw licorice is about 20% of the raw licorice raw material; and the solid content of the condensate of American ginseng is about 24.3% of the American ginseng raw material. The solid content of the
20 condensate of pinellia, raw licorice, or American ginseng is the condensate excluding the water.

(5) An adequate amount of corn starch (as excipient) was added to each of the condensate then granulated by spray-drying method to produce individual granules.

25 (6) The individual granules, together with the granules from lantern tridax, were mixed together to form mixed granules.

III. For Ophiopogon:

The tuber of ophiopogon was condensed in accordance with the procedures of II (1)-(4). The only difference between the condensate of ophiopogon and those of the pinellia,
30 raw licorice, and American ginseng was that the ophiopogon condensate contained a significant amount of water while the rest of the condensates were almost deprived of water.

For 9 g of ophiopogon raw material, the condensate was about 5.4 g in which about 2.25 g was solid content and 3.15 g was water.

The liquid condensate of ophiopogon was mixed with the rest of the herbal granules.

The gram weights of the herbs in the raw materials, STA-36, and STA-4 are

5 illustrated in the following Table 3a as a comparison:

Table 3a. Content and Weight of STA-36 and STA-4

	<i>Ophiopogon</i>	<i>Pinellia</i>	<i>Raw Licorice</i>	<i>American ginseng</i>	<i>Lantern tridax</i>	<i>Total Weight (Oral Dosage Form)</i>	<i>Ratio (Dosage) of STA-36/STA-4</i>
Raw Material	9 g	6 g	3 g	3 g	3 g	24 g	
STA-36	5.4 g (2.25 SC* + 3.15 g water)	6 g (herbal powders)	3 g (herbal powders)	3 g (herbal powders)	3 g (herbal powders)	20.4 (herbal powders)	2
STA-4	5.40 g condensate (2.25 g SC* + 3.15 g water)	1.50 g granules (1.20 g SC* + 0.30 g Corn Starch)	0.63 g granules (0.60 g SC* + 0.03 g Corn Starch)	0.96 g granules (0.73 g SC* + 0.23 g Corn Starch)	1.25 g granules (0.23 g SC* + 1.02 g Corn Starch)	10.20 g (9.74 g of granules and ophiopo gon condens ate + 0.46 g of Corn Starch)	1

*SC = Solid Content (which is the dried condensate [condensate excluding water or solvent]).

In Table 3a, the "raw material" line shows the weight of each herb before processing.

10 The "STA-36" and "STA-4" lines show (1) what the final form of each of the herbs was; and (2) what the final weight of each of the herbs was. For example, in STA-36, except for ophiopogon which was in the form of condensate, pinellia, raw licorice, American ginseng, and lantern tridax were all in the form of herbal powders. Also, except for ophiopogon which contained less weight than its raw material, the herbal powders of the rest of the herbs

15 in STA-36 maintained the same weight as their raw materials.

In STA-4, only ophiopogon maintained the same final form as that in STA-36, i.e., as condensate. The gram weight of the rest of the herbs, after gone through extraction, condensation, and granulation, had been substantially reduced, as indicated in Table 3a. In addition, to assist in stabilizing the condensate during granulation, an excipient (e.g., corn starch) was added to each of the herbal condensate. The amount of corn starch which was added to each of the condensates varied depending on the stability of the condensates. The end product of STA-4, which was in the form of granules, was about half of the weight of that of STA-36, even though both were derived from the same amount of raw materials. The reduction in total gram weight in STA-4, while maintaining the same or better therapeutic effects on immunological disorders as STA-36, constituted another improvement from STA-36, because it meant that the patients would not have to take in twice amount of the herbs to get the same or better therapeutic effects.

The following examples are illustrative, and should not be viewed as 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

Preparation of STA-31, STA-32, STA-33, STA-34, STA-35, and STA-36

The contents of the pharmaceutical compositions are shown in Table 1, and the quantities by weight of the herbs in the combinations are illustrated in Table 3.

Table 3. Quantity (by Weight) of each Herbs Used In STA-3

Exp. No.	Name	Ophiopogon (kg)	Tang-shen, or Notoginseng, or American Ginseng (kg)	Pinellia (kg)	Licorice (kg)	Lantern tridax, or Taiwan adenostema, or Heartleaf houttuynia (kg)
1	STA-31	3.00	1.00 (Tang-shen)	2.00	1.00	1.00 (Taiwan adenostema)
2	STA-32	3.00	1.00 (Tang-shen)	2.00	1.00	1.00 (Heartleaf Houttuynia)
3	STA-33	3.00	1.00 (Tang-shen)	2.00	1.00	1.00 (Lantern tridax)
4	STA-34	3.00	1.00 (American Ginseng)	2.00	1.00	1.00 (Taiwan adenostema)
5	STA-35	3.00	1.00 (American Ginseng)	2.00	1.00	1.00 (Heartleaf Houttuynia)
6	STA-36	3.00	1.00 (American Ginseng)	2.00	1.00	1.00 (Lantern tridax)

The method for preparing the pharmaceutical compositions was primarily the same as the general method for preparing the compositions (*supra*).

(1) All applicable herbs except ophiopogon, in the amounts as indicated in Table 3, were cut, dehydrated, ground, and passed through a sieve (preferably 40-120 mesh) to produce herbal powder.

(2) Ophiopogon in the amount as indicated in Table 3 was immersed in water in a frying-boiling container where the water volume was at least 10 cm above the top of ophiopogon. The submerged ophiopogon was heated at 97 to 103°C for about sixty (60) minutes twice to produce an ophiopogon extract solution. Then, the ophiopogon extract solution was passed through a sieve (approximately 100 mesh). The filtrate was collected. The ophiopogon filtrate was then condensed at a temperature of 50 to 60°C under vacuum condition with pressure between 400 and 650 mm-Hg to obtain a liquid condensate with a desirable concentration. It was preferred that the liquid condensate was about 1/14 of the ophiopogon filtrate.

(3) The liquid condensate of ophiopogon was mixed with the herbal powders of the rest of the herbs. The mixture was then transferred to the fluidized granulator and placed in a spraying tube made of filtered fabric. The granulator was set at 90 to 110°C. After pre-heating for about five (5) minutes, the mixture was sprayed at about 40 to 55 seconds per time for an appropriate amount of times and the spraying tube was patted at a frequency of once in every 10 seconds. After granulation, the granules were dried and then cooled off. The granules were passed through sieves and packaged in PE bags. The preferred water content of the granules was less than 6%. The granules can be further encapsulated in any of the conventional capsules, including, but not limited to, natural gelatin, pectin, casein, collagen, protein, modified starch, and polyvinyl pyrrolidone.

EXAMPLE 2

Quality Controls of the Pharmaceutical Compositions Using High Performance Liquid Chromatography (HPLC)

The present invention used HPLC techniques to ensure the quality of the herbs was well under control. Three HPLC methods were developed to accommodate the chemical and physiological characteristics of each herb. The three HPLC methods were described as follows:

(A) Method 1 of the HPLC Analysis

Method 1 used a precolumn Lichrospher RP-18 endcapped (5µm, 4.0 ID X 10 mm, Merck), and a Cosmosil 5C18-MS column (5 µm, Nacalai tesque 5C18-MS, 4.6 ID X 250

mm) at room temperature. The mobile phase of the column was a gradient containing (A) H₂O: KH₂PO₄: 10% H₃PO₄ (1000 ml : 2.72 g : 1 ml, v/w/v), (B) CH₃CN, (C) H₂O, and (D) CH₃OH. The elution gradient was described in Table 4. The flow rate was 1.0 ml/min. The detection wavelength was 250 nm. The total retention time of the analysis was 60 minutes.

5 *Table 4. Mobile Phase Gradient of Method 1*

Time	(A)	(B)	(C)	(D)
Initial	90	10	0	0
30	75	25	0	0
40	65	35	0	0
55	0	75	25	0
60	0	10	90	0

Post Run: 15 min.

(B) Method 2 of the HPLC Analysis

10 Method 2 of the HPLC analysis used a precolumn Lichrospher RP-18 endcapped (5 μm, 4.0 ID X 10 mm, Merck), and a Cosmosil 5C18-MS column (5 μm, Nacalai tesque 5C18-MS, 4.6 ID X 250 mm) at 35°C. The mobile phase of the column was a gradient containing (A) 10 mM KH₂PO₄ + 10 mM K₂HPO₄ + 0.01% H₃PO₄ (KH₂PO₄ : K₂HPO₄ : 10% H₃PO₄ : H₂O = 1.36 g : 1.74 g : 1 ml : 1000 ml), (B) CH₃CN, (C) H₂O, and (D) CH₃OH. The elution gradient was described in Table 5. The flow rate was 1.0 ml/min. The
15 detection wavelength was 260 nm. The total retention time of the analysis was 60 minutes.

Table 5. Mobile Phase Gradient of Method 2

Time (min)	(A)	(B)	(C)	(D)
Initial	100	0	0	0
25	60	0	20	20
30	20	0	20	60
35	100	0	0	0

Post run: 15 min.

(C) Method 3 of the HPLC Analysis

20 Method 3 of the HPLC analysis used a precolumn Lichrospher RP-18 endcapped (5 μm, 4.0 ID X 10 mm, Merck), and a Cosmosil 5C18-MS column (5 μm, Nacalai tesque

5C18-MS, 4.6 ID X 250 mm) at 35°C. The mobile phase of the column was a gradient containing (A) H₂O: KH₂PO₄ (1000 ml : 2.72g, v/w), (B) CH₃CN, (C) H₂O, and (D) CH₃OH. The elution gradient was described in Table 6. The flow rate was 1.0 ml/min. The detection wavelength was 203 nm. The total retention time of the analysis was 60 minutes.

5 *Table 6. Mobile Phase Gradient of Method 3*

Time (min)	(A)	(B)	(C)	(D)
Initial	80	20	0	0
40	65	35	0	0
55	0	80	20	0
60	0	20	80	0

Post run: 15 min.

The HPLC analysis of the herbs used in the compositions, which includes the method, major marker(s), and detection wavelength of each herb, are summarized in Table 7. The HPLC chromatograms of the STA-36 herbs are shown in Figures 1-5.

10 *Table 7. HPLC Analysis of Herbs Used in STA-36*

Herbal Name	HPLC Analytical Method	Marker(s)	Retention Time (min)	Wavelength (nm)
Ophiopogon	Method 1	6115-1	11.5	214
		6115-2	13.0	214
Pinellia	Method 2	Adenine	14.8	250
		Guanosine	17.0	250
<i>Radix Glycyrrhizae</i>	Method 1	Glycyrrhizin	46.4	250
American Ginseng	Method 3	Ginsenoside Rb ₁	37.5	203
Lantern Tridax	Method 1	FY-1	28.0	250
		FY-2	31.6	250
		FY-3	33.5	250
		FY-4	18.3	250

Before HPLC analysis, 0.5 g of the herbal sample was mixed with 20 ml solution containing 70% of methanol. The sample solution was sonicated for 15 min at room temperature and placed in a rotating water bath at 40°C for 20 min with the rotation speed set at 160 rpm. The sample solution was then left to sit for about 30 min to allow the debris to precipitate. Ten (10) ml of the supernatant was then pipetted out and passed through a

0.45 μm filter. The filtrate was ready for HPLC analysis. Twenty (20) μl of the filtrate was taken for HPLC analysis.

Results:

The HPLC chromatogram of ophiopogon is shown in Figure 1. As shown in Table 7, method 1 of the HPLC analysis was used to detect ophiopogon at 214 nm wavelength. Two distinctive peaks, 6115-1 and 6115-2, were identified on the ophiopogon HPLC chromatogram. 6115-1 had a retention time of 11.5 min. 6115-2 had a retention time of 13 min. Although the chemical components in these two peaks were not yet identified, they could be used for the purpose of ensuring the quality of ophiopogon.

The HPLC chromatogram of pinellia is shown in Figure 2. As shown in Table 7, using method 2 of the HPLC analysis to detect pinellia at 250 nm wavelength, pinellia had shown two distinctive chemical markers, i.e., adenine and guanosine, both are constituents of nucleic acids. Adenine also has effect of elevating serum aldolase. Adenine had a retention time of 14.8 min. Guanosine had a retention time of 17 min.

The HPLC chromatogram of *Radix Glycyrrhizae* is shown in Figure 3. As shown in Table 7, using method 1 of the HPLC analysis to detect *Radix Glycyrrhizae* at 250 nm wavelength, a chemical marker "glycyrrhizin" was found in the *Radix Glycyrrhizae* HPLC chromatogram. Glycyrrhizin ($\text{C}_{42}\text{H}_{62}\text{O}_{16}$) is a very sweet substance. It is known to have detoxification, anti-inflammation, and haemolysis effects, and has been used to treat Addison's disease. The retention time for glycyrrhizin is 46.4 min.

The HPLC chromatogram of American ginseng is shown in Figure 4. As shown in Table 7, using method 3 to detect American ginseng at 203 nm wavelength, a chemical marker "ginsenoside Rb_1 " was found in the American ginseng HPLC chromatogram. Ginsenoside Rb_1 has medicinal effects on dilating blood vessel, relieving fatigue, and avoiding haemolysis.

The HPLC chromatogram of lantern tridax is shown in Figure 5. As indicated in Table 7, using method 1 of the HPLC analysis to detect lantern tridax at 250 nm wavelength, four (4) distinctive peaks, FY-1 to FY-4, were identified. FY-1 had a retention time of 28.0 min. FY-2 had a retention time of 31.6 min. FY-3 had a retention time of 33.5 min. FY-4 had a retention time of 18.3 min. Due to the consistent appearance of these four peaks, even

though the chemical contents associated with these four peaks were still not yet identified, they could be used for the purpose of identifying and ensuring the quality of lantern tridax.

EXAMPLE 3

5 Thin-Layer Chromatography (TLC) Characterization of Raw Licorice, Ophiopogon, Pinellia, and Lantern Tridax

10 In addition to HPLC, some of the herbs used in the STA-36 compositions, including raw licorice, ophiopogon, pinellia, and lantern tridax, can be characterized by thin-layer chromatography (TLC) methods.

 The TLC method used a stationary phase of silica gel 60 F254 (which was bound to the TLC plate) and three mobile phase (or dissociation) solvents, depending upon the characteristics of the herbs. The first solvent contained ethyl acetate, 4 N ammonia water, and ethanol in a volume ratio of 4 : 1 : 2 (v/v/v). This first solvent was particularly useful
15 for characterizing raw licorice. The second solvent contained butanol, 4 N ammonia water, and ethanol in a volume ratio of 5 : 2 : 1 (v/v/v). This second solvent was particularly useful for characterizing ophiopogon and American ginseng. The third solvent contained chloroform (CHCl₃) and methanol at a volume ratio of 5 : 1 (v/v). This third solvent was particularly useful for characterizing lantern tridax. For each TLC determination, 5 µl of
20 sample was added at the starting point of the TLC plate. The plate was placed in a TLC chamber within which the mobile phase solvent was applied. The solvent was allowed to migrate up to 9 cm. The results of the TLC determination were described as follows:

(A) Raw Licorice

 Raw licorice was analyzed by TLC using a stationary phase of silica gel 60 F254
25 attaching to a TLC plate, and a mobile phase solvent containing ethyl acetate : 4N ammonia water : Ethanol = 4:1:2 (v/v/v). After the application of the sample, the TLC plate was placed into the TLC chamber which contained the mobile phase solvent with the upper liquid mark not to exceed the sample application point. The solvent was allowed to migrate up to 9 cm from the sample application point. Then, the TLC plate was taken out of the
30 TLC chamber and air-dried. The dried TLC plate was sprayed with a spraying reagent containing vanillin/H₂SO₄. The plate was then activated under heat at 105°C for two (2) minutes. A yellow spot which represented raw licorice appeared on the plate at

approximately $R_f=0.5$. R_f = (the distance [cm] between the spot and the sample application point)/total migration distance [i.e., 9 cm]).

(B) Ophiopogon

Ophiopogon was analyzed by TLC using a stationary phase of Silica gel 60 F254
5 attaching to a TLC plate and a mobile phase solvent containing butanol: 4N ammonia water
: ethanol = 5:2:1 (v/v/v). After the application of the sample, the TLC plate was placed into
the TLC chamber which contained the mobile phase solvent with the upper liquid mark not
to exceed the sample application point. The solvent was allowed to migrate up to 9 cm from
the sample application point. Then, the TLC plate was taken out of the TLC chamber and
10 air dried. The dried TLC plate was sprayed with a spraying reagent containing
vanillin/ H_2SO_4 . The plate was then activated under heat at $105^\circ C$ for two (2) minutes. A
brown spot which represented ophiopogon appeared on the plate at approximately $R_f=0.4$.

(C) American Ginseng

American ginseng was analyzed by TLC using a stationary phase of Silica gel 60
15 F254 attaching to a TLC plate and a mobile phase solvent containing butanol: 4N ammonia
water : ethanol = 5:2:1 (v/v/v). After the application of the sample, the TLC plate was
placed into the TLC chamber which contained the mobile phase solvent with the upper
liquid mark not to exceed the sample application point. The solvent was allowed to migrate
up to 9 cm from the sample application point. Then, the TLC plate was taken out of the
20 TLC chamber and air dried. The dried TLC plate was sprayed with a spraying reagent
containing vanillin/ H_2SO_4 . The plate was then activated under heat at $105^\circ C$ for two (2)
minutes. A purplish spot which represented American ginseng appeared on the plate at
approximately $R_f=0.3$.

(D) Lantern Tridax

Lantern tridax was analyzed by TLC using a stationary phase of Silica gel 60 F254
25 attaching to a TLC plate and a mobile phase solvent containing chloroform : methanol = 5 :
1 (v/v). After the application of the sample, the TLC plate was placed into the TLC chamber
which contained the mobile phase solvent with the upper liquid mark not to exceed the
sample application point. The solvent was allowed to migrate up to 9 cm from the sample
30 application point. Then, the TLC plate was taken out of the TLC chamber and air dried.
The dried TLC plate was placed under a UV detector and detected at 366 nm wavelength.

A pale yellow fluorescent spot which represented lantern tridax appeared on the plate at approximately $R_f=0.3$.

EXAMPLE 4

5 Effects of Five Traditional Chinese Herbal Decoction Formulations 10 on Allergen-Induced IgG, IgE, and IL-4 In Mice

The effects of five traditional Chinese herbal decoction formulations on suppression of allergen-induced immunoglobulin G (IgG), immunoglobulin E (IgE) synthesis, and expression of interleukin-4 (IL-4) gene were investigated using BALB/c mice sensitized with D.P. (*Dermatophagoides pteronyssimus*) allergen "Der p 5". *Dermatophagoides pteronyssimus* is the house dust mite which acts as an antigen and produces an allergic asthmatic reaction in atopic person.

The formulations of these traditional Chinese decoctions are shown in Table 8.

15 These traditional Chinese decoction groups are known to have special effects on treating patients with lung-related ailments, such as asthma.

Table 8. Formulations of Five Traditional Chinese Herbal Decoctions

Name of Traditional Chinese Herbal Decoctions	Herbal Ingredients (with weight ratio)
White-Draining Powder	licorice (2.25), bark of mulberry root (4.5), cortex of wolfberry root (4.5), polished round-grained nonglutinous rice/japonica rice (6).
Little Green-Blue Dragon Decoction	pinellia (4), ephedra (4), Chinese herbaceous peony (4), Chinese wild ginger (1.5), licorice (4), dried ginger (4), cinnamon twig (4), schisandra fruit (1.5).
Ophiopogon Decoction	ginseng (2), polished round-grained nonglutinous rice/japonica rice (4), pinellia (2), Chinese date/jujube (2), licorice (2), ophiopogon (8).
Dryness-Clearing Lung-Rescuing Decoction	white mulberry leaf (7.5), ass-hide glue/gelatin (2), ginseng (2), gypsum (6.5), licorice (2.5), ophiopogon (3), apricot seed/kernel (2), black sesame seeds (2.5), loquat leaf (2).
Perilla Fruit Qi-Downbearing Decoction	purple perilla fruit (5), tangerine peel (3), pinellia (5), Chinese angelica root (2), hogfennel root (2), licorice (2), cinnamon bark (3), magnolia bark (2), fresh ginger rhizome

(2), Chinese date/jujube (1).

Experimental Design:(A) Preparation of Herbal Materials:

Fifty (50) mg of each of the above indicated herbal formulations (all from Sun Ten Sci. Pharm. Corp., Taipei, Taiwan) were mixed with 180 μ l of Tween 80, respectively, and homogenized (homogenizer DC-3S, Xinguang Precision Instrument Industrial Limited, Inc., Taiwan). Water was added to the homogenate to bring the final volume to 2 ml, and the final concentration 25 mg/ml. Each mouse received 0.4 ml/per 20 g body weight of one of the herbal composition solution or placebo every other day for fourteen (14) days.

10 (B) Animals

Female BALB/c mice aged between 4 and 6 weeks were obtained from the National Animal-breeding Center, Republic of China and used in the studies. Mice were age- and sex- matched for each experiment.

(C). Allergen Derp5 (Dermatophagoides pteronyssinus group 5 allergen) Purification

15 pGEX-2T plasmid was used to express Der p 5-glutathion S-transferase (GST) in *E. coli*. The molecular weight of the fusion protein was about 42 kD. The protein was purified by glutathione-conjugated agarose gel column chromatography. A single colony of the Der p 5-GST positive-bacterial strain was selected by growing the transfected *E. coli* on LB broth containing Ampicillin (100 μ g/ml). The Der p 5-GST positive *E. coli* colony was
20 further cultured to produce substantial amount of Der p 5-GST. Then, the bacterial culture was collected and centrifuged. The supernatant was discarded, and the pellet was washed with TBS (pH 7.5) and collected in a centrifuge tube. Immediately following the TBS wash, 0.1 M phenylmethylsulfonyl fluoride was added to the pellet, followed by the addition of DNase I, Tween 20, and lysozyme. The pellet was lyzed by the freeze-and-thaw process to
25 release Der p 5 + GST protein. Subsequently, EDTA was added to the cell lysate, which was then centrifuged. The pellet was discarded, and the supernatant was poured onto a glutathione-conjugated agarose gel adsorption column where Der p 5-GST protein was adsorbed onto the column. The column was washed first by TBS buffer solution at 4°C, and then by reduced glutathione in Tris-base (pH 8.0) to separate the Der p 5-GST from the
30 agarose gel column. The purity of the Der p 5-GST protein was confirmed by SDS-PAGE. The quantity of the protein was measured by conventional protein analytical methods.

(D) Animal Sensitization

BALB/c mice were initially intraperitoneally sensitized with 10 μ g of *Dermatophagoides pteronyssinus* group 5 ("Der p 5") allergen and 4 mg of aluminium hydroxide (Wyeth Pharmaceuticals, Punchbowl, Australia). Seven (7) days after the first sensitization, mice, except the placebo control group, were fed with Chinese herbal decoctions.

Three (3) weeks after the first sensitization, mice were boosted with Der p 5 again. Three (3) days after the second booster injection, mice were placed in a round plastic container where the mice were further challenged by 0.1% Der p 5 spray.

Each time after the booster injection, 50 μ l of blood were collected from the tail vein of each mouse in the following day. The blood was allowed to sit at room temperature for 1 hour and then centrifuged. The serum was collected and stored at -80°C for ELISA (see below) analysis.

(E) Determination of Der p 5-specific IgG and IgE

The amount of Der p 5-specific IgG and IgE were determined by Enzyme Linked Immunosorbent Assay (ELISA). Protein high binding plates were coated with 100 μ l purified Der p 5 diluted in coating buffer (0.1 M NaHCO₃, pH 8.2) to a concentration of 5 μ g/ml. After overnight incubation at 4°C, plates were washed three times and blocked with 3% (wt/vol) BSA-PBS buffer for 2 hr at 25°C. Sera were used at 1:100 dilution for IgG measurement and 1:10 dilution for IgE measurement in duplicate. After overnight incubation at 4°C, either biotin-conjugated monoclonal rat anti-mouse IgE mAb, or rat anti-mouse IgG mAb diluted in 0.05% gelatin buffer, was added for an additional hour. Avidin-alkaline phosphatase (1:1000) was then added and incubated for 1 hour at 25°C, followed by six (6) washes. The color reaction was developed with the addition of phosphatase substrate *p*-nitrophenyl phosphate, disodium. Plates were read in a microplate autoreader (Metertech, Taiwan) at 405 nm. Readings were referenced to commercial isotypes standards which were mouse anti-TNP mAb, IgG1 (107.3), IgG2a (G155-178), and IgE (IgE-3).

(F) Expression of IL-4 gene in splenocytes by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction)

The splenocytes were isolated from BALB/c mice after the animals were sensitized and challenged with Der p 5 allergen followed by treatment, respectively, of five herbal decoctions as listed in Table 8 or placebo. The splenocytes were incubated with Der p 5 (15 μ g/ml) for 4 hours. RNA was extracted using Trizol reagent (Gibco BRL, Life

Technologies, U.S.A). The extracted RNA was used for the production of cDNA using Ready to go T-Primed First-Strand Kit (Pharmacia Biotch, USA). The cDNA was used as template for polymerase chain reaction (PCR). A pair of IL-4 specific primers having the sequences of:

- 5 5'-GAATGTACCAGGAGCCATATC-3' (SEQ ID NO:1); and
5'-CTCAGTACTACGAGTAATCCA-3' (SEQ ID NO:2);

was used for PCR amplification and detection of IL-4 gene. A pair of HPRT (hypoxanthine-guanine phosphoribosyltransferase) specific primers having the sequences of:

- 10 5'-GTTGGATACAGGCCAGACTTTGTTG-3' (SEQ ID NO:3); and
5'-GATTCAACTTGCGCTCATCTTAGGC-3' (SEQ ID NO:4);

was used for PCR amplification and detection of HPRT. The PCR was performed under the following conditions: 95°C denaturation for 5 minutes, followed by 35 cycles of 95°C for 1 minute, 72°C for 1 minute, and 55°C for 3 minutes in a Thermo-cycler. The PCR products were then electrophorized on a 2% agarose gel.

15 (G) Statistical Analysis

To assess changes of Der p 5- specific IgG, IgE, and IL-4/HPRT ratio after Der p 5 challenge, repeated measures for ANOVA were performed to compare the differences among groups. Following analysis of variance, Duncan multiple range test was used to differentiate differences between experimental and control groups. A $p < 0.05$ was used to
20 indicate statistical significant difference.

Results:

As shown in Figure 6, the levels of IgG and IgE in mice after Der p 5 challenge followed by treatment of the five traditional Chinese herbal decoctions were not significantly different from the control (placebo) group. The ELISA units for IgG in White-
25 Draining Powder Group (A), Green-Blue Dragon Decoction Group (B), Ophiopogon Decoction Group (C), Dryness-Clearing Lung-Rescuing Decoction Group (D), Perilla Fruit Qi-Downbearing Decoction Group (E), and Placebo Group (F) were 70.9 ± 42 , 62.5 ± 44 , 63.3 ± 44 , 63.3 ± 43 , 59.7 ± 38 , and 67.4 ± 42 , respectively. The ELISA units for IgE in White-
30 Draining Powder Group (A), Green-Blue Dragon Decoction Group (B), Ophiopogon Decoction Group (C), Dryness-Clearing Lung-Rescuing Decoction Group (D), Perilla Fruit Qi-Downbearing Decoction Group (E), and Placebo Group (F) were 99.3 ± 23 , 106.3 ± 34 , 104.9 ± 23 , 105.7 ± 23 , 93.9 ± 21 , and 97.8 ± 19 , respectively.

The results of Figure 6 demonstrate that the five traditional Chinese herbal decoctions did not suppress the production of IgE.

As shown in Figure 7, except for ophiopogon decoction which shows a significant decrease in IL-4/HPRT, the rest of the traditional Chinese herbal decoctions have no effect with respect to the suppression of IL-4 gene production.

EXAMPLE 5

Effects of Individual Herbs in the STA-31, STA-32, STA-33, STA-34, STA-35, and STA-36 on Allergen-Induced IgG, IgE, and IL-4 In Mice

The effects of individual herb, including ophiopogon, pinellia, raw licorice, and tang-shen, and the STA-36 on allergen-induced IgG, IgE, and IL-4 were investigated using BALN/c mice sensitized with D.P. allergen "Der p 5". The experimental designs of this example were the same as those described in Example 5 (*supra*).

Results:

The IgG levels in mice serum after challenged by allergen Der p 5 and then treated by ophiopogon, tang-shen, pinellia, and raw licorice are shown in Figure 8. The results indicated that the IgG levels were not significantly different between ophiopogon, tang-shen and pinellia treatment and placebo. The IgG level after raw licorice treatment was significantly lower than that of placebo treatment.

However, as shown in Figure 9, the IgE levels in mice serum after challenged by Der p 5 and then treated by ophiopogon, tang-shen, and the STA-36 composition were significantly lower than that of the placebo. In fact, after treatment with the STA-36 composition, the level of IgE was reduced by more than 2.5 times. The IgE levels after treatment of pinellia and raw licorice were insignificantly different from that of the placebo treatment.

As shown in Figure 10, the expressions of IL-4 gene (measured by RT-PCR and expressed as IL-4/HPRT ratio) in mice splenocytes after challenged by Der p 5 and then treated by ophiopogon, tang-shen, pinellia, and raw licorice, respectively, were not significantly different from that of the placebo. However, the expression of IL-4 gene ratio) after treatment of STA-36 was significantly lower than that of the placebo, indicating that the STA-36 could down-regulate the expression of IL-4 gene.

EXAMPLE 6

Methods of Determining IgG, IgE, IL-4 and IFN- γ In Animals

The effects of the STA-36 and STA-4 herbal composition as well as individual herbs on suppression of allergen-induced immunoglobulin G (IgG), immunoglobulin E (IgE), interleukin-4 (IL-4), and interferon γ (IFN- γ) were investigated using BALB/c mice sensitized with D.P. (*Dermatophagoides pteronyssinus*) allergen "Der p 5".

Dermatophagoides pteronyssinus is the house dust mite which acts as an antigen and produces an allergic asthmatic reaction in atopic person.

Experimental Design:

(A) Animals

Female BALB/c mice aged between 4 and 6 weeks and weighed about 20 g were obtained from the National Animal-breeding Center, Republic of China and used in the studies. Mice were age- and sex- matched for each experiment.

(B). Allergen Derp5 (*Dermatophagoides pteronyssinus* group 5 allergen) Purification

pGEX-2T plasmid was used to express Der p 5-glutathion S-transferase (GST) in *E. coli*. The molecular weight of the fusion protein was about 42 kD. The protein was purified by glutathione-conjugated agarose gel column chromatography. A single colony of the Der p 5-GST positive-bacterial strain was selected by growing the transfected *E. coli* on LB broth containing Ampicillin (100 μ g/ml). The Der p 5-GST positive *E. coli* colony was further cultured to produce substantial amount of Der p 5-GST. Then, the bacterial culture was collected and centrifuged. The supernatant was discarded, and the pellet was washed with TBS (pH 7.5) and collected in a centrifuge tube. Immediately following the TBS wash, 0.1 M phenylmethylsulfonyl fluoride was added to the pellet, followed by the addition of DNase I, Tween 20, and lysozyme. The pellet was lyzed by the freeze-and-thaw process to release Der p 5 + GST protein. Subsequently, EDTA was added to the cell lysate, which was then centrifuged. The pellet was discarded, and the supernatant was poured onto a glutathione-conjugated agarose gel adsorption column where Der p 5-GST protein was adsorbed onto the column. The column was washed first by TBS buffer solution at 4°C, and then by reduced glutathione in Tris-base (pH 8.0) to separate the Der p 5-GST from the agarose gel column. The purity of the Der p 5-GST protein was confirmed by SDS-PAGE. The quantity of the protein was measured by conventional protein analytical methods.

(C) Animal Sensitization

BALB/c mice were initially intraperitoneally sensitized with 10 µg of *Dermatophagoides pteronyssinus* group 5 ("Der p 5") allergen and 4 mg of aluminium hydroxide (Wyeth Pharmaceuticals, Punchbowl, Australia). Seven (7) days after the first sensitization, mice, except the placebo control group, were fed with STA-36 and STA-4
5 herbal compositions until the end of the twenty-first day.

At the end of the twenty first day, the mice were given a second booster injection of Der p 5. Forty eight (48) hours after the second booster injection, mice were placed in a round plastic container where the mice were further challenged by 0.1% Der p 5 spray.

Several hours after the challenge, the mouse was anaesthetized using 100 mg/ml
10 phenobarbital. Blood was collected from the tail vein or heart. The blood was allowed to sit at room temperature for 1 hour and then centrifuged at 1000 rpm for about 10 minutes to collect serum, which was stored at -80°C for ELISA (see below) analysis of IgE and IgG2a. The fluid collected from washing the pulmonary alveoli was centrifuged at 1000 rpm for 10 minutes. The supernatant of the fluid was used for analysis of cytokine, interleukin-4 (IL-4)
15 and interferon-γ (IFN-γ). The pellet of the fluid (containing the cells) were re-dissociated using PBS and proceed with cytospin count for inflammatory cells.

(D) ELISA Determination of Der p 5-specific IgG and IgE

The amount of Der p 5-specific IgG and IgE were determined by Enzyme Linked Immunosorbent Assay (ELISA). High protein high binding microwell plates (Costar, No.
20 3590) were coated with 100 µl (10 mg/ml) purified Der p 5 diluted in coating buffer (PBS solution containing 3% BSA, pH 7.4) to a concentration of 5 µg/ml. After overnight incubation at 4°C, plates were washed three times in PBS solution (pH 7.4) containing 0.05% Tween 20 and blocked with 3% (wt/vol) BSA-PBS buffer for 2 hr at 25°C.

Sera were used at 1:100 dilution for IgG measurement and 1:10 dilution for IgE
25 measurement in duplicate. After overnight incubation at 4°C, either biotin-conjugated monoclonal rat anti-mouse IgE mAb (PharMinge 02122D), or rat anti-mouse IgG mAb (PharMinge, 02022D) diluted in 0.05% gelatin buffer, was added for an additional 2 hours. Avidin-alkaline phosphatase (1:1000) (Strptavidin-AP, PharMinge 13043E) was then added and incubated for 1 hour at 25°C, followed by six (6) washes. The color reaction was
30 developed with the addition of phosphatase substrate *p*-nitrophenyl phosphate, disodium (pNPP alkaline phosphatase substrate, Sigma N-2770). Plates were read in a microplate autoreader (ELISA reader, Dynex MRX) at 405 nm. Readings were referenced to

commercial isotypes standards which were mouse anti-TNP mAb, IgG1 (107.3), IgG2a (G155-178), and IgE (IgE-3).

(E) ELISA Determination of cytokines (IL-4 and IFN- γ)

High protein high binding microwell plates (Costar, No. 3590) were coated with purified anti-cytokine antibody (for IL-4, 2 μ g/ml of IL-4 antibody from PharMinge 18191D was used; for IFN- γ , 3 μ g/ml of IFN- γ antibody, PharMinge 18181D was used). The plates were coated with PBS solution containing 3% BSA, pH 7.4 and then washed with PBS solution containing 0.05% Tween-20, pH 7.4.

One hundred (100) μ l of the supernatant from the fluid of pulmonary alveoli were then added to the microwells, together with cytokine standard solution, for overnight reaction at 4°C. Then, 100 μ l of biotin-anti-mouse-IL-4 (PharMinge 18042D, 1 μ g/ml) or biotin-anti-mouse-IFN- γ (PharMinge 18112D, 1 μ g/ml) were added to the microwells. After two (2) hours of reaction, 100 μ l of avidin-alkaline phosphatase (1:1000) (streptavidin-AP, PharMinge 13043E) were added to microwells with vibration for 1 hour. Two hundred (200) μ l of phosphatase substrate *p*-nitrophenyl phosphate, disodium (pNPP alkaline phosphatase substrate, Sigma N-2770) were added to microwells for color development. Plates were read in a microplate autoreader (ELISA reader, Dynex MRX) at 405 nm.

(F) Measuring of Lung Function.

After iv injection of 100 mg/ml of phenobarbital to the mouse, a PE 50 tube was inserted into the trachea. The tube was connected to a pressure detection device to measure the change in respiratory pressure during inhalation and exhalation. Another PE 50 tube was inserted into the esophagus to measure the esophagus pressure as substitute for transpulmonary pressure (TPP). When the signal of pressure was transmitted to Bio-Recorder BR8000, the signal was amplified and converted into volume of breath and transpulmonary pressure to calculate the trachea pressure. During the experiment, a needle was maintained at the tail vein to provide for acetylcholine (Ach) injection so as to induce change in trachea pressure.

4. Statistics.

All of the data contained in the experiments were calculated as "mean $\pm$ SEM." Trachea resistance change induced by acetylcholine after converted to PC 100 was measured as μ g/kg. Ig E and Ig G_{2a} measurement were shown based upon value of optical absorption.

The unpaired student's t test was used to determine the significance of the differences among the groups. A $p < 0.05$ was used to indicate statistical significant difference.

EXAMPLE 7

5 Effects of Aqueous Extraction of the Herbs on IgG and IgE

Various aqueous extraction methods were tested on American ginseng and lantern tridax to determine the optimal form of aqueous extract to be used in the herbal composition.

American ginseng in (1) powder, (2) 95% alcohol extract, (3) water extract, and (4) 50% alcohol extract was tested for its medicinal effects on suppression of IgE and IgG_{2a}. As
10 shown in Figure 11, American ginseng extracted with 50% alcohol produced the best IgE suppression effect, even though its effect on IgG_{2a} reduction was not as good as that extracted in 95% alcohol. Because antigen-induced immunological disorders were primarily IgE mediated diseases, it appears that using 50% alcohol extraction for American ginseng in the herbal composition should provide the best IgE reduction result for the composition.
15 However, when the granules produced from the alcohol extract of American ginseng were added to the rest of the granules, the granules produced from the water extract of American ginseng provided better effects on both IgE inhibition and IgG elevation. Thus, in the STA-4 composition, the American ginseng was extracted from water.

Lantern tridax in (1) powder, (2) 95% alcohol extract, (3) water extract, and (4) 50%
20 alcohol extract was also tested for its medicinal effects on suppression of IgE, IgG_{2a}, IL-4 and IFN- γ . As shown in Figure 12, lantern tridax extracted with 50% alcohol produced the best IgE and IgG_{2a} suppression effect, even though the water extract of lantern tridax had demonstrated the best suppression effect on IL-4 and IFN- γ . Nevertheless, because the water extract of lantern tridax had induced blood clotting in animals during the studies, it
25 was not under consideration for use in humans.

EXAMPLE 8

Comparison of the Effects of STA-36 and STA-4 on IgE, IgG, IL-4, IFN- γ and Lung Functions

30 A comparative study between the medicinal effects of herbal compositions of STA-36 and STA-4 on IgE, IgG, IL-4, and IFN- γ , as well as on lung functions was conducted in experimental animals as described in Example 3 (*supra*). The results of this study were shown in Figures 13-15.

Figure 13 showed a comparative study of the effects of the herbal compositions of STA-36 and STA-4 on IgG and IgE in mice. As shown in Figure 13, both the herbal compositions of STA-36 and STA-4 demonstrated suppression of IgE, as compared with the control, with the effect of STA-4 much greater than that of STA-36. The inhibitory effect on IgE by STA-4 was statistically significant over the control. However, neither STA-36 nor STA-4 demonstrated an inhibitory effect on IgG. In fact, on the contrary, the level of IgG increased in both STA-36 and STA-4 treatment groups, as compared with the control.

Figure 14 showed the effects of STA-36 and STA-4 on lung functions of the mice. Both STA-36 and STA-4 showed significant improvement on lung functions than the control ($p < 0.05$). Among the two, STA-4 showed better improvement on lung functions than STA-36, although the values between STA-4 and STA-36 were not significantly different probably due to insufficient numbers (n) of animals in each experimental group.

Figure 15 compared the effects of STA-4 with conventional asthma drugs, i.e., ketotifen, prednisolone, and aminophline, on IgE, IgG, IL-4 and IFN- γ . The results showed that only STA-4 demonstrated significant decrease in IgE and significant increase in IgG, IL-4, and IFN- γ , thus, suggesting that STA-4 possessed better therapeutic effects in treating asthma than ketotifen, prednisolone, and aminophline.

The results in Figure 15 also suggested that the therapeutic effects of STA-4 might be different from the conventional mechanism where the drug suppressed IL-4 synthesis which then indirectly decreased the level of IgE. In the present case, both IL-4 and IFN- γ increased substantially after the intake of STA-4 so that the reduction in IgE was not due to decrease in IL-4. On the contrary, the amount of IFN- γ (produced and secreted by TH1 cell) increased significantly after the treatment of STA-4. IFN- γ stimulates B cells to synthesize and secrete IgG_{2a}, which explained why the amount of IgG_{2a} increased during the STA-4 treatment. Thus, it is postulated that the STA-4 effects were at least partially derived from IgG_{2a}, which, upon second contact with the allergens, interacted with the allergens so as to reduce the allergic reaction.

EXAMPLE 9

Test of Toxicity of the Herbal Compositions of STA-36 and STA-4

The herbal compositions were tested for toxicity based on a 28-day sub-acute oral

toxicity study in which the mortality, ophthalmologic examination, clinical signs, and gross necropsy were determined. The results were shown in Tables 9-11:

Table 9. Mortality of Mice After Intake of STA-36 and STA-4

	2 % Tween-80 Aqueous Solution	STA-36	STA-4
Dose (mg/kg/day)		5000	5000
Sex	Male	Male	Male
Number of mice	10	10	10
Mortality	0/10	0/10	0/10

5

Table 10. Ophthalmologic Examination and Clinical Signs of Mice After Intake of STA-36 and STA-4

	2 % Tween-80 Aqueous Solution	STA-36	STA-4
Dose (mg/kg/day)		5000	5000
Sex	Male	Male	Male
Number of mice	10	10	10
Before Initiation (Day 0)	0/10	0/10	0/10
Before necropsy (Day 28)	0/10	0/10	0/10
Clinical signs	0/10	0/10	0/10

10

Table 11. Gross Necropsy After Intake of STA-36 and STA-4

	2 % Tween-80 Aqueous Solution	STA-36	STA-4
Dose (mg/kg/day)		5000	5000
Sex	Male	Male	Male
Number of mice	10	10	10
Organs abnormal	0/10	0/10	0/10

The results of Tables 9-11 demonstrated that both STA-36 and STA-4 showed no sign of sub-acute toxicity in the study.

Having described the invention in detail and by reference to the preferred
embodiments it will be apparent to those skilled in the art that modifications and variations
are possible without departing from the scope of the invention as defined in the following
appended claims.

SEQUENCE LISTING

<110> HSU, CHING-HSIANG
SHEU, SHUENN-JYI

5

<120> HERBAL PHARMACEUTICAL COMPOSITIONS FOR TREATING IMMUNOLOGICAL
DISORDERS

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WO 02/047704

PCT/US01/45136

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<220>

<223> Description of Artificial Sequence: Primer

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25

25

We claim:

1. A pharmaceutical composition for treating immunological disorders comprising an effective amount of:

Tuber Ophiopogonis;

5 *Tuber Pinelliae*;

Radix Glycyrrhizae; and

an herb which is one selected from the group consisting of *Herba Tridacis procumbentis*, *Herba Adenostemmatidis*, and *Herba Houttuyniae*.

10 2. The pharmaceutical composition according to claim 1, wherein said herb is *Herba Tridacis*.

3. The pharmaceutical composition according to claim 2, wherein *Tuber Ophiopogonis* is in an aqueous extract,

said *Tuber Pinelliae*, said *Radix Glycyrrhizae* and said *Herba Tridacis procumbentis* are in powder form.

15 4. The pharmaceutical composition according to claim 2, wherein *Tuber Ophiopogonis*, *Tuber Pinelliae*, *Radix Glycyrrhizae* and *Herba Tridacis procumbentis* are at a weight ratio of about 3 : 2 : 1 : 1.

5. The pharmaceutical composition according to claim 2, further comprising an effective amount of *Radix Codonopsitis*.

20 6. The pharmaceutical composition according to claim 2, wherein said *Radix Codonopsitis* is in powder form.

7. The pharmaceutical composition according to claim 5, wherein said *Tuber Ophiopogonis*, said *Tuber Pinelliae*, said *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and said *Radix Codonopsitis* are at a weight ratio of about 3 : 2 : 1 : 1 : 1.

25 8. The pharmaceutical composition according to claim 2, further comprising an effective amount of *Radix Pancis Quinquifolii*.

9. The pharmaceutical composition according to claim 8, wherein said herb is said *Radix Pancis Quinquifolii* is in powder form.

30 10. The pharmaceutical composition according to claim 8, wherein said *Tuber Ophiopogonis*, said *Tuber Pinelliae*, said *Radix Glycyrrhizae*, said *Herba Tridacis procumbentis*, and said *Radix Pancis Quinquifolii* are at a weight ratio of 3 : 2 : 1 : 1 : 1.

11. The pharmaceutical composition according to claim 8, wherein said *Tuber Ophiopogonis*, said *Tuber Pinelliae*, said *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and said *Radix Pancis Quinquefolii* are in aqueous extract form.

12. The pharmaceutical composition according to claim 11, wherein each of said aqueous extracts of *Tuber Ophiopogonis*, *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and *Radix Pancis Quinquefolii* is separately filtered and condensed to form each of condensates of *Tuber Ophiopogonis*, *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and *Radix Pancis Quinquefolii*.

13. The pharmaceutical composition according to claim 12, wherein each of said condensates of *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and *Radix Pancis Quinquefolii* is separately granulated to form individual granules; wherein said granules from *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and *Radix Pancis Quinquefolii* are mixed with said condensate of *Tuber Ophiopogonis* to form mixed granules.

14. The pharmaceutical composition according to claim 13, wherein an excipient is added to each of said condensates of *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Herba Tridacis procumbentis*, and *Radix Pancis Quinquefolii* before granulation.

15. The pharmaceutical composition according to claim 14, wherein said excipient is corn starch.

16. The pharmaceutical composition of claim 11, wherein said aqueous extract of *Tuber Ophiopogonis* is a water extract of *Tuber Ophiopogonis*.

17. The pharmaceutical composition of claim 11, wherein said aqueous extract of *Tuber Pinelliae* is a water extract of *Tuber Pinelliae*.

18. The pharmaceutical composition of claim 11, wherein said aqueous extract of *Radix Glycyrrhizae* is a water extract of *Radix Glycyrrhizae*.

19. The pharmaceutical composition of claim 11, wherein said aqueous extract of *Herba Tridacis procumbentis* is an alcohol extract of *Herba Tridacis procumbentis*.

20. The pharmaceutical composition of claim 19, wherein said alcohol extract of *Herba Tridacis procumbentis* is a 50% alcohol extract.

21. The pharmaceutical composition of claim 11, wherein said aqueous extract of *Radix Pancis Quinquefolii* is water extract of *Radix Pancis Quinquefolii*.

22. The pharmaceutical composition according to claim 12, wherein a solid content of said condensate of *Tuber Ophiopogonis* is about 20-30% by weight of *Tuber Ophiopogonis*; a solid content of said condensate of *Tuber Pinelliae* is about 15-25% by weight of *Tuber Pinelliae*; a solid content of said condensate of *Radix Glycyrrhizae* is about 15-25% by weight of *Radix Glycyrrhizae*, a solid content of said condensate of *Radix Pancis Quinquefolii* is about 19-29% by weight of *Radix Pancis Quinquefolii*, and a solid content of said condensate of said *Herba Tridacis procumbentis* is about 5-10% of *Herba Tridacis procumbentis*.

23. The pharmaceutical composition according to claim 13, wherein said granules of *Tuber Pinelliae* is about 10-20% by weight of *Tuber Pinelliae*, said granules of *Radix Glycyrrhizae* about 2-10% of *Radix Glycyrrhizae*, said granules of *Radix Pancis Quinquefolii* is about 5-15% by weight of *Radix Pancis Quinquefolii*, and said granules of *Herba Tridacis procumbentis* is about 9-15% by weight of *Herba Tridacis procumbentis*.

24. The pharmaceutical composition according to claim 1, wherein said pharmaceutical composition treats patients with immunological disorders.

25. The pharmaceutical composition according to claim 24, wherein said immunological disorders comprises at least one selected from the group consisting of allergic rhinitis, allergic conjunctivitis, allergic asthma, atopic eczema, atopic dermatitis, food allergy, hyper IgE syndrome, and rheumatoid arthritis.

26. A method for preparing the pharmaceutical composition according to claim 3, comprising:

grinding *Tuber Pinelliae*, *Radix Glycyrrhizae*, and *Herba Tridacis procumbentis* to form herbal powders;

boiling *Tuber Ophiopogonis* in water to form the aqueous extract of *Tuber Ophiopogonis*;

mixing said herbal powders with said aqueous extract of *Tuber Ophiopogonis* to form a herbal mixture.

27. The method according to claim 26, further comprising grinding *Radix Codonopsitis* to form *Radix Codonopsitis* powders; and mixing said *Radix Codonopsitis* powders with said herbal mixture.

28. The method according to claim 26, further comprising grinding *Radix Pancis Quinquefolii* to form *Radix Pancis Quinquefolii* powders; and mixing said *Radix Pancis Quinquefolii* powders with said herbal mixture.

29. The method according to claim 26, wherein said herbal mixture is granulated.

5 30. The method according to claim 29, wherein said granulated herbal mixture is encapsulated.

31. A method of treating patients with immunological disorder comprising:
administering an efficient amount of the pharmaceutical composition according to claim 2 to said patients.

10 32. The method according to claim 31, wherein said immunological disorder is one selected from the group consisting of allergic rhinitis, allergic conjunctivitis, allergic asthma, atopic dermatitis, food allergy, hyper IgE syndrome, atopic eczema, and rheumatoid arthritis.

15 33. An immunological disorder drug comprising an effective amount of the pharmaceutical composition according to claim 2.

34. A method for preparing a pharmaceutical composition according to claim 11 comprising:

extracting *Tuber Ophiopogonis* in water by decocting; said water extract of *Tuber Ophiopogonis* being filtered and concentrated to form a condensate of *Tuber Ophiopogonis*;

20 extracting *Tuber Pinelliae* in water by decocting; said water extract of *Tuber Pinelliae* being filtered and concentrated to form a condensate of *Tuber Pinelliae*; said condensate of *Tuber Pinelliae* being granulated by spray-drying;

extracting *Radix Glycyrrhizae* in water by decocting; said water extract of *Radix Glycyrrhizae* being filtered and concentrated to form a condensate of *Radix Glycyrrhizae*;
25 said condensate of *Radix Glycyrrhizae* being granulated by spray-drying;

extracting *Herba Tridacis procumbentis* in alcohol under refluxing; said *Herba Tridacis procumbentis* being filtered and concentrated to form a condensate of *Herba Tridacis procumbentis*; said condensate of *Herba Tridacis procumbentis* being granulated by spray-drying;

30 mixing the granules of *Tuber Pinelliae*, *Radix Glycyrrhizae*, *Radix Pancis Quinquefolii*, and *Herba Tridacis procumbentis* and the condensate of *Tuber Ophiopogonis* together to form mixed granules.

35. The method according to claim 34, wherein said alcohol extract of *Herba Tridacis procumbentis* is 50% alcohol extract.
36. The method according to claim 34, wherein an excipient is added to each of said condensate of *Tuber Pinelliae*, *Radix Glycyrrhizae*, and *Herba Tridacis procumbentis* before granulation.
37. The method according to claim 34, wherein said excipient is corn starch.
38. The method according to claim 34, further comprising:
extracting *Radix Pancis Quinquefolii* in water by decocting; said water extract of *Radix Pancis Quinquefolii* being filtered and concentrated to form a condensate of *Radix Pancis Quinquefolii*; said condensate of *Radix Pancis Quinquefolii* being granulated by spray-drying; and mixing said granules of said *Radix Pancis Quinquefolii* with the mixed granules.
39. The method according to claim 34, wherein a solid content of said condensate of said *Tuber Ophiopogonis* is about 20-30% by weight of said *Tuber Ophiopogonis*; a solid content of said condensate of said *Tuber Pinelliae* is about 15-25% by weight of said *Tuber Pinelliae*; a solid content of said condensate of said *Radix Glycyrrhiza* is about 15-25% by weight of said *Radix Glycyrrhiza*; a solid content of said condensate of said *Radix Pancis Quinquefolii* is about 20-30% by weight of said *Radix Pancis Quinquefolii*; and a solid content of said condensate of said *Herba Tridacis procumbentis* is about 5-10% by weight of said *Herba Tridacis procumbentis*.
40. The method according to claim 34, wherein said granules of *Tuber Pinelliae* is about 10-20% by weight of said *Tuber Pinelliae*, said granules of said *Radix Glycyrrhizae* about 2-10% of said *Radix Glycyrrhizae*, said granules of said *Radix Pancis Quinquefolii* is about 5-15% by weight of said *Radix Pancis Quinquefolii*, and said granules of said *Herba Tridacis procumbentis* is about 9-15% by weight of said *Herba Tridacis procumbentis*.
41. The method according to claim 34, wherein each of said condensate is condensed by decompressed condensation at a temperature of about 55°C and a vacuum of about 30 torr.
42. The method according to claim 34, wherein each of said condensate is spray-dried at an inlet temperature of about 105°C and an outlet temperature of about 76-77°C.
43. A method of treating patients with immunological disorder comprising:

administering an efficient amount of the pharmaceutical composition according to claim 11 to said patients.

44. The method according to claim 43, wherein said immunological disorder is one selected from the group consisting of allergic rhinitis, allergic conjunctivitis, allergic asthma, atopic dermatitis, food allergy, hyper IgE syndrome, atopic eczema, and rheumatoid arthritis.

45. An immunological disorder drug comprising an effective amount of the pharmaceutical composition according to claim 11.