

CINNAMOMI AND PORIA COMPOSITION AND USES THEREOF

[0001] This application claims priority of U.S. provisional application No. 60/829,945, filed October 18, 2006. The entire contents and disclosures of the preceding application are incorporated by reference into this application.

FIELD OF THE INVENTION

[0002] The present invention is related to the making and using of a composition comprising Chinese herbal medicines *Ramulus cinnamomi* and *Poria cocos* (Chinese name: Guizhi and Fuling).

BACKGROUND OF THE INVENTION

[0003] Traditional Chinese medicine employs herbal formulations to treat bodily ailments. In some cases, single herbs or herb derivatives are used. More commonly, however, "formulas", or specific combinations of several particular herbs, are administered. The following five herbal materials have been used in Traditional Chinese medicine for more than 2000 years. They have been recorded in many historical Chinese medical textbooks, and are cited in many articles published in contemporary scientific journals.

<u>COMMON NAME</u>	<u>LATIN NAME</u>	<u>CHINESE NAME</u>
Cassia Twig	<i>Ramulus cinnamomi</i>	Guizhi
Indian Bread	<i>Poria cocos</i>	Fuling
Peach Seed	<i>Semen persicae</i>	Tao Ren
White Peony Root	<i>Radix paeoniae alba</i>	Bai Shao
Tree Peony Bark	<i>Cortex moutan</i>	Mu Dan Pi,

[0004] Cassia Twig is the dried young stem of *Cinnamomum cassia Presl* (Fam. *Lauraceae*). The plant is collected in spring or summer. It is dried in the sun after collection and removal of its leaves. It can also be chopped into slides. The medicinal preparation has cylindrical body, multi-branched, 30-75 cm in length, with a thick end of 0.3-1 cm in diameter. It

is brown or reddish-brown on the surface, with longitudinal lines, fine wrinkles, dotted with leaf, branch or bud scars. Hard and fragile, it is easily broken. For slices, it is 2-4 mm thick, cut surface showing reddish-brown in the bark area, 5 yellowish-white to pale yellowish-brown in the wood part. It has a characteristic aromatic odor, and is tasted sweet or slightly pungent (especially in the bark). The medicinal preparation is the clean scraps of *Cinnamomi* without visible impurities. The important ingredients of *Cinnamomi* is 10 cinnamaldehyde and cinamic acid.

[0005] Indian Bread is the dried sclerotium of the fungus, *Poria cocos* (Fam. *Polyporaceae*). The plant is collected from July to September. After collection, it was piled up and spread 15 about for air-drying repeatedly until wrinkles appears on the surface and its inner moisture evaporated. The whole dried sclerotium is known as "Fulingge". If the fresh sclerotium is peeled before drying, the separated parts are called "Fulingpi" (peel) and "Fulingkuai" (flesh).

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[0006] The outer skin is thin and rough, brown to blackish brown, conspicuously shrivelled and striated. The texture is hard and compact. It is odourless, tastes weak and becomes sticky when chewed. The medicinal preparation is the clean 25 scraps of *Poria* without visible impurities. Its important active ingredients are Pachman and Pachymic acid.

[0007] A number of United States Patents and literatures have taught methods of composing curative compositions from herbs 30 like *Cinnamomi*, *Poria* etc. A number of investigators from China have also reported on animal or clinical research using certain *Cinnamomi* and *Poria* composition.

[0008] In U.S. Patent No. 6,093,403, a formula consists of 35 *Poria* extract up to 20% in weight is been used to treat or prevent disorders in sugar balance, diabetes, and blood circulation diseases such as Angina Pectoris.

[0009] *Poria cocos* wolf is being use for treating cardio-, cerebro-vascular diseases, Alzheimer's and depression. A composition containing *Poria cocos* wolf up to 20% of its weight has been disclosed in U.S. Patent No. 5,589,182.

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[0010] He et al. has published an article in 1994 on clinical study of a *Cinnamomi* and *Poria* composition in 100 cases of gynecopathies: dysfunctional uterine bleeding caused by irregular shedding of uterine endometrim, chronic pelvic inflammations, dysmenorrhea and small intramural hysteromyoma. In comparison with 50 cases of patients that were treated with another medicinal preparation (Bolus of *Cinnamomi* and *Poria*, BCP), observations based on multiple complaining symptoms and physical signs showed no significant difference between the two groups.

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[0011] Shi et al. reported in 2000 the therapeutic effects of a *Cinnamomi* and *Poria* composition in 60 patients with hysteromyoma. Patients accepted physical gynecology examination, ultrasonic examination and hematochrome test. The Shi's study reported an effective rate of 91.7%, and among which, 10% of the cases was of significant improvement. The effectiveness of treatment was defined as "significant improvement" if the tumor was reduced 3-5 cm and enlarged menstrual flow was reduced 50% or more; or "improvement" if the tumor was reduced 2-3 cm and menstrual flow was reduced 25% or more.

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[0012] The present invention provides a herbal composition comprising *Cinnamomi* and *Poria* that would be effective in relieving symptoms of a number of diseases such as those discussed above. In contrast to the more traditional practice in Chinese medicine, the present invention is produced by updated technologies which could better preserve the effective ingredients of *Cinnamomi* and *Poria*.

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[0013] Throughout this application, various publications are referenced and full citations for these publications may be found in the references at the end of the specifications preceding the claims. The disclosures of these publications are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to the skilled therein as of the date of the invention described and claimed herein.

SUMMARY OF THE INVENTION

[0014] The present invention provides a composition comprising Chinese herbal medicines *Ramulus cinnamomi* and *Poria cocos* (Chinese name: Guizhi and Fuling). In one embodiment, the herbal composition is formulated into capsule as described in U.S. Patent 7,052,700, which is hereby incorporated by reference.

[0015] The herbal composition of the present invention is defined by a HPLC fingerprint that displays 12 peaks. Extracting the herbal composition with methanol and analyzed against paeoniflorin as a standard, the 12 peaks have the following characteristic:

Common Peak No.	Relative Retention Time Range	Relative Peak Area Range
1	0.209~0.314	0.25~0.89
2	0.456~0.683	
3	0.733~0.999	
S	1	1
4	0.871~1.306	
5	0.921~1.382	
6	0.968~1.451	
7	1.009~1.514	
8	1.058~1.587	0.69~1.50
9	1.152~1.728	0.30~0.85
10	1.690~2.536	
11	1.771~2.657	
12	1.943~2.915	1.56~3.06

[0016] In one embodiment, the present invention provides a herbal composition comprising *Ramulus cinnamomi* and *Poria cocos*, wherein the composition produces 12 peaks when subjected to a method comprising the steps of: a) extracting the composition with an appropriate organic solvent; and b) performing chromatographic analysis. The relative retention time ranges for peaks 1-12 as compared to paeoniflorin are 0.209-0.314, 0.456-0.683, 0.733-0.999, 0.871-1.306, 0.921-1.382, 0.968-1.451, 1.009-1.514, 1.058-1.587, 1.152-1.728, 1.69-2.536, 1.771-2.657, and 1.943-2.915 respectively.

[0017] In another embodiment, the present herbal composition further comprises paeoniflorin and paeonol. Preferably, there are 1.26~1.90% paeoniflorin and 0.71~1.07% paeonol.

[0018] In another embodiment, the present invention provides a composition produced by a method comprising the steps of: (a) preparing *Poria* powder; (b) preparing extracts of *Cortex moutan*; (c) preparing extracts of *Ramulus cinnamomi*; (d) preparing extracts of *Radix paeoniae alba*, *Semen persicae* and *Poria*; (e) combining the extracts obtained from steps (b)-(d); (f) mixing the *Poria* powder with the extracts obtained from step (e), and generating fine powders; and (g) mixing the fine powders with inclusion substances comprising extracts of *Cortex moutan* and *Ramulus cinnamomi*.

[0019] The present invention also provides a method of producing a composition comprising *Ramulus cinnamomi* and *Poria cocos*, the method comprises the steps of: (a) preparing *Poria* powder; (b) preparing extracts of *Cortex moutan*; (c) preparing extracts of *Ramulus cinnamomi*; (d) preparing extracts of *Radix paeoniae alba*, *Semen persicae* and *Poria*; (e) combining the extracts obtained from steps (b)-(d); (f) mixing the *Poria* powder with the extracts obtained from step (e), and generating fine powders; and (g) mixing the fine powders with inclusion substances comprising extracts of *Cortex moutan* and *Ramulus cinnamomi*.

[0020] The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the herbal composition described herein.

5 [0021] The present invention also provides uses of the composition described herein in the manufacture of medicament for treating a disease or disorder selected from the group consisting of primary dysmenorrheal, secondary dysmenorrheal, dysfunctional uterine bleeding, chronic pelvic inflammations,
10 and small intramural hysteromyoma

DETAILED DESCRIPTION OF THE FIGURES

[0022] Figure 1 shows the HPLC fingerprint of a *Cinnamomi* & *Poria* capsule composition as described herein.

DETAILED DESCRIPTION OF THE INVENTION

15 [0023] The present invention provides a composition comprising Chinese herbal medicines Guizhi and Fuling (*Ramulus cinnamomi* and *Poria cocos*). In one embodiment, the herbal composition can be formulated into capsule as described in U.S. Patent 7,052,700, the disclosure of which is hereby incorporated by reference.

25 [0024] In one embodiment, the present invention provides a herbal composition comprising *Ramulus cinnamomi* and *Poria cocos*, wherein the herbal composition is defined by a 12-peaks fingerprint (Figure 1) obtained by a fingerprinting method comprising the steps of extracting the composition with an appropriate organic solvent, and performing chromatographic
30 analysis. In one embodiment, the chromatographic analysis is high performance liquid chromatography (HPLC). In general, the present herbal composition is extracted with the organic solvent methanol, and then subjected to HPLC with a paeoniflorin standard according to procedures well-known in the art.

[0025] The relative peak area ranges for peaks 1, 8, 9, and 12 as compared to paeoniflorin are 0.25-0.89, 0.69-1.5, 0.3-0.85, and 1.56-3.06 respectively.

5 [0026] The relative retention time ranges for peaks 1-12 as compared to paeoniflorin are 0.209-0.314, 0.456-0.683, 0.733-0.999, 0.871-1.306, 0.921-1.382, 0.968-1.451, 1.009-1.514, 1.058-1.587, 1.152-1.728, 1.69-2.536, 1.771-2.657, and 1.943-2.915 respectively.

10 [0027] The herbal composition of the present invention further comprises paeoniflorin and paeonol. In one embodiment, there are 1.26~1.90% paeoniflorin and 0.71~1.07% paeonol.

15 [0028] The present invention also provides a pharmaceutical composition comprising the herbal composition described herein and a pharmaceutically acceptable carrier. In general, the herbal composition can be formulated into a pill, capsule, tablet, suspension, or syrup according to standard procedures.

20 [0029] Pharmaceutical compositions for use in accordance with the present invention may include a pharmaceutically acceptable excipient or carrier. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert
25 solid, semi-solid or liquid filler, diluent, encapsulating material or formulation. Representative examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives
30 such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; glycols, such as propylene glycol; esters, such
35 as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; and

phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition according to the uses of the herbal composition.

[0030] The present invention also provides a method of treating a subject having a disease or disorder, comprising the step of administering to the subject an effective amount of the herbal composition disclosed herein. The "effective amount" of an agent or composition refers to the amount necessary to elicit a desired biological response. The effective amount of the active components of an herb or herbal remedy is the amount necessary to decrease a particular sign and/or symptom. One of ordinary skill in the art could readily determine an effective amount of the present herbal composition through conventional methods and techniques.

[0031] The herbal composition of the present invention can be used to treat a variety of disease or disorder. A number of applications have been discussed in U.S. Patent 7,052,700. For example, the *Cinnamomi* & *Poria* capsule can be used to treat primary or secondary dysmenorrhea, dysfunctional uterine bleeding caused by irregular shedding of endometrium, chronic pelvic inflammations with inflammatory lower abdomen masses or small intramural hysteromyoma, and many other common pelvic disorders. Further pharmacological studies, pre-clinical as well as clinical studies using the herbal composition of the present invention can be carried out as described in U.S. Patent 7,052,700.

[0032] Preferably, the inventive pharmaceutical compositions of the present invention can be administered orally. In another embodiment of the invention, pharmaceutical compositions may be delivered to mucous membranes, for example, by inhalation or injection. In general, the present inventive pharmaceutical

compositions can be administered to humans and/or other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), buccally, or as an oral or nasal spray.

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[0033] The invention being generally described, will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

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EXAMPLE 1

Cinnamomi & Poria Capsule

[0034] The present example provides a herbal composition comprising *Cinnamomi & Poria* in capsule form (*Cinnamomi & Poria* capsule). The process of making *Cinnamomi & Poria* capsule has been described in U.S. Patent 7,052,700, which is hereby incorporated by reference. The *Cinnamomi & Poria* capsule contains yellowish brown granule which tasted slightly bitter. In one embodiment, each capsule containing 0.31 g of the granule can be stored for 24 months at room temperature.

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[0035] In one embodiment, the *Cinnamomi* and *Poria* capsules can be produced by a process described below:

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- Step 1:** Weigh (50%-90% of the formulation quantity) *Poria*
- Step 2:** Grind the *Poria* into fine powder, and pass through a 100-mesh sieve.
- Step 3:** Sterilize the *Poria* powder by microwave. This is designated as *Poria* Powder.
- Step 4:** Weigh *Cortex moutan*.
- Step 5:** Extract *Cortex moutan* by steam distillation.
- Step 6:** Collect the liquid distillate. The Liquid Distillate is refrigerated, filtered and cool dried to achieve an extract. The dried extract is designated as *Paconol*.
- Step 7:** Save the extracted raw materials (Solid Residue I).
- Step 8:** Extract Solid Residue I with 80~95% ethanol twice (3~5 times volume for the first time and 2~4 times for the second). Boil for 1~3 hours each time.
- Step 9:** Combine the ethanol extracts. This is Extract I.
- Step 10:** Extract the solid residue with water twice (5~7 times volume for the first time and 3~5 times for the second). Boil for 1~3 hours each time.
- Step 11:** Combine the aqueous extracts. This is Extract II.

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- Step 12:** Weigh *Ramulus cinnamomi*.
- Step 13:** Extract the volatile oil by steam distillation. Collect the yield of the volatile oil
- 5 **Step 14:** Save the extracted raw materials (Solid Residue II).
- Step 15:** Extract Solid Residue II with 80~95% ethanol twice (3~5 times volume for the first time and 2~4 times for the second). Boil for 1~3 hours each time.
- 10 **Step 16:** Combine the ethanol extracts. This is Extract III.
- Step 17:** Extract the solid residue with water twice (5~7 times volume for the first time and 3~5 times for the second). Boil for 1~3 hours each time.
- Step 18:** Combine the aqueous extracts. This is Extract IV.
- 15 **Step 19:** Weigh *Radix paeoniae alba*, *Semen persicae*, and *Poria* (10%-50% of the formulation quantity) .
- Step 20:** Add 3~5 times of 80~95% ethanol and leave at room temperature for 20~40 minutes and then boil for 1~3 hours.
- Step 21:** Extract the solid again with 2~4 times of ethanol, and boil for 1~3 hours.
- 20 **Step 22:** Combine the ethanol extracts. This is Extract V.
- Step 23:** Extract the solid residue with water twice (5~7 times volume for the first time and 3~5 times for the second). Boil for 1~3 hours each time.
- Step 24:** Combine the aqueous extracts. This is Extract VI.
- 25 **Step 25:** Combine Extract I (Step 9), III (Step 16) and V (Step 22). Concentrate under vacuum till no ethanol smell. This is Liquid A.
- Step 26:** Combine Extract II (Step 11), IV (Step 18) and VI (Step 24). Concentrate under vacuum. This is Liquid B.
- Step 27:** Combine Liquid A and B. Concentrate under vacuum till the relative density equal to 1.15~1.35(60~90°C). The concentrated liquid extract is designated as Semisolid Extract.
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- Step 28:** Weigh β -cyclodextrin and add into 2~3 times of water. Heat the β -cyclodextrin solution to 50~70°C.
- 35 **Step 29:** Dissolve the total paeonol extract from Step 6 into 4~6 times of 80~95% ethanol.
- Step 30:** Add the paeonol solution into the β -cyclodextrin solution.
- Step 31:** Grind for 10~30 minutes.
- Step 32:** Then place into refrigerator for 12~36 hours.
- 40 **Step 33:** Filter under vacuum.
- Step 34:** Dry at 40~60°C. The resulted white powder is Inclusion Substance I.
- Step 35:** Weigh β -cyclodextrin and add into 4~6 times of 10~30% ethanol solution. Add the all volatile oil from Step 13 into the β -cyclodextrin solution under continuous stirring
- 45 **Step 36:** Keep grind for 20~40 minutes.
- Step 37:** Then place into refrigerator for 12~36 hours.
- Step 38:** Filter under vacuum.
- Step 39:** Dry at 40~60°C. The resulted white powder is Inclusion Substance II.
- 50 **Step 40:** Mix the *Poria* Powder with all the Semisolid Extract.
- Step 41:** Dry the mixture at 70~100°C, and grind into fine powders
- Step 42:** Mix Fine Powder I with Inclusion Substances I and Inclusion Substances II.

Step 43: Add 50~80% ethanol solution to make a dough-like mass.

Step 44: Pass the wet material through a 16-24mesh sieve to obtain the granules.

Step 45: Dry the granules at 40~60°C.

Step 46: Pass the dried granules through the sieve again.

Step 47: Mix the dried granules well. This is Final Granules.

Technical Requirements

[0036] (1) The *Poria* fine powder needs to be screened by 100 meshes of screen (inner diameter $150 \pm 6.6 \mu\text{m}$.)

[0037] (2) The concentration of crude paeonol should be no less than 90%.

[0038] (3) Concentration must be performed under vacuumed conditions.

[0039] (4) The volatile matter is tested for the presence of cinnamaldehyde.

[0040] (5) Material mass balance should be $\pm 5\%$.

Quality Control

[0041] Creamed extractive: the creamed extractive is dark brown in color. Its relative density should be 1.15~1.35 (60~70°C), and the content of paeoniflorin should be 1.36~2.04%

[0042] Soft powder after mixing of *Poria cocos*: yellowish brown in color with a slightly bitter taste. The microbe limit: number of bacteria can not exceed 5000 entries/g; fungus can not exceed 100 entries/g; living acarid, acarid egg or coliform should not be detected. Content of paeoniflorin: (1.23~0.87%). (1.23~1.87%)

[0043] Final granules: yellowish brown with no more than 6.0% water content.

[0044] Capsules: clean, not sticky or mutilated in shape. Differences in content should be no more than $\pm 10\%$ (0.31 g/capsule). Dissolving time is within 30 minutes. Water

content should be no more than 7.0%. Content of paeoniflorin is at 1.26~1.90% and paeonol 0.71~1.07%.

EXAMPLE 2

Fingerprinting of *Cinnamomi* & *Poria* Capsule

[0045] The test was carried out according to the HPLC method (Chinese Pharmacopoeia 2000 Ed, Vol. One, Appendix VI D) and the requirements for fingerprints.

HPLC Conditions and System Suitability

[0046] Agilent 1100 Liquid Chromatograph; Alltima C18 5µm, 250mm X 4.6mm column. Mobile Phase A contains 0.1% phosphoric acid and 5% acetonitrile aqueous solution; Mobile Phase B contains 0.1% phosphoric acid and 50% acetonitrile aqueous solution. Flow rate: 1ml/min; detection wavelength: 230nm. Based on the peak of paeoniflorin, the number of theoretical plates of the column should be no less than 6000. The gradient elution program runs as follows:

<u>Time (min)</u>	<u>A%</u>	<u>B%</u>
0	100	0
70	0	100

Preparation of Reference Solution

[0047] Weigh accurately certain amount of paeoniflorin standard reference, and then add suitable amount of methanol to make a solution of 50µg/ml.

Sample Preparation

[0048] Take the content of the samples for Weight Variation Test, mix thoroughly, and grind into fine powder. Weigh accurately about 0.25 g of the mixture and add 25 ml of 50% methanol. Sonicate the mixture for 30 min, and then filter. Discard the first part of the filtrate. Collect the rest filtrate as the test solution.

Assay

[0049] Inject 10µl of each the reference solution and the test solution into the HPLC system, and run the procedure.

5 **Results**

[0050] As shown in Figure 1 and Table 1, 13 peaks (No. 1-12 and standard, S) were detected, and their relative retention time ranges were specified. In addition, Peaks 1, 8, 9 and 12 were specified for the range of relative peak area (Table 1).

10 Such specifications were based on the combined analysis on HPLC from many batches of *Cinnamomi* & *Poria* composition.

[0051] According to the results presented herein, the HPLC chromatogram of any batch of *Cinnamomi* & *Poria* composition
 15 should have the 13 characteristic peaks. The relative retention time of the 13 peaks and the relative peak area of Peaks 1, 8, 9 and 12 should be in the specified range. Through such control, the reproducibility and internal quality of *Cinnamomi* & *Poria* composition can be controlled and ensured. HPLC data from ten
 20 different batches of *Cinnamomi* & *Poria* capsules have been confirmed to display the fingerprint profile presented herein.

TABLE 1
Common Peaks and Their Limits in HPLC Fingerprint

Common Peak No.	Relative Retention Time Range	Relative Peak Area Range
1	0.209~0.314	0.25~0.89
2	0.456~0.683	
3	0.733~0.999	
S	1	
4	0.871~1.306	0.69~1.50
5	0.921~1.382	
6	0.968~1.451	
7	1.009~1.514	
8	1.058~1.587	0.30~0.85
9	1.152~1.728	
10	1.690~2.536	
11	1.771~2.657	
12	1.943~2.915	1.56~3.06

[0052] The fingerprint profile presented herein is different from the ones shown in U.S. Patent 7,052,700. In the '700 patent, the aqueous samples for HPLC were prepared by water reflux extraction. The contents of 3 *Cinnamomi* & *Poria* capsules
5 were mixed with 200 ml distilled water for heat reflux for 30 min (start timing at boiling). The solution was cooled down, centrifuged for 10 min, and filtered with a 0.45 µm filtration membrane. The first part of the filtrate was discarded and the rest of the filtrate was taken as the test solution.
10 Consequently, the '700 patent presents four chromatograms, i.e. one for water-soluble components, two for lipid-soluble components (of different chromatographic conditions and detection wavelengths), and one for gas Chromatography. The first three chromatograms exhibit poor reproducibility and have
15 some peaks overlapping with each other.

[0053] In the instant invention, this processing method has been changed to use 50% methanol to extract the sample. The content of the samples for Weight Variation Test was mixed
20 thoroughly and grinded into fine powder. Twenty five ml of methanol was added to 0.25 g of the mixture, which was then sonicated for 30 min and then filtered. The first part of the filtrate was discarded, and the rest of the filtrate was collected as the test solution. It was found that extraction
25 with 50% methanol resulted in more peaks, revealing not only more components of the *Cinnamomi* & *Poria* capsules, but also shows lipid-soluble and volatile components in the product. Therefore, the current method produces only one chromatogram, which can be used in combination with other quality control
30 procedures to control the quality of the *Cinnamomi* & *Poria* capsules.

[0054] Hence, the difference between these two methods is that the former method of water reflux extraction extracted
35 components that were mainly water-soluble, and the major peaks in the chromatogram were the water-soluble compounds in the product. In contrast, the method of the instant invention uses

50% methanol as the extraction solvent to extract more water-soluble components, lipid-soluble components as well as the volatile compound paeonol, and these compounds can be presented in one chromatogram that shows a higher paeonol peak based on equal sample amount.

EXAMPLE 3

Specificity, Reproducibility, and Stability of *Cinnamomi* & *Poria* Capsule Fingerprinting

[0055] The evaluation parameters for the fingerprints of Traditional Chinese medicines are usually the ratios of the retention time and area of the characteristic peaks to those of the references. The relative retention time and peak area of each peak are used for the characterization of the fingerprint. In standard fingerprinting, the relative retention time and relative peak area of the feature peaks are specified.

[0056] Such evaluation parameters can eliminate variations caused by differences in instruments, columns, mobile phase and environment that can change the retention time and peak area. Therefore, these parameters can be used objectively and comprehensively to represent the actual conditions of the constituents in and the internal quality of the product.

Specificity:

[0057] The fingerprint characteristics presented herein are unique to the *Cinnamomi* & *Poria* Capsule of the present invention. The characteristic peaks of the fingerprint reveal constituents of every batch of *Cinnamomi* & *Poria* Capsule processed according to the disclosed manufacturing procedure.

Reproducibility:

[0058] The current fingerprint standard was derived from optimization of assay conditions and data collected from many batches. Results from repeated assays of the same batch, as well as data from ten different batches (Tables 2-3) show that

the current method can accurately fingerprint *Cinnamomi & Poria* Capsule with good reproducibility. The results also show that the constituents' contents are similar in different batches, the relative retention time and peak area of all the characteristic peaks being in the specified range.

Stability:

[0059] The fingerprints of consecutive three batches have been studied from 0 to 12 months. The results show that there is no obvious change of the characteristic peaks in 12 months, demonstrating the stability of the quality of the product as well as the fingerprint assay method (Tables 4-6).

TABLE 2
Finger Print Data From Five Batches

Peak No.	Specifications from the Standard Chromatogram		Batch No.									
			051201		051202		060101		060102		060301	
	Relative Retention Time (RRT)	Relative Peak Area (RPA)	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.209~ 0.314	0.25~ 0.89	0.304	0.555	0.304	0.521	0.304	0.478	0.312	0.850	0.313	0.625
2	0.456~ 0.683		0.620		0.621		0.620		0.634		0.635	
3	0.733~ 0.999		0.929		0.929		0.929		0.931		0.931	
4	0.871~ 1.306		1.066		1.066		1.066		1.062		1.062	
5	0.921~ 1.382		1.115		1.116		1.115		1.106		1.106	
6	0.968~ 1.451		1.155		1.155		1.155		1.144		1.143	
7	1.009~ 1.514		1.234		1.234		1.234		1.227		1.226	
8	1.058~ 1.587	0.69~ 1.50	1.286	1.030	1.287	0.917	1.286	1.061	1.274	0.888	1.272	0.915
9	1.152~ 1.728	0.30~ 0.85	1.368	0.581	1.368	0.548	1.368	0.421	1.355	0.744	1.354	0.679
10	1.690~ 2.536		1.954		1.957		1.954		1.933		1.928	
11	1.771~ 2.657		2.038		2.040		2.038		2.011		2.009	
12	1.943~ 2.915	1.56~ 3.06	2.222	1.998	2.224	1.888	2.222	2.473	2.189	2.698	2.186	1.825
S	1	1	1	1	1	1	1	1	1	1	1	1

TABLE 3
Finger Print Data From Five Other Batches

Peak No.	Specifications from the Standard Chromatogram		Batch No.									
			060302		060401		060402		060501		060502	
	Relative Retention Time (RRT)	Relative Peak Area (RPA)	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.209~ 0.314	0.25~ 0.89	0.291	0.488	0.291	0.485	0.291	0.607	0.313	0.556	0.313	0.662
2	0.456~ 0.683		0.612		0.612		0.611		0.635		0.635	
3	0.733~ 0.999		0.926		0.926		0.926		0.931		0.931	
4	0.871~ 1.306		1.067		1.067		1.066		1.062		1.062	
5	0.921~ 1.382		1.114		1.114		1.113		1.106		1.106	
6	0.968~ 1.451		1.154		1.153		1.153		1.143		1.143	
7	1.009~ 1.514		1.242		1.242		1.242		1.226		1.226	
8	1.058~ 1.587	0.69~ 1.50	1.292	0.899	1.292	0.901	1.292	0.924	1.272	0.841	1.272	0.828
9	1.152~ 1.728	0.30~ 0.85	1.380	0.631	1.380	0.636	1.380	0.658	1.355	0.624	1.355	0.806
10	1.690~ 2.536		1.995		1.995		1.996		1.929		1.930	
11	1.771~ 2.657		2.076		2.075		2.076		2.008		2.010	
12	1.943~ 2.915	1.56~ 3.06	2.266	2.643	2.265	2.627	2.266	2.344	2.187	1.803	2.188	2.870
S	1	1	1	1	1	1	1	1	1	1	1	1

TABLE 4
Stability Data

Peak No.	Batch No. 050301									
	0 Month		3 Month		6 Month		9 Month		12 Month	
	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.270	0.630	0.252	0.632	0.304	0.639	0.274	0.636	0.267	0.637
2	0.599		0.553		0.622		0.607		0.597	
3	0.925		0.912		0.927		0.921		0.921	
4	1.061		1.086		1.066		1.054		1.057	
5	1.096		1.139		1.115		1.088		1.091	
6	1.135		1.187		1.156		1.121		1.128	
7	1.195		1.293		1.237		1.196		1.194	
8	1.276	0.803	1.349	0.805	1.286	0.810	1.261	0.808	1.272	0.811
9	1.375	0.645	1.453	0.648	1.368	0.641	1.360	0.649	1.371	0.652
10	2.014		2.094		1.957		1.997		2.024	
11	2.076		2.205		2.045		2.029		2.065	
12	2.270	2.021	2.416	2.028	2.222	2.022	2.223	2.029	2.262	2.026
S	1	1	1	1	1	1	1	1	1	1

5

TABLE 5
Stability Data

Peak No.	Batch No. 050302									
	0 Month		3 Month		6 Month		9 Month		12 Month	
	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.273	0.579	0.260	0.577	0.304	0.572	0.274	0.571	0.267	0.570
2	0.600		0.562		0.621		0.606		0.597	
3	0.925		0.914		0.929		0.921		0.921	
4	1.062		1.090		1.066		1.054		1.057	
5	1.096		1.130		1.116		1.086		1.091	
6	1.134		1.176		1.155		1.121		1.129	
7	1.195		1.247		1.234		1.192		1.196	
8	1.275	0.796	1.334	0.803	1.287	0.806	1.261	0.795	1.272	0.801
9	1.375	0.539	1.458	0.542	1.368	0.543	1.360	0.536	1.371	0.545
10	2.014		2.144		1.957		1.996		2.029	
11	2.076		2.255		2.040		2.029		2.065	
12	2.270	1.915	2.476	1.923	2.224	1.925	2.222	1.919	2.264	1.920
S	1	1	1	1	1	1	1	1	1	1

TABLE 6
Stability Data

Peak No.	Batch No. 050303									
	0 Month		3 Month		6 Month		9 Month		12 Month	
	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
1	0.273	0.692	0.260	0.692	0.304	0.698	0.274	0.696	0.267	0.694
2	0.601		0.562		0.622		0.606		0.597	
3	0.925		0.915		0.929		0.921		0.921	
4	1.062		1.090		1.066		1.056		1.056	
5	1.096		1.129		1.116		1.086		1.091	
6	1.135		1.175		1.155		1.121		1.128	
7	1.195		1.283		1.236		1.193		1.195	
8	1.276	0.963	1.333	0.969	1.287	0.967	1.261	0.968	1.272	0.967
9	1.375	0.721	1.457	0.726	1.369	0.730	1.361	0.727	1.371	0.730
10	2.014		2.144		1.957		1.996		2.027	
11	2.076		2.255		2.041		2.029		2.066	
12	2.270	2.530	2.477	2.535	2.224	2.539	2.222	2.538	2.262	2.537
S	1	1	1	1	1	1	1	1	1	1

What is claimed is:

1. A composition comprising *Ramulus cinnamomi* and *Poria cocos*,
wherein the composition produces 12 peaks when subjected to
a method comprising the steps of:
 - a) extracting the composition with an appropriate organic
solvent; and
 - b) performing chromatographic analysis.
2. The composition of claim 1, wherein the relative peak area
ranges for peaks 1, 8, 9, and 12 as compared to
paeoniflorin are 0.25-0.89, 0.69-1.5, 0.3-0.85, and 1.56-
3.06 respectively.
3. The composition of claim 1, wherein the relative retention
time ranges for peaks 1-12 as compared to paeoniflorin are
0.209-0.314, 0.456-0.683, 0.733-0.999, 0.871-1.306, 0.921-
1.382, 0.968-1.451, 1.009-1.514, 1.058-1.587, 1.152-1.728,
1.69-2.536, 1.771-2.657, and 1.943-2.915 respectively.
4. The composition of claim 1, wherein the organic solvent is
methanol.
5. The composition of claim 1, wherein paeoniflorin is used as
a standard in the chromatographic analysis.
6. The composition of claim 1, wherein the chromatographic
analysis is high performance liquid chromatography (HPLC).
7. The composition according to any one of claims 1-6, further
comprising paeoniflorin and paeonol.
8. The composition of claim 7, wherein the paeoniflorin is
present at 1.26~1.90% and the paeonol is present at
0.71~1.07%.

9. The composition according to any one of claims 1-8, wherein the composition is produced by a method comprising the steps of:

- (a) preparing *Poria* powder;
- 5 (b) preparing extracts of *Cortex moutan*;
- (c) preparing extracts of *Ramulus cinnamomi*;
- (d) preparing extracts of *Radix paeoniae alba*, *Semen persicae* and *Poria*;
- (e) combining the extracts obtained from steps (b)-(d);
- 10 (f) mixing the *Poria* powder with the extracts obtained from step (e), and generating fine powders; and
- (g) mixing the fine powders with inclusion substances comprising extracts of *Cortex moutan* and *Ramulus cinnamomi*.

15 10. A pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier.

20 11. The pharmaceutical composition of claim 10, wherein the composition is formulated into a form selected from the group consisting of a pill, capsule, granule, tablet, suspension, and syrup.

25 12. A method of producing a composition comprising *Ramulus cinnamomi* and *Poria cocos*, the method comprises the steps of:

- (a) preparing *Poria* powder;
- (b) preparing extracts of *Cortex moutan*;
- (c) preparing extracts of *Ramulus cinnamomi*;
- 30 (d) preparing extracts of *Radix paeoniae alba*, *Semen persicae* and *Poria*;
- (e) combining the extracts obtained from steps (b)-(d);
- (f) mixing the *Poria* powder with the extracts obtained from step (e), and generating fine powders; and
- 35 (g) mixing the fine powders with inclusion substances comprising extracts of *Cortex moutan* and *Ramulus cinnamomi*.

13. Use of the composition of claim 1 or claim 10 in the manufacture of medicament for treating a disease or disorder selected from the group consisting of primary
5 dysmenorrheal, secondary dysmenorrheal, dysfunctional uterine bleeding, chronic pelvic inflammations, and small intramural hysteromyoma.
14. The use of claim 13, wherein the composition is
10 administered orally, rectally, parenterally, intravaginally, intraperitoneally, topically, or as an oral or nasal spray.