

Supplementary Material

1 **1 Supplementary methods**

2 *Standard production process of the decocting-free granules of two herbal formulas*

3 **1.1 The STYHCD herbal formula**

4 The STYHCD herbal formula is composed of ten different herbs, as detailed in Supplementary Table
5 S1. The primary components of each herb were extracted following the specified processing procedures
6 outlined below. Subsequently, these ten extracts were thoroughly mixed for 30 minutes to achieve
7 uniformity. The homogenized mixture was then granulated to produce granules with a size range of
8 12-40 mesh. Finally, the granules were packaged into four aluminum foil bags.

9 **1.1.1 Bupleurum chinense DC.**

10 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
11 filter, and concentrate the filtrate to a clear paste with a relative density of 1.10 to 1.12 ($65\pm 5^\circ\text{C}$). 3.
12 Spray-dry, sift, and mix.

13 **1.1.2 Glycyrrhiza uralensis Fisch. ex DC.**

14 Decoct the pieces with water twice, first for 2.5 hours and second for 1.5 hour. 2. Combine the
15 decoctions, filter, and concentrate the filtrate to a clear paste with a relative density of 1.09 to 1.11
16 ($65\pm 5^\circ\text{C}$). 3. Spray-dry, sift, and mix.

17 **1.1.3 Astragalus mongholicus Bunge**

18 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
19 filter, and concentrate the filtrate to a clear paste with a relative density of 1.11 to 1.13 ($65\pm 5^\circ\text{C}$). 3.
20 Spray-dry, sift, and mix.

21 **1.1.4 Atractylodes macrocephala Koidz.**

22 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
23 filter, and concentrate the filtrate to a clear paste with a relative density of 1.09 to 1.11 ($65\pm 5^\circ\text{C}$). 3.
24 Spray-dry, sift, and mix.

25 **1.1.5 *Hansenia weberbaueriana* (Fedde ex H.Wolff) Pimenov & Kljuykov**

26 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
27 filter, and concentrate the filtrate to a clear paste with a relative density of 1.10 to 1.12 (65±5°C). 3.
28 Spray-dry, sift, and mix

29 **1.1.6 *Actaea heracleifolia* (Kom.) J.Compton**

30 Decoct the pieces with water for 2.5 hours. 2. Filter and concentrate the filtrate to a clear paste with a
31 relative density of 1.08 to 1.10 (65±5°C). 3. Spray-dry, sift, and mix

32 **1.1.7 *Codonopsis pilosula* (Franch.) Nannf.**

33 Decoct the pieces with water twice, first for 1.5 hours and second for 0.5 hour. 2. Combine the
34 decoctions, filter, and concentrate the filtrate to a clear paste with a relative density of 1.03 to 1.05
35 (65±5°C). 3. Spray-dry, sift, and mix

36 **1.1.8 *Scutellaria baicalensis* Georgi**

37 Decoct the pieces with water twice, first for 1 hour and second for 1 hour. 2. Combine the decoctions,
38 filter, and concentrate the filtrate to a clear paste with a relative density of 1.15 to 1.17 (65±5°C). 3.
39 Spray-dry, sift, and mix

40 **1.1.9 *Coptis chinensis* Franch.**

41 Decoct the pieces with water twice, first for 1 hour and second for 1 hour. 2. Combine the decoctions,
42 filter, and concentrate the filtrate to a clear paste with a relative density of 1.09 to 1.11 (65±5°C). 3.
43 Spray-dry, sift, and mix

44 **1.1.10 *Pothos chinensis* (Raf.) Merr.**

45 Decoct the pieces with water for 3 hours. 2. Filter and concentrate the filtrate to a clear paste with a
46 relative density of 1.10 to 1.11 (65±5°C). 3. Spray-dry, sift, and mix

47 **1.2 The CSQBD herbal formula**

48 The CSQBD herbal formula is composed of 8 different herbs, as detailed in Supplementary Table S2.
49 The primary components of each herb were extracted following the specified processing procedures
50 outlined below. Subsequently, these ten extracts were thoroughly mixed for 30 minutes to achieve
51 uniformity. The homogenized mixture was then granulated to produce granules with a size range of
52 12-40 mesh. Finally, the granules were packaged into four aluminum foil bags.

53 **1.2.1 Astragalus mongholicus Bunge**

54 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
55 filter, and concentrate the filtrate to a clear paste with a relative density of 1.11 to 1.13 (65±5°C). 3.
56 Spray-dry, sift, and mix.

57 **1.2.2 Glycyrrhiza uralensis Fisch. ex DC.**

58 Decoct the pieces with water twice, first for 2.5 hours and second for 1.5 hour. 2. Combine the
59 decoctions, filter, and concentrate the filtrate to a clear paste with a relative density of 1.09 to 1.11
60 (65±5°C). 3. Spray-dry, sift, and mix.

61 **1.2.3 Codonopsis pilosula (Franch.) Nannf.**

62 Decoct the pieces with water twice, first for 1.5 hours and second for 0.5 hour. 2. Combine the
63 decoctions, filter, and concentrate the filtrate to a clear paste with a relative density of 1.03 to 1.05
64 (65±5°C). 3. Spray-dry, sift, and mix.

65 **1.2.4 Angelica sinensis (Oliv.) Diels**

66 Decoct the pieces with water for 2.5 hours. 2. Filter and concentrate the filtrate to a clear paste with a
67 relative density of 1.07 to 1.09 (65±5°C). 3. Spray-dry, sift, and mix.

68 **1.2.5 Citrus reticulata Blanco**

69 Decoct the pieces with water for 1 hours. 2. Filter and concentrate the filtrate to a clear paste with a
70 relative density of 1.05 to 1.07 (65±5°C). 3. Spray-dry, sift, and mix.

71 **1.2.6 Actaea heracleifolia (Kom.) J.Compton**

72 Decoct the pieces with water for 2.5 hours. 2. Filter and concentrate the filtrate to a clear paste with a
73 relative density of 1.08 to 1.10 (65±5°C). 3. Spray-dry, sift, and mix.

74 **1.2.7 Bupleurum chinense DC.**

75 Decoct the pieces with water twice, first for 2 hours and second for 1 hour. 2. Combine the decoctions,
76 filter, and concentrate the filtrate to a clear paste with a relative density of 1.10 to 1.12 (65±5°C). 3.
77 Spray-dry, sift, and mix.

78 **1.2.8 Atractylodes macrocephala Koidz.**

79 Decoct the pieces with water twice, first for 1.5 hours and second for 1 hour. 2. Combine the decoctions,
80 filter, and concentrate the filtrate to a clear paste with a relative density of 1.05 to 1.07 (65±5°C). 3.
81 Spray-dry, sift, and mix.

82 **1.3 Detection of the main chemicals in the two herbal formulas**

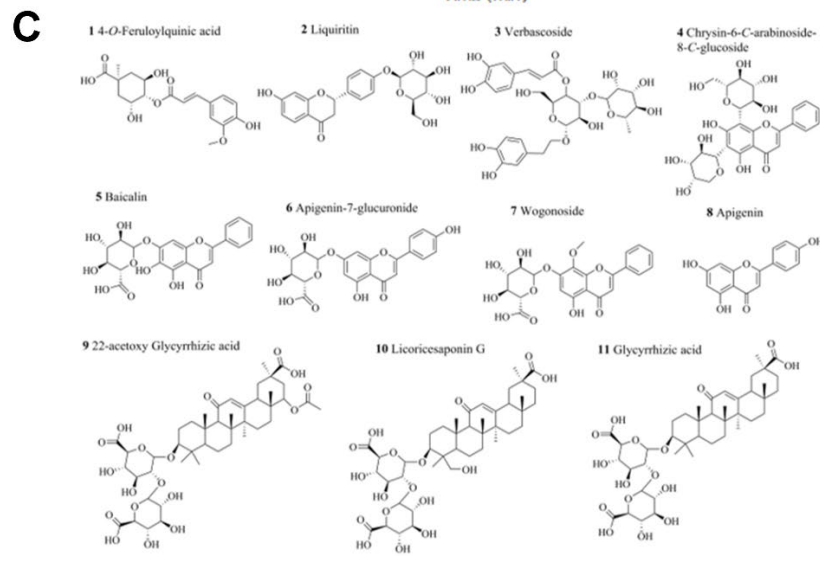
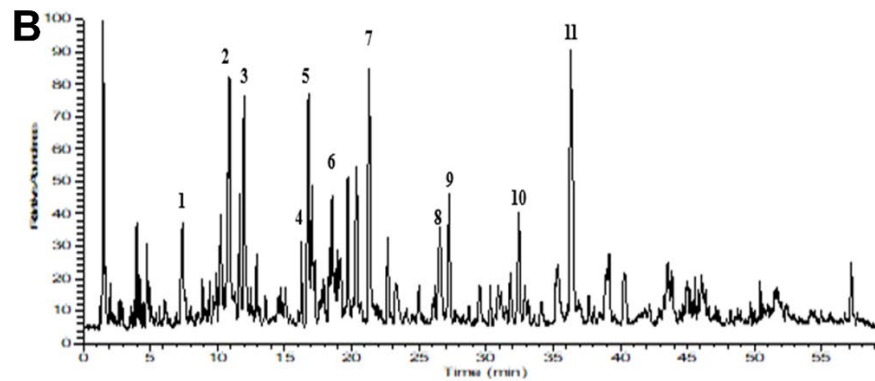
83 The samples were dissolved in hot water and then extracted once with an equal volume of butyl alcohol.
84 The butyl alcohol fraction was concentrated until dry, and subsequently dissolved in methanol. This
85 methanol solution was then filtered through 0.22 μm micropore filter for further analysis.

86 High Performance Liquid chromatography (HPLC) was performed using an Accela system
87 (Thermo Scientific, USA). The chromatographic separation was conducted on an Agilent Eclipse Plus
88 C18 column (4.6 mm \times 100 mm, 2.7 μm). The mobile phase consisted of 0.05% formic acid solution
89 (A) and acetonitrile (B). The gradient elution program was set as follows: 0 min, A:B 90:10; 15 min,
90 A:B 75:25; 40 min, A:B 60:40; 55 min, A:B 30:70; 56 min, A:B 90:10; 60 min, A:B 95:5. The flow
91 rate was 0.3 mL/min and the injection volume was 2 μL .

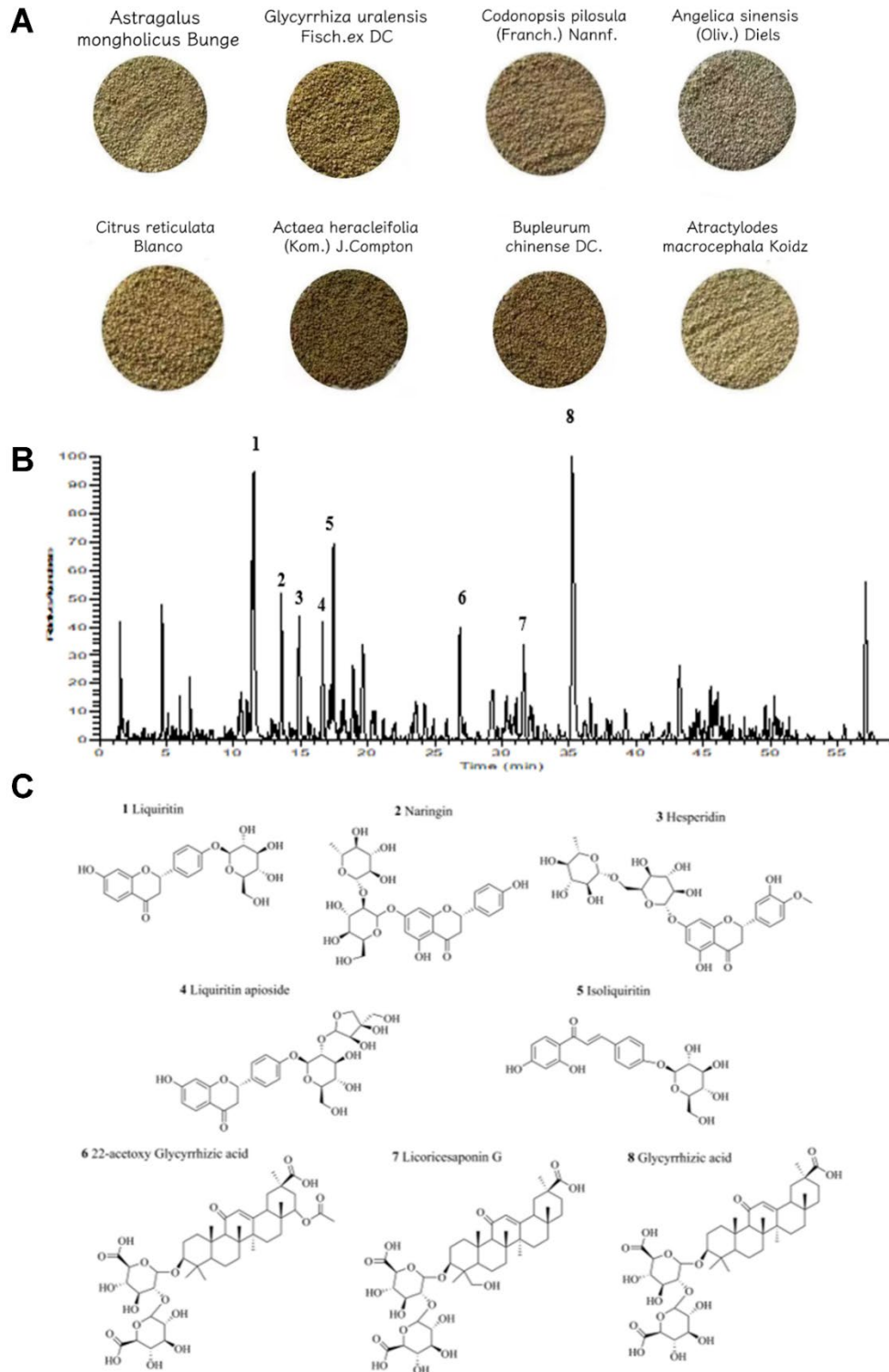
92 LC-MS analysis was performed on LTQ-Orbitrap XL Mass Spectrometer (Thermo Scientific,
93 USA) equipped with an ESI source in negative ionization mode. The capillary temperature was set at
94 350°C. Sheath, auxiliary, and sweep gas flow rates were set at 30, 4, and 0 units, respectively. Source
95 voltage was set at 3.8 kV, capillary voltage at -18 V, and tube lens voltage at -67 V. The data evaluation
96 was performed with Xcalibur 2.0 software. A Collision-Induced Dissociation (CID) energy of 35 eV
97 was used in a data dependent scan mode in MS/MS analysis.

98

99 2 Supplementary Figures and Supplementary Tables

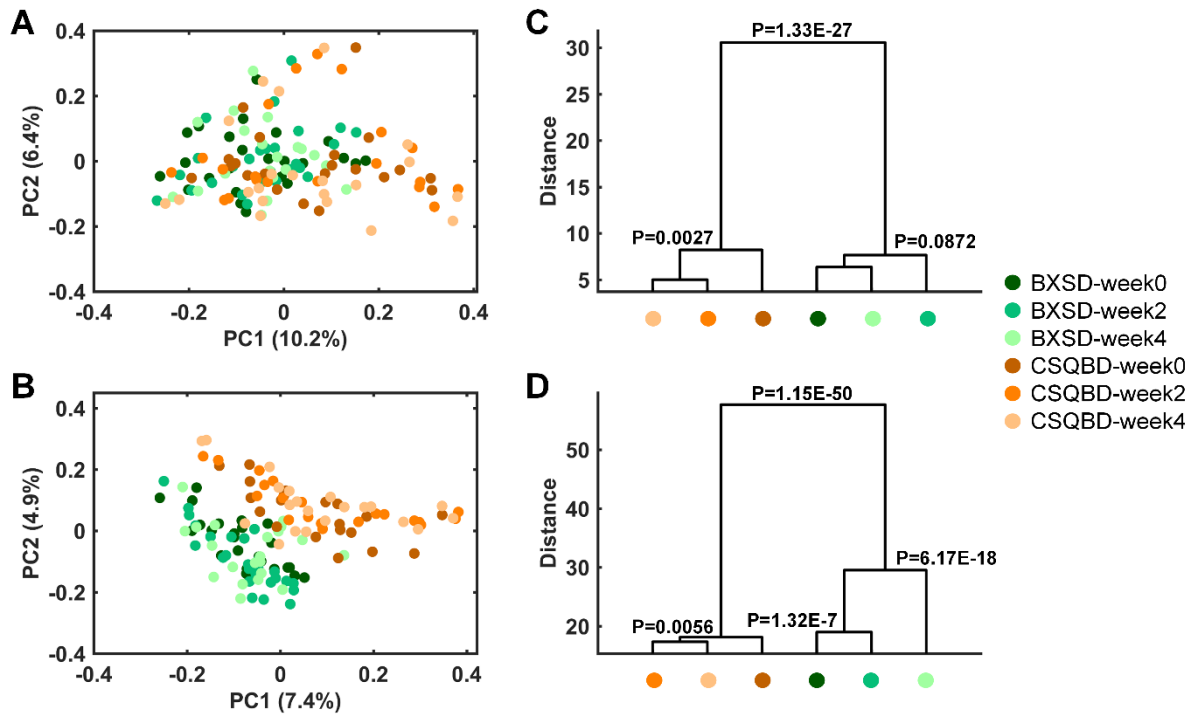


101 Supplementary Figure S1. The STYHCD herbal formula. (a) Photos of the decocting-free granules of
 102 the ten herbs in the STYHCD herbal formula. (b) HPLC-TIC-MS finger-printing of STYHCD herbal
 103 formula. (c) The eleven main chemicals in STYHCD.



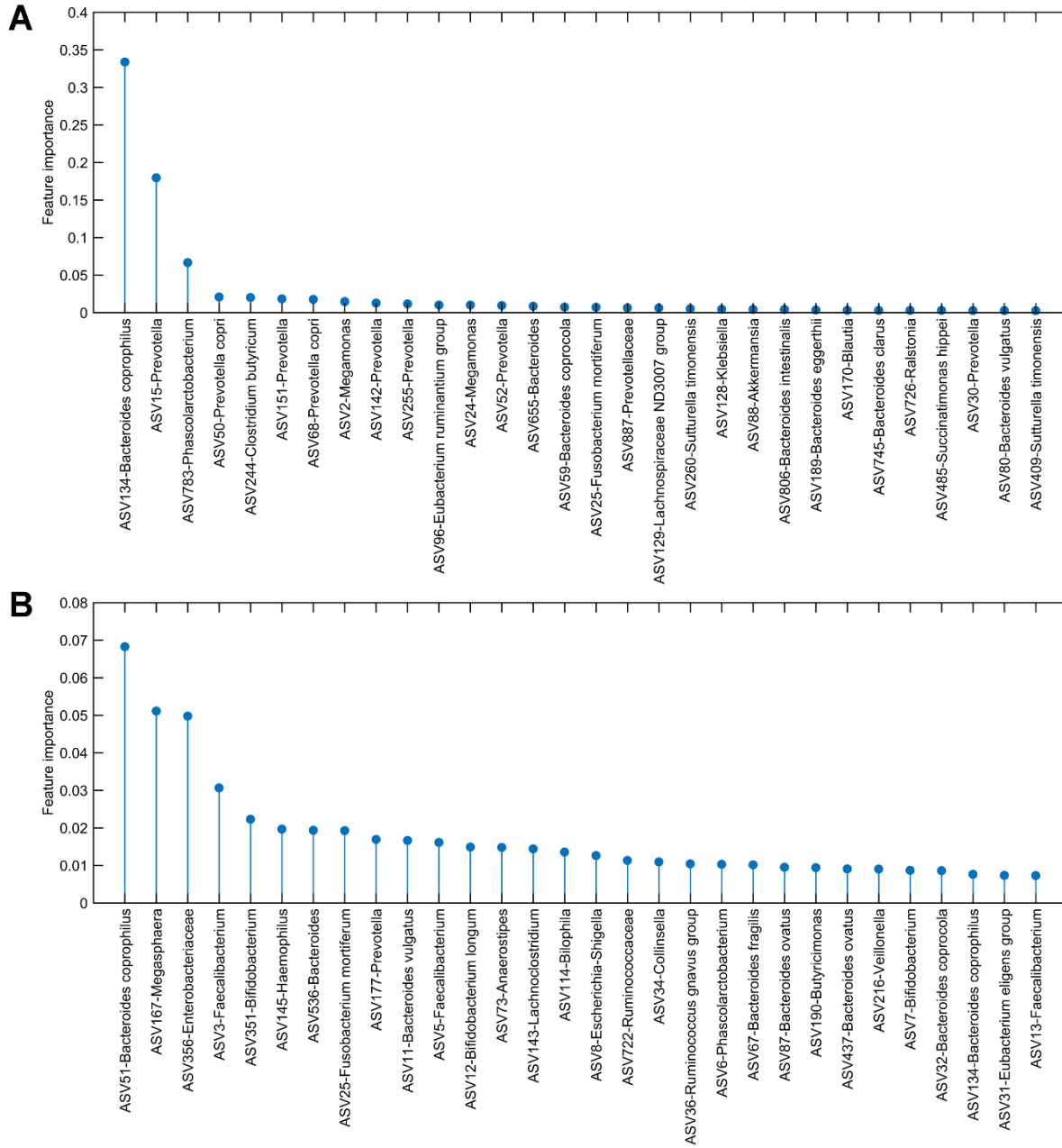
105 Supplementary Figure S2. The CSQBD herbal formula. (a) Photos of the decocting-free granules of
 106 the eight herbs in the CSQBD herbal formula. (b) HPLC-TIC-MS finger-printing of CSQBD herbal
 107 formula. (c) The eight main chemicals in CSQBD. STYHCD, spleen-tonifying and yin heat-clearing
 108 decoction; CSQBD, center-supplementing and qi-boosting decoction.

109



110

111 Supplementary Figure S3. The temporal alteration of the gut microbiome in insomnia patients received
 112 two different herbal formula treatments over time. Principal coordinate analysis based on (A) Bray-
 113 Curtis dissimilarity distance and (B) Jaccard distance. Clustering of gut microbiota based on inter-
 114 group distances obtained through MANOVA test based on (C) Bray-Curtis dissimilarity distance and
 115 (D) Jaccard distance.



116

117 Supplementary Figure S4. Feature importance of the top 30 gut microbial species identified through
 118 nested 10-fold cross-validated random forest regression for each herbal formula. A. STYHCD, Spleen-
 119 Tonifying and Yin Heat-Clearing Decoction. B. CSQBD, Center-Supplementing, and Qi-Boosting
 120 Decoction.

121

122

123 Supplementary Table S1. Herbal ingredients of the Spleen-Tonifying and Yin Heat-Clearing
124 Decoction.

Herb	Weight of the herb (g)	Herbal decocting-free granule (g)
<i>Bupleurum Chinese</i> DC.	15	3.9
<i>Glycyrrhiza uralensis</i> Fisch. ex DC.	10	4.3
<i>Astragalus mongholicus</i> Bunge	10	3.7
<i>Atractylodes macrocephala</i> Koidz.	10	5.8
<i>Hansenia weberbaueriana</i> (Fedde ex H. Wolff) Pimenov & Kljuykov	10	2.5
<i>Actaea heracleifolia</i> (Kom.) J. Compton	5	0.7
<i>Codonopsis pilosula</i> (Franch.) Nannf.	5	4.9
<i>Scutellaria baicalensis</i> Georgi	5	1.9
<i>Coptis chinensis</i> Franch.	5	0.9
<i>Gypsum fibrosum</i>	5	1
Total weight	80	29.6

125

126 Supplementary Table S2. Herbal ingredients of the Center-Supplementing, and Qi-Boosting
127 Decoction.

Herb	Weight of the herb (g)	Herbal decocting-free granule (g)
<i>Astragalus mongholicus</i> Bunge	30	10
<i>Glycyrrhiza uralensis</i> Fisch. ex DC	15	5.8
<i>Atractylodes macrocephala</i> Koidz.	15	9.5
<i>Codonopsis pilosula</i> (Franch.) Nannf.	10	8.9
<i>Angelica sinensis</i> (Oliv.) Diels	10	6.4
<i>Citrus reticulata</i> Blanco	10	3.7
<i>Actaea heracleifolia</i> (Kom.) J. Compton	10	1.3
<i>Bupleurum chinense</i> DC.	10	2.3
Total weight	110	47.9

128

129 Supplementary Table S3. Baseline alpha-diversity for the insomnia patients with different TCM
130 symptoms.

alpha-diversity metric	Coefficient	SEM	p-value	FDR_BH
shannon_entropy	0.0011	0.0004	0.0072	0.0249
pielou_evenness	0.0002	6.76E-05	0.0083	0.0249
chao1	-0.0006	0.0013	0.6654	0.9489
observed_features	-0.0003	0.0012	0.7907	0.9489
menhinick	1.41E-05	4.20E-05	0.7390	0.9489
margalef	-7.86E-06	0.0002	0.9662	0.9662

131 STYHCD group was used as the reference.

132 FDR_BH, FDR-corrected p-value were obtained with the Benjamini-Hochberg method to adjust for
133 multiple comparisons.

134

135 Supplementary Table S4. Longitudinal effects of the two herbal formulas on stress and systematic
136 inflammation over time.

Treatment	Cytokine	Coefficient	SEM	p-value	FDR_BH
STYHCD	Cortisol (ng/mL)	0.0194	0.0477	0.6876	0.8251
	IL-1 β (pg/mL)	0.1909	0.0642	0.0064	0.0166
	IL-6 (pg/mL)	0.1435	0.0364	0.0008	0.0037
	TNF- α (pg/mL)	0.2013	0.0615	0.0033	0.0098
	IFN- α (pg/mL)	-0.0846	0.0721	0.2471	0.3706
	IL-10 (pg/mL)	0.1291	0.0456	0.0102	0.0229
	TNF- β (pg/mL)	0.0186	0.0741	0.8026	0.8498
CSQBD	Cortisol (ng/mL)	-0.1372	0.0760	0.0788	0.1772
	IL-1 β (pg/mL)	-0.0419	0.0779	0.5965	0.6316
	IL-6 (pg/mL)	0.1026	0.0725	0.1730	0.3115
	TNF- α (pg/mL)	0.0688	0.0794	0.3914	0.5419
	IFN- α (pg/mL)	-0.1606	0.0748	0.0381	0.1142
	IL-10 (pg/mL)	0.2042	0.0730	0.0081	0.0362
	TNF- β (pg/mL)	-0.0655	0.0816	0.4278	0.5500

137 FDR_BH, FDR-corrected p-value were obtained with the Benjamini-Hochberg method to adjust for
138 multiple comparisons.

139 STYHCD, spleen-tonifying and yin heat-clearing decoction; CSQBD, center-supplementing and qi-
140 boosting decoction.

141

142 Supplementary Table S5. Longitudinal effects of the two herbal formulas on the alpha-diversity of the
143 gut microbiome over time.

Treatment	alpha-diversity metric	Coefficient	SEM	p-value	FDR_BH
STYHCD	shannon_entropy	-1.64E-04	1.35E-04	0.2270	0.5537
	pielou_evenness	-3.12E-05	2.01E-05	0.1247	0.5537
	chao1	6.31E-04	6.54E-04	0.3374	0.5537
	observed_features	-5.51E-04	6.09E-04	0.3691	0.5537
	menhinick	2.09E-05	3.16E-05	0.5104	0.6125
	margalef	1.43E-05	1.20E-04	0.9055	0.9055
CSQBD	shannon_entropy	3.85E-04	3.00E-04	0.2040	0.6120
	pielou_evenness	6.77E-05	5.09E-05	0.1887	0.6120
	chao1	-1.17E-04	5.45E-04	0.8302	0.8302
	observed_features	-1.52E-04	5.02E-04	0.7623	0.8302
	margalef	-4.56E-05	8.63E-05	0.5994	0.8302
	menhinick	-4.52E-06	2.04E-05	0.8257	0.8302

144 FDR_BH, FDR-corrected p-value were obtained with the Benjamini-Hochberg method to adjust for
145 multiple comparisons.

146 STYHCD, spleen-tonifying and yin heat-clearing decoction; CSQBD, center-supplementing and qi-
147 boosting decoction.