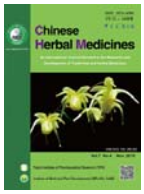

Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Comparison on Traditional and Machinery Decoctions for Da-cheng-qi Decoction Based on Chemical Ingredients, Pharmacological Functions, and Clinical Trials

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ARTICLE INFO

Article history

Received: June 15, 2015

Revised: July 30, 2015

Accepted: September 18, 2015

Available online: November 10, 2015

DOI: 10.1016/S1674-6384(15)60064-8

ABSTRACT

Objective Patients and doctors often have questions about the equivalence of traditional and machinery decoctions. In this article, using Da-cheng-qi Decoction (DCQD) as a model of formula, traditional decoction (TD), machinery decoction under high pressure (MDHP), and machinery decoction under normal pressure (MDNP) were compared. **Methods** For chemical components, HPLC fingerprints were established and evaluated using AHP combined with CRITIC weighing method; For animals' effects, the experiments of small intestinal propulsion were conducted; For clinical effects, a randomized clinical trial (RCT) was designed and performed. **Results** Although there were some differences between TD and MDNP in chemical ingredients, there was no significant difference in animal experiments and clinical trials ($P > 0.05$). **Conclusion** The traditional and machinery decoctions of DCQD could be used bioequivalently.

Key words

chemical ingredients; clinical trials; Da-cheng-qi Decoction; machinery decoction under high pressure; machinery decoction under normal pressure; pharmacological functions; traditional decoction

1. Introduction

Traditional decocting method for Chinese herbs has been applied in China for thousands of years. In details, herbs are soaked in water for a while, extracted twice to thrice in ceramic pot, filtrated, and mixed. The mixture liquid can be orally taken directly twice to thrice daily. However, this traditional approach is more and more unsuitable for the modern life style because of its inconvenience. For recent years machinery decocting method came into our daily life. This process is to extract herbs under certain temperature and pressure in a sealed stainless steel container and quantitatively

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Funds: Longhua Medical Project (LYTD-14), National special research foundation of TCM (No. 201007010)

packed by automatic pack machine in vacuum. The products are sterile and convenient to be stored and administrated. Although this decocting performance has been widely accepted by clinics, some patients and doctors still have questions about the method. For example, sometimes the color and taste of traditional and machinery decoctions are different. Furthermore, the effects of temperature and pressure on the active ingredients are unclear. However, up to now, few studies on the machinery decocting method have been reported.

Da-cheng-qi Decoction (DCQD) is a classical purgative Chinese medicinal formula which fights against ailments such as acute intestinal obstruction without complications, acute cholecystitis, and acute appendicitis (Liu et al, 2009; Xu et al, 2009; Jiang et al, 2015; Yuan, 2015). It is also often used for prophylaxis and treatment of postoperative paralytic ileus (Qi et al, 2007). The major components include polyphenol acid and anthraquinones originated from *Rhei Radix* et *Rhizoma* (*e.g.* sennoside, rhein, emodin, aloe-emodin, and chrysophanol), lignans, phenylethanoid glycosides from *Magnoliae Officinalis Cortex* (*e.g* magnolol and honokiol), and flavonoids from *Aurantii Immaturus Fructus* (*e.g.* hesperidin, rheochrysidin, naringin, and naringenin) (Xu et al, 2008; Yu et al, 2009) (Figure 1). Among these ingredients, polyphenol acid and anthraquinones play the essential roles in their purgative functions (Xie et al, 2013). Some papers reported that the contents of total anthraquinones and polyphenol acid could be decreased if *Rhei Radix* et *Rhizoma* was boiled for more than 15 min (Takayama et al, 2012). Other articles showed that global chemical differences between traditional and modern decoctions did exist (Wang et al, 2013). These indicated that decocting process could impact the chemical ingredients in DCQD. But it is not confirmed by animal and clinical trials.

In the present study, using DCQD as model formula, traditional and machinery decocting methods were compared based on chemical components, pharmacological functions, and clinical trials.

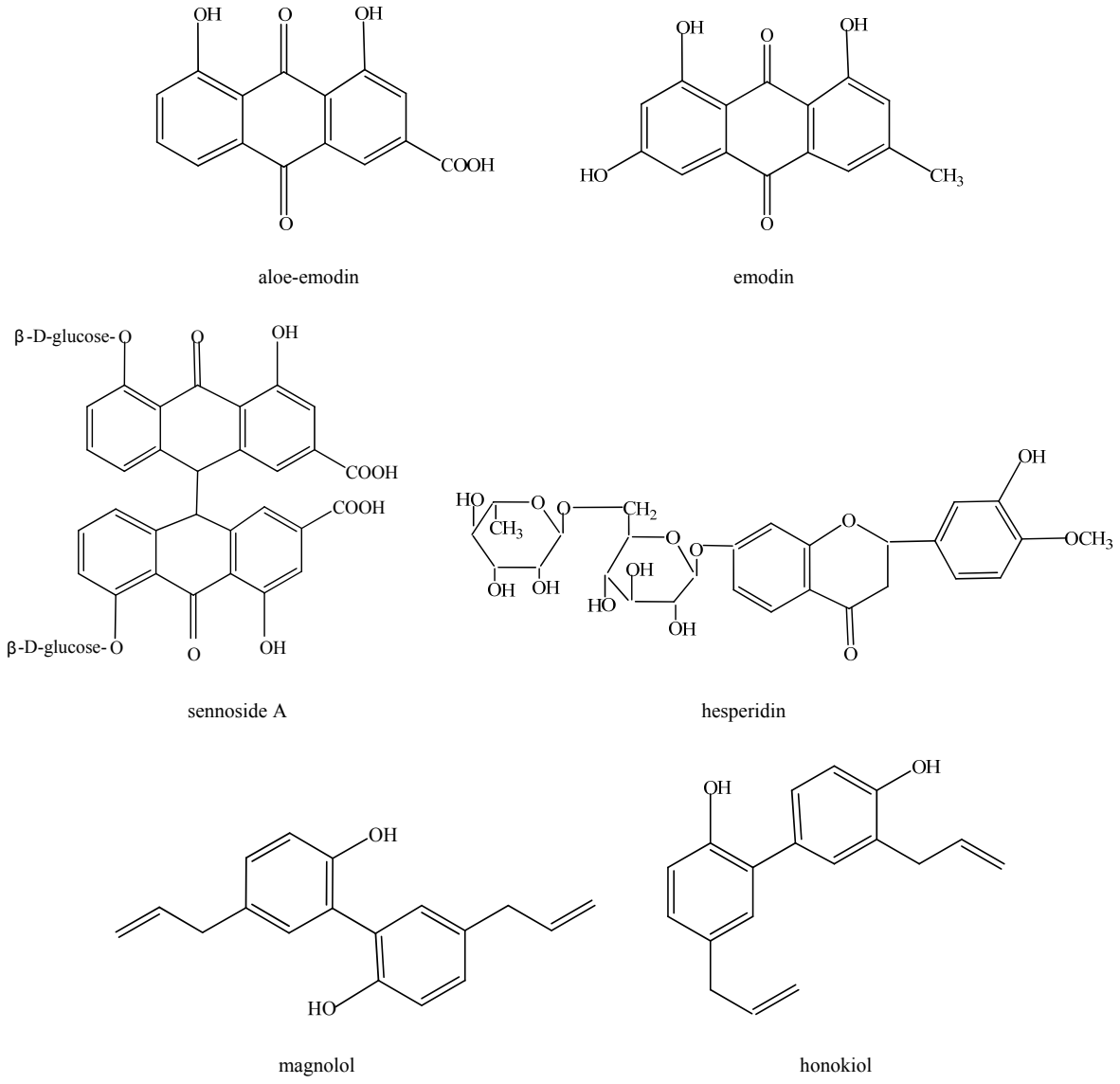

Figure 1 Structures of main components in DCQD

2. Materials and methods

2.1 Chemical ingredients analysis

2.1.1 Instrumentation

The analysis was carried out on an Agilent 1100 Series HPLC System (Agilent Corporation, Germany) consisting of a G1315B Diode Array Detector (DAD), a G1311A Low-pressure Quatpump, a G1379A Online Degasser, a G1316A Thermostat Column Compartment, and a G1313A Automatic Sample Injector. SB2200 Ultrasonic Bath (Bineng Ultrasonic Instrument Company, Shanghai, China), YFDL20 Automatic Decocting Machine of Multi-function, YF−20 Donghua Automatic Decocting Machine, YBS250E Liquid Packing Machine, and YBS Liquid Packing Machine were purchased from Beijing Donghuayuan Medical Equipment Co., Ltd.

2.1.2 Reagents, chemicals, and materials

The methanol and phosphate acid of HPLC-grade were purchased from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q Water System (Millipore, USA) for preparing samples and mobile solution. Other reagents were of analytical grade. All solvents were filtered through 0.22 μm membrane filters before analysis.

The reference standards of hesperidin, aloe-emodin, honokiol, magnolol, emodin, and sennoside A were obtained from Chinese Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The purities of all the standards were not less than 98%.

Rhei Radix et *Rhizoma* (Lot: 110519), *Magnoliae Officinalis Cortex* (Lot: 110326), *Aurantii Immaturus Fructus* (Lot: 110519), and *Natrii Sulfas* (Lot: 110307) were supplied by Nanjing Haiyuan Herbs Product Co., Ltd. All materials were stored at room temperature in the absence of light in a well-ventilated room. Chief pharmacist Qiu-feng Shi (Longhua Hospital, Shanghai, China) authenticated the plant materials and the voucher specimens were dried at room temperature in the absence of light in a well-ventilated room.

2.1.3 Decocting procedures of DCQD

After investigating the effects of solvent volume, duration and frequency of extraction on the chemical markers (details not shown in this paper), parameters of decocting procedure were optimized as follows.

1) Traditional decocting process

All materials were weighed respectively according to the ratio of formula. Among them, *Magnoliae Officinalis Cortex* and *Aurantii Fructus* were soaked in water for 40 min and extracted with nine folds volume of water for 35 min. *Rhei Rhizoma* was soaked for 40 min, added into above two herbs and extracted together for 5 min. Then the obtained decoctions were poured out. The rest of herbs were extracted twice with seven folds volume of water for 40 min. All the decoctions were mixed, filtrated through 6# mesh sieve, and concentrated. Finally, *Natrii Sulfas* was dissolved in concentrated solutions, filtrated through 7# mesh sieve, and packed using machine with 170−200 mL/bag.

2) Machine decocting process under high pressure

Magnoliae Officinalis Cortex and *Aurantii Fructus* were soaked for 45 min and extracted with nine folds volume of water in the decocting machine under the temperature of 140 °C till the pressure arriving at 0.25 MPa. Then a part of hot decoctions were excreted via the valve to soak *Rhei Radix* et *Rhizoma* and *Natrii Sulfas* for 5 min. The liquids were filtrated and poured back into decocting machine again. Finally, all the decoctions were mixed, filtrated, and packed with 170−200 mL/bag.

3) Machine decocting process under normal pressure

Magnoliae Officinalis Cortex and *Aurantii Fructus* were soaked for 60 min and extracted with nine folds volume of water in the decocting machine under the normal pressure for 35 min. *Rhei Radix* et *Rhizoma*, was soaked for 60 min, added into above two herbs and extracted together for 10 min. Then a part of hot decoctions were excreted via the valve to dissolve *Natrii sulfas*. These liquids were filtrated and poured back to machine. Finally, all the decoctions were mixed, filtrated, and packed with 170−200 mL/bag.

2.1.4 Preparation of standard solutions and samples

1) Preparation of standard solutions

Each accurately weighed standard was respectively dissolved in methanol. A set of standard solutions were prepared by appropriate dilutions of the stock solutions with methanol, in order to establish the calibration curves.

2) Preparation of samples

Approximately 2 to 4 mL of DCQD was measured accurately and diluted to 10 mL with methanol, respectively. The samples were extracted under the ultrasound for 20 min, then centrifuged and filtered through syringe filters (0.22 μm). All samples were stored at 4 °C and brought to room temperature before use.

2.1.5 Method validation

1) Calibration and detection limits

Calibration standard solution of 10 μL was injected into HPLC instrument. Calibration curves were constructed by plotting the chromatographic peak area versus the compound amount injected and there was good linear relationship when the correlation coefficient was above 0.9. The limits of detection (LOD) and quantification (LOQ) for marker compounds under the present chromatographic conditions were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively.

2) Precision, repeatability, and accuracy

To assess the intra-day variations, the same solution was determined in triplicate within a day; The inter-day precision was with the same solution over three consecutive days by three injections per day. To estimate the reproducibility, five different working solutions were prepared from the same decoction and analyzed. To evaluate the accuracy of this

method, the known amounts of standards were respectively spiked to the same sample at the low, medium, and high amounts and analyzed as described above. Average recoveries were calibrated by the formula: recovery = (amount found − original amount)/amount spiked, and relative standard deviation or RSD = SD/mean.

2.1.6 Content determination of samples

The DCQD were decocted respectively by traditional process, machinery processes under high and normal pressure. Each method was repeated for six times. The samples of the obtained decoctions were prepared as above described and analyzed under above HPLC conditions. The contents of marked ingredients were calculated according to the corresponding curves.

2.1.7 Data analysis

1) Evaluation of similarity

The similarity tests among samples were performed using the professional software named Similarity Evaluation System for Chromatographic Fingerprint of TCM (2004A). The matching amongst the fingerprints of samples was performed by a multipoint calibration mode based on the retention time and spectra. In this paper, all samples were examined to generate a reference chromatogram as the representative standard fingerprint and the similarity of each chromatogram against this standard chromatogram was then calculated using the nearest neighbor and cosine method (equations 1 and 2).

$$C_{xy} = \frac{(x,y)}{\|x\|\|y\|} \quad (1)$$

$$\cos\theta = \frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2}\sqrt{\sum_{i=1}^{n} y_i^2}} \quad (2)$$

2) Evaluation of HPLC fingerprints

Synthetic weighing method (AHP combined with CRITIC) was carried out to handle the relative importance of different constituents in the HPLC fingerprints of DCQD based on our previous research (Zhao et al, 2011). The synthetic scores of three decocting methods were calculated according to obtained weight values and compared using One-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

2.2 Animal pharmacological study

2.2.1 Animals and raising conditions

Fifty of Kunming mice (weighing 18−22 g), half male and half female, were purchased from Silaike Experimental Animal Co., Ltd. [Licensed No. SCXK (hu) 2007−0005, Shanghai, China] and housed in Animal Laboratory of Longhua Hospital [Licensed No. SYXK (hu) 2005−0002, Shanghai, China]. The room temperature maintained at 20−25 °C under a 12 h light/12 h dark cycle (07:00−19:00) and humidity was between 40% and 70%. The animals were raised in mice cages with five per cage. Food and water were given *ad libitum.* The protocol of the animal study was in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. The Experimental Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine Care Guidelines was complied with to ensure that the mice received human care (ethics approval number: SCXK2010−0005, 2010.7.30)

2.2.2 Protocol of experiments

Before the experiment, the animals were fasted for 12 h with free to access to water. Next morning, they were randomly assigned to five groups (five male and five female per group) and respectively ig administered by normal saline (negative control group), 32.5 μg/mL mosapride solution (positive control group), traditional decoction (TD group), machinery decoction of high pressure (MDHP) group and machinery decoction of normal pressure (MDNP) group. The concentration of DCQD was 0.312 g/mL. The dosage of administration was 20 mL/kg animal weight. Half an hour later, 0.3 mL of 1% charcoal powder suspension (dissolved in 1% sodium carboxymethyl cellulose (CMC-Na) aqueous solution) was ig given to each mouse. After 60 min, the mouse was sacrificed and the intestine was collected. The length of pylorus to the frontier of black carbon powder paste and pylorus to cecum were respectively measured and the ratio of both lengths was calculated as the rate of small intestinal propulsion.

2.2.3 Data analysis

The rates of small intestinal propulsion for different groups were presented as $\bar{x} \pm s$ and the data were evaluated by One-way ANOVA. $P < 0.05$ was considered statistically significant. Above statistical analysis was carried out adopting SPSS 17.0 software for Windows.

2.3 Clinical trials

2.3.1 Subjects

This trial was a randomized and double-blind study. The protocol of the clinical study was approved by Medical Ethics Committee of Longhua Hospital (201007010). It was conducted between October 2010 and January 2011 in the outpatient TCM clinic of Longhua Hospital, Shanghai, China. The following measurements were taken for determining the eligibility of the patients at baseline: (1) age, height, body weight, respiration, heart rate, and so on; (2) present illness.

After the eligibility assessments, subjects who were diagnosed with chronic functional constipation in Longhua Hospital were enrolled. The inclusions were as follows: (1) adults; (2) consistence with functional constipation criteria (Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders); (3) written consent was obtained from the subject stating that the subject was comfortable to complete the study. Potential participants were excluded at screening if the

participants (1) were unwilling to accept the assigned herbs treatment; (2) were participating in another research project; (3) suffered from the constipation was induced by other causes such as cancers, endocrine, metabolic diseases, drugs and so on, or (4) had metabolic, renal, anaphylactic or endocrine disease, or suffered from primary hypertension, primary hypotension, chronic anemia, tuberculosis, a mental disorder or a chronic affection, and so on.

2.3.2 Interventions

Subjects were randomly divided into three groups: TD group, MDNP group, and MDHP group. The decoctions were packed as 170−200 mL/bag.

One bag of DCQD was orally given twice daily for continuous 7 d. During therapy, the enrolled patients were required regular diet, absence of alcohol and avoiding big changes in their lifestyles. Symptoms of bowel and other signs were evaluated at days 1 and 7. Adverse events were also recorded during the treatment period.

2.3.3 Statistical analysis

Measurement data were expressed as $\bar{x} \pm s$. The differences of quantitative data between two groups were analyzed with *t* test. Paired *t*-test was used to compare the data before and after therapy. ANOVA was performed to compare measurement data among the three groups. Enumeration data were analyzed using χ^2 test. If the number of samples was less than 40, the Fisher's exact method was used to calculate the exact *P* value. All analyses were conducted using SPSS for Windows version 17.0 software. All tests were two sided and $P < 0.05$ was defined as the significant level.

3. Results and discussion

3.1 Selection of model formula

In order to investigate different decocting methods, a model formula is needed to be chosen. DCQD is a classic purgative formula and has been widely applied in our country for hundreds of years. Furthermore, this formula has other characteristics.

(1) In this prescription there are only four herbs, but the medicinal parts are variable. The official parts of *Rhei Radix* et *Rhizoma* (*Dahuang*), *Magnoliae Officinalis Cortex* (*Houpu*), and *Aurantii Immaturus Fructus* (*Zhishi*) are respectively rhizomes, barks, and fruits. *Natrii Sulfas* (*Mangxiao*) is mineral.

(2) The requirements of processes are various. *Rhei Radix* et *Rhizoma* should be added after other herbs, because purgative components such as anthraquinone glycosides may be hydrolyzed to anthraquinones if the extracting period is too long; The chemical molecular of *Natrii Sulfas* is sodium sulfate which should be dissolved in hot water.

(3) The pharmacological functions of DCQD are unambiguous (Xu et al, 2010a; 2010b; 2010c).

(4) The assessment of efficacy is feasible. The animal model has been well established and generally accepted. Disease response in clinics can be evaluated according to criteria.

All these reasons indicate DQCT is a representative formula and convenient for us to study the decocting process.

3.2 Comparison on chemical ingredients for three decocting methods

3.2.1 Optimization of chromatographic conditions

The chromatographic column, wavelength, and gradient eluting programs were optimized in order to obtain as many peaks as possible in a single run and achieve a good baseline separation. After lots of trials, the chromatographic conditions were finally confirmed as follows:

The chromatographic separation was performed on a XDB-C_{18} column (250 mm × 4.6 mm, 5 μm) at 25 ^{o}C. The mobile phase consisted of methanol (A) and 0.1% phosphate acid water (B) with a gradient elution program of 25% (A) in 0−5 min, 25%−40% (A) in 5−23 min, 40% (A) in 23−35 min, 40%−80% (A) in 35−48 min, 80%−85% (A) in 48−55min, 85% (A) in 55−64 min, 25% (A) in 64−69 min and then re-equilibration of the column with 25% (A) for 7 min. The flow rate was 1.0 mL/min, and the injection volume was 10 μL. The analytes were detected at 294 nm. From the chromatograms of DCQD (Figure 2), it was concluded that no other peaks from impurities disturbed the separation process.

3.2.2 Validation of HPLC method

1) Calibration and detection limits

Under the developed method, hesperidin, aloe-emodin, honokiol, magnolol, emodin, and sennoside A (Figure 1) were well separated and a good linearity of each marker ingredient was observed in a relatively wide concentration with correlation coefficient above 0.999. Limits of LOD and LQD were also satisfied (Table 1).

2) Precision, reproducibility, and accuracy

As shown in Table 2, RSDs of the intra- and inter-day precision were found not exceeding 4%, suggesting that the instrument have a good precision. The recovery test for six compounds showed mean recovery rates were between 89% and 110%, indicating that accuracy of the method was acceptable. The RSDs of reproducibility were below 4% (Table 3), demonstrating that the extracting methods of samples were stable.

3.2.3 Similarity analysis of HPLC fingerprints for DCQD

Six batches of decoctions adopting three cooking methods were analyzed using above established HPLC-DAD method. Among characteristic peaks, the peak abundance of hesperidin was found generally consistent in all 18 chromatograms (Figure 2). Besides, hesperidin also eluted at a reasonable time within the chromatographic windows and possessed known pharmacological activities. As a result, it

was chosen as reference peak.

The correlation coefficients of six batches decoctions for three decocting methods were all more than 0.9914 (RSD < 1%) (Table 4). And this proved that six batches had satisfied similarities. Therefore, six batches could be applied for followed animal and clinical experiments.

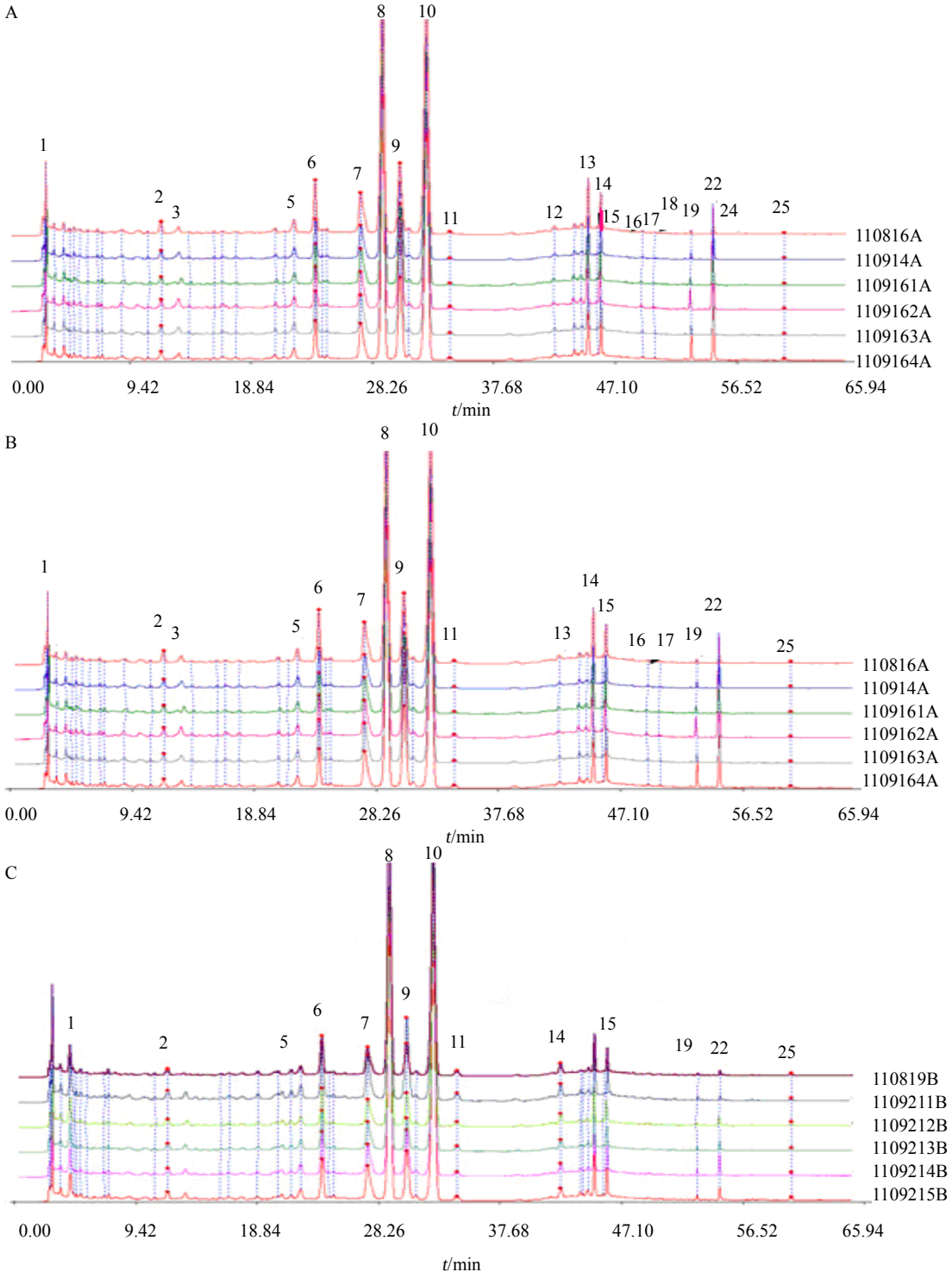

Figure 2 HPLC fingerprint of six batches of DCQD in TD (A), MDHP (B), and MDNP (C) groups

In Figure 2A, Peak 9 is hesperidin (30.44 min), Peak 18 is aloe-emodin (51.16 min), Peak 19 is honokiol (52.95 min), Peak 22 is magnolol (54.70 min), Peak 24 is emodin (57.22 min), and Peak 25 is chrysophano (60.23 min); In figure 2B, Peak 9 is hesperidin, Peak 17 is honokiol, Peak 18 is magnolol, and Peak 19 is chrysophano; In figure 2C, Peak 9 is hesperidin (30.31 min), Peak 19 is honokiol (52.91 min), Peak 22 is magnolol (54.68 min), and Peak 25 is chrysophano (60.19 min).

Table 1 Calibration curves, LODs, and LOQs of six compounds by HPLC-DAD

Compounds	Retention time	Regression equation	Correlation coefficients	Linear ranges / μg	LOD / ng	LOQ / ng
Hesperidin	30.45	$Y = 1184.7X - 6.8027$	1.0000	0.056-2.800	5	9
Aloe-emodin	51.15	$Y = 513.86X - 0.3438$	1.0000	0.030-0.600	16	30
Honokiol	52.95	$Y = 1846.2X + 3.3171$	0.9999	0.013-1.300	4	11
Magnolol	54.69	$Y = 1432.1X - 4.0437$	1.0000	0.020-2.000	7	20
Emodin	57.19	$Y = 2503.8X + 3.941$	1.0000	0.004-0.560	2	3
Sennoside A	60.23	$Y = 359.42X - 3.2472$	0.9997	0.030-0.300	15	30

Y is peak area in UV chromatograms monitored at detection wavelengths, *X* is compound amount injected

Table 2 Precision, repeatability, and accuracy of six chemical structures

Compounds	Spiked mean / μg	Intra-day precision ($n = 3$)		Inter-day precision ($n = 3$)		Accuracy / %
		Measured mean / μg	RSD / %	Measured mean / μg	RSD / %	($n = 9$)
hesperidin	6.339	13.517 ± 0.020	0.15	13.815 ± 0.300	2.17	89.98
	7.924	15.334 ± 0.072	0.47	15.672 ± 0.298	1.90	95.21
	9.509	17.356 ± 0.126	0.72	17.679 ± 0.318	1.80	100.52
aloe-emodin	1.100	3.713 ± 0.078	2.10	3.784 ± 0.076	2.01	109.83
	1.250	3.769 ± 0.040	1.05	3.806 ± 0.073	1.92	109.96
	1.600	4.287 ± 0.147	3.42	4.320 ± 0.112	2.59	99.52
honokiol	5.500	3.553 ± 0.016	0.44	3.630 ± 0.070	1.93	105.44
	7.160	3.948 ± 0.015	0.39	3.998 ± 0.050	1.25	107.47
	12.200	4.667 ± 0.060	1.30	4.751 ± 0.080	1.68	93.36
magnolol	5.500	3.553 ± 0.016	0.44	3.630 ± 0.070	1.93	105.44
	5.500	3.553 ± 0.016	0.44	3.630 ± 0.070	1.93	105.44
	7.160	3.948 ± 0.015	0.39	3.998 ± 0.050	1.25	107.47
emodin	2.554	5.547 ± 0.066	1.20	5.611 ± 0.060	0.01	93.73
	3.192	6.540 ± 0.101	1.54	6.528 ± 0.081	1.24	104.66
	3.830	7.721 ± 0.120	1.55	7.699 ± 0.083	1.07	107.90
	3.530	7.832 ± 0.061	0.78	7.725 ± 0.097	1.26	95.10
sennoside A	4.412	9.035 ± 0.093	1.03	9.041 ± 0.069	0.77	104.90
	5.294	10.354 ± 0.159	1.53	10.504 ± 0.166	1.58	103.90

Table 3 Reproducibility of six compounds ($n = 5$)

Compounds	Concentration / ($mg \cdot g^{-1}$)	SD	RSD / %
hesperidin	1.512	0.049	3.25
aloe-emodin	0.626	0.002	0.27
honokiol	0.314	0.009	2.74
magnolol	0.612	0.011	1.74
emodin	0.104	0.001	1.19
sennoside A	0.400	0.004	1.00

Table 4 Similarities of different batches of decoctions for three decocting methods ($n = 6$)

Batch No.	TD		MDHP		MDNP	
	Correlation coefficients	RSD / %	Correlation coefficients	RSD / %	Correlation coefficients	RSD / %
1	0.9991 ± 0.0007	0.07	0.9943 ± 0.0041	0.41	0.9974 ± 0.0039	0.39
2	0.9985 ± 0.0011	0.11	0.9978 ± 0.0017	0.17	0.9914 ± 0.0039	0.40
3	0.9991 ± 0.0006	0.06	0.9979 ± 0.0016	0.16	0.9978 ± 0.0037	0.37
4	0.9992 ± 0.0005	0.05	0.9956 ± 0.0038	0.38	0.9975 ± 0.0040	0.40
5	0.9988 ± 0.0009	0.09	0.9964 ± 0.0029	0.29	0.9975 ± 0.0033	0.33
6	0.9982 ± 0.0013	0.13	0.9974 ± 0.0025	0.25	0.9977 ± 0.0030	0.30

3.2.4 Comparison on HPLC fingerprints for three decocting methods

Among acquired chromatograms of decoctions, 21 common peaks were separated for TD while 18 and 14 common peaks were respectively identified for MDHP and MDNP (Figure 2). This implied three decocting methods might impact on the ingredients of DCQD.

The concentration of main components in three kinds of decoctions further revealed that different methods were related to the variations of contents (Figure 3). The contents of hesperidin and magnolol in MDHP were the most abundant in three methods ($P < 0.01$); For honokiol and chrysophano, there were no obvious differences between traditional and machinery decoctions; Meanwhile, the amounts of aloe-emodin and emodin in machinery decoctions were limited. To our knowledge, hesperidin and magnolol respectively belonged to flavones and phenols (Figure 1). Under the high temperature and pressure, these kinds of components could be more easily extracted, which might contribute to the phenomena of the richest contents in MDHP. In the other side, MDHP was executed only once and immediately terminated when the pressure arriving at 0.25 MPa while other two decoctions were required twice to thrice and extracting times were 35 to 40 min for each time. The longer the extracting time was, the more opportunities the active purgative anthraquinone glycosides were hydrolyzed into anthraquinones whose purgative functions were mild and antibiotic actions were major (Li et al, 2009).

In order to systematically evaluate the information of fingerprints, AHP combined with CRITIC weighing methods was proposed. The primary ranking order was decided by the subjective judgments of analysts. Based on the theory of TCM, among four materials of DCQD, *Rhei Radix* et *Rhizoma* was the most important herb like the monarch, so the principal pharmacologic active components such as chysophano in *Rhei Radix* et *Rhizoma* were the first critical layer elements; *Magnoliae Officinalis Cortex* and *Aurantii Fructus* as assistance, hesperidin in *Aurantii Fructus*, magnolol and honokiol in *Magnoliae Officinalis Cortex*, were the parameters of second layer; Although still unidentified, Peaks 8 and 10 had the richest amounts and were marked as the third layer; Other common peaks were decided as the four layer; uncommon peaks were thought as the least important factors. With respect to this sequence, the weights were summarized in Table 5. And subsequent synthetic scores for three kinds of decoctions were represented in Figure 4. Among three decoctions, the synthetic scores of MDHP were the largest, but did not appear statistical significance compared to TD ($P > 0.05$). The value of MDNP was significantly less than TD ($P < 0.01$). That was to say, from the view of whole chemical constituents, MDHP was consistence with TD, but MDNP was not satisfied choice.

3.3 Comparison of pharmacologic functions for three decocting methods

To evaluate the efficacy of three decoctions, small intestinal propulsion trial was conducted. It was indicated in Figure 5 that both mosapride and decoctions could improve significantly the movement of intestinal ($P < 0.05$); decoctions of DCQD had the similar functions as positive group; and there were no obvious therapeutic differences among three decoctions ($P > 0.05$).

3.4 Comparison of clinical efficacy for three decocting methods

3.4.1 Baseline characteristics of study subjects

Fifty-three patients with chronic functional constipation were enrolled between October and January 2012 according to criteria (Tables 6). They showed the symptoms such as mouth bitter and bad breath, burning and turbid urination, red tongue, greasy yellow tongue coating and slippery pulse However, six patients dropped out because they did not follow-up on time, two patients did not complete the treatment as they felt better and stopped taking medicine. So a total of 45 patients were analyzed: 14 in MDHP group, 15 in MDNP group and 16 in TD group. For all the patients, no obvious adverse events happened.

Baseline characteristics of the participants are summarized in Table 7. No statistically significant difference was found in mean age, height, weight, breath, heart rate, and disease states ($P > 0.05$), demonstrating the baseline of enrolled cases was consistent.

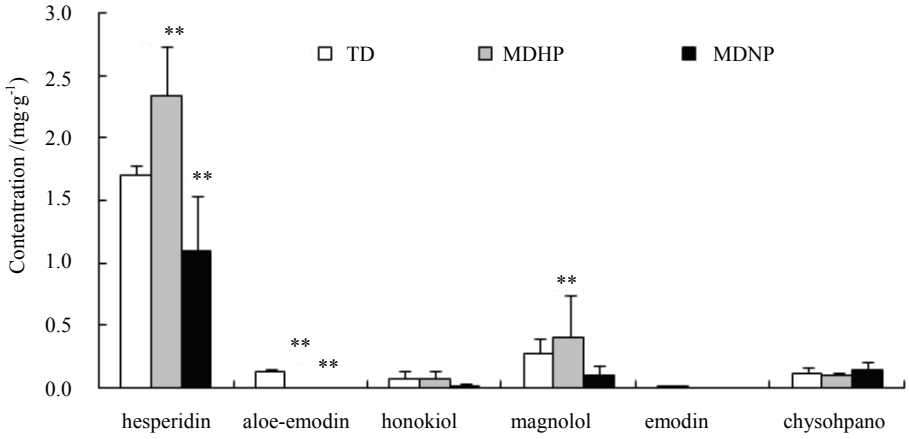

Figure 3 Comparison of principal ingredients from three decocting methods ($\bar{x} \pm s$, $n = 6$)

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* TD group, same as below

Table 5 AHP, CRITIC, and synthetic weights

Peaks	Compounds	AHP	CRITIC	AHP combined with CRITIC
1		0.0234	0.0518	0.0367
2		0.0126	0.0574	0.0218
3		0.0126	0.0534	0.0203
4		0.0126	0.1104	0.0419
5		0.0234	0.0273	0.0193
6		0.0234	0.0302	0.0213
7		0.0234	0.0263	0.0186
8		0.0381	0.0300	0.0346
9	hesperidin	0.0634	0.0584	0.1117
10		0.0381	0.0286	0.0329
11		0.0126	0.0487	0.0185
13		0.0234	0.0470	0.0333
14		0.0234	0.0356	0.0252
15		0.0234	0.0329	0.0233
16		0.0126	0.0452	0.0172
17		0.0126	0.0412	0.0156
18	aloe-emodin	0.0983	0.0514	0.1527
19	honokiol	0.0634	0.0567	0.1085
22	magnolol	0.0634	0.0714	0.1366
23		0.0126	0.0482	0.0183
25	sennoside A	0.0634	0.0479	0.0917

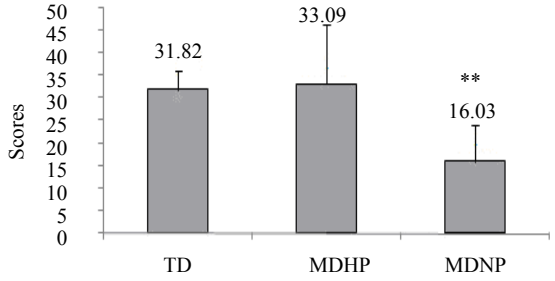

Figure 4 Synthetic scores of three methods ($\bar{x} \pm s$, $n = 6$)

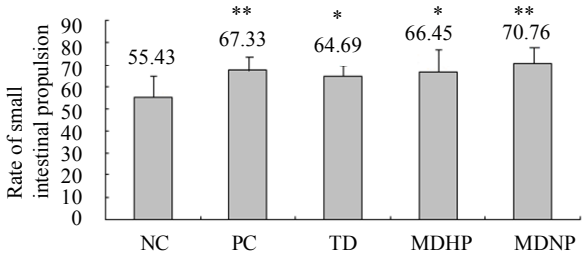

Figure 5 Comparison of small intestinal propulsion rates from three decocting methods ($\bar{x} \pm s$, $n = 10$)

Table 6 Criteria of primary TCM symptoms

Items	Normal (0)	Mild (1)	Moderate (2)	Severe (3)
Time interval of poop	≤ 2 /d	2–3/ d	3–5/d	> 5/d
Stool frequency	≤ 2 /d	2–3 /d	3–5/d	> 5/d
Stool sense	Extremely	Sometimes	Occasionally	Not at all
Hardness of stool	Soft	Hard, dry stools	Hard, dry stool	Hard, dry granular stool
Abdominal distension	Not at all	Occasionally, not obvious	Frequently, obvious	Accompanied by each stool
Exertion of poop	Not at all	Occasionally	Frequently	Each time
Incompletion of poop	Not at all	Occasionally	Frequently	Each time
Anal bulge	Not at all	Occasionally	Frequently	Each time

3.4.2 Efficacy evaluation

In all groups, scores of TCM symptoms after treatment were significantly lower than those before treatment ($P < 0.01$). In details, for a large part of symptom items, there were significant differences before and after therapy with $P < 0.05$ (Table 8). This implied all groups were effective in the treatment of chronic functional constipation.

Furthermore, effective rates were assessed. The calculation of effective rates included completely cured, significant curative and improved cases. That was to say, therapy was considered effective when scores of TCM symptoms were improved 30%. Results showed (Table 9): the effective rates of TD, MDNP, and MDHP were respectively 93.75%, 93.33%, and 71.43%. This confirmed that decoctions of DCQD using different decocting protocols were effective.

3.4.3 Comparison on treatment in each group

The ANOVA was employed to analyze the difference between before and after treatment. The results displayed as shown in Table 10 there was no obvious difference ($P > 0.05$) in total score of patients and the score of other items except the defecation interval. This illustrated there was no significant difference in effect among three groups.

The results of Chi-square test revealed that there was no obvious difference in efficacy rates between TD and MDNP groups ($P = 0.962 > 0.05$) as well as TD and MDHP groups ($P = 0.102 > 0.05$). This confirmed that TD group had no apparent differences in the clinical effect with MDNP and MDHP groups.

Table 7 Baseline characteristics of subjects

Groups	MDPH (n = 14)	MDNP (n = 15)	TD (n = 16)	P
Age	35.00 ± 15.37	36.73 ± 15.23	37.63 ± 11.32	0.875
Height /cm	162.35 ± 4.84	163.67 ± 7.96	163.75 ± 5.63	0.801
Body weight /kg	55.50 ± 9.67	57.80 ± 7.82	58.25 ± 7.09	0.627
Breath	17.64 ± 1.55	17.36 ± 1.74	17.81 ± 1.87	0.771
Heart Rate	78.29 ± 6.33	75.20 ± 7.44	75.19 ± 8.10	0.435
Time interval of poop	1.43 ± 1.02	1.20 ± 0.77	2.00 ± 0.63	0.026
Stool frequency	1.50 ± 1.09	1.27 ± 0.80	1.88 ± 0.62	0.143
Stool sense	1.29 ± 1.20	1.60 ± 0.99	1.75 ± 0.86	0.456
Hardness of stool	1.71 ± 1.20	1.73 ± 1.03	1.38 ± 1.09	0.602
Abdominal distension	1.21 ± 1.25	0.47 ± 0.74	0.81 ± 0.91	0.135
Exertion of poop	2.36 ± 0.74	2.40 ± 0.63	1.88 ± 0.81	0.098
Incompletion of poop	1.79 ± 0.97	1.80 ± 1.26	1.06 ± 1.06	0.119
Anal bulge	1.43 ± 1.22	1.27 ± 1.03	0.88 ± 0.89	0.335
Total scores	12.71 ± 3.63	11.73 ± 2.69	11.62 ± 4.14	0.663

Table 8 Comparison of symptom scores of patients between before and after treatment

Groups	MDHP (n = 14)			MDNP (n = 15)			TD (n = 16)		
	Before treatment	After treatment	P	Before treatment	After treatment	P	Before treatment	After treatment	P
Time interval of poop	1.43 ± 1.02	0.71 ± 0.99	0.055	1.20 ± 0.77	0.40 ± 0.51	0.001	2.00 ± 0.63	0.31 ± 0.48	0.000
Stool frequency	1.50 ± 1.09	0.64 ± 1.01	0.040	1.27 ± 0.80	0.40 ± 0.51	0.000	1.88 ± 0.62	0.38 ± 0.50	0.000
Stool sense	1.29 ± 1.20	0.86 ± 0.86	0.111	1.60 ± 0.99	0.53 ± 0.83	0.000	1.75 ± 0.86	0.50 ± 0.73	0.000
Hardness of stool	1.71 ± 1.20	0.86 ± 1.03	0.012	1.73 ± 1.03	0.67 ± 1.11	0.006	1.38 ± 1.09	0.38 ± 0.62	0.005
Abdominal distension	1.21 ± 1.25	1.07 ± 0.92	0.547	0.47 ± 0.74	0.33 ± 0.62	0.000	0.81 ± 0.91	0.31 ± 0.60	0.027
Exertion of poop	2.36 ± 0.74	1.36 ± 1.22	0.010	2.40 ± 0.63	0.73 ± 0.88	0.334	1.88 ± 0.81	0.91 ± 0.85	0.011
Incompletion of poop	1.79 ± 0.97	1.43 ± 0.85	0.315	1.80 ± 1.26	0.87 ± 1.06	0.000	1.06 ± 1.06	0.38 ± 0.62	0.011
Anal bulge	1.43 ± 1.22	0.71 ± 0.91	0.117	1.27 ± 1.03	0.40 ± 0.83	0.005	0.88 ± 0.89	0.44 ± 0.73	0.089
Total scores	12.71 ± 3.63	7.64 ± 4.83	0.000	11.73 ± 2.69	4.33 ± 3.44	0.001	11.63 ± 4.15	3.63 ± 2.92	0.000

Table 9 Effective rates of three groups

Groups	Effective cases	Ineffective cases	Total cases	Effective rates / %
TD	15	1	16	93.75
MDNP	14	1	15	93.33
MDHP	10	4	14	71.43
Total	39	6	45	86.67

Table 10 Effects of three groups of drugs on each symptom

Difference before and after treatment (per day)	MDHP	MDNP	TD	P values
Time interval of poop	0.71 ± 1.26	0.80 ± 0.77	1.69 ± 0.70	0.010
Stool frequency	0.86 ± 1.40	0.87 ± 0.74	1.50 ± 0.73	0.132
Stool sense	0.43 ± 0.94	1.07 ± 0.88	1.25 ± 1.06	0.066
Hardness of stool	0.86 ± 1.10	1.07 ± 1.28	1.00 ± 1.21	0.892
Abdominal distension	0.14 ± 0.86	0.13 ± 0.52	0.50 ± 0.82	0.308
Exertion of poop	1.0 ± 1.24	1.67 ± 0.90	0.94 ± 1.29	0.171
Incompletion of poop	0.36 ± 1.28	0.93 ± 1.10	0.69 ± 0.95	0.383
Anal bulge	0.75 ± 1.53	0.87 ± 0.83	0.44 ± 0.96	0.583
Total scores	5.07 ± 2.95	7.40 ± 3.54	8.00 ± 4.70	0.126

4. Conclusions

In this paper, DCQD as a formula model, chemical components and therapeutic functions of traditional decoction are compared with machinery decoctions under normal and high pressure. Results show that there are some differences in chemical ingredients between traditional and machinery decoctions, but no statistic variations for pharmacological functions and clinical effects. While more materials and formula are required to be compared in order to judge the equivalence of traditional and machinery decoctions.

Acknowledgments

The authors wish to acknowledge Dr. Lin-lin Chen (Pharmacology, Yale University, USA) and You-hua Wang for their assistances in the preparation of this manuscript.

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