Phytomedicine 19 (2012) 1263–1269

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

# Danshensu is the major marker for the antioxidant and vasorelaxation effects of Danshen (*Salvia miltiorrhiza*) water-extracts produced by different heat water-extractions

Xuelin Zhou[a,1], Shun Wan Chan[b,1], Hui Ling Tseng[c,1], Yan Deng[a], Pui Man Hoi[c], Pou Seng Choi[c], Penelope M.Y. Or[a], Jia-ming Yang[d], Francis F.Y. Lam[a], Simon Ming Yuen Lee[c], George Pak Heng Leung[e], Siu Kai Kong[f], Ho Pui Ho[g], Yiu Wa Kwan[a,**], John H.K. Yeung[a,*]

[a] *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*
[b] *State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China*
[c] *Institute of Chinese Medical Sciences, University of Macau, Macao, China*
[d] *School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China*
[e] *Department of Pharmcology and Pharmacy, The University of Hong Kong, Hong Kong, China*
[f] *School of Life Sciences, Faculty of Sciences, The Chinese University of Hong Kong, Hong Kong, China*
[g] *Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong, Hong Kong, China*

ARTICLE INFO

*Keywords:*
*Salvia miltiorrhiza*
Phenolic acid
Tanshinones
Microwave water extraction
Antioxidant
Vascular relaxation

ABSTRACT

Some of the major components of Danshen (*Salvia miltiorrhiza*), a widely used Chinese herbal medicine rich in phenolic acids, are thermosensitive and may degrade to other phenolic acids during extractions with heating. The chemical profiles of Danshen water-extract may vary with different heat water extraction at different temperatures, affecting the composition and bioactivity of the extracts. In this study, six water-extracts of Danshen obtained from heat reflux water extraction and microwave-assisted extraction with water (MAE-W) at different temperatures were tested for their composition and pharmacological effects. Among these extracts, the third-round MAE-W (100 °C) extract had the highest phenolic acids and tanshinones contents, with the strongest antioxidant activity in 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay and ferric reducing/antioxidant potential (FRAP) assay. This extract also showed the strongest inhibitory effects on 2,2′-azobis-2-amidinopropane (AAPH)-induced hemolysis in human red blood cells, hydrogen peroxide-induced apoptosis in rat heart H9c2 cells and the highest relaxation effects on rat basilar artery. The antioxidant effects of Danshen water-extracts linearly correlated to their relaxation effects ($r = 0.895–0.977$). Through multiple linear regression analysis, danshensu was found to be the most significant marker in the antioxidant and vasodilation effects of Danshen water-extract, while tanshinone IIA as the marker on hydrogen peroxide-induced apoptosis in rat heart H9c2 cells. Danshensu is, therefore, a useful marker for the quality control of Danshen water-extracts in antioxidant and vasodilation, while tanshinone IIA for anti-apoptotic potential of different extracts.



## Introduction

Danshen, the dried root of *Salvia miltiorrhiza*, is a widely used Chinese herbal medicine in China, East Asia, European and USA. Over 35 formulations and concoctions containing Danshen as the principal drug or assistant drug are indexed in 2010 *Chinese Pharmacopoeia* (Comission 2010). Promoting blood circulation and removing blood stasis mainly contribute to the wide medicinal uses of Danshen, especially for its functions on cardiovascular and cerebrovascular systems (Zhou et al. 2012). Danshen is rich in water-soluble phenolic acids including protocatechuic aldehyde, protocatechuic acid, danshensu, rosmarinic acid, salvianolic acid B and other minor constituents, as well as about 40 diterpene quinones including four lipid-soluble diterpene quinones (tanshinone I, tanshinone IIA, cryptotanshinone and dihydrotanshinone) (Zhou 1993; Liu et al. 2007). The chemical structures of the major compounds isolated from Danshen are illustrated in Fig. 1. Phenolic acids and tanshinones have similar individual pharmacological effects such as antioxidant, anti-apoptosis and vasodilation,

* Corresponding author. Tel.: +852 39436864; fax: +852 26035139.
** Corresponding author. Tel.: +852 39436884.
*E-mail addresses:* yiuwakwan@cuhk.edu.hk (Y.W. Kwan), johnyeung@cuhk.edu.hk (J.H.K. Yeung).
[1] These authors contributed equally to this work.

0944-7113/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2012.08.011

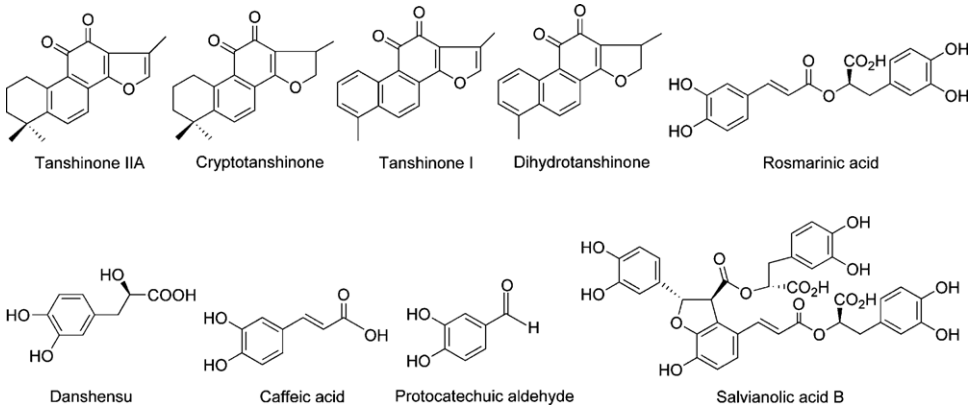


**Fig. 1.** Structures of major components in Danshen (*Salvia miltiorrhiza*) extract.

respectively (Zhou et al. 2005; Lam et al. 2007, 2008a; Wang et al. 2011a). The HPLC methods for chemical quality control of Danshen extracts are recommended in *Chinese Pharmacopoeia* with danshensu as the marker for the products obtained by single water extraction, and tanshinone IIA or salvianolic acid B for ethanolic extracts or a combination of an ethanolic extract and water extract from its ethanolic extraction residues (Zhou et al. 2012). The chemical compositions of Danshen preparations are critical for their pharmacological effects and medicinal usage.

The chemical compositions of herbal medicine may be affected by different growth conditions and extraction methods. It is therefore important to establish both chemical and bioactive markers during the extraction of herbal medicine. Some of major components in Danshen, like salvianolic acid B, are thermosensitive and degrade to form phenolic acids during heat water-extraction (Xintian and Haibin 2012). When heat water-extraction at different temperatures was used, the chemical profiles of Danshen water-extract would be different, leading to quantitative difference in the contents of the bioactive components and consequently different pharmacological effects. Some conventional extraction methods with water or solvent solution, including traditional heat boiling extraction (Chen et al. 2010), ultrasonic extraction (Yang et al. 2007; Wu et al. 2009) and microwave extraction (Pan et al. 2001; Fang et al. 2009), have shown different extraction yields of the major components, as well as other methods such as supercritical fluid extraction (Wang et al. 2008) and high-speed counter-current chromatography (Sun et al. 2011). However, the pharmacological effects of different Danshen extracts obtained from different extraction methods have not been investigated.

The aim of this study was to investigate how different extraction methods may affect the chemical profiles of different Danshen water-extracts. The pharmacological effects of the extracts including antioxidant, anti-apoptosis and vasodilation activities were investigated using phenolic acids/tanshinones as the bioactive markers. Different Danshen water-extracts were individually prepared from heat reflux water extraction (HRWE) and different microwave-assisted extraction with water (MAE-W) methods, whose chemical compositions were analyzed by a validated HPLC method. The pharmacological effects of the extracts were tested using different pharmacological models including 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay, ferric reducing/antioxidant potential (FRAP) assay, 2,2′-azobis-2-amidinopropane (AAPH)-induced hemolysis in human red blood cells (RBCs), hydrogen peroxide-induced apoptosis in rat heart H9c2 cells, and vasodilation effects on U46619-pre-contracted rat basilar artery. The relationships between the chemical profiles and pharmacological effects were analyzed by multiple linear regression method.

## Materials and methods

### *Materials and apparatus*

Dried root of Danshen herb was bought from Eu Yan Sang Co., Ltd. (Hong Kong, China). Authentic standards (purities more than 95%) of danshensu and salvianolic acid B, tanshinone I, tanshinone IIA, cryptotanshinone and dihydrotanshinone were supplied by Sichuan Chengdu Congon Biotech Co., Ltd. (China). HPLC grade acetonitrile and methanol were purchased from Labscan Analytical Sciences (Bangkok, Thailand). Other unspecified chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Pure water was produced by Milli-Q purification system (Millipore, MA). Microwave-assisted extraction (MAE) was performed with MAS-II microwave oven (Shanghai SINEO Microwave Co. Ltd., China), which can be fitted with a vacuum pump (WP61 22050, Millipore, USA) for vacuum MAE. Water-extracts were freeze-dried by Scanlaf Coolsafe 110-4 Freeze-dried machine (LaboGene, Denmark).

### *Extraction procedures*

50 g powder (40–120 mesh) of crude herbs was used each time for heat reflux water extraction (HRWE) and microwave-assisted extraction with water (WAE-W). For HRWE, the powder was boiled with 300 ml distilled water in a glass beaker by a hotplate at 100 °C for 2 h. For WAE-W, the powder was put into a round-bottom flask with 300 ml distilled water, and the extraction was performed with a microwave power of 300 W in the microwave oven connected with a condenser, under normal atmosphere at 100 °C or a negative pressure of −50 kPa at 50 and 75 °C for 2 h, respectively. After extraction, all the mixtures were centrifuged at 5000 × *g* for 20 min. The pellet was dried at 49 °C for at least 72 h, and reused for the next round of extraction; the supernatant was collected for freeze-dry, weighed for extraction yield, then ground into powder and stored in a dry and cold place in darkness. The extraction of MAE-W was carried out for three cycles with same crude herbs. The abbreviations of the samples produced by different methods are listed in Table 1.

**Table 1**
Abbreviations for the samples produced by different extraction methods.

| Sample abbreviations | Extraction methods |
|---|---|
| *HRWE* | Heat reflux water extraction at 100 °C |
| *MAE-W 100 (1)* | 1 st round MAE-W at 100 °C |
| *MAE-W 100 (2)* | 2nd round MAE-W at 100 °C |
| *MAE-W 100 (3)* | 3rd round MAE-W at 100 °C |
| *MAE-W 75* | MAE-W at 75 °C |
| *MAE-W 50* | MAE-W at 50 °C |

MAE-W: microwave-assisted extraction with water.