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The Traditional Processing Method Enriches the Anti-liver Fibrosis Active Compounds in *Salvia Miltiorrhiza*

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Abstract. Processing, an important concept in oriental medicine theory, is a way to prepare Chinese medicine according to its own characteristics and the usage for clinical treatment. *Salvia miltiorrhiza* (SM) has known as a potent anti-liver fibrosis medicine that is commonly processed by rice wine. In this study, SM was processed by different concentrations of ethanol compared to the one without processing and their anti-liver fibrosis activity were analyzed in a human hepatic stellate cells model. The concentrations of Cryptotanshinone, Tanshinone I and IIA, the active compounds in SM, are increased pronouncedly after 18% ethanol (rice wine) processing. In addition, the SM in Taiwan contains enriched active compounds than SM in China. In conclusion, SM processed by rice wine is a beneficial method for enriching the anti-liver fibrosis active compounds for the medicinal purposes.

Keywords: Anti-liver fibrosis, *Salvia miltiorrhiza*, Cryptotanshinone, Tanshinone I and IIA, Rice wine processing

1. Introduction

Processing, based on traditional concept and theory, is a way to prepare raw materials of Chinese medicine. However, the scientific rationale of processing still needs to be clarified in different aspects, such as chemistry, toxicity and bioactivity. *Salvia miltiorrhiza* (SM) has known to possess anti-liver fibrosis activity.[1,2] In this study, fresh roots of SM, grown in Taiwan, are harvested and further processed by rice wine [18% ethanol (EtOH)] according to the traditional processing method[3] and compared with 95% EtOH processing.

Liver fibrosis is one of the prevalent chronic liver diseases in the world and is caused by hepatic injury from virus, alcohol or metabolic disorders. Hepatic stellate cells (HSCs) play as key mediators in the progression of liver fibrosis. The activation of HSCs contributes to fibrogenesis and altered matrix degradation as a result of phenotypic changes of proliferation, contractility and chemokine signalling. Thus, the resolution of anti-liver fibrosis might be achieved through inhibiting HSCs activation or promoting apoptosis of activated HSCs.

2. Result and Discussion

Based on traditional method, the fresh roots of SM were processed with rice wine (18% EtOH, WPSM) and compared with 95% EtOH processing (EPSM). The chemical constituent analysis was identified by HPLC to evaluate the differences among two processed samples and fresh dry roots. In TLC and HPLC analysis, Cryptotanshinone, Tanshinone I and IIA, the active compounds of SM, were found to increase in WPSM but not in EPSM or fresh dry roots (Fig.1A, lane 3~5; Fig. 1B). As exemplified in Fig. 2, the contents of those active compounds of SM in Taiwan are increases three folds in WPSM compared to SM or

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EPSM. In addition, the active compounds are much more enriched from the SM in Taiwan than in China-A or –B (Fig. 2).

LX-2 cells, the human HSC cell line, are activated by transforming growth factor-beta 1 (TGF-β1) treatment to serve as the fibrosis cell model[4]. The up-regulation of α 1(I) pro-collagen, a fibrogenesis-related gene, responds to the activation of LX-2 cells (aLX-2) that is performed to evaluate the anti-liver fibrosis activity of compounds. As exemplified in Fig. 3, the SM in Taiwan processed by WPSM diminishes the mRNA level of collagen I, indicating that it possesses the anti-liver fibrosis activity. The vehicle control as well as other processed samples in China-A or –B show no significant influences on collagen I level compared to TGF-β control.

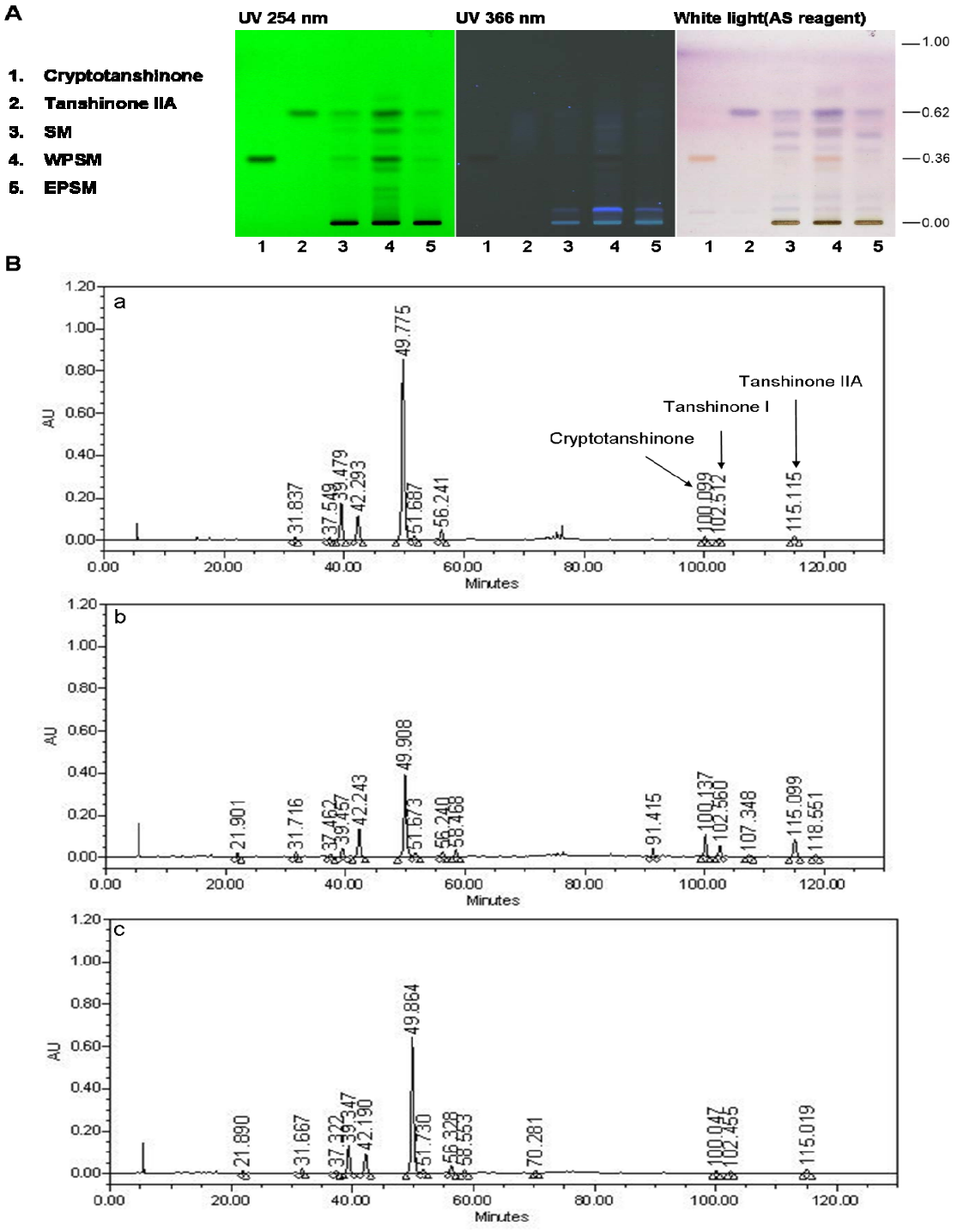

Fig. 1: The TLC and HPLC analysis of the fresh SM and two processed SM. A: the TLC profiles of Cryptotanshinone, Tanshinone IIA, SM, WPSM, and EPSM. B: the HPLC chromatograms of SM (a), WPSM (b), and EPSM (c). SM: fresh Salvia miltiorrhiza, WPSM: SM processed with rice wine, EPSM: SM processed with EtOH.

In conclusion, the contents of Cryptotanshinone, Tanshinone I and IIA are increased under rice wine processing condition. It suggests that the traditional processing method for SM is beneficial to its anti-liver fibrosis activity for clinical purposes. Those constituents may be considered as markers for the quality control of SM processing.

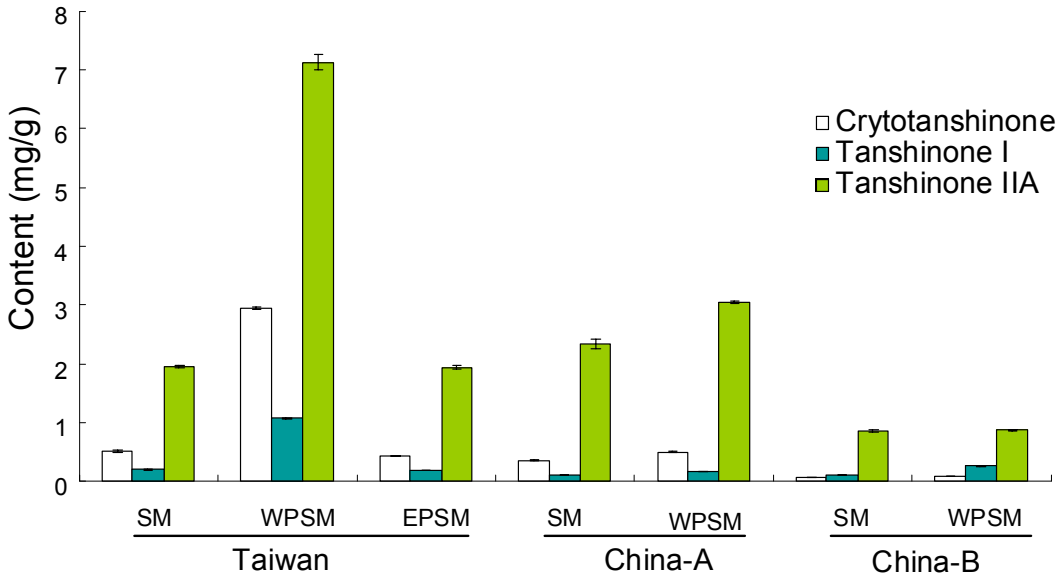

Fig. 2: The content analysis of SM from Taiwan, China-A and -B. Crytotanshinone, Tanshinone I and IIA from Taiwan are enriched in WPSM not in China-A and -B.

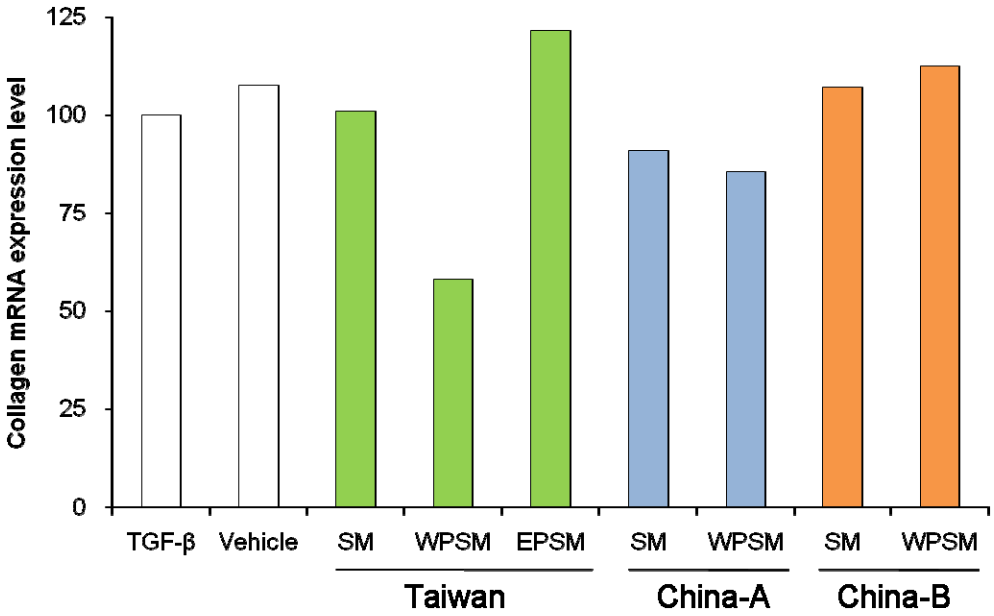

Fig. 3: The mRNA level of collagen I in response to different treatments. LX2 is activated by TGF-β treatment. Vehicle serves as a solvent control. The collagen I gene expression level of WPSM in Taiwan is significant decreased.

3. Acknowledgements

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