



Research Paper

Chemical and biological comparison of raw and vinegar-baked Radix Bupleuri

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ABSTRACT

Ethnopharmacological relevance: Radix Bupleuri (RB) is a commonly used herbal drug in Traditional Chinese Medicine (TCM), and it can be baked with vinegar to afford vinegar-baked Radix Bupleuri (VBRB), which is used in TCM for liver diseases treatment. In this study, the chemical compositions and biological effects between raw and two processed RBs by different vinegars were systematically compared.

Materials and methods: The chemical compositions of raw and two processed RBs were analyzed by ¹H NMR spectroscopy coupled with multivariate analysis. Two different extraction procedures were used, including direct extraction and liquid–liquid partition. Then HPLC was applied to determine the changes of saikosaponin contents. In addition, their liver protective effects against CCl₄ induced liver injury were also investigated, and the biochemical parameters and histopathology were measured after treatment of mice with raw RB and two processed RBs (5 g/kg/day) for 14 days.

Results: Multivariate analysis showed clear differences between the raw and the two processed RBs, and the vinegar-baking process induced elevated contents of ssb1, ssb2, acetic acid, malic acid, citric acid, 5-HMF, and ligustrazine, as well as the decreased contents of ssa, ssd, sucrose, glycine, succinic acid etc. In addition, both raw and processed RBs showed liver protective effects against CCl₄ induced liver injury, and the vinegar-baked RBs showed better effects than that of raw RB.

Conclusions: The raw and vinegar-baked RBs differed not only in the chemical compositions but also in the pharmacological effects. And two processed RBs also showed chemical differences, suggesting that the type of vinegar had an important effect on vinegar baking. In order to ensure the therapeutic effect and safety of TCM, the effect of different vinegars on processing of herbal drugs should be further studied.

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1. Introduction

Radix Bupleuri (RB), also named Chaihu in China, is derived from the roots of *Bupleurum chinense* DC. or *B. scorzonifolium* Willd. (Apiaceae) according to the Chinese Pharmacopoeia, and has been widely used to treat influenza, fever, inflammation, malaria and menstrual disorders (Committee for the Pharmacopoeia of People's Republic of China, 2010). Previous chemical studies on RB have suggested the presence of saponins, flavonoids, coumarins,

fatty acids, steroids, polysaccharides, and polyacetylenes (Huang et al., 2011; Lee et al., 2011).

The processing of herbal drugs has a long history in Traditional Chinese Medicine (TCM), and the importance of processing has been documented in the Huang Di Nei Jing for 2000 years. Depending on the therapeutic effect applications, the same herbal drug can be processed differently. When RB is mixed thoroughly with vinegar, and then baked to dry, it is changed to vinegar-baked Radix Bupleuri (VBRB). The surface of VBRB is light tan, with slight vinegar aroma

Abbreviations: RB, Radix Bupleuri; TCM, Traditional Chinese Medicine; VBRB, vinegar-baked Radix Bupleuri; S-VBRB, Shanxi vinegar-baked Radix Bupleuri; R-VBRB, rice vinegar-baked Radix Bupleuri; ssa, saikosaponin a; ssd, saikosaponin d; ssc, saikosaponin c; ssb1, saikosaponin b1; ssb2, saikosaponin b2; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MvDA, multivariate data analysis; PCA, principal component analysis; PLS-DA, Partial Least Squares Discriminant Analysis; OPLS-DA, Orthogonal Projection to Latent Structure with Discriminant Analysis; BMRB, Biological Magnetic Resonance Data Bank

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and tastes bitter, and it has better soothing liver and choleric effects (Zhao and Gao, 2013). The pharmacological effects and chemical compositions of RB will change after the vinegar-baking process. It was demonstrated that VBRB had a much stronger effect on acesodyne and bile secretion than that of the RB (Nie et al., 2002), and VBRB was extensively used in TCM for liver diseases treatment (Zhao et al., 2009, 2010). Previous reports showed that the contents of saikosaponin b1 (ssb1) and saikosaponin b2 (ssb2) were increased, while the saikosaponin a (ssa), saikosaponin c (ssc), and saikosaponin d (ssd) were decreased (Xu et al., 2012; Meng and Guo, 2013; Jiang et al., 2009) after the vinegar-baking process. However, synergistic effects of different components are vital for the therapeutic efficacy of phytomedicine (Williamson, 2001; Liu et al., 2007). It may be misleading to confine the analysis to limited metabolites for quality evaluations of TCM. Thus, a non-targeted chemical fingerprinting, which can cover a wide range of metabolites, is needed to reveal the processing effect on RB. According to Chinese Pharmacopoeia, RB should be baked with rice vinegar to give VBRB. However, there are many kinds of vinegars produced in China, and most of them were used in the vinegar-baking process of herbal drugs. The influence of different vinegars on VBRB remains unknown.

NMR based metabolic fingerprinting approach, has been proven to be a state of art technique in food and medicinal plants research. NMR has shown some distinct advantages, such as rapidity, non-selectiveness, reproducibility, and stability compared with GC–MS and LC–MS (Lindon et al., 2004; Tang and Wang, 2006). In addition, detailed structural information of metabolites, including chemical shifts and coupling constants, can be directly obtained. Recently, NMR based metabolic fingerprinting, in combination with multivariate data analysis (MvDA), has been successfully used to study the processing effect of several herbal drugs in TCM, such as Kansui (Tang et al., 2014), Rehmanniae Radix (He et al., 2014), Polygalae Radix (Wang et al., 2012).

The liver plays a pivotal role in metabolism and detoxification of endogenous and exogenous hepatotoxicants in the body, and these metabolic reaction can potentially lead to liver injury (Wang et al., 2014). Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins for inducing liver injury in experimental animal studies. CCl_4 causes hepatic injury through several pathway (Hung et al., 2006). Elevated lipid peroxidation due to increased free radical formation generated from CCl_4 is thought to be one of the mechanisms leading to hepatotoxicity (Shen et al., 2009). CCl_4 also causes the activation of immune systems through the infiltration of inflammatory cells to the site of injury. Immune cells may be responsible for the release of pro-inflammatory cytokines such as IL-6 and TNF- α , which further enhance hepatotoxicity through repeated cycle of inflammation (Al-Harbi et al., 2014).

In this study, two different vinegars (rice vinegar and Shanxi vinegar) were used to prepare VBRB. Then the chemical difference between raw and two processed RBs were compared by ^1H NMR based fingerprinting approach. In addition, their liver protective effects were also investigated using CCl_4 induced liver injury animal models.

2. Materials and methods

2.1. Plant materials

The Radix Bupleuri was purchased from Shanxi Weikangtang Chinese herbal pieces limited company and authenticated by Prof. Xue-Mei Qin as *Bupleurum chinense* DC. A voucher specimen (Lot no. CH-46) was deposited at Modern Research Center for Traditional Chinese Medicine of Shanxi University.

2.2. Solvents and chemicals

Analytical grade methanol, chloroform, ethyl acetate (EtOAc), petroleum ether, n-butanol (n-BuOH) and CCl_4 were purchased from Beijing Chemical Works (Beijing, China). Rice vinegar was bought from Tongwanzhenji Food Company (Hebei, China) and Shanxi vinegar was from Shanxi Donghu Vinegar Group (Shanxi, China). Deuterated chloroform (CDCl_3 , 99.8%D) containing tetramethylsilane (TMS, 0.03%, m/v) and methanol- d_4 (99.8% D) were obtained from Merck (Darmstadt, Germany). D_2O was bought from Norell (Landisville, USA). Sodium 3-trimethylsilyl [2, 2, 3, 3- d_4] propionate (TSP) was from Cambridge Isotope Laboratories Inc. (Andover, MA), and NaOD was purchased from Armar (Dottingen, Switzerland). Ssa, ssd, ssb1, ssb2 were purchased from Aladdin Industrial Corporation (purity > 98%, Shanghai, China). Chromatographic acetonitrile was bought from Fisher Scientific (Thermo Fisher, USA).

2.3. VBRB preparation

According to Chinese Pharmacopoeia, the crude RB (100 g) was incubated with Shanxi vinegar or rice vinegar (20 g), respectively. Then the material was dried by stir-firing to obtain S-VBRB (by Shanxi vinegar) or R-VBRB (by rice vinegar) after vinegar was totally absorbed into raw RB. Six different batches of S-VBRB and R-VBRB were prepared with each kind of vinegar.

2.4. ^1H NMR based metabolomic profiling

2.4.1. Extraction of plant samples

Two different extraction procedures were used in this study. In the first procedure (M1), dry material powder (200 mg) was transferred into 10 mL glass centrifuge tube. 3 mL of 50% methanol and 3 mL of chloroform were added to the tube followed by vortexing for 1 min and ultrasonication for 20 min. The mixture was then centrifuged at 3500 rpm for 25 min. The chloroform

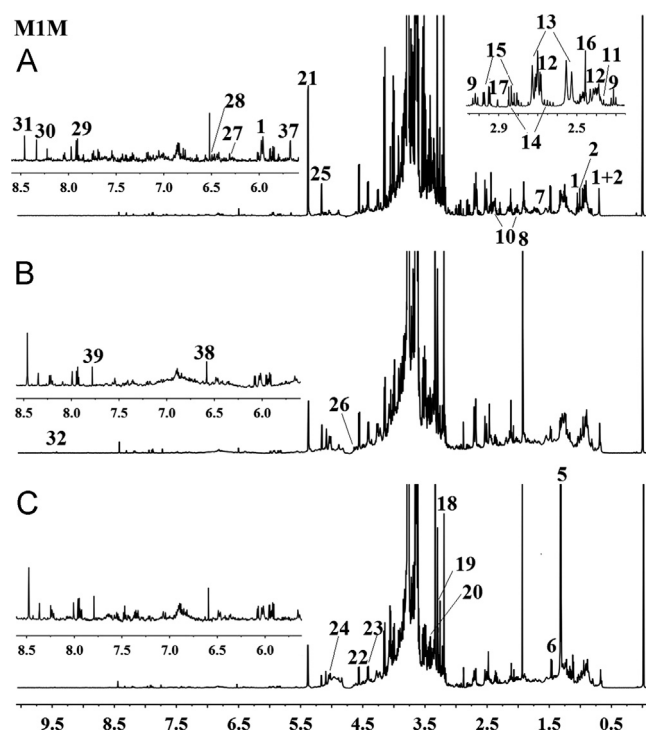


Fig. 1. Representative ^1H NMR spectrum of RB (A), S-VBRB (B) and R-VBRB (C) of M1M (the aqueous methanol extracts in M1 procedure).

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