

Application of a strategy based on metabolomics guided promoting blood circulation bioactivity compounds screening of vinegar-Institution of Basic Theory-China Academy of Chinese Medical Sciences- China

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Abstract

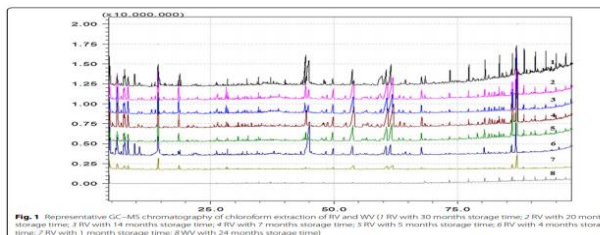
Vinegar has been adopted as favoring dating from around 3000 BC in Asian, European and other traditional cuisines of the world .As evidences accumulated, vinegar was proved to exhibit therapeutic properties, including blood pressure reduction , antioxidant activity , antibacterial activity , reduction in the effects of diabetes and prevention of cardiovascular disease . It is also used as a kind of accessory documented in Lei's treatise on processing of drugs (LeigongpaozhiLun) (618–907 AD). Numerous Chinese medicines such as Frankincense, Rhizoma Corydalis were believed to enhance the promoting blood circulation therapeutic efficiency after preparation by vinegar . Fruitful researches have been carried on the herbal enhancement of therapeutic efficiency after processing ,but there are few relative reports concerning the blood circulation bioactivity of vinegar Vinegar accumulate an overwhelming variety of metabolites that play nondeductible roles in health benefit. During recent years, many studies employed GC–MS technique for quality control and determination of vinegar. Alcohols, organic acids, amino acids, carbohydrates, esters and various micro-constituents were proved to present in vinegar [8]. The previous results showed that the contents of most conventional ingredients (organic acids, free amino acids, carbohydrates) were increased during aging process. Tetramethylpyrazine (TMPZ), a kind of alkaloid metabolites yielding during aging process of vinegar, was used in clinical trials

since the 1970s [9]. Reports indicate that TMPZ reduces arterial resistance and increases coronary and cerebral blood flow . A number of alkaloid metabolites are developed as clinical drugs found to have significant biological activities (e.g berberine and paclitaxel) . Hence variation of alkaloid metabolites should not be overlooked for their exhibit notable function properties.

Rice vinegar (RV) and white vinegar (WV) as daily favoring, have also used as accessory in traditional Chinese medicine processing. As we know, the promoting blood circulation efficiency could be enhanced when herbs processed by vinegar. Number of reports focused on health benefits derived by consumption of vinegar. However, few concerned the blood circulation bioactivity.

In this paper, a metabolomics guided strategy was proposed to elaborate on the chemical constituents' variation of two kinds of vinegar. GC–MS coupled with multivariate statistical analysis were conducted to analyze the chemical components in RV and WV and discriminate these two kinds of vinegar. The anti-platelet activities in vitro were investigated by whole blood aggregometry platelet test. And the anticoagulant activities were monitored by the whole blood viscosity, plasma viscosity, packed cell volume, prothrombin time, and four coagulation tests (PT, TT, APTT, FIB) in vivo .Results were found by keen observation of GC–MS conditions Chromatographic parameters such as column type, carrier gas flow, temperature rate, and ion source temperature were adjusted to be able to obtain the best separation for the

compounds. The Rxi-50 capillary column obtained the best separation. The carrier gas at flow rate of 1.0 mL/min and the 250 °C ion source temperature were proved to be the most suitable. Established chromatographic conditions and mass spectra conditions are listed in “Gas chromatography–mass spectrometry analysis”. Metabolic profiles of RV and WV Five batches of WV and RV with aging time of 1, 4, 5, 7, 14, 20, 30 months were analyzed. Representative GC–MS fingerprints are presented in Fig. 1. A total of 53 compounds were detected, including different kinds of alcohol, organic acids, amino acids, aldehydes, phenols, ketones, heterocyclic, which were same as those reported in literatures [1, 20]. PCA and OPLS-DA were utilized to classify the metabolic phenotypes and identify the differentiating metabolites. A PCA score plot for first and second principal components was utilized to depict the general variation among the samples of two dosage forms ($R^2 X = 0.78, Q^2 = 0.987$).



Conclusions, In this work, a strategy of bioactivity compounds screening based on metabolomics guided was established. The chemical analysis and multivariate statistical analysis were conducted for classification of RV and WV. Constituents of RV and WV were globally characterized by GC–MS and 33 potential biomarkers were identified. Alkaloid metabolites were proved to be the main compounds contributing to discrimination of two kinds of vinegar and verified only in RV. TMPZ, dihydroergotamine, harmine and 1,2,3,4-tetrahydroharmine were screened and the contents of the four potential biomarkers increased with aging time by semi-quantitative analysis and trends of time-series analysis.

With the guidance of metabolomics research, alkaloid metabolites were isolated. The anti-platelet in vitro confirmed an effect of RV and its alkaloids metabolites preliminarily. RV and its alkaloids metabolites further were endowed with in vivo by monitoring WBV, PV, ESR, PCV, and four coagulation tests. WV failed to exhibit the effect of promoting blood circulation. Both the tests of bioactivity in vitro and in vivo are validated the results of metabolomics research. Promoting blood circulation activity of RV may make it to assist the several promoting blood circulation therapeutic efficiency of traditional Chinese medicines after processing. Compared with the traditional isolation and purification method, the established strategy combined of metabolomics and bioactivity screening we proposed should . a Effects of RV and WV on antiplatelet in vitro (1 Aspirin group; 2 2–3 year aging processed RV group; 3 1–2 year aging processed RV group; 4 0–1 year aging processed RV group; 5 WV group; 6 Control group); b alkaloids metabolites of different vinegar inhibition of AA induced platelet aggregation in vitro (1 Aspirin group; 2 Alkaloids metabolites of 2–3 year aging processed RV; 3 Alkaloids metabolites of 1–2 year aging processed RV; 4 Alkaloids metabolites of 0–1 year aging processed RV; 5 Alkaloids metabolites of WV; 6 Control group); c AUC value of different vinegars inhibition of ADP induced platelet aggregation in vitro; d AUC value of alkaloids metabolites of different vinegar inhibition of AA induced platelet aggregation in vitro. ‘*’ and ‘***’, $p < 0.05$ and $p < 0.01$ respectively, comparison with the normal control group .Valid the promoting blood circulation activity of vinegars in vivo Data represent mean \pm SD $n = 8$ N Normal group, M Model group, W WV group, RL RV low dosage group, RH RV high dosage group, AERO Alkaloid extraction of RV * p

Biography

Zhenli Liu has her expertise in “The quality control of Chinese herbal medicine using analytical methods and in separating active ingredients from herbal medicine using chemical separation methods”. She has published more than 100 journal articles in both Chinese and English.

Note: This work is partly presented at 4th World Congress on Chromatography during August on 07-09, 2017 held at Rome, Italy