Isolation and identification of the constituents absorbed into blood of rats after oral administration of *Citrus aurantium* in Dachengqi decotion

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**ABSTRACT**

To isolate and identify the constituents absorbed into blood of rats after oral administration of *citrus aurantium* in Dachengqi Decotion (DT) based on the preliminary research of Serum Pharmacochemistry of DT. The isolation and purification of constituents were performed on silica gel column based on serum fingerprint profiles, their retention time and UV absorption spectra, and the structures of constituents were identified according to relative spectral data. The two kinds of constituents absorbed into blood from *citrus aurantium* in DT were identified as Naringenin and 1H-Pyrrole-2-carboxylic acid (Minaline). Both of them are the original ingredients from *citrus aurantium*. Serum pharmaco-chemistry was introduced to determine the bioactive constituents of Chinese medicine, and the constituents absorbed into blood after oral administration of *citrus aurantium* in DT were systematically isolated and identified.

**Keywords:** *Citrus aurantium*; Constituents absorbed into blood; Isolation and identification; Serum pharmacochemistry

**INTRODUCTION**

*Citrus aurantium*, dry young fruit of *citrus aurantium* L. and *Citrus sinensis* Osbeck, is an important part of Dachengqi decotion (DT). Learn from the literatures, it mainly contains volatile oil, flavonoids, coumarins, a small amount of alkaloids and other components[1]. It is generally believed that the medicines should be absorbed into the blood circulation before they play the role of biological activity (drugs for the direct role of gastrointestinal and external use are excluded)[2]. In preliminary study, the HPLC method was used to identify those blood components’ source. Chromatographic behavior in vivo and vitro of DT, DT without *citrus aurantium* and *citrus aurantium* were contrasted. Based on the comparison of retention time and UV absorption spectra, 13 ingredients in the serum after administration of DT from DT were identified, which included two components of the prototype components from *citrus aurantium*[3].

**EXPERIMENTALSECTION**

**2.1 Materials**

*Citrus aurantium* L. (Lot number 0808044), purchased from Chengdu Xinhehua Drug Company (Chengdu,China) and authenticated by Prof. Liangke Song of the Pharmacognosy Department, School of Life science & Engineering of Southwest Jiaotong University. Acetonitrile and methanol (HPLC grade), purchased from FISHER Technology Inc.(USA). Analytical grade phosphoric acid, purchased from Chengdu Jinshan Chemical Company (Chengdu, China). Wahaha purified water.

**2.2 Instrument**

Shimadzu LC-10AT system which was equipped with a LC-6AD solvent delivery, SPD-M10Avp diode array detector
(DAD) and CTO-10Asvp Column Oven. System control and data-analysis were carried out by Class-vp software (Version 6.1, Shimadzu, JP). The chromatographic separation of samples was achieved by a reversed-phase HPLC column (AKZO NOBEL Kromasil C18, 250mm×4.6mm, 5um, Sweden) protected by a pre-column (Chromguard C18 column, Shimadzu, JP), 0.45µm microporous membrane. Sartorius BS224S electronic analytical balance (Beijing Sartorius Instrument Systems, Inc.). Flash-type extractor JHBE-50 (Technology Development Co., Ltd. Henan gold-nie). Silica gel H(200-300 mesh) and thin silica gel plates (TLC, GF254) were produced by Qingdao marine Chemical Factory.

2.3 Methods
In this experiment, the isolation and purification of constituents were performed on silica gel column based on serum fingerprint profiles and their retention time and UV absorption spectra, and the structures of constituents were identified according to relative spectral data.

RESULTS AND DISCUSSION

3.1 Determination of the constituents absorbed into blood of citrus aurantium
In our preliminary study, serum samples after administration of DT, DT without citrus aurantium and citrus aurantium were prepared and analysed by HPLC, and the results were shown in Fig1. It indicated that the No.1,2,3,4 peaks were constituents absorbed into blood from citrus aurantium. 1, 2 peaks have no significant absorption in the chromatogram of crude drugs in vitro, but significant absorption in the chromatogram of serum samples after administration of citrus aurantium. It shows that components 1 and 2 may be the metabolites after oral administration of citrus aurantium. Peaks 3 and 4 absorbed into blood and peaks 3 and 4 in crude drug of citrus aurantium have the same retention time and UV absorption spectra (Fig2). It indicated that 3 and 4 are original constituents absorbed into blood from citrus aurantium. In this study, compounds 3 and 4 will be isolated from extract of citrus aurantium and their structures will be identified.
3.2 Isolation of the constituents absorbed into blood of *citrus aurantium*

4Kg Citrus meal was extracted by Flash-type extractor with anhydrous ethanol. The total extract was concentrated and extracted by petroleum ether, chloroform, ethyl acetate and n-butanol in turn. Then the parts with target components were screened by using HPLC. After analysis of HPLC, the target compounds were concentrated in the ethyl acetate part.

Ethyl acetate extract was dissolved in a small amount of methanol, adsorbed with the amount of silica gel, dried, levigated, and set on a silica gel column. Gradient elution chromatography with dichloromethane - methanol(200:1, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, pure methanol) under the HPLC trace detection was applied. By HPLC screening, the target components were concentrated in dichloromethane- methanol (200:1) and (100:1) elution part. These two parts were collected, and then gradient elution and column chromatography with petroleum ether (60~90 °C)-acetic acid acetate (15:1) was applied, and compounds A and B were achieved.

After analysis of HPLC, according to the retention time and UV absorption spectra, compounds A and B are confirmed to be constituents absorbed into blood from *citrus aurantium* of DT, and also the peak 3 and 4 in Fig 1.

3.3 Identification of the Constituents Absorbed into Blood of *citrus aurantium*

1H-NMR, 13C-NMR and MS spectra of Compound A and Compound B were detected, the results are as follows.

**Compound A:** C_{5}H_{5}NO_{2}, white needle-like crystals. ESI-MS m/z (%): 112 [M+1]+ (40); 1H NMR (600 MHz, DMSO) δ: 11.64 (br s, N-H ), 6.93 (1H, m, H-5), 6.70 (1H, m, H-3), 6.11 (1H, m, H-4); 13C NMR (150 MHz, DMSO) δ: 162.3 (s, -COOH), 123.8 (d, C-5), 123.4 (s, C-2), 115.1 (d, C-3), 109.7 (d, C-4). Consistent with the literature [4], so the compound A was identified as 1H-Pyrrole-2-carboxylic acid. Analyzed under the HPLC conditions established in our preliminary study, the chromatographic behavior on HPLC and UV spectral data of Compound A was same to peak 3, so compound A was confirmed to be constituent absorbed into blood of *citrus aurantium*.

**Compound B:** C_{15}H_{12}O_{5}, yellow powder. ESI-MS m/z (%): 295 [M+Na]+ (45), 273 [M+1]+ (100), 258 (15); 1H NMR (400 MHz, CD3OD) δ: 7.30 (2H, d, J = 8.2 Hz, H-2',-6'), 6.80 (2H, d, J = 8.2 Hz, H-3', -5'), 5.89 (1H, d, J = 2.4 Hz, H-8), 5.87 (1H, d, J = 2.0 Hz, H-6), 5.33 (1H, dd, J = 12.8, 2.8 Hz, H-2), 3.11 (1H, dd, J = 17.2, 13.2 Hz, H-3a), 2.68 (1H, dd, J = 17.2, 3.2 Hz, H-3b). Consistent with the literature [5], so the compound B was identified as Naringenin. Analyzed under the HPLC conditions established in our preliminary study, the chromatographic behavior on HPLC and UV spectral data of Compound B was same to peak 4, so compound B was confirmed to be constituent absorbed into blood of citrus aurantium.

**CONCLUSION**

Guided with "Chinese Serum pharmaco-chemistry", this paper prepared and isolated the two constituents absorbed into blood of *citrus aurantium* of DT directionally. These two constituents are identified as pyrrole-2-carboxylic acid and naringenin, which are original ingredients from *citrus aurantium* absorbed into the blood directly. Pyrrole-2-carboxylic acid is isolated from *citrus aurantium* for the first time, and detected as the constituent absorbed into blood, which lays the foundation for the further study of the efficacy and pharmacology of these two compounds.

When it comes to the identification of the crude drug origin of constituents absorbed into blood, it is carried out
under condition of the the UV identification; however, some copositons of *citrus aurantium* may not have UV absorption, which may be the reason why only two constituents absorbed into blood are detected. In addition, due to the complexity of Chinese herbal medicinal ingredients, whether some of them can reactivate after the metabolism in vivo waits for future study.

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