Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):478-481

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Isolation and identification of the constituents absorbed in rat serum of *Citrus aurantium* L. after oral administration of dachengqi decoction

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ABSTRACT

To isolate and identify the prototype constituents absorbed in rat serum of citrus aurantium L. after oral administration of Dachengqi Decoction (DT) based on the preliminary research of Serum Pharmacochemistry of DT. Extracts of citrus aurantium L. were isolated and purified targeted by silica gel chromatography based on serum fingerprints. The prototype constituents absorbed in rat serum of citrus aurantium L. were screening and determined according to their retention time and UV absorption spectra and their structures were identified according to relevant spectra data. Two original ingredients absorbed in blood of citrus aurantium L. from Dachengqi Decoction were isolated and identified as Naringenin and 1H-Pyrrole-2-carboxylic acid (Minaline). The original ingredients absorbed in rat serum of citrus aurantium L. after oral administration of Dachengqi Decoction were isolated and identified successfully by introducing Serum Pharmacochemistry into the field of compound separation and preparation.

Keywords: *citrus aurantium L.*; constituents absorbed in rat serum; isolation and identification; Serum pharmacochemistry

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INTRODUCTION

Citrus aurantium L. is dry young fruit of *citrus aurantium L.* and its variants or *Citrus sinensis Osbeck.* which is an important part of Dachengqi decoction (DT). Learned from literatures, it mainly contains volatile oil, flavonoids, coumarins, a small amount of alkaloids and other components (Z Hong, S Ming-jiang, W Ling. Journal of Chinese Medicinal Material.2009, 32（11）:1787-1789).

It is generally believed that medicines should be absorbed in blood before they play the role of biological activity (drugs for gastrointestinal direct acting and external use are excluded (W Xi-jun. China J Chin Mater Med. 2006,31（10）:789-792). 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. And two of these 13 ingredients were the original ingredients from *citrus aurantium L.* (X Ying-sheng. Master's degree thesis of Southwest Jiaotong University,2010).

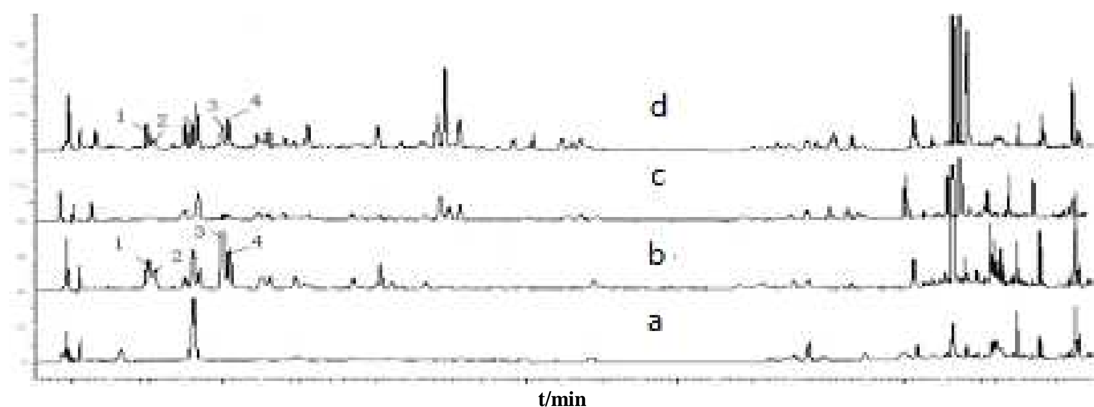
In this study, extracts of *citrus aurantium L.* were isolated and purified targeted by silica gel chromatography based on serum fingerprints. The prototype constituents absorbed in blood of *citrus aurantium L.* were screening and determined according to their retention time and UV absorption spectra and their structures were identified according to relevant spectra data.

EXPERIMENTAL SECTION

2.1. Instrumentals
Shimadzu LC-10AT system 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) which was 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.

2.2. Medicine and Reagents
Citrus aurantium L. (Lot number 0808044) was purchased from Chengdu Xinhehua Drug Company (Sichuan Chengdu, China) and authenticated by Prof. Liangke Song who works in the Pharmacognosy Department, School of Life science and Engineering, Southwest Jiaotong University. Acetonitrile and methanol (chromatographic grade) were purchased from FISHER Technology Inc.(USA). Analytical grade phosphoric acid was purchased from Chengdu Jinshan Chemical Company (Sichuan Chengdu, China).Wahaha purified water.

3.Determination of the constituents absorbed in rat serum of citrus aurantium L.
In our preliminary study, serum samples after oral administration of DT, DT without *citrus aurantium L.* and *citrus aurantium L.* were prepared and analysed by HPLC, and the results were shown in Figure1[3]. It indicates that No.1,2,3,4 peaks are constituents migrating in blood from *citrus aurantium L.* 1,2 peaks have no significant absorption in the chromatogram of crude drugs in vitro, but have significant absorption in the chromatogram of serum samples after oral administration of *citrus aurantium L.* It shows that components 1 and 2 may be the metabolites after oral administration of *citrus aurantium L.* Peaks 3 and 4 in the chromatogram of serum samples after administration of *citrus aurantium L.* and peaks 3 and 4 in the chromatogram of *citrus aurantium L.* crude drug have the same retention time and UV absorption spectra (Figure2). It indicates that 3 and 4 are original constituents absorbed in blood from *citrus aurantium L.* This study is to isolate compounds 3 and 4 from extract of *citrus aurantium L.* and identify their structures.



t/min

Fig 1. a.The chromatogram of blank serum
b.The chromatogram of serum after oral administration of *citrus aurantium*
c.The chromatogram of serum after oral administration of DT Decoction without *Citrus aurantium*
d.The chromatogram of serum after oral administration of DT Decoction

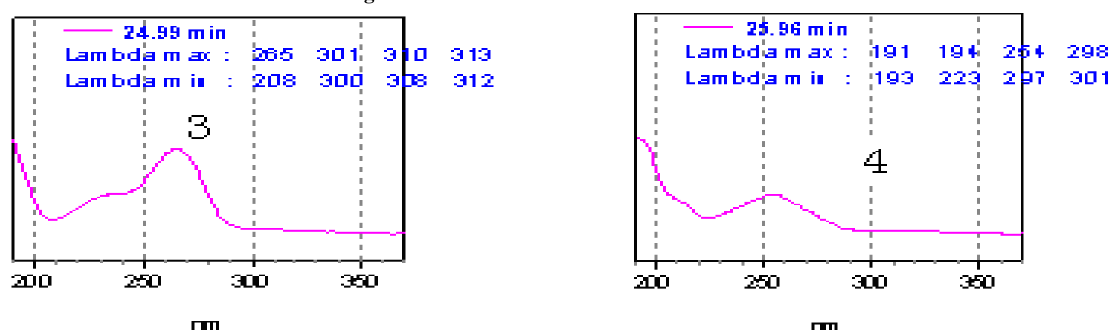


Fig 2. UV absorption spectra of peaks 3 and 4

4. Isolation of the constituents absorbed in blood of *citrus aurantium L.*

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

Ethyl acetate extract was dissolved in a spot of methanol, then adsorbed with appropriate amount of silica gel , dried, grinded, 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. The target components were concentrated in dichloromethane- methanol (200:1) and (100:1) elution parts by HPLC screening. These two parts were collected, and compounds A and B were obtained by silica gel column chromatography with petroleum ether (60 ~ 90 □)-ethyl acetate (15:1) .

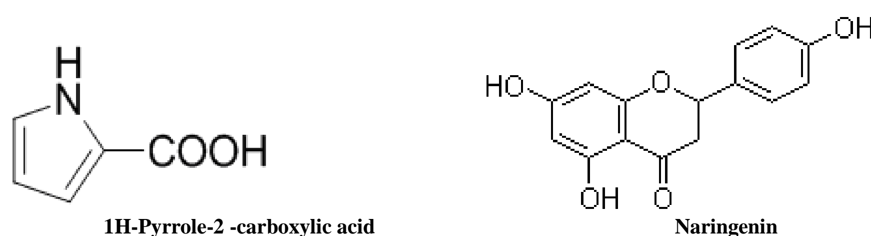
After analysis of HPLC, according to the retention time and UV absorption spectra, compounds A and B were confirmed as constituents absorbed in blood of *citrus aurantium L.* from DT, which also were the constituents that peaks 3 and 4 in Figure 1 stand for.

5.Identification of the Constituents Absorbed In Blood of citrus aurantium L.

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

Compound A: $C_5H_5NO_2$, white acicular crystals. ESI-MS m/z (%): 112 $[M+1]^+$ (40); ^{1}H 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)； ^{13}C 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). Compound A was identified as 1H-Pyrrole-2 -carboxylic acid which was in agreement with literatures (T Ning-hua, Z Shou-xun, C Chang-xiang, et a1 .Acta Botanica Yunnanica.1991,13(4)：440). Analysed 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 as constituent absorbed into blood of *citrus aurantium L.*

Compound B:$C_{15}H_{12}O_5$, faint yellow powder. ESI-MS m/z (%): 295 $[M+Na]^+$ (45), 273 $[M+1]^+$ (100), 258 (15); ^{1}H NMR (400 MHz, CD_3OD) 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). Compound B was identified as Naringenin which was in agreement with literatures (Q Lei,Y Jiu-zhi,C Hai-yan,et a1. Journal of Chinese Medicinal Material.2007,30（10）:1242-1244.). Analysed 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 as constituent absorbed into blood of *citrus aurantium L.*



1H-Pyrrole-2 -carboxylic acid **Naringenin**

RESULTS AND DISCUSSION

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

In our preliminary study, the crude drug origin of constituents absorbed in blood was identified only by UV. However, some compositions of *citrus aurantium L.* may not have UV absorption, which may be the reason why only two constituents absorbed in blood were detected. In addition, due to the complexity of Chinese herbal medicinal ingredients, whether some of them can generate activity after the metabolism in vivo waits for futher study.

Acknowledgments
This research was supported by National Natural Science Fund Project in China (No.30873365).The funders had no Involvement in the preparation or writing up of this research.

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