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Flavonoids of Fructus aurantii processed using the Zhangband system.

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Abstract

Background: To analyse the flavonoid components of Fructus Aurantii processed using the Zhangband method. Fructus Aurantii, the dried immature fruit of *Citrus aurantium* L., achieves its highest medicinal efficacy using the Zhangband method of processing, a specialized system of traditional Chinese medicine developed in Jiangxi Province, China. The flavonoids responsible for the medicinal qualities have never been analysed.
Methods: Fructus aurantii was processed according to traditional methods. Flavonoids were isolated by chromatography and identified spectrophotometrically.
Results: Four principal flavonoids were isolated: naringin (I), hesperindin (II), neohesperindin (III), and hesperetin 7-O-β-D-glucopyranoside (IV).
Conclusions: Hesperetin 7-O-β-D-glucopyranoside was newly identified in Fructus Aurantii; the compound was present in Fructus aurantii after, but not before, processing using the Zhangband method. Processing appears to attenuate the drying characteristics of Fructus aurantii and to enhance its medicinal efficacy.

Keywords: Fructus aurantii, Processing, Chemical components, Zhangband method, Flavonoids, Chinese medicine.

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Introduction

Fructus Aurantii (FA), the dried immature fruit of *Citrus aurantium* L. and cultivars thereof, is a medicinal herb produced locally in Jiangxi Province, China. The fruit is bitter, sour, and pungent, and the processed herb has a warming effect on the body; FA acts on the spleen, stomach, and large intestine to regulate and activate Qi and relieve distention. Clinically, FA is generally used to treat Qi stagnation in the chest, intestinal distention and pain, dyspepsia, phlegm and fluid retention, gastroptosis, uterine prolapse, and rectocele. In recent years, FA has been found to be effective in the treatment of gallstones [1], cancer [2], anxiety [3], diabetes [4], and obesity [5], and thus shows a broad range of applications. Fructus Aurantii contains principally alkaloids, flavonoids, and volatile oils, with the flavonoids being the most important ingredients medically. In addition, FA contains smaller amounts of coumarins, tannins, saccharides, organic acids, and microelements.

Medicinal herbs, including those derived from FA, are abundant in Jiangxi Province, and the region has a long history of involvement in traditional Chinese medicine. The famous herb-processing system known as the Zhangband system was established in the region of Zhangshu, and it is said that the medicinal herbs processed in Zhangshu using the Zhangband system are particularly powerful and efficacious. The Chinese herbs processed by the Zhangband method are characterized by unique colours and remarkable levels of efficacy, and they are famous worldwide. Details of the Zhangband method, which is unique to Jiangxi Province, are known to only a small number of advanced herbal technicians who are considered as masters of the technique. Moreover, the long-term survival of the system is uncertain, as it depends on the passage of the techniques from one generation to the next. In addition, no systematic study of the herbs using modern methodologies has yet been conducted, and quality control of processed herbs is based solely on the experience of the herbal technicians.

After Zhangband processing, the skin of FA is green, and the fruit contains a thick pulp that is whitish in colour and has a strong flavour and a small pulp sac in the shape of a chrysanthemum. The fruit is of high quality and is very efficacious, being the best form of FA available. In this study, we compared the FA materials before and after Zhangband processing using high-performance liquid chromatography (Figure 1), which showed that two new peaks (peaks 5 and 6, Figure 2) developed during processing. We identified the substance of peak 6 as hesperetin7-O-β-D-glucopyranoside.

Materials and Methods

Instruments and reagents

Samples were analysed using: a rotary evaporator (model RF-52; Shanghai Ya-rong Biochemical Instruments); an X-4

digital melting point microdetector (Beijing Tech Instrument Co. Ltd.); a magnetic resonance spectrometer (Bruker av400; Switzerland); D101 large-pore adsorbent resin (Cangzhou Bon Adsorber Technology Co. Ltd., catalogue no. 080912); 200-300 mesh silica gel for column chromatography (Qingdao Yinhai Chemicals); 60-90 mesh polyamide 20080331 (Yangyu, Hengjie Town, Lu-qiao District, Taizhou City, Zhejiang Province); silica gel plates with dimensions of 100 × 100 mm, 100 × 200 mm, and 100 × 50 mm (Qingdao Yinhai Chemical Co.); 10 × 20 cm of polyamide film (Taizhou Lu-qiao Sijia Biochemical Plastics). All reagents were analytically pure.

Herbal materials

Fructus Aurantii material was purchased from Jiangxi Tianqitang Pharmaceuticals Co. Ltd. and was confirmed to be the dried immature fruit of *Citrus aurantium* L. by Professor Liu Qing-hua of the Chinese Herb Identification Laboratory, Jiangxi University of Traditional Chinese Medicine, Jiangxi, China.

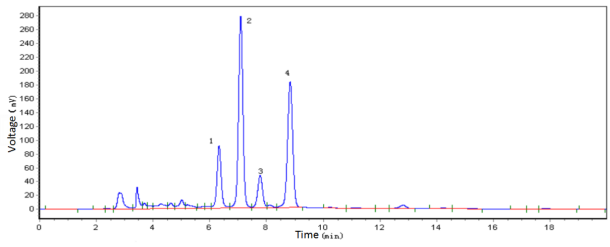

Figure 1. *High-performance liquid chromatography plot of flavonoids from unprocessed Fructus aurantii.*

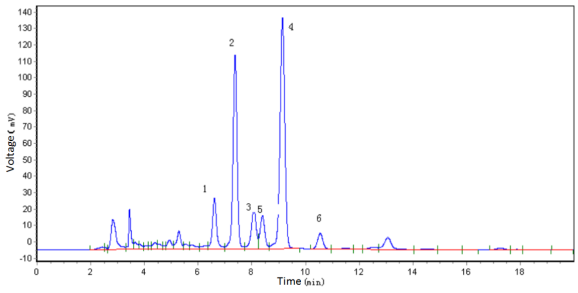

Figure 2. *High-performance liquid chromatography plot of Fructus aurantii flavonoids after Zhangband processing: 2, naringin; 3, hesperindin; 4, neohesperindin; 6, hesperetin 7-O-β-D-glucopyranoside.*

Zhangband processing of FA

Pulp was removed from FA using a knife, and was washed with water to remove sand and mud. The pulp was collected and moistened overnight (12 h), flattened using an iron weight, and subject to further pressure using a specially designed frame for 3-5 days. When mould spots started to develop, the pressed cake was folded in half and sliced into pieces in the shape of a "phoenix eye"; the pieces were then sun dried. Next, wheat bran was placed into a pot and, when whitish smoke arose upon heating, dried FA pieces were added and stirred continuously until they became light yellow in colour. The FA pieces were then removed from the pot, allowed to cool, and the scorched bran was mesh-filtered. The filtrate was added to the cooled FA pieces. A total of 5-10 kg of wheat bran was used to process each 100 kg batch of FA pieces.

Extraction and isolation

Pieces of FA (mass, 2 kg) were coarsely ground and extractions were performed twice, for 2 h each time, using 70% (v/v) ethanol at a ratio of 1:8 FA:ethanol (v/v). The extracts were combined and evaporated to dryness under reduced pressure. The dried powders were dissolved in water, absorbed into D-101 large-pore resin, and eluted with water to remove soluble impurities; elutions were performed sequentially using 10%, 30%, 50%, 70%, and 95% (v/v) ethanol. The fractions were combined, evaporated to dryness, re-dissolved in water, re-absorbed into the resin, re-eluted with 50% (v/v) ethanol, and subjected to silica gel column chromatography.

Results: Structural Identifications

Silica gel column chromatography yielded four compounds: naringin (compound I), hesperindin (compound II), neohesperindin (compound III), and hesperetin 7-O-β-D-glucopyranoside (compound IV).

Compound I: naringin

A white powder collected upon evaporation of methanol, mp 238-240˚C; AlC13 significantly enhanced the fluorescence intensity of the material. ^{1}H-NMR (DMSO-d6, 400 MHz); δ: 11.96 (1H, s, 5-OH), 9.55 (1H, s, 4-OH), 7.24 (2H, d, J=8.24 Hz, H-3', 5'), 6.70 (2H, d, J=7.72 Hz, H-2', 6'), 6.02 (1H, s, H-6), 5.99 (1H, s, H-8), 5.25 (1H, d, J=4.6 Hz, H-2), 3.3 (1H, d, J=9.6 Hz, H-3), 2.62 (1H, d, J=9.6 Hz, H-3), 5.06 (1H, d, J=5.04 Hz, H-1''), 5.0 (1H, s, H-1'''), 1.05 (3H, d, J=5.64 Hz, 6'''-CH_3). ^{3}C-NMR (DMSO-d6, 100 MHz); δ: 78.77 (C-2), 42.06 (C-3), 197.23 (C-4), 162.88 (C-5), 96.25 (C-6), 164.81 (C-7), 95.07 (C-8), 162.7 (C-9), 103.27 (C-10), 128.51 (C-1'), 128.4 (C-2'), 115.17 (C-3'), 157.76 (C-4'), 115.17 (C-5'), 128.4 (C-6'), 100.36 (C-1''), 76.8 (C-2''), 78.58 (C-3''), 69.55 (C-4''), 77.07 (C-5''), 60.4 (C-6''), 97.25 (C-1'''), 70.43 (C-2'''), 70.33 (C-3'''), 71.77 (C-4'''), 68.25 (C-5'''), 18.0 (C-6'''). These data indicate that the compound is naringin [6].

Compound II: hesperindin

A white powder on evaporation of absolute ethyl alcohol, mp 256-263˚C; AlC13 significantly enhanced the fluorescence intensity of the material. ^{1}H-NMR (DMSO-d6, 400 MHz); δ: 12.03 (1H, s, 5-OH), 9.15 (1H, s, 3'-OH), 6.95-6.89 (3H, m, H-2' , 5', 6'), 6.14 (1H, s, H-6), 6.12 (1H, s, H-8), 5.44 (1H, d, J=4.8 Hz, H-2), 3.28 (1H, d, J=4.5 Hz, H-3), 3.14 (1H, d, J=5.76 Hz, H-3), 3.77 (3H, s, -OCH_3), 1.07 (3H, d, J=7.4 Hz,

6’’’-CH_3); ^{13}C-NMR (DMSO-d6, 100 MHz); δ: 78.32 (C-2), 41.99 (C-3), 196.97 (C-4), 162.98 (C-5), 96.34 (C-6), 165.08 (C-7), 95.51 (C-8), 162.44 (C-9), 103.28 (C-10), 130.84 (C-1’), 114.08 (C-2’), 146.39 (C-3’), 147.92 (C-4’), 112.0 (C-5’), 117.93 (C-6’), 99.41 (C-1’’), 75.47 (C-2’’), 72.02 (C-3’’), 69.56 (C-4’’), 76.2 (C-5’’), 65.97 (C-6’’), 100.54 (C-1’’’), 70.22 (C-2’’’), 70.65 (C-3’’’), 72.94 (C-4’’’), 68.27 (C-5’’’), 17.77 (C-6’’’), 55.65 (C-OCH_3). These data indicate that the compound is hesperindin [7].

Compound III: neohesperindin

A white powder upon evaporation of absolute ethyl alcohol, mp 246-247˚C; AlC13 significantly enhanced the fluorescence intensity of the material. ^{1}H-NMR (DMSO-d6, 400 MHz) δ: 12.05 (1H, s, 5-OH), 9.17 (1H, s, 3’-OH), 6.95-6.87 (3H, m, H-2’, 5’, 6’), 6.11 (1H, s, H-6), 6.09 (1H, s, H-8), 3.77 (3H, s, 4OCH_3), 5.36 (1H, d, J=6.84 Hz, H-2), 3.38 (1H, s, H-3), 2.51 (1H, s, H-3), 5.17 (1H, d, J=5.32 Hz, H-1’’), 5.1 (1H, s, H-1’’’), 1.16 (1H, d, J=6.2 Hz, H-6’’’); ^{13}C-NMR (DMSO-d6, 100 MHz); δ: 76.53 (C-2), 41.56 (C-3), 196.41 (C-4), 162.33 (C-5), 95.68 (C-6), 164.25 (C-7), 94.57 (C-8), 162.0 (C-9), 102.75 (C-10), 130.3 (C-1’), 113.52 (C-2’), 145.89 (C-3’), 147.41 (C-4’), 111.44 (C-5’), 117.25 (C-6’), 96.83 (C-1’’), 77.83 (C-2’’), 76.3 (C-3’’), 67.7 (C-4’’), 75.53 (C-5’’), 59.84 (C-6’’), 99.81 (C-1’’’), 69.78 (C-2’’’), 99.81 (C-3’’’), 71.22 (C-4’’’), 69.0 (C-5’’’), 17.43 (C-6’’’), 55.1 (C-OCH_3). These data indicate that the compound is neohesperindin [8].

Compound IV: hesperetin 7-O-β-D-glucopyranoside

A white powder upon evaporation of absolute ethyl alcohol, mp 223-225˚C; AlC13 significantly enhanced the fluorescence intensity of the material. ^{1}H-NMR (DMSO-d6, 400 MHz); δ: 12.05 (1H, s, 5-OH), 9.16 (1H, s, 3’-OH), 6.96-6.87 (3H, m, H-2’, 5’, 6’), 6.16 (1H, s, H-6), 6.14 (1H, s, H-8), 5.50 (1H, dd, J=3.97 Hz, H-2), 2.76 (1H, d, J=14.5 Hz, H-3), 3.29 (1H, d, J=12.9 Hz, H-3), 3.76 (3H, s, 4’-OCH_3), 4.97 (1H, d, J=7.41 Hz, H-1’’); ^{13}C-NMR (DMSO-d6, 100 MHz); δ: 78.43 (C-2), 42.13 (C-3), 196.98 (C-4), 162.9 (C-5), 96.46 (C-6), 65.28 (C-7), 95.46 (C-8), 162.57 (C-9), 103.26 (C-10), 130.89 (C-1’), 112.01 (C-2’), 147.95 (C-3’), 146.47 (C-4’), 114.13 (C-5’), 117.77 (C-6’), 99.59 (C-1’’), 73.0 (C-2’’), 76.29 (C-3’’), 69.49 (C-4’’), 77.07 (C-5’’), 60.55 (C-6’’), 55.68 (C-OCH_3). These physicochemical characteristics and spectrophotometric data indicate that the compound is hesperetin 7-O-β-D-glucopyranoside [9].

Discussion and Conclusions

Four flavonoids were isolated from Zhangband-processed FA using large-pore adsorbent resin and repeated silica gel column chromatography [10,11]. Of these, hesperetin7-O-β-D-glucopyranoside was present after, but not before, Zhangband processing, and is herein described for the first time from FA.

Disorders of gastrointestinal motility are important features of Qi stagnation of the spleen and stomach. Fructus aurantii effectively regulates Qi and relieves stagnation in these areas, and is also used to treat Qi stagnation of the chest and abdomen, abdominal distention, and dyspepsia. However, raw FA exerts excessive drying of tissues. Pharmacological experiments have shown that a decoction of FA processed using the Zhangband method exhibits milder effects on *ex-vivo* rabbit intestines and the gastrointestinal motility of live mice, as compared to raw FA. Thus, processing attenuates the drying characteristics of FA and enhances the ability of the material to strengthen the spleen and stomach. Such changes in the pharmacological effects of processed FA may be associated with chemical changes that occur during processing. Thus, the present study provides evidence that Zhangband processing may change the pharmacological actions of FA.

Acknowledgments

None

Declaration of Interest

The authors report no declarations of interest.

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