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Isolation and identification of new chemical constituents from Chinese chive (*Allium tuberosum*) and toxicological evaluation of raw and cooked Chinese chive

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ABSTRACT

Chinese chive (*jiu cai*) is a popular vegetable in China and has a unique flavour and aroma. The molecular basis of the characteristic fragrance and nutritional properties of Chinese chive has not been previously identified. Sequential extractions in a series of solvents and high-performance liquid chromatography were used to isolate 40 compounds from Chinese chive. The compounds were identified based on high-resolution electrospray ionization mass spectra, 1D and 2D nuclear magnetic resonance techniques, and circular dichroism spectra. Eight novel compounds were identified—four new pyrazines, which have distinctive flavour; one new lignan; and three new flavonoids—together with 32 known compounds. Several of these compounds have potential applications as health-promoting dietary supplements, food additives, or seasonings. Additionally, the volatile organic compounds in fresh and steamed Chinese chive were compared, and the toxicological activity of extracts from fresh and steamed Chinese chive was tested in normal rat liver (IAR20) and kidney (NRK) cells. The results showed that Chinese chive is toxic to liver and kidney cells when fresh, but is safe after heating. This could explain why it is traditional to eat cooked Chinese chive. A possible metabolic rule regarding pyrazines is postulated based on this data, and a human metabolic pathway is suggested for two of the novel compounds which have the highest amount of Chinese chive extracts.

1. Introduction

Chinese chive (*Allium tuberosum* Rottl. ex Spreng.), named *jiu cai* in Mandarin, is a very popular vegetable in China (China Flora Editorial Board, 1980). It has been consumed for more than 3000 years (Gao and Wu, 2004), and is a healthy food that is believed to support kidney function by practitioners of traditional Chinese medicine (Block and Naganathan, 1992; Maga and Sizer, 1973; Masuda and Mihara, 1988).

Chinese chive has a unique aroma. According to previous reports, the fragrant components of Chinese chive are thioether compounds (Block and Putman, 1992). However, the main fragrant thioether compounds that have been identified in Chinese chive have a pungent odour that is not characteristic of Chinese chive. Thus, the molecular basis of Chinese chive's flavour is not yet thoroughly understood.

Studies on the chemical constituents and bioactivity of Chinese chive reveal the presence of sulphur ethers, sterols, and flavonoids, but these analyses have mainly used gas chromatography–mass spectrometry (GC-MS) or liquid chromatography–mass spectrometry (LC-MS) (China Flora Editorial Board, 1980; Gao and Wu, 2004), which may call the results into question. A large number of unknown constituents of Chinese chive have not been identified correctly and their characteristics and bioactivity are not clear.

Abbreviations: δ, chemical shift; CD, circular dichroism; ECD, electronic circular dichroism; GC-MS, gas chromatography–mass spectroscopy; HPLC, high performance liquid chromatography; HRESIMS, high resolution electrospray ionization mass spectra; IAR20, normal rat liver cells; *J*, coupling constant; NMR, nuclear magnetic resonance; NRK, normal rat kidney cells; PHPLC, preparative high performance liquid chromatography; UV, ultraviolet.

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Phytochemistry methods have the potential to more precisely reveal the chemical constituents of Chinese chive.

In the present study, sequential extractions in a series of solvents, high-performance liquid chromatography (HPLC), and preparative high-performance liquid chromatography (PHPLC) were used to isolate 40 compounds from Chinese chive. High-resolution electrospray ionization mass spectra (HRESIMS), 1D and 2D nuclear magnetic resonance (NMR) techniques, and circular dichroism (CD) spectra were used to gain insight into the molecular composition of the obtained fractions.

The Chinese traditionally cook Chinese chive before eating it. To explore the chemical changes that take place in Chinese chive upon cooking, the volatile organic compounds in fresh and steamed Chinese chive were analysed and compared using GC-MS. The toxicity of these compounds to normal rat liver and kidney cells was evaluated. Based on the research findings reported here, a novel plant metabolic rule is proposed, and possible human metabolic pathways of two highest amount compounds in Chinese chive is postulated.

2. Materials and methods

2.1. Plant materials

Chinese chive (*A. tuberosum*) samples were purchased from Beijing Xinfadi vegetable market in March of 2016. The species was identified by Professor Yan-su Li (Institute of Vegetables and Flowers, Chinese Academy of Agriculture Sciences).

2.2. Extraction and isolation

Chinese chive samples (30.00 kg) were extracted 4 times with 75% EtOH (30 L) at room temperature for 3 d each time. The extraction solution was concentrated in vacuo, suspended in water, and lyophilized using a freeze dryer to obtain a brown residue (865.50 g). The residue was then sequentially extracted with petroleum ether, ethyl acetate, and *n*-butyl alcohol. The extraction solutions were concentrated in vacuo to yield a petroleum ether−soluble fraction (85.80 g), an ethyl acetate−soluble fraction (100.50 g), and an *n*-butyl alcohol−soluble fraction (158.50 g).

Ultraviolet spectra of the fractions were obtained on a Waters 2695 HPLC with a photo diode array detector. PHPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector, using a YMC-Pack ODS-A column. The *n*-butyl alcohol−soluble fraction was eluted with H_2O/C_2H_5OH (100:0, 85:15, 70:30, 50:50, 0:100, v/v) through a macroporous resin column to obtain 5 fractions, Fr.1 (15.70 g), Fr.2 (31.50 g), Fr.3 (20.30 g), Fr.4 (22.60 g), and Fr.5 (32.30 g). The fractions were then further purified into compounds using PHPLC (supplementary material). Fr.2 was eluted through a gel column (300 g, 8 cm × 100 cm) with H_2O to give 6 sub-fractions (Fr.2.1−Fr.2.6), and the same method was used with Fr.3 to obtain 5 sub-fractions (Fr.3.1−Fr.3.5). Then Fr.2.1 was eluted with MeOH/H_2O (22:78) to yield compound **1** (6.86 mg). Compound **2** (13.52 mg) was obtained from Fr.2.2 using a mobile phase of 23% MeOH. Fr.2.3 was eluted with MeOH/H_2O (24:76) to yield compounds **3** (17.63 mg), **4** (8.73 mg), **5** (18.62 mg), **6** (25.63 mg), and **7** (14.85 mg). Fr.2.4 was eluted with MeOH/H_2O (25:75) to isolate compounds **8** (30.72 mg) and **9** (29.80 mg). Fr.2.5 was eluted with MeOH/H_2O (26:74) to give compounds **10** (9.30 mg) and **11** (8.3 mg). Compounds **12** (7.90 mg) and **13** (6.84 mg) were obtained from Fr.2.6 with 27% MeOH in the mobile phase. Fr.3.1 was eluted with MeOH/H_2O (40:60) to yield compounds **14** (7.95 mg), **15** (8.70 mg), **16** (7.25 mg), **17** (8.52 mg), **18** (7.82 mg), **19** (9.68 mg), **20** (12.50 mg), and **21** (7.34 mg). Fr.3.2 was eluted with MeOH/H_2O (45:55) to yield compounds **22** (6.50 mg), **23** (7.83 mg), **24** (9.20 mg), **25** (7.15 mg), **26** (783.21 mg), **27** (1250.82 mg), **28** (45.80 mg), **29** (34.60 mg), and **30** (28.12 mg). Fr.3.3 was eluted with MeOH/H_2O (48:52) to give compounds **31** (7.12 mg), **32** (7.60 mg), **33** (6.95 mg), **34** (9.11 mg), **35** (10.83 mg), and **36** (6.55 mg). Fr.3.4 was eluted with MeOH/H_2O (50:50) to yield compounds **37** (6.86 mg) and **38** (7.50 mg). Compounds **39** (8.50 mg) and **40** (8.12 mg) were obtained from Fr.3.5 by using 52% MeOH in the mobile phase.

2.3. Identification of extracted compounds

NMR spectra were obtained using Bruker AV-500 spectrometers (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) (Bruker, Switzerland). The chemical shift (δ) values are given in ppm with tetramethylsilane (TMS) as an internal standard, and coupling constants (J) are given in Hz. High-resolution electrospray ionization mass spectra were obtained using an Agilent 6540 high-resolution quadrupole time-of-flight mass spectrometer (Agilent, U.S.A). Circular dichroism spectra were recorded on a JASCO J-815 CD spectrometer (JASCO, Japan).

2.4. Extraction method of volatile components from fresh and steamed Chinese chive

To compare the volatile components of fresh and steamed Chinese chive, 250.00 g of fresh Chinese chive was steamed in water vapour for 20 min. Both fresh and steamed Chinese chive (250.00 g each) were minced and placed in separate round-bottom flasks, and each was mixed with distilled water to a solid−liquid ratio of 1:10. After that, 5 mL *n*-hexane was added to the volatile oil extractor and a 6 h distillation process was performed. Then the *n*-hexane layer of each flask was transferred to a brown bottle for storage, and the constituents were detected by GC-MS.

2.5. Analysis of volatile components in fresh and steamed Chinese chive using GC-MS

The volatile components of fresh and steamed Chinese chive were analysed using an Agilent 6890N/5973i GC-MS (Agilent, U.S.A) with a 7683 series injector under the following conditions: capillary column, HP-5 (30.00 m × 0.25 mm, with a 0.25 μm film, Dikma); initial temperature 50 °C maintained for 2 min, raised to 160 °C at 4 °C/min, then raised to 250 °C at 8 °C/min; turnaround time, 38 min; injection temperature, 240 °C; carrier, He gas (99.999%) (Praxair, U.S.A); flow rate, 1.0 mL/min; pressure, 56 kPa; spilt ratio, 1:1; MS ionization, EI, 70eV; interface temperature, 280 °C; MS quad, 150 °C; MS source, 230 °C; solvent delay, 3 min; scan area, 40−550 amu; EM voltage, 2470.6 V; MS matching library, NIST G1041A.

2.6. Toxicity studies

2.6.1. Cell lines and media

Normal rat kidney cells (NRK) and normal rat liver cells (IAR20) were obtained from China Infrastructure of Cell Line Resources (Beijing, China). High-glucose foetal bovine serum, penicillin, streptomycin, and trypsin-EDTA were purchased from Gibco by Life Technologies (U.S.A). Dimethyl sulphoxide was purchased from Sigma (U.S.A). Dulbecco's phosphate-buffered saline was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

2.6.2. Cell culture and treatment

NRK were cultured in Dulbecco's modified Eagle's medium with 10% foetal bovine serum. IAR20 were cultured in minimum essential medium with 20% foetal bovine serum. Both cell media

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