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Comparison of antioxidant activities of different parts from snow chrysanthemum (*Coreopsis tinctoria* Nutt.) and identification of their natural antioxidants using high performance liquid chromatography coupled with diode array detection and mass spectrometry and 2,2′-azinobis(3-ethylbenzthiazoline-sulfonic acid)diammonium salt-based assay

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ABSTRACT

Snow chrysanthemum (*Coreopsis tinctoria* Nutt.), a world-widely well-known flower tea material, has attracted more and more attention because of its beneficial health effects such as antioxidant activity and special flavor. In this study, a high performance liquid chromatography coupled with diode array detection and mass spectrometry (HPLC-DAD-MS) and 2,2′-azinobis(3-ethylbenzthiazoline-sulfonic acid)diammonium salt (ABTS) based assay was employed for comparison and identification of antioxidants in different samples of snow chrysanthemum. The results showed that snow chrysanthemum flowers possessed the highest while stems presented the lowest antioxidant capacities. Fourteen detected peaks with antioxidant activity were temporarily identified as 3,4′,5,6,7-pentahydroxyflavanone-O-hexoside, chlorogenic acid, 2R-3′,4′,8-trihydroxyflavanone-7-O-glucoside, flavanomarein, flavanocorepsin, flavanokanin, quercetagitin-7-O-glucoside, 3′,5,5′,7-tetrahydroxyflavanone-O-hexoside, marein, maritimein, 1,3-dicaffeoylquinic acid, coreopsin, okanin and acetyl-marein by comparing their UV spectra, retention times and MS data with standards or literature data. Antioxidants existed in snow chrysanthemum are quite different from those reported in *Chrysanthemum morifolium*, a well-known traditional beverage in China, which indicated that snow chrysanthemum may be a promising herbal tea material with obvious antioxidant activity.

1. Introduction

Overproduction of free radicals can induce many human diseases such as diabetes, cancer, stroke, rheumatoid arthritis and atherosclerosis [1–3]. Antioxidants can alleviate the oxidative stress, which is beneficial for human health [4]. However, some currently used synthetic free radical scavengers have been demonstrated various side effects [5,6]. Therefore, functional foods become a promising source of natural antioxidants [7,8].

Coreopsis tinctoria, belongs to the Compositae family, is native from North America and has world-widely distributed now. In China, it has been found around the snowline (above 3000 m) of Mountain Kunlun, which locates in the center of the Eurasian land bridge [9,10]. Therefore, it is also called snow chrysanthemum, *Xueju* in Chinese (Fig. 1). There is a long history for snow chrysanthemum used as a herbal tea or medicine for treatment of diseases such as diabetes, diarrhea and bleeding both in China and other countries [11]. Pharmacological studies have shown that snow chrysanthemum possesses multiple activities, and antioxidant activity is the most concerned [3,12–14]. Indeed, several

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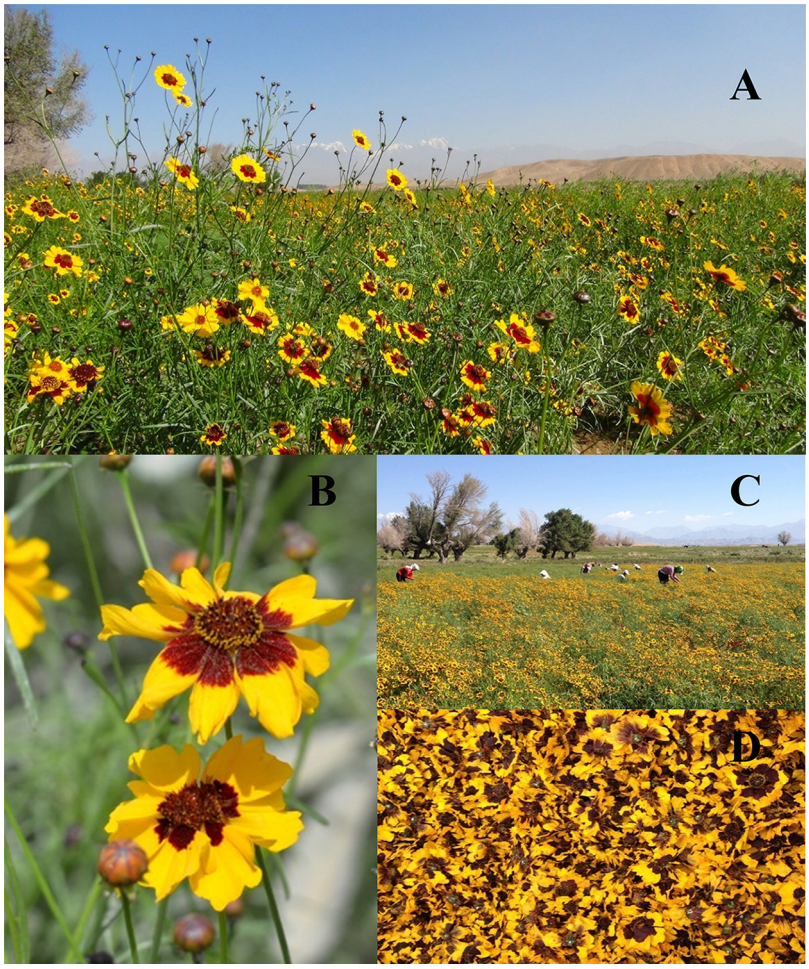

Fig. 1. The growth environment (A), plant with flower (B), flower collection (C) and the raw materials (D) of snow chrysanthemum.

studies have reported the antioxidant activity of snow chrysanthemum extract [15,16]. However, which compounds contribute to this effect are still unknown.

In this study, antioxidant activities of different parts of snow chrysanthemum from different geographical areas of China were investigated and compared using HPLC-DAD-MS coupled with online ABTS-based analysis. The antioxidants in snow chrysanthemum were also identified.

2. Materials and methods

2.1. Materials and reagents

The different parts (flowers, buds, seeds, leaves and stems) of snow chrysanthemum (*Coreopsis tinctoria* Nutt.) were collected from Da Bancheng, Xinjiang Autonomous Region, China. The other flower samples of snow chrysanthemum were from Ke Liyang, Lao Yeke and A Ye, Xinjiang Autonomous Region, respectively. The detailed sample information was shown in Table 1. Voucher specimens of these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China.

ABTS was purchased from International Laboratory (San Bruno, CA). Potassium persulfate was from Fluka (Seelze, Germany). Chlorogenic acid (≥98%) was purchased from Aladdin (Shanghai, China). Marine and okanin (≥98%) were separated and purified in our laboratory, their structure were also confirmed based on their MS and NMR data. HPLC grade formic acid, acetonitrile and ethanol were purchased from Merck (Darmstadt, Germany). Nylon membrane filters (0.45 μm) were purchased from Millipore (Billerica, MA). Deionized water was prepared using a Millipore Milli Q-Plus system (Millipore, Billerica, MA).

Table 1
Summary of the analyzed samples.

Codes	Varieties	Parts	Locations
S1	*Coreopsis tinctoria* Nutt. (Yellow)	Flowers	Da Bancheng, Xinjiang
S2	*C. tinctoria* Nutt. (Red)	Flowers	Da Bancheng, Xinjiang
S3	*C. tinctoria* Nutt. (Yellow)	Buds	Da Bancheng, Xinjiang
S4	*C. tinctoria* Nutt. (Yellow)	Seeds	Da Bancheng, Xinjiang
S5	*C. tinctoria* Nutt. (Yellow)	Leaves	Da Bancheng, Xinjiang
S6	*C. tinctoria* Nutt. (Yellow)	Stems	Da Bancheng, Xinjiang
S7	*C. tinctoria* Nutt. (Yellow)	Flowers	Ke Liyang, Xinjiang
S8	*C. tinctoria* Nutt. (Yellow)	Flowers	Ke Liyang, Xinjiang
S9	*C. tinctoria* Nutt. (Yellow)	Flowers	Ke Liyang, Xinjiang
S10	*C. tinctoria* Nutt. (Yellow)	Flowers	Lao Yeke, Xinjiang
S11	*C. tinctoria* Nutt. (Yellow)	Flowers	A Ye, Xinjiang

2.2. Preparation of sample extract

The dried sample powder (0.1 g, 40 mesh) and 2 mL of ethanol–water (55:45, v/v) were transferred into 5 mL extraction vessels made of borosilicate glass. The microwave-assisted extraction was carried out in Multiwave 3000 (Anton Paar GmbH, Graz, Austria), which was performed at 400 W and 80 °C for 5 min. Then, the extract was compensated the loss of weight with the solvent and subsequently centrifuged at 5000 × *g* for 5 min. After