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Chemical constituents, antioxidant and gastrointestinal transit accelerating activities of dried fruit of *Crataegus dahurica*

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ABSTRACT

Crataegus dahurica Koehne is an edible wild fruit mainly distributed in Northeast China. The purpose of this study was to elucidate the chemical constituents and investigate the bioactivities of dried fruit of *C. dahurica* methanol extract (CdME). Through various chromatographic methods, thirty-five compounds were isolated from CdME for the first time and their structures were identified on the basis of physicochemical properties and spectroscopic data. The main structural types of these compounds were triterpenoids and polyphenolics. Pharmacological experiments results showed that CdME had potently antioxidant capacity and ethyl acetate fraction was the active part with the greatest antioxidant activities. Moreover, CdME especially *n*-butanol fraction significantly accelerated the gastrointestinal transit in mice (acceleration rate: 78.5 ± 1.5% vs. 69.9 ± 3.2% at a dose of 250 mg/kg, compared to the control group, $P < .01$). On the basis of these results, *C. dahurica* may be considered as a good resource of antioxidants and digestion-improving agents.

1. Introduction

Some edible wild fruits including berries are the important sources of functional foods and drinks. These fruits have various bioactivities, such as antioxidant, anti-inflammatory, gastro-protective, hypolipidaemic, and antibacterial activities, thus people can benefit from daily consumption of them to prevent or delay the onset of chronic age-related diseases or life-style diseases (Kong, Chia, Goh, Chia, & Brouillard, 2003; Krikorian et al., 2010). Due to the health functions derived from the phytochemicals in them, edible wild fruits especial berries have attracted more and more attention worldwide. In Europe and America, many wild fruits have been systematically investigated and exploited (Grace, Esposito, Dunlap, & Lila, 2014). By contrast, in China, although the resources are diverse and abundant, most of wild fruits have not been investigated and utilized. Nowadays, with more and more emphasis on healthy life styles and foods, it is a waste to remain these vast quantities of wild fruits with health-promoting effects to be studied and exploited.

Northeast China harbors the largest natural distribution area of wild fruits in China and is the richest in terms of berry species (Zhang, Wei, Ma, & Xu, 2006; Zhou, Zhu, & Yu, 2012). However, few of them have been completely researched and exploited. Fruit of *Crataegus dahurica* Koehne is one of the wild fruits mainly distributed in Heilongjiang and Jilin provinces and often collected and eaten by local people in autumn. Different from other fruits of hawthorn genus, e.g., *C. pinnatifida* Bge. and *C. pinnatifida* Bge. var. major N.E.Br., the two main species distributed in China, the fruit of *C. dahurica* is smaller and has softer pulp and more delicate textures after maturation without white or yellow dots on the pericarp, which is more like a berry. Meanwhile, the fruit of *C. dahurica* also as an ethnic medicine of Oroqen minority people, dominantly living in the Great Xing'an Mountain region, is used for the treatment of dyspepsia and infantile indigestion (Sun & Liu, 2007). However, systematic chemical constituents study and pharmacological activities of this wild edible and medicinal fruit have not been reported so far.

The aim of this present study was to elucidate the chemical

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constituents and determine the antioxidant activity and digestion improving effect of *C. dahurica* methanol extract (CdME). By using various chromatographic methods, the chemical components research on the fruit of *C. dahurica* was conducted, and structures of the isolated compounds were unambiguously characterized on the basis of their physicochemical properties and spectroscopic data. Furthermore, 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) assay, ferric-reducing antioxidant power (FRAP) assay, the hydroxyl free radical scavenging assay and anti-lipid peroxidation assay were used to evaluate the antioxidant activities of the extract and fractions. Additionally, gastrointestinal transit (GIT) assay in mice was used to measure the digestion enhancing effect of the wild fruit. To the best of our knowledge, this is the first report on the systematic chemical research on the fruit of *C. dahurica* from Northeast China, as well as antioxidant activities and GIT acceleration activities. This work will be useful to develop and exploit this wild fruit as a healthy food or drink. Meanwhile, it may also have a reference value in the development of healthy beneficial food products.

2. Materials and methods

2.1. Instrumentation and reagents

^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III-400 spectrometer (Bruker, Germany), using tetramethylsilane (TMS) as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. Semi-preparative HPLC was done on a Shimadzu LC-6AD pump and RID-10A detector (Shimadzu, Kyoto, Japan). Ultraviolet absorption (UV) were measured on a UV-1200 spectrophotometer (Mapada, Shanghai, China).

The chromatographic packing materials such as ODS (Merck Co., Germany), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) and silica gel (Qingdao Haiyang Chemical Co., Shandong, China) were used in isolation. The solvents used in extraction and isolation were analytical or chromatographic grade purchased from Fuyu Fine Chemical Co. (Tianjin, China). 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), thiobarbituric acid (TBA) and ascorbic acid were obtained from Energy Chemical Co. (Shanghai, China). 1, 10-phenanthroline, DPPH and other chemicals were purchased from Sigma-Alorich Co. (St. Louis, MO, USA).

2.2. Plant material

The fruits of *C. dahurica* were collected in August 2015, Tongbei, Heilongjiang province and identified by professor Xiaofeng Niu, of School of Pharmacy, Health Science Center, Xi'an Jiaotong University. A voucher specimen (MLN150816) has been deposited in the herbarium of School of Pharmacy, Health Science Center, Xi'an Jiaotong University.

2.3. Preparation of extract and fractions of C. dahurica

The dry fruit of *C. dahurica* (4.1 kg) was smashed and extracted with methanol (20 L) three times under ambient temperature. After removing the solvent in reduced pressure, the residue (1374.1 g, 30.3%) was obtained as the methanol extract (CdME). Part of the CdME (1225.2 g) was suspended in water (6 L) and extracted successively with petroleum ether (PE), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), respectively. After evaporation of the solvent in vacuum, the PE fraction (18.9 g, 0.5%), EtOAc fraction (41.2 g, 1.1%), *n*-BuOH fraction (160.8 g, 4.4%) and H_2O fraction (907.1 g, 24.8%) were respectively obtained.

2.4. Isolation and purification of chemical constituents

A part of PE fraction (17.0 g) was subjected to silica gel column chromatography (CC) gradiently eluting with PE/EtOAc (1:0, 80:1, 50:1, 20:1, 10:1, *v/v*) and then $CHCl_3/CH_3OH$ (1:0, 80:1, 50:1, 20:1, 10:1, 5:1, 1:1, 0:1, *v/v*) successively to yield twenty nine fractions (Fr. PE1 to Fr. PE29). Fr. PE12 was then separated by silica gel column, eluting with PE/EtOAc (20:1, *v/v*) to yield eighteen subfractions (Fr. PE12-1 to Fr. PE12-18). The crystal in Fr. PE12-12 was purified by recrystallization with $CHCl_3/CH_3OH$ to afford compound **1** (60.8 mg). Compound **2** (15.0 mg) was obtained from Fr. PE12-5 by silica gel CC eluting with PE/EtOAc (50:1 to 1:1, *v/v*) and preparative TLC using *n*-hexane/EtOAc (3:1, *v/v*) as the developer. The samples of Fr. PE16 to Fr. PE18 were respectively purified by Sephadex LH-20 column eluting with $CHCl_3/CH_3OH$ (1:1, *v/v*). And then seven subfractions (Fr. PE16-1 to Fr. PE16-7) were obtained on the basis of TLC analysis. Compound **3** (27.5 mg) was obtained by preparative TLC developed by the solvent of PE/EtOAc (2:1, *v/v*) from Fr. PE16-5 subfraction. Fr. PE21 was separated by repeated silica gel column to afford compound **4** (12.7 mg).

The EtOAc fraction (36.2 g) was separated by silica gel (200–300 mesh, 500 g) CC eluting with $CHCl_3/CH_3OH$ (1:0, 80:1, 50:1, 20:1, 15:1, 10:1, 5:1, 1:1, 1:5, *v/v*) to yield twenty three fractions (Fr. EA1 to Fr. EA23). Fr. EA12 was divided into nine subfractions (Fr. EA12-1 to Fr. EA12-9) by silica gel CC gradiently eluting with $CHCl_3/CH_3OH$ (1:0, 50:1, 20:1, 10:1, 5:1, 1:1, *v/v*). Fr. EA12-4 was further isolated and purified by silica gel CC with PE/EtOAc (8:2, *v/v*) as eluent to afford compound **4** (8.7 mg) and compound **5** (7.9 mg). Compound **6** (7.2 mg), compound **7** (3.4 mg), compound **8** (6.2 mg) and compound **9** (8.3 mg) were obtained from Fr. EA12-5 which was separated by silica gel CC and eluted by *n*-hexane/EtOAc (7:3, *v/v*), and then purified by HPLC with 80% methanol in 0.5% acetic acid as the mobile phase. Compound **10** (6.2 mg), compound **11** (21.7 mg) and compound **12** (5.6 mg) were purified from Fr. EA3 by silica gel CC eluting with PE/EtOAc (8:2, 7:3, 1:1, 0:1, *v/v*) and by preparative HPLC eluting with 80% methanol in 0.5% acetic acid. The solid in Fr. EA17 was repeatedly recrystallized by methanol to afford compound **13** (215.7 mg).

The *n*-BuOH fraction (115.7 g) was separated by silica gel (200–300 mesh, 1000 g) CC using gradient solvent of $CHCl_3/CH_3OH$ (1:0, 80:1, 50:1, 20:1, 10:1, 5:1, 2:1, 0:1, *v/v*) as eluent to yield thirty six fractions (Fr. Bu1 to Fr. Bu36). Fr. Bu13 was further separated into nine subfractions (Fr. Bu13-1 to Fr. Bu13-9) through Sephadex LH-20 CC eluting with methanol. Compound **14** (12.9 mg), compound **15** (9.6 mg) were obtained from Fr. Bu13-4 by preparative HPLC with 30% methanol in 0.5% acetic acid as the mobile phase. The same method was used to obtain compound **16** (4.6 mg), compound **17** (11.8 mg), compound **18** (13.2 mg) from Fr. Bu13-5 subfraction. Fr. Bu14 was separated by ODS (212 g) CC which was eluted by $MeOH/H_2O$ (15:85, 30:70, 45:55, 60:40, 80:20, 100:0, *v/v*) to yield fourteen subfractions (Fr. Bu14-1 to Fr. Bu14-14). Samples of Fr. Bu14-6 to Fr. Bu14-8 were purified by HPLC with 35% methanol in 0.5% acetic acid as the mobile phase to afford compound **19** (45.6 mg), compound **20** (42.7 mg) and compound **21** (24.3 mg), respectively. Fr. Bu16 was divided into sixteen subfractions (Fr. Bu16-1 to Fr. Bu16-16) by ODS CC eluting with $MeOH/H_2O$ (25:75, 34:66, 44:56, 57:43, 74:26, 100:0, *v/v*). Compound **22** (4.8 mg) and compound **23** (6.6 mg) were purified by HPLC with 40% methanol in 0.5% acetic acid as the mobile phase from the Fr. Bu16-8. Fr. Bu17 was firstly separated by the silica gel CC eluting with $CHCl_3/CH_3OH/H_2O$ (9:1:0.1, 8:2:0.2, 7:3:1, 6:4:1, *v/v*) and thirteen subfractions (Fr. Bu17-1 to Fr. Bu17-13) were obtained. The Fr. Bu17-8 was further subdivided into thirteen parts (Fr. Bu17-8-1 to Fr. Bu17-8-13) by ODS CC and purified by HPLC with 35% methanol in 0.5% acetic acid at the rate of 3.0 mL/min, and compound **24** (7.6 mg), compound **25** (6.4 mg), compound **26** (4.3 mg), compound **27** (4.0 mg), compound **28** (6.8 mg) and compound **29** (14.6 mg) were obtained. Fr. Bu18 was separated into ten subfractions (Fr. Bu18-1 to Fr. Bu18-10) by the ODS column eluting with the solvent of $MeOH/H_2O$ (32:68, 45:55, 57:43, 100:0, *v/v*). The subfractions Fr. Bu18-2, Fr. Bu18-3 and Fr. Bu18-6 were further purified by HPLC using the mobile phase of 40% methanol in 0.5% acetic acid and compound **30** (14.4 mg), compound **31** (18.7 mg), compound **32** (17.3 mg), compound **33** (8.5 mg) and compound **34** (11.4 mg) were afforded. Compound **35** (11.2 mg) was