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Phenolic contents, cellular antioxidant activity and antiproliferative capacity of different varieties of oats

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ABSTRACT

The objectives of this research were to determine the phenolic contents, oxygen radical absorbance capacities (ORAC), cellular antioxidant activities (CAA), and antiproliferative capacities of nine oat varieties and four brans in China. Of all varieties, Longyan 3 and Beiyan 1 exhibited the highest total avenanthramides (146.94 ± 7.31 and 120.95 ± 6.66 μg/g, respectively) and ORAC values (21.03 ± 0.56 and 21.18 ± 1.45 μM Trolox/g, respectively), while Shaotong exhibited the highest total phenolic acids (143.52 ± 9.42 μg/g) and CAA values (33.38 ± 1.74 μM quercetin/100 g). The EC_{50} of antiproliferative capacities ranged from 167.31 ± 6.42 to 233.42 ± 21.31 mg/mL, with the lowest in Beixiao 8 while the highest in Jinyan 8. ORAC values correlated with avenanthramides while CAA values correlated with phenolic acids. Moreover, phenolic contents, antioxidant properties, and antiproliferative capacities of oat brans was higher than that of corresponding whole oats in most cases.

1. Introduction

Reactive oxygen species (ROS) are a series of metabolites involved in pathological and degenerative processes in the human body. Superfluous ROS induce free radical damage of organs and tissues, which accelerates the development of many diseases, such as cardiovascular disease, diabetes mellitus, and cancer (Babbar, Oberoi, Uppal, & Patil, 2011). Therefore, various kinds of phytochemicals have been identified as natural antioxidants to remove ROS (Adom & Liu, 2002). Of these phytochemicals, phenolic compounds have been brought into focus due to an inverse correlation between the incidence of chronic diseases and the intake of phenolic-rich foods (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993).

Polyphenolic and phenolic compounds are bioactive non-nutrient secondary metabolites present in fruits, vegetables, and cereals. These compounds protect the plant against pathogen attack or ultraviolet radiation. In food, these metabolites can contribute not only to antioxidant activity but also to color, astringency, or bitterness (Hitayezu, Baakdah, Kinnin, Henderson, & Tsopmo, 2015). Previous researches have shown that the consumption of some cereal products protect against the development of chronic diseases such as diabetes, cancers, and cardiovascular diseases (Slavin, Jacobs, & Marquart, 1997). The protective influence of grains is mainly attributed to the existence of both polyphenols and dietary fibers. As antioxidants, phenolic compounds prevent oxidative damage to cellular organelles, proteins, lipids, DNA, and RNA (Peterson, 2001).

Oat, a kind of whole-grain which is commonly consumed by human being, contains many phytochemicals that display antioxidant activities, such as tocols, flavonoids, phytic acid, and phenolic acids (Peterson, 2001). In addition to these common antioxidants, there is a group of unique, low-molecular-weight, substituted *N*-cinnamoylanthranilic acids which have been only found in oats, called avenanthramides (AVs) (Collins, 1989). Among more than two dozen AVs that differ in substituent groups on the anthranilic acid and cinnamic acid rings, there are three predominant in oat: AV 2c, AV 2f and AV 2p (Bratt et al., 2003). In vitro experiments have shown that they have significant antioxidant capabilities, with 10–30 times higher radical scavenging activities than caffeic acid, ferulic acid, and vanillin (Emmons & Peterson, 1999). Moreover, AVs were found to have an antiproliferative influence on vascular smooth muscle cells (Nie, Wise, Peterson, & Meydani, 2006a), a process known to be a major limiting factor to the development of restenosis and atherosclerosis after angioplasty. Oat bran is the

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comestible, outer layer of whole oat and is produced by grinding oat groats to isolate the oat flour by sieving, bolting, and other suitable methods into different kinds of sub-fractions (Alakhrash, Anyanwu, & Tahergorabi, 2016). Most phenolics are located in the outer bran layer of whole oats, which could be an important food source of these phenolic compounds (Peterson, 2001).

There are two main kinds of oats in the world, one is *Avena sativa* L., which is cultivated more widely than that of the other one (*Avena nuda* L.). *Avena sativa* L. is widely distributed in North America, Europe, and Asia, while *Avena nuda* L. is more common in China (Zheng, Lu, Tian, & Zhao, 2006). Emmons and Peterson (2001) reported the influence of location and cultivar on antioxidant activities and phenolic contents of ethanol extracts from oat groats (*Avena sativa* L). Their research proposed a method of improving the phenolic concentrations and antioxidant activity of oats as quantitative character in the development program of cultivars. However, there are very few researches that reported phenolic compositions, antioxidant activities and antiproliferative effects of different cultivars of *Avena nuda* L.

The cellular antioxidant activity assay, CAA, is a newly developed method that quantifies the antioxidant activities of bioactive compounds in cultured cells (Wolfe & Liu, 2007). In this approach, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) is used as probe and taken up by cells when oxidized to fluorescent (DCF) by reactive nitrogen species and reactive oxygen. After the treatment of HepG2 cells, extracts or pure phytochemical compounds show the abilities of inhibiting the generation of DCF and quenching peroxyl radical. Compared with other antioxidant method, CAA assay provides a better understanding on how phytochemical compounds will be transported into cells under physiological conditions. The antioxidant activity of oat extract has been studied by chemical methods. However, no information is known about the cellular antioxidant properties and antiproliferative effects on cancer cells of different varieties of oats as far as we know. Therefore, the present study was aimed to measure the phenolic profiles, total phenolic content (TPC), antiproliferative effects, and antioxidant activities of two varieties of *Avena sativa* L, six varieties of *Avena nuda* L, one variety of wild oat (*Avena fatua* L.), and four oat brans using oxygen radical absorption capacity (ORAC) and cellular antioxidant activity (CAA) assays.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, protocatechuic acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, avenanthramide 2p, avenanthramide 2c, avenanthramide 2f, 2′,7′-Dichlorofluorescein diacetate (DCFH-DA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-Azobis (2-amidino-propane) dihydrochloride (AAPH) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Acetic acid and acetonitrile were of chromatographic grade and were obtained from Fisher Scientific Co. (Nepean, ON, Canada). Petroleum ether, Folin-Ciocalteu reagent, ethanol, potassium phosphate dibasic (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4), and sodium carbonate (Na_2CO_3) were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Human HepG2 liver cancer cell line were obtained from Beijing Cuizhu Biological Technology Co., Ltd (Beijing, China). Dulbecco's Modified Eagle's Medium-high glucose (DMEM) medium, fetal bovine serum (FBS), penicillin, streptomycin, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), hank's balanced salt solution (HBSS), and dimethyl sulfoxide (DMSO) were purchased from Gibco Biotechnology Company (Carlsbad, CA, USA).

2.2. Sample preparation

Nine varieties of oat were studied. Longyan 3 and Beiyan 1 were of *Avena sativa* L. and were purchased from local farmers in Guyuan, the Ningxia Hui Autonomous Region, China, and Hohhot, the Inner Mongolia Autonomous region, China, respectively. Likewise, six varieties of *Avena nuda* L. (Beixiao 1, Jinyan 8, Beixiao 8, Shaotong, Baiyan 2, and Dingxiao 7) and one wild oat (Zhenjiang) were also obtained from the local farm product market. Four varieties of oats (Longyan 3, Beiyan 1, Baiyan 2, and Dingxiao 7) were processed in a mill (Landert-Motoren AG Company, Buelach, Switzerland) to separate the bran and flour. The weight of bran was approximately 20–25% of the original oat groats. All samples were crushed into flour on a mill, then passed through a 60-mesh sieve. After milling, the oat flour was stored at −20 °C before use.

2.3. Extraction of phenolic compounds

Oat flour was mixed with petroleum ether in a ratio of 1:10 (w/v) for 16 h at room temperature. Subsequently, the mixture was centrifuged (15 min at 4000× rpm) and filtered through a filter paper. The residue was then re-extracted twice following the same method. Finally, the residue was gathered, dried using an oven for 3 h at 40 °C to remove any residual petroleum ether and stored at −20 °C before extraction.

Phenolic compounds were extracted following the method of Chen et al. (2016) with some modifications. Briefly, 4 g of sample was mixed with 40 mL of chilled 80% ethanol using a powerful electric blender (JB300D, Shanghai Specimen and Model Factory, Shanghai, China) for 20 min, and then the mixture was centrifuged for 20 min at 4000 rpm. The supernatant was collected and the remains was re-extracted twice using the same procedure as mentioned above. Finally, the supernatants were collected and evaporated using a rotary evaporator at 45 °C under reduced pressure, followed by lyophilization. The freeze-dried ethanol extract was added to 20 mL distilled water and mixed for 2 h, followed by extracting three times sequentially with 20 mL of *n*-butanol. This *n*-butanol fraction was centrifuged, evaporated, and lyophilized as described above. Finally, the extracts were resuspended in 70% methanol to a final volume of 4 mL and stored at −20 °C until use. Extractions for each sample were performed in triplicate.

2.4. Determination of total phenolic content (TPC)

The TPC of all extracts was determined by the colorimetric Folin-Ciocalteu method with slight modifications (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, each aliquot (1 mL) of oat extracts was diluted with 9 mL of distilled water. Then 0.05 mL of diluted extracts was blended with 0.05 mL of Folin-Ciocalteu's reagent. After mixing for 6 min in dark, 0.4 mL of deionized water and 0.5 mL of Na_2CO_3 solution (7:100, w/v) were added. The reaction mixture was kept for 90 min in dark and the absorbance of the final solution was determined at 760 nm using a microplate reader (SH-1000, Corona Electric Co. Ltd, Lethbridge, Canada). A standard curve was prepared using gallic acid, and the TPC was determined in terms of milligrams gallic acid equivalents per 100 g defatted sample (mg GAE/100 g, DW).

2.5. Determination of oxygen radical absorbance capacity (ORAC)

The ORAC of oat sample was measured as described by Huang, Ou, Hampschwoodill, Flanagan, and Prior (2002) with slight modifications. Briefly, all the reagents and extracts were made fresh and diluted with phosphate buffer (75 mM, pH 7.4). Trolox was used as the standard and diluted to a range of solutions with differ-