Food Chemistry 221 (2017) 997–1003

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Comparative assessment of phytochemical profiles, antioxidant and antiproliferative activities of Sea buckthorn (*Hippophaë rhamnoides* L.) berries

Ruixue Guo [a], Xinbo Guo [a,b,*], Tong Li [b], Xiong Fu [a], Rui Hai Liu [b,*]

[a] School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China
[b] Department of Food Science, Cornell University, Ithaca, NY 14853, United States

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 14 November 2016
Accepted 14 November 2016
Available online 15 November 2016

Keywords:
Sea buckthorn
Phytochemicals
Phenolics
Antioxidant activity
Antiproliferative activity

ABSTRACT

Phytochemical profiles, antioxidant and antiproliferative activities of berry extracts were evaluated and compared in four subspecies of Sea buckthorn (*Hippophaë rhamnoides* L.). Among the subspecies, *Hippophaë rhamnoides* L. subsp. *sinensis* exhibited highest total phenolics content (38.7 ± 1.3 mg GA equiv./g DW) and corresponding total antioxidant activity. Whereas maximum cellular antioxidant and antiproliferative activities were determined in *Hippophaë rhamnoides* L. subsp. *yunnanensis*. Total antioxidant activity was significantly associated to total phenolics, isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside. The cellular antioxidant activity and antiproliferative activity of phytochemicals were fairly correlated to phenolic acids and flavonoid aglycones. Lower median effective dose (EC_{50}) of individual compounds against human liver cancer HepG2 cells proliferation studies confirmed the better correlation between antiproliferative activity of Sea buckthorn extracts and flavonoid aglycones, including isorhamnetin, quercetin and kaempferol.

1. Introduction

Previous epidemiological studies have shown that significant associations among additive and synergistic interactions of phytochemicals especially phenolics and flavonoids contributed to consumers health and well-being (Liu, 2003). Advice to consumers is that a diet rich in bioactive compounds from a wide variety of foods may help in the reduction of risks associated with major chronic diseases, such as cardiovascular disease, cancer, diabetes and age-related function decline (Liu, 2013a). Like other food resources, berries have been reported as a rich source of phenolics, flavonoids, phenolic acids, anthocyanins and tannins. These naturally occurring bioactive constituents are powerful antioxidant, anticancer, anti-aging, antimicrobial, anti-inflammatory and anti-neurodegenerative ingredients (Liu, 2013b; Nile & Park, 2014).

Sea buckthorn *(Hippophaë rhamnoides* L.), is a deciduous shrub belong to botanical family Elaeagnaceae. It is wildly distributed in Asia and Europe as a pioneer plant used in water and soil conservation, and for land reclamation because of nitrogen-fixing root nodules (Khan, Akhtar, & Mahmood, 2010). Sea buckthorn berries are widely used as functional food supplement, source of jam and food coloring material (i.e. "Sea buckthorn yellow" pigment obtained from berries after juice extraction) in food industry (Beveridge, Li, Oomah, & Smith, 1999).

Sea buckthorn berries are rich in natural antioxidants including phenolics, flavonoids, ascorbic acid, tocopherols, fatty acids, carotenoids and organic acids (Tiitinen, Hakala, & Kallio, 2005). The berries' extract has been utilized for nutritional and medicinal purposes for centuries in Asia and Russia, such as nutraceuticals, cosmeceuticals and marketed herbal dietary supplements for prevention of cardiovascular and cerebrovascular diseases (Bal, Meda, Naik, & Satya, 2011; Beveridge et al., 1999; Xu, Kaur, Dhillon, Tappia, & Dhalla, 2011). Recently, research was mostly focused on the identification of compounds in Sea buckthorn extracts. The main identified components are ascorbic acid, carotenoids and various phenolics, including proanthocyanidins, gallic acid, ursolic acid, caffeic acid, cumaric acid, ferulic acid, catechin and epicatechin derivatives, quercetin, kaempferol, and isorhamnetin glycoside derivatives (Arimboor, Kumar, & Arumughan, 2008; Bal et al., 2011; Ma et al., 2016; Teleszko, Wojdylo, Rudzinska, Oszmianski, & Golis, 2015). *In vitro* antioxidant activity was reported to be closely related to the high content of ascorbic acid and total phenolics (Gao, Ohlander, Jeppsson, Björk, &

* Corresponding authors at: School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China (X. Guo), Department of Food Science, Cornell University, Ithaca, NY 14853, United States (R.H. Liu).
E-mail addresses: xbg720@gmail.com (X. Guo), rl23@cornell.edu (R.H. Liu).

http://dx.doi.org/10.1016/j.foodchem.2016.11.063
0308-8146/

Trajkovski, 2000; Kim, Kwon, Sa, & Kim, 2011; Rosch, Bergmann, Knorr, & Kroh, 2003), whereas the anticancer activity has been explored towards human liver cancer cells, breast cancer cells and colon cells and the effects were dramatically diversified depending on different composition of extracts (Grey, Widen, Adlercreutz, Rumpunen, & Duan, 2010; Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004).

Like other plant species, genetic variation, growth condition, degree of maturity and harvesting season can affect on phytochemical concentration, *in vitro* and *in vivo* activities in Sea buckthorn (Gao et al., 2000; Zheng, Kallio, & Yang, 2016). Though, phytochemical composition and *in vitro* antioxidant activity have been reported in Sea buckthorn, little is know about the cellular antioxidant and the antiproliferative activity of phytochemical extracts especially the phenolic fraction. The association among bioactivities of total phenolics and identified compounds has not been investigated. Furthermore, there is a scarcity of literature on the comparison of phytochemicals and their bioactivities from different subspecies of Sea buckthorn. Thus, the present work is aimed to make a comprehensive comparison of the phytochemical composition of four different subspecies of Sea buckthorn and to link these findings with extracellular and cellular antioxidant activity and antiproliferative activity against human liver cancer cells HepG2 combined with a correlation analysis.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin (QE), gallic acid (GA), Folin-Ciocalteu reagent, 2,2′-azobis-amidinopropane (ABAP), and dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich Inc. (St. Louis, USA). Protocatechuic acid (PA), ferulic acid (FA), epicathin (Epi), catechin (CE), QE glycosides, isorhamnetin (IS) glycosides and kaempferol (KA) glycosides were purchased from Weikeqi Biological (Chengdu, China). Human liver cancer cells HepG2 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Williams' medium E (WME), fetal bovine serum and other cell culture reagents were purchased from Gibico U.S. Biotechnology Co. All the other chemicals and solvents were of analytical grade.

2.2. Sample preparation

Four subspecies of Sea buckthorn *(Hippophaë rhamnoides* L.), viz: *H. rhamnoides* L. subsp. *sinensis* (Sinensis), *H. rhamnoides* L. subsp.*yunnanensis* (Yunnanensis), *H. rhamnoides* L. subsp. *mongolica* (Mongolica) and *H. rhamnoides* L. subsp. *turkestanica* (Turkestanica) were used in the present study. The berries of all four subspecies of Sea buckthorn were supplied by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. Berries were washed in running tap water and freeze-dried before extraction.

2.3. Extraction of free and bound phytochemicals

Phytochemicals of Sea buckthorn berries were extracted using method reported previously by Guo, Li, Tang, and Liu (2012) with modification. Berries were skimmed before extraction, and 80% acetone was applied to extract the free phytochemicals. All extracts were reconstituted using 10% methanol. For the bound fraction, 4 M NaOH was added into the dry residua for digestion. The mixture was acidified to pH 2 using concentrated hydrochloric acid, and supernatants were re-extracted by ethyl acetate. The ethyl acetate fraction was evaporated to dryness, followed by the addition of 10% methanol to reconstitute bound phytochemicals. Both extractions were stored at −40 °C for further analysis.

2.4. Determination of total phenolics

A Folin-Ciocalteu colorimetric method was adopted to determine the total phenolics following the method explained previously (Liu & Sun, 2003; Zhang & Liu, 2015). Data is expressed as milligram gallic acid equivalent per gram of dry weight of berries (mg GA equiv./g DW) in triplicate.

2.5. Determination of total flavonoids

The flavonoids in free and bound fractions were determined by the borohydride/chloranil protocol (SBC) as reported before (He, Liu, & Liu, 2008). Final values were reported in milligram catechin equivalent per gram of dry weight (mg catechin equiv./g DW) of berries by measuring the absorbance at 490 nm using a UV Visible Spectrophotometer.

2.6. Determination of phytochemical composition by RP-HPLC

The phytochemical composition of Sea buckthorn was assessed by RP-HPLC technique using a Waters 2998 Photodiode Array Detector (Waters Co., USA) at 370 nm and 280 nm wavelengths with a C18 column (250 × 4.6 mm, 5 μm) maintained at 35 °C (Ma et al., 2016; Teleszko et al., 2015). The flow rate of the binary elution phase (A: 0.1% trifluoroacetic acid in water, B: 50% acetonitrile-49.8% water-0.2% trifluoroacetic acid) was 1.0 mL/min using gradient elution as follows: 0–5 min (95% A), 5–40 min (95–75% A), 40–47 min (75–62% A), 47–49 min (62–55% A), 49–51 min (55% A), 51–70 min (55–20% A), 70–75 min (20–5% A), 75–77 min (5–95% A), 77–90 min (95% A). Measured values were expressed as milligrams per 100 g of dry weight of berries (mg/100 g DW).

2.7. Quantification of in vitro antioxidant activity

The total antioxidant activity were evaluated by the oxygen radical absorbance capacity (ORAC) and the peroxyl radical scavenging capacity (PSC) assays as described previously (Adom & Liu, 2005; Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). ORAC value was expressed as micromoles of Trolox equivalent per gram of dry weight of berries (μmol Trolox equiv./g DW), whereas to determine PSC, vitamin C was employed as calibration standard. Results were expressed as micromoles of vitamin C (Vit. C) equivalent per gram of dry weight of berries (μmol Vit. C equiv./g DW).

2.8. Cell culture

Human liver cancer cells HepG2 were cultured in WME medium supplemented with 5% fetal bovine serum, 10 mM Hepes, 2 mM L-glutamine, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 50 units/mL penicillin, 50 μg/mL streptomycin and 100 μg/mL gentamycin as described previously (Liu et al., 1994). HepG2 cultures were maintained at 37 °C in humidified atmosphere of 5% CO_2.

2.9. Cellular antioxidant activity of phytochemical extracts of Sea buckthorn

The cellular antioxidant activity (CAA) assay was used to quantify the cellular antioxidant capacity of Sea buckthorn berry extracts as explained previously (Wolfe & Liu, 2007). The CAA