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Inhibition of proliferation of human carcinoma cell lines by phenolic compounds from a bearberry-leaf crude extract and its fractions

Ryszard Amarowicz[a], *Ronald B. Pegg*[b,*]

[a]*Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland*
[b]*Department of Food Science & Technology, College of Agricultural and Environmental Sciences, The University of Georgia, 100 Cedar Street, Athens, GA 30602-2610, USA*

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ABSTRACT

Phenolic compounds were extracted from the leaves of bearberry, a potential functional food ingredient, using 80% (v/v) aqueous ethanol after which the resultant crude extract was applied to a Sephadex LH-20 column. A fraction comprising low-molecular-weight phenolics (LMW fraction) and sugars was eluted from the column with 95% (v/v) ethanol. A tannin fraction was then obtained after switching the mobile phase to acetone/water (1:1, v/v). Phenolic compounds present in the crude extract and its two fractions showed antiproliferative activities in a concentration-dependent manner against five carcinoma cell lines, namely MCF-7 (estrogen receptor-positive breast carcinoma), DU-145 (androgen receptor-negative prostate carcinoma), HT-29 (colon carcinoma), SK-MEL-5 and MDA-MB-435 (melanoma; skin carcinoma). IC_{50} data revealed that the tannin fraction was best at retarding cell proliferation in the tested cancer cell lines. The greatest inhibition at 1.5 μg fraction/mL assay was observed for the HT-29 colon carcinoma cell line. The proliferation of SK-MEL-5 skin carcinoma cells was also strongly inhibited by both the crude extract and LMW fraction. MDA-MB-435 cells were found to be the least sensitive for the materials tested, particularly for the LMW fraction.

1. Introduction

Bearberry (*Arctostaphylos uva-ursi* L. Sprengel) is a ubiquitous trailing evergreen shrub often forming mats 50–100 cm wide (Willard, 1992). It is found throughout North America, Asia, as well as Europe and serves little purpose other than as wildlife forage and an occasional ornamental species, despite its presence as an active ingredient in many commercial products. The plant grows preferentially on sandy and well-drained soil, and is common in open woodlands, rocky hills, and eroded slopes throughout the North American Prairies (Simonot, 2000). Also referred to as uva-ursi, kinnikinnik, mealberry, or bear's grape, bearberry has an official classification as a phytomedicine in various regions of Europe. Its commercial importance is based on astringent properties and beneficial effects in nephritis, kidney stones, and other diseases of the urinary tract. The indigenous peoples of North America prepare a functional tea from bearberry for such a purpose. In chronic inflammation of the bladder and kidneys, bearberry has no equal (Willard, 1992). The leaves are oval, leathery, and evergreen. The main compounds of bearberry leaf are arbutin (5–15%), methylarbutin (variable and up to 4%), and small quantities of the free aglycones. Other constituents include ursolic acid, tannic acid, gallic acid, *p*-coumaric acid, syringic acid, galloylarbutin, and up to 20% gallotannins, as well as some flavonoids, notably glycosides of quercetin, kaempferol, and myricetin (Barl, Loewen, & Svendsen, 1996).

* *Corresponding author.* Tel.: +1 706 542 1099; fax: +1 706 542 1050.
E-mail address: rpegg@uga.edu (R.B. Pegg).
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Due to the abundance of phenolic compounds, the bearberry plant is a warehouse of bioactives. Antioxidant and antimicrobial activities of the extracts of bearberry leaves have been reported: Amarowicz, Barl, and Pegg (1999) as well as Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil (2004) demonstrated that a bearberry-leaf extract possessed very strong reducing power, antioxidant properties in a β-carotene-linoleate model system, and antiradical properties using the DPPH radical scavenging assay and an EPR spin-trapping technique. Using the PHOTOCHEM® device to measure the scavenging potential of phenolic constituents against the superoxide anion radical, an ethanolic extract from bearberry leaves exhibited high antioxidative activity at inhibiting the photo-induced chemiluminescence (PCL) of luminol; that is, 5.93 mmol ascorbic acid equiv/g extract and 10.4 mmol Trolox equiv/g extract for the water- (ACW) and lipid-soluble (ACL) compounds assays, respectively (Pegg, Amarowicz, Naczk, & Shahidi, 2007). In cooked pork systems (*i.e.*, a true food system), the crude bearberry-leaf extract, its low-molecular-weight phenolics, and tannin fraction inhibited TBARS formation when added at a 200-ppm level even after 7 days of refrigerated storage by 97%, 49%, and 100%, respectively (Pegg, Amarowicz, & Naczk, 2005). The observed retardation in lipid oxidation/autoxidation by the bearberry-leaf extract in cooked pork patties demonstrates thermal stability of bioactive constituents in the extract which impart antioxidant activity. In the study of Carpenter, O'Grady, O'Callaghan, O'Brien, and Kerry (2007), addition of the bearberry-leaf extract impeded lipid oxidation (TBARS) in raw pork patties even on days 9 and 12 of refrigerated storage, relative to controls.

The inhibitory effects of bearberry-leaf extracts against *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* were reported by Cervenka et al. (2006). It was established by Annuk et al. (1999) that aqueous extracts of bearberry and cowberry leaves enhance cell aggregation of *Helicobacter pylori* strains tested by the salt aggregation test, and that the extract of bearberry possesses a remarkable bacteriostatic activity. In the investigation of Dykes, Amarowicz, and Pegg (2003), the bearberry-leaf extract displayed no antimicrobial activity on its own but enhanced the activity of nisin, as determined by minimum inhibitory concentrations against many Gram-positive, but none of the Gram-negative, bacteria. The bearberry-leaf extract strongly protected against hydrogen peroxide- and *tert*-butylhydroperoxide-induced DNA damage in U937 cells (Carpenter, O'Callaghan, O'Grady, Kerry, & O'Brien, 2006). Application of an extract from bearberry leaves has also been reported in cosmetic applications for skin lightening (Marks, 1997).

In this paper, we report on the inhibitory efficacy of a bearberry-leaf crude extract and its fractions against the proliferation of selected human carcinoma cell lines.

2. Materials and methods

2.1. Plant materials

Authenticated leaves of bearberry (*Arctostaphylos uva-ursi* L. Sprengel) were acquired from the Department of Plant Sciences, University of Saskatchewan in Saskatoon, SK, Canada.

2.2. Extraction

In a coffee mill (Model Tipo 203, Krups, New York, NY, USA), bearberry leaves were ground for 30 s with intermittent pulses. A crude extract of phenolic compounds was prepared using 80% (v/v) ethanol according to Amarowicz, Karamać, Weidner, Abe, and Shahidi (2002). Following evaporation of the ethanol at <45 °C using a Büchi Rotavapor/Water bath (Models EL 131 and 461, respectively, Brinkmann Instruments (Canada) Ltd., Toronto, ON, Canada), the residual water was removed by lyophilization with a FreeZone 12 L Freeze Dry System (Model 77540, Labconco Corporation, Kansas City, MO). The dried extract was stored in a refrigerator at 4 °C until analyzed further.

2.3. Column chromatography

Phenolic compounds present in the bearberry crude extract were separated into two dominant fractions using a Sephadex LH-20 column (bead size: 25–100 μm; Chromaflex column, 40 × 250 mm [I.D. × length], Kontes, Vineland, NJ, USA) (Strumeyer & Malin, 1975). The fractions comprised basically (i) low-molecular-weight phenolics (LMW fraction) and (ii) high-molecular-weight polyphenolics (*i.e.*, tannin fraction). The LMW fraction was eluted from the column first with 95% (v/v) ethanol, whereas the tannin fraction was obtained using the more polar 50% (v/v) aqueous acetone as the mobile phase. Organic solvents were evaporated *in vacuo* at <45 °C and residual water in the tannin fraction was removed by lyophilization. The dried fractions were stored in a refrigerator at 4 °C until analyzed further.

2.4. In vitro assay of antiproliferative activity

Cell culture medium and fetal bovine serum were purchased from Invitrogen (Life Technologies, Grand Island, NY, USA). Cell culture plasticware, including 75 cm^2 canted-neck cell culture flasks, 96-well flat-bottomed tissue-culture-treated plates, cell scrappers, sterile pipettes and tips, was acquired from Fisher Scientific Company (Ottawa, ON, Canada), VWR International (Mississauga, ON), and Invitrogen. The five cell lines against which the bearberry-leaf crude extract and its fractions were tested were representative of a variety of human tumor types and included MCF-7 (estrogen receptor-positive breast carcinoma), DU-145 (androgen receptor-negative prostate carcinoma), HT-29 (colon carcinoma), SK-MEL-5 (melanoma; skin carcinoma) and MDA-MB-435 (melanoma; not an estrogen receptor-negative breast carcinoma as originally proposed). All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cultured cell lines were maintained in minimum essential medium with nucleosides plus 10% endotoxin-free, heat-inactivated fetal bovine serum, penicillin (50k U/L), and streptomycin (50 mg/L) (*i.e.*, the growth medium). Cultures were incubated in a humidified atmosphere of 5% (v/v) CO_2 at 37 °C. Rapidly proliferating cells were utilized for establishing cultures of experimental cells, which were allowed to plate overnight in 96-well plates or 75 cm^2 flasks before manipulation (Ferguson, Kurowska, Freeman, Chambers, & Koropatnick, 2004).