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Original Article

Phenolic composition, antioxidant and anti-proliferative activities of edible and medicinal plants from the Peruvian Amazon

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ABSTRACT

Among 23 extracts of medicinal and edible plants tested, *Mauritia flexuosa* L.f., Arecaceae, showed significant antioxidant ability (DPPH and ORAC = 1062.9 and 645.9 ± 51.4 μg TE/mg extract, respectively), while *Annona montana* Macfad., Annonaceae, demonstrated the most promising anti-proliferative effect (IC_{50} for Hep-G2 and HT-29 = 2.7 and 9.0 μg/ml, respectively). However, combinatory antioxidant/anti-proliferative effect was only detected in *Oenocarpus bataua* Mart., Arecaceae (DPPH = 903.8 and ORAC = 1024 μg TE/mg extract; IC_{50} for Hep-G2 and HT-29 at 102.6 and 38.8 μg/ml, respectively) and *Inga edulis* Mart., Fabaceae (DPPH = 337.0 and ORAC = 795.7 μg TE/mg extract; IC_{50} for Hep-G2 and HT-29 at 36.3 and 57.9 μg/ml, respectively). Phenolic content was positively correlated with antioxidant potential, however not with anti-proliferative effect. None of these extracts possessed toxicity towards normal foetal lung cells, suggesting their possible use in development of novel plant-based agents with preventive and/or therapeutic action against oxidative stress-related diseases.

Introduction

It is widely accepted that oxidative stress is involved in the development and/or secondary pathology of various human diseases (Halliwell and Gutteridge, 2007). Several studies show evidence that regular consumption of plant foods is associated with lowered risk of incidence of these (Alasalvar and Shahidi, 2013). It is believed that health beneficial effect of plants foodstuffs can mainly be credited to number of phenolic compounds and their ability to promote antioxidant effect (Brewer, 2011). Currently, antioxidant activity is primarily examined in common food plants such as fruits and vegetables. However, recent studies indicate that other plant categories, such as medicinal plants, also possess significant antioxidant efficacy (Jaberian et al., 2013).

Previously it was proposed that progression of cancer is strongly related to oxidative stress. Thus, validation of antioxidant effect of tested plant material is nowadays routinely supplemented with analysis of anti-proliferative activity against various types of carcinoma cell lines (Loizzo et al., 2014; da Costa et al., 2015). In case of phenylpropanoids, the compounds toxic to normal cells (*e.g.* podophyllotoxin) may be responsible for this anti-carcinomatous effect (Dewick, 2009). However, more recent studies are showing that dietary phenolics (*e.g.* flavonoids) may exert anti-proliferative effect as well (Ferry et al., 1996; Anter et al., 2011). Despite the fact that medicinal plants are regarded as the main sources of anti-neoplastic agents, there is now an increased interest in research of edible plants' anti-proliferative effects (De la Rosa et al., 2014).

Even though plants are generally considered as very important factor for maintaining food and health security (mainly in third world countries), health-promoting properties of majority of these plants have not been properly verified *via* modern scientific methods. Despite the well-documented traditional use of plants from

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that region for treatment of diseases related to oxidative stress such as cancer, diabetes, cardiovascular, inflammatory and neurodegenerative diseases (Duke and Vásquez, 1994; Duke et al., 2009), to our best knowledge, only a very small proportion of edible and medicinal plants from the Peruvian Amazon have ever been assessed for their combinatory antioxidant/anti-proliferative properties (Neri-Numa et al., 2013). In addition, for a majority of these plants, the phytochemical profile was never fully characterized (Newman and Cragg, 2012).

Proceeding from these facts, this study provides detailed information on *in vitro* antioxidant and anti-proliferative potential of 23 methanol extracts from twelve Peruvian medicinal and edible plant species which were additionally analyzed by UHPLC-MS/MS with the aim to determine the relationship between biological activity and phenolic compound content.

Materials and methods

Plant material

Selection of plant material was based on previously reported data on traditional use for treatment of diseases likely to be associated with oxidative stress (Table 1). Plants were collected from farms in areas surrounding Pucallpa city in the Peruvian Amazon, between March and June 2013. Voucher specimens were authenticated by Ymber Bendezu Flores and deposited at herbarium of IVITA-Pucallpa, Universidad Nacional Mayor de San Marcos (UNMSM).

Sample preparation

Fresh plant samples were frozen and lyophilized in FreeZone 1 freeze dry system (Labconco, Kansas City, USA). Samples were finely grounded in IKA A 11 electric mill (IKA Werke GMBH&Co.KG, Staufen, Germany). Subsequently, 2 g of plant material were extracted in a Soxhlet-like IKA 50 extractor (IKA Werke GMBH&Co.KG, Staufen, Germany) in 70% ethanol in a 1/20 (w/v) proportion during three 7-min cycles at 130 °C followed by cooling to 50 °C. Extracts were subsequently filtered through a Teflon (PTFE) syringe filter (17 × 0.45 mm) and evaporated to dryness using a rotary evaporator R-3000 (Büchi, Flawil, Switzerland) *in vacuo* at 40 °C. Dry residues were dissolved in 80% methanol to create 50 mg/ml stock solutions and subsequently stored at −20 °C. Extracts for UHPLC–MS/MS analysis were evaporated to dryness and re-dissolved at a concentration of 0.4 g dry weight per ml.

Chemicals and reagents

The following chemicals and reagents, purchased from Sigma–Aldrich (Prague, Czech Republic), were used in this study: 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiazolyl blue tetrazolium bromide (MTT), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Dulbecco's modified Eagle's medium (DMEM), Eagle's minimum essential medium (EMEM), fluorescein (FL), Folin-Ciocalteu reagent, Griess reagent and penicillin–streptomycin solution. Analytical standards (given in Table 2) were purchased from Indofine Chemical Company (Hillsborough, USA) or Sigma–Aldrich. Formic acid, methanol and water of HPLC-grade were purchased from Merck (Darmstadt, Germany); ethanol and dimethyl sulfoxide (DMSO) from Penta (Prague, Czech Republic).

Cell culture

Liver carcinoma cell line Hep-G2 and normal foetal lung cells MRC-5 (ATCC, Rockville, USA) were maintained in EMEM supplemented with foetal bovine serum (10%), penicillin–streptomycin solution (1%), non-essential amino acids (1%) and glutamine (4 mM and 2 mM for Hep-G2 and MRC-5, respectively). Colon carcinoma cell line HT-29 (ATCC, Rockville, USA) was maintained in DMEM solution and otherwise were treated identically as Hep-G2 and MRC-5. Cultures were incubated in 5% CO_2 atmosphere at 37 °C using MCO 170AIC-PE CO_2 incubator (Panasonic Corporation, Osaka, Japan).

In vitro *antioxidant activity*

DPPH radical-scavenging assay

Slightly modified method described by Sharma and Bhat (2009) was used for evaluation of samples' ability to inhibit DPPH radical. Concentrations and volumes of samples, standard and reagent were adjusted in order to be used in a microplate format. Two-fold serial dilution of each sample (final concentration range: 1.25–5120 μg/ml) was prepared in absolute methanol (175 μl) in 96-well microtiter plates. Subsequently, 25 μl of freshly prepared 1 mM DPPH in methanol was added to each well in order to start the radical-antioxidant reaction. Mixture was kept in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using Infinite 200 reader (Tecan, Männedorf, Switzerland). Trolox (at concentrations 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 254 and 512 μg/ml) was used as a positive control and methanol as a blank. Results were expressed as Trolox equivalents (μg TE/mg extract).

Oxygen radical absorbance capacity (ORAC) assay

Adjusted ORAC method was used for determination of samples' ability to protect FL from AAPH-induced damage (Cao and Prior, 1998; Ou et al., 2001). Outer wells of black absorbance 96-wellmicrotiter plates were filled with 200 μl of distilled water, in order to provide better thermal mass stability, as suggested by Held (2005). Stock solutions of AAPH radical (153 mM) and FL (540 μM) were prepared in 75 mM phosphate buffer (pH 7.0). Afterwards, 25 μl of each sample at final concentration range of 6.4–32 μg/ml were diluted in 150 μl FL (54 nM) and incubated at 37 °C for 10 min. Reaction was started by adding 25 μl AAPH Standard calibration curves of positive control Trolox were acquired at five concentration levels (0.5, 1, 2, 4, 8 μg/ml). The 75 mM phosphate buffer was used as a blank. Fluorescence changes were measured in 1-min intervals for 120 min using an Infinite 200 reader with emission and absorbance wavelengths set at 494 nm and 518 nm, respectively. Results were expressed as Trolox equivalents (μg TE/mg extract).

Total phenolic content (TPC)

TPC was measured using the method developed by Singleton et al. (1998). Firstly, each sample (diluted in water; final concentration ranging from 16 to 80 μg/ml) with a volume of 100 μl was added to 96-well microtiter plates. Thereafter, 25 μl of pure Folin-Ciocalteu reagent was added. Plate was inserted in an orbital shaker at 40 rpm for 10 min. Reaction was started by adding 75 μl of 12% Na_2CO_3 (w/v). Mixture was kept in dark at 37 °C for 2 h. Absorbance was measured at 700 nm (Infinite 200 reader). Nine concentration levels of gallic acid (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 μg/ml) were used to create the standard calibration curve. Results were expressed as gallic acid equivalents (μg GAE/mg extract).

Cell viability assay

Modified method based on metabolization of MTT to blue formazan by mitochondrial dehydrogenases in living cells previously

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