



Original Article

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



Jan Tauchen^a, Ludvik Bortl^{b,c}, Lukas Huml^{c,d}, Petra Miksatkova^{d,e}, Ivo Dosekocil^f, Petr Marsik^a, Pablo Pedro Panduro Villegas^g, Ymber Bendezu Flores^h, Patrick Van Damme^{b,i}, Bohdan Lojka^b, Jaroslav Havlik^f, Oldrich Lapcik^d, Ladislav Kokoska^{b,*}

^a Department of Quality of Agricultural Products, Faculty of Agrobiolgy, Food and Natural Resources, Czech University of Life Sciences Prague, Prague 6-Suchdol, Czech Republic

^b Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague 6-Suchdol, Czech Republic

^c Students for the Living Amazon, o.s.p., Prague 6 - Bubenec, Czech Republic

^d Department of Chemistry of Natural Compounds, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Prague 6-Dejvice, Czech Republic

^e Forensic Laboratory of Biologically Active Substances, University of Chemistry and Technology, Prague, Prague 6-Dejvice, Czech Republic

^f Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiolgy, Food and Natural Resources, Czech University of Life Sciences Prague, Prague 6-Suchdol, Czech Republic

^g Escuela de Ingenieria Agroforestal Acuicola, Facultad de Ingenieria y Ciencias Ambientales, Universidad Nacional Intercultural de la Amazonia, Pucallpa, Peru

^h Instituto Nacional de Investigación Agraria, Lima, Peru

ⁱ Department of Plant Production, Faculty of Bio-Science Engineering, Ghent University, Ghent, Belgium

ARTICLE INFO

Article history:

Received 2 December 2015

Accepted 23 March 2016

Available online 14 June 2016

Keywords:

Antioxidant

Anticarcinogenic

Phenolic compounds

Plant extracts

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 (IC₅₀ 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; IC₅₀ 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; IC₅₀ 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.

© 2016 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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

* Corresponding author.

E-mail: kokoska@ftz.czu.cz (L. Kokoska).

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 Free-Zone 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-methylpropionamide) 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% CO₂ atmosphere at 37 °C using MCO 170AIC-PE CO₂ 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-well microtiter 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% Na₂CO₃ (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 metabolism of MTT to blue formazan by mitochondrial dehydrogenases in living cells previously

Table 1
Ethnobotanical data of tested plant species.

Botanical name [voucher specimen]	Family	Vernacular name ^a	Part(s) tested	Way of consumption	Traditional medicinal use ^b	Reference ^d
<i>Annona montana</i> Macfad. [LB0037]	Annonaceae	Guanabana	Leaf	Infusion/decoction	Cancer	(Duke and Vásquez, 1994; Barbalho et al., 2012)
<i>Bertholletia excelsa</i> Bonpl. [LB0120]	Lecythidaceae	Castaña	Leaf	Infusion/decoction	Cancer	(Duke et al., 2009)
<i>Bunchosia armeniaca</i> (Cav.) DC. [LB0044]	Malpighiaceae	Ciruela (china)	Seed, pericarp	Fruit eaten fresh or in processed form	n/a ^c	(Lim, 2012)
<i>Genipa americana</i> L. [LB0032]	Rubiaceae	Huito, Lana	Whole fruit	Fruit eaten fresh or in processed form	Cancer	(Duke et al., 2009)
<i>Inga edulis</i> Mart. [LB0013]	Fabaceae	Guaba	Leaf, pericarp, aril, seed	Pulp eaten fresh or used for flavouring; leaves used as infusion	Rheumatoid arthritis	(Lim, 2012)
<i>Mauritia flexuosa</i> L. f. [LB0084]	Arecaceae	Aguaje	Exocarp, mesocarp	Processed into juices	Neurodegenerative diseases	(Duke et al., 2009)
<i>Myrciaria dubia</i> (Kunth) McVaugh [LB0095]	Myrtaceae	Camu camu	Leaf, pericarp	Processed into juices; leaves used as infusion	Neurodegenerative diseases	(Duke et al., 2009)
<i>Oenocarpus bataua</i> Mart. [LB0123]	Arecaceae	Ungurahui	Exocarp + mesocarp	Fruit eaten fresh or in processed form	Cancer	(Sosnowska and Balslev, 2009)
<i>Solanum sessiliflorum</i> Dunal [LB0046]	Solanaceae	Cocona	Whole fruit	Fruit eaten fresh or cooked	Diabetes	(Duke and Vásquez, 1994; Lim, 2012)
<i>Theobroma bicolor</i> Humb. & Bonpl. [LB0073]	Malvaceae	Macambo	Pericarp, aril + seed	Pulp is eaten fresh, seeds are consumed roasted	Cardiovascular diseases, cancer, diabetes	(Lim, 2012)
<i>Theobroma cacao</i> L. [LB0016]	Malvaceae	Cacao	Leaf, pericarp, aril + seed	Pulp is eaten fresh, seeds are consumed roasted; leaves used as infusion/decoction	Cardiovascular diseases, cancer, diabetes	(Lim, 2012)
<i>Theobroma grandiflorum</i> (Willd. ex Spreng.) K.Schum. [LB0052]	Malvaceae	Copoazú	Leaf, pericarp, aril	Pulp is eaten fresh, seeds are consumed roasted; leaves used as infusion/decoction	Cardiovascular diseases, cancer, diabetes	(Lim, 2012)

^a Vernacular names apply in the area of collection (Ucayali region, Peruvian Amazon).

^b Only diseases or conditions likely to be associated to oxidative stress are recorded.

^c To our best knowledge, documentation on traditional use as remedy in the Amazon region is not available.

^d References are related to plant parts tested in this study.

described by Mosmann (1983) was used to test cell viability. Cells were pre-incubated (24 h) in a 96-well plate at a density of 2.5×10^3 cells per well and afterwards treated with two-fold serial dilutions of plant extracts in range of 0.24–500 $\mu\text{g/ml}$ for 72 h. After addition of MTT reagent (1 mg/ml) in EMEM or DMEM solution, plates were incubated for an additional 2 h. Media were then removed, and the intracellular formazan product was dissolved in 100 μl of DMSO. Absorbance was measured at 555 nm (Infinite 200 reader) and percentage of viability calculated when compared to untreated control. Results were expressed as 50% inhibitory concentration (IC_{50}) in $\mu\text{g/ml}$.

Characterization of phenolic compounds by UHPLC-MS/MS

UHPLC-MS/MS analysis of 30 phenolic acids, flavonoids and related compounds was carried out using modified method previously described by Miksatkova et al. (2014). Instrument was composed of Agilent 1290 Infinity instrument (Agilent, Santa Clara, USA) equipped with a binary pump (G4220B), autosampler (G4226A), autosampler thermostat (G1330B), column compartment thermostat (G1316C), coupled to an Agilent triple quadrupole mass spectrometer (6460A) with a Jet Stream ESI ion source. A Kinetex PFP column (2.6 μm , 100 A, 150.0 \times 3.0 mm) from Phenomenex (Torrance, USA) was used for the chromatographic separation of extracts. Column temperature was set at 35 °C and injection volume at 3 μl . Gradient elution was carried out employing mobile phase A (10 mM formic acid) and B (100% methanol) as follows: 0 min, 60:40 (A:B); 10 min, 0:100; 14 min, 0:100; 15 min, 60:40, 19 min, 60:40 to reach starting conditions. Flow rate was set at 0.3 ml/min. The MS/MS apparatus was operating in positive and negative mode in the same analysis. Conditions of Jet Stream Ion Source were: drying

gas temperature 290 °C; drying gas flow 4 l/min; sheath gas temperature 380 °C; sheath gas flow 10 l/min; nebulizer pressure 35 psi; nozzle voltage 2.0 kV and 1.8 kV; and capillary voltage was set at 3.5 and 5.0 kV in positive and negative acquisitions, respectively. Nitrogen was used as collision gas. Multiple reaction monitoring (MRM) mode was used for the detection. Peak areas of standards (eleven concentration levels ranging from 0.1 to 1000 ng/ml – i.e. 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 500 and 1000 ng/ml) were plotted against the corresponding response using weighed linear regression to generate calibration curves. Specific parameters of MS/MS method are given in Table 2. Agilent Mass Hunter (Agilent, Santa Clara, USA) was used for data acquisition and quantification of samples.

Statistical analysis

All *in vitro* assays were performed in three separated experiments, each in duplicate. UHPLC-MS/MS data were acquired in two separate experimental measurements. Results were expressed as mean values with standard deviations. Linear correlation coefficients (r^2) were established using Pearson product moment correlation between TPC and (i) antioxidant assay (plotted against DPPH and ORAC values) and (ii) anti-proliferative assay (plotted against IC_{50} values for Hep-G2 and HT-29). Statistical analysis was performed in Statistica 7.1 (StatSoft Inc., Tulsa, USA) software.

Results

Six plant extracts out of total 23 tested, namely leaves of *Annona montana*, *Inga edulis*, *Myrciaria dubia* and *Theobroma grandiflorum*; exocarp of *Mauritia flexuosa* and fruit without seed of *Oenocarpus bataua* showed significant antioxidant and/or anti-proliferative

Table 2
Transitions and MS/MS parameters of analyzed compounds.

Compound	Ionization mode	Retention time (min) ^a	Fragmentor (V)	Precursor ion (m/z)	Product (m/z)				LOD (ng/ml) ^c	LOQ (ng/ml) ^d
					Quantification transition	E (eV) ^b	Confirmation transition	E (eV) ^b		
Anisic acid	ESI+	5.35 (0.5)	72	153.06	77.2	5	109.1	9	3.6	11.9
Apigenin	ESI–	7.98 (0.6)	108	269.04	117.0	3	151.1	7	0.1	0.3
Apigenin-7-glucoside	ESI+	5.54 (0.5)	109	433.12	271.0	3	153.0	60	0.2	0.8
Caffeic acid	ESI–	3.60 (0.4)	81	179.00	89.0	30	135.1	3	2.3	7.7
Chlorogenic acid	ESI–	3.01 (1.0)	81	353.09	191.1	9	e	e	0.6	1.9
p-Coumaric acid	ESI+	4.37 (0.5)	60	165.05	147.1	9	e	e	0.9	3.0
(–)-Epicatechin	ESI–	3.10 (0.5)	111	289.07	109	3	245.1	5	0.7	2.4
Ferulic acid	ESI+	4.65 (0.5)	63	195.07	145.0	3	177.0	5	1.0	3.2
Flavone	ESI+	8.80 (0.5)	119	223.10	77.2	1	121.1	25	0.2	0.8
Gallic acid	ESI–	2.43 (0.6)	75	169.01	124.9	9	169.0	5	0.7	2.2
Hesperetin	ESI–	7.30 (0.5)	108	301.07	164.0	17	286.0	9	0.1	0.3
Isoquercitrin	ESI–	4.87 (0.5)	150	463.09	300.3	18	271.0	42	0.4	1.2
Kaempferol	EIS+	7.55 (0.6)	161	287.06	153.0	1	69.1	3	1.1	3.6
Luteolin	ESI–	7.23 (0.8)	128	285.04	133.0	33	151.0	0	0.4	1.4
Luteolin-7-glucoside	ESI–	4.92 (0.6)	151	447.09	285.0	25	133.0	0	1.3	4.4
Morin	ESI+	6.22 (0.8)	141	303.05	152.9	0	69.1	4	22.0	73.2
Myricetin	ESI–	5.72 (0.8)	113	317.03	151.0	7	137.0	1	30.3	101.0
Naringenin	ESI–	7.05 (0.5)	93	271.06	119.0	1	151.0	9	0.1	0.1
Naringenin-7-glucoside	ESI–	4.76 (0.5)	117	433.11	271.1	0	119.0	0	0.1	0.4
Naringin	ESI–	4.57 (0.5)	166	579.17	271.1	9	151.0	9	3.0	10.0
Pterostilbene	ESI–	9.07 (0.5)	102	255.10	240.1	3	197.1	5	0.3	1.0
Quercetin	ESI–	6.70 (0.9)	106	301.03	151.0	3	121.1	4	1.3	4.2
Quercetin-3-arabinoside	ESI–	5.18 (0.5)	114	433.07	300.0	7	271.0	7	0.3	0.9
Resveratrol	ESI–	5.25 (0.5)	102	227.10	143.0	5	185.1	3	0.1	0.2
Rutin	ESI–	4.69 (0.5)	163	609.14	271.0	1	300.0	5	0.3	0.9
Salicylic acid	ESI–	5.22 (0.6)	72	137.02	93.1	3	65.1	9	0.5	1.8
Scopoletin	ESI–	4.84 (0.5)	81	191.03	176.0	0	104.0	2	0.4	1.2
Sinapic acid	ESI–	4.80 (0.4)	81	223.06	208.1	9	149.0	7	0.1	0.2
syringic acid	ESI+	3.99 (0.5)	60	199.06	140.1	3	77.2	5	0.5	1.7
Vanillic acid	ESI+	3.79 (0.5)	78	169.05	65.2	2	125.1	5	1.4	4.6

^a Retention time window (minutes) is given in brackets.^b Collision energy.^c Limits of detection (signal-to-noise ratio of 3).^d Limits of quantification (signal-to-noise ratio of 10).^e Only one transition was used for detection.

activity (Table 3). None of the tested plants exhibited toxicity to normal cells. Gallic, chlorogenic, salicylic and vanillic acids, (–)-epicatechin, myricetin, quercetin and its derivatives (isoquercitrin, quercetin-3-arabinoside and rutin) were the most predominant constituents in all analyzed extracts. Complete results for antioxidant efficacy and cytotoxicity are given in Table 3, whereas for UHPLC-MS/MS analysis in Tables 4–6.

Antioxidant activity

In DPPH assay, *M. flexuosa* (exocarp) extract possessed higher antioxidant potential than positive control Trolox (1062.9 µg TE/mg). The promising antioxidant efficacy was also detected for *O. bataua* fruit, *T. grandiflorum* leaves, *M. dubia* leaves and pericarp (903.8, 714.8, 641.9, and 440.9 µg TE/mg, respectively). Other extracts showed only weak to moderate free radical scavenging ability (range 0.2–337.0 µg TE/mg). In ORAC assay, *O. bataua* fruits showed highest antioxidant activity (1024.4 µg TE/mg), being stronger than Trolox. Leaf extracts of *T. grandiflorum*, *I. edulis* and *M. dubia*; extracts of *M. flexuosa* exocarp and *I. edulis* pericarp, also showed promising results with µg TE/mg values at 821.9, 795.7, 642.6, 645.9 and 645.7, respectively. The rest of the tested plants showed weak to moderate efficacy (from 10.7 to 613.3 µg TE/mg). Highest content of phenolic compounds (TPC assay) was observed in *O. bataua* fruit, *M. flexuosa* (exocarp), *T. grandiflorum* (leaves) and *M. dubia* (leaves and pericarp) with values at 672.3, 461.5, 400.6 and 342.0 µg GAE/mg, respectively (Table 3). The rest of plant extracts tested exhibited only low to moderate quantities of phenolic compounds (range 3.6–266.4 µg GAE/mg). Strong correlation was found

between TPC and both antioxidant assays used: DPPH ($r=0.946$) and ORAC ($r=0.899$).

Cell viability assay

A. montana (leaves) demonstrated to be the plant extract with the most-promising anti-proliferative effect to Hep-G2 cell line ($IC_{50}=2.7$ µg/ml), followed by extracts of *I. edulis* (leaves), *O. bataua* (fruit), *M. dubia* (pericarp, leaves), *T. grandiflorum* (leaves) and *I. edulis* (seed) (IC_{50} 's at 36.3, 102.6, 124.0, 149.5, 140.4 and 179.1 µg/ml, respectively). The other samples exhibited very low anti-proliferative activity to carcinoma cells with IC_{50} values higher than 500 µg/ml. In tests performed on HT-29 cell line, leaves of *A. montana* proved again to be the most-effective plant extract, with IC_{50} value at 9 µg/ml, followed by extracts of *O. bataua* fruit, leaves of *Bertholletia excelsa*, *T. grandiflorum*, *I. edulis*, *Theobroma cacao* and pericarp of *T. grandiflorum*: IC_{50} 's at 38.8, 41.3, 46.5, 57.9, 82.6, and 83.9 µg/ml, respectively. The other plants possessed IC_{50} values in a range of 137.6–294.0 µg/ml or exhibited non-toxic effect ($IC_{50} > 500$ µg/ml). Toxicity assessment on normal MRC-5 cells revealed all plant extracts to be non-toxic ($IC_{50} > 500$ µg/ml) (Table 3). Weak correlation was found between phenolic content and cell viability assays, whereas correlation coefficients of TPC vs. IC_{50} 's for Hep-G2 and HT-29 were 0.050 and 0.230, respectively.

UHPLC-MS/MS analysis

With regard to quantity of phenolic compounds identified by UHPLC-MS/MS in individual species, the highest amount was

Table 3
Total phenolic content, antioxidant and anti-proliferative activity of tested plant extracts.

Species	Plant part(s) ^a	Antioxidant assay/mean ± SD ^b			Cell type/mean IC ₅₀ ± SD ^b		
		DPPH ^c	ORAC ^c	TPC ^d	Hep-G2 ^e	HT-29 ^e	MRC-5 ^e
<i>A. montana</i>	L	186.9 ± 16.7	608.3 ± 18.8	196.8 ± 10.7	2.7 ± 0.2	9.0 ± 1.3	>500
<i>B. excelsa</i>	L	258.8 ± 6.4	613.3 ± 26.8	266.4 ± 14.1	>500	41.3 ± 3.4	>500
<i>B. armeniaca</i>	P	1.5 ± 0.1	10.7 ± 1.0	6.5 ± 0.7	>500	>500	>500
	S	□0.2	27.3 ± 2.6	3.6 ± 0.7	>500	>500	>500
<i>G. americana</i>	FW	20.6 ± 4.7	113.9 ± 4.7	28.0 ± 2.2	>500	>500	>500
<i>I. edulis</i>	A	21.2 ± 3.2	69.9 ± 3.9	20.8 ± 1.8	>500	>500	>500
	L	337.0 ± 26.3	795.7 ± 25.4	262.3 ± 11.8	36.3 ± 15.7	57.9 ± 2.1	>500
	P	288.0 ± 8.8	645.7 ± 33.9	207.2 ± 13.8	>500	190.9 ± 1.1	>500
	S	7.9 ± 0.5	51.5 ± 2.8	17.2 ± 3.3	179.1 ± 13.7	148.5 ± 41.7	>500
<i>M. flexuosa</i>	E	1062.9 ± 163.9	645.9 ± 51.4	461.5 ± 32.5	>500	>500	>500
	M	130.8 ± 15.4	244.5 ± 7.5	87.0 ± 3.9	>500	262.6 ± 2.2	>500
<i>M. dubia</i>	L	641.9 ± 127.9	642.6 ± 32.7	342.0 ± 18.7	149.5 ± 23.8	>500	>500
	P	440.9 ± 62.7	333.0 ± 21.6	275.8 ± 13.2	124.0 ± 12.3	>500	>500
<i>O. bataua</i>	FO	903.8 ± 158.1	1024.4 ± 69.3	672.3 ± 46.9	102.6 ± 4.2	38.8 ± 5.4	>500
<i>S. sessiliflorum</i>	FW	8.8 ± 1.2	88.9 ± 6.0	18.1 ± 2.0	>500	>500	>500
<i>T. bicolor</i>	A+S	107.4 ± 13.6	243.0 ± 20.7	102.9 ± 4.3	>500	294.0 ± 34.9	>500
	P	152.4 ± 3.8	217.9 ± 16.8	104.9 ± 4.7	388.5 ± 22.2	156.8 ± 11.4	>500
<i>T. cacao</i>	A+S	329.9 ± 59.5	587.3 ± 48.8	217.2 ± 5.5	407.8 ± 4.6	137.6 ± 12.0	>500
	L	152.2 ± 7.5	542.7 ± 23.1	149.8 ± 4.4	>500	82.6 ± 5.5	>500
	P	51.6 ± 6.1	179.7 ± 12.3	49.4 ± 3.6	>500	>500	>500
<i>T. grandiflorum</i>	A	25.9 ± 4.2	145.5 ± 9.5	57.6 ± 3.2	>500	>500	>500
	L	714.8 ± 111.3	821.9 ± 65.6	400.6 ± 25.9	140.4 ± 3.0	46.5 ± 0.2	>500
	P	188.2 ± 10.9	434.9 ± 38.8	163.0 ± 8.1	218.6 ± 26.2	83.9 ± 0.7	>500

A, aril; E, exocarp; FO, fruit without seed; FW, whole fruit; M, mesocarp; L, leaves; P, pericarp; S, seed.

^a Abbreviation refers to plant part(s).

^b Standard deviation.

^c µg TE/mg extract.

^d µg GAE/mg extract.

^e µg/ml.

evidenced in *M. flexuosa* (exocarp) and *Solanum sessiliflorum* with values of 0.003% of dry weight. Noticeable results were also observed for *M. flexuosa* (mesocarp), pericarps of *T. bicolor* and *T. grandiflorum*, *O. bataua* (fruit without seed), and leaves of *I. edulis*, whose phenolic compound content in dry weight was detected at 0.002%. Remaining species had 0.001% or lower percentages of phenolic compounds on a dry weight basis.

Predominant constituents identified in *M. flexuosa* (exocarp) and *S. sessiliflorum*, which are expressed as percentage of phenolic compounds quantity, were chlorogenic acid, rutin and isoquercitrin (36, 23, and 23% for *M. flexuosa* and 50, 12, and 16% for *S. sessiliflorum*, respectively). Similar to the exocarp of *M. flexuosa*, its mesocarp predominantly contained chlorogenic acid, rutin and isoquercitrin, although in slightly different ratios (48, 19, and 27%, respectively); (–)-epicatechin (31%) and chlorogenic acid (27%) were regarded as principal constituents in pericarp of *T. bicolor*, while isoquercitrin (42%), quercetin-3-O-arabinoside (22%), (–)-epicatechin (15%) and vanillic acid (14%) were most in evidence in pericarp of *T. grandiflorum*. Fruit without seed of *O. bataua* showed relatively high levels of epicatechin (45%), chlorogenic acid (12%) and isoquercitrin (11%). Leaves of *I. edulis* were shown to be mostly composed of myricetin (21%), isoquercitrin (21%) and salicylic acid (13%) (Tables 4–6).

Discussion

In this study, we investigated potential of Peruvian edible and medicinal plants for elimination of oxidative stress-related diseases using innovative approach based on determination of their combinatory antioxidant and anti-proliferative effects (Tauchen et al., 2015). As a result of our experiments, *O. bataua* and *I. edulis* possessed the best antioxidant/anti-proliferative properties. Although previous studies on chemistry of *O. bataua* have suggested high contents of anthocyanins (Rezaire et al., 2014), a compounds known to produce antioxidant and anticancer activity (Prior and Wu, 2006; Wang and Stoner, 2008), this is the first report on combined

antioxidant and anti-proliferative effects of this plant. In contrast to earlier demonstrated relatively low cytotoxic efficacy of *I. edulis* towards various carcinoma cell lines (UACC-62, MCF-7, 786-O, NCI-460, PCO-3, OVCAR-03, HT-29 and K-562) including multidrug-resistant variants (NCI-ADR) (Pompeu et al., 2012), we recorded moderate anti-proliferative activity against Hep-G2 and HT-29 cells of this plant. Differences between results of these experiments can be caused by dissimilar response of cancer cells to active compounds present in *I. edulis* as it has previously been observed for various classes of natural compounds (Sak, 2014). Since the kojic acid, recently found in leaves of *I. edulis* (Tchuenmogne et al., 2013), have exerted significant antioxidant as well as anti-proliferative activities (Novotny et al., 1999; Kusumawati and Indrayanto, 2013) it might considerably contribute to combined biological effect of the plant.

The most-interesting results regarding selectivity of anti-proliferative effect towards carcinoma and normal cells were observed for *A. montana*. Despite the existence of records on anti-proliferative efficacy of various Annonaceous species (such as *A. muricata*, *A. squamosa* or *A. reticulata*) (Barbalho et al., 2012), the cytotoxicity has not previously been recorded for *A. montana*. Acetogenins are regarded as being chiefly responsible for prominent anticancer effect of Annonaceous species (Smith et al., 2014). Hence, supposedly these constituents are also responsible for the cytotoxic effect of *A. montana* observed in this study. Contrary to the fact that our results suggests *A. montana* extract to be safe, a study of Potts et al. (2012) describes present acetogenins (e.g. annonacin) as the induction factor for neurotoxicity. Additional studies regarding toxicological profile of this plant and its constituents are thus required. Low correlation between TPC and anti-proliferative activity in the rest of tested plant extracts, as well as similar findings in literature (Yang et al., 2009), suggest only partial responsibility of phenolic compounds for anticancer effect.

Among the plant species tested in this study, *I. edulis*, *M. dubia*, *M. flexuosa*, *O. bataua* and *T. grandiflorum* have been found to be

Table 4
Concentrations of phenolic acids in tested plant extracts.

Species	Plant part(s) ^a	Compound (ng/g DW) ^{b,c}									
		Anisic acid	Caffeic acid	Chlorogenic acid	p-Coumaric acid	Ferulic acid	Gallic acid	Salicylic acid	Sinapic acid	Syringic acid	Vanillic acid
<i>A. montana</i>	L	116.3 ± 3.0	115.7 ± 4.3	267.1 ± 9.6	153.4 ± 4.2	81.8 ± 2.4	253.2 ± 1.4	64.3 ± 0.6	17.7 ± 0.5	94.2 ± 2.7	317.7 ± 7.2
<i>B. excelsa</i>	L	35.3 ± 0.7	25.5 ± 0.4	45.6 ± 2.3	391.5 ± 7.0	84.5 ± 1.5	3929.8 ± 25.4	665.9 ± 0.1	35.4 ± 1.1	123.9 ± 1.0	183.6 ± 5.4
<i>B. armeniaca</i>	P	ND	<LOQ	65.4 ± 3.1	48.1 ± 0.3	10.6 ± 0.0	ND	37.1 ± 0.4	1.6 ± 0.0	43.9 ± 1.0	37.2 ± 0.5
	S	ND	ND	<LOQ	25.3 ± 0.6	22.7 ± 0.3	ND	16.2 ± 0.3	ND	ND	ND
<i>G. americana</i>	FW	197.5 ± 2.4	46.9 ± 1.1	ND	56.1 ± 1.8	591.2 ± 5.4	ND	104.2 ± 0.1	155.6 ± 3.4	87.7 ± 2.2	6642.9 ± 86.1
<i>I. edulis</i>	A	ND	<LOQ	16.8 ± 0.9	33.6 ± 0.7	19.5 ± 0.5	647.5 ± 11.1	681.7 ± 5.2	26.8 ± 0.6	18.6 ± 0.4	43.9 ± 1.1
	L	ND	46.7 ± 2.0	ND	272.8 ± 0.4	32.1 ± 0.4	829.5 ± 3.5	2158.9 ± 2.5	17.8 ± 0.6	107.1 ± 2.2	1270.1 ± 29.6
	P	ND	<LOQ	5.7 ± 0.3	60.4 ± 1.4	15.0 ± 0.1	789.0 ± 19.3	1985.2 ± 4.1	1.0 ± 0.0	47.9 ± 1.9	456.7 ± 10.3
	S	ND	<LOQ	<LOQ	42.0 ± 1.2	277.8 ± 8.6	62.4 ± 1.1	43.1 ± 0.9	66.1 ± 0.4	16.9 ± 0.5	87.1 ± 2.1
<i>M. flexuosa</i>	E	<LOQ	162.7 ± 4.9	11,767.9 ± 75.0	52.3 ± 0.5	98.9 ± 2.9	159.0 ± 0.9	13.9 ± 0.1	188.0 ± 1.4	177.3 ± 6.4	390.5 ± 7.9
	M	ND	53.8 ± 1.8	10,354.6 ± 73.5	58.8 ± 1.6	93.4 ± 3.5	61.7 ± 1.0	16.5 ± 0.1	347.3 ± 3.4	48.6 ± 1.5	115.1 ± 2.1
<i>M. dubia</i>	L	37.6 ± 0.3	<LOQ	66.3 ± 0.1	159.9 ± 5.8	<LOQ	4087.7 ± 10.1	111.8 ± 0.8	5.2 ± 0.1	82.9 ± 1.2	108.0 ± 1.4
	P	<LOQ	<LOQ	15.3 ± 0.4	165.2 ± 5.1	19.0 ± 0.1	163.8 ± 4.1	51.3 ± 1.3	ND	10.7 ± 0.1	38.3 ± 3.1
<i>O. batava</i>	FO	ND	256.3 ± 8.1	2324.7 ± 45.2	501.8 ± 14.7	351.6 ± 1.2	15.4 ± 0.4	39.6 ± 0.1	52.2 ± 0.5	704.2 ± 4.1	980.1 ± 24.0
<i>S. sessiliflorum</i>	FW	ND	235.4 ± 5.3	15,066.5 ± 106.2	295.0 ± 7.2	99.6 ± 1.1	ND	432.8 ± 3.6	103.0 ± 2.6	24.1 ± 0.3	59.8 ± 1.2
<i>T. bicolor</i>	A+S	49.1 ± 0.9	23.8 ± 0.3	ND	47.9 ± 1.0	34.7 ± 0.6	ND	153.8 ± 4.3	134.7 ± 3.0	166.7 ± 4.5	261.7 ± 6.8
	P	36.6 ± 0.5	59.1 ± 0.7	5318.4 ± 29.1	100.0 ± 3.3	178.1 ± 2.3	15.3 ± 1.0	51.9 ± 1.2	90.9 ± 1.5	165.7 ± 3.8	1066.3 ± 31.7
<i>T. cacao</i>	A+S	20.9 ± 0.8	42.6 ± 1.0	<LOQ	22.6 ± 0.3	8.5 ± 0.0	<LOQ	13.5 ± 0.1	1.0 ± 0.0	7.2 ± 0.1	121.1 ± 2.0
	L	ND	180.9 ± 4.9	6678.4 ± 38.5	748.0 ± 15.7	198.1 ± 5.1	6.1 ± 0.3	350.6 ± 1.3	274.7 ± 8.2	170.1 ± 3.8	593.6 ± 8.5
	P	ND	66.3 ± 2.0	<LOQ	29.5 ± 1.2	620.0 ± 11.7	<LOQ	53.8 ± 0.2	87.3 ± 2.7	54.7 ± 1.5	307.6 ± 5.9
<i>T. grandiflorum</i>	A	20.2 ± 0.7	<LOQ	9.5 ± 0.8	112.0 ± 2.6	52.5 ± 1.0	8.4 ± 0.9	146.3 ± 1.5	3.7 ± 0.1	240.5 ± 7.9	1179.8 ± 25.5
	L	<LOQ	27.9 ± 0.7	8.7 ± 0.4	142.6 ± 3.3	54.7 ± 0.5	28.2 ± 1.0	1853.9 ± 1.8	102.7 ± 2.3	379.9 ± 6.4	948.1 ± 9.8
	P	ND	<LOQ	16.9 ± 0.8	148.8 ± 3.9	76.8 ± 0.2	6.7 ± 0.2	121.6 ± 0.4	13.1 ± 0.2	497.5 ± 0.7	2723.8 ± 36.9

A, aril; E, exocarp; FO, fruit without seed; FW, whole fruit; M, mesocarp; L, leaves; P, pericarp; S, seed.

^a Abbreviation refers to plant part(s).

^b ND, compound not detected.

^c <LOQ, compound presented in sample under limit of quantification.

Table 5
Concentrations of flavonoids in tested plant extracts.

Species	Plant part(s) ^a	Compound (ng/g DW) ^{b,c}									
		Apigenin	(–)-Epicatechin	Flavone	Hesperetin	Kaempferol	Luteolin	Morin	Myricetin	Naringenin	Quercetin
<i>A. montana</i>	L	ND	602.2 ± 4.9	ND	ND	54.8 ± 1.1	<LOQ	ND	473.4 ± 0.7	<LOQ	55.3 ± 0.6
<i>B. excelsa</i>	L	<LOQ	137.1 ± 3.9	ND	ND	271.9 ± 7.7	<LOQ	<LOQ	500.0 ± 2.3	5.7 ± 0.0	293.3 ± 0.3
<i>B. armeniaca</i>	P	ND	11.5 ± 0.4	ND	2.0 ± 0.1	<LOQ	<LOQ	ND	ND	1.6 ± 0.0	12.8 ± 0.2
	S	ND	9.8 ± 0.3	ND	ND	ND	ND	ND	ND	ND	ND
<i>G. americana</i>	FW	<LOQ	126.3 ± 3.8	ND	1.5 ± 0.0	34.3 ± 0.1	<LOQ	ND	ND	1.6 ± 0.1	29.2 ± 0.8
<i>I. edulis</i>	A	32.6 ± 0.4	1284.7 ± 24.9	ND	ND	<LOQ	167.6 ± 1.9	ND	ND	0.6 ± 0.0	127.9 ± 2.5
	L	18.4 ± 0.0	298.3 ± 9.9	ND	ND	32.2 ± 0.6	692.1 ± 19.3	ND	3593.1 ± 29.5	1.2 ± 0.0	934.0 ± 8.5
	P	7.7 ± 0.2	2229.3 ± 22.4	ND	1.4 ± 0.0	ND	600.4 ± 5.2	ND	ND	1.6 ± 0.0	153.8 ± 1.6
	S	16.5 ± 0.1	14.1 ± 0.9	ND	ND	51.5 ± 1.4	281.5 ± 8.3	ND	569.7 ± 1.4	0.3 ± 0.0	134.4 ± 5.3
<i>M. flexuosa</i>	E	528.6 ± 0.4	228.8 ± 7.5	ND	8.7 ± 0.1	158.6 ± 3.7	477.8 ± 3.2	454.2 ± 1.2	471.2 ± 0.0	171.0 ± 2.6	252.6 ± 4.9
	M	15.0 ± 0.1	186.1 ± 6.7	ND	<LOQ	ND	5.5 ± 0.1	ND	ND	7.1 ± 0.1	32.7 ± 0.5
<i>M. dubia</i>	L	ND	<LOQ	ND	ND	247.2 ± 3.5	ND	ND	1147.8 ± 8.0	8.7 ± 0.3	375.9 ± 8.9
	P	ND	ND	6.0 ± 0.2	ND	27.7 ± 0.7	ND	ND	1010.4 ± 2.3	0.7 ± 0.0	161.9 ± 1.1
<i>O. bataua</i>	FO	54.4 ± 0.4	8628.5 ± 36.8	ND	2.4 ± 0.1	82.3 ± 2.7	30.9 ± 0.2	ND	473.7 ± 0.7	21.9 ± 0.1	687.2 ± 8.3
<i>S. sessiliflorum</i>	FW	7.1 ± 0.0	ND	ND	ND	302.0 ± 8.9	9.2 ± 0.1	ND	ND	1449.8 ± 17.0	124.0 ± 2.2
<i>T. bicolor</i>	A+S	ND	6495.1 ± 7.4	ND	0.7 ± 0.0	16.4 ± 0.3	7.3 ± 0.6	ND	6282.9 ± 38.8	4.2 ± 0.2	358.4 ± 3.5
	P	1.2 ± 0.0	6055.4 ± 46.3	ND	7.9 ± 0.0	60.1 ± 0.6	24.9 ± 0.5	ND	597.6 ± 4.5	440.8 ± 2.2	343.9 ± 4.0
<i>T. cacao</i>	A+S	<LOQ	6672.0 ± 51.1	ND	ND	9.4 ± 0.0	62.4 ± 0.8	ND	<LOQ	14.0 ± 0.3	948.0 ± 4.9
	L	29.5 ± 0.3	4128.6 ± 36.8	ND	ND	<LOQ	88.6 ± 0.5	ND	ND	ND	21.7 ± 0.1
	P	21.3 ± 0.1	1324.1 ± 58.5	ND	<LOQ	<LOQ	194.6 ± 2.2	ND	ND	5.5 ± 0.0	30.5 ± 0.1
<i>T. grandiflorum</i>	A	3.1 ± 0.2	3635.7 ± 23.0	ND	ND	33.7 ± 0.1	266.4 ± 7.7	ND	471.2 ± 0.1	7.7 ± 0.2	134.6 ± 3.3
	L	1.9 ± 0.0	1100.7 ± 15.0	ND	<LOQ	844.8 ± 0.8	128.7 ± 3.0	ND	474.7 ± 0.0	4.9 ± 0.1	1011.8 ± 1.7
	P	<LOQ	2905.7 ± 37.4	ND	ND	44.8 ± 0.1	<LOQ	ND	ND	8.4 ± 0.1	296.6 ± 0.0

A, aril; E, exocarp; FO, fruit without seed; FW, whole fruit; M, mesocarp; L, leaves; P, pericarp; S, seed.

^a Abbreviation refers to plant part(s).

^b ND, compound not detected.

^c <LOQ, compound presented in sample under limit of quantification.

Table 6
Concentrations of flavonoid derivatives, stilbenes and other phenolic compounds in tested plant extracts.

Species	Plant part(s) ^a	Compound (ng/g DW) ^{b,c}									
		Apigenin-7-glucoside	Luteolin-7-glucoside	Naringenin-7-glucoside	Quercetin-3-arabinoside	Naringin	Isoquercitrin	Rutin	Pterostilbene	Resveratrol	Scopoletin
<i>A. montana</i>	L	2.0 ± 0.0	22.2 ± 0.6	1.2 ± 0.1	2.6 ± 0.0	ND	1486.8 ± 21.0	8086.8 ± 92.4	ND	ND	<LOQ
<i>B. excelsa</i>	L	8.7 ± 0.1	<LOQ	2.5 ± 0.0	603.9 ± 5.7	ND	4156.2 ± 46.5	3007.1 ± 43.0	ND	1.9 ± 0.0	ND
<i>B. armeniaca</i>	P	ND	ND	11.0 ± 0.0	2.3 ± 0.0	ND	1112.7 ± 3.0	7024.2 ± 52.1	2.4 ± 0.0	0.6 ± 0.0	<LOQ
	S	ND	ND	2.5 ± 0.0	<LOQ	ND	<LOQ	8.1 ± 0.1	ND	ND	ND
<i>G. americana</i>	FW	<LOQ	ND	1.5 ± 0.1	23.3 ± 0.1	28.1 ± 0.6	378.7 ± 2.0	3046.7 ± 0.3	2.5 ± 0.0	<LOQ	<LOQ
<i>I. edulis</i>	A	22.5 ± 0.4	49.6 ± 0.7	7.1 ± 0.1	2386.0 ± 37.2	ND	6241.1 ± 2.5	849.8 ± 4.5	2.5 ± 0.0	ND	<LOQ
	L	121.0 ± 1.5	416.5 ± 7.4	1.9 ± 0.1	1588.6 ± 28.0	36.8 ± 0.8	3478.6 ± 59.4	838.7 ± 6.1	ND	4.5 ± 0.1	6.4 ± 0.0
	P	35.2 ± 0.4	132.8 ± 2.2	7.9 ± 0.1	848.3 ± 9.4	ND	2892.0 ± 4.9	81.2 ± 0.8	2.4 ± 0.0	<LOQ	4.1 ± 0.1
	S	195.9 ± 2.8	310.0 ± 3.6	4.4 ± 0.1	20.2 ± 0.0	ND	458.2 ± 5.0	90.2 ± 1.4	ND	ND	ND
<i>M. flexuosa</i>	E	333.6 ± 9.5	501.5 ± 1.3	45.9 ± 0.9	70.0 ± 2.3	85.9 ± 2.5	7417.6 ± 22.2	7435.8 ± 70.8	184.3 ± 0.7	590.1 ± 13.8	ND
	M	20.8 ± 0.1	54.4 ± 0.1	30.3 ± 0.3	28.2 ± 0.6	<LOQ	5858.6 ± 80.0	3998.2 ± 106.2	5.8 ± 0.0	6.5 ± 0.1	ND
<i>M. dubia</i>	L	<LOQ	ND	11.2 ± 0.0	1392.0 ± 3.9	ND	3345.6 ± 2.5	ND	ND	1.9 ± 0.1	ND
	P	ND	ND	2.1 ± 0.1	124.0 ± 2.7	ND	170.3 ± 1.5	ND	ND	<LOQ	ND
<i>O. bataua</i>	FO	68.9 ± 0.4	14.2 ± 0.1	5.0 ± 0.0	<LOQ	ND	2128.0 ± 16.5	650.6 ± 4.7	39.1 ± 0.8	907.3 ± 15.3	ND
<i>S. sessiliflorum</i>	FW	26.9 ± 0.1	35.0 ± 1.0	3186.4 ± 35.1	76.6 ± 2.9	ND	4823.8 ± 5.1	3659.3 ± 87.3	ND	<LOQ	21.1 ± 0.0
<i>T. bicolor</i>	A+S	ND	ND	1.6 ± 0.1	54.0 ± 1.5	ND	695.5 ± 6.1	7.3 ± 0.2	ND	8.2 ± 0.1	84.3 ± 0.7
	P	<LOQ	12.3 ± 0.3	437.3 ± 5.2	90.2 ± 1.5	198.2 ± 9.6	2064.9 ± 5.0	1936.1 ± 2.5	2.6 ± 0.8	7.5 ± 0.1	384.9 ± 9.4
<i>T. cacao</i>	A+S	3.3 ± 0.0	71.3 ± 2.6	15.4 ± 0.0	3269.1 ± 85.9	<LOQ	10,259.3 ± 2.6	2.4 ± 0.1	<LOQ	1.5 ± 0.0	<LOQ
	L	747.9 ± 4.3	1239.8 ± 2.2	5.1 ± 0.2	281.5 ± 2.1	<LOQ	917.4 ± 6.6	<LOQ	ND	<LOQ	3.2 ± 0.0
	P	69.7 ± 0.6	295.6 ± 0.7	7.6 ± 0.1	298.9 ± 5.4	<LOQ	360.1 ± 7.5	2.9 ± 0.1	ND	<LOQ	ND
<i>T. grandiflorum</i>	A	10.2 ± 0.1	29.8 ± 0.6	2.7 ± 0.0	285.1 ± 3.2	40.8 ± 1.0	1753.9 ± 17.8	44.7 ± 0.7	ND	1.1 ± 0.0	35.3 ± 0.4
	L	2.5 ± 0.1	<LOQ	<LOQ	422.3 ± 6.4	<LOQ	2474.5 ± 13.1	255.4 ± 5.8	ND	1.6 ± 0.0	82.5 ± 0.9
	P	ND	ND	7.2 ± 0.1	4184.2 ± 17.0	114.1 ± 0.1	8149.4 ± 2.4	25.6 ± 0.9	2.6 ± 0.1	0.7 ± 0.0	46.5 ± 0.3

A, aril; E, exocarp; FO, fruit without seed; FW, whole fruit; M, mesocarp; L, leaves; P, pericarp; S, seed.

^a Abbreviation refers to plant part(s).

^b ND, compound not detected.

^c <LOQ, compound presented in sample under limit of quantification.

the most effective antioxidants. Despite the existence of previous records on antioxidant effect of these species (Souza et al., 2008; Fracassetti et al., 2013; Koolen et al., 2013; Pugliese et al., 2013; Rezaire et al., 2014), to our best knowledge, majority of these were not using ORAC assay, regarded as one of the most biological relevant methods to determine antioxidant activity *in vitro* (MacDonald-Wicks et al., 2006). Our results from phytochemical and statistical analyses suggested phenolics to be major constituents responsible for the observed antioxidant effect of all five above-mentioned species that is corresponding with earlier published studies (De Sousa Dias et al., 2010; Fracassetti et al., 2013; Pugliese et al., 2013; Bataglion et al., 2014; Rezaire et al., 2014).

Conclusion

The current study provides novel information on *in vitro* antioxidant activity and/or anti-proliferative activity of six plant species, namely *A. montana*, *I. edulis*, *M. dubia*, *M. flexuosa*, *O. bataua* and *T. grandiflorum*. None of the tested extracts exerted significant toxicity towards normal MRC-5 cells, pointing their relative safety. We conclude that the above-noted plant extracts could serve as prospective material for further development of novel plant-based antioxidant and/or anti-proliferative agents. Particularly *O. bataua* and *I. edulis*, the only extracts exhibiting combinatory antioxidant and anti-proliferative efficacy in this study, deserve deeper research attention. Detailed analysis of their chemical composition and *in vivo* antioxidant/anti-proliferative activity should be carried out in order to verify their potential practical use.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

LB, PPPV and YBF collected the plant samples and organized the botanical identification and confection of herbarium specimens. JT performed the antioxidant assays and drafted the paper. ID organized the anti-proliferative test. LH and PMik did the UHPLC–MS/MS analysis. PMar provided statistical analysis of gained data. PVD, BL, JH and OL contributed to critical reading of the manuscript. LK designed the study, supervised the laboratory work and revised the final version of the paper. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research was supported by the Internal Grant Agency of the Czech University of Life Sciences Prague (Project Nos. CIGA 20142012, CIGA 20132035 and IGA 20165009). Study was also supported by a grant of Czech Ministry of Education, Youth and Sports (MSMT No. 20/2015). Authors are very grateful to Mirella Zoyla

Clavo (UNMSM) for deposition of herbarium specimens of plant species tested in this study.

References

- Alasalvar, C., Shahidi, F., 2013. *Dried fruits: Phytochemicals and Health Effects*, 1st ed. Wiley-Blackwell, Hoboken, USA.
- Anter, J., Romero-Jiménez, M., Fernández-Bedmar, Z., Villatoro-Pulido, M., Analla, M., Alonso-Moraga, A., Muñoz-Serrano, A., 2011. Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid. *J. Med. Food* 14, 276–283.
- Barbalho, S.M., de Alvares Goulart, R., Farinazzi-Machado, F.M.V., de Souza, M.S.S., dos Santos Bueno, P.C., Guiguer, É.L., Araújo, A.C., Groppo, M., 2012. *Annona* sp: plants with multiple applications as alternative medicine – a review. *Curr. Bioact. Compd.* 8, 277–286.
- Bataglion, G.A., da Silva, F.M.A., Eberlin, M.N., Koolen, H.H.F., 2014. Simultaneous quantification of phenolic compounds in buriti fruit (*Mauritia flexuosa* L.f.) by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. *Food Res. Int.* 66, 396–400.
- Brewer, M.S., 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr. Rev. Food Sci. Food Saf.* 10, 221–247.
- Cao, G., Prior, R.L., 1998. Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol.* 299, 50–62.
- da Costa, L.S., Andrezza, N.L., Correa, W.R., Cunha, I.B.S., Ruiz, A.L.T.G., de Carvalho, J.E., Schinor, E.C., Dias, D.A., Salvador, M.J., 2015. Antiproliferative activity, antioxidant capacity and chemical composition of extracts from the leaves and stem of *Chresta sphaerocephala*. *Rev. Bras. Farmacogn.* 25, 369–374. <http://dx.doi.org/10.1016/j.bjpp.2015.04.005>.
- De la Rosa, L.A., Vazquez-Flores, A.A., Alvarez-Parrilla, E., Rodrigo-García, J., Medina-Campos, O.N., Ávila-Nava, A., González-Reyes, S., Pedraza-Chaverri, J., 2014. Content of major classes of polyphenolic compounds, antioxidant, antiproliferative, and cell protective activity of pecan crude extracts and their fractions. *J. Funct. Foods* 7, 219–228.
- De Sousa Dias, A.L., De Souza, J.N.S., Rogez, H., 2010. Purification of phenolic compounds from *Inga edulis* leaves using solid-phase extraction: major compounds quantification and antioxidant capacity evaluation. *Quim. Nova* 33, 38–42.
- Dewick, P.M., 2009. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed. Wiley & Sons, Chichester, UK.
- Duke, J.A., Bogenschutz-Godwin, M.J., Ottesen, A.R., 2009. *Duke's Handbook of Medicinal Plants of Latin America*, 1st ed. CRC Press, Boca Raton, USA.
- Duke, J.A., Vásquez, R., 1994. *Amazonian Ethnobotanical Dictionary*, 1st ed. CRC Press, Boca Raton, USA.
- Ferry, D.R., Smith, A., Malkhandi, J., Fyfe, D.W., DeTakats, P.G., Anderson, D., Baker, J., Kerr, D.J., 1996. Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for *in vivo* tyrosine kinase inhibition. *Clin. Cancer Res.* 2, 659–668.
- Fracassetti, D., Costa, C., Moulay, L., Tomás-Barberán, F.A., 2013. Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). *Food Chem.* 139, 578–588.
- Halliwell, B., Gutteridge, J.M., 2007. *Free Radicals in Biology and Medicine*, 4th ed. Oxford University Press, Oxford, UK.
- Held, P., 2005. Performing oxygen radical absorbance capacity assays with synergy™HT: ORAC antioxidant tests. In: Application Note. Biotek Instruments, Winooski, USA.
- Jaberian, H., Piri, K., Nazari, J., 2013. Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants. *Food Chem.* 136, 237–244.
- Koolen, H.H., da Silva, F., Gozzo, F.C., de Souza, A.Q., de Souza, A.D., 2013. Antioxidant, antimicrobial activities and characterization of phenolic compounds from Buriti (*Mauritia flexuosa* L. f.) by UPLC-ESI-MS/MS. *Food Res. Int.* 51, 467–473.
- Kusumawati, I., Inprayanto, G., 2013. Natural antioxidants in cosmetics. *Stud. Nat. Prod. Chem.* 40, 485–505.
- Lim, T.K., 2012. *Edible Medicinal and Non Medicinal Plants: Fruits*. Springer, Dordrecht, Netherlands.
- Loizzo, M.R., Rashed, K., Said, A., Bonesi, M., Menichini, F., Tundis, R., 2014. Antiproliferative and antioxidant properties of *Alhagi maurorum* Boiss (Leguminosae) aerial parts. *Ind. Crops Prod.* 53, 289–295.
- MacDonald-Wicks, L.K., Wood, L.G., Garg, M.L., 2006. Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *J. Sci. Food Agric.* 86, 2046–2056.
- Miksatkova, P., Ancheeva, E., Hejtmanikova, K., Teslov, L., Lapcik, O., 2014. Determination of Flavonoids in *Stellaria* by High-Performance Liquid Chromatography-Tandem Mass Spectrometry. *Anal. Lett.* 47, 2317–2331.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63.
- Neri-Numa, I.A., Carvalho-Silva, L.B., Morales, J.P., Malta, L.G., Muramoto, M.T., Ferreira, J.E.M., de Carvalho, J.E., Ruiz, A.L.T.G., Maróstica Junior, M.R., Pastore, G.M., 2013. Evaluation of the antioxidant, antiproliferative and antimutagenic potential of araçá-boi fruit (*Eugenia stipitata* Mc Vaugh – Myrtaceae) of the Brazilian Amazon Forest. *Food Res. Int.* 50, 70–76.
- Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75, 311–335.
- Novotny, L., Rauko, P., Abdel-Hamid, M., Vachalková, A., 1999. Kojic acid – a new leading molecule for a preparation of compounds with an anti-neoplastic potential. *Neoplasma* 46, 89–92.

- Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* 49, 4619–4626.
- Pompeu, D.R., Rogez, H., Monteiro, K.M., Tinti, S.V., Carvalho, J.E., 2012. Antioxidant capacity and pharmacologic screening of crude extracts of *Byrsonima crassifolia* and *Inga edulis* leaves. *Acta Amaz.* 42, 165–172.
- Potts, L.F., Luzzio, F.A., Smith, S.C., Hetman, M., Champy, P., Litvan, I., 2012. Annonacin in *Asimina triloba* fruit: implication for neurotoxicity. *Neurotoxicology* 33, 53–58.
- Prior, R.L., Wu, X., 2006. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radic. Res.* 40, 1014–1028.
- Pugliese, A.G., Tomas-Barberan, F.A., Truchado, P., Genovese, M.I., 2013. Flavonoids, proanthocyanidins, vitamin C, and antioxidant activity of *Theobroma grandiflorum* (Cupuassu) pulp and seeds. *J. Agric. Food Chem.* 61, 2720–2728.
- Rezaire, A., Robinson, J.-C., Bereau, D., Verbaere, A., Sommerer, N., Khan, M.K., Durand, P., Prost, E., Fils-Lycaon, B., 2014. Amazonian palm *Oenocarpus bataua* ("patawa"): Chemical and biological antioxidant activity – phytochemical composition. *Food Chem.* 149, 62–70.
- Sak, K., 2014. Cytotoxicity of dietary flavonoids on different human cancer types. *Pharmacogn. Rev.* 8, 122–146.
- Sharma, O.P., Bhat, T.K., 2009. DPPH antioxidant assay revisited. *Food Chem.* 113, 1202–1205.
- Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1998. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299, 152–178.
- Smith, R.E., Tran, K., Richards, K.M., 2014. Bioactive annonaceous acetogenins. *Stud. Nat. Prod. Chem.* 41, 95–117.
- Sosnowska, J., Balslev, H., 2009. American palm ethnomedicine: a meta-analysis. *J. Ethnobiol. Ethnomed.* 5, <http://dx.doi.org/10.1186/1746-4269-5-43>.
- Souza, J.N.S., Silva, E.M., Loir, A., Rees, J.-F., Rogez, H., Larondelle, Y., 2008. Antioxidant capacity of four polyphenol-rich Amazonian plant extracts: a correlation study using chemical and biological in vitro assays. *Food Chem.* 106, 331–339.
- Tauchen, J., Dorskocil, I., Caffi, C., Lulekal, E., Marsik, P., Havlik, J., Damme, P.V., Kokoska, L., 2015. In vitro antioxidant and anti-proliferative activity of Ethiopian medicinal plant extracts. *Ind. Crops Prod.* 74, 671–679.
- Tchuenmogne, A.M., Donfack, E.V., Kongue, M.D., Lenta, B.N., Ngouela, S., Tsamo, E., Sidhu, N., Dittrich, B., Laatsch, H., 2013. Ingacamerounol, a new flavonol and other chemical constituents from leaves and stem bark of *Inga edulis* Mart. *Bull. Korean Chem. Soc.* 34, 3859–3862.
- Wang, L.-S., Stoner, G.D., 2008. Anthocyanins and their role in cancer prevention. *Cancer Lett.* 269, 281–290.
- Yang, J., Liu, R.H., Halim, L., 2009. Antioxidant and antiproliferative activities of common edible nut seeds. *LWT Food Sci. Technol.* 42, 1–8.