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Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region

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Abstract

The polyphenolic compound content has been determined in 15 Amazonian plant species (leaves, bark, stems, fruits, and seeds) used in folk medicine, using two complementary spectrophotometric methods. In addition, the antioxidant activity of the corresponding plant extracts has been determined by TEAC (trolox equivalent antioxidant capacity) and $ORAC_{Fluorescein}$ (oxygen radical antioxidant capacity using fluorescein as fluorescent probe) assays to identify naturally-rich sources of antioxidants. The plants under investigation showed a great range of TEAC (1.0 up to 347.1 μmol of trolox equiv./g) and ORAC (6.7 up to 1396.4 μmol of trolox equiv./g) values. These values were highly correlated to the concentration in total phenolics obtained by the Folin–Ciocalteau procedure (TEAC: $r^2 = 0.88$, $n = 65$; ORAC: $r^2 = 0.70$, $n = 62$), and in total flavanoids, quantified using the chromogen reagent *p*-dimethylaminocinnamaldehyde (TEAC: $r^2 = 0.75$, $n = 54$; ORAC: $r^2 = 0.74$, $n = 51$). The high antioxidant capacities found in leaves and bark of *Byrsonima crassifolia*, *Inga edulis*, *Davilla kunthii* and *Cecropia palmata* and also their great biomasses in the forest should stimulate further studies regarding the characterization, purification and concentration of their phenolic compounds.

Keywords: ORAC; TEAC; Polyphenols; Flavanoids; Amazonian plants

1. Introduction

The interest in polyphenolic antioxidants has increased remarkably in the last decade because of their elevated capacity in scavenging free radicals associated with various diseases. This property has been evidenced by a large number of tests measuring the antioxidant activity in vitro (Cao & Prior, 1999; Hirayama, Takagi, Hukumoto, & Katoh, 1997; Ou, Hampsch-Woodill, & Prior, 2001; Re et al., 1999; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). In vivo, the antioxidant protection attributed to the polyphenols can be checked through the level of biomarkers, such as malondialdehyde, which is associated with lipid peroxidation (Nielsen, Mikkelsen, Nielsen, Andersen, & Grandjean, 1997), or 8-oxo-7,8-dihydroguanine, which indicates oxidative damage to the DNA bases (Cadet, Douki, Gasparutto, & Ravanat, 2003). The protective capacity of polyphenols is also supported by a number of studies indicating an effect of dietary polyphenols on coronary heart disease (CHD) (Weisburger, 1999), cancer (Duthie, Duthie, & Kyle, 2000; Yang, Landau, Huang, & Newmark, 2001), gene regulation (Myhrstad, Carlsen, Nordström, Blomhoff, & Moskaug, 2002), and neurodegenerative diseases (Sun & Chen, 1998). These phytochemicals are ubiquitous in the plant kingdom. They originate from the shikimate and acetate–malonate pathways and share a basic carbon skeleton: the phenylpropanoid unit (C_6–C_3). The large group of phenolic compounds includes the simple phenols (C_6; catechol, resorcinol, etc.), phenolic acids (C_6–C_2; *p*-hydroxybenzoic acids, etc.), hydroxycinnamic acids (C_6–C_3; caffeic and ferrulic acids, etc.), stilbenes (C_6–C_2–C_6; resveratrol, etc.), flavo-

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noids (C_6–C_3–C_6; quercetin, cyanidin, etc.), biflavonoids ((C_6–C_3–C_6)$_2$; ormocarpin, etc.), and proanthocyanidins ((C_6–C_3–C_6)$_n$; epicatechin-(4β → 8)-catechin) (Robards & Antolovich, 1997; Wollenweber, 1993). The flavonoids are among the most efficient antioxidant molecules (Rice-Evans, Miller, & Paganga, 1997; Robards et al., 1999) and more than 6,400 structures have been established (Harborne & Williams, 2000). They are subdivided into various families such as flavonols, flavan-3-ols, flavones, flavanones, anthocyanins, chalcones, etc., with the flavonols, flavan-3-ols and anthocyanins being quantitatively dominant in plants (Robards & Antolovich, 1997). The most traditionally consumed polyphenol sources are fruits, juices, vegetables, red wine, green and black teas, and chocolate.

Amazonia, with its enormous biodiversity, has a great potential in providing new nutritional compounds. For instance, the açaí fruit (*Euterpe oleracea*) has an elevated antioxidant capacity due to its high content of anthocyanins and tocopherols (Rogez, 2000). In the Amazonian flora, many plants unknown to scientists have been used for years by Indian people to cure degenerative diseases, such as CHD and diabetes. The study of these medicinal plants, often rich in polyphenols, may lead to the discovery of new, useful antioxidant sources, providing an incentive for the preservation of these plants and sustainable development within this region.

Due to the multifunctional characteristics of phytochemicals, the antioxidant efficacy of a plant extract is best evaluated based on results obtained by commonly accepted assays, taking into account different oxidative conditions, system compositions, and antioxidant mechanisms (Frankel & Meyer, 2000; Prior, Wu, & Schaich, 2005). Accordingly, we have used in the present study two fast spectrophotometric assays to assess the antioxidant activity of extracts from a set of 15 plant species potentially rich in polyphenols and native to the Brazilian Amazonian forest. These assays were the ORAC (oxygen radical absorbance capacity) test that measures the capacity of antioxidants in protecting a substrate (fluorescein) against peroxyl radical attacks, and the TEAC (trolox equivalent antioxidant capacity) test, a method measuring the direct scavenging of the radical cation $ABTS^{+\bullet}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)).

2. Materials and methods

2.1. Plant materials

Fifteen plants (Table 1) were selected from a data bank of 300 native species of the Amazonian region, on the basis of both interviews with traditional healers and data from the Brazilian scientific literature. The plants that were collected were taxonomically identified by a pharmacognosy expert of the Botanical Museum Emilio Goeldi (Belém, Brazil). The parts of the plants to be studied were chosen according to their traditional use in folk medicine (Table 1) and were collected in the countryside of the State of Pará (Brazil). The plant samples were carried to the laboratory within a maximum of 12 h after harvest. Otherwise, the material was stored under refrigeration at 4 °C.

2.2. Extraction

The plant parts were thoroughly rinsed in water and cut into very tiny pieces. Twelve grams of leaf, 22 g of bark or 14 g of stem were immersed in 100 ml of an extraction solution, composed of methanol, ethanol, distilled water, and hydrochloric acid (69:20:10:1, v:v:v:v). The duration of the extraction was 24 h, without agitation and in the dark. The crude extracts were concentrated using a rotary evaporator (40 °C) to a final volume of 30 ml. This volume was divided into three equal fractions and poured into brown glass bottles, which were then saturated with gaseous nitrogen and stored at −20 °C. One of these fractions was shipped in dry

Table 1
Latin names, families, plant parts and uses in Amazonia of the 15 plants studied

Botanical name	Family	Plant parts	Medicinal uses in Amazonia
Arrabidaea chica var. *angustifolia* Bureau and K. Schum.	Bignoniaceae	Leaf/stem	Analgesic, anti-inflammatory and anti-anaemic
Bauhinia forficata var. *latifolia* Benth.	Caesalpiniaceae	Leaf/bark/stem	Antidiabetic, anti-cholesterol and anti-elephantiasis
Bauhinia macrostachya Benth.	Caesalpiniaceae	Leaf/stem	Antidiabetic, anti-cholesterol and anti-elephantiasis
Byrsonima crassifolia H.B. and K.	Malpighiaceae	Leaf/bark/fruit	Diuretic and anti-inflammatory
Cecropia obtusa Trécul.	Moraceae	Leaf/stem	Anti-CHD, anti-rheumatic and tranquilizer
Cecropia palmata Willd.	Moraceae	Leaf/bark/stem	Anti-CHD, anti-rheumatic and tranquilizer
Cedrela odorata L.	Meliaceae	Bark	Anti-cancer, anti-ulcer, anti-inflammatory and anti-gastritis
Cordia exaltata Lam.	Boraginaceae	Leaf/fruit	Anti-inflammatory
Dalbergia monetaria L.f.	Leguminosae	Bark	Anti-ulcer, anti-inflammatory and anti-anaemic
Dalbergia subcymosa Ducke	Leguminosae	Bark	Anti-ulcer, anti-inflammatory and anti-anaemic
Davilla kunthii St. Hil.	Dilleniaceae	Leaf/bark	Anti-ulcer, anti-inflammatory and purgative
Davilla rugosa Poir.	Dilleniaceae	Leaf/bark	Anti-ulcer, anti-inflammatory and purgative
Eugenia patrisii Vahl.	Myrtaceae	Leaf	Anti-inflammatory
		Fruit	Edible
Inga edulis Mart.	Leguminosae	Leaf/bark/seed	Anti-inflammatory and anti-diarrheic
		Fruit	Edible
Stryphnodendron barbadetimam Mart.	Leguminosae	Bark	Anti-ulcer, anti-inflammatory and anti-cancer

ice to the Université Catholique de Louvain (Belgium), where it was submitted to ultracentrifugation at 27,000*g* for 15 min, giving a clear supernatant for the next analyses.

2.3. Determination of total phenolics (TP)

The concentration of total phenolics was determined by the Folin–Ciocalteau colorimetric method (Singleton & Rossi, 1965). Measurements were carried out in triplicate and calculations based on a calibration curve obtained with gallic acid (Extrasynthèse, Genay, France). The total phenolics were expressed as milligram of gallic acid equivalents (GAE) per gram of fresh weight (FW).

2.4. Determination of total flavanoids (TF)

Total flavanoids (compounds containing *meta*-oriented di- or tri-hydroxy substituted benzene rings, with a single bond at the 2,3 position of the ring C, such as flavan-3-ols, flavan-4-ols, flavan-3,4-diols, flavanones and derivatives) were estimated in the plant extracts using the chromogen *p*-dimethylaminocinnamaldehyde (DMACA) (Sigma, St. Louis, MO), following the protocol proposed by Delcour and Janssens de Varebeke (1985). All determinations were made in triplicate and values were calculated from a calibration curve obtained with catechin (Extrasynthèse, Genay, France). Final results were expressed as milligram of catechin equivalent (CE) per gram of FW.

2.5. ORAC assay

The ORAC assay used fluorescein as fluorescent probe and was an adaptation of the protocols proposed by Ou et al. (2001), Huang, Ou, Hampsch-Woodill, Judith, and Prior (2002). Fluorescein sodium salt (16.7 mg) (Sigma, St. Louis, MO) was dissolved in 10 ml of phosphate buffer solution (PBS) (75 mM, pH 7.0) to get a stock solution. The working solution (60 nM) was obtained by subsequent dilution in PBS. A 10 ml solution of AAPH (2,2-azinobis (2-amidinopropan) dihydrochloride), purchased from WAKO Pure Chemical Industries (Orokama, Japan), was prepared each day at a concentration of 153 mM and maintained in ice before automatic injection. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma, St. Louis, MO) solutions used for the calibration curve (8, 16, 24, 32 and 40 μM) were prepared each day by dilution in PBS of a 1 mM stock solution made in the same solvent and stored at −80 °C.

The analysis was performed using microplates (96-well, opaque white, Greiner Bio-One, Wemmel, Belgium) and a fluorimeter Ascent F.L. (Fluoroscan Labsystems, Helsinki, Finland). Sample (25 μl) was mixed with 250 μl of fluorescein (60 nM) and incubated for 10 min at 37 °C in the microplate. AAPH (25 μl) solution was then automatically injected and the microplate was shaken. The fluorescence ($\lambda_{excitation} = 485$ nm, $\lambda_{emission} = 520$ nm) was registered each minute over 50 min. All samples were analysed at three dilutions and the mean value was taken for ORAC, as recommended by Huang et al. (2002). The quantification of the antioxidant activity was based on the calculation of the area under the curve, as proposed by Cao and Prior (1999). The antioxidant activity by ORAC was expressed as μmol of trolox equivalents (TE) per gram of FW.

2.6. TEAC assay

The antioxidant activity was determined by the TEAC assay according to the procedure proposed by Re et al. (1999), using the radical cation $ABTS^{+\bullet}$ (Sigma, St. Louis, MO). The analysis was adapted to be conducted in a microwell plate (96-well, transparent, Nalge Nunc International, Rochester, NY), with a spectrophotometer Spectra Max 190 (Molecular Devices, Sunnyvale, CA). The $ABTS^{+\bullet}$ stock solution (7 mM) was prepared with the due recommendations (Re et al., 1999), using $K_2S_2O_8$ (Sigma, St. Louis, MO) as the oxidant agent. The working solution of $ABTS^{+\bullet}$ was obtained by diluting the stock solution in ethanol to give an absorption of 0.70 ± 0.02 at $\lambda = 734$ nm. Extract or trolox (10 μl) diluted in ethanol was put in the well and the reaction began with the addition of 290 μl of the $ABTS^{+\bullet}$ working solution. The trolox solution (50 mM) was prepared in ethanol and was stored at −20 °C under an atmosphere of nitrogen. For each session of measurements, a standard curve with trolox was plotted.

The TEAC value was calculated by the measurement of the area under the curve, derived from plotting the percentage inhibition of the absorbance as a function of time. The absorbance was recorded every 10 s for 6 min, to allow a close monitoring of the reaction rate, which is important for those antioxidants that show a time dependency when submitted to this assay. The calculation of the area under the curve was performed for one sample dilution which had a final percentage inhibition between 20% and 80%. Each extract was analysed in triplicate. The antioxidant activity for the plant extracts was expressed as μmol of TE per litre (Van den Berg, Haenen, Van den Berg, & Bast, 1999), and was calculated by the equation

$$\text{TEAC (TE, } \mu\text{mol/L)} = 30 \times k \times \frac{\text{AUC}_{\text{sample}}}{\text{r.c.}_{\text{trolox}}}, \quad (1)$$

where k is the dilution of the sample, 30 is a dilution factor originated from the protocol, $\text{r.c.}_{\text{trolox}}$ is the regression coefficient calculated from the calibration curve:

$$\text{AUC}_{\text{trolox}} = \text{r.c.}_{\text{trolox}} \times [\text{trolox}] \quad (2)$$

$\text{AUC}_{\text{sample}}$ and $\text{AUC}_{\text{trolox}}$ are the areas produced respectively by the sample and trolox and are calculated by the following equation:

$$\text{AUC} = \left(\%\text{Inh}_{(t=0)} \times 0.5 + \sum_{i=1}^{36} \%\text{Inh}_{(t=10\times i)} \right) \times 10, \quad (3)$$

where $\%\ \text{Inh}_{(t)}$ is the inhibition percentage at t seconds.

3. Results and discussion

3.1. Polyphenolic contents

Prior to the polyphenolic measurements, all the 65 extracts from the 15 species were submitted to a spectrophotometric scanning between 200 and 800 nm and exhibited an absorption band between 200 and 325 nm, which is characteristic of polyphenol absorption. However, no sample showed an absorption band between 465 and 560 nm, which is typical of the anthocyanins (Robards & Antolovich, 1997).

Table 2 reports the results of TP and TF analyses. It should be emphasized that these results are estimation of total phenolic content and total flavanoid content in their chemical equivalents (gallic acid and catechin, respectively), since different phenolic compounds contribute differently to the readings using the Folin–Ciocalteau reagent for TP results, and DMACA for TF results. Mean TP and TF varied from 0.3 to 45.5 mg_{GAE}/g_{FW} and from 0.06 to 11.71 mg_{CE}/g_{FW}, respectively. A high linear relation between TP and TF was verified ($r^2 = 0.82$, $p < 0.001$, $n = 54$) (Fig. 1). The slope of Fig. 1 indicates that the mean TF value corresponds to 27% of the mean TP value. TF were not detected in the leaves and fruits of *Cordia exaltata*, the bark of *Bauhinia forficata,* the stems of *Arrabidaea chica* and the seeds of *I. edulis* (Table 2). *Dalbergia subcymosa* presented high TP (35.8 mg_{GAE}/g_{FW}) in the bark but low TF (1.43 mg_{CE}/g_{FW}), making this point the most distant from the regression line (Fig. 1).

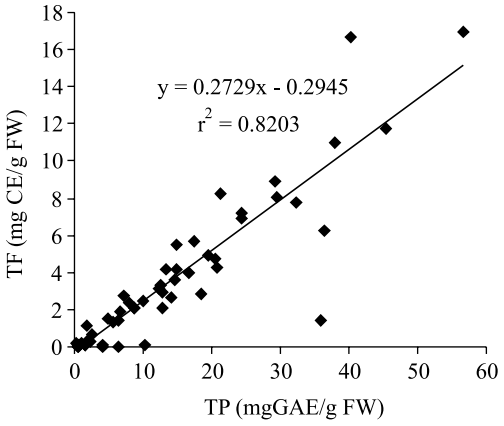

Fig. 1. Relationship between the total phenolics (TP) and the total flavanoids (TF) ($n = 54$).

Polyphenols were found in higher concentrations in leaves, confirming that the biosynthesis of polyphenols is accelerated by light exposure and serves as a filtration

Table 2
Total phenolics (TP), total flavanoids (TF), TEAC and ORAC in 15 Amazonian plants

Plant		TP (mg_{GAE}/g_{FW})	TF (mg_{CE}/g_{FW})	TEAC ($\mu mol_{TE}/g_{FW}$)	ORAC ($\mu mol_{TE}/g_{FW}$)
Species	Part				
Arrabidaea chica	Leaf	10.2 ± 0.1	0.06 ± 0.00	64.3 ± 0.2	261.4 ± 0.7
	Stem	4.5 ± 0.1	ND	20.0 ± 2.2	104.9 ± 22.2
Bauhinia forficata	Leaf	16.6 ± 2.6	3.25 ± 0.54	35.9 ± 22.6	255.2 ± 34.8
	Bark	0.3 ± 0.0	ND	1.6 ± 0.0	6.7 ± 0.3
	Stem	1.5 ± 0.1	0.12 ± 0.00	9.0 ± 6.4	24.6 ± 3.8
Bauhinia macrostachya	Leaf	14.1 ± 1.0	2.69 ± 0.10	133.3 ± 2.6	227.1 ± 7.2
	Bark	24.3 ± 0.8	6.87 ± 0.15	82.1 ± 3.1	564.5 ± 40.7
Byrsonima crassifolia	Leaf	45.5 ± 1.9	11.71 ± 0.02	347.1 ± 0.7	778.8 ± 54.4
	Bark	38.0 ± 1.6	11.00 ± 0.21	261.3 ± 16.3	590.8 ± 17.7
	Fruit	0.8 ± 0.4	0.12 ± 0.11	4.2 ± 1.3	11.8 ± 5.6
Cecropia obtusa	Leaf	12.8 ± 0.4	3.49 ± 0.59	46.9 ± 13.0	375.3 ± 57.7
	Bark	12.3 ± 0.2	3.14 ± 0.04	56.6 ± 1.7	209.6 ± 23.5
Cecropia palmata	Leaf	19.6 ± 0.1	4.95 ± 0.06	108.9 ± 0.9	489.2 ± 10.7
	Bark	4.1 ± 2.4	0.78 ± 0.72	13.9 ± 1.1	100.1 ± 24.4
Cedrela odorata	Bark	15.0 ± 8.7	5.13 ± 4.36	171.5 ± 52.7	1038.1 ± 189.6
Cordia exaltata	Leaf	10.2 ± 0.3	ND	50.1 ± 0.5	424.8 ± 37.2
	Fruit	1.9 ± 0.0	ND	10.1 ± 0.4	66.6 ± 7.4
Davilla kunthii	Leaf	36.4 ± 3.8	6.29 ± 0.44	282.9 ± 3.5	212.4 ± 15.7
	Bark	9.9 ± 0.4	2.46 ± 0.10	59.6 ± 3.7	137.8 ± 10.0
Davilla rugosa	Leaf	29.6 ± 0.6	8.02 ± 0.20	193.3 ± 7.5	582.4 ± 17.0
	Bark	5.5 ± 3.3	1.80 ± 0.61	35.1 ± 13.2	141.4 ± 60.2
Dalbergia monetaria	Bark	4.1 ± 0.2	0.07 ± 0.03	12.5 ± 0.7	83.1 ± 8.8
Dalbergia subcymosa	Bark	35.8 ± 0.9	1.43 ± 0.06	197.4 ± 42.3	1396.8 ± 36.8
Eugenia patrisii	Leaf	14.8 ± 0.3	4.21 ± 0.35	100.4 ± 2.5	354.0 ± 15.0
	Fruit	2.6 ± 0.7	0.69 ± 0.02	27.3 ± 0.2	32.3 ± 4.0
Inga edulis	Leaf	9.8 ± 6.7	3.31 ± 2.10	58.1 ± 44.9	239.5 ± 47.4
	Bark	14.8 ± 0.2	5.50 ± 0.14	31.4 ± 1.8	200.7 ±14.2
	Fruit	0.7 ± 0.2	0.12 ± 0.02	2.2 ± 0.4	17.5 ± 0.4
	Seed	0.4 ± 0.0	ND	1.0 ± 0.2	8.9 ± 0.0
Stryphnodendron barbadetimam	Bark	16.6 ± 5.4	3.4 ± 1.90	90.8 ± 23.5	242.2 ± 64.9

Results are means ± SD. ND: not detected.

mechanism against UV-B radiation (Harborne & Williams, 2000). Bark samples were found with the second highest values in TP and TF, due to the presence of flavanol derivatives (condensed tannins), which have a defence function through their antimicrobial activity (Chattopadhyay et al., 2001) and a pronounced bitter taste. The results of TP in mg_{GAE}/g_{FW} of the leaves and barks (Table 2) are similar to or higher than those found in green and black teas, one of the richest sources of polyphenols, which can vary from 16 to 73.5 mg_{GAE}/g_{FW} (Prior & Cao, 1999).

3.2. Antioxidant activities

The antioxidant activities in the plant extracts were focused on phenolic compounds and the two assays used to assess their antioxidant activities are based on different radicals and mechanisms of reaction. Results of TEAC assay are summarized in Table 2. The range of TEAC values obtained was very large, from 1.0 up to 347.1 $\mu mol_{TE}/g_{FW}$ (Table 2), with TEAC values of leaves generally greater than those in barks. TEAC of most of the plants under investigation are similar to or higher than those of other plants rich in antioxidants such as strawberry (25.9), raspberry (18.5), red cabbage (13.8), broccoli (6.5), spinach (7.6) (Proteggente et al., 2002), holm oak leaves (*Quercus ilex*) (<50) (Omari et al., 2003), and pulps (2.4), skins (12.8), seeds (281.3), and leaves (236.1) of Muscadine grapes (Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003).

As illustrated in Fig. 2, a high correlation was found between TEAC and TP ($r^2 = 0.88$, $p < 0.001$, $n = 65$) when analyzing the group of the studied species together, as already reported in the literature (Cai, Luo, Sun, & Corke, 2004; Javanmardi, Stushnoff, Locke, & Vivanco, 2003). As a consequence, TEAC was also significantly correlated with TF ($r^2 = 0.75$, $p < 0.001$, $n = 54$).

With regard to ORAC measures, $ORAC_{Fluorescein}$ was the only method adapted to measure the scavenging activity of peroxyl radicals in this study. In fact, the $ORAC_{\beta\text{-PE}}$, which uses the protein β-phycoerythrin (β-PE) as fluorescent probe (Cao & Prior, 1999), did not work appropriately with several of the samples (results not shown) since the β-PE fluorescence was completely inactivated, which might be explained by the capacity of flavanol polymers (proanthocyanidins or condensed tannins) to precipitate proteins (Hagerman & Butler, 1981). Furthermore, an absence of linearity was observed between extract concentrations and initial relative fluorescences, which can be attributed to interactions between β-PE and the polyphenols, as was also observed by Ou et al. (2001) for grape seed extracts. Fortunately, the $ORAC_{FL}$ which uses the fluorescein (FL) as fluorescent probe overcame all these troubles, and all samples could be measured properly.

Results of ORAC assay are shown in Table 2. The values ranged from 6.7 to 1396.4 $\mu mol_{TE}/g_{FW}$, a greater range than in TEAC. ORAC values of leaves and barks were the most elevated, as for TEAC. Their values were higher than well-known antioxidant-rich sources such as the fruits plum (62.4), strawberry (35.8), blueberry (62.2), raspberry (49.3), the vegetables radish (9.5), potato (russet) (13.2), carrot (12.2), broccoli (15.9), the nuts almond (44.5), pecan (179.4), pistachio (79.8), the dried fruits date (39.0), raisin (30.4), prune (85.8), and the grain brans rice bran (242.9) (Wu et al., 2004).

As illustrated in Fig. 3, the correlations between ORAC values and polyphenol contents were high. However, for the correlation analysis, the samples of *Cedrela odorata* and *D. subcymosa* were not taken into account, since the simple removal of these samples improved the coefficient of determination (r^2) from 0.57 to 0.70 when relating ORAC with TP, and from 0.39 to 0.74 when relating ORAC with TF ($p < 0.001$).

The correlation between ORAC and TEAC was moderate ($r^2 = 0.55$, $n = 65$, $p < 0.001$), and demonstrates how a single assay is not sufficient to evaluate the total antioxidant activity (Frankel & Meyer, 2000). The present approach using both ORAC and TEAC assays is to be recommended when seeking for new antioxidant-rich plants. From a mechanistic point of view, ORAC should be considered better than TEAC (Ou et al., 2001). The ORAC assay follows a hydrogen atom transfer mechanism (the H of the polyphenols neutralizes the radicals formed), measuring, thus, the capacity of an antioxidant to break chain reactions. The

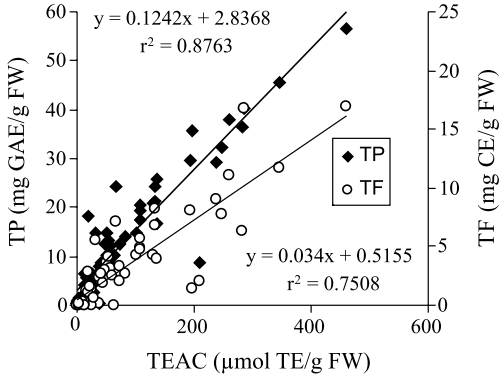

Fig. 2. Correlation between the antioxidant activity (TEAC) and total phenolics (TP) ($n = 65$) and total flavanoids (TF) ($n = 54$).

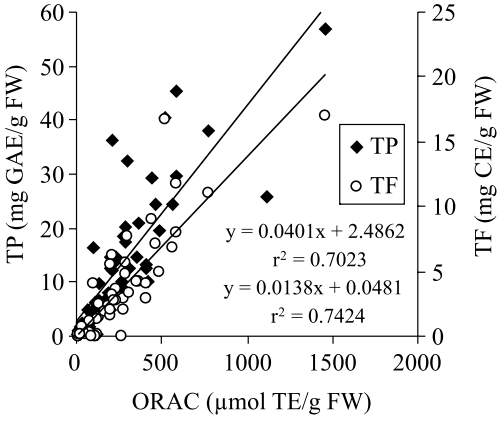

Fig. 3. Correlation between the antioxidant activity (ORAC) and total phenolics (TP) ($n = 62$) and total flavanoids (TF) ($n = 51$).

TEAC assay is considered a method that only measures the redox power of the antioxidant mixture (as an extract) in relation to the radical cation $ABTS^{+\bullet}$. The increase in the correlation between ORAC values and polyphenol contents by removing *D. subcymosa and C. odorata* samples from the regression analysis suggests that ORAC measures the activity of antioxidants other than the phenolics. The same was not observed with TEAC.

Overall, an elevated antioxidant activity was associated with both high TP and TF. This has also been observed for *Rubus* berries in which anthocyanins and TP both account for their antioxidant activity (Deighton, Brennan, Finn, & Davies, 2000).

4. Conclusions

In this study, results of TP content, TF content, TEAC and ORAC of 15 Amazonian plants were obtained for the first time. A high correlation was found between TP and TF, as well as between the polyphenolic contents and the antioxidant activities. Due to the elevated values of their antioxidant activity, the species *B. crassifolia*, *Bauhinia macrostachya*, *C. palmata, C. odorata*, *C. exaltata*, *D. kunthii*, *Davilla rugosa*, *D. subcymosa*, *I. edulis*, and *Stryphnodendron barbadetimam* may be considered as interesting sources of antioxidants. These should be further analysed, regarding their chemical and technological aspects, such as the identification of polyphenolic structures, extractability of compounds, and stability of polyphenols. Further analyses regarding their biological properties, such as preventive capacity against LDL oxidation, biodisponibility, and toxicity of the crude extracts should be performed as well.

Acknowledgments

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References

Cadet, J., Douki, T., Gasparutto, D., & Ravanat, J. L. (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 531*, 5–23.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences, 74*, 2157–2184.

Cao, G., & Prior, R. L. (1999). Measurement of oxygen radical absorbance capacity in biological samples. *Methods in Enzymology, 299*, 50–62.

Chattopadhyay, D., Maiti, K., Kundu, A. P., Chakraborty, M. S., Bhadra, R., Mandal, S. C., & Mandal, A. B. (2001). Antimicrobial activity of *Alstonia macrophylla*: a folklore of bay islands. *Journal of Ethnopharmacology, 77*, 49–55.

Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild *Rubus* species. *Journal of the Science of Food and Agriculture, 80*, 1307–1313.

Delcour, J. A., & Janssens de Varebeke, D. (1985). A new colorimetric assay for flavonoids in pilsner beers. *Journal of the Institute of Brewing, 91*, 37–40.

Duthie, G. G., Duthie, S. J, & Kyle, J. A. M. (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews, 13*, 79–106.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Hagerman, A. E., & Butler, L. G. (1981). The specificity of proanthocyanidin-protein interactions. *Journal of Biological Chemistry, 256*, 4494–4497.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481–504.

Hirayama, O., Takagi, M., Hukumoto, K., & Katoh, S. (1997). Evaluation of antioxidant activity by chemiluminescence. *Analytical Biochemistry, 247*, 237–241.

Huang, D., Ou, B., Hampsch-Woodill, M. F., Judith, A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437–4444.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry, 83*, 547–550.

Myhrstad, M. C. W., Carlsen, H., Nordström, O., Blomhoff, R., & Moskaug, J. O. (2002). Flavonoids increase the intracellular glutathione level by transactivation of the γ-glutamylcysteine synthetase catalytical subunit promoter. *Free Radical Biology and Medicine, 32*, 386–393.

Nielsen, F., Mikkelsen, B. B., Nielsen, J. B., Andersen, H. R., & Grandjean, P. (1997). Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry, 43*, 1209–1214.

Omari, B., Fleck, I., Aranda, X., Abadía, A., Cano, A., & Arnao, M. B. (2003). Total antioxidant activity in *Quercus ilex* resprouts after fire. *Plant Physiology and Biochemistry, 41*, 41–47.

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. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Pastrana-Bonilla, E., Akoh, C. C., Sellappan, S., & Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal of Agricultural and Food Chemistry, 51*, 5497–5503.

Prior, R. L., & Cao, G. H. (1999). Antioxidant capacity and polyphenolic components of teas: implications for altering in vivo antioxidant status. *Proceedings of the Society for Experimental Biology and Medicine, 220*, 255–261.

Prior, R. L., Wu, X. L., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., et al. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*, 217–233.

Re, R., Pelligrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Robards, K., & Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids. A review. *Analyst, 122*, 11R–34R.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their roles in oxidative process in fruits. *Food Chemistry, 66*, 401–436.

Rogez, H. (2000). *Açaí: Preparo, Composição e Melhoramento da Conservação*. Belém (Brazil): Edufpa.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Sun, A. Y., & Chen, Y. M. (1998). Oxidative stress and neurodegenerative disorders. *Journal of Biomedical Science, 5*, 401–414.

Van den Berg, R., Haenen, G. R. M. M., Van den Berg, H., & Bast, A. A. L. T. (1999). Applicability of an improved trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chemistry, 66*, 511–517.

Weisburger, J. H. (1999). Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food and Chemical Toxicology, 37*, 943–948.

Wollenweber, E. (1993). Flavones and flavonols. In J. B. Harborne (Ed.), *The flavonoids, advances in research since 1986* (pp. 259–335). London: Chapman & Hall.

Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., et al. (2004). Development of a database for total antioxidant capacity in foods: a preliminary study. *Journal of Food Composition and Analysis, 17*, 407–422.

Yang, C. S., Landau, J. M., Huang, M. T., & Newmark, H. L. (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review of Nutrition, 21*, 381–406.