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Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use

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ABSTRACT

The total phenol and flavonoid contents of 19 Amazonian plants and their related antioxidant activities were determined. The extracts from the plant leaf, bark, root, fruit and/or stem were prepared as infusions, as are traditionally used in popular medicine. Total phenols ranged from 0.8 to 22.2 mg gallic acid equivalents/g and flavonoids from 0.0 to 10.2 mg catechin equivalents/g, by using Folin–Ciocalteau and aluminium chloride colourimetric methods. Differences were observed in phenol and flavonoid contents at the organic level, the leaf presenting greater values than the stem. All the extracts showed different degrees of antioxidant activity with TEAC, 1.1 up to 117.4 and ORAC, 7.8 up to 359.1 μmol Trolox equivalents/g. These values correlated with total phenol content (r^2 = 0.90) and flavonoid content (r^2 = 0.70 for TEAC; r^2 = 0.76 for ORAC). *Piper putumayoense*, *Piper glandulosissimum*, *Piper krukoffii* and *Senna reticulata* leaves and *Brownea rosademonte* bark showed elevated antioxidant activities, thus representing promising plant-sources of medicine.

1. Introduction

There is increasing interest in naturally occurring antioxidants for use in foods to replace synthetic antioxidants. The advantages of their use are numerous. Thus, the combined action of two or more components in a vegetable often potentiates a specific therapeutic action (Liu, 2004), and with no observed secondary or collateral effects, as is the case with chemically synthesised compounds (Velioglu, Mazza, Gao, & Oomah, 1998). Many of the therapeutic actions of phytochemicals are ascribed to their biologically active polyphenol components, such as flavonoids and phenolic acids, which possess powerful antioxidant activities (Croft, 1998; Pietta, 2000).

The use of plants in traditional medicine since ancient times has led to a very important selection of vegetables with specific actions in health. Many other plants with toxic or innocuous actions have been discarded in this process. Plants with therapeutic actions are commonly used, particularly in phytogeographic regions, such as Amazonia. The biodiversity-rich rainforest in the Amazonian Northwest (Ecuador, Colombia, Peru) is a source of autochthonous plant species, which are used by the indigenous communities (Vandebroek, Van, Van, Arrazola, & De, 2004). The herbs, prepared by different ways (cooking, infusion or maceration), are traditionally used in food preparations and are particularly known in folklore for their therapeutic potential. For instance, the *Brownea rosademonte* bark has been used for its neutralisation and anticoagulant abilities against snake venom (Nuñez et al., 2004; Otero et al., 2000); the plant species *Solanum grandiflorum* is widely used for its relaxant, sedative, and anti-spasmodic properties (de Angelis Pereira et al., 2003); and the leaves of *Uncaria guianensis* possess anti-inflammatory and anti-allergic activities (Carvalho, Penido, Siani, Valente, & Henriques, 2006). Yet, data related to the antioxidant activity as well as phenol content of these and other Amazonian species are unknown. In view of there being limited or no data to this respect, and both the herbal ethnobotanical and pharmacological interest, the aim of the present study was to determine total phenol and flavonoid content and the antioxidant activities of 19 Amazonian plants from Colombia prepared as traditionally used in folk medicine. The antioxidant activities were evaluated by the Trolox equivalent antioxidant activity (TEAC) and oxygen radical absorbance capacity (ORAC) assays.

2. Materials and methods

2.1. Materials

The phenolic standards, catechin and gallic acid, potassium persulfate and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma–Aldrich®, USA. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and fluorescein sodium salt were from Sigma, USA, 2,2-azinobis

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(2-amidinopropan) dihydrochloride (AAPH) was from Wako Pure Chemical Industries, Japan. All other used reagents were from the highest purity available.

2.2. Plant samples

Diverse plants of native Amazonian species with known therapeutic actions were collected from the Macagual Research Centre forest in Florencia, Caquetá (Colombia) (Table 1). The plants were selected on the basis of data from their traditional use in Colombian folk medicine. The plants were taxonomically identified by botanical experts and deposited at the Herbarium of the Botanical Garden of Amazonia University – HUAZ (Florencia, Colombia).

The plant samples were processed in the laboratory within a maximum of 24 h after harvesting. Otherwise, the material was stored under refrigeration at 4 °C.

2.3. Preparation of plant extracts

Plant extracts were obtained from infusions prepared as generally used in traditional medicine. For this purpose, the different parts of the fresh plants were rinsed in water, cut into tiny pieces and boiled in 500 ml water with constant shaking for 15 min. The mixture was allowed to settle for 10 min. Aliquots were taken and stored at −20 °C. The samples were carried to the Physiology Department at the Medicine and Dentistry School of the University of the Basque Country (Spain). Once defrosted, samples were centrifuged at 1200*g* for 5 min at 4 °C, and aliquots of supernatants were frozen at −80 °C. On the day of assay, the extracts were cleared by centrifugation at 3600*g* for 5 min at room temperature, and the supernatant used.

2.4. Determination of total phenols

Total phenols were estimated by a colourimetric assay based on procedures described by Velioglu et al. (1998) with some modifications. Basically, 100 μl of the sample were mixed with 750 μl of 1/10 diluted (in Milli-Q water) Folin–Ciocalteau phenol reagent. After 5 min in the dark at room temperature, 750 μl of sodium bicarbonate (60 g/l) were added to the mixture. The tubes were kept in the dark for 90 min at 30 °C, after which the absorbance was read at 725 nm. Gallic acid (6–32 μg) was used for constructing the standard curve. Results were expressed as mg of gallic acid (GA) per gram of the fresh plant part.

2.5. Determination of total flavonoids

Flavonoids were determined by a colourimetric method described by Barreira, Ferreira, Oliveira, and Pereira (2008) with some modifications. Hundred microlitres of the plant extracts were mixed with 500 μl Milli-Q water and 30 μl of a 5% $NaNO_2$ solution. After 5 min, 60 μl of a 10% $AlCl_3$ solution was added. After 6 min incubation at room temperature, 200 μl of 1 M NaOH and 110 μl of Milli-Q water were added. Tubes were incubated for 15 min at room temperature. The absorbance was read at 510 nm. The absorbance of each blank, consisting of the same mixture in which $AlCl_3$ solution was substituted by Milli-Q water, is subtracted from the test absorbance (Joubert, Manley, & Botha, 2008). Catechin (0.42–27.15 μg diluted in ethanol) was used as standard. Results were expressed as mg of catechin (C) per gram of the fresh plant part.

2.6. Trolox equivalent antioxidant capacity (TEAC)

The antioxidant activity was determined by the TEAC assay, using the radical cation $ABTS^{+\cdot}$, according to the procedure described by Re et al. (1999).

The reaction was started with the addition of 1 ml of the $ABTS^{+\cdot}$ solution (0.70 ± 0.01 at λ = 734 nm) to 10 μl of the plant extract in a cuvette maintained at 30 °C. The absorbance readings were taken 30 s after initial mixing and every min up to 12.5 min, using a Uvikon 943 spectrophotometer. A calibration curve for the TEAC was built by using different Trolox concentrations (0.25–2.0 mM) in phosphate buffer saline. The TEAC of the plant extracts was calculated comparing the area under the curve, derived from plotting the percentage inhibition of the absorbance as a function of time, with the area under the curve for Trolox standard. Different dilutions of the plant extracts, showing a final percentage inhibition of 40–60%, were used. All the determinations were carried out in duplicate. The TEAC was expressed as μmol of Trolox equivalents per gram of the plant part used. The intra- and inter-assay coefficients were 2.2% and 2.7%, respectively.

2.7. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed using fluorescein as a fluorescent probe by the method described by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002).

The analysis was carried out using 96-well microplates, in which 25 μl of the plant extracts were mixed with 150 μl of

Table 1
Botanical names, families, plant parts and uses in Amazonia of the 19 plants studied.

Botanical name	Family	Plant part	Medicinal uses in Amazonia
Abuta grandiflora	Menispermaceae	Bark	Antimicrobial, anti-plasmodium, anti-cytotoxic against snake bites, anti-diabetic
Anacardium excelsum	Anacardiaceae	Leaf, stem	Anti-diarrheic, stomach pain
Anacardium occidentale	Anacardiaceae	Fruit	Anti-diarrheic, against topical diseases (dermatitis), cephalalgia
Bellucia grossularioides	Melastomataceae	Fruit	Anti-fungal, anti-infectious
Brownea rosademonte	Caesalpiniaceae	Bark	Anti-haemorragic and anti-cytotoxic against snake bites
Crescentia cujete	Bignoniaceae	Fruit	Smooth muscle relaxant in respiratory diseases, anti-inflammatory, analgesic
Cyperus prolixus	Cyperaceae	Root	Purgative, anti-anaemic
Eugenia stipitata	Myrtaceae	Fruit	Anti-hypertensive
Irlbachia alata	Gentianaceae	Leaf, stem	Anti-malarial, anti-fungal, anti-inflammatory
Justicia pectoralis	Acanthaceae	Leaf, stem	Anti-diabetic, smooth muscle relaxant in respiratory diseasses, against prostate diseases, anti-bacterial, sedative
Piper glandulosissimum	Piperaceae	Leaf, stem	Anti-inflammatory
Piper krukoffii	Piperaceae	Leaf, stem	Anti-inflammatory
Piper putumayoense	Piperaceae	Leaf, stem	Anti-inflammatory
Senna reticulata	Fabaceae	Leaf	Anti-rheumatic, hypoglycaemic, against topical diseases (dermatitis)
Solanum cf grandiflorum	Solanaceae	Stem	Anti-cancer, sedative
Solanum sessiliflorum	Solanaceae	Fruit	Against snake bites and scorpion stings, skin infections, anti-hypertensive
Spathiphyllum sp.	Araceae	Leaf	Anti-inflammatory, anti-cancer
Uncaria guianensis	Rubiaceae	Stem	Anti-inflammatory, anti-rheumatic, anti-cancer
Vismia baccifera	Clusiaceae	Stem	Against topical diseases (dermatitis)

fluorescein (8.16×10^{-5} mM in 75 mM phosphate buffer, pH 7.4) and incubated for 15 min at 37 °C in the microplate. Then, 25 μl of 153 mM AAPH were added and the microplate was shaken for 30 s. The fluorescence was recorded ($\lambda_{excitation}$ = 485 nm, $\lambda_{emission}$ = 520 nm) every 75 s for 50 min in the fluorimeter microplate reader (Synergy HT, Biotek). All the plant samples were analysed at four dilutions and the mean value was taken for ORAC (Huang et al., 2002). Trolox solutions (1.25–50 μM), prepared daily in phosphate buffer, were used for the calibration curve. Results were calculated based upon differences in areas under the fluorescence decay curve between the blank, samples and standards. Final ORAC values were expressed as μmol of Trolox equivalents per gram of the fresh plant part. All the determinations were carried out in triplicate and at least two independent experiments were performed for each sample. The intra- and inter-assay coefficients were 3.2% and 4.8%, respectively.

2.8. Statistical analysis

Results were expressed as the mean ± standard deviation (SD). SPSS (version 16.0) statistical program was used for data analysis (Pearson's correlation coefficient).

3. Results and discussion

The total phenol and flavonoid contents and antioxidant activity of the different parts of the Amazonian plants studied are shown in Table 2. Total phenols and flavonoids were expressed in terms of gallic acid (GA) and catechin (C) equivalents per gram of the fresh plant part used. The mean values of phenols ranged from 0.8 to 22.2 mg GA/g and flavonoids from 0.0 to 10.2 mg C/g. The leaves from *Piper putumayoense* and *Piper krukoffii* and *B. rosademonte* bark showed the highest levels of total phenols (22.2, 16.8 and 17.2 mg GA/g, respectively) and flavonoids (10.2, 8.7 and 4.5 mg C/g, respectively). The rest of the extracts showed phenol values below 11 mg GA/g, with a mean value of 3.7 mg GA/g. The plant organs had different total phenol contents, the leaf containing about 4-fold higher phenol concentrations than the stem. *Irlbachia alata* stem (0.8 mg GA/g) and *Anacardium occidentale* fruit (0.8 mg GA/g) showed the lowest phenol content. Regarding flavonoids, the mean value of catechin equivalents was 0.8 mg/g, excluding values from the three extracts with the highest flavonoid content and those from *Bellucia grossularioides* fruit and *I. alata* stem, where flavonoids were undetected. Differences in flavonoid content at the organic level were also observed, the leaf presenting higher values than the stem. These results are in agreement with previous works reporting high leaf/stem polyphenol proportions in other plants (Grubesic, Vukovic, Kremer, & Vladimir-Knezevic, 2005), thus confirming that leaf function serves as defence mechanism against UV damage (Harborne & Williams, 2000). Exposure to increased levels of UV radiation causes the leaves to redden and increases the concentrations of total phenols and the main flavonoids (Garcia-Macias et al., 2007).

Table 2 also shows the percentage contribution of flavonoids to total phenols. As can be seen, high phenol content was not always accompanied by high flavonoid concentrations. Thus, for instance, *Senna reticulata* and *Piper glandulosissimum* leaves contained similar total polyphenol concentrations (near 10 mg AG/g), but the percentage contribution of flavonoids to total phenols varied significantly (7% in *S. reticulata* and 35% in *P. glandulosissimum*). The flavonoid/polyphenol proportion varied considerably, ranging from undetected in *B. grossularioides* fruit and *I. alata* stem to higher than 50% in *P. putumayoense* extracts.

Total antioxidant activity of the plant extracts was determined by TEAC and ORAC assays. As can be seen in Table 2, all the extracts tested had TEAC to different degrees (from 1.1 to 117.4 μmol Trolox/g). *B. rosademonte* bark and leaves from *P. krukoffii* and *P. putumayoense* showed the greatest TEAC values, as was the case of total phenols. For a particular plant, TEAC values were higher in leaves than in stem. TEAC of the plants under investigation were similar to or much higher than those reported for other plants containing polyphenols, as in fruits such as kiwi (2.7), apple (1.6), apricot (1.4), peach (1.2), strawberry (33) (Scalzo, Politi, Pellegrini, Mezzetti, &

Table 2
Total phenols and flavonoids, TEAC and ORAC in the studied Amazonian plants.

Species	Plant part	Phenols (mg GA/g)	Flavonoids (mg C/g)	Flav./phenols (%)	TEAC (μmol Trolox/g)	ORAC (μmol Trolox/g)
Abuta grandiflora	Bark	0.99 ± 0.00	0.39 ± 0.00	36.3	3.7 ± 0.5	12.9 ± 0.7
Anacardium excelsum	Leaf	9.90 ± 0.08	1.49 ± 0.03	15.1	56.6 ± 3.7	65.3 ± 3.6
	Stem	4.58 ± 0.03	0.74 ± 0.03	16.2	29.3 ± 0.9	17.5 ± 0.5
Anacardium occidentale	Fruit	0.84 ± 0.01	0.01 ± 0.00	1.6	5.3 ± 0.3	7.8 ± 0.2
Bellucia grossularioides	Fruit	3.58 ± 0.01	ND	0.0	26.6 ± 1.1	26.8 ± 2.4
Brownea rosademonte	Bark	17.18 ± 0.24	4.54 ± 0.18	26.4	117.4 ± 4.9	289.4 ± 9.3
Crescentia cujete	Fruit	0.86 ± 0.01	0.02 ± 0.00	2.6	1.1 ± 0.1	11.8 ± 0.4
Cyperus prolixus	Root	3.15 ± 0.00	0.80 ± 0.04	25.4	18.5 ± 1.3	31.6 ± 1.4
Eugenia stipitata	Fruit	1.57 ± 0.00	0.05 ± 0.00	3.4	5.5 ± 0.3	12.1 ± 0.4
Irlbachia alata	Leaf	2.29 ± 0.02	0.14 ± 0.00	6.0	4.9 ± 0.3	59.8 ± 3.5
	Stem	0.81 ± 0.01	ND	0.0	1.7 ± 0.2	12.5 ± 1.1
Justicia pectoralis	Leaf	5.65 ± 0.01	0.75 ± 0.04	13.3	18.8 ± 0.7	89.7 ± 3.7
	Stem	1.18 ± 0.00	0.12 ± 0.00	9.7	5.3 ± 0.7	21.9 ± 1.0
Piper glandulosissimum	Leaf	10.62 ± 0.02	3.76 ± 0.01	35.4	34.8 ± 1.2	139.6 ± 4.1
	Stem	1.71 ± 0.01	0.46 ± 0.01	26.7	5.1 ± 0.1	44.3 ± 1.6
Piper krukoffii	Leaf	16.84 ± 0.07	8.69 ± 0.06	51.6	92.0 ± 3.8	247.1 ± 18.5
	Stem	5.26 ± 0.08	2.66 ± 0.04	50.6	29.1 ± 2.1	128.6 ± 5.0
Piper putumayoense	Leaf	22.20 ± 0.11	10.20 ± 0.03	45.9	91.0 ± 12.3	359.1 ± 44.4
	Stem	3.70 ± 0.08	1.66 ± 0.00	44.9	13.2 ± 3.5	55.6 ± 2.8
Senna reticulata	Leaf	9.32 ± 0.01	0.67 ± 0.00	7.2	34.0 ± 4.4	222.6 ± 14.8
Solanum cf grandiflorum	Stem	1.49 ± 0.00	0.25 ± 0.00	17.1	5.9 ± 0.2	20.5 ± 1.0
Solanum sessiliflorum	Fruit	0.96 ± 0.03	0.07 ± 0.00	7.4	1.9 ± 0.1	12.3 ± 0.7
Spathiphyllum sp.	Leaf	3.99 ± 0.02	0.67 ± 0.03	16.8	11.1 ± 1.2	46.8 ± 1.3
Uncaria guianensis	Stem	4.66 ± 0.03	1.49 ± 0.01	31.9	26.8 ± 1.3	69.4 ± 2.9
Vismia baccifera	Stem	4.01 ± 0.01	0.73 ± 0.01	18.2	20.5 ± 1.7	58.8 ± 3.7

Results are mean ± SD. ND, not detected.

Battino, 2005), and vegetables including onion (5.3), red cabbage (13.8), spinach (7.6), lettuce (1.7) and tomato (2.6) (Proteggente et al., 2002). It is noteworthy that the herbs were not extracted by organic solvents, but were prepared as infusions in water, as normally used in traditional medicine. The antioxidant activities we have registered in this study indicate that, for instance, a 200 ml cup of *B. rosademonte* bark infusion (20 g/500 ml H_2O) represents a TEAC of 939 μmol Trolox equivalents, the same antioxidant activity as 293 ml of orange juice (Pellegrini, Del Rio, Colombi, Bianchi, & Brighenti, 2003), 170 ml of grape juice (van den Berg, Haenen, van den Berg, & Bast, 1999) or 250 ml of black tea (Pellegrini et al., 2003).

ORAC results showed similar trends to TEAC data for the different plants studied (Table 2). ORAC values ranged from 7.8 to 359.1 μmol Trolox/g, the leaves of *P. krukoffii* and *P. putumayoense* and *B. rosademonte* bark showing the highest values. *S. reticulata* and *P. glandulosissimum* leaves also recorded high ORAC values, although their TEAC were not particularly high. This discrepancy in total antioxidant activity values depending on the method used indicates that both assays determine different aspects of the antioxidant capacity. Although both assays are based on antioxidant inhibition properties, different radicals and mechanisms of reaction are occurring. Thus, the TEAC assay uses exogenous $ABTS^+$ radicals, whereas the ORAC assay uses more physiologically relevant peroxyl radicals. In addition, different antioxidants contribute differently to the total antioxidant activity in a fluid, depending on the TEAC or ORAC assay used. For instance, uric acid contributed 7.1% to serum ORAC and 19.3% to serum TEAC (Cao & Prior, 1998). Our results showed a correlation value r^2 of 0.70 between both methods for the 25 plant extracts studied (data not shown).

The TEAC and ORAC antioxidant activity values increased with increasing polyphenol content of the plant extracts analysed (Fig. 1A). Thus, total polyphenol content correlated ($r^2 = 0.898$) with both measures of total antioxidant activity, in agreement with previous works (Corral-Aguayo, Yahia, Carrillo-Lopez, & Gonzalez-Aguilar, 2008; Proteggente et al., 2002). It should be noted that, although we observed a correlation between antioxidant values and polyphenol content, the Amazonian plant extracts might contain other compounds that could contribute to their overall antioxidant potential. In this sense, low correlations between phenolic content in Tunisian olives and their total antioxidant capacity have been described in the literature (Ben Othman, Roblain, Thonart, & Hamdi, 2008). In our system, the total flavonoid content and the antioxidant activity of the plant extract, measured by TEAC ($r^2 = 0.698$) and ORAC ($r^2 = 0.757$) also correlated significantly, but to a lower extent compared with total polyphenols (Fig. 1B). As indicated above, not only non-phenolic components of the plant extracts with antioxidant properties (carotenoids, alkaloids,...), but also phenolic species other than flavonoids, such as coumarins, tannins and/or phenolic acids, could also contribute to the overall antioxidant potential.

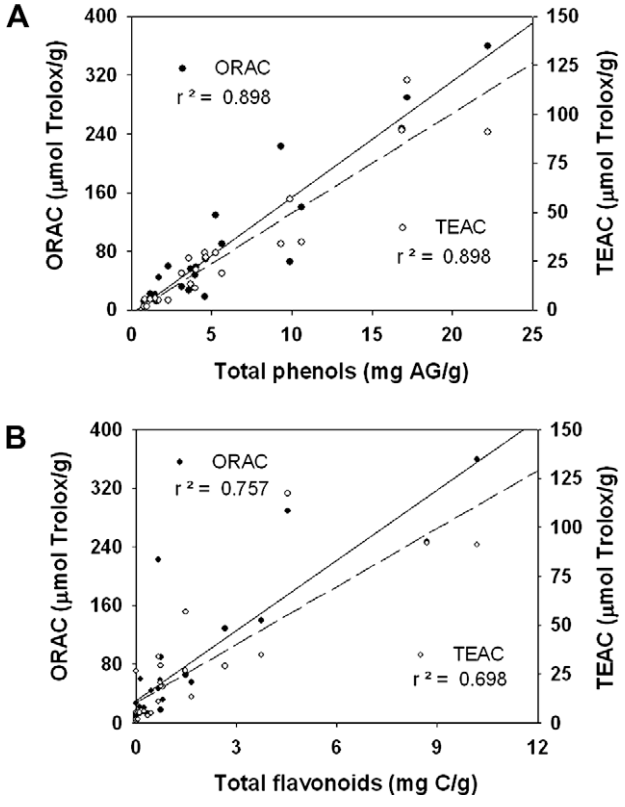

Fig. 1. Correlation between antioxidant activity for ORAC and TEAC methods and (A) total phenols ($N = 25$); and (B) total flavonoids ($n = 23$).

4. Conclusions

In this work, the phenol and flavonoid contents of 19 Amazonian plants and their related antioxidant activities are demonstrated for the first time. We highlight the observed total antioxidant activities of the plant extracts prepared as infusions, as are used in folk medicine. *P. glandulosissimum*, *P. krukoffii*, *P. putumayoense* and *B. rosademonte* species showed elevated antioxidant activities, thus representing promising sources of plant-based medicine. A detailed analysis of their chemical composition, with regards not only to polyphenols, but also other phytochemicals, as well as their *in vitro* toxicity potential in cell systems merits further investigation.

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