

Antioxidant Properties of Medicinal Plants from Peru

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ABSTRACT

There is a wide diversity of plants and seasonal crops in Peru, due to the presence of many climatic zones. Numerous plants are used to cure or prevent diseases. These plants are promising candidates for functional foods products. The most frequent form in which they are used is an aqueous infusion or decoction. In this study, we compared the antioxidant properties of ten Peruvian plants infusions and investigated their relation to the phenolic content. The studied plants were: *Uncaria tomentosa* (cat's claw), *Lepidium meyenii* (maca), *Berberis vulgaris* L. (barberry, agracejo), *Phyllanthus niruri* (chanca piedra), *Annona muricata* L. (graviola, soursop), *Gentianella alborosea* (hercampure), *Geranium dielsianum* (pasuchaca), *Tabebuia ochracea* (tahuari), *Notholaena nivea* ("cuti cuti") and *Tiquilia paronychioides* ("flor de arena"). Infusions of all studied plants have shown antioxidant activity, though there was a large diversity between the results. The antioxidant properties, determined with DPPH and ABTS scavenging assays as well as FRAP test, were strongly correlated with total phenolic content, while there was no correlation with the carotenoid content.

Keywords: Antioxidant; Total Polyphenols; Medicinal Plants

1. Introduction

There is a wide diversity of plants and seasonal crops in Peru, due to the presence of many climatic zones, including the unique areas such as Amazonian rainforest or Andean mountains. Numerous plants are used in medicine, to cure or prevent diseases. Widely known are *Uncaria tomentosa* (cat's claw), *Phyllanthus niruri* (chanca piedra) or *Lepidium meyenii* (maca) which were already shown to have therapeutic or prophylactic potential. These plants are promising candidates for functional foods products.

U. tomentosa, a vine growing in the Amazon region, has been used medicinally by native tribes for at least 2000 years in treating inflammation, arthritis, bone pain, asthma, deep wounds, and cancer [1]. It is probably the best known medicinal plant of South America. Extracts from the bark were extensively studied [2] and found to exhibit immunostimulating, antiinflammatory and antioxidant properties [3].

L. meyenii, a tuber from the central Andes consumed by native Peruvians has also multipharmacological functions such as fertility improvement and the protection of cells against oxidative stress [4,5]. It has been demonstrated that different types of *L. meyenii* (differentiated

by size and colour) have distinct biological properties [6,7]. *L. meyenii* infusion may act as an antioxidant by radical scavenging or by maintenance of intracellular ATP production in conditions of oxidative stress [8] as well as an immunomodulator [9].

Barberry (*Berberis vulgaris* L.) is known in Peru under the name "agracejo". Its fruits, leaves, root and stem are used for ailments of kidneys, liver, urinary and gastrointestinal tract, for the relieve of respiratory tract discomforts, and as a stimulant for the circulatory system [10, 11]. Berberis species were active antioxidants in many tests evaluating antioxidant activity, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, β -carotene bleaching, prevention of lipid peroxidation and oxyhaemoglobin bleaching, or protection from DNA damage [12,13].

Chanca piedra (*P. niruri*), a small herb indigenous to the tropical areas is widely used as medicinal plant. In Ayurveda and Traditional Chinese Medicine it was used for treating dysentery, influenza, diabetes, kidney stones, diuretics and tumours, as well as hepatotoxicity and hyperglycaemia [14]. The biologically active compounds of *P. niruri* are flavonoids, alkaloids, lignans, tannins, coumarins, terpenes, saponins and phenylpropanoids [15,16]. Research on this plant revealed that its aqueous extract inhibited HIV-1 reverse transcriptase [17], as well as re-

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duced urinary calcium in patients with hypercalcuria [18]. It showed antioxidant properties in *in vitro* tests [19], namely free radical-scavenging, inhibition of reactive oxygen species production and peroxidation of lipids, as well as hepatoprotective properties against the paracetamol-induced injury in mice model [20].

Annona muricata L. (graviola, soursop) is a tree widely distributed in most of tropical countries. Its leaves have been traditionally used to treat headaches, hypertension, cough, asthma and as sedative [21,22]. In a model of skin papilloma in mice, the *A. muricata* leaves extract was able to suppress tumor initiation and tumor promotion even at lower dosage [23]. It showed antioxidant [24,25], antibacterial [26], antifungal [27] and anti-inflammatory [28] properties.

Gentianella alborosea (hercampure) is used in folk medicine for obesity treatment, in liver ailments and as colagogue, coleretic and digestive [29]. The active compounds are flavonoids, alkaloids, saponins and glycosides [30]. The extract exhibited moderate antioxidant activity and apoptotic properties on HeLa cell line [1].

Plants from the Geranium genus, to which *Geranium dielsianum* (pasuchaca) belongs, have been shown to have anti-influenza virus activity (*G. sanguineum* L.) [31], as well as antioxidant and radical scavenging capacities (*G. macrorrhizum*) [32]. Those properties have been attributed to their polyphenolic constituents. In traditional medicine *G. dielsianum* is used as blood purifier and hypoglycemic herb.

Antioxidant properties were also observed for *Tabebuia ochracea* (tahuari) [33]. It exhibited also antibacterial activity against *Staphylococcus aureus*.

Notholaena nivea (cuti cuti) is used in South America mainly as a herbal tea with the hypoglycaemic effect [34]. The lipophilic extract from aerial parts of this plant exhibited antioxidant properties [35].

T. paronychioides (flor de arena) is used in traditional medicine for treating inflammation of the ovaries [36] and the antioxidant mechanism is often at least partly responsible for antiinflammatory action. To our best knowledge, there is no data on its antioxidant properties.

The common feature of all mentioned above plants is their antioxidant activity. It is usually ascribed to the presence polyphenols, since a high content of phenolic compounds in a plant is usually connected with high antioxidant properties [37]. However, popular and the most frequent form in which those plants are used is an aqueous infusion or decoction. Its preparation can induce the degradation of polyphenols. Therefore, in this study, we compared the antioxidant properties of ten Peruvian plants infusions and investigated their relations to the phenolic content.

The antioxidant properties were studied with DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sul-

phonic acid)) radical scavenging and ferric reducing antioxidant power (FRAP) tests. For DPPH test we have chosen EPR spectroscopy technique, as it gives more reliable results than spectrophotometry [38]. We determined also the carotenoid content of those plants, to check whether this group of antioxidants can be also partly responsible for antioxidant properties.

2. Experimental

2.1. Plant Material

Ten samples of dried Peruvian plants: *Uncaria tomentosa* (cat's claw), *Lepidium meyenii* (maca), *Berberis vulgaris* L. (barberry, agracejo), *Phyllanthus niruri* (chanca piedra), *Annona muricata* L. (graviola, soursop), *Gentianella alborosea* (hercampure), *Tabebuia ochracea* (tahuari), were obtained from Uncaria Institute (Warsaw, Poland).

2.2. Infusion Preparation

The 2.5 g of dry powdered plant material was weighted and 250 ml of distilled boiling water was added. Then infusions were left for 10 hours at darkness.

2.3. DPPH Scavenging (EPR Test)

100 μ l of an infusion was mixed with 1 ml of 1.3 mM DPPH methanolic solution. After vortexing the samples were kept for 30 minutes at darkness and then EPR spectra were recorded. The samples with distilled water (100 μ l) in place of an infusion were prepared as intensity standards. The intensity was taken as the double integral of the spectra. Results were expressed as Trolox equivalents (TEAC, milimoles per 100 ml) with the use of previously prepared standard curve. All experiments were performed in triplicate.

ESR measurements were performed on a Miniscope MS200 spectrometer (Magnettech GmbH). Parameters were as follows: central field 334 mT, sweep range 8 mT, sweep time 30 s, microwave power 10 mW, modulation amplitude 0.1 mT.

2.4. ABTS Scavenging

ABTS assay was performed according to Re et al [39] with small modifications. Briefly, 1500 μ l of ABTS cation radical solution, prepared by mixing 7 mM ABTS reagent and 2.45 mM potassium persulfate in equal volumes, equilibrating the mixture for 16 hours and diluting with ethanol to absorbance value of 0.70, was added to 15 μ l of plant infusion or standard (trolox) solution. The absorbance reading was taken at 734 nm in sixth minute after adding the radical solution. All experiments were performed in triplicate and results were expressed as milimoles of trolox for 100 ml (TEAC) of an aqueous infusion.

2.5. FRAP Assay

FRAP assay was done according to Benzie and Strain procedure [40]. Briefly, 50 μ l of plant infusion or 50 μ l of freshly prepared FeSO₄ standard solution was mixed with 1500 μ l of working FRAP reagent, and absorbance reading at 593 nm was taken after 4 minutes of thermostating at 37°C. The working FRAP reagent was prepared daily by mixing FeCl₃ and TPTZ (2,4,6-Tripyridyl-s-Triazine) solutions with acetate buffer (pH 3.6). The results were taken as a mean of three replicates and expressed as milimoles of reduced Fe³⁺ per 100 ml of plant infusion.

2.6. Total Phenolic Content

Total phenolic content (TP) was determined by modified Folin-Ciocalteu colorimetric method [41]. Briefly, to 20 μ l of an infusion 1580 μ l of Millipore water and 100 μ l of Folin-Ciocalteu reagent was added. After 5 minutes at room temperature, 300 μ l of 20% sodium carbonate was added, and the reaction mixture was thermostated for 20 minutes at 37°C and the absorbance at 765 nm was taken using Evolution 60S spectrophotometer (Thermo Scientific). Results were expressed as gallic acid equivalents (GAE [mg/100 ml]) with the use of the standard curve, prepared in parallel with measurements. All experiments were performed in triplicate.

2.7. Total Carotenoid Content

Total carotenoid content (TC) determination was carried out as beforehand reported [42] with small modifications. The extraction was realized by adding 30 mL n-hexane-acetone mixture (6:4) to 2.5 g of the powdered sample. After shaking for 10 minutes it was filtered through a paper filter and the absorbance at 450 nm was measured immediately with Evolution 60S spectrophotometer (Thermo Scientific), with up to 10 fold dilution where appropriate. Results were expressed as μ g of carotenoids per 1 g of plant material, calculated with average carotenoid absorbance coefficient A_{1%} of 2500 [43]. All experiments were performed in duplicate.

2.8. Statistical Analysis

Pearson correlation analysis was performed using a Statistica (Statistical Statsoft, Tulsa, OK) software; *P*-values < 0.05 were considered significant.

3. Results and Discussion

Among studied plants, the best DPPH-scavenging properties were observed for *U. tomentosa* and *G. dielsianum* (1.327 \pm 0.034 and 1.234 \pm 0.031 mmol trolox/100 ml, respectively), followed by *N. nivea*, *T. paronychioides* and *A. muricata* L. (**Figure 1(a)**). The DPPH-TEAC va-

lue obtained for those samples was over three times higher than the value for the weakest DPPH scavengers, *i.e.* *L. meyenii* and *T. ochracea* (0.434 \pm 0.005 and 0.413 \pm 0.016, respectively). In case of the ABTS radical, *G. dielsianum* and *U. tomentosa* still showed the highest scavenging activity (0.645 \pm 0.027 and 0.513 \pm 0.061 mmol trolox/100 ml, respectively), although all results obtained from this test were lower than those obtained from the DPPH test (**Figure 1(b)**). However, the best result in the ABTS test (for *G. dielsianum*) was over six times better than those for the weakest scavengers, *i.e.* *L. meyenii* and *T. ochracea* (0.067 \pm 0.010 and 0.079 \pm 0.006 mmol trolox/100 ml, respectively). The result for *U. tomentosa* was also quite high, but for other plants it was distinctly lower. The difference in results from those two tests can be tentatively ascribed to the different structure of those radicals and especially to different charge, since the DPPH molecule has no charge and the ABTS radical is a cation. This can result in different reaction mechanism both for active compounds in plant infusions and for standard antioxidant, *i.e.* trolox.

For the FRAP test results, the highest value was obtained for *T. paronychioides* infusion (0.607 \pm 0.022 mmol Fe/100 ml), followed by *G. dielsianum* (0.562 \pm 0.017 mmol Fe/100 ml) and *U. tomentosa* (0.507 \pm 0.016 mmol Fe/100 ml) (**Figure 1(c)**). The lowest result was given by *L. meyenii* (0.011 \pm 0.001 mmol Fe/100 ml), similar to the radical scavenging tests. In this test the variability among studied samples was distinctly bigger than for previous tests, with the value obtained for *T. paronychioides* over fifty times higher than the value for *L. meyenii*.

The compounds responsible for DPPH and ABTS scavenging, as well as for iron-reducing activity, are mainly polyphenols, as can be seen from coefficients of correlations (*r*) between results of those tests and polyphenol content (**Table 1**), ranging from 0.803 to 0.930. It is worth stressing that correlations between FRAP and ABTS tests results and total polyphenols was significant with *p* < 0.005, and between DPPH test results and polyphenol content with *p* = 0.005.

The polyphenol amount per 100 ml of an infusion varies from 4.6 GAE/100 ml for *L. meyenii* to 75.7 GAE for *T. paronychioides* (**Figure 2(a)**). The high polyphenol content and good antioxidant properties of *U. tomentosa* bark infusion is consistent with results obtained by Gonçalves *et al.* [1], Pilarski *et al.* [44] and Ranilla *et al.* [17]. These works also have shown that potent radical scavenging activity strongly correlated with the presence of proanthocyanidins and phenolic acids. The main phenolic acid was either caffeic acid [1,32] or chlorogenic acid [32].

In our study the total phenolic content obtained for *P. niruri* (14.9 GAE/100 ml) was lower than that for *U.*

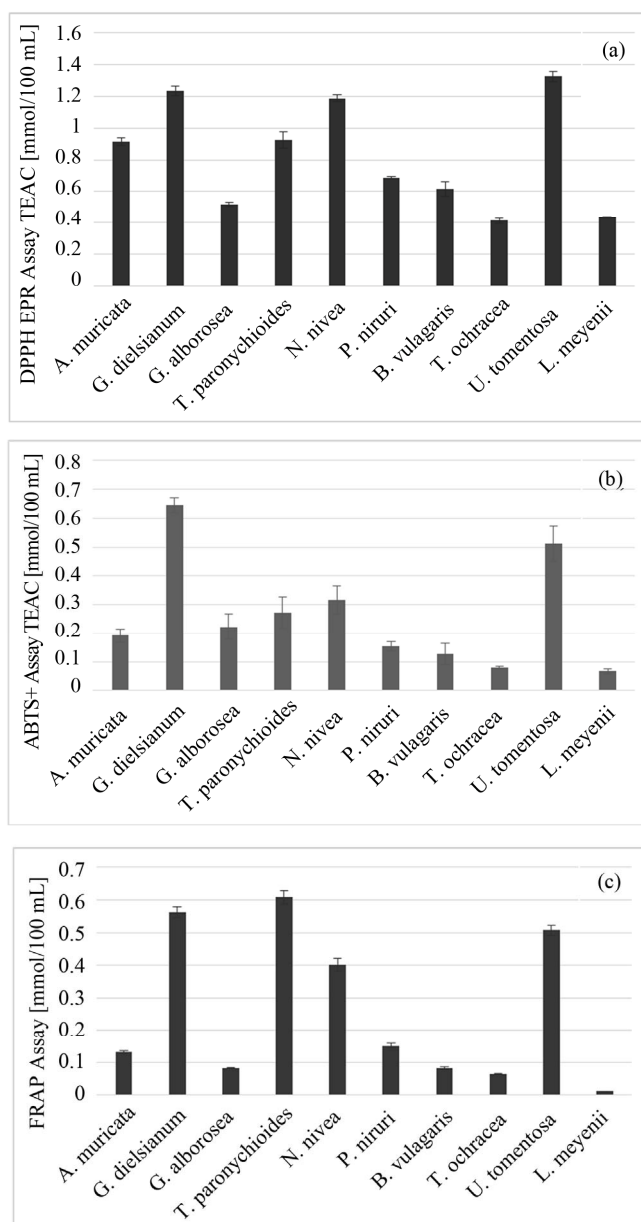


Figure 1. Antioxidant properties of Peruvian plants infusions. (a) DPPH-EPR; (b) ABTS; (c) FRAP. Results are presented as means of three experiments results with standard deviation.

tomentosa (58.9 GAE/100 ml), as opposed to the results of Ranilla [17]. However, this difference can be due to different methods of extraction.

The highest total carotenoid content was observed for *A. muricata* L., the lowest for *T. ochracea* (Figure 2(b)). However, it should be noted that for all studied plants the carotenoid content in plant material was low (0.001 - 0.310 $\mu\text{g}/1\text{ g}$). Whatmore, carotenoids are lipophilic compounds and therefore they would be extracted only in very small part into aqueous infusion. It can be the reason behind the lack of correlation between carotenoids content of plant material and antioxidant properties of aqueous infusions (Table 1).

Table 1. Pearson's correlation coefficients. An asterisk indicates significant correlations.

	ABTS	DPPH	TP	TC
FRAP	0.824*	0.851*	0.930*	-0.289
ABTS		0.868*	0.819*	-0.297
DPPH			0.803*	0.012
TP				-0.297

Overall, the studied plant infusions could be divided into three groups: good antioxidant sources (*U. tomentosa*, *P. niruri*, *T. paronychioides* and *N. nivea*), which

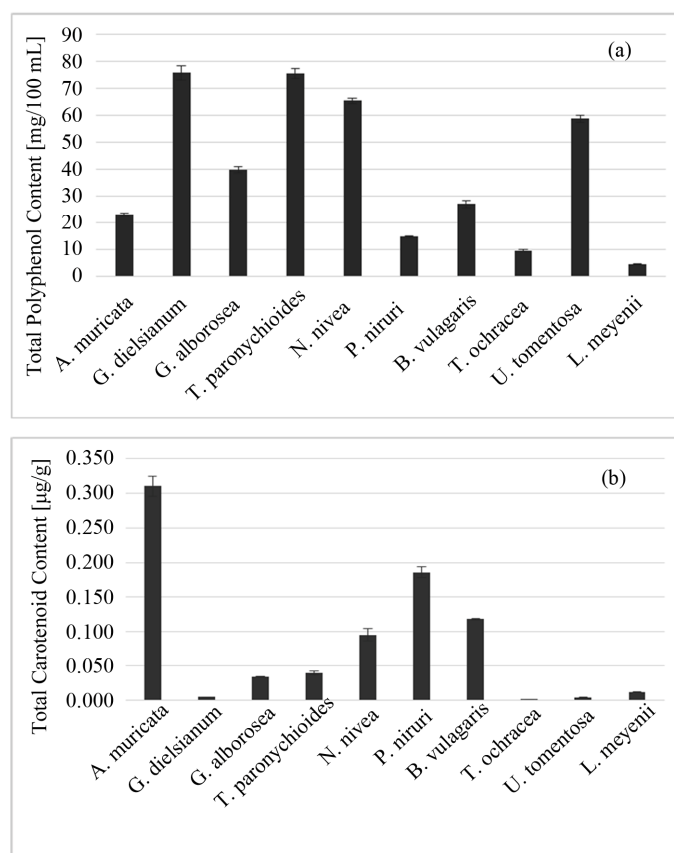


Figure 2. Total phenolic (a) and total carotenoid; (b) content of Peruvian plants infusions and in plant material, respectively. Results are presented as means of three or two respectively experiment results with standard deviation.

gave high results in all antioxidant tests (DPPH, ABTS and FRAP), weak antioxidant sources, with low values obtained from all those tests (*L. meyenii*, *T. ochracea*, *B. vulgaris* L., *P. niruri*) and selective antioxidant sources, which had high or at least moderate values only in one or two tests (*A. muricata* L., *G. alborosea*). Good scavengers have also a high content of total polyphenols, while the phenolic content of plants infusions with low TEAC values is also low. The exception is hercampuri (*G. alborosea*), which is a weak scavenger of ABTS radical and has low FRAP value despite its relatively high polyphenol content.

4. Conclusions

Infusions of all ten studied plants have shown antioxidant activity, though there was a large diversity between the results. Therefore, they can be used as a valuable antioxidant component of human diet not only in areas where they are endemic to, but in the whole world.

The antioxidant properties were correlated with polyphenols content; there was no correlation with the carotenoids content. It supports the hypothesis that the polyphenol group is the dominating group of antioxidants in those plants, at least in their most popular serving form,

i.e. aqueous infusion.

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