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), *Phyllantus niruri* (chanca piedra), *Annona muricata* L. (graviola, soursop), *Gentianella alborosea* L. (graviola, soursop), *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), *Phyllantus 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 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].

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), *Phyllantus niruri* (chanca piedra), *Annona muricata* L. (graviola, soursop), *Gentianella alborosea* L. (graviola, soursop), *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.

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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 paracetal-mol-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 apotopic 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. macorrhizum*) [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. paronychioiodes* (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 anti-inflammatory 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-sulphonic 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), *Phyllantus 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 μl 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 μl) 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 μl 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 μl 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 µl of plant infusion or 50 µl of freshly prepared FeSO4 standard solution was mixed with 1500 µl 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 µl of an infusion 1580 µl of Millipore water and 100 µl of Folin-Ciocalteu reagent was added. After 5 minutes at room temperature, 300 µl 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). 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].
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.

The highest total carotenoid content was observed for *A. muricata* L., the lowest for *T. ochrasea* (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 µg/1 g). Whatsmore, 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.**

<table>
<thead>
<tr>
<th></th>
<th>ABTS</th>
<th>DPPH</th>
<th>TP</th>
<th>TC</th>
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<tbody>
<tr>
<td>FRAP</td>
<td>0.824</td>
<td>0.851</td>
<td>0.930</td>
<td>-0.289</td>
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<tr>
<td>ABTS</td>
<td>0.868</td>
<td>0.819</td>
<td></td>
<td>-0.297</td>
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<tr>
<td>DPPH</td>
<td></td>
<td>0.803</td>
<td>0.012</td>
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<tr>
<td>TP</td>
<td></td>
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<td>-0.297</td>
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Overall, the studied plant infusions could be divided into three groups: good antioxidant sources (*U. tomentosa*, *P. niruri*, *T. paronychioides* and *N. nivea*), which
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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.

REFERENCES


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