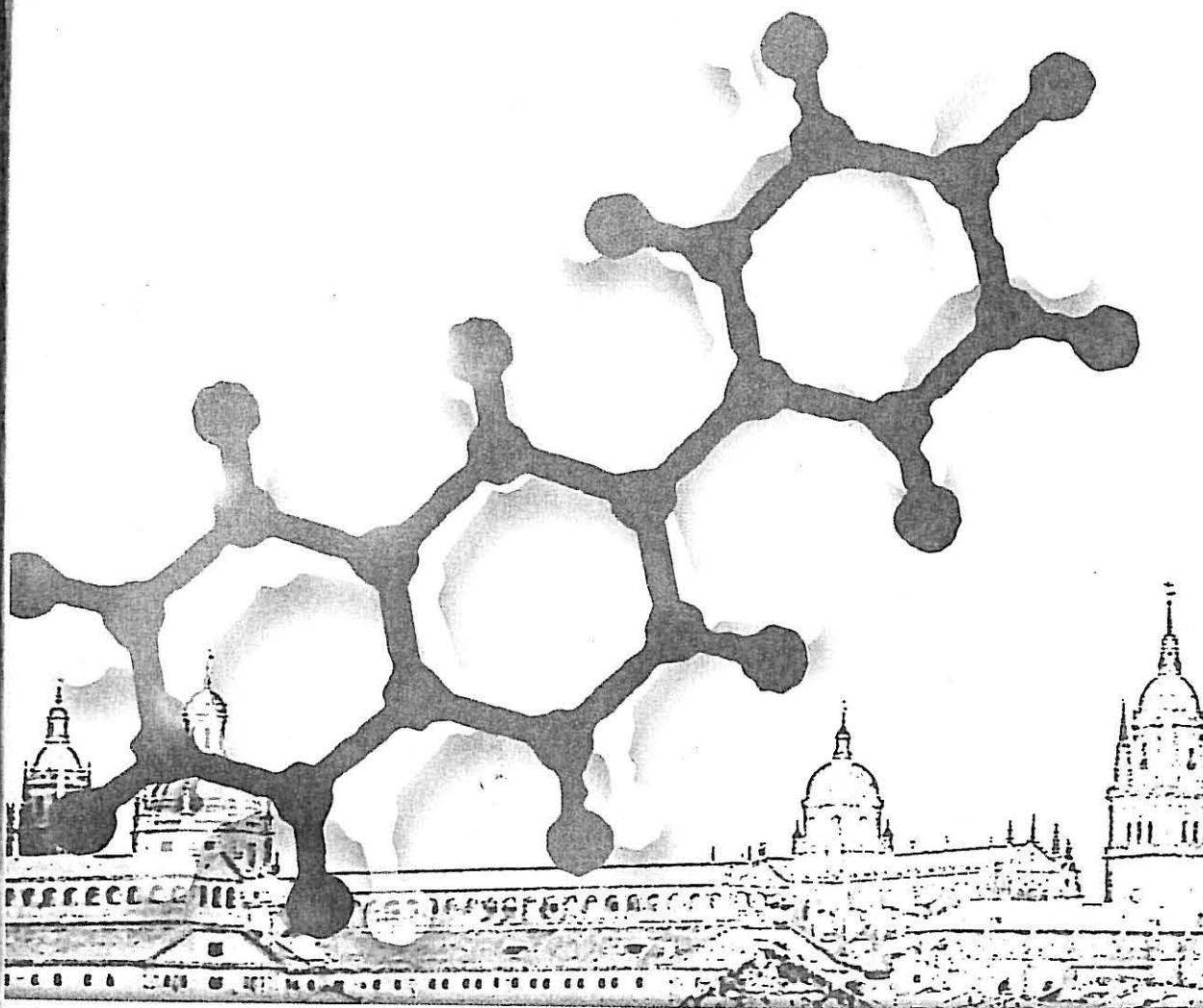


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Antioxidant activity of methanolic extracts from *Cydonia oblonga* leaves

Márcia Carvalho^{1,2*}, Rossana M. Costa¹, José A. Pereira³, Paula B. Andrade⁴, Patrícia Valentão⁴, Rosa M. Seabra⁴, Branca M. Silva^{1,4}. ¹CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, R. Carlos da Maia, 296, 4200-150 Porto, Portugal; ²REQUIMTE, Serviço de Toxicologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 4050-047 Porto, Portugal; ³CIMO, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal; ⁴REQUIMTE, Serviço de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 4050-047 Porto, Portugal; * mcarv@ufp.pt

Abstract. The purpose of this study was to evaluate the total phenolic content and antioxidant activity of *Cydonia oblonga* Miller leaf in comparison to quince fruit and *Camellia sinensis* leaf. For this objective, methanolic extracts were prepared. The total phenolic contents were determined by Folin-Ciocalteu method and the antiradical activities evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) microassay.

All extracts showed similar antioxidant activities and IC₅₀ values (mean IC₅₀ of 19.8 µg/ml) were correlated with total phenolic content (mean value of 225.1 g of 5-*O*-caffeoylquinic acid / kg dry matter). *C. oblonga* leaf is presented as an excellent source of natural antioxidants, similar to green tea (IC₅₀ of 12.7 µg/ml) and much better than quince fruit (pulp, peel and seed).

Introduction. Several studies have showed that *Cydonia oblonga* Miller species is a good source of phenolic acids and flavonoids, which are considered potent antioxidants and its fruit is recognized as an important dietary source of health promoting compounds, due to its antioxidant, antimicrobial and antiulcerative properties [1-3]. Several studies suggest that hydroxycinnamic acids derivatives, are the main responsible for these activities and consequently by the possible health benefits [1,3].

Considering the high phenolic content of quince leaf [2], its capacity to scavenge the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH[•]) was studied and compared with that of green tea (*Camellia sinensis*) and quince fruit.

Materials and Methods. Samples. Six healthy *C. oblonga* leaves samples were collected in the beginning of June (2006) in three different places of two geographical origins of Portugal. Each quince leaves sample was dried in a stove at 30 ± 2°C for 5 days (in the dark). *C. sinensis* dried leaves sample was purchased on the Portuguese market. **Preparation of methanolic extracts.** For each sample, three methanolic extracts were prepared. Briefly, ca. 0.25 g of dried sample was thoroughly mixed with methanol (3 x 25 mL) (40°C). The methanolic extract was filtered, concentrated to dryness under reduced pressure (40°C). **Determination of total phenolic content.** The content of total phenolics in dried leaves was determined by using the Folin-Ciocalteu's phenol reagent. 100 µl of methanolic extract was mixed with 1 ml of Folin-Ciocalteu's phenol reagent and 5 ml of sodium carbonate solution (20%) and the mixture adjusted to 10 ml with water. The reaction was kept in the dark for 20 min, after which the absorbance was read at 735 nm. The total phenolic content was determined from a standard curve using 5-*O*-caffeoylquinic acid as standard. **Determination of DPPH radical scavenging activity.** The antiradical activity of the extracts was determined spectrophotometrically, by monitoring the disappearance of DPPH, according to a previously described procedure [3]. For each extract, a dilution series (five different concentrations) was prepared in a 96 well plate. The reaction mixtures in the sample wells consisted of 25 µL of extract and 200 µL of 150 mM DPPH (dissolved in methanol). The reaction was conducted at room temperature, until no variation of the absorbance was observed. Ascorbic and 5-*O*-caffeoylquinic acids were used as reference compounds. Four experiments were performed in triplicate. The antiradical activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC₅₀). The IC₅₀ value for each extract was determined graphically by plotting the percentage of DPPH scavenging as a function of extract concentration.

Results and Discussion. The study of phenolic content present in the different quince leaves samples revealed high values, between 186.3 and 249.1 g of 5-*O*-caffeoylquinic acid / kg dry matter (mean value of 225.1 g of 5-*O*-caffeoylquinic acid / kg dry matter) (Table 1).

Methanolic extracts of all samples of quince leaves presented a strong concentration-dependent antioxidant activity (Figure 1). The scavenging activity on DPPH radicals was very high at low extract concentrations and IC₅₀ values varied from 14.5 to 23.3 µg/ml (mean value of 19.8 µg/ml) (Table 1). These antioxidant activities are much higher than those previously found for quince fruit [3]. In those studies, peel methanolic extract showed the strongest antioxidant activity, with an IC₅₀ of 600 µg/mL, followed by pulp and seed extracts, with IC₅₀ of 1700 and 2000 µg/ml, respectively.

Table 1. IC₅₀ values and total phenolic contents of quince leaf samples.

| Samples | IC ₅₀ (µg/ml) | SD (µg/ml) | Total phenolic content (g/kg dry matter) | SD (g/kg dry matter) |
|---------|--------------------------|------------|------------------------------------------|----------------------|
| 1 | 17.6 | 0.4 | 245.1 | 2.8 |
| 2 | 21.8 | 1.8 | 237.5 | 1.0 |
| 3 | 20.4 | 1.2 | 249.1 | 2.4 |
| 4 | 14.5 | 1.5 | 205.3 | 3.3 |
| 5 | 21.3 | 0.8 | 227.3 | 5.5 |
| 6 | 23.3 | 0.6 | 186.3 | 1.3 |
| Mean | 19.8 | | 225.1 | |
| SD | 3.2 | | 24.6 | |

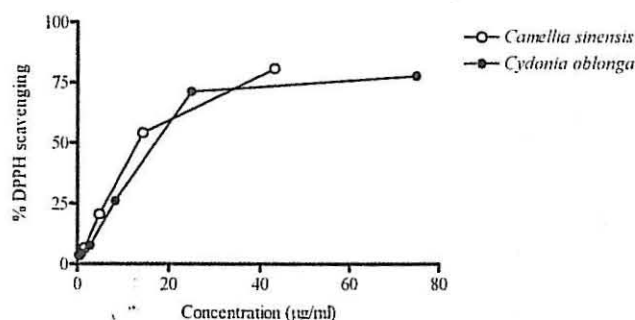


Figure 1. Antiradical activity of the methanolic extracts from *C. oblonga* (sample 4) and *C. sinensis* leaves.

The obtained results demonstrate that quince leaves exhibit interesting antioxidant properties, expressed by their capacity to effectively scavenge DPPH radical, although with a slightly lower activity than that observed for green tea (IC₅₀ of 12.7 µg/ml).

In conclusion, *C. oblonga* leaf is presented as a possible new source of natural antioxidants and may have relevance in the prevention of diseases in which free radicals are implicated. However, further investigation of *in vivo* activity is warranted to confirm these promising results.

References

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