DOI:10.1021/jf902093m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Antioxidant and Biological Properties of Bioactive Phenolic Compounds from *Quercus suber* L.

Ana Fernandes,[†] Iva Fernandes,[†] Luís Cruz,[†] Nuno Mateus,[†] Miguel Cabral,[‡] and Victor de Freitas*,[†]

[†]Chemistry Investigation Centre (CIQ), Department of Chemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal, and [‡]Amorim & Irmãos, S. A., Rua de Meladas 380, 4536-902 Mozelos VFR, Portugal

Phenolic compounds, namely, hydrolyzable tannins and low molecular weight phenolic compounds, were isolated and purified from Portuguese cork from *Quercus suber* L. Some of these compounds were studied to evaluate their antioxidant activity, including free-radical scavenging capacity (DPPH method) and reducing capacity (FRAP method). All compounds tested showed significant antioxidant activity, namely, antiradical and reducing properties. The antiradical capacity seemed to increase with the presence of galloyl groups. Regarding the reducing capacity, this structure−activity relationship was not so clear. These compounds were also studied to evaluate the growth inhibitory effect on the estrogen responsive human breast cancer cell line (ER+) MCF-7 and two other colon cancer cell lines (Caco-2 and HT-29). Generally, all the compounds tested exhibited, after a continuous exposure during a 48 h period, a dose-dependent growth inhibitory effect. Relative inhibitory activity was primarily related to the number of phenolic hydroxyl groups (galloyl and HHDP moieties) found in the active structures, with more groups generally conferring increased effects, except for HHDP-di-galloyl-glucose. Mongolicain B showed a greater potential to inhibit the growth of the three cell lines tested, identical to the effect observed with castalagin. Since these compounds are structurally related with each other, this activity might be based within the *C*-glycosidic ellagitannin moiety.

KEYWORDS: Antitumor activity; cork; phenolic compounds; hydrolyzable tannins; *Quercus suber* L.

INTRODUCTION

Cork is produced by the cork cambium in the outer bark of *Quercus suber* L. To date, the cork industry and the general public have viewed cork mainly in terms of wine-bottling stoppers. However, innovation is increasingly occurring in this traditional industry (*1*). Cork has specific properties (physical, chemical and mechanical) that confer great potential for new applications. This raw material is composed essentially of suberin, lignin, and cellulose and also contains a small amount of extractives (*2*). These extractives, which are unbounded or loosely bounded to the cork cell wall, can be easily extractable with solvents (*3*). In fact, some of these components can be responsible for the organoleptic properties of wine contributing to wine color, flavor, astringency, and bitterness (*4*−*6*). Others may play an important role in wine oxidation processes (*7*), and they can undergo numerous chemical transformations, e.g., reacting with flavanols (*8*). Two of the most important extractable components are waxes and phenolic compounds (*9*). Waxes are composed of diverse aliphatic and aromatic compounds (*1*). Besides phenolic acids, phenolic aldehydes, and coumarins (*10*), the group of phenolic compounds also includes the chemical families of flavonoids and tannins (*1*). Tannins may be monomeric or polymeric and can be divided into condensed and hydrolyzable tannins. The term hydrolyzable tannin refers to both ellagitannins and gallotannins (*11*−*13*). Gallotannins consist of a sugar core, usually D-glucose, acylated partially or wholly by gallic acid and/or *m*-depsidically linked gallic acid units. Ellagitannins are esters of hexahydroxydiphenoyl (HHDP) groups with a sugar core, usually again D-glucose, and often contain galloyl groups (*14*). In flavanoellagitannins, a hydrolyzable tannin unit is connected through a carbon−carbon linkage to a flavan-3-ol moiety (*8*, *11*, *12*).

The pharmacological potential of cork lies in its low molecular weight components. However, to date, only triterpenic compounds have been studied (fridelin and friedelin derivatives) (*15*, *16*). Recently, there has been an increasing interest in ellagitannins because they have been implicated in numerous biological properties, including antioxidant, anticancer, anti-inflammatory, antibacterial, and anti-HIV replication activities (*13*). The remarkable antitumor-promoting effect of these compounds in various animal models and tumor systems lead Gali-Muhtasib *et al.* to postulate that these polyphenols are universal antitumor agents (*17*).

In light of the above findings, the aim of this work was the isolation and identification of different bioactive phenolic compounds with pharmacological potential that can be extracted

*Author to whom correspondence should be addressed. E-mail: vfreitas@fc.up.pt. Fax: +351.220402659. Tel.: +351.220402558.

 Published on Web 11/04/2009

Table 1. LC-MS Data for Phenolic Compounds Tentatively Identified in Cork of *Quercus suber*[a]

fraction	elution time (min)	postulated phenolic compounds (*m*/*z*; $[M-H]^-$)
I	0−150	**137** (Protocatechuic aldehyde); **151** (Vanillin); **153** (Protocatechuic acid); **169** (Gallic acid); **177** (Conyferaldehyde); **179** (Caffeic acid); **193** (Ferrulic acid); **301** (Ellagic acid); **433** (Ellagic acid−pentose); **447** (Ellagic acid−deoxyhexose); **463**(Ellagic acid−hexose); **481** (HHDP-glucose); **465** (unknown)
II	150−230	**301** (Ellagic acid); **469** (Valoneic acid dilactone); **505** (Valoneic acid); **613** (dehydrated tergallic-*C*-glucoside); **635** (trigalloyl-glucose); **595** (unknown)
III	230−400	**633** (HHDP-galoyl-glucose); **783** (di-HHDP-glucose); **785** (HHDP-digalloyl-glucose); **787** (tetragalloy-glucose); **873** (unknown)
IV	400−460	**783** (di-HHDP-glucose); **785** (HHDP-digalloyl-glucose); **915** (unknown); **977** (unknown)
V	460−520	**939** (pentagalloyl-glucose); **807** (unknown); **963** (unknown)
VI	520−610	**935** (di-HHDP-galloyl-glucose); **807** (unknown)
VII	610−730	**933** (Vescalagin); **947** (unknown)
VIII	730−820	**933** (Castalagin); **937** (HHDP- trigalloyl-glucose); **1175** (Mongolicain B)

[a] HHDP: Hexahydroxydiphenoyl group.

from cork from *Quercus suber* L. The antioxidant properties, namely, antiradical and reducing properties, were evaluated as well as the growth inhibitory activity against three human tumor cell lines (MCF-7 (breast cancer), Caco-2 (colon cancer), and HT-29 (colon cancer)).

MATERIALS AND METHODS

Reagents. Toyopearl gel was purchased from Tosoh (Tokyo, Japan). Antibiotic/antimycotic solution (100x), 2,2 diphenyl-1-picrylhydrazyl (DPPH), $FeCl_3$, fetal bovine serum (FBS), minimum essential medium eagle (MEME), RPMI-1640, 0.25% trypsin-EDTA, trypan blue, PBS, sulforhodamine B (SRB), tris-HCl, and trolox were supplied from Sigma-Aldrich (Madrid, Spain).

Acetic glacial acid, dimethyl sulfoxide (DMSO), and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Fluka (Buchs, Switzerland). Trichloacetic acid (TCA) was purchased from Merck (Darmstadt, Germany). Tissue culture supports were supplied by TPP (Trasadingen, Switzerland).

Extraction of *Quercus suber* Phenolic Compounds. Grounded cork from *Quercus suber* L, free of outer corkback, was obtained by grinding and sieving high-quality Portuguese cork kindly supplied by Amorim & Irmãos. Grounded cork (0.5−1 mm particle size) was extracted with a wine model solution (12% ethanol, 5.0 g/L tartaric acid buffered to pH 3.2) for 72 h at room temperature with occasional agitation. The suspension was filtered by gravity, with a filter membrane, and the ethanol was removed by vacuum distillation. The aqueous residue was then spray dried on a Büchi Mini Spray Drier B-290, and the powder obtained was extracted three times with ethyl acetate. The organic fractions were combined and evaporated to dryness under vacuum. The residue was dissolved in H_2O/MeOH (9:1; v/v) and then freeze-dried. Fractionation of cork phenolic compounds was carried out according to the method described elsewhere (*18*). A portion of the above freeze-dried residue was dissolved in methanol and chromatographed on a TSK Toyopearl HW-40(s) column (250 × 16 mm i.d.) for 14 h using methanol as eluent, at 0.8 mL/min. Phenolic compounds were separated based on molecular weight, and each fraction obtained (**I**−**VIII**) was freeze-dried after eliminating the solvent. The resulting solids were analyzed by HPLC and LC-DAD/ESI-MS.

HPLC-DAD Analysis. The samples were analyzed by HPLC (Merck Hitachi Elite Lachrom) on a 150 × 4.6 mm i.d. reversed-phase C18 column (Merck) thermostatted at 25 °C (Merck Hitachi Column Oven L-2300), according to an adaptation of a method described elsewhere (*19*). Detection was carried out at 280 nm using a diode array detector (Merck Hitachi Diode Array Detector L-2455). Solvents were (A) H_2O/CH_3COOH (99:1; v/v) and (B) CH_3COOH/CH_3CN/H_2O (1:20:79; v/v/v) with the gradient 80−20% A over 55 min, 20−10% A from 55 to 70 min, and 10−0% A from 70 to 90 min, at a flow rate of 0.3 mL/min. The sample injection volume was 20 μL. The chromatographic column was washed with 100% B for 10 min and then stabilized with the initial conditions for another 10 min.

LC-DAD/ESI-MS Analysis. A Finnigan Surveyor series liquid chromatograph, equipped with a Thermo Finnigan (Hypersil Gold) reversed-phase column (150 mm × 4.6 mm, 5 μm, C18) thermostatted at 25 °C, was used. The samples were analyzed using the same solvents, gradients, injection volume, and flow rate referred above for the HPLC analysis. Double-online detection was done by a photodiode spectrophotometer and mass spectrometry. The mass detector was a Finnigan LCQ DECA XP MAX (Finnigan Corp., San Jose, CA) quadrupole ion trap equipped with an atmospheric pressure ionization (API) source, using electrospray ionization (ESI) interface. The vaporizer and the capillary voltages were 5 kV and 4 V, respectively. The capillary temperature was set at 325 °C. Nitrogen was used as both sheath and auxiliary gas at flow rates of 80 and 30, respectively (in arbitrary units). Spectra were recorded in the negative ion mode between *m*/*z* 120 and 2000. The mass spectrometer was programmed to do a series of three scans: a full mass, a zoom scan of the most intense ion in the first scan, and a MS−MS of the most intense ion using relative collision energies of 30 and 60. The samples were also directly injected into the MS spectrometer with a pump at a flow rate of 3 μL/min. The capillary temperature and voltage used were 275 °C and 15 V, respectively, and spectra were obtained in the positive ion mode between *m*/*z* 120 and 2000. The mass spectrometer was programmed to do a series of three scans: a full mass, a zoom scan of the most intense ion in the first, and an MS−MS of the most intense ion using relative collision energies of 30 and 60.

Isolation of *Quercus suber* Phenolic Compounds by Preparative HPLC. Cork phenolic compounds were purified by preparative HPLC (Knauer K-1001) fitted with a PrepPak Cartridge C18 reversed-phase column 100 mm × 25 mm i.d (Prep LC Universal Base); detection was carried out at 280 nm using a UV−vis L-2420 Merck Hitachi (Elite) detector. The injection volume was 2 mL at a flow rate of 10 mL/min. The solvents were: A, H_2O/CH_3COOH (98:2; v/v); and B, CH_3COOH/CH_3CN/H_2O (2:20:78; v/v/v). The gradient consisted in 90−20% (A) in 45 min, 20−10% (A) for 45−55 min, and an isocratic gradient at 10% A for 15 min. The column was washed with 100% of B during 20 min and then stabilized with the initial conditions for another 20 min.

The isolated phenolic compounds were purified with C18 gel to remove the HPLC solvents and then frozen and freeze-dried. The purity of the isolated compounds was confirmed by HPLC-DAD, MS, and NMR.

Antioxidant Activity Evaluation. Two methods were employed to evaluate the antioxidant activity of cork phenolic compounds, including one based on the evaluation of the free-radical scavenging capacity (DPPH) and another based on measuring their ion-reducing capacity (FRAP).

In addition to the cork phenolic compounds, the antioxidant activity of trolox (water-soluble analogue of Vitamin E) was evaluated. The results were expressed in terms of ratio between these compounds and trolox antioxidant capacities.

Radical DPPH Scavenging Activity. Following the method described in the literature (*20*) with some modifications, radical scavenging activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) as the free radical. In a 96-well micro plate, 30 μL of antioxidant (100 μM dissolved in methanol) was added to 270 μL of radical DPPH (60 μM in methanol). The reaction was carried out at 25 °C, and the decrease in absorbance was measured at 515 nm, at 0 and 20 min in a microplate reader (Biotek Instruments Inc., Powerwave XS, Winooski, USA). The antiradical activity was expressed in micromolar Trolox equivalents, determined using a calibration curve of Trolox (2.5−50 μM).

Ferric Reducing Antioxidant Power (FRAP). The FRAP method was developed to measure the ferric reducing ability of plasma at low pH (*21*). An intense blue color is formed when the ferric (Fe^{3+})−tripyridyltriazine complex is reduced to the ferrous (Fe^{2+}) complex. In a 96-well micro plate,

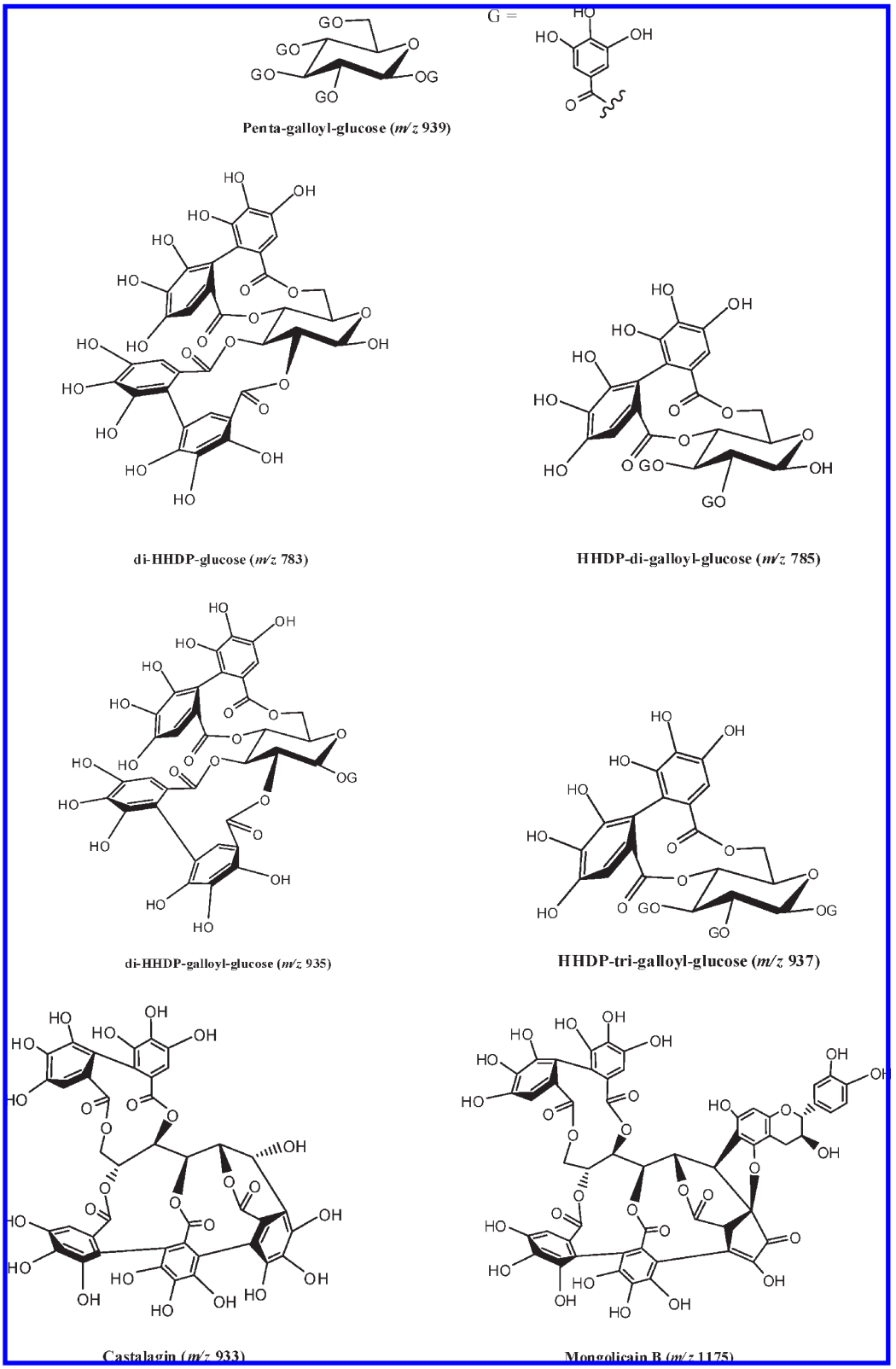

Figure 1. Chemical structures of di-HHDP-glucose, HHDP-di-galloyl-glucose, di-HHDP-galloyl-glucose, HHDP-tri-galloyl-glucose, penta-galloyl-glucose, castalagin, and mongolicain B.

30 μL of antioxidant (100 μM dissolved in methanol) was added to 270 μL of FRAP reagent [(10 volumes of 300 mM acetate buffer, pH 3.6 + 1 volume of 10 mM TPTZ in 40 mM HCl + 1 volume of 20 mM $FeCl_3$), diluted to one-third with acetate buffer and preheated at 37 °C]. The blank assay was performed using 270 μL of FRAP reagent and 30 μL of methanol. The reaction was made at 37 °C, and the absorbance was measured at 0 and 4 min at 593 nm in a microplate reader (Biotek Instruments Inc., Powerwave XS, Winooski, USA). The reducing power was expressed in micromolar Trolox equivalents determined using a calibration curve of Trolox (2.5−50 μM).

Cell Culture Conditions. One human breast cancer tumor cell line, the ER (+) MCF-7, and two human colon adenocarcinoma cell lines (Caco-2 and HT-29) were grown as monolayer, from passage number 38-48.

For routine maintenance, cells were cultured in 25 cm^2 as monolayer and maintained in MEME and/or RPMI-1640 and supplemented with 10 or 15% heat-inactivated FBS and 1% antibiotic/antimycotic solution (100 units/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B) at 37 °C in a humidified atmosphere with 5% CO_2.

Cells were harverested by trypsinization (0.25% (w/v) trypsin-EDTA$_4$Na) twice a week. The exponentially growing cells were obtained by plating 1.5×10^5 cells/mL followed by 24 h incubation. One day later, the seeding medium was replaced by fresh medium containing the tested compounds for 48 h. The effect of the vehicle solvent (DMSO) was evaluated in all experiments by exposing untreated control cells to the maximum concentration (0.1%) of DMSO used in each assay. A stock solution of each compound was prepared in DMSO and kept at −20 °C. Appropriate dilutions of each compound were freshly prepared just prior to every assay.

Sulforhodamine B Assay. The effect of the compounds on the growth of the three cell lines was evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the "*In vitro* Anticancer Drug Discovery Screen" that uses the protein-binding dye SRB to assess cell growth (*22*, *23*).

Briefly, exponentially growing cells in 96-well plates were exposed for 48 h to five serial concentrations of compound (6.3, 12.5, 25, 50, and 100 μM). Following this incubation period, adherent cells were fixed in situ, washed, and stained with SRB. The bound stain was solubilized, and absorbance was measured at 492 nm in a microplate reader (Biotek Instruments Inc., Powerwave XS, Winooski, USA).

Statistical Analysis. Values are expressed as the arithmetic means ± standard deviation (DPPH and FRAP assays) and the arithmetic means ± standard error mean (SRB assay). Statistical significance of the difference between various groups was evaluated by one-way analysis of variance (ANOVA), followed by the Bonferroni test. Statistical significance of the difference between samples was evaluated by Student's *t* test. Differences were considered to be statistically significant when $^*p < 0.05$ *vs* control.

RESULTS AND DISCUSSION

Phenolic Compounds Identification. Each fraction obtained (**I**−**VIII**) after Toyopearl fractionation was analyzed by HPLC and LC-DAD/ESI-MS. This analysis revealed the presence of several phenolic compounds, including low molecular weight phenolic compounds like phenolic acids and aldehydes, ellagic acid derivatives, and hydrolyzable tannins (**Table 1**). The structures of the low molecular weight phenolic compounds were postulated by the comparison of their retention times in HPLC with standards. The structures of the ellagic acid derivatives and hydrolyzable tannins were postulated by the UV−vis spectrum, MS fragmentation pattern, and comparison with data already described in the literature (*24*, *25*). Mongolicain B (flavanoellagitannin) structure was assessed by 2D NMR by comparison with the data already described in the literature (*26*). Some of these compounds were isolated by preparative HPLC for the further antioxidant and biological studies, and their purity was confirmed by HPLC-DAD and MS. Low molecular weight phenolic compounds, ellagic acid derivatives, and several molecular structures of ellagitannins had already been reported on cork of *Q. suber* (*4*, *27*). Despite that several hydrolyzable tannins and mongolicain A had already been reported in leaves and acorns from *Quercus suber* (*24*, *28*), to our knowledge there is no information regarding the presence of this group of compounds in cork from *Q. suber*.

Antioxidant Activity. The antioxidant features of several *Quercus suber* L. hydrolyzable tannins and one flavanoellagitannin (**Figure 1**) were studied by means of two different *in vitro* techniques: DPPH and FRAP assay.

DPPH. Hydrolyzable tannins are powerful antioxidant agents because they possess a great number of hydroxyl groups, especially many galloyl groups (*29*, *30*). These hydroxy functions exhibit high ability to donate hydrogen atoms and to support the unpaired electron (*31*). As shown in **Figure 2**, all the compounds tested were demonstrated to possess significant antiradical capacity. Among the various hydrolyzable tannins tested, the following trend was obtained: an increase of galloyl groups significantly enhanced the radical scavenging activity of these molecules. The bigger the tannin molecules were and the more galloyl groups they possessed, the stronger their activities were. Indeed, the antiradical capacity was higher for HHDP-tri-galloyl-glucose and penta-galloyl-glucose, while simpler structures like di-HHDP-glucose and HHDP-di-galloyl-glucose demonstrated a lower antiradical capacity. Ellagitannins with a galloyl group plus a HHDP group, like di-HHDP-galloyl-glucose, tend to exhibit a more potent radical scavenging effect than those with only a HHDP group, like di-HHDP-glucose. These results are in agreement with those reported in the literature (*29*).

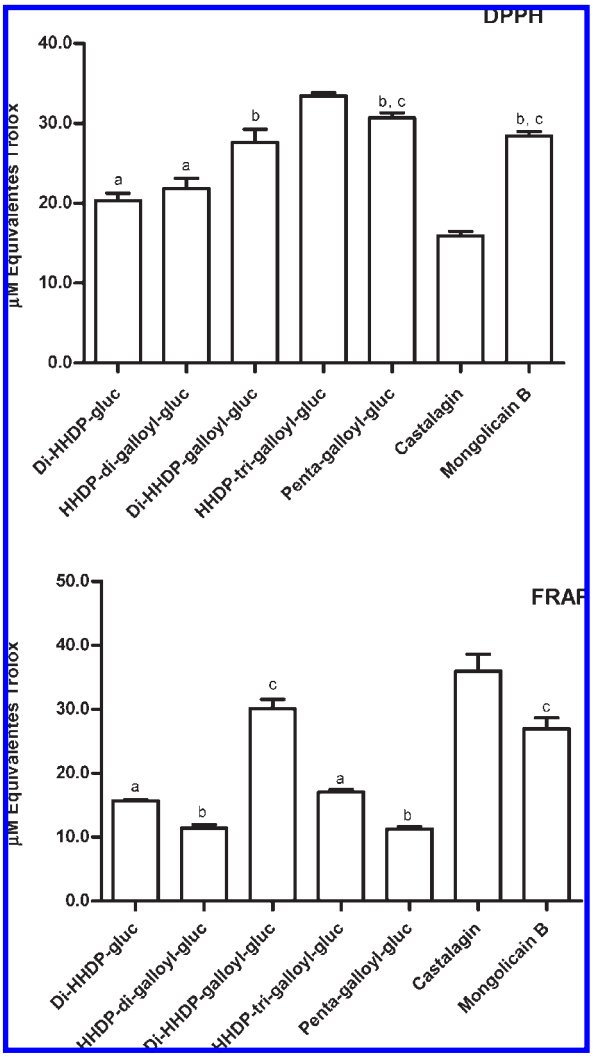

Figure 2. Radical scavenging activity (DPPH) and reducing capacity (FRAP) of 10 μM di-HHDP-glucose, HHDP-digalloyl-glucose, di-HHDP-galloyl-glucose, HHDP-trigalloyl-glucose, pentagalloyl-glucose, castalagin, and mongolicain B (micromolar Trolox Equivalents). Columns represent mean ± standard deviation. Columns with the same letter do not differ statistically ($^*p < 0.05$).

Mongolicain B was also found to have a strong antiradical capacity. This compound is structurally derived from ellagitannin-*C*-glycosides and belongs to the group of flavanoellagitannins in which an ellagitannin unit is connected to a flavan-3-ol moiety. Compared to castalagin, a *C*-glycosidic ellagitannin, mongolicain, B demonstrated a much higher antiradical capacity.

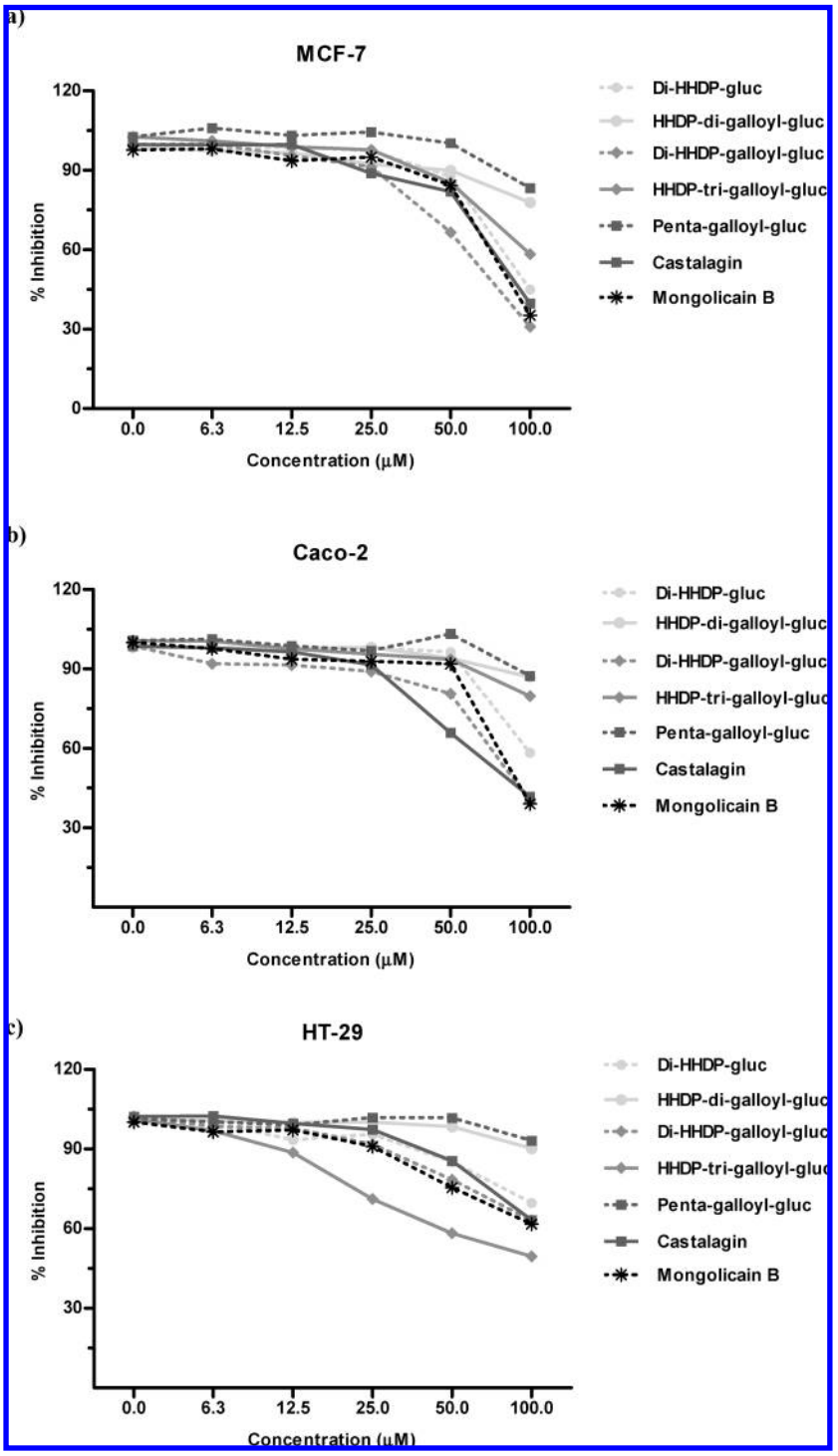

Figure 3. Effect of phenolic compounds on (a) MCF-7, (b) Caco-2, and (c) HT-29 cell proliferation evaluated by SRB assay. Cells, seeded in 96-well plates, were treated with a broad concentration range (6.3−100.0 μM) of each compound for 48 h.

The presence of this additional flavan-3-ol moiety in the mongolicain B structure could be responsible for this enhanced capacity.

FRAP. Concerning the reducing capacity of these phenolic compounds, the trend obtained was different from that of the DPPH assay, and it was not possible to establish a structure/activity relationship within cork hydrolyzable tannins. As previously stated for the antiradical capacity, the reducing capacity should also increase with the number of hydroxyl groups. However, the results obtained appear to be somehow unexpected. As seen in **Figure 2**, there was no difference between di-HHDP-glucose and HHDP-tri-galloyl-glucose and between HHDP-di-galloyl-glucose and penta-galloyl-glucose. In both cases, the second compound presents in its structure more galloyl groups than the first compound, which should have thus enhanced the reducing power of these tannins. Nevertheless, there is a possible explanation for this outcome. The presence of a higher number of galloyl groups in the molecular structure could allow ferric ion chelation. Since the FRAP assay includes the presence of ferric ions ($FeCl_3$), those may be more easily chelated by HHDP-tri-galloyl-glucose and penta-galloyl-glucose, which are thus less able to reduce the TPTZ complex yielding a lower FRAP

value. Indeed, this behavior has already been reported for other phenolic compounds in similar conditions (*32*).

Mongolicain B was found to possess a strong ion-reducing capacity, but lower than that of castalagin.

Effect on the Growth of Human Tumor Cell Lines. The effects of these phenolic compounds on the growth of three human tumor cell lines, MCF-7 (breast cancer), Caco-2 (Caco-2 cells are derived from a human colonic adenocarcinoma but differentiate into small intestinal-like cells after confluence), and HT-29 (colon cancer), were evaluated after a continuous exposure to a serial range of concentrations from 6.3 to 100.0 μM for 48 h. The growth inhibitory effects expressed as the dose−response profiles are shown in **Figure 3**. Generally, all the compounds tested exhibited a dose-dependent growth inhibitory effect after a continuous exposure during a 48 h period.

In contrast to the results of other studies (*33*), the representative compound of gallotannins, penta-galloyl-glucose, caused only a weak inhibition (>100 μM) on the growth of the three cell lines, probably because of its poor solubility in aqueous medium. Penta-galloyl-glucose has formally six hydrogens more than castalagin; these two molecules possess five aromatic nuclei and 15 phenolic hydroxyl groups. However, castalagin is highly soluble in water, while penta-galloyl-glucose has a very limited solubility (*34*).

Among the ellagitannins studied, di-HHDP-glucose, castalagin, and di-HHDP-galloyl-glucose showed great cytotoxic activity against all cell lines, while HHDP-di-galloyl-glucose was found to be the less active. HHDP-tri-galloyl-glucose exhibited significant activity against HT-29 cells but was less effective on the growth of MCF-7 and Caco-2 cells. This different cell line response can reflect a possible tumor type-specific sensitivity of this compound.

A previous work had already reported potent cytotoxicity effects of castalagin and casuarinin (structurally identical to di-HHDP-galloyl-glucose, but with a glucose open ring) (*33*). It has also been found that apoptosis was induced in leukemia cell line HL-60 cells treated with castalagin and casuarinin from *Eugenia jambos* (*35*).

A comparison of the cytotoxicity of these compounds suggests that the presence of the HHDP group seems to be important for the cytotoxicity displayed, and the combination of HHDP with the other phenolic carboxylic acid groups might be essential for this feature.

Several reports have indicated that ellagitannins and ellagic acid inhibit the proliferation of cells by inhibiting cell cycle progression and by inducing apoptosis (*36*, *37*). On the other hand, ellagitannins containing galloyl groups, such as woodfordin I and rugosin E, induced apoptosis through the activation of caspase 3 (*38*), and woodfordin C induced tumor cell death and inhibited DNA topoisomerase II (*39*). Gallic acid has also revealed antioxidation activity (*40*) and cytotoxicity (*41*). Therefore, it may be suggested that ellagic acid and gallic acid hydrolyzed from the compounds tested herein may be important products of cytotoxicity in tumor cells.

Relative inhibitory activity was primarily related to the number of phenolic hydroxyl groups (galloyl and hexahydroxydiphenoyl moieties) found in the active structures, with more groups generally conferring increased effects, except for HHDP-digalloyl-glucose that showed weaker selective cytotoxicity than all the other ellagitannins tested.

Mongolicain B, which represents the flavanoellagitannins, showed a greater potential to inhibit the growth of the three cell lines tested when compared with the flavan-3-ol catechin (data not shown), which could point to the previously demonstrated importance of the gallic or hexahydroxydiphenoyl groups present in the structure in association with the flavanol moiety. Acutissimin, the flavanoellagitannin precursor of mongolicain B, had already been described as a strong and selective cytotoxic agent against melanoma cells (*26*, *33*). In the same study, an identical cytotoxical effect was observed with castalagin. Since these compounds are structurally related to each other, this activity might be due to the *C*-glycosidic ellagitannin moiety.

Some of the phenolic compounds present in cork and tested herein showed significant antioxidant activity, namely, antiradical and reducing properties, besides having a relevant antiproliferative potential against breast and colon cancer cell lines. Interestingly, the most effective antioxidants tested were also the most cytotoxic and effective antiproliferative agents. This could be due to a dual antioxidant/prooxidant effect of these polyphenols. Altogether, cork has shown to be an important source of bioactive molecules with putative medicinal applications.

LITERATURE CITED

(1) Silva, S. P.; Sabino, M. A.; Fernandes, E. M.; Correlo, V. M.; Boesel, L. F.; Reis, R. L. Cork: Properties, capabilities and applications. *Int. Mater. Rev.* **2005**, *50*, 345–365.

(2) Pereira, H. Chemical composition and variability of cork from *quercus suber* l. *Wood Sci. Technol.* **1988**, *22*, 211–218.

(3) Rocha, S. M.; Ganito, S.; Barros, A.; Carapuça, H. M.; Delgadillo, I. Study of cork (from *quercus suber* l.)-wine model interactions based on voltammetric multivariate analysis. *Anal. Chim. Acta* **2005**, *528*, 147–156.

(4) Conde, E.; Cadahía, E.; García-Vallejo, M. C.; Fernández de Simón, B.; González Adrados, J. R. Low molecular weight polyphenols in cork of *quercus suber*. *J. Agric. Food Chem.* **1997**, *45*, 2695–2700.

(5) Boudaoud, N.; Eveleigh, L. A new approach to the characterization of volatile signatures of cork wine stoppers. *J. Agric. Food Chem.* **2003**, *51*, 1530–1533.

(6) Varea, S.; García-Vallejo, M. C.; Cadahía, E.; Fernández De Simón, B. Polyphenols susceptible to migrate from cork stoppers to wine. *Eur. Food Res. Technol.* **2001**, *213*, 56–61.

(7) Vivas, N.; Glories, Y. Role of oak wood ellagitannins in the oxidation process of red wines during aging. *Am. J. Enol. Vitic.* **1996**, *47*, 103–107.

(8) Quideau, S.; Jourdes, M.; Lefeuvre, D.; Montaudon, D.; Saucier, C.; Glories, Y.; Pardon, P.; Pourquier, P. The chemistry of wine polyphenolic C-glycosidic ellagitannins targeting human topoisomerase II. *Chem. Eur. J.* **2005**, *11*, 6503–6513.

(9) Pereira, H. Constituição química da cortiça. Estado actual dos conhecimentos. Boletim do instituto dos produtos florestais. *Cortiça* **1979**, *483*, 259–264.

(10) Conde, E.; Cadahia, E.; Garcia-Vallejo, M. C.; Fernandez de Simon, B.; González Adrados, J. R. Low molecular weight polyphenols in cork of *Quercus suber*. *J. Agric. Food Chem.* **1997**, *45*, 2695–2700.

(11) Haslam, E.; Cai, Y. Plant polyphenols (vegetable tannins): Gallic acid metabolism. *Nat. Prod. Rep.* **1994**, *11*, 41–66.

(12) Quideau, S.; Feldman, K. S. Ellagitannin chemistry. *Chem. Rev.* **1996**, *96*, 475–503.

(13) *Chemistry and biology of ellagitannins: An underestimated class of bioactive*; Quideau, S., Ed.; World Scientific: Singapore, 2009.

(14) Barry, K. M.; Davies, N. W.; Mohammed, C. L. Identification of hydrolysable tannins in the reaction zone of *eucalyptus nitens* wood by high performance liquid chromatography-electrospray ionisation mass spectrometry. *Phytochem. Anal.* **2001**, *12*, 120–127.

(15) Moiteiro, C.; Justino, F.; Tavares, R.; Marcelo-Curto, M. J.; Florencio, M. H.; Nascimento, M. S. J.; Pedro, M.; Cerqueira, F.; Pinto, M. M. M. Synthetic secofriedelane and friedelane derivatives as inhibitors of human lymphocyte proliferation and growth of human cancer cell lines in vitro. *J. Nat. Prod.* **2001**, *64*, 1273–1277.

(16) Queiroga, C. L.; Silva, G. F.; Dias, P. C.; Possenti, A.; de Carvalho, J. E. Evaluation of the antiulcerogenic activity of friedelan-3-β-ol and friedelin isolated from *maytenus ilicifolia* (celastraceae). *J. Ethnopharmacol.* **2000**, *72*, 465–468.

(17) Gali-Muhtasib, H. U.; Yamout, S. Z.; Sidani, M. M. Plant tannins as inhibitors of hydroperoxide production and tumor promotion induced by ultraviolet b radiation in mouse skin in vivo. *Oncol. Rep*. **1999**, *6*, 847–53.
(18) de Freitas, V.; Glories, Y.; Bourgeois, G.; Vitry, C. Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. *Phytochemistry* **1998**, *49*, 1435–1441.
(19) Pena-Neira, A.; Hernandez, T.; Garcia-Vallejo, M. C.; Cadahia, E.; De Simon, B. F.; Suarez, J. A. Low molecular weight phenols in cork stoppers. *Am. J. Enol. Vitic*. **1999**, *50*, 285–290.
(20) Bondet, V.; Brand-Williams, W.; Berset, C. Kinetics and mechanisms of antioxidant activity using the dpph. Free radical method. *Lebensm.-Wiss. Technol*. **1997**, *30*, 609–615.
(21) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (frap) as a measure of "Antioxidant power": The frap assay. *Anal. Biochem*. **1996**, *239*, 70–76.
(22) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*. **1990**, *82*, 1107–12.
(23) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A..; et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst*. **1991**, *83*, 757–66.
(24) Cantos, E.; Espin, J. C.; Lopez-Bote, C.; De la Hoz, L.; Ordonez, J. A.; Tomás-Barberán, F. A. Phenolic compounds and fatty acids from acorns (*Qquercus spp*.), the main dietary constituent of free-ranged iberian pigs. *J. Agric. Food Chem*. **2003**, *51*, 6248–6255.
(25) Mämmelä, P.; Savolainen, H.; Lindroos, L.; Kangas, J.; Vartiainen, T. Analysis of oak tannins by liquid chromatography-electrospray ionisation mass spectrometry. *J. Chromatogr. A* **2000**, *891*, 75–83.
(26) Nonaka, G. C.; Ishimaru, K.; Mihashi, K.; Iwase, Y.; Ageta, M.; Nishioka, I. Tannins and related compounds. Lxiii.: Isolation and characterization of mongolicains a and b, novel tannins from *quercus* and *castanopsi*s species. *Chem. Pharm. Bull*. **1988**, *36*, 857–869.
(27) Cadahía, E.; Conde, E.; Fernández de Simón, B.; García-Vallejo, M. C. Proanthocyanidins and ellagitannins in cork of *Quercus suber*. *Proceedings of the 18th International Conference on Polyphenols, Bordeaux*, **1996**, *I*, 215−216.
(28) Ito, H.; Yamaguchi, K.; Kim, T. H.; Khennouf, S.; Gharzouli, K.; Yoshida, T. Dimeric and trimeric hydrolyzable tannins from *quercus coccifera* and *quercus suber*. *J. Nat. Prod*. **2002**, *65*, 339–345.
(29) Yokozawa, T.; Chen, C. P.; Dong, E.; Tanaka, T.; Nonaka, G. I.; Nishioka, I. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochem. Pharmacol*. **1998**, *56*, 213–222.
(30) Bouchet, N.; Barrier, L.; Fauconneau, B. Radical scavenging activity and antioxidant properties of tannins from *guiera senegalensis* (combretaceae). *Phytother. Res*. **1998**, *12*, 159–162.
(31) Nicoli, M. C.; Anese, M.; Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci. Technol*. **1999**, *10*, 94–100.
(32) Paiva-Martins, F.; Gordon, M. H. Interactions of ferric ions with olive oil phenolic compounds. *J. Agric. Food Chem*. **2005**, *53*, 2704–2709.
(33) Kashiwada, Y.; Nonaka, G.; Nishioka, I.; Chang, J. J.; Lee, K. H. Antitumor agents, 129. Tannins and related compounds as selective cytotoxic agents. *J. Nat. Prod*. **1992**, *55*, 1033–43.
(34) Haslam, E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod*. **1996**, *59*, 205–215.
(35) Yang, L.-L.; Lee, C.-Y.; Yen, K.-Y. Induction of apoptosis by hydrolyzable tannins from eugenia jambos l. On human leukemia cells. *Cancer Lett*. **2000**, *157*, 65–75.
(36) Mertens-Talcott, S. U.; Percival, S. S. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. *Cancer Lett*. **2005**, *218*, 141–151.
(37) Constantinou, A.; Stoner, G. D.; Mehta, R.; Rao, K.; Runyan, C.; Moon, R. The dietary anticancer agent ellagic acid is a potent inhibitor of DNA topoisomerases in vitro. *Nutr. Cancer* **1995**, *23*, 121–130.
(38) Liu, M. J.; Wang, Z.; Li, H. X.; Wu, R. C.; Liu, Y. Z.; Wu, Q. Y. Mitochondrial dysfunction as an early event in the process of apoptosis induced by woodfordin i in human leukemia k562 cells. *Toxicol. Appl. Pharmacol*. **2004**, *194*, 141–155.
(39) Kuramochi-Motegi, A.; Kuramochi, H.; Kobayashi, F.; Ekimoto, H.; Takahashi, K.; Kadota, S.; Takamori, Y.; Kikuchi, T. Woodfruticosin (woodfordin C), a new inhibitor of DNA topoisomerase II. Experimental antitumor activity. *Biochem. Pharmacol*. **1992**, *44*, 1961–1965.
(40) Reddy, M. K.; Gupta, S. K.; Jacob, M. R.; Khan, S. I.; Ferreira, D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *punica granatum* l. *Planta Med*. **2007**, *73*, 461–467.
(41) Sakagami, H.; Kuribayashi, N.; Iida, M.; Sakagami, T.; Takeda, M.; Fukuchi, K.; Gomi, K.; Ohata, H.; Momose, K.; Kawazoe, Y.; Hatano, T.; Yoshida, T.; Okuda, T. Induction of DNA fragmentation by tannin- and lignin-related substances. *Anticancer Res*. **1995**, *15*, 2121–2128.

Received for review June 18, 2009. Revised manuscript received October 21, 2009. Accepted October 21, 2009. This work was supported by a research project grant funding from ARCP (Associação Rede de Competência em Polímeros) from Portugal. This work was also supported by FCT (Fundação para a Ciência e Tecnologia) (POCI 2010, FSE), by one Ph. D. student grant (SFRH/BD/38883/2007) and one project grant (PTDC/QUI/65501/2006).