Volume 105, issue 3, 2007 ISSN: 0308-8146

FOOD CHEMISTRY

Available online at
ScienceDirect
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ScienceDirect
Food Chemistry 105 (2007) 1018–1025
Food Chemistry
www.elsevier.com/locate/foodchem

Hazel (*Corylus avellana* L.) leaves as source of antimicrobial and antioxidative compounds

Ivo Oliveira [a], Anabela Sousa [a], Patrícia Valentão [b], Paula B. Andrade [b], Isabel C.F.R. Ferreira [a], Federico Ferreres [c], Albino Bento [a], Rosa Seabra [b], Letícia Estevinho [a], José Alberto Pereira [a,*]

[a] *CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Sta Apolónia, Apartado 1 172, 5301-855 Bragança, Portugal*
[b] *REQUIMTE/Serviço de Farmacognosia, Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal*
[c] *Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus Univ. Espinardo, Murcia, Spain*

Received 14 November 2006; received in revised form 21 February 2007; accepted 29 April 2007

Abstract

Aqueous extracts of leaves of different hazel (*Corylus avellana* L.) cultivars (Cv. M. Bollwiller, Fertille de Coutard and Daviana), were analysed by reversed-phase HPLC/DAD for the definition of their phenolic composition. Antioxidant potential was assessed by the reducing power assay, and the scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and β-carotene linoleate model system. Their antimicrobial capacity was also tested against Gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*). Eight phenolic compounds were identified: 3-, 4- and 5-caffeoylquinic acids, caffeoyltartaric acid, *p*-coumaroyltartaric acid, myricetin-rhamnoside, quercetin 3-rhamnoside and kaempferol 3-rhamnoside. A *p*-coumaric acid, three myricetin and one quercetin derivatives were also detected. The hazel leaves extract presented high antioxidant activity in a concentration-dependent way, in general with similar behaviour of all cultivars. Gram positive bacteria revealed to be very sensitive to hazel leaf extract (MIC 0.1 mg/ml for *B. cereus* and *S. aureus* and 1 mg/ml for *B. subtilis*). However, Gram negative and the fungi displayed much lower sensitivity, being *P. aeruginosa* and *C. albicans* resistant at 100 mg/ml. Cv. M. Bollwiller exhibited the most potent antimicrobial activity.

Keywords: Hazel leaves; Phenolics; Antioxidant potential; Antimicrobial activity

1. Introduction

Although a number of *Corylus* species are found throughout the world, *C. avellana* and its hybrids, are the most important as regards nut production. Hazel is a tree or bush which may grow to 6 m high, exhibiting deciduous leaves that are rounded, 6–12 cm long and across, softly hairy on both surfaces, and with a double-serrate margin. It grows wild in Europe, and western Asia (Vaughan & Geissler, 1997). Despite its wide cultivation for nuts collection, hazel leaves are also largely consumed as an infusion. They are used in folk medicine for the treatment of haemorrhoids, varicose veins, phlebitis and lower members' oedema, as consequence of its astringency, vasoprotective and anti-oedema properties (Valnet, 1992).

Nowadays there is considerable evidence that the antioxidants contained in fruits, vegetables and beverages play an important role in the maintenance of health and in the prevention of disease. In fact, plant-derived products contain a wide range of phytochemicals, namely phenolic compounds, with antioxidant capacity, providing protection against the harmful effects resultant of oxidative stress (Pereira et al., 2006; Proestos, Chorianopoulos, Nychas,

* Corresponding author. Tel.: +351 273 303277; fax: +351 273 325405.
E-mail address: jpereira@ipb.pt (J.A. Pereira).

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doi:10.1016/j.foodchem.2007.04.059

& Komaitis, 2005; Seabra et al., 2006). Furthermore, there is growing interest in using natural antimicrobial compounds because of consumer pressure on the food industry to avoid chemical preservatives and the increasing resistance to antibiotics. With this regard the antimicrobial capacity of phenolic compounds has also been reported (Pereira et al., 2006; Proestos et al., 2005; Puupponen-Pimiä et al., 2001; Rauha et al., 2000; Zhu, Zhang, & Lo, 2004).

Previous studies on hazel leaves concerned the measurement of the bidirectional reflectance distribution function (Bousquet, Lachérade, Jacquemoud, & Moya, 2005) and the determination of organochlorine pesticides (Barriada-Pereira et al., 2004) hormone contents (Andrés, Fernández, Rodríguez, & Rodríguez, 2002), nitrate accumulation (Stams & Lutke Schipholt, 1990), polycyclic aromatic hydrocarbons (Howsam, Jones, & Ineson, 2000; Howsam, Jones, & Ineson, 2001) and free polyamines (Rey, Díaz-Sala, & Rodríguez, 1998). In addition, the phenolic composition has already been described by our (Amaral et al., 2005) and another research group (Fraisse, Carnat, Carnat, & Lamaison, 1999) but, as far as we know, nothing has been reported about their antioxidant and antimicrobial potential.

The aim of this work was to determine the phenolics composition of the leaves of three hazel cultivars (Cv. M. Bollwiller, Fertille de Coutard and Daviana) grown in Portugal, and to assess their antimicrobial and antioxidant abilities. The phenolic compounds were identified and quantified by HPLC/DAD. The antimicrobial activity was evaluated against different microorganisms, namely Gram positive (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*) bacteria and fungi (*Candida albicans* and *Cryptococcus neoformans*). The antioxidant potential was tested in three distinct assays: reducing power, scavenging of DPPH radicals and β-carotene bleaching.

2. Materials and methods

2.1. Samples

Hazel leaves were obtained from three *Corylus avellana* L. cultivars: M. Bollwiller, Fertille de Coutard and Daviana, and were collected on 3rd July 2006 in Bragança, northeast of Portugal (6°46′W, 41°49′N, 670 m a.s.l.). The orchard has a planting density of 3.5 × 7 m. The trees were ten years old, and had been pruned when necessary. No phytosanitary treatments were applied. The leaves were collected from the middle third of branches exposed to sunlight, put in plastic bags and immediately frozen at −20 °C. The plant material was then freeze dried.

2.2. Extract preparation

For each cultivar, three powdered sub samples (~5 g; 20 mesh) were extracted with 250 ml of boiling water for 45 min and filtered through Whatman no. 4 paper. The aqueous extract was frozen, lyophilized and redissolved in water at concentrations of 100 mg/ml and 10 mg/ml for antimicrobial and antioxidant activities assays, respectively.

2.3. Phenolic compounds analysis

2.3.1. Standards and reagents

The standards used were from Sigma (St. Louis, MO, USA) or Extrasynthèse (Genay, France). Methanol and formic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA) before use.

2.3.2. HPLC-DAD analytical conditions

Chromatographic separation was achieved as previously reported (Amaral et al., 2005) with an analytical HPLC unit (Gilson), using a reversed-phase Spherisorb ODS2 (250 × 4.6 mm, 5 μm particle size, Merck, Darmstadt, Germany) column. The solvent system used was a gradient of water/formic acid (19:1) (A) and methanol (B), starting with 15% methanol and installing a gradient to obtain 30%B at 15 min, 45%B at 30 min, 52.5%B at 40 min and 100%B at 42 min. The flow rate was 1 ml min^{-1}, and the injection volume was 20 μl. Detection was accomplished with a diode array detector (DAD) (Gilson), and chromatograms were recorded at 320 and 350 nm. Spectral data from all peaks were accumulated in the 200–400 nm range. Data were processed on Unipoint system software (Gilson Medical Electronics, Villiers le Bel, France).

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, with detection at 320 nm for phenolic acids and at 350 nm for flavonoids. 3- and 4-Caffeoylquinic acids were quantified as 5-caffeoylquinic acid, caffeoyltartaric acid as caffeic acid, *p*-coumaric acid derivative and *p*-coumaroyltartaric acid as *p*-coumaric acid, myricetin 3-hexoside + myricetin derivative as myricetin-rhamnoside, quercetin 3-hexoside + myricetin derivative as quercetin 3-rhamnoside and kaempferol 3-rhamnoside as kaempferol 3-glucoside. The other compounds were quantified as themselves.

2.4. Antioxidant activity

2.4.1. Reagents

BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (tert-butyl hydroquinone) and α-tocopherol were purchased from Sigma (St. Louis, MO, USA). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was obtained from Alfa Aesar. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, USA). Methanol was obtained from Pronalab (Lisboa, Portugal). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

2.4.2. Reducing power assay

The reducing power was determined according to a described procedure (Oyaizu, 1986). Various concentrations of sample extracts (2.5 ml) were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After incubation 2.5 ml of 10% tricloroacetic acid (w/v) were added and then the mixture was centrifuged at 1000 rpm in a refrigerated centrifuge (Centorion K24OR-2003), for 8 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration. BHA and α-tocopherol were used as reference compounds.

2.4.3. Scavenging effect assay

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to a method reported before (Hatano, Kagawa, Yasuhara, & Okuda, 1988). Various concentrations of sample extracts (0.3 ml) were mixed with 2.7 ml of methanolic solution containing DPPH radicals (6×10^{-5} mol/l). The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discolouration using the equation: % scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration. BHA and α-tocopherol were used as reference compounds.

2.4.4. β-Carotene linoleate model system

The antioxidant activity of hazel leaf extracts was evaluated according to a described procedure (Mi-Yae, Tae-Hun, & Nak-Ju, 2003). β-Carotene solution was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. Two millilitres of this solution were placed in a 100 ml round-bottom flask. After chloroform removal, at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 ml of distilled water were added to the flask under vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of different concentrations of hazel leaf extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance at 470 nm was measured. Absorbance readings were then recorded until the control sample had changed colour. A blank assay, devoid of β-carotene, was prepared for background subtraction. Antioxidant activity was calculated using the following equation: Antioxidant activity = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant percentage against extract concentration. TBHQ was used as reference compound.

2.5. Antimicrobial activity

2.5.1. Reagents

Ampicillin and cycloheximide were of the highest available quality, and purchased from Merck (Darmstadt, Germany). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.5.2. Microorganisms and culture conditions

Microorganisms CECT were obtained from the Spanish type culture collection (CECT) of Valencia University, while microorganisms ESA were clinically isolated strains identified in Microbiology Laboratory of Escola Superior Agrária de Bragança. Gram + (*B. cereus* CECT 148, *B. subtilis* CECT 498 and *S. aureus* ESA 7 isolated from pus) and Gram – (*E. coli* CECT 101, *P. aeruginosa* CECT 108 and *K. pneumoniae* ESA 8 isolated from urine) bacteria, and fungi (*C. albicans* CECT 1394 and *C. neoformans*ESA 3 isolated from vaginal fluid) were used to screen antimicrobial activity of the three hazel leaves cultivars. Microorganisms were cultured aerobically at 37 °C (Scientific 222 oven model, 2003) in nutrient agar medium for bacteria, and at 30 °C (Scientific 222 oven model, 2003) in sabouraud dextrose agar medium for fungi.

2.5.3. Test assays for antimicrobial activity

The screening of antibacterial activities against Gram + and Gram-bacteria and fungi and the determination of the minimal inhibitory concentration (MIC) were achieved by an adaptation of the agar streak dilution method based on radial diffusion (Hawkey & Lewis, 1994; Ferreira, Calhelha, Estevinho, & Queiroz, 2004; Sousa et al., 2006). Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/ml, and the plates containing agar medium were inoculated (100 μl; spread on the surface). Each sample (50 μl) was placed in a hole (3 mm depth, 4 mm diameter) made in the centre of the agar. Under the same conditions, different solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The assays with the standards were carried out using DMSO solutions, which was chosen as the best solvent. After comparative toxicity assays this solvent was shown to be non-toxic. The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria or fungi, after 24 h. The diameters of the inhibition zones corresponding to the MICs were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition zone diameter was measured three

times (three different plates) and the average was considered. A control using only inoculation was also carried out.

3. Results and discussion

3.1. Phenolic compounds in hazel leaves

The HPLC-DAD analysis of hazel leaves aqueous extracts (Fig. 1) revealed the presence of several hydroxycinnamic acid and flavonoid derivatives. The three analysed cultivars exhibited a common qualitative composition, in which eight phenolic compounds were identified: 3-, 4- and 5-caffeoylquinic acids, caffeoyltartaric acid, *p*-coumaroyltartaric acid, myricetin-rhamnoside, quercetin 3-rhamnoside and kaempferol 3-rhamnoside (Fig. 2). In addition, another *p*-coumaric acid, three myricetin and one quercetin derivative were also detected (Fig. 1). All these compounds were previously reported to occur in hazel leaves (Amaral et al., 2005), with the exceptions of 4-caffeoylquinic acid and of the unidentified *p*-coumaric acid derivative.

The quantification of the phenolics present in the different aqueous extracts revealed a high amount of these compounds, ranging from ca. 38 to 44 g/kg, dry basis (Table 1), which are considerably higher than the values found before for methanolic extracts of the same and other hazel cultivars (Amaral et al., 2005). As observed before, flavonoids were always the major compounds, varying between 71% and 80% of total phenolics (Table 1). Cv. M. Bollwiller showed the highest content of phenolic compounds (Table 1).

All samples exhibited a similar phenolic profile, in which myricetin-rhamnoside was the major compound, representing ca. 62.2% of total phenolics (Fig. 3). However, in what concerns phenolic acids, some quantitative differences were noticed: in Fertille Coutard leaves the main phenolic acid is 3-caffeoylquinic acid, while in M. Bollwiller and Daviana cultivars 5-caffeoylquinic and caffeoyltartaric acids are the major phenolic acid, respectively. Thus, it seems that the nature of the cultivar influences the phenolic acid composition. The pair quercetin 3-hexoside plus myricetin derivative presented the smallest content in all cultivars, corresponding to ca. 0.9% of total phenolics (Fig. 3).

When comparing the results with those previously obtained for Fertille Coutard and M. Bollwiller cultivars (Amaral et al., 2005) it could be noticed that the increase

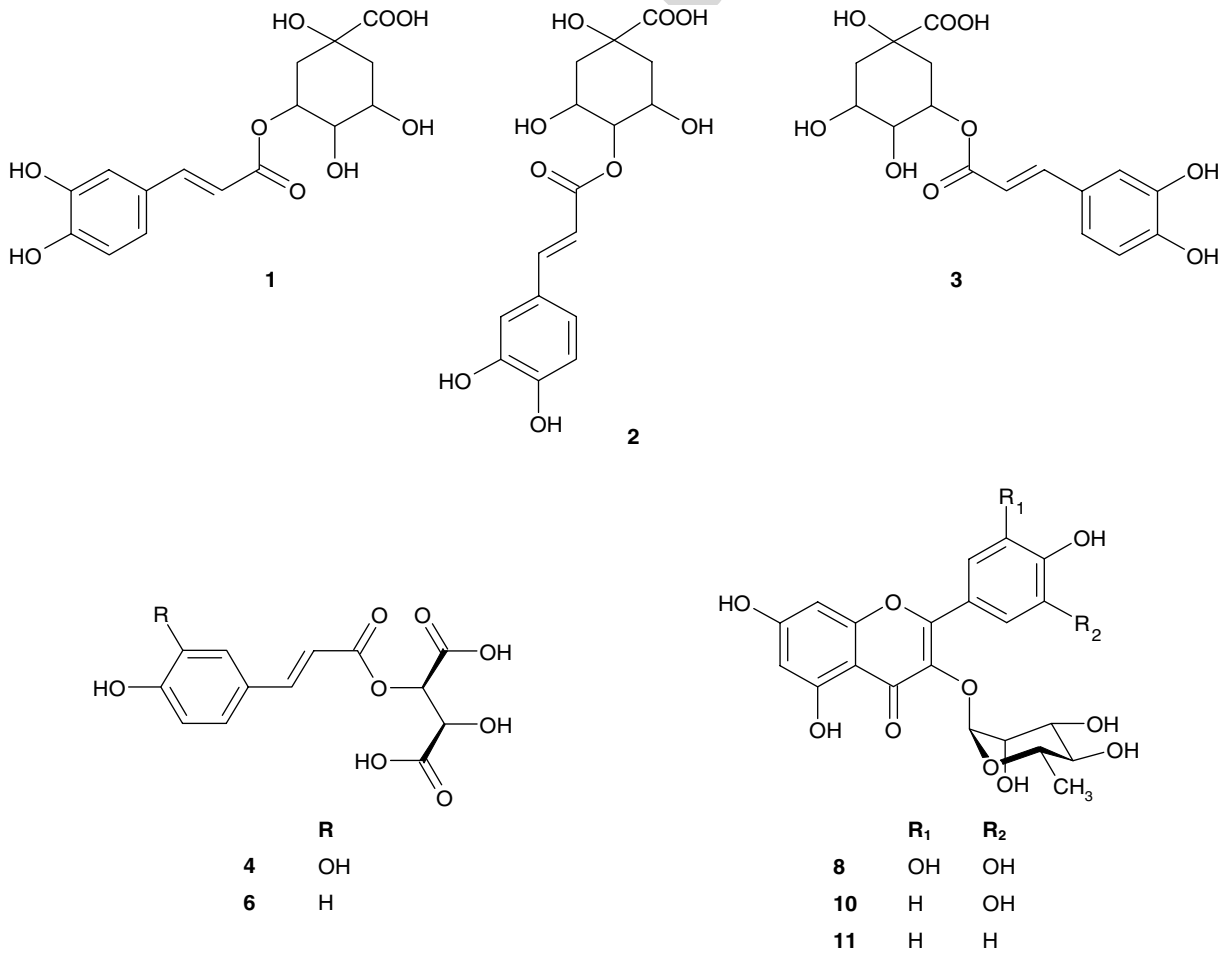

Fig. 1. Chemical structures of identified phenolic compounds from hazel leaves. (1) 3-Caffeoylquinic acid; (2) 4-caffeoylquinic acid; (3) 5-caffeoylquinic acid; (4) caffeoyltartaric acid; (6) *p*-coumaroyltartaric acid; (8) myricetin 3-rhamnoside; (10) quercetin 3-rhamnoside; (11) kaempferol 3-rhamnoside.

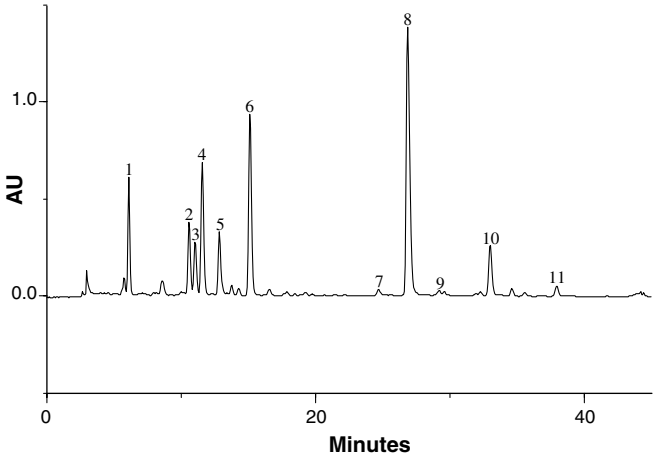

Fig. 2. HPLC-DAD of phenolic compounds in hazel leaves (Cv. Fertille de Coutard). Detection at 320 nm. Peaks: (1) 3-Caffeoylquinic acid; (2) 4-caffeoylquinic acid; (3) 5-caffeoylquinic acid; (4) caffeoyltartaric acid; (5) *p*-coumaric acid derivative; (6) *p*-coumaroyltartaric acid; (7) myricetin 3-hexoside + myricetin derivative; (8) myricetin 3-rhamnoside; (9) quercetin 3-hexoside + myricetin derivative; (10) quercetin 3-rhamnoside; (11) kaempferol 3-rhamnoside.

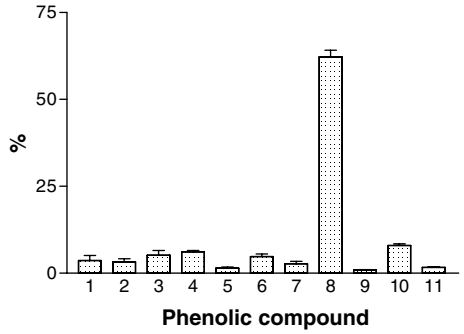

Fig. 3. Phenolic compounds profile of hazel leaves. (1) 3-Caffeoylquinic acid; (2) 4-caffeoylquinic acid; (3) 5-caffeoylquinic acid; (4) caffeoyltartaric acid; (5) *p*-coumaric acid derivative; (6) *p*-coumaroyltartaric acid; (7) myricetin 3-hexoside + myricetin derivative; (8) myricetin 3-rhamnoside; (9) quercetin 3-hexoside + myricetin derivative; (10) quercetin 3-rhamnoside; (11) kaempferol 3-rhamnoside.

of the total phenolics content is mainly due to an increase of phenolic acid derivatives contents. This could be attributed to the environmental factors that allowed an increased production of these compounds, since samples' geographical origin is different from that of the previous work. Nevertheless, this rise could also be partially explained by the drying process of the samples: in this work the leaves were dried by lyophilisation, which is faster and less drastic than the use of a ventilated stove at 30 °C reported before (Amaral et al., 2005), that allows enzymatic reactions, with possible alteration and loss of compounds. In addition, 3-caffeoylquinic and caffeoyltartaric acids have now been detected in Fertille Coutard and M. Bollwiller cultivars, respectively.

3.2. Antioxidant activity

Although several publications dealing with antioxidant activity of hazelnut kernel (Alasalvar, Karamacă, Amarowicz, & Shahidi, 2006; Duraka et al., 1999; Krings & Berger, 2001; Moure et al., 2001; Sivakumar & Bacchetta, 2005) and green leafy cover (Alasalvar et al., 2006) have appeared, no such information is available about antioxidant properties of their leaves. Recently, Sivakumar and Bacchetta (2005) reported the determination of natural vitamin E, a potent antioxidant, from Italian hazel leaves but did not present antioxidant activity studies. In the present study, the antioxidant potential of hazel leaves samples was measured by different biochemical assays: reducing power, scavenging activity on DPPH radicals and lipid peroxidation inhibition by the β-carotene linoleate system.

From the analysis of Fig. 4, we can conclude that the reducing power of the extracts increased with increasing concentration and were excellent, presenting high reducing powers at very low concentrations (<1 mg/ml), and being even more potent than BHA ($A_{700} = 0.12$ at 3.6 mg/ml) and α-tocopherol ($A_{700} = 0.13$ at 8.6 mg/ml) standards. The reducing power of the different cultivars was very similar and followed the order Fertille Coutard > Daviana ~M. Bollwiller.

The scavenging effect of hazel leaves extracts on DPPH radicals also increased with concentration, specially for

Table 1
Phenolic compounds in hazel leaf samples (mg/kg, dry basis)[a]

Sample	Compound[b]											Total
	1	2	3	4	5	6	7	8	9	10	11	
M. Bollwiller	1241.0 (10.1)	1396.8 (5.4)	3466.1 (50.6)	2939.7 (41.7)	443.5 (0.5)	1551.9 (14.5)	1105.3 (23.5)	26655.9 (76.4)	356.9 (9.6)	3967.0 (27.6)	867.8 (17.1)	43991.9
Fertille Coutard	2438.7 (12.5)	1825.3 (13.3)	1442.6 (0.7)	2031.4 (21.8)	741.0 (0.1)	2355.3 (30.4)	642.7 (25.8)	22629.9 (400.9)	303.4 (9.2)	2760.3 (63.8)	545.0 (7.9)	37715.6
Daviana	632.7 (7.0)	708.0 (19.2)	1471.0 (14.6)	2480.1 (1.5)	544.9 (2.7)	1802.7 (17.8)	1560.1 (12.8)	25771.1 (348.2)	436.8 (7.3)	2979.5 (27.1)	606.5 (7.6)	38993.4

[a] Results are expressed as mean (standard deviation) of three determinations.

[b] (1) 3-Caffeoylquinic acid; (2) 4-caffeoylquinic acid; (3) 5-caffeoylquinic acid; (4) caffeoyltartaric acid; (5) *p*-coumaric acid derivative; (6) *p*-coumaroyltartaric acid; (7) myricetin 3-hexoside + myricetin derivative; (8) myricetin 3-rhamnoside; (9) quercetin 3-hexoside + myricetin derivative; (10) quercetin 3-rhamnoside; (11) kaempferol 3-rhamnoside.

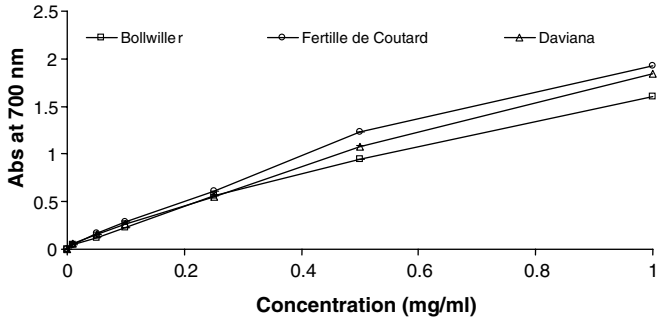

Fig. 4. Reducing power values of different cultivars of hazel leaf extracts. Each value is expressed as mean ± standard deviation.

concentrations below 0.5 mg/ml (Fig. 5). Fertille Coutard and M. Bollwiller cultivars showed the highest and the lowest activities, respectively, being the obtained results much better than those obtained for BHA (96% at 3.6 mg/ml) and α-tocopherol (95% at 8.6 mg/ml). For all the tested hazel cultivars DPPH scavenging activity values were higher than 93.1% at 0.5 mg/ml. These results are much better than DPPH radical scavenging effects described in the literature (Moure et al., 2001) for methanol extracts from hazel fruits (14.2% at 2 mg/mL). Nevertheless, ethanol extract from hazelnut was reported as possessing higher radical scavenging effects than other roasted foods such as almond (Krings & Berger, 2001).

The antioxidant activity of hazel leaves extracts measured by the bleaching of β-carotene is shown in Fig. 6. The results obtained indicated a concentration-dependent antioxidant capacity, following the order Fertille Coutard > Daviana > M. Bollwiller which presented values at 2 mg/ml of 61.3%, 51.3% and 50.3%, respectively. It is probable that the antioxidative components in the extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system (Mi-Yae et al., 2003). The protection of β-carotene bleaching provided by TBHQ standard reached 82.2% at 2 mg/ml and was slightly more efficient than the samples. Nevertheless, this and other synthetic antioxidants applied in fat and oily foods to prevent oxida-

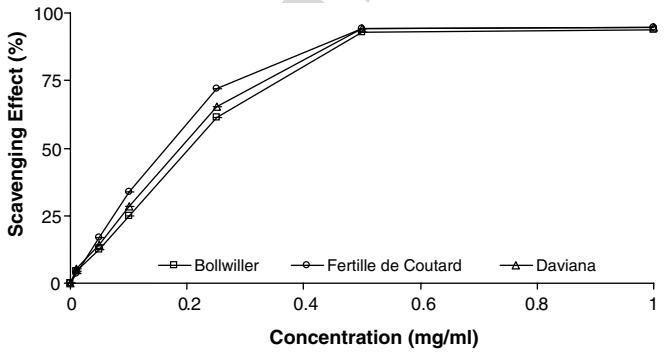

Fig. 5. Scavenging effect on DPPH of different cultivars of hazel leaf extracts. Each value is expressed as mean ± standard deviation.

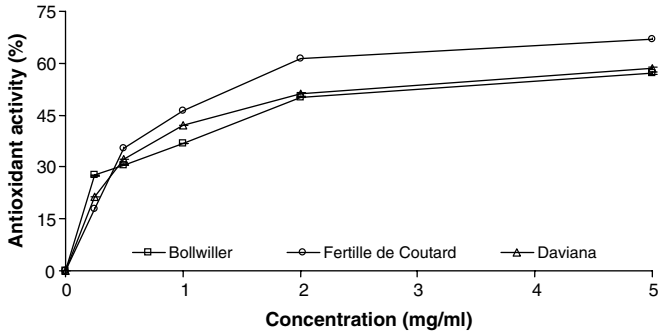

Fig. 6. Antioxidant activity (%) by β-carotene bleaching method of different cultivars of hazel leaf extracts. Each value is expressed as mean ± standard deviation.

tive deterioration were found to be anticarcinogenic as well as carcinogenic in experimental animals (Loliger, 1991).

For an overview of the results, at Table 2 there are presented the EC_{50} values for the antioxidant activity assays obtained from each hazel leaves sample. Fertille Coutard cultivar revealed better antioxidant properties (lower EC_{50} values) than the other samples, for all the biochemical assays used in the antioxidant activity screening. The EC_{50} values obtained for reducing power and scavenging effects on DPPH radicals (<0.3 mg/ml) were better than for β-carotene bleaching inhibition (>1.2 mg/ml).

3.3. Antimicrobial activity

The antimicrobial capacity of phenolic compounds, in a general way, is well-known (Pereira et al., 2006; Proestos et al., 2005; Puupponen-Pimiä et al., 2001; Rauha et al., 2000; Zhu et al., 2004) and we propose, for the first time, the use of hazel leaves extracts as a source of antimicrobials. As previously described, individual phenolic compounds present in the hazel leaves extracts were identified and quantified, but we choose to submit the entire extracts to antimicrobial activity studies. In fact, food extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Borchers, Keen, & Gerstiwin, 2004). The minimal inhibitory concentration (MIC) values for bacteria (*B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa, K. Pneumoniae*) and fungi (*C. albicans* and *C. neoformans*) were determined as an evaluation of the antimicrobial activity of the hazel samples and were presented in Table 3.

The tested samples revealed antimicrobial activity against all the microorganisms apart from *P. aeruginosa*

Table 2
EC_{50} values (mg/ml) of hazel leaf samples

Samples	Reducing power (EC_{50})	DPPH (EC_{50})	β-Carotene bleaching (EC_{50})
M. Bollwiller	0.224	0.203	1.981
Fertille Coutard	0.199	0.164	1.243
Daviana	0.223	0.188	1.861

Table 3
Antimicrobial activity of hazel leaf extracts

MIC (mg/ml)								
Cultivar	*B. cereus*	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. neoformans*
M. Bollwiller	0.1 (++++)	1 (++++)	0.1 (++)	100 (–)	50 (++++)	1 (++)	100 (–)	100 (++++)
Fertille	0.1	1	0.1	100	100	10	100	100
Coutard	(++)	(+++)	(++++)	(–)	(–)	(++++)	(–)	(–)
Daviana	0.1	1	0.1	100	100	50	100	100
	(++)	(++)	(++)	(–)	(++++)	(++++)	(–)	(–)

No antimicrobial activity (–), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (++), inhibition zone 4–5 mm. High antimicrobial activity (+++), inhibition zone 6–9 mm. Strong antimicrobial activity (++++), inhibition zone > 9 mm. Standard deviation ± 0.5 mm.

and *C. albicans,* which were resistant to the extracts at a concentration of 100 mg/ml. M. Bollwiller cultivar proved to be the most promising hazel cultivar to inhibit microbial growth, presenting lower MICs and higher growth inhibition zones. Gram positive bacteria were more susceptible than either Gram negative bacteria or fungi, presenting MICs of 0.1 mg/ml for *B. cereus* and *S. aureus*, and 1 mg/ml for *B. subtilis*. These results are of a great importance particularly in the case of *S. aureus* which is well-known for being resistant to a number of phytochemicals and for the production of several types of enterotoxins that cause gastroenteritis (Halpin-Dohnalek & Marth, 1989). The antifungicide activity of the hazel leaves was weak, being *C. albicans* resistant to all the samples and *C. neoformans* susceptible only to samples from M. Bollwiller cultivar but in a high concentration (100 mg/ml). Nevertheless, the MIC values obtained for these extracts were even better than the results obtained by us in previous works with table olives (Pereira et al., 2006) and "alcaparras" (Sousa et al., 2006).

In conclusion, the results obtained in this study demonstrate that hazel leaves may be a good candidate for employment as antimicrobial agent against bacteria responsible for human gastrointestinal and respiratory tract infections. It may also constitute a good source of healthy compounds, namely phenolic compounds, suggesting that it could be useful in the prevention of diseases in which free radicals are implicated.

Acknowledgements

The authors are grateful to INTERREG III A Program, Project PIREFI for financial support of this work.

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