

Laboratoire de Pharmacognosie¹, Faculté de Pharmacie, Département d'Athérosclérose², Institut Pasteur, Lille, France, Laboratoire de Physiologie Cellulaire et Morphogénèse Végétales³, UFR de Biologie, Université des Sciences & Technologies de Lille, Villeneuve d'Ascq, France

Hawthorn extracts inhibit LDL oxidation

C. QUETTIER-DELEU^{1,3}, G. VOISELLE², J.-C. FRUCHART², P. DURIEZ², E. TEISSIER², F. BAILLEUL¹, J. VASSEUR³, F. TROTIN¹

Received December 12, 2002, accepted January 15, 2003

Professeur Francis Trotin, Laboratoire de Pharmacognosie, Faculté des Sciences Pharmaceutiques et Biologiques, BP 83, F-59006 Lille
frotin@phare.univ-lille2.fr

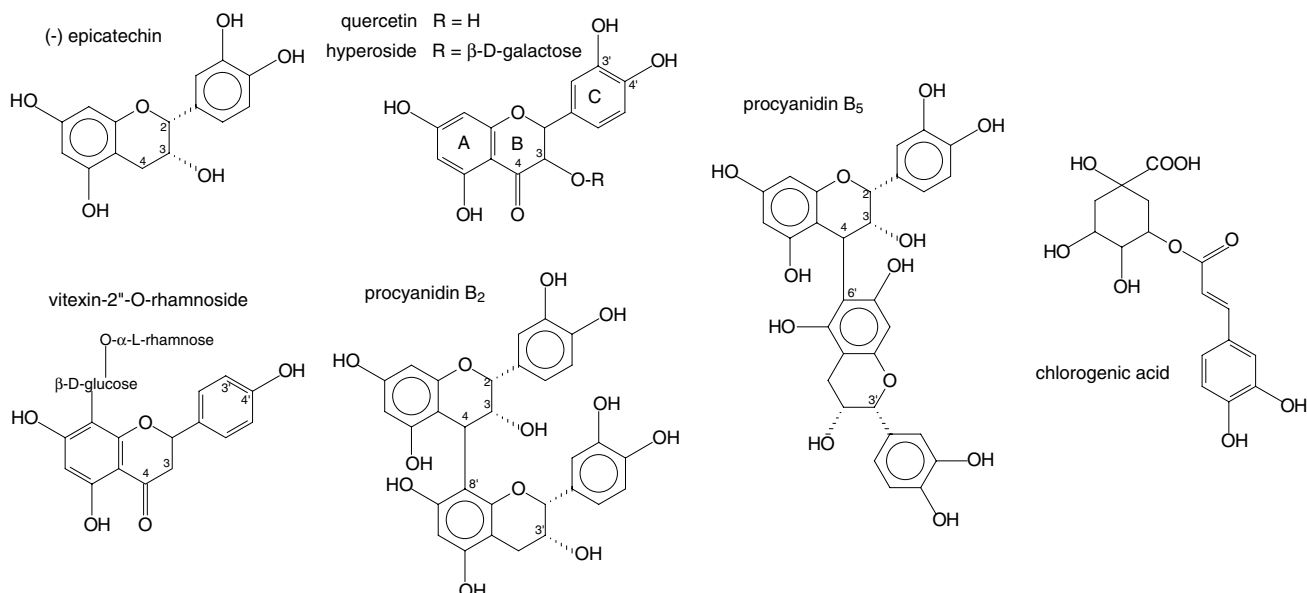
Pharmazie 58: 577–581 (2003)

Polyphenol-rich diet decreases cardiovascular risk. LDL oxidation is the primary event in atherosclerosis plaque formation and antioxidants such as polyphenols were shown to inhibit LDL oxidation and atherosclerosis development. Hawthorn (*Crataegus*) and derived pharmaceuticals are rich in polyphenols and already prescribed to treat moderate heart failure, nervousness and sleep disorders. Extracts either from fresh plant parts (flower buds, flowers, young leaves or green fruits) or from dried pharmaceutical parts (flowers and flowering tops) were previously shown to be effective inhibitors of lipoperoxidation and scavengers of oxygen species. In this study, the capacity of total and ethyl-acetate extracts from dried pharmaceutical flowers, tops and fruits to inhibit Cu²⁺-induced LDL oxidation was tested. This capacity was positively linked to their content in total polyphenols, proanthocyanidins (global and oligomeric forms), as well as to their content in two individual phenolics: a flavanol, the dimeric procyanidin B₂ and a flavonol glycoside, hyperoside. Flavanol-type phenolics showed to be higher active than the majority of the flavonoids tested in inhibiting Cu²⁺-induced LDL peroxidation. This study suggests that hawthorn could be a source of polyphenols able to inhibit LDL oxidation.

1. Introduction

Interest in the pharmacological properties of hawthorn (*Crataegus*) is growing in therapeutics, since derived pharmaceuticals and extracts are known and recognized to improve the coronary blood flow and cardiac contraction in moderate heart failure not requiring a major cardiotonic therapy (Stages I and II according to NYHA), as well as in

the ageing heart [1, 2]. These interests and uses are particularly evident in Germany owing to the classification of hawthorn – based pharmaceuticals among the cardiac remedies. Hawthorn is also generally prescribed as a sedative to treat nervousness and sleep disorders, property receiving preference in prescription and automedication in France. The plant parts registered in many Pharmacopoeias (flowers, tops – i.e. flowers with leaves –, and fruits) are rich in



polyphenols [1], mainly flavonoids (ca 1–2% in dried tops) and proanthocyanidins (ca. 1–3% in dried tops). The major flavonoids in hawthorn are flavonol derived glycosides like hyperoside (quercetin-3-*O*-galactoside), predominant in flowers and fruits, or like the flavone glycoside vitexin-2''-*O*-rhamnoside. The main flavanols are (–) epicatechin and the dimeric procyanidins B₂ and B₅.

During a long period, reports on the pharmacological properties of hawthorn in animals could mainly be found, but in the last years clinical trials in human therapy become more numerous. In both animals and humans the capacities of hawthorn extracts to avert heart failure, improve coronary blood flow and protect the vascular wall have been related to their polyphenol content [3–5, 6]. In these reviews, hyperoside was reported to enhance the coronary blood flow, but the fraction of low-polymerized oligomeric proanthocyanidins (OPC) proved to be more potent [7]. Furthermore, hawthorn extracts and particularly their OPCs were highly active *per os* in inducing protection against cardiac ischemia in rats [7].

Numerous polyphenols are active antioxidants: hydroxycinnamic acids, caffeic and phenylpropanoid derivatives, flavonoids, flavanols including catechins and proanthocyanidins [8–13]. Hawthorn is rich in flavonoids and flavanols [14].

In a previous work, we showed that the antilipoperoxidative effects of extracts from different fresh hawthorn parts were linked to their phenolic content and particularly to their proanthocyanidin concentration [15]. Oxygen species scavenging by different extracts from dry flowers, fresh plant parts, cell cultures, and hawthorn-based pharmaceuticals were shown to depend on their total phenolic and proanthocyanidin yields [16, 17].

The aim of the present work is to study the protective effects of extracts obtained from the dried pharmaceutical hawthorn parts, to establish a relation with the phenolic contents and with a potentially more active category of phenolics.

2. Investigations and results

2.1. Activity of reference phenolics

The Fig. shows that the flavanolic-type derivatives (epicatechin, procyanidins B₂ and B₅) were highly active inhibitors of Cu²⁺-induced LDL oxidation: epicatechin [(–)E] drastically inhibited LDL oxidation (ED₅₀ = 0.79 μM) while procyanidin B₂ and B₅ dimers [B₂, B₅] were less active (respective ED₅₀ = 1.49 and 1.42 μM) (Fig.). The flavonoid derivatives quercetin [Q.] and hyperoside [Hyp.] were also efficient against LDL oxidation, but not as effective as flavanols since their ED₅₀ ranged from 1.92 (quercetin) to 1.96 μM (hyperoside). Vitexin-2''-*O*-rhamnoside (not shown) was a very poor inhibitor (ED₅₀ = 847 μM). It should be noted that one flavonoid derivative, isoquercitroside [IQ.] differed from the two others and was a potent inhibitor of LDL oxidation (ED₅₀ = 1.08 μM). The ED₅₀ of the hydroxycinnamic derivative chlorogenic acid was 2.6 μM.

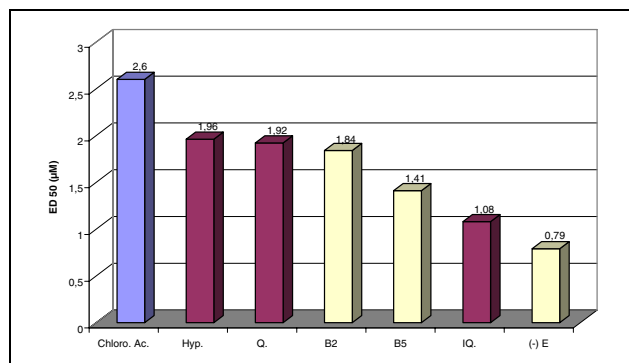


Fig.: Inhibition of LDL oxidation by reference phenolics (μM). Abbreviations for all fig. and tables: Chloro. Ac. = chlorogenic acid, Hyp. = hyperoside, Q. = quercetin, IQ. = isoquercitroside, B₂ = B₂ procyanidin, B₅ = B₅ procyanidin, (–) E = (–) epicatechin

2.2. Total extract contents and activities

All polyphenols were more abundant in total extracts (TE) of flowers and tops (Table 1) than in TE of fruits. In flowers, total phenols, global proanthocyanidin (GPC) and flavonoid (Flav.) yields were approximately twice as high than in tops. Total phenols, GPC and flavonoid concentrations were very low in the fruit TE. There was a strong relationship between the high polyphenol concentrations in the flowers and tops TEs and their strong capacity to inhibit LDL oxidation. Conversely low polyphenol concentrations in the fruit TE were clearly linked to their poor capacity to decrease LDL oxidation.

It should be noted that the references B₂ procyanidin and hyperoside, which strongly inhibited LDL oxidation as individually tested, were predominant in total extracts. B₂ procyanidin concentration (Table 2) was higher in the flowers TE than in tops TE and very low in the TE of fruits. Similarly, hyperoside concentrations were much higher in flowers TE than in tops and fruit TEs. Therefore, this study suggests a positive relationship in the different TEs between B₂ dimer and hyperoside concentrations and their capacity to inhibit LDL oxidation. At the same time, low concentrations of isoquercitroside characterized extracts from flowers, tops and fruits. The same observation was made for chlorogenic acid except in flower TE (flowers: 0.873; tops 0.029 g/100 g D.W. and absent in fruits; not shown) and quercetin was present at negligible levels in TEs (0.030 g/100 g D.W. in

Table 1: Comparative yields of total phenols, global procyanidins, flavonoids (g/100 g D.W.) determined by colorimetry in hawthorn total extracts and activities (ED₅₀ in mg D.W./ml)

	T. phen.	GPC	Flav.	ED ₅₀
Flowers	13.251 ± 0.576	3.011 ± 0.115	2.912 ± 0.071	0.003
Tops	6.522 ± 0.223	1.799 ± 0.048	1.367 ± 0.036	0.015
Fruits	1.518 ± 0.051	0.392 ± 0.012	0.186 ± 0.006	0.04

Table 2: Comparative yields of the main phenolics in total extracts of hawthorn (HPLC determination, g/100 g D.W.) and activities (mg D.W./ml)

	(–) E	B ₂	B ₅	Hyp.	IQ.	ED ₅₀
Flowers	0.240 ± 0.008	0.828 ± 0.026	0.382 ± 0.013	0.918 ± 0.032	0.136 ± 0.004	0.003
Tops	0.107 ± 0.004	0.447 ± 0.001	0.141 ± 0.005	0.268 ± 0.009	0.149 ± 0.005	0.015
Fruits	0.097 ± 0.004	0.087 ± 0.002	0.037 ± 0.001	0.047 ± 0.001	0.052 ± 0.002	0.04

Table 3: Comparative yields of total phenols, oligomeric procyanidins, flavonoids (colorimetric determination, g/100 g D.W.) of hawthorn ethylacetate extracts and activities (mg D.W./ml)

	T. Phen.	OPC	Flav.	ED ₅₀
Flowers	3.692 ± 0.015	0.543 ± 0.118	1.663 ± 0.021	0.018
Tops	3.148 ± 0.02	0.516 ± 0.095	1.854 ± 0.014	0.02
Fruits	0.935 ± 0.423	0.119 ± 0.03	0.125 ± 0.004	0.423

flowers, absent elsewhere; not shown). The contents in vitexin-2''-O-rhamnoside and vitexin were very weak except in flower extracts (0.249 and 0.0873 g/100 g D.W. in flowers TE), absent in others (not shown in all following Tables).

2.3. Ethyl acetate extracts contents and activities

Total phenols and OPC yields (Table 3) were of a similar order in the ethyl acetate extracts (EAEs) of both flowers and tops while being lower in the EAE of fruit. As for OPC, the flavonoid contents were of similar order in EAEs of tops and flowers, the tops extract being a little more rich. They were very low in the fruit EAE. The ED₅₀ of these three EAEs extend in a wider range (from 18×10^{-3} mg D.W./ml to 423×10^{-3} mg D.W./ml) than the range of their corresponding ED₅₀ in TEs (from 3×10^{-3} mg D.W./ml to 40×10^{-3} mg D.W./ml). The ED₅₀ of EAEs from flowers and tops were not very different, respectively 18 and 20×10^{-3} mg D.W./ml while the ED₅₀ of fruits EAE was very high (423×10^{-3} mg D.W./ml).

The B₂ procyanidin (Table 4), efficient antioxidant as demonstrated in this assay, was more abundant in EAE of flowers than in the corresponding top extract and negligible in fruits EAE. Hyperoside concentrations in EAE were low whichever part of the plant was extracted. It should be noted that the content in the highly antioxidant epicatechin was greater in the EAE of tops than in the corresponding flower and fruit extracts.

3. Discussion

The phenolic compounds tested as references and the different hawthorn extracts inhibited Cu²⁺-induced LDL oxidation *in vitro*. Owing to the activities displayed by the reference phenolics, it could be considered that the capacity of the extracts to inhibit LDL oxidation mainly depends on their flavanolic contents, particularly in epicatechin and in B₂ and/or B₅ dimers, since these molecules are more active in decreasing LDL peroxidation than the other principal phenolics present in these extracts, particularly the flavonoids. The similarity of ED₅₀ in B₂ and B₅ procyanidins, two dimers combining epicatechin units (respectively bound C₄–C₈ and C₄–C₆) could reflect their close chemical structure. These data also corroborate an observation made in previous results about *Crataegus* ex-

tracts where we showed that flavanols (B₂, epicatechin) were more active inhibitors of lipoperoxidation than flavonoids (hyperoside, rutin) [15]. Finally, we must mention that OPC were demonstrated to be more effective antioxidants and radical-scavengers than the polymeric proanthocyanidins in *Crataegus* extracts [7].

Among the flavonoids, quercetin is already known to highly inhibit LDL oxidation [8, 10, 18–20] and frequently taken as a standard. In our study the flavonol heteroside hyperoside (quercetin-3-O-galactoside) and the flavonol aglycone quercetin displayed similar ED₅₀ values, though being a little less active than flavanols. Hyperoside is the major flavonoid contained in hawthorn flowers, flower buds and fruits [21]. The poor capacity of vitexin-2''-O-rhamnoside to inhibit LDL oxidation could depend on the absence of O-dihydroxyl substitution on its B ring since this chemical structure has been shown to greatly increase the antioxidant and radical-scavenging capacities of flavonoids [9, 10]. It should be noted that isoquercitrin (quercetin-3-O-glucoside) highly reduced LDL oxidation in our study, marking an exception among the tested flavonoids.

The capacity of total and ethyl acetate extracts to inhibit LDL oxidation decreased from flowers to tops and fruit and clearly seemed to depend on their general phenolic contents. So, TEs of flowers were markedly more active than TEs of tops and their chemical analysis suggests that this difference could be linked to the higher concentrations measured for total phenols, GPC and flavonoids in flowers. This study also shows that both ethyl acetate extracts (EAEs) of flowers and tops exert a similar protection of LDL against oxidation. As before, this could depend on their nearly identical total phenol and OPC yields and on the limited difference between their flavonoid contents. In the same order, the markedly lower activities displayed by fruit TEs and EAEs seem clearly linked to their lower contents in total phenols, OPC or flavonoids.

All EAEs were less active protectors of LDL than TEs as expressed in the corresponding plant dry weight. This is easily explained by the well known elimination of nearly all the highly polymerized proanthocyanidins (PPC) after ethyl acetate fractionation [14, 15]. PPCs are generally known as less active antioxidants than OPC when tested individually or as PPC-defined fractions [7, 11, 12, 15], but their proportion is not negligible in total extracts and positively influences the activities. The interest in EAEs is their lesser complex composition which can better evidence the role played either by particular classes such as OPCs or by individual phenolics.

The participation of the different phenolics in extract activities could both be evaluated on the basis of their individual capacity to inhibit LDL peroxidation and on their concentrations in the extracts. B₂ dimer, one of the most active compounds, is the major flavanol in TEs as well as in EAEs and its role could be pre-eminent. Simultaneously, we have shown that epicatechin is the most potent tested molecule to inhibit LDL oxidation. Its yields in the extracts were generally limited but a fact is in favour

Table 4: Comparative yields of the main phenolics in ethylacetate extracts (g/100 g D.W.) of hawthorn and activities (mg D.W./ml)

	(–)-E	B ₂	B ₅	Hyp.	IQ.	ED ₅₀
Flowers	0.158 ± 0.008	0.346 ± 0.012	0.080 ± 0.003	0.032 ± 0	0.048 ± 0.001	0.018
Tops	0.257 ± 0.006	0.216 ± 0.003	0.070 ± 0.002	0.018 ± 0	0.055 ± 0.002	0.020
Fruits	0.049 ± 0.001	0.011 ± 0	0.001 ± 0	0.009 ± 0	0.003 ± 0	0.423

of its positive influence: in the EAE of tops which display an activity not very different of the TE, a lower yield in B₂ dimer compared to that of flowers was observed, while the content in epicatechin was higher. It could be suggested that the capacity of the tops EAE to protect LDL was maintained similar to that of flowers since epicatechin concentration was higher and counterbalanced the lack in B₂ dimer.

The use of hawthorn fruit has more recently been introduced in Europe than in the USA. Our study shows that its antioxidant capacity is lower than that of flowers and tops, which have been registered in different Pharmacopoeias for a longer time. Previous studies have shown that the astringent and unpleasant-tasting green fruit contains higher GPC and OPC concentrations and that its extracts are more active than the red ripe fruit ones as inhibitors of lipoperoxidation [14, 15].

This study shows that hawthorn extracts, known as rich in phenols, particularly in flavanols, are highly active in inhibiting LDL oxidation through this specific chemical composition. Therefore hawthorn could be useful as a source of antioxidant polyphenols which have already been involved in the capacity of different nutrients (fruits, grape juice, wine, tea) to decrease cardiovascular risk factors [22–24]. Moreover, previous studies reported that hawthorn preparations improved the objective and subjective symptoms of moderate cardiovascular disease [2].

4. Experimental

4.1. Plant material

Dried pharmaceutical flowers, flowering tops (flowers plus leaves) and fruits were purchased from Herboristerie Cailleau, Chemillé, France.

4.2. Chemicals and biochemicals

(–)-Epicatechin, cyanidin chloride, hyperoside, isoquercitrin, quercetin, chlorogenic acid and gallic acid came from Extrasynthese (Genay, France). Proanthocyanidin B₂ and B₃ dimers were isolated by us according to a previously developed method according to Moumou in our laboratories from *Crataegus monogyna* leaves, by CC on Sephadex LH–20 and identified by ¹H- and ¹³C NMR [25]. Folin-Ciocalteu and other reagents were analytical grade from Merck (Darmstadt, Germany).

4.3. Plant extraction

All samples were crushed in a blender, 30 g macerated at 4 °C in 4 × 400 ml (first 24 h, then 3 × 2 h) of methanol/acetone/water (7/7/3, v/v/v). The filtrates, low-pressure concentrated, left a water-phase which was divided in two parts: one half directly freeze-dried gave the “total extract” (TE), the other half was extracted by ethyl acetate, 8 × 300 ml. The ethyl acetate phases, concentrated to dryness under low-pressure, were dissolved in 20 ml methanol, then 80 ml water were added. Methanol was eliminated at low-temperature and the remaining water was freeze-dried to give the so-called “ethyl acetate extract” (EAE).

4.4. Phenolic content

4.4.1. Total phenolics

A Folin-Ciocalteu derived method, according to Singleton was used [26]. Lyophilisates (0.5 mg) from extracts were dissolved in 0.5 ml distilled water; 7 ml distilled water and 0.5 ml Folin-Ciocalteu reagent (Merck) were added. After 3 min, 2 ml of 20% Na₂CO₃ were added and the solution was heated at 100 °C for 1 min in a water-bath, then cooled in darkness. Absorbance at 685 nm was compared to that of a gallic acid standard solution and the results expressed in mg gallic acid/100 g dry weight.

4.4.2. Proanthocyanidin content

One mg lyophilisate of total or ethyl acetate extract (TE or EAE) was dissolved in 0.5 ml methanol, then 6 ml of n-butanol/conc. HCl (95/5, v/v) and 0.2 ml of a 2% solution of NH₄Fe(SO₄)₂ · 12 H₂O in 2 M HCl were added. The mixed solution was heated in a sealed tube in water-bath (95 ± 2 °C) for 40 min [27]. After cooling, absorbance was read at 550 nm, and the results expressed in mg cyanidin chloride/100 g dry weight, comparatively to a cyanidin chloride standard solution in butanol/HCl.

Values obtained with total extracts (TE) correspond to global proanthocyanidins (polymeric plus oligomeric forms = GPC), while the values from ethyl acetate extracts (EAE) represent the oligomers (OPC).

4.4.3. Flavonoid content

Flavonoid evaluation by colorimetry was performed using AlCl₃ complexation [16]. Two mg lyophilisate in 1 ml methanol was added to 1 ml of 2% methanolic AlCl₃, 6 H₂O and left at room temperature for 10 min. Absorbance was read at 430 nm comparatively to a methanolic solution of hyperoside [21].

4.4.4. HPLC analysis

Twenty µl of methanolic extract (TE or EAE) filtered on Millipore (0.45 µm) were analyzed on a Lichrospher 100 RP 18 (5 µm) C₁₈ column (4.6 × 250 mm) with an acidic CH₃CN/H₂O gradient. The elution program at a 0.7 ml/min flow rate was: 0–5 min, 0–7.5% B in A (linear gradient); 5–15 min, 7.5% B in A (isocratic); 15–20 min, 7.5–20% B in A; 20–25 min, 20–32.5% B in A; 25–30 min, 32.5–40% B in A; 30–45 min, 40–55% B in A; 45–50 min, 55–100% B in A; 50–55 min, 100–100% B in A; 55–60 min, 100–0% B in A. Solvents (A = CH₃CN/H₂O, 1/9; B = CH₃CN/H₂O, 9/1, both adjusted to pH 2.6 with phosphoric acid); temperature = 30 °C. Flavanols and phenolics acids were detected at 280 nm and flavonoids at 365 nm. Filtered methanolic solutions of pure reference phenolics: (–)-epicatechin, B₂ and B₃ dimer, quercetin, hyperoside and isoquercitrin were also injected.

4.5. Pharmacological assay

4.5.1. LDL preparation

Human LDL were isolated from freshly drawn blood from healthy, normolipidemic, and fasting volunteers. Blood was collected into EDTA and the plasma was separated by low speed centrifugation. LDL were isolated by sequential density gradient ultracentrifugation in sodium bromide density solutions in the density range 1.019–1.063 g/ml as previously reported [28]. LDL were then dialyzed against 0.01 M PBS (phosphate buffered saline: 0.15 M NaCl, 0.01 M Na-phosphate, pH 7.4) containing 0.01% EDTA and sterilized by filtration through a 0.22 µm pore-size filter and stored at 4 °C before use. The protein concentration was determined by Peterson's method using bovine serum albumin as standard [29].

4.5.2. Induction of LDL oxidation by copper

Solutions of reference polyphenols were prepared at 10^{–2} M in ethanol and diluted in 0.01 M PBS to give final concentrations of 0.1–100 µM in a total ethanol concentration of 1% (v/v). The same amounts of pure ethanol were added to blanks. Total and acetyl-acetate extracts were diluted in ethanol. The same amounts of pure ethanol were added to blanks.

Prior to oxidation, EDTA was removed by extensive dialysis of LDL solution against 0.01 M PBS under N₂, then oxidation was induced at 30 °C by adding 100 µl of 16.6 µM CuSO₄ to 800 µl of LDL (125 µg protein/ml) and 100 µl of chemicals in 0.01M PBS. During copper-induced LDL oxidation, diene conjugate formation was followed by measurement of optical density (OD) at 234 nm every 10 min for 8 h with a thermostated Kontron Uvikon 930 spectrophotometer equipped with a 10-position sample changer. Analyses were performed in triplicate. Three phases can be distinguished from the OD change pattern: 1) a lag-phase nearly deprived of OD increase corresponding to a resistance of LDL to copper-induced oxidation; 2) a propagation phase where OD rapidly increases; 3) a degradation phase starting at the point at which the OD increase reaches its plateau [8].

As the antioxidant capacity of extracts and isolated polyphenols can be characterized by the evolution of LDL oxidation curves according to their concentration, each pure polyphenol was tested at concentrations of 0.1–100 µM and each extract at concentrations of 0.1–100 × 10^{–3} mg/ml. Extracts and pure polyphenol antioxidant activities were expressed in percentages of their lag phase increase compared to that of the control. We considered that the drug concentration had 100% activity (efficiency dose 100%: ED₁₀₀) when it doubled the control lag phase duration and we defined the efficiency dose at 50% (ED₅₀) the concentration of the drug that increased this control lag phase by 1.5 times. Increasing concentrations of compounds were used with a logarithmic scale to calculate the ED₅₀ of antioxidant activity in µM.

References

- 1 Wichtl, M.; Anton, R.: *Plantes thérapeutiques. Technique et Documentation*, Paris, 1999
- 2 Loew, D.: *Phytomedicine* **4**, 267 (1997)
- 3 Ammon, H. P. T.; Kaul, R.: *Dtsch. Apoth. Ztg.* **134**, 2433 (1994)
- 4 Ammon, H. P. T.; Kaul, R.: *Dtsch. Apoth. Ztg.* **134**, 2521 (1994)
- 5 Ammon, H. P. T.; Kaul, R.: *Dtsch. Apoth. Ztg.* **134**, 2631 (1994)
- 6 Schüssler, M.; Hölzl, J.; Fricke, U.: *Arzneim.-Forsch./Drug Res.* **45**, 842 (1995)

- 7 Chatterjee, S. S.; Koch, E.; Jaggy, H.; Krzeminski, T.: *Arzneim.-Forsch./Drug Res.* **47**, 821 (1997)
- 8 Seidel, V.; Verholle, M.; Malard, Y.; Tillequin, F.; Fruchart, J.-C.; Duriez, P.; Bailleul, F.; Teissier, E.: *Phytother. Res.* **14**, 93 (2000)
- 9 Rice-Evans, C. A.; Miller, N. J.; Paganga, G.: *Trends Pharmacol. Sci.* **2**, 152 (1997)
- 10 Van Acker, S. A. B. E.; Van Den Berg, D. J.; Tromp, M. N. J. L.; Griffioen, D. H.; Van Bennekom, W. P.; Van Der Vijgh, W. J. F.; Bast, A.: *Free Rad. Biol. Med.* **20**, 331 (1996)
- 11 Hatano, T.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Fujita, Y.; Yasuhara, T.; Yoshida, T.; Okuda, T.: *Chem. Pharm. Bull.* **37**, 2016 (1989)
- 12 Ricardo Da-Silva, M.; Darmon, N.; Fernandez, Y.; Mitjavila, S.: *Agric. Food Chem.* **39**, 1549 (1991)
- 13 Quettier-Deleu, C.; Gressier, B.; Vasseur, J.; Dine, T.; Brunet, C.; Luyckx, M.; Cazin, M.; Cazin, J.-C.; Bailleul, F.; Trotin, F.: *J. Ethnopharmacol.* **72**, 35 (2000)
- 14 Thompson, R. S.; Jacques, D.; Haslam, E.; Tannner, R. J. N.: *J. Chem. Soc., Perkin Trans I*, 1387 (1972)
- 15 Bahorun, T.; Trotin, F.; Pommery, J.; Vasseur, J.; Pinkas, M.: *Planta Med.* **60**, 323 (1994)
- 16 Rakotoarison, D. A.; Gressier, B.; Trotin, F.; Brunet, C.; Dine, T.; Luyckx, M.; Vasseur, J.; Cazin, M.; Cazin, J. C.; Pinkas, M.: *Pharmazie* **52**, 60 (1997)
- 17 Bahorun, T.; Gressier, B.; Trotin, F.; Brunet, C.; Dine, T.; Luyckx, M.; Vasseur, J.; Cazin, M.; Cazin, J.-C.; Pinkas, M.: *Arzneim.-Forsch./Drug Res.* **46**, 1086 (1996)
- 18 Vinson, J. A.; Jang, J.; Dabbagh, Y. A.; Serry, M. M.; Cai, S.: *J. Agric. Food Chem.* **43**, 2798 (1995)
- 19 Hollman, P. C. H.; Katan, M. B.: *Biomed. Pharmacother.* **51**, 305 (1997)
- 20 Brown, J. E.; Khodr, H.; Hider, R. C.; Rice-Evans, C. A.: *Biochem. J.* **330**, 1173 (1998)
- 21 Lamaison, J. L.; Carnat, A.: *Pharm. Acta Helv.* **65**, 315 (1990)
- 22 Cambou, J. P.; Arveiler, D.; Amouyel, P.; Ruivadt, J. B.; Haas, B.; Montaye, M.; Bingham, A.; Richard, J. L.: *Rev. Epidem. et Santé Publ.* **44**, S46 (1996)
- 23 Renaud, S.; De Lorgeril, M.: *Lancet* **339**, 1523 (1992)
- 24 Hertog, M. G. L.; Fersens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D.: *Lancet* **342**, 1007 (1993)
- 25 Moumou, Y.; Trotin, F.; Vasseur, J.; Vermeersch, G.; Guyon, R.; Dubois, J.; Pinkas, M.: *Planta Med.* **58**, 516 (1992)
- 26 Singleton, V. L.; Joseph, A.; Rossi, J. R.: *Am. J. Enol. Vitic.* **16**, 144 (1965)
- 27 Porter, L. J.; Hrstich, L. N.; Chan, B. G.: *Phytochem.* **25**, 223 (1986)
- 28 Havel, R. J.; Eder, H. S.; Bragdon, J. H.: *J. Clin. Invest.* **3**, 1345 (1955)
- 29 Peterson, G.: *Anal. Biochem.* **83**, 346 (1977)