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Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas

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Abstract

The present paper presents the antioxidant, anti-inflammatory and antiproliferative capabilities of 16 plants. These plants can be found in the Limousin countryside and most of them are used in popular medicine as herbal tea. The biological properties of the water-soluble fractions were measured. Antioxidant properties were evaluated by the ESR method in order to visualize the inhibition of the DPPH, superoxide and hydroxyl radicals. Some extracts were good antioxidants by comparison with reference molecules, e.g. vitamin E and quercetin. Antioxidant effects were correlated with the total amount of phenolic compounds contained in the extracts. Also measured were the anti-inflammatory activities of the 16 water-soluble fractions, by evaluating inhibition of lipoxygenase activity. Finally the effects of these plants on the proliferation of melanoma B16 cells were studied.

Keywords: Plant extracts; Antioxidants; Free radical scavengers; 15-Lipoxygenase; B16 mouse melanoma cells; Phenolic compounds

1. Introduction

Antioxidant properties are among the first links between chemical reactions and biological activity and have been extensively studied for the past 10 years. Many molecules, such as phenolic compounds, are well known to possess this activity (Packer, 1999; Rice-Evans, Miller, & Paganga, 1996) as well as other biological activity, including DNA protective effects (Kelly, Xu, Alexander, & Loo, 2001; Lopaczynski & Zeisel, 2001), and enzyme inhibition (Carlo, Mascolo, Izzo, & Capasso, 1999; Le Bail, Laroche, Marre-Fournier, & Habrioux, 1998). Many phenolic compounds have been identified in extensively studied plants such as *Vitis vinifera* (Bombardelli & Morazzoni, 1995; Santos-Buelga, Francia, & Escribano-Bailon, 1995), *Ginkgo biloba* (Logani, Chen, Tran, Le, & Raffa, 2000), *Pinus maritima* (Packer, Rimbach, & Virgili, 1999). Determining plant composition is very tedious and preliminary studies of antioxidant capacities must be performed (Lee, Mitchell, & Shibamoto, 2000; Liu & Ng, 2000). We selected plants used in folk medicine, which are known to contain phenolic compounds (Table 1); however, their biological properties are actually not completely elucidated. Sixteen water-soluble extracts were screened: *Filipendula ulmaria*, *Lithospermum officinal, Alchemilla vulgaris, Rosmarinius officinalis, Achillea millefolium, Betula pendula, Hieracium pilosella, Equisetum arvense, Lamium album, Cynara scolymus, Humulus lupulus, Vaccinium myrtillus, Chamomilla recutita, Lotus corniculatus, Melilotus officinalis, Urtica dioïca*. These plants grow in France and most of them in the Limousin countryside. The phenolic compounds which are contained in these extracts are known to have some biological effects (Bruneton, 1999; Wichtl &

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Anton, 1999) and are the major components of some traditional medicinal plants. Many epidemiological and experimental studies actually suggest that these compounds play an important role in the prevention of various diseases : they are associated with a lower incidence of coronary heart diseases (Visioli & Galli, 1995) and certain tumors (Visioli, Bellomo, & Galli, 1998), diseases for which uncontrolled free radical production has been postulated (Duthie, Wahle, & James, 1989). It is well known that polyphenolic extracts act as free radical scavengers, and as antilipoperoxidants and are helpful in protecting collagen from degradation caused by superoxide anion radicals (Chen & Ho, 1997). Phenolic compounds also possess an array of potentially beneficial lipoxygenase inhibitory and antioxidant properties; they have been used for the treatment of inflammatory diseases (Sreejayan & Rao, 1996).

In vitro bioassay systems have been extensively used to monitor biological activities of medicinal plant extracts used in traditional medicines. In this study, we have demonstrated a correlation between antioxidant efficiency and phenolic composition of the different water-soluble extracts. The anti-inflammatory action was determined by a bioassay system that tested the inhibitory effect on soybean 15-lipoxygenase. Finally, the effects of the extracts on B16 cell proliferation were measured.

2. Materials and methods

2.1. Plant extracts

Hydro-alcoholic plant extracts (*Filipendula ulmaria*, *Lithospermum officinal, Alchemilla vulgaris, Rosmarinius officinalis, Achillea millefolium, Betula pendula, Hieracium pilosella, Equisetum arvensse, Lamium album, Cynara scolymus, Humulus lupulus, Vaccinium myrtillus, Chamomilla recutita, Lotus corniculatus, Melilotus officinalis, Urtica dioïca*) were from "Pharma & Plantes Laboratories". The water-soluble fractions only were used in the measurements.

2.2. Extraction procedure

For each plant, 5 g of hydro-alcoholic plant extracts were extracted by water addition (50 ml) and filtration. The solvent was evaporated under reduced pressure at 45 °C to yield dry extracts. They were stored under vacuum at room temperature and protected against UV irradiation.

2.3. Analyses of phenolic compounds

Total phenol and tannin concentrations were determined, according to the Folin-Ciocalteu method, using pyrogallol as a standard. The absorbance was measured at 760 nm on a Uvikon 930 UV/VIS spectrophotometer (Kontron instruments) and the results were expressed as pyrogallol equivalents in g per 100 g dry material.

2.4. Lipoxygenase assay

Soybean 15-lipoxygenase (15-LOX) was from Sigma. Inhibition experiments were run by measuring the loss of soybean 15-lipoxygenase activity in the presence of various concentrations of plant extracts. Activity of this enzyme was assayed with linoleic acid (Sigma) as the substrate under conditions previously described (Poca, Rabinovitch-Chable, Cook-Moreau, Pagès, & Rigaud, 1990). Briefly, lipoxygenase activity was tested by measuring oxygen consumption using a Clark-oxygen electrode with a Gilson oxygraph, assuming a 240 μM O_2 concentration in air-saturated buffer at 25 °C. The standard assay mixtures contained soybean 15-LOX in 200 mM phosphate buffer (pH 7.4). The reaction was started by addition of linoleic acid using Michaelis saturation conditions for this enzyme. The extracts were added at 2, 1.25, 0.625 and 0.1 mg/ml. Three determinations were made for each extract.

2.5. DPPH scavenging test

The antioxidant activity of plant extracts was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2picrylhydrazyl (DPPH, from Sigma) free radical (Fatimi et al., 1993) using ESR (Electron Spin Resonance) spectroscopy (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). Reaction mixtures contained 100 μl test samples and 100 μl DPPH ethanolic solution (5×10^{-4} M). Inhibition ratio was determined by comparison with a water-treated control group. ESR spectra were obtained with a Bruker ESP300E spectrometer using micro-sampling pipettes at room temperature under the following conditions: modulation frequency, 100 kHz; modulation amplitude, 0.197 mT; scanning field, 349.7 mT; receiver gain, 1.25×10^5; sweep time, 11 s; microwave power, 4 mW; microwave frequency, 9.78 GHz. All spectra were recorded at 3 min after homogenization by agitation (Fig. 1). The inhibition percentage was calculated by using the double integral of the signal and by using Eq. (1) :

$$\text{inhibition ratio} = \frac{\text{ref-extract}}{\text{ref-bg}} \quad (1)$$

where *ref* is the reference signal (DPPH + water), *extract* is the test signal, *bg* is the background signal. The data were the means of five measurements.

2.6. Hydroxyl radical scavenging test

Hydroxyl radicals were generated by Fenton reaction and reacted rapidly with nitrone spin-trap 5,5-dimethyl

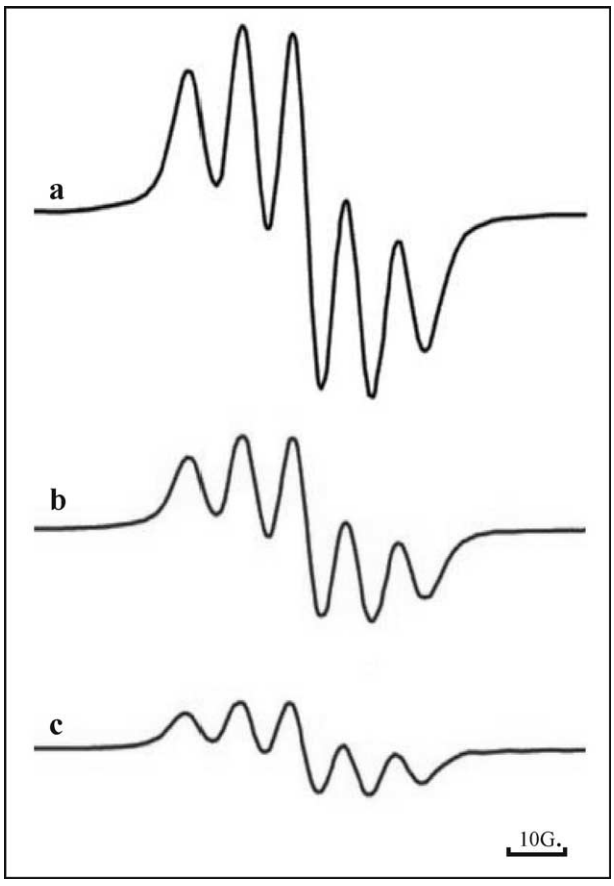

Fig. 1. Modification of DPPH radical ESR spectra by various concentrations of plant extract. Example for *M. officinalis*: (a) reference signal; (b) 1.25 mg/ml and (c) 2 mg/ml.

N-oxide pyrroline (DMPO, Sigma). The resultant DMPO-OH adduct was detected by means of ESR spectrometry (Gao, Huang, Yang, & Xu, 1999) using a Bruker ESP300E spectrometer set under the following conditions: modulation frequency, 100 kHz; modulation amplitude, 0.0987 mT; scanning field, 349.5 mT; receiver gain, 2×10^5; sweep time, 5 s; microwave power, 6.36 mW; microwave frequency, 9.78 GHz. ESR spectra were recorded at room temperature, 3 min after 50 μl of water (reference) or extract was mixed with H_2O_2 (10 mM, 25 μl), iron (II) sulfate (2 mM, 25 μl), and DMPO (48 mM, 50 μl) in a phosphate buffer solution (PBS, pH 7.4). The percentage inhibition was calculated by using the amplitude of the two central lines of the spectra and by using Eq. (1). The data were the mean of five measurements.

2.7. Superoxide radical scavenging test

Oxidation of lucigenin by superoxide radicals was assayed by the chemiluminescence (CL) response of lucigenin (Cotelle et al., 1996). Superoxide radicals ($O_2^{\cdot-}$) were generated by the action of 50 μl xanthine (2 mM) on 20 μl xanthine oxidase (0.5 U) in a reaction mixture containing 300 μl phosphate buffer (pH 7.4), 60 μl lucigenin (1 mM) and 100 μl test reagent (water as reference signal or extract). The mixture was quickly vortexed, and CL was measured in a luminometer analyser (Lumat LB 9507—Berthold) at room temperature for 60 s using 75×12 mm tubes. The inhibition percentage was calculated by using Eq. (1). The data were the means of five measurements.

2.8. Cell lines and culture conditions

B16, a mouse melanoma cell line derived from a spontaneous skin tumor in C57BI/6 mice, was provided from the Institut de Cancérologie et Immunogénétique, Villejuif, France. Stock cells were maintained as monolayers in 25 cm^2 culture flasks in Eagle's minimum essential medium (Gibco, Paisley, UK) supplemented with 10% heat-inactived fetal calf serum (Sigma Chemical Co.), vitamin (100x, Gibco), 100mM sodium pyruvate (Gibco), non-essential amino-acids (100×, Gibco), 200 μM L-glutamine and gentamycin (Gibco). The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. Under these conditions, the doubling time was 15 h. B16 cells were plated at a concentration of 0.5×10^6 and were grown in a 25 cm^2 flask in 4 ml of complete culture medium. Cell counts were made with a hemocytometer and viability of cells assessed by trypan blue exclusion. Each test was performed six times. The extract concentration which gives 50% growth inhibition is referred to as the IC_{50}.

3. Results and discussion

3.1. Types of plants

The antioxidative, anti-inflammatory activities and anti-tumor properties of the following sixteen plant extracts were evaluated: *Filipendula ulmaria, Alchemilla vulgaris, Rosmarinius officinalis, Equisetum arvense, Betula pendula, Hieracium pilosella, Achillea millefolium, Lithospermum officinal, Cynara scolymus, Lamium album, Vaccinium myrtillus, Humulus lupulus, Chamomilla recutita, Melilotus officinalis, Urtica dioïca*, and *Lotus corniculatus*. The healing virtues of these plants are very well known in French popular medicine. Some of them are also commonly consumed in the form of herbal tea. The known properties are listed in Table 1.

3.2. Antioxidant tests

Oxygen free radicals are involved in the pathophysiology of many diseases, such as inflammation, ischemic heart diseases, cancer, and many more.

Table 1
Details of the chemical constituents of the 16 extracts with their traditional uses in France

Species (English common name)	Major pytochemicals	Traditional uses in France
Achillea millefolium (Yarrow)	phenolic acids, tannins, flavonoids, essential oil, sesquiterpenoids lactones	stomachic, spasmolytic, diuretic, anti-inflammatory.
Alchemilla vulgaris (Lady's mantle)	Phenolic acids, tannins, flavonoids	Astringent,antihaemorrhagic, antidiarrheal.
Betula pendula (Birch)	Flavonoids, tannins, triterpenes (betulinol), arylbutanoids, glycosidic diarylheptanoids, essential oil	Diuretic.
Chamomilla reculita (Matricaria)	Phenolic acids, flavonoids, essential oil, coumarins, sesquiterpenoid lactones, mucilage	Anti-inflammatory, antispasmodic, appetite stimulant.
Cynara scolymus (Artichoke)	Phenolic acids, phenolic alcohols, flavonoids, sesquiterpenoid lactones	Choleretic, stomachic.
Equisetum arvense (Field horsetail)	Phenolic acids, flavonoids, sterols minerals (15–20%, particularly in silicon)	Diuretic.
Filipendula ulmaria (Meadowsweet)	glycosides of phenolic acids (derivatives of salicylic acid), tannins, flavonoids	Diuretic, antalgic, anti-inflammatory.
Hieracium pilosella (Mouse-Ear)	Phenolic acids, flavonoids, coumarins (umbelliferone)	Diuretic.
Humulus lupulus (Hops)	Tannins, flavonoids, derivatives of 1-acylphloroglucinol (lupulone, humulone), essential oil	Sedative, appetite stimulant.
Lamium album (White dead nettle)	Phenolic acids, flavonoids, mucilage, iridoids	Diuretic, enhance renal and digestive elimination function
Lithospermum officinale (Gromwell)	Phenolic acids, tannins, naphtoquinone	Gonadotropin antagonist, contraceptive.
Lotus corniculatus (Bird's-foot)	Flavonoids, organic substances	Sedative, spasmolytic.
Melilotus officinalis (Sweet clover)	Phenolic acids, flavonoids, coumarin	Vascular protective and antiedemic activity, minor sedative.
Rosmarinus officinalis (Rosemary)	Phenolic acids, flavonoids, tricyclic diterpenes (rosmaridiphenol, rosmanol, ...), essential oil	Choleretic, diuretic, spasmolytic antioxidant, stomachic and carminative.
Urtica dioïca (Nettle)	Phenolics (phenolic acids, lignans ...), sterols (sitosterols and derivatives)	Beneficial effect on prostatic adenoma, diuretic.
Vaccinium myrtillus (Blueberry)	Phenolic acids, flavonoids, proanthocyanidins, anthocyanins, sugars, organic acids	Vascular protective and antiedemic activity, antidiarrheal.

Possibly these plants possess good antioxidant properties due to the presence of polyphenolic compounds. The antioxidant and the free radical scavenging capacities of their water-soluble fractions were therefore evaluated.

Fig. 2 shows the concentration of DPPH which gave 50% inhibition (IC_{50}). There were large differences between the least effective extract (*Urtica dioïca*, $IC_{50} = 3.48$ mg/ml) and the most effective one (*Filipendula ulmaria*, $IC_{50} = 0.058$ mg/ml). By comparison, the IC_{50} of vitamin E was, under the same experimental conditions, 0.025 mg/ml. Quercetin IC_{50} values were used as a reference (Rice-Evans et al., 1996). In the DPPH test, quercetin IC_{50} was 0.012 mg/ml. The extract IC_{50} was the result of a mixture of molecules, with or without antioxidant activity. Thus, it is not unreasonable to speculate that the most active molecules which are contained in this extract, might have a low IC_{50} compared to vitamin E.

Fig. 3 shows the results of the superoxide test. The potential for inhibiting this radical is very important. Indeed, with the hydroxyl radical, the superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, the superoxide radical is normally formed first and is a precursor which leads to the formation of cell-damaging free radicals and oxidizing

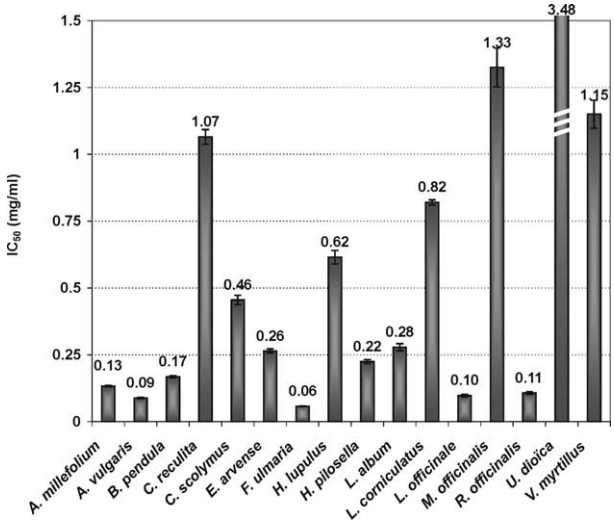

Fig. 2. Scavenging effects of the water-soluble part of 16 extracts on DPPH radicals. Data points represent means ± S.D. ($n = 5$).

agents. Here, similar results to those previously observed on Fig. 2 were obtained: *Filipendula ulmaria* was the most active extract in both tests with DPPH and superoxide ($IC_{50} = 0.14$ μg/ml); while *Urtica dioïca* was the least active ($IC_{50} = 31.2$ μg/ml). The IC_{50} for quercetin was 0.15 μg/ml and was comparable to those obtained for *Filipendula ulmaria* and *Betula pendula*. A recent study identified some diarylheptanoids and some flavonoids, from the bark of *Betula platyphylla*, with $O_2^{\bullet-}$ scavenging activity (Matsuda et al., 1998).

While superoxide is a precursor radical, the hydroxyl radical is the most toxic for cell components (DNA, membranes). The results obtained for this test are given in Fig. 4. The *Filipendula ulmaria* extract always showed the greatest activity ($IC_{50} = 0.11$ mg/ml). By comparison, quercetin had an IC_{50} of 0.034 mg/ml. For this test, the differences between the activities of the sixteen extracts were lower than for the DPPH test and the $O_2^{\bullet-}$ test.

Three classical antioxidant tests were carried out. The first one, which evaluates the inhibition of the stable radical DPPH, is known to give reliable information concerning the antioxidant ability of the tested compounds. The other two measure the inhibition of two oxygen radicals naturally produced in cells. There are some fundamental differences between these three tests. First, the redox potentials of the three radicals are not the same. Furthermore, superoxide radicals were generated by an enzymatic reaction (see Materials and Methods) and the signal inhibition could be attributed to inhibition of the enzyme or to inhibition of the radical. Similarly, the hydroxyl test, using the Fenton reaction, did not distinguish between free radical scavenging and the ferrous chelating effects of the extracts. The correlation between the three assays showed good results between the DPPH and the superoxide tests ($r^2 = 0.91$), while the correlations between the DPPH and the hydroxyl radical or superoxide anion and the hydroxyl tests were not satisfactory ($r^2 = 0.54$ or 0.33, respectively). As above, the hydroxyl test used Fenton reactions, and the chelating effects of some compounds in extracts could explain the lack of correlation between the hydroxyl test and the other two. When this ferrous chelation was observed in plant studies, it could often be attributed to the presence of flavonoids, which are well known to be chelator compounds (Calliste, Trouillas, Allais, Simon, & Duroux, 2001; Zhonghon, Kaixun, Xiangliang, & Huibi, 1999).

3.3. Antioxidant activities and phenol composition

The antioxidant and free radical scavenging powers of phenolic compounds have been extensively studied for the past 10 years. The roles of OH groups and π electron delocalizations in flavonoids have been largely discussed (Rice-Evans et al., 1996).

Many plants which contain flavonoids are known to possess good antioxidant activities by comparison with reference molecules such as Trolox and vitamin E.

In this study, it was first speculated that these plants might be a source of natural antioxidants. The tests performed confirmed the ability of some extracts to scavenge DPPH, $O_2^{\bullet-}$, $OH^{\bullet}$ radicals. The idea that these activities were due to the presence of phenolic compounds led us to investigate the phenolic composition of the extracts studied. Amounts of minerals, glucides and vitamins contained in these extracts were not estimated.

Table 2 shows the total phenolic composition and distinguishes between tannins and other phenolic compounds. Fig. 5 shows the correlation between the three tests and the total amount of phenolic compounds.

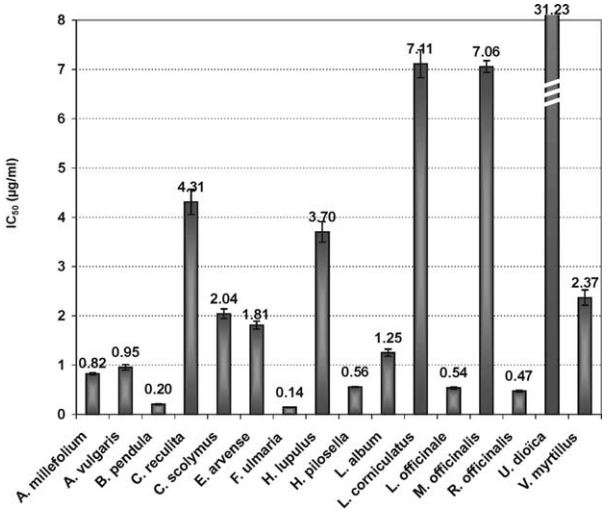

Fig. 3. Scavenging effects of the water-soluble part of 16 extracts on superoxide radicals generated by XO/X system. Data points represent means ± S.D. ($n = 5$).

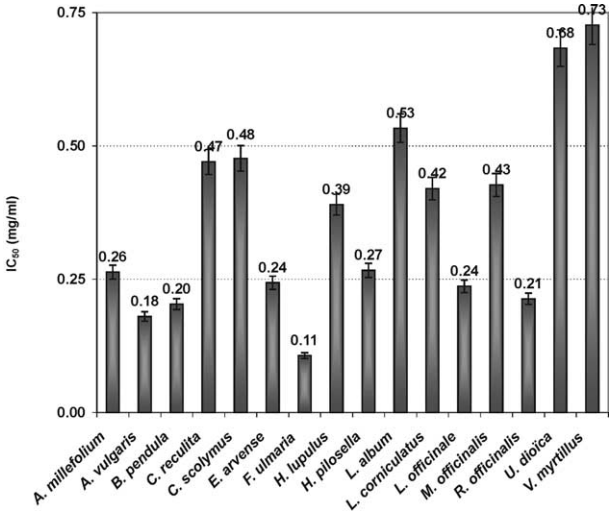

Fig. 4. Scavenging effects of the water-soluble part of 16 extracts on hydroxyl radicals generated by Fenton reaction. Data points represent means ± S.D. ($n = 5$).

Table 2
Phenolic compounds determined as pyrogallol equivalents in g per 100 g by Folin-Ciocalteu method

	Phenolic compounds (g/100 g)		
	Total	Tannins	Others
A. millefolium	6.20	0.90	5.30
A. vulgaris	6.25	2.19	4.06
B. pendula	5.64	1.35	4.29
C. reculita	1.42	–	1.42
C. scolymus	2.66	0.05	2.61
E. arvense	3.51	0.36	3.14
F. ulmaria	9.20	2.73	6.47
H. lupulus	2.05	0.10	1.95
H. pilosella	4.62	0.18	4.44
L. album	4.12	0.43	3.69
L. corniculatus	2.34	–	2.34
L. officinale	6.74	1.18	5.56
M. officinalis	1.92	–	1.92
R. officinalis	7.94	1.19	6.75
U. dioïca	0.88	–	0.88
V. myrtillus	0.92	–	0.92

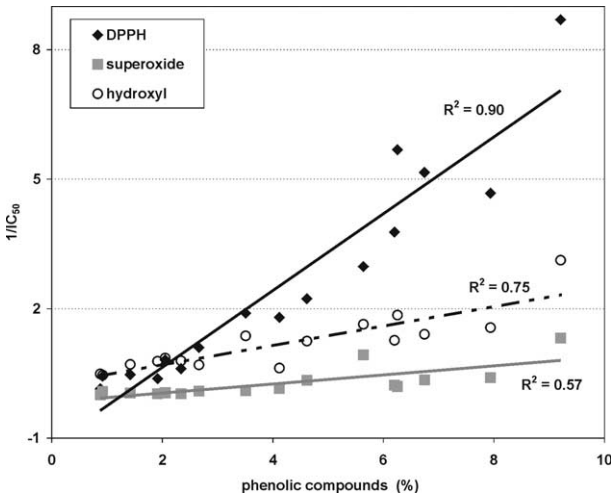

Fig. 5. Correlation between the activity (represented by $1/IC_{50}$) of the three antioxidant tests and the concentration of total phenolic compounds.

These calculations were made using $1/IC_{50}$ which was representative of activity. In fact, when activity increases, $1/IC_{50}$ decreases, and $1/IC_{50}$ is exactly proportional to the activity. $1/IC_{50}$ was plotted against the concentration of total phenols. Good correlation was observed between composition and anti-DPPH activity ($r^2 = 0.90$). We established a linear correlation between $1/IC_{50}$ and the amount of antioxidant molecules (which means that 1 molecule inhibits 1 radical, 2 molecules inhibit 2 radicals, ..., n molecules inhibit n radicals). This was because this test only involves direct radical scavenging, which occurs by "simple" redox reactions.

The relationship between the amount of phenolic compounds and anti-hydroxyl and anti-superoxide activities was less obvious ($r^2 = 0.75$ and 0.57, respectively). There is a large diversity of phenolic compounds, and many antioxidant behaviours can be attributed to these compounds, particularly mechanisms other than direct scavenging actions, including enzymatic inhibition or iron chelation. Chelation reactions and especially enzymatic reactions are very complex (receptor binding, complex formation). For these reasons, it was difficult to establish a simple linear correlation between the amount of phenolic compounds and the anti-hydroxyl and anti-superoxide tests.

The presence of natural antioxidant phenolic compounds in these plants is demonstrated by the present results, and the IC_{50} measured seemed to show that some of these plants (*Filipendula ulmaria, Alchemilla vulgaris, Lithospermum officinal, Rosmarinius officinalis, Betula pendula*) contain molecules with better antioxidant capacity than quercetin or vitamin E.

While it cannot be concluded from this study that the medicinal values of these plants are due to the presence of natural antioxidants, it is not unreasonable to speculate that some of these plants may lead to the development of potential drugs of considerable therapeutic value against many diseases that involve cytotoxic oxygen free radicals.

3.4. Anti-inflammatory activities

Since arachidonic acid metabolites are important mediators of inflammation, especially lipoxygenase products, the inhibition of arachidonate 5-lipoxygenase, an enzyme which catalyzes the oxygenation of arachidonate to 5-hydroperoxyeicosatetraenoic acid (a polyunsaturated fatty acid) (Borgeat & Samuelson, 1979) was determined. This is the first product of the arachidonate cascade, leading to the leukotriene biosynthesis pathway. Leukotrienes are considered to be involved in the initiation and the maintenance of a variety of inflammatory diseases; inhibition of leukotriene synthesis may, at least in part, be responsible for the anti-inflammatory action (Ammon, Annazodo, Safayhi, Dhawan, & Scrimal, 1992). Thus, the inhibitory effect of the plant extracts on soybean 15-lipoxygenase was measured in order to evaluate their anti-inflammatory activities.

Fig. 6 shows the results of the screening of all sixteen plant extracts for their ability to inhibit lipoxygenase activity. Three groups could be distinguished. *Filipendula ulmaria, Alchemilla vulgaris, Rosmarinius officinalis,* belonged to the group with the highest activity ($IC_{50} \sim 0.5$ mg/ml); *Equisetum arvense, Betula pendula, Hieracium pilosella, Achillea millefolium, Lithospermum officinal, Cynara scolymus, Lamium album, Vaccinium myrtillus, Chamomilla recutita* and *Humulus lupulus* had the next highest anti-inflammatory activity ($IC_{50} \sim 1.5$ mg/ml); while *Melilotus officinalis,*

Urtica dioïca and *Lotus corniculatus* were the least active extracts ($IC_{50} > 2$ mg/ml). By comparison, the IC_{50} of ursolic acid, chosen as a reference compound for this test, was 0.14 mg/ml (Simon, Najid, Chulia, Delage, & Rigaud, 1992).

Both antioxidant and anti-inflammatory activities were studied. Oxygen radicals are well known to be produced during the inflammatory processes, so correlations of two activities were attempted for the sixteen extracts. *Filipendula ulmaria, Alchemilla vulgaris* and *Rosmarinius officinalis* were the most effective while the *Urtica dioïca* extract was the least active in all four bioassays. Moreover, the correlation coefficient was calculated between lipoxygenase and antioxidant activities. There was good correlation with the DPPH and superoxide tests (0.80 and 0.72, respectively). No conclusion could be drawn because the composition of extracts is very complex. For example, was this double activity due to the same molecules or was it due to different molecules with specific activities?

Antioxidants are, in general, known to inhibit plant lipoxygenases (Ammon, Safayhi, Mack, & Sabieraj, 1993). Studies have implicated oxygen free radicals in the process of inflammation and phenolic compounds may block arachidonic acid metabolism by inhibiting lipoxygenase activity, or may serve as a scavenger of reactive free radicals which are produced during arachidonic acid metabolism (Sreejayan & Rao, 1996). The anti-inflammatory activities of the sixteen plant extracts could be explained by the potent inhibitory effects of their phenolic compounds on arachidonic acid metabolism through the lipoxygenase pathway. It is possible that the correlation observed between the two tests is due to the same family of molecules: phenolic compounds.

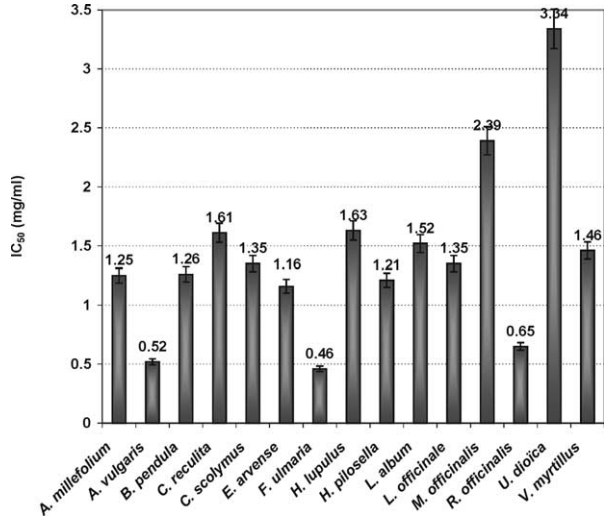

Fig. 6. Antilipoxygenase activities of the water-soluble part of 16 extracts on soybean 15-LOX (no activity was measured for *Lotus corniculatus*). Data points represent means ± S.D. ($n = 3$).

3.5. Cytotoxic effects on B16 cells

The cytotoxic effects of all sixteen plant extracts were evaluated on B16 mouse melanoma cells. B16 is a cell line derived from C57BI/6 mouse spontaneous skin tumor cells. Fig. 7 shows the effects of the extracts on cell proliferation after two days. A large difference is observable between the extracts. For low concentrations (< 0.25 mg/ml) there was no effect except for *Filipendula ulmaria, Betula pendula, Urtica dioïca, Achillea millefolium, Lithospermum officinal, Rosmarinius officinalis, Hieracium pilosella* and *Cynara scolymus* which showed increased proliferation. For high concentrations (> 0.5 mg/ml), six extracts showed a significant antiproliferative effect (*Filipendula ulmaria, Alchemilla vulgaris, Equisetum arvense, Humulus lupulus, Achillea millefolium* and *Lamium album*).

Previous studies suggested that compounds with antioxidant or anti-inflammatory activities, especially phenolic compounds, inhibit tumor promotion and cell proliferation (Huang et al., 1994; Simon et al., 1998). The mechanism underlying the antiproliferative activity of phenolic compounds is not fully understood. Previous studies concerning caffeic acid phenyl ester (CAPE) demonstrated that it was cytostatic; it also inhibited 5-LOX in the micromolar concentration range and exhibited antioxidant properties. It blocked production of reactive oxygen species in human neutrophils and the xanthine/xanthine oxidase system. The effectiveness of CAPE and caffeic acid as lipoxygenase inhibitors was correlated with their antioxidant properties. The authors speculated that the antioxidant inhibitors blocked the reduction of the peroxyl radical by Fe(II)

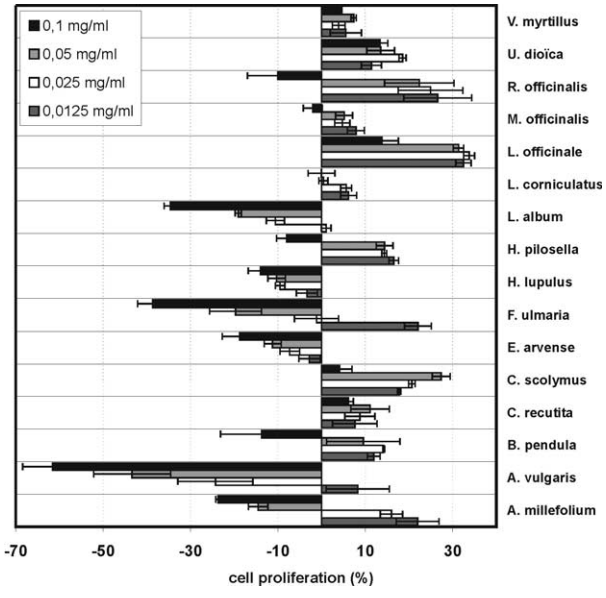

Fig. 7. Proliferative and antiproliferative, dose-dependent effects of the water-soluble part of 16 extracts on B16 cells after 2 days of growth. Data points represent means ± S.D. ($n = 6$).

and, therefore, formation of the activated Fe(III) form of the enzyme and fatty acid peroxide (Sud'ina et al., 1993).

Studies performed in our laboratory have demonstrated that ursolic acid, a lipoxygenase inhibitor, suppressed the proliferation of HL60 leukemic cells (Simon et al., 1992), of HGT, a human gastric tumor cell line (Es-Saady et al., 1994), of MCF-7, a breast human cell line (Es-Saady, Simon, Jayat-Vignoles, Chulia, & Delage, 1996) and of B16, a mouse melanoma cell line (Es-Saady, Simon, Ollier, et al., 1996).

However a possible correlation, for some extracts, between the antiproliferative and the antioxidant effects is specific to each cell line. In the present report the mechanism could not be studied because the effects observed could be due to several molecules and molecules which are not yet identified. In fact, the plant extracts studied are used in herbal and traditional medicinal preparations in France and the purpose of the present study was to evaluate some of their unknown activities.

Effective antioxidant activities were observed by comparison with two reference molecules, vitamin E and quercetin. These activities are possibly due to the presence of phenolic compounds. However, further investigations are required to determine the specific components that may have high antioxidant activity. Also observed were anti-inflammatory properties and cytotoxic effects on B16 mouse melanoma cells. *Filipendula ulmaria* extract, which exhibited the highest antioxidant and anti-inflammatory activities, is particularly used as a herbal tea in France; it also had antiproliferative effects on B16 cells. However, these were in vitro tests and it is well known that phenolic compounds lead to the formation of metabolites, after digestion, which could have different antioxidant activities. Moreover, agronomic practices and environmental conditions could influence the plant contents. Nevertheless, the present phytochemical study provides some information about the virtues of these extracts, used in folk medicine.

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