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Antioxidant Capacity Changes and Phenolic Profile of *Echinacea purpurea*, Nettle (*Urtica dioica* L.), and Dandelion (*Taraxacum officinale*) after Application of Polyamine and Phenolic Biosynthesis Regulators

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The changes of the antioxidant (AOA) and antiradical activities (ARA) and the total contents of phenolics, anthocyanins, flavonols, and hydroxybenzoic acid in roots and different aerial sections of *Echinacea purpurea*, nettle, and dandelion, after treatment with ornithine decarboxylase inhibitor, a polyamine inhibitor (*O*-phosphoethanolamine, KF), and a phenol biosynthesis stimulator (carboxymethyl chitin glucan, CCHG) were analyzed spectrophotometrically; hydroxycinnamic acids content was analyzed by RP-HPLC with UV detection. Both regulators increased the AOA measured as inhibition of peroxidation (IP) in all herb sections, with the exception of *Echinacea* stems after treatment with KF. In root tissues IP was dramatically elevated mainly after CCHG application: 8.5-fold in *Echinacea*, 4.14-fold in nettle, and 2.08-fold in dandelion. ARA decrease of *Echinacea* leaves treated with regulators was in direct relation only with cichoric acid and caftaric acid contents. Both regulators uphold the formation of cinnamic acid conjugates, the most expressive being that of cichoric acid after treatment with CCHG in *Echinacea* roots from 2.71 to 20.92 mg g^{-1}. There was a strong relationship between increase of the total phenolics in all sections of *Echinacea*, as well as in the studied sections of dandelion, and the anthocyanin content.

KEYWORDS: ***Echinacea purpurea*; nettle; dandelion; antioxidant and antiradical activities; phenolics profile; polyamine inhibitor; carboxymethyl chitin glucan; photospectrometry; HPLC analysis**

INTRODUCTION

Free radicals present in the human organism cause oxidative damage to different molecules, such as lipids, proteins, and nucleic acids, and thus are involved in the initiation phase of some illnesses. Oxidative stress appears to provide a critical link between environmental factors and endogenous ones including genetic risk factors in the pathogenic mechanisms of diseases. Reactive oxygen species are highly reactive oxidant molecules that are also generated endogenously through regular metabolic activity, and their production depends on lifestyle and diet.

Antioxidants are protective agents that inactivate reactive oxygen species and thus significantly delay or prevent oxidative damage. As a consequence, the antioxidant compounds may play a major role in the prevention of certain diseases, such as carcinogenesis, cellular aging, mutagenesis, cataracts, coronary heart disease, diabetes, cerebral pathologies, and rheumatoid arthritis. Some of them also reveal antiulcer effects and antiallergic, antiviral, and anti-inflammatory properties (*1*).

However, from a health perspective of the plant antioxidants, one must be aware that these moleucles can also display prooxidant activities, leading to oxidative damage of cellular components (*2*, *3*).

Herbs have been used for a large range of purposes. They are found to be potent sources of natural antioxidants. Some

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have been used for hundreds of years, and their clinical and pharmacological effects have been extensively studied from various viewpoints. Some of the clinical effects of herbs are closely related to their antioxidant activities. We have selected for our research the plants used in folk medicine, which are known to contain phenolic compounds. *Echinacea purpurea* serves as an immunomodulator for innate immune responses by stimulating macrophages and natural killer cells (*4*). The physiologically beneficial effects of *Echinacea* are exerted by the multitude of constituents present in the preparations. Preparations of *Echinacea* species are used as immunostimulants to prevent or cure the common cold and infections in the upper respiratory tract. *Echinacea*'s polysaccharide and phytosterol constituents support the immune system by activating white blood cells (*5*). *Echinacea* reduces the spread of infection agents by attacking hyaluronidase. Cichoric acid (phenylpropanoid) has been shown to stimulate phagocytosis—viral and bacterial cell destruction (*6*). In *Echinacea* four groups of substances are referred to as the most important: phenylpropanoids, alkamides, polysaccharides, and glycoproteins (*7*). This herb contains also gums, polyacetylenes, higher fatty acids, unsaturated aliphatic sesquiterpenes, etc. Dandelion serves mainly as a diureticum, and at the same time as a cleanser of the blood and liver. An active substance of dandelion reduces serum cholesterol and triglycerides because it intensifies bile secretion. Dandelion improves the function of the liver, pancreas, and stomach. It is used for anemia, cirrhosis of the liver, hepatitis, and rheumatism, and the leaves, for assembling salads, are also recommended as a natural source of vitamin C in the early spring. Radix has hypoglycemic effects. Dandelion water extract has antitumor activity attributed to polysaccharides. The most important biologically active components are sesquiterpenic lactones, biotin, inositol, and vitamins B, C, D, E, and P. The leaves possesses a higher content of β-carotene than carrot and more Fe and Ca than spinach, along with other macro- and micro-elements. The content of phenolics in dandelion is low (*8*). All parts of nettle are used to prevent or cure many diseases. Therapeutic effects are shown by possesses numerous phytochemicals. In radix there are mainly sterols and coumarin derivatives (*9*). All parts of nettle contain lignans, lower and fatty acids, tannins, mono- and triterpenes, vitamins, acid and neutral polysaccharides, choline, xanthophylls and related compounds, lectins, phenylpropanoid alcohols and aldehydes, phenolic acids, and other phenolics (*10*).

Phenolics are an ecologically significant class of secondary metabolites expressed in all higher plants. The most common group of plant phenolics is the flavonoid family. Flavonoids with the high chemopreventive potential (*11*) are subdivided into several families according to molecular structure: flavonols, flavanols, flavones, isoflavones, and anthocyanidins. The other phenolic compounds occurring in plants are non-flavonoid compounds such as hydroxycinnamic acids, hydroxybenzoic acids, and stilbenes (*12*). Phenolic acids are present in plants mostly in bound form. Hydroxycinnamic acids occur frequently as simple esters with quinic acid, glucose, in Asteraceae with succinic acid (*13*). The key enzyme for the phenolic metabolism is phenylalanine ammonia-lyase (PAL) (*14*). PAL activity was found to vary greatly with the stage of plant development (*15*). There are some reports (*16*, *17*) showing that stress conditions increase either PAL synthesis or activity in plants; other authors (*18*) have found that some stress treatments delay the increase in wound-induced PAL activity.

The higher level of phenolics is a component of the defensive mechanism of plants. Exogenous phenolcarboxylic acid application has been controversial in relation to PAL activity (*19*, *20*). In the effort to improve dietery antioxidant content of crop plants, phenolics as antioxidant have also been targets for enhancement in crops (*21*). Several strategies have proved to be successful in enhancing their level. One is the treatment of plants with biologically active compounds acting on the molecular level (*22*). Others use gene manipulations. Both structural genes and transcription factors have been manipulated, the latter because they play a prominent part in the overall regulation of the branches in the pathway (*23*).

To potentially raise the levels of phenolics and antioxidant capacity of the studied herbs, we applied two regulators of the different biosynthesis pathways. The first compound was *O*-phosphoethanolamine (KF), which acts as the ornithine decarboxylase inhibitor, a polyamine biosynthesis inhibitor. KF indirectly influenced the phenolics content and significantly increased the total content of anthocyanins and total phenolics in black chokeberry (*24*). The second compound was water-soluble carboxymethyl chitin glucan (CCHG), because according to Vander et al. (*25*) and others (*26*−*28*) chitin and chitosan, like other elicitors, such as β-glucan and yeast extract, could induce secondary metabolites through the phenylpropanoid pathway in edible and medical plants without any stresses or genetic modification. The major products of the phenylpropanoid pathway are the hydroxycinnamic acids, which can accumulate as esters or serve as precursors for other phenolic metabolites including flavonoids and lignin.

In this paper, we present the study results of the effect of *O*-phosphoethanolamine and water-soluble carboxymethyl chitin glucan on the total content of phenolics, flavonols, anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids, dry matter, and antioxidant and antiradical activities of the root and aerial parts (stem, leaf, and flower head) of *Echinacea purpurea*, nettle, and dandelion.

MATERIALS AND METHODS

Apparatus. The total phenolics, anthocyanins, flavonols, and hydroxybenzoic acids, and also antiradical activity and antioxidant activity, were determined using the UV−vis spectrophotometer (model Mini 1240, Shimadzu Corp., Tokyo, Japan); some cinnamic acids, for example, cichoric acid, caftaric acid, and chlorogenic acid, were determined using a Waters Breeze HPLC system.

Reagents and Standard Solutions. Linoleic acid (99%), Tween 20, β-carotene (95%), 2,2-diphenyl-1-picrylhydrazyl radicals (90%), quercetin dihydrate (98%), chlorogenic acid, gallic acid, caffeic acid (98%), methanol (spectrophotometric grade), and Folin−Ciocalteu's phenol reagent were obtained from Sigma-Aldrich. Kuromanin chloride (cyanidin-3-*O*-glucoside chloride, HPLC grade) was obtained from Extrasynthese (Genay, France). Ethanol (96%) and natrium carbonate (p.a. grade) were obtained from Microchem Ltd. (Pezinok, Slovakia). *O*-Phosphoethanolamine (98%) was synthesized at the Petrochemistry Research Institute (Prievidza, Slovakia). Carboxymethyl chitin glucan was separated from *Penicilium chrizogenum* mycelia at the Institute of Chemistry, Slovak Academy of Sciences (Bratislava, Slovakia). Cichoric acid and caftaric acid were identified after HPLC separation from *E. purpurea* extracts (Semi-Prep column Waters Spherisorb, 10 μm ODS-2, 10 × 250 mm) using NMR analysis (Varian, AS 600, Oxford).

Plant Material. *E. purpurea* (L.) Moench, nettle (*Urtica dioica* L.), and dandelion (*Taraxacum officinale*) were cultivated in fields located in the same area of Nitra (Slovakia). Dandelion before flowering, nettle (height = 0.15 m), at the beginning of May 2006, and *E. purpurea* (3-year-old field), at the beginning of July 2006 (fourth stage of development, bud enlargement), were foliarly treated for each of the treatments on an area of 3 m^2 (small parcel = 1 m^2, 3-fold repetition of treatment). The treatment of variants was as follows: control treatment, water (400 L ha^{-1}); KF treatment, *O*-phosphoethanolamine

(5.6 g ha^{-1}) dissolved in 400 L of water; CCHG treatment, carboxymethyl chitin glucan (8.0 g ha^{-1}) dissolved in 400 L of water.

Samples. Four plants from each small parcel were separately harvested 15 days after treatment. The whole plants with the roots and surrounding soil were transferred to the laboratory and processed immediately. Roots, stems, leaves, and flowers were cut off and rinsed with water; after homogenization in a blender, 5 g of sample was taken and extracted with 34.25 mL of 70% ethanol, by maceration at room temperature in sealed flasks. All extractions from each repetition of treatments were done in duplicate (total of six analyses/treatment). Flasks were placed in darkness and shaken every day for 5 min; after 72 h, the contents were filtered and then stored at −20 °C before analysis.

Analytical Procedures. On the basis of the chemical reactions involved, major antioxidant capacity assays are roughly divided into two categories: (1) hydrogen atom transfer (HAT) reaction based assays and (2) single-electron transfer (ET) reaction based assays (*29*). The extracts were examined for their phenolic contents (ET) and phenolic profiles as well as antioxidant activity in a β-carotene−linoleate model system (HAT) and scavinging of DPPH radicals (ET). Both methods give a measure of antioxidant activity. The antioxidant efficacy of the samples was evaluated on the basis of their abilities inhibit the β-carotene bleaching, caused by free radicals generated during the peroxidation of linoleic acid (*30*). The values of antioxidant activity are classified as high (>70% inhibition), intermediate (40−70% inhibition), and low (<40% inhibition), at 11.68 mg of dry matter of samples.

Inhibition of Peroxidation. One milliliter of β-carotene (0.2 mg/mL) dissolved in chloroform was added to an Erlenmeyer flask containing linoleic acid (0.02 mL) and Tween 20 (0.2 mL). The mixture was then dosed with 0.1−1.8 mL of extracts (so 11.68 mg of dry matter/sample was used) and 1.9−0.2 mL of 70% ethanol. Fifty milliliters of distilled water, saturated for 15 min with oxygen, was added to the flask. The resulting mixture was shaken and kept for 2 h at 50 °C. The absorbance of the samples was measured spectrophotometrically at 470 nm, immediately after their preparation ($t = 0$ min) and at the end of the experiment ($t = 120$ min). The inhibition of peroxidation (IP) was calculated as percent inhibition of oxidation versus control sample (2 mL of 70 mL ethanol), using the equation

$$\% \text{ IP} = 100 \times [1 - (A_s^0 - A_s^{120})/(A_c^0 - A_c^{120})]$$

where A_s^0 is the absorbance of sample at 0 min, A_s^{120} is the absorbance of sample at 120 min, A_c^0 is the absorbance of control sample at 0 min, and A_c^{120} is the absorbance of control sample at 120 min. Not only in this method, but also in the DPPH method, radical scavenging takes place—the differences are in the radicals.

Antiradical Activity Determination. Free radical scavenging potentials were tested in a methanolic solution of DPPH (*31*). The degree of decoloration of the solution indicates the scavenging efficiency of the added extract. Amounts from 0.1 to 1.8 mL of extracts and from 1.9 to 0.2 mL of 70% ethanol were added to 4 mL of DPPH solution (10 mg/L). Thirty minutes later, the absorbance was measured at 517 nm. A reference sample was prepared with 2 mL of 70% ethanol. The ARA was calculated as a percentage of DPPH decoloration using the equation

$$\% \text{ ARA} = 100 \times (1 - A_s/A_r)$$

where A_s is the absorbance of the sample and A_r is the absorbance of the reference. All values of IP and ARA were expressed as mean ± standard deviation (SD).

Total Phenolics Determination. The Folin−Ciocalteu (FC) method (*32*) based on the reduction of a phosphowolframate−phosphomolybdate complex by phenolics to blue reaction products was used to determine phenolic compounds. Briefly, 5 mL of water, 0.1 mL (*Echinacea*) or 1.0 mL (dandelion, nettle) of extracts, and 1.0 mL of FC reagent were added to a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5 min at room temperature. Next, 5 mL of a 7.5% of sodium carbonate solution and water filled to volume was added. After 2.5 h of standing at room temperature, the absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents in milligrams per gram of dry matter.

Phenolic Profile Determination. A modified version of the Glories' method (*33*) was used to estimate the phenolic profiles of the same samples as were used for the FC method. The absorbance (A) at 280 nm was used to estimate hydroxybenzoic acids, A_{360nm} was used to estimate flavonols, and A_{520nm} was used to estimate anthocyanins. Standards used were gallic acid in 10% ethanol for hydroxybenzoic acids, quercetin in 95% ethanol for flavonols, and cyanidin-3-glucoside in 10% ethanol for anthocyanins. Phenolic subclasses were expressed as milligrams per gram of dry matter.

The mean sample of each plant section/treatment was used (owing to data verification) for determination of total anthocyanins (results are not in the tables) according to the method previously reported by Hudec et al. (*24*). Small numeric insignificant differences between the two methods did not change the relative relationships among the plant sections and treatments.

High-Performance Liquid Chromatography (HPLC). Analysis of hydroxycinnamic acids in *Echinacea* extracts was performed using a Waters Breeze HPLC system equipped with a Waters UV-2487 detector. Separation was achieved on a reverse phase Spherisorb ODS 2 column of 250 × 4.6 mm i.d. The column temperature was maintained at 35 °C, and a flow rate of 1.0 mL/min was used. Solvent A was 0.2% (v/v) phosphoric acid, and solvent B was methanol. The elution conditions were as follows: linear gradient from 90% A to 10% A/90% B, 0−25 min; to 90% A/10% B, 25−27 min; isocratic elution 90% A/10% B, 27−30 min; post-time 15 min before next injection. The retention times for caftaric acid, chlorogenic acid, and cichoric acid measured at 330 and 254 nm were 8.51, 10.25, and 13.33 min, respectively.

Date Analysis. Three repetitions of treatments and two different samples from each repetition produced six values. All values of antioxidant and antiradical activity in the tables are expressed as mean (from six analyses) ± SD. The LSD method was used to determine the significant differences of phenolic contents between the treatments at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

Several epidemiological studies have been published showing an inverse correlation between phenolic-rich diets and the incidence of several diseases. Therefore, phenolics as antioxidants have also been targets for enhancement in crops. Our two expected phenol biosynthesis regulators significantly influenced the various studied parameters.

E. purpurea. The antioxidant activities of extracts measured by the β-carotene bleaching method (IP) was observed to be highest in leaves. In control plants IP then followed by stems, flowers, and roots in decreasing order. Extracts from the control roots possess almost no activity, which is in accordance with Turianica et al. (*34*). They state that the inhibition of peroxidation of *E. purpurea* flower heads during stages 5−7 of development radically decreases, but in leaves it slowly increases. According to Brovelli et al. (*35*) the concentration of cichoric acid (in the aerial parts of *E. purpurea*) as a strong antioxidant in the final stage of development decreases. Phenolic substances such as caffeic acid belong to the most efficient antioxidants from natural sources. Substitution of the aromatic ring in the ortho or para position will enhance the antioxidant efficacy because of the possible resonance structures leading to increased stability of the antioxidant radical formed upon scavenging of other radicals. Caffeic acid and caftaric acid have one ortho-dihydroxy phenyl group, whereas cichoric acid is composed of two molecules of caffeic acid (*36*). An overall antioxidant activity of *E. purpurea* measured as IP was intermediate in leaves and low in other aerial sections. The IP of leaves at 11.68 mg of dry matter of samples was determined to be 55.96%, whereas for stems, flower heads, and roots at

Table 1. Effect of Phenolic Metabolism Regulators on Antioxidant and Antiradical Activities and Phenolic Content[a] in Different Sections of *E. purpurea*

treatment	plant section	antioxidant activity[b]	antiradical activity[c]	total phenols[d]	hba[e]	flavonols[f]	anthocyanins[g]	flavonols/ anthocyanins	dry matter[h]
control	root	4.59 ± 1.36	82.44 ± 3.11	20.13 a	1.73 a	1.79 a	9.34 a	0.192	30.08 a
	stem	22.48 ± 1.94	64.89 ± 3.07	19.11 c	1.69 c	1.62 d	7.33 d	0.221	30.85 c
	leaf	55.96 ± 2.78	20.61 ± 1.54	39.96 d	8.14 e	6.99 f	19.62 f	0.356	23.39 e
	flower[i]	22.02 ± 1.61	64.12 ± 2.86	46.56 f	8.35 f	5.55 h	22.67 h	0.245	22.82 f
KF	root	10.55 ± 1.32	83.21 ± 3.41	44.95 b	11.10 b	5.89 b	19.11 b	0.308	34.77 b
	stem	−1.80 ± 0.98	66.41 ± 2.97	19.71 c	5.83 d	1.53 d	8.38 d	0.183	32.69 d
	leaf	58.26 ± 3.19	−22.77 ± 1.88	54.24 e	8.16 e	7.00 f	28.62 g	0.245	23.34 e
	flower	30.73 ± 1.96	64.12 ± 3.14	59.25 g	15.95 g	8.46 i	27.45 i	0.308	24.21 g
CCHG	root	38.99 ± 1.91	82.44 ± 3.77	47.00 b	11.56 b	3.80 c	24.10 c	0.158	33.40 b
	stem	33.94 ± 1.75	64.12 ± 3.36	20.71 c	1.67 c	4.07 e	9.99 e	0.407	31.19 c
	leaf	64.68 ± 2.69	6.11 ± 1.01	43.28 d	8.33 e	4.12 g	20.96 f	0.197	22.88 e
	flower	41.74 ± 2.26	62.60 ± 3.18	64.80 h	15.97 g	6.75 j	28.33 i	0.238	24.18 g

[a] Means followed by the same letter compared root, stem, leaf, and flower per se are not significantly different by LSD test at 5% level. [b] Percent. [c] Percent. [d] Spectrophotometrically (mg of gallic acid g^{-1} of DW,[i] 765 nm). [e] Hydroxybenzoic acid (mg of gallic acid g^{-1} of DW, 280 nm). [f] Milligrams of quercetin g^{-1} of DW, 360 nm. [g] Milligrams of cyanidin-3-glucoside, 520 nm. [h] Percent. [i] Dry weight. [j] Flower with seeds (flower head).

Table 2. Content (Milligrams per Gram of Dry Weight) of Hydroxycinnamic Acid Derivatives[a] in Different Sections of *E. purpurea* after Treatment with Phenolic Metabolism Regulators

	leaves			stems			flowers[b]			roots		
acid	control	KF	CCHG	control	KF	CCHG	control	KF	CCHG	control	KF	CCHG
caftaric	4.25 a	3.74 b	2.62 c	1.24 a	1.95 b	2.00 b	3.53 a	5.41 b	5.45 b	0.44 a	1.52 b	2.7 c
chlorogenic	ta[c]	ta	ta	0.31 a	0.26 a	0.31 a	0.31 a	0.40 a	0.37 a	0.54 a	0.36 ab	0.32 b
cichoric	14.91 a	13.24 b	9.61 c	1.63 a	2.77 b	3.52 b	13.79 a	19..58 b	18.91 b	2.71 a	13.53 b	20.92 c

[a] Determined by HPLC. Means followed by the same letter compared root, stem, leaf, and flower per se are not significantly different by LSD test at 5% level. [b] Flowers with seeds (flower head). [c] Trace amounts.

the same concentration IP was 22.47, 22.02, and 4.59%, respectively. Both regulators with the exception of stems after treatment with KF increased IP. In root tissue IP was dramatically elevated mainly after derivative application (8.5-fold). Carboxymethyl chitin glucan also increased IP of stems, leaves, and flowers by 151, 115.6, and 189.6%, respectively, versus control.

The inverse relationship between IP and ARA can be seen with the evaluation in all sections of control plants (**Table 1**). Of course, many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH. According to Joubert et al. (*3*) this apparent discrepancy could be explained if pro-oxidation, due to the high concentration of total flavonoids, dihydrochalcones, and/or total polyphenols, is considered. Frankel et al. (*37*) also showed that a high concentration of green tea extract resulted in pro-oxidation, but Burda and Oleszek (*38*) refer especially to the significant effect of the structure of phenolic compounds in the plant. The radical scavenging activity in water−ethanolic extracts of roots, stems, and flower heads after treatment with regulators was unchanged with the exception of leaves. ARA of these leaves after treatment with KF was dramatically decreased (>42-fold), and these tissues showed pro-radical activities (**Table 1**). This may result if a significant increase in weaker-reducing-agent phenolics, still measured in the antioxidant assay, and total phenols occurrs or the sample contains other compounds of high absorbance. From the chromatograms we could see two new peaks (retention times of 9.45 and 11.30 min) not assigned to known compounds (compared to the control leaves) that were significantly different in their size after treatment with KF versus CCHG. Similarly, one new peak in stem extract after treatment with KF (comparison to the control stem extract) was found (retention time of 8.16 min). ARA decrease of leaves after treatment with regulators was in direct relation only with hydroxycinnamic acids (cichoric acid and caftaric acid) contents, because chlorogenic acid was determined to be in trace amount. Cichoric acid is an efficient scavenger of free radicals and comparable to flavonoids (*36*). Therefore, its content decreases only in leaves (**Table 2**) and participates in the reduction of ARA only in leaves (**Table 1**). Our target is not to compare the antioxidant activity measured as IP or ARA, because both represent the results of the antioxidant activity assays achieved in two different systems (*29*).

The average total phenol contents in *Echinacea* control root, stem, leaf, and flower head were found to be 20.13, 19.11, 33.96, and 46.56 mg g^{-1} of dry weight, respectively. It has been reported that the flowers and roots of *Echinacea* have the highest contents of polyphenols and alkamides, whereas the leaves and stems are rich in polysaccharides (*39*). In our study, the highest phenolic contents in *Echinacea* flower heads and leaves were determined. Naturally, this result is the function of development stage. The inconsistencies among different sections of plants in phenolic profiles content and IP or ARA results are deceptive. That is to say, the antioxidant activity of phenolic compounds depends on several factors: the oxidation system, the degree of glycosylation, concentration, partition coefficient (phase distribution in assay conditions), and parameter measured. Phenolics generally considered to be antioxidants can also exhibit pro-oxidant activity under certain conditions. Hydrogen atom donation by *o*-diphenols (the basis for antioxidant activity) results in the formation of a phenoxyl radical that can undergo secondary reactions of a pro-oxidant nature. Certain phenolics are prone to autoxidation. The combination of these reactions accounts for the redox cycling activity of some flavonoids and non-flavonoid phenolics. These compounds often demonstrate pro-oxidant activity in the presence of alkaline pH, transition metals, and oxidative enzymes (*40*, *41*). Some authors (*42*) have shown a high correlation between antioxidant activity and

Table 3. Effect of Phenolic Metabolism Regulators on Antioxidant and Antiradical Activities and Phenolics Content[a] in Different Sections of Nettle

treatment	plant section	antioxidant activity[b]	antiradical activity[c]	total phenols[d]	hba[e]	flavonols[f]	anthocyanins[g]	flavonols/ anthocyanins	dry matter[h]
control	root	9.86 ± 1.62	44.77 ± 2.92	7.82 a	0.27 a	3.00 a	0.93 a	0.323	19.99 a
	stem	37.56 ± 2.91	50.58 ± 2.73	9.91 c	0.50 c	4.18 d	4.17 d	1.002	11.12 c
	leaf	76.06 ± 3.04	26.75 ± 3.01	7.62 e	2.67 e	1.92 f	6.36 f	0.302	21.33 e
KF	root	31.46 ± 2.14	27.33 ± 1.37	7.32 a	0.90 b	3.82 b	2.19 b	1.744	26.04 b
	stem	57.28 ± 2.93	43.60 ± 1.92	11.94 d	1.15 d	4.28 d	5.27 e	0.812	10.70 c
	leaf	81.22 ± 3.70	15.12 ± 1.05	7.61 e	2.67 e	2.89 g	6.91 g	0.418	21.41 e
CCHG	root	40.85 ± 3.13	62.79 ± 4.92	10.25 b	1.00 b	4.80 c	3.12 c	1.538	29.76 b
	stem	51.17 ± 3.06	75.00 ± 4.60	10.71 c	1.22 d	5.83 e	4.33 d	1.346	16.70 d
	leaf	85.92 ± 3.42	20.93 ± 2.34	9.61 f	2.74 e	2.95 g	6.60 fg	0.447	25.65 f

[a] The symbols are identical with those of **Table 1**.

phenolic content, whereas other authors (*43*) have found a low correlation or no correlation (*44*). Furthermore, herbs contain a wide array of chemical compounds working together. Most often there is a rather incomplete knowledge of their cammon effects, and some of the active components may have remained undetected, but some of them may create an inhibiting or stimulating effect from the point of different analyzed parameters, which complicates the understanding of the mechanisms and relations.

The group of unidentified alkamides with retention times between 22 and 24 min and free caffeic acid were presented in roots only. Several authors (*45*, *46*) attribute specific alkamides the retention time of 22−24 min to the same analytical conditions. This result together with the other undetermined compounds could be in relation to the highest ARA of roots and contributes to the synergistic antioxidant effects of *Echinacea* constituents as shown by Dalby-Brown et al. (*7*). On the contrary, Hu and Kitts (*47*) found no radical scavenging activity in the chloroform extracts of *Echinacea* species, and therefore they declare the alkamides do not contribute to free radical scavenging activity. The group of alkamides with retention times between 26 and 28 min was present in all sections of *E. purpurea*. KF and CCHG treatment significantly increased the total phenol contents with the exception of stem. The total phenols content in root tissue was elevated 2.33-fold after treatment with CCHG, 1.36-fold in leaf after treatment with KF, and 1.39-fold in flower after treatment with CCHG. There was a strong relationship between the increase of the total phenolic content in all sections of *Echinacea* and the anthocyanin content (**Table 1**). The findings presented in this study on the total phenol content of *Echinacea* after treatment with KF are consistent with previous results obtained for black chokeberry (*24*). The results ascribed to hydroxybenzoic acids (measuring the absorbance at 280 nm) are not the absolute values, but the higher one, because it reflects contributions from a wider range of polyphenols (flavanols, hydroxycinnamates, etc.). The effect of both regulators mainly on the higher content of hydroxybenzoic acids in roots and flowerheads is unambiguous.

Both regulators, KF indirectly (for possible ways see ref *24*) and CCHG directly induced the accumulation of phenolic compounds. Both regulators expressively changed the conditions of synthesis or translocation of phenolic compounds in *E. purpurea*. They increased the content of cinnamic acid derivatives in stems by almost 1.9-fold (CCHG), in roots by 6.5-fold (CCHG), and in flowers by 44% (KF) and decreased their content in leaves by 36% versus control. The maximum elevation of cichoric acid was from 2.71 to 20.92 mg g^{-1} in roots after treatment with CCHG (**Table 2**). Both regulators increased the formation and accumulation of cinnamic acid conjugates in all plant sections with the exception of leaves.

KF treatment increased the dry matter content of the roots, stems, and flowers, whereas CCHG increased the dry matter content of only the roots and flowers.

Nettle. The results are given in **Table 3**. Extracts from control roots possessed almost no antioxidant capacity (9.86%), control stem IP was intermediate (37.56%), and leaves showed high AOA (76.06%). Both applied regulators increased IP in all plant sections, on average more considerably after treatment with CCHG. CCHG increased IP in roots 4.14-fold and in leaves by 13% versus control. The leaves just after treatment with CCHG showed the highest IP (85.92%) in all nettle sections.

The antioxidant activity of stems measured as IP was more strongly increased by KF of 52.5% compared to the control. Both the leaf and the nettle root are used pharmaceutically, although use in benign prostatic hyperplasia is limited to the root (*48*). Moreover, antiprostatic effects, according to present knowledge, are possessed by many substances, which act by 15 different mechanisms (*49*). One of them is an antioxidant mechanism. Strategies aimed at reducing the generation or increasing the scavenging of reactive oxygen species offer exciting promise for the prevention of prostate cancer. An expressive elevation of IP in roots after treatment with our regulators could interact with the probably most active components of root, β-sitosterol and lectin, to provide more effective prevention or therapy of prostate cancer or benign prostatic hyperplasia, respectively.

The regulators had an expressively different effect on ARA in all nettle sections. KF decreased the antiradical activity in all plant sections. After treatment with CCHG, the antiradical activity of roots increased by 40.25% and that of stems increased by 48.28% versus control, but that of leaves decreased by 21.76%. These results (the decrease of ARA after treatment with KF and the increase of ARA after treatment with CCHG) could be explained by differences in the mechanisms of the action of regulators. CCHG as chitin derivative acts as the stimulator of PAL activity (*26*, *27*) and thus directly influences the phenolic biosynthesis, but KF as the inhibitor of ornithine decarboxylase (*50*) only indirectly (*24*) induces this biosynthesis.

CCHG significantly increased the dry matter content of all nettle plant sections. In the case of KF only the dry matter of the roots was significantly increased in comparison to the control. The dry matter was the most expressively increased in roots (by 30.3% after treatment with KF and by 48.9% after treatment with CCHG). The higher lignification of the cell walls related to the higher phenolic compounds accumulation after treatment with CCHG is in accordance with Tan et al. (*51*) and attests at the same time that CCHG has a positive effect on

Table 4. Effect of Phenolic Metabolism Regulators on Antioxidant and Antiradical Activities and Phenolics Content[a] in Different Sections of Dandelion

treatment	plant section	antioxidant activity[b]	antiradical activity[c]	total phenols[d]	hba[e]	flavonols[f]	anthocyanins[g]	flavonols/ anthocyanins	dry matter[h]
control	root	13.22 ± 1.23	83.43 ± 2.96						15.31 ± 0.68
	stem	35.00 ± 2.45	78.86 ± 2.31						8.80 ± 0.28
	leaf	31.43 ± 2.10	61.14 ± 2.40	5.38 a	1.14 a	1.30 a	2.56 a	0.51	14.36 ± 0.28
	flower	7.14 ± 0.59	82.29 ± 2.24	12.66 c	0.68 c	4.50 b	7.79 c	0.58	14.79 ± 0.32
KF	root	18.57 ± 1.06	80.57 ± 2.98						24.47 ± 0.66
	stem	52.14 ± 2.68	72.57 ± 2.92						10.31 ± 0.40
	leaf	61.07 ± 2.41	56.57 ± 2.30	8.91 b	1.09 a	0.94 a	2.48 a	0.38	16.15 ± 0.33
	flower	53.93 ± 2.33	78.86 ± 2.27	15.24 d	0.21 d	3.40 c	7.38 d	0.46	15.94 ± 0.37
CCHG	root	27.50 ± 1.71	76.00 ± 2.71						24.89 ± 0.83
	stem	52.86 ± 2.98	74.29 ± 2.13						10.11 ± 0.43
	leaf	60.00 ± 2.65	60.00 ± 2.46	9.22 b	1.59 b	1.19 a	3.00 b	0.40	15.60 ± 0.40
	flower	50.38 ± 2.07	80.00 ± 2.29	15.47 d	0.72 c	4.08 d	8.14 c	0.50	17.59 ± 0.48

[a] The symbols are identical with those of **Table 1**.

PAL activity, because its decrease, as also an another enzyme from the phenylpropanoid pathway, reduces the lignin level (*52*). This fact corresponds with the results of Li et al. (*16*) because chitin oligomers are more intensive inducors of PAL expression than the stress from the wheat leaves infection. The total phenolic content of the roots (compare KF vs control) does not support the statement that the higher lignification of the cell walls is related to the higher phenolic compounds, but indicates that KF acts by the other mechanism to the phenolic formation. The more expressive effect on the total phenols was shown by CCHG, although after treatment with KF the highest content (11.94 mg of gallic acid equivalent g^{-1} of dry weight) was determined in nettle stem.

After treatment with CCHG, especially in roots, the content of hydroxybenzoic acids was 3.7-fold, the content of anthocyanins almost 3.4-fold, and the content of flavonols 1.6-fold increased.

The statement that KF expressively prefers an anthocyanin synthesis and accumulation in black chokeberry (*24*) was not unambiguously confirmed in all sections of herbs. Obviously, the other plants may possess different transport methods and accumulation places of the active compounds.

Dandelion. The extracts of the control dandelion plant sampling at the stage of flowering were shown to be weak antioxidants and were more effective as scavengers of DPPH. Both regulators increased IP in all plant sections. CCHG stimulated an increase of IP the most in flowers (an increase of 70.56%), in roots (an increase of 108.0%), and stems (an increase of 51.0% compared to the control) (**Table 4**). KF elevated IP the most in flowers (7.55-fold) and in leaves (of 94.3%). The low antioxidant activity of control flowers (7.14%, practically without activity) in the development stage of full flowering could be related to the relatively higher level of conjugates, especially in flowers, including polyamine conjugates with hydroxycinnamic acids. The conjugates content during bud formation and development of flower was dramatically changed (*53*). Flavonoid conjugates owing to substantial structural changes in comparison with aglycons have got a very low antioxidant activity measured as IP and often also prooxidant activity (*38*, *54*). KF inhibits polyamine synthesis, mainly putrescine and spermidine formation, so part of the phenolics cannot be bound in polyamines and remain unconjugated and in this way contribute to the higher antioxidant activity.

The radical scavenging activity of extracts from roots, flowers, and stems was high (the most active was root, 83.43%) and that from leaves was intermediate (61.14%). Both regulators slightly reduced ARA and increased the dry matter, most expressively in roots (as in *Echinacea* and nettle roots). This fact is the next indirect evidence that not only CCHG but also KF affects the phenylpropanoid pathway. The mechanism of action is not clear. Alternatives are described by Hudec et al. (*24*).

The major phenolic fraction in dandelion was anthocyanins. This phenolic fraction of the flowers was reduced by KF, whereas CCHG had no effect on their anthocyanin content, but it did increase the anthocyanin content of the leaves. Both regulators reduced the flavonol content of the flowers. Despite this, both treatments increased the total phenol content of the leaves and flowers, the most after treatment with CCHG, in flowers to 15.47 mg g^{-1}, which is an increase of 22.2%, and in leaves to 9.22 mg g^{-1}, which is an increase of 71.4% in comparison to the control. The effects of KF and CCHG did not differ significantly. The total phenolic contents were in a tighter relationship with ARA than IP, which supports the notion that phenolics act through an electron transfer mechanism and that their content reflects the reducing capacity of the antioxidatively affecting source (*29*, *55*).

The results of this project suggest that herbs found in nature with low or intermediate antioxidant activity after treatment with *O*-phosphoethanolamine or carboxymethyl chitin glucan as the stimulator of PAL activity might be potential sources of higher antioxidant content and can play an important role in chemoprevention and also are more desirable for processing in the pharmaceutical industry.

ABBREVIATIONS USED

AOA, antioxidant activity; ARA, antiradical activity; IP, inhibition of peroxidation; CCHG, carboxymethyl chitin glucan; KF, *O*-phosphoethanolamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl radicals; FC, Folin−Ciocalteu; ODC, ornithine decarboxylase; PAL, phenylalanine ammonia-lyase.

LITERATURE CITED

(1) Moskovitz, J.; Yim, K. A.; Choke, P. B. Free radical and diseases. *Arch. Biochem. Biophys.* **2002**, *397*, 354−359.

(2) Galati, G.; O'Brien, P. J. Potential toxicity of flavonoids and other dietary phenolic : significance for their chemopreventive and anticancer properties. *Free Radical Biol. Med.* **2004**, *37*, 287−303.

(3) Joubert, E.; Winterton, P.; Britz, T. J.; Wentzel, C. A.; Gelderlom, W. C. A. Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (*Aspalathus linearis*). *J. Agric. Food Chem.* **2005**, *53*, 10260−10267.

(4) Currier, N. L.; Miller, S. C. *Echinacea purpurea* and melatonin augment natural-killer cells in leukemic mice and prolong life span. *J. Altern. Complementary Med.* **2001**, *7*, 241−251.

(5) Sel, D. M.; Broumand, N.; Sahl, L.; Tilles, J. G. In vitro effect of *Echinacea* and ginseng on natural killer and antibody-dependent cell cytotoxicity in health subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. *Immunopharmacology* **1997**, *35*, 229−235.

(6) Presser, A. M. *Pharmacist's Guide to Medical Herbs*; Smart Publications: Petaluma, CA, 2000; pp 131−132.

(7) Dalby-Brown, L.; Barsett, H.; Landbo, A.-K. R.; Meyer, A. S.; Mølgaard, P. Synergistic antioxidative effects of alkamides, caffeic acid derivatives, and polysaccharide fraction from *Echinacea purpurea* on in vitro oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **2005**, *53*, 9413−9423.

(8) Bruneton, J. *Pharmacognosy, Phytochemistry, Medicinal Plants*; Lavoisier Publishers: Paris, France, 1995; 915 pp.

(9) Belaiche, P.; Lievoux, O. Clinical studies on the palliative treatment of prostatic adenoma with extract of *Urtica* root. *Phytother. Res.* **1991**, *5*, 267−269.

(10) Bombardelli, E.; Morazzoni, P. *Urtica dioica* L.—review. *Fitoterapia* **1997**, *68*, 387−401.

(11) Sueiro, L.; Yousef, G. G.; Seigler, D.; De Meija, E. G.; Grace, M. H.; Lila, M. A. Chemopreventive potential of flavonoid extracts from plantation-bred and wild *Aronia melanocarpa* (black chokeberry) fruits. *J. Food Sci.* **2006**, *71* (8), C 480−488.

(12) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; pp 1−25.

(13) Shahidi, F.; Naczk, M. *Food Phenolics*; Technomic Publishing: Basel, Switzerland, 1995.

(14) Camm, E. L.; Towers, G. H. N. Review article, phenylalanine ammonia-lyase. *Phytochemistry* **1973**, *12*, 961−973.

(15) Hahlbrock, K.; Sheel, D. Physiology and molecular biology of phenylpropanoids. *Annu. Rev. Plant Mol. Biol.* **1989**, *40*, 347−369.

(16) Benoit, M. A.; D'Aprano, G.; Lacroix, M. Effect of γ-irradiation on phenylalanine ammonia-lyase activity total phenolic content, and respiration of mushrooms (*Agaricus bisporus*). *J. Agric. Food Chem.* **2000**, *48*, 6312−6316.

(17) Li, He-P.; Fischer, R.; Liao, Yu-C. Molecular evidence for induction of phenylalanine ammonia-lyase during *Puccinia graminis* infection and elicitation in wheat. *Can. J. Plant Pathol.* **2001**, *23*, 286−291.

(18) Campos-Vargas, R.; Nonogaki, H.; Suslow, T.; Salveit, M. E. Heat shock treatments delay the increase in wound-induced phenylalanine ammonia-lyase activity by its expression, not its induction in Romaine lettuce (*Lactusa sativa*) tissue. *Physiol. Plant.* **2005**, *123*, 82−91.

(19) Shann, J. R.; Blum, U. The utilization of exogenously supplied ferulic acid in lignin biosynthesis. *Phytochemistry* **1987**, *26*, 2977−2981.

(20) Politycka, B. Phenolics and the activities of phenylalanine ammonia-lyase, phenol-β-glucosytransferase and β-glucosidase in cucumber roots as affected by phenolic allelochemicals. *Acta Physiol. Plant.* **1998**, *20*, 405−410.

(21) Parr, A. J.; Bolwell, G. P. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.* **2000**, *80*, 985−1012.

(22) Mirdehghan, S. H.; Rahemi, M.; Serano, M.; Guillén, F.; Martinez-Romero, D.; Valero, D. The application of polyamines by pressure or immersion as a tool to maintain functional properties in stored pomegranate arils. *J. Agric. Food Chem.* **2007**, *55*, 755−760.

(23) Vom Endt, D.; Kijne, J. W.; Memelink, J. Transcription factors controlling plant secondary metabolism: what regulates the regulators? *Phytochemistry* **2002**, *61*, 107−114.

(24) Hudec, J.; Bakoš, D.; Mravec, D.; Kobida, L.; Burdova, M.; Turianica, I.; Hlušek, J. Content of phenolic compounds and free polyamines in black chokeberry (*Aronia melanocarpa*) after application of polyamine biosynthesis regulators. *J. Agric. Food Chem.* **2006**, *54*, 3625−3628.

(25) Vander, P.; Värum, K. M.; Domard, A.; El Guedari, N. E.; Moerschbacher, B. M. Comparism of the ability of partially N-acetylated chitosans and chitooligosaccharides, to elicit resistance reactions in wheat leaves. *Plant Physiol.* **1998**, *118*, 1353−1359.

(26) Kim, H. J.; Chen, F.; Wang, X.; Rajapakse, N. C. Effect of chitosan on the biological properties of sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* **2005**, *53*, 3696−3701.

(27) Kogan, G.; Machova, E.; Chorvatovičová, D.; Slováková, L.; Šoltés, L.; Šandula, J. Chitin−glucan complex of *Aspergillus niger* and its derivatives: antimutagenic, antiinfective and antiviral activity. In *Advances in Chitin Science*; Domard, A., Roberts, G. A. F., Varum, K. M., Eds.; Jacques André Publishers: Lyon, France, 1998; Vol. II, pp 641−647.

(28) Wu, T.; Zivanovic, S.; Draughon, F. A.; Conway, W. S.; Sams, C. E. Physicochemical properties and bioactivity of fungal chitin and chitosan. *J. Agric. Food Chem.* **2005**, *53*, 3888−3894.

(29) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841−1856.

(30) Pratt, D. E. Natural antioxidants from plant material. In *Phenolic Compounds in Food and Their Effect on Health, Vol. II; Antioxidants and Cancer Prevention*; Huang, M.-T, Ho, C.-T., Lee, C., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC, 1992; pp 54−71.

(31) Lee, S. K.; Mbwambo, Z. H.; Chung, H.-S.; Luyengi, L.; Games, E. J. C.; Mehta, R. G.; Kinghorn, A. D.; Pezzuto, J. M. Evaluation of the antioxidant potential of natural products. *Combin. Chem. High Throughput Screen.* **1998**, *1*, 35−46.

(32) Waterman, P. G.; Mole, S. *Analysis of Phenolic Plant Metabolites*; Blackwell Scientific: Oxford, U.K., 1994; pp 83−91.

(33) Romani, A.; Mancini, P.; Tatti, S.; Vincieri, F. F. Polyphenols and polysaccharides in Tuscan grapes and wines. *Ital. J. Food Sci.* **1996**, *1*, 13−24.

(34) Turianica, I.; Burdova, M.; Rostoka, L. M.; Hudec, J.; Balint, L. I. Antioxidant activity of various parts of *Echinacea purpurea* as the index of its prophylactic medicinal value. *Med. Chem. (Ukraine)* **2006**, *8*, 70−72.

(35) Brovelli, E. A.; Rua, D.; Roh-Schmidt, H.; Chandra, A.; Lamont, E.; Noratto, G. D. Human gene expression as a tool to determine horticultural maturity in a bioactive plant (*Echinacea purpurea* L. Moench). *J. Agric. Food Chem.* **2005**, *53*, 8156−8161.

(36) Thygesen, L.; Thulin, J.; Mortensen, A.; Skibsted, L. H.; Molgard, P. Antioxidant activity of cichoric acid and alkamides from *Echinacea purpurea*, alone and in combination. *Food Chem.* **2007**, *101*, 74−81.

(37) Frankel, E. N.; Huang, S.-W.; Aeschbach, R. Antioxidant activity of green teas in different lipid systems. *J. Am. Oil. Chem. Soc.* **1997**, *74*, 1309−1315.

(38) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774−2779.

(39) Stuart, D. L.; Wills, R. B. H.; Dickenson, T. M. Optimization of polysaccharides in processed *Echinacea purpurea.* In *A Report for the Rural Industries Research and Developmental Corporation*; RIRDC Publication 04/118; Centre for the Advancement of Food Technology and Nutrition, University of Newcastle: Australia, 2004; pp 8−9.

(40) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure−activity relationship. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(41) Sakihama, Y.; Cohen, M. F.; Grace, S. C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: phenolic-induced oxidative damage mediated by metals in plants. *Toxicology* **2002**, *177*, 67−80.

(42) Sun, J.; Chu, Y. F.; Wu, X.; Liu, R. H. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* **2002**, *50*, 7449−7454.

(43) Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.* **1998**, *46*, 25−31.

(44) Hassimoto, N. M. A.; Genovese, M. I.; Lajolo, F. M. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem.* **2005**, *53*, 2928−2935.

(45) Luo, Xu-B.; Chen, Bo; Yao, S.-Z.; Zeng, J.-G. Simultaneous analysis of caffeic acid derivatives and alkamides in roots and extracts of *Echinacea purpurea* by high-performance liqiud chromatography−photodiode array detection − electrospray mass spectrometry. *J. Chromatogr., A* **2003**, *986*, 73−81.

(46) Mølgaard, P.; Johnsen, S.; Christensen, P.; Cornett, C. HPLC method validated for the simultaneous analysis of cichoric acid and alkamides in *Echinacea purpurea* plants and products. *J. Agric. Food Chem.* **2003**, *51*, 6922−6933.

(47) Hu, C.; Kitts, D. D. Studies on the antioxidant activity of *Echinacea* root extract. *J. Agric. Food Chem.* **2000**, *48*, 1466−1472.

(48) Barnes, J. Benign prostatic hyperplasia. *Pharm. J.* **2002**, *269*, 250−252.

(49) Crawford, E. D. Complementary medicine, chemoprevention, and staging of prostate cancer. *Rev. Urol.* **2003**, *5* (Suppl. 6), S 23−S 32.

(50) Gilad, G. M.; Gilad, V. H. Inhibition of ornithine decarboxylase and glutamic acid decarboxylase activities by phosphorylethanolamine and phosphorylcholine. *Biochem. Biophys. Res. Commun*. **1984**, *122*, 277−282.

(51) Tan, K. S.; Hoson, T.; Masuda, Y.; Kamisaka, S. Effect of ferulic acid and *p*-coumaric acids in *Oryza* coleoptyle growth and the mechanical properties of cell walls. *J. Plant Physiol.* **1992**, *140*, 460−465.

(52) Sewalt, V. J. H.; Ni, W.; Blount, J. W.; Jung, H. G.; Masoud, S. A.; Howles, P. A.; Lamb, C.; Dixon, R. A. Reduced lignin content and altered lignin composition in transgenic tobacco down-regulated in expression of L-phenylalanine ammonia-lyase or cinnamate-4-hydroxylase. *Plant Physiol.* **1997**, *115*, 41−50.

(53) Burtin, D.; Martin-Tanguy, J.; Paynot, M.; Rossin, N. Effect of the suicide inhibitors of arginine and ornithine decarboxylase activities on organogenesis, growth, free polyamine and hydroxycinnamoyl putrescine levels in leaf explants of *Nicotiana Xanthii n.c.* cultivated in vitro in a medium producing callus formation. *Plant Physiol.* **1989**, *89*, 104−110.

(54) Fiorucci, S.; Golebiowski, J.; Cabrol-Bass, D.; Antonczak, S. DFT study of quercetin activated forms involved in antiradical, antioxidant, and prooxidant biological processes. *J. Agric. Food Chem*. **2007**, *55*, 903−911.

(55) Connor, A. M.; Luby, J. J.; Hancock, J. F. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *J. Agric. Food Chem.* **2002**, *50*, 893−898.

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