Eur Food Res Technol (2015) 240:549–557
DOI 10.1007/s00217-014-2353-2

ORIGINAL PAPER

Flavone *C*-glycosides from *Capsicum annuum* L.: relationships between antioxidant activity and lipophilicity

Małgorzata Materska

Received: 4 July 2014 / Revised: 23 September 2014 / Accepted: 29 September 2014 / Published online: 11 October 2014

Abstract Three flavonoid *C*-glycosides isolated from pepper fruit (*Capsicum annuum* L.), var. Capel Hot (luteolin 6-*C*-glucoside, luteolin 6,8-di-*C*-glucoside and apigenin 6-*C*-glucoside-8-*C*-arabinoside), were investigated to determine their antioxidant activity and lipophilicity. The antioxidant activity was evaluated using in vitro methods to generate free radicals in hydrophilic (superoxide radical) and lipophilic (2,2-diphenyl-1-picrylhydrazyl (DPPH) and peroxide radicals) media. Parameters characterising the lipophilicity (log *P*) of the studied compounds were calculated using three commonly available software programs. The relationships between the calculated log *P* of the investigated compounds and the experimentally determined antioxidant activities are discussed. The chemical activity of the flavonoid *C*-glycosides and aglycones was variable depending on the type of assay used. Luteolin and its *C*-6 glucoside showed the greatest ability to scavenge superoxide radicals (generated in the enzymatic and non-enzymatic systems) and DPPH radicals and also had the strongest activity in inhibiting xanthine oxidase activity, followed by apigenin-*C*-glycoside and apigenin. A high positive correlation coefficient was found between the ability of tested *C*-glycosides to inhibit xanthine oxidase and the calculated log *P* values. The highest correlation coefficient was obtained for the ACD/log *P* (Advanced Chemistry Development, Inc.) method ($R = 0.790$). In contrast, negative correlations were obtained between the abilities to scavenge superoxide, DPPH and lipid peroxyl radicals. The highest negative correlation coefficient was obtained in a β-carotene/linoleic acid model system and the Ghose–Crippen method ($R = -0.725$).

Keywords *C*-glycosides of phenolic compounds · Antioxidant activity · Lipophilicity

M. Materska (✉)
Research Group of Phytochemistry, Department of Chemistry, University of Life Sciences, ul. Akademicka 15, 20-950 Lublin, Poland
e-mail: malgorzata.materska@up.lublin.pl

Introduction

Pepper fruits (*Capsicum annuum* L.) belong to vegetables popular in human diet because of dietary benefits and a good sensory properties. They have high levels of vitamins C, E and the provitamin A [1, 2]. In addition, there is a high diversity of carotenoid pigments, which do not have provitamin A activity but which show antioxidant properties and may protect the lipid membranes of cells against free radicals. The antioxidant profile is completed by phenolic compounds as phenolic acids and flavonoids, which occur in peppers bound to sugars [3, 4].

Many studies have proven that flavonoids are compounds with broad bioactivity [5]. Among them, luteolin and apigenin have been described as compounds with anticancerogenic and antimutagenic abilities in vitro and in vivo [5–7]. Luteolin has a vasodilation effect on the thoracic aortas of rats [8], and apigenin, which also has the effect of vasodilation, can suppress skin tumour growth [9]. Although the biological and health properties of apigenin and luteolin have been extensively studied, very limited information is available about their derivatives bioactivity, especially on *C*-glycosides. In the case of peppers, mainly luteolin and apigenin occur as *C*-glycosidic flavonoid derivatives [2, 10, 11], and only fragmented information is available in the literature regarding the bioactivity of *C*-flavonoid glycosides. These compounds appear to be attractive natural derivatives of flavonoids, which, due to their

specific chemical structure (the presence of free hydroxyl groups), are expected to exhibit a beneficial antioxidant potential. These compounds would also be expected to show a variable lipophilicity, which is influenced by the sugar substituents present in the molecule, and should be active both in lipo- and hydrophilic systems.

The antioxidant activity (AA) of the compounds is determined in a number of in vitro systems, which generate free radicals in both the aqueous and the lipid phases. Superoxide radicals are formed in the hydrophilic phase. These are considered the principal precursors of DNA damage and specific forms of cancer, as well as many other diseases [12]. In in vitro assays, superoxide radicals are generated by both enzymatic and non-enzymatic methods. In the enzymatic method, xanthine oxidase (XOD) catalyses the oxidation of xanthine to uric acid. During re-oxidation of XOD, molecular oxygen acts as an electron acceptor and free radicals are produced [13]. In the non-enzymatic method, β-nicotinamide adenine dinucleotide (NADH) is a precursor of superoxide radicals, which reduces PMS, and this reaction involving oxygen generates superoxide radicals [14]. It has been found that flavonoids and their derivatives have the ability to neutralise of free radicals and inhibit the activity of XOD [13, 15]. Analysis with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is popular laboratory test to provide rapid information about the potential activity of the tested compound [16]. Disadvantages of this method include the use of methanol as the reaction mixture and the fact that the DPPH radical does not occur in in vivo systems. However, this method is often used to assess the potential of natural antioxidant substances in the in vitro systems due to the simplicity and low cost of the analyses [16, 17]. The method using the emulsion phase is the β-carotene–linoleic acid model system. In this method, linoleic acid, upon reacting with hydrogen peroxide, generates lipid peroxide radicals. These generated radicals react with a molecule of β-carotene, causing a loss of double bonds, which can be monitored as a decrease in absorbance at a wavelength of 470 nm [18].

In the last decade, significant help in predicting the bioactivity of compounds has been given in the form of simulation computer programs. These programs use the chemical structure of the compound to calculate theoretical physico-chemical parameters such as lipophilicity, partition coefficients or the solvation energy [19–21]. The data obtained in this way are useful when screening for substances that may have beneficial physiological activity or may be used as nutraceuticals with many health benefits. The basic parameter used in such simulations is compound lipophilicity, which is expressed as log *P* [19]. However, the relationship between the lipophilicity and bioactivity of the compound is ambiguous. First, a number of oxidants, which cause unwanted cellular events, are hydrophilic, such as a large group of free radicals (superoxide, hydroxyl and nitrate). Furthermore, despite the absorption of the lipophilic compounds on cell membranes, their transport within the cytosol is limited. In addition, the calculated values of log *P* are often different between the algorithms used and they differ from those determined experimentally [19, 22].

In many experimental studies regarding the search for biologically active compounds, the AA of compounds is determined at the beginning. In the case of theoretical considerations, a parameter determining the activity of the compounds is their lipophilicity. In the world literature, there is little information regarding the comparison of experimental results with theoretical calculations. For this reason, the aim of the present study was to compare the experimentally determined AA of three *C*-glycosidic flavonoid derivatives with values of log *P* calculated by computer algorithms. The AA was determined in enzymatic and non-enzymatic systems that generated both hydro- and lipophilic radicals. For the calculation of log *P* values, three computer programs were used.

Materials and methods

General

The mass spectra were recorded on a Thermo Finnigan LCQ Advantage Max ion-trap spectrometer using an electrospray ion source (Thermo Electron Corp., Bellefonte, PA). The spray voltage was 4.2 kV at the capillary inlet and 60 V at the outlet of the capillary. The temperature of the capillary was 220 °C, and the samples were injected in methanol. The full scan mass covered the range from *m/z* 200 to 2,000. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas. MS data were acquired in the negative ionisation mode and in the positive mode.

High-performance liquid chromatography (HPLC) was performed using an Empower-Pro chromatograph (Waters, Milford, MA, USA) consisting of a quaternary pump (M2998 Waters) with a degasser and a UV–Vis diode array detection (DAD) system. Separation was performed on a column filled with a modified silica gel RP-18 (Atlantis T3 Waters, 3 μm, 4.6 mm × 150 mm). The gradient elution consisted of mobile phases A (1 % H_3PO_4 in water) and B (40 % acetonitrile in solvent A) in a proportion in which the concentration of solvent B was as follows: until the tenth min–20 %, during the 25th min–25 % and during the 40th min–40 %; the flow speed was 1 cm^3 min^{-1}. The detection was at 330 nm.

Spectrophotometric analyses were performed using a Cary 50 (Varian, Palo Alto, CA, USA) spectrophotometer.

Plant material

The experimental materials used were the pericarps of hot pepper, *C. annuum* L., cv. Capel Hot. The fruits were purchased at a Seminis farm near Lublin in Poland in 2010. Immediately after harvest, the fruits were shipped to the laboratory (25 km) where they were washed and drained and the placenta with seeds were separated from the pericarp. The pericarps were chopped, mixed and averaged, and their dry matter content was evaluated by drier and subjected to the freeze-drying process. Lyophilised fruits were stored at −20 °C prior to further analysis.

Extraction and isolation

Extract preparation

Lyophilised pericarps of pepper fruits (360 g) were divided into four parts, and each part was homogenised in 80 % aqueous ethanol solution (3 × 500 cm^3) using a Diax 900 homogeniser. The ethanolic extracts (EE) were filtered, combined and concentrated in a rotary evaporator at 40 °C to remove solvents, which yielded 290 g of a glassy material that was subjected to the solid-phase extraction procedure as follows. The condensed EE was diluted with redistilled water and was put into a sintered funnel filled with conditioned modified silica gel C18 (LichroPrep, Merck, 40–63 μm). Hydrophilic substances were eluted with redistilled water, and next, the phenolic compound fraction (PCF) was eluted with 40 % aqueous methanol solution in a quantity required to produce a colourless filtrate. Nitrogen was passed through the filtrate to remove air. The PCF was diluted in redistilled water to reduce the methanol concentration and enriched on C18 gel. PCF was eluted from the gel matrix with a small volume of 80 % methanol–water solution, and the eluate was evaporated in a rotary evaporator at 40 °C to dryness. Dry residue (7 g) was dissolved in water (50 cm^3) and subjected to preparative separation.

Preparative chromatography

The PCF was separated by medium pressure liquid chromatography (MPLC) using the Büchi system consisting of a medium pressure pump (C-601), a manual injector unit, a preparative column, a UV detector (C-635) and a fraction collector (C-660). A preparative column with a diameter of 2.5 cm and a length of 50 cm and filled with modified silica gel C18 (LichroPrep, Merck 40–63 μm) was used. The column was washed first with H_2O and then with a linear gradient of methanol–H_2O (starting with 0 and ending with 100 % methanol). The fractions eluted from the column were collected into 15-cm^3 test tubes. The absorbance at $\lambda = 330$ nm of each collected fraction was recorded, and the phenolic profile in the tubes with the maximum and half-maximum absorbance was confirmed using the HPLC method. Samples with similar phenolic profiles were combined, and their purity was analysed using the HPLC method. Fractions containing one compound were concentrated under vacuum and dried under a stream of nitrogen. Multicomponent fractions were purified on an RP-18 column (0.8 × 25 cm, 25–40 μm, Merck) in an isocratic system (CH_3–CN—1 % H_3PO_4) with the concentration selected for each fraction on the basis of the HPLC separation. Finally, the isolated compounds were identified using the ESI–MS method.

Antioxidant activity assessments

Stock solutions with a concentration of 0.5 mg mL^{-1} of the tested compound were prepared. The compounds were dissolved in small amounts of dimethyl sulfoxide (DMSO) and equilibrated in water. Next, a series of compound dilutions were prepared from the stock solutions, yielding concentrations ranging from 0.5 to 0.01 mg mL^{-1}. The diluted compound samples were analysed according to antioxidant activity. Ascorbic acid was used as a positive control.

Superoxide radical scavenging activity: enzymatic assay

Antiradical activity was determined spectrophotometrically by monitoring the effect of the tested substances on the reduction of nitro blue tetrazolium (NBT) to blue chromogen formazan by $O_2^{\cdot -}$. Superoxide radicals were generated using the xanthine/XOD system as previously described with some modification [13, 15]. The reaction mixture in the sample wells consisted of xanthine (55 μM), XOD (0.02 U mL^{-1}), NBT (80 μM) and the tested compounds (0–70 μM). Flavonoids were dissolved in DMSO, and all other components were dissolved in 50 mM phosphate buffer (pH 7.4). The reaction was initiated by the addition of XOD and proceeded at 37 °C for 30 min; the formation of NBT^+ was measured at $\lambda = 560$ nm. Different concentrations of flavonoids were analysed, and the half-maximal inhibitory concentration (IC_{50}) was calculated using a statistical regression method.

Effect on XOD activity

The effect of the analysed compounds on XOD activity was evaluated by measuring the formation of uric acid from xanthine. The reaction mixtures contained the same proportion of components as in the enzymatic assay for superoxide radical scavenging activity, except NBT. The absorbance was measured at 295 nm after 30 min of incubation at 37 °C. Uric acid production was calculated from

the differential absorbance with a blank solution in which the XOD was replaced with the buffer solution.

Superoxide radical scavenging activity: non-enzymatic assay

Superoxide radicals were generated in the NADH/PMS system [23]. The reaction mixture consisted of a reduced form of NADH (10.64 mM), NBT (0.24 mM), phenazine methyl sulphate (PMS; 5.39 μM) and the tested sample (0.1 mL). The components were dissolved in 19 mM phosphate buffer with a pH of 7.4. The reaction was initiated by the addition of PMS. The reaction cuvettes were maintained at 37 °C for 30 min. The absorbance was measured at 560 nm using a Cary 50 (Varian) spectrophotometer. The AA was calculated based on formula (1). The IC_{50} values, i.e., the concentrations at which fractions demonstrated 50 % activity, were determined based on the correlations between the AA and fraction concentrations.

$$\%\mathrm{AA} = 1 - \frac{\text{sample absorbance}}{\text{control absorbance}} \cdot 100\% \quad (1)$$

DPPH scavenging activity

Evaluation of the scavenging effect on DPPH radicals is described in detail in Materska et al. [10]. A methanol stock solution of DPPH at a concentration of 0.1 M was prepared. The absorbance of the mixture of the analysed samples and the DPPH working solution was measured at 517 nm. The reference was a test tube containing methanol instead of the sample. Antioxidant activity was determined as the percentage of discoloration of the DPPH solution once the plateau was reached in all samples (30 min.) compared with the reference. The calculations were performed using formula (1). Next, IC_{50} values were evaluated on the basis of the dependence between the antiradical activity and the sample concentration f(c) = %AA.

β-Carotene–linoleic acid bleaching test

The antioxidant properties on the basis of joint oxidation of β-carotene and linoleic acid were determined as previously described [10, 18], with specific modifications. A β-carotene solution in chloroform (0.12 mg mL^{-1}) was prepared. Next, a 3 mL aliquot was obtained and added to a round-bottom flask containing 40 mg of linoleic acid in 400 mg of Tween 20. Chloroform was removed using a vacuum evaporator at 30 °C, and 100 cm^3 of 30 % hydrogen peroxide was subsequently added. After thorough mixing, 3 mL of the emulsion was added to 0.5 mL of the tested compound; the control was 0.5 mL methanol instead of pepper extract. Oxidation of the β-carotene emulsion was monitored spectrophotometrically at 470 nm. The measurements were performed every 20 min for 3 h starting at the moment of the addition of hydrogen peroxide. Between the measurements, the tubes were incubated at a temperature of 50 °C. The AA was expressed as the % inhibition of β-carotene emulsion bleaching compared with the control.

Lipophilicity calculations

Parameters characterising lipophilicity (log *P*) of the studied compounds were calculated using of three algorithms. First, the on-line Virtual Computational Chemistry Laboratory was used, which provides interactive prediction of log *P* utilising the Molinspiration algorithm implemented in DragonX software (milog *P*). Second, the calculations of log *P* were performed with Spartan Pro 1.08 (Wavefunction, Inc.) using ab initio methods at the Hartree–Fock level with 6–31 +G** basis set. Third, the ACD/log *P* was calculated on the basis of a versatile, fragment-based algorithm provided by Advanced Chemistry Development, Inc. (ACD/Labs).

Relationships between antioxidant activity and lipophilicity

To assess the relationship between the antioxidant activities and lipophilicity of the investigated compounds, the correlation analysis was performed and the Pearson correlation coefficient was calculated. A p value of <0.01 was taken to be significant.

Statistical analysis

Statistical analyses were conducted to compare the results obtained in three replications, and the data were expressed as the mean ± SD. The dose–response curve was obtained by plotting the percentage of inhibition versus concentration. The inhibitory concentration of 50 % (IC_{50}) was calculated by linear and nonlinear regression analyses. Statistical comparisons were performed using the Statgraphic Centurion software, version XVI.

Results and discussion

Isolation and identification of phenolic compounds derivatives

During the separation of the PCF in a preparative scale, three pure flavonoid *C*-glycosides were obtained in amounts sufficient for further analysis. These compounds were luteolin 6-*C*-glucoside (8.2 mg, purity: 98.2 %), luteolin 6,8-di-*C*-glucoside (5.3 mg, purity: 98.7 %) and apigenin 6-*C*-glucoside-8-*C*-arabinoside (2.3 mg, purity: 97.4 %). The structures of the isolated compounds were

Table 1 t_R, UV, and ESI–MS data for compounds isolated from pepper fruit

Compound	t_R (min)	UV (nm)	$[M-H]^-$ (*m/z*)	$-MS^2$ $[M-H]^-$ (*m/z*) (%)					
				−18	−60	−90	−120	Agl + 113	Agl + 83
L-6,8-di-*C*-Glc	9.0	270, 296sh, 349	609	591 (8)	549 (1)	519 (24)	489 (100)	399 (5)	369 (2)
L-6-*C*-Glc	18.4	269, 296sh, 350	447	429 (33)	387 (6)	357 (100)	327 (93)	399 (5)	369 (3)
A-6-*C*-Glc-8-*C*-Ara	20.8	271, 295sh, 336	563	545 (11)	503 (2)	473 (52)	443 (100)	383 (11)	353 (16)

A: apigenin, Ara: arabinoside, Glc: glucoside, L: luteolin

established using chromatography and spectroscopic analyses (UV, ESI–MS), and the results obtained are summarised in Table 1 and Fig. 1. Comparison of the HPLC–DAD retention data and results of our previous analyses [3, 4], as well as the fragmentation patterns obtained using the MS method by other groups [2, 11], confirmed the structure of the isolated compounds.

Antioxidant activity of phenolics isolated from pepper and standards of their aglycones

To determine a wide range of factors that affect the chemical properties of these compounds, five model systems that generate free radicals in both the hydrophilic and lipophilic media were analysed.

Superoxide radical scavenging activity: enzymatic and non-enzymatic assays

The superoxide radical scavenging activity and the inhibitory effect of the tested compounds on XOD activity were concurrently assessed with an enzymatic assay. Simultaneously, the antiradical activity of phenolic compounds in relation to the superoxide radical generated with the non-enzymatic assay (NADH/PMS) was evaluated. It was found that all analysed compounds exhibited concentration-dependent activity that varied depending on the model system employed (Table 2; Fig. 2). In the enzymatic assay generating superoxide radicals, the highest antiradical activity was noted for luteolin ($IC_{50} = 8.9 \pm 0.14$ μM) (Table 2; Fig. 2) and from the compounds isolated from the pericarp of the pepper fruits for luteolin 6-*C*-glucoside ($IC_{50} = 207.8 \pm 11.52$ μM). Luteolin also had the highest XOD inhibitory activity ($IC_{50} = 11.6 \pm 0.41$ μM), whereas among the glycosides isolated from the pepper fruits, apigenin 6-*C*-glucoside-8-*C*-arabinoside, which exhibited higher activity than the free apigenin ($IC_{50} = 51.4 \pm 0.57$ μM), was the most potent inhibitor of the enzyme. In the non-enzymatic assay (NADH/PMS), the highest AA was found for L-ascorbic acid ($IC_{50} = 10.6 \pm 0.49$ μM), and among the compounds derived from the pepper fruits, the highest AA was found for luteolin 6-*C*-glucoside ($IC_{50} = 76.2 \pm 0.14$ μM) (Table 2). The results obtained by other research teams were divergent. According to Cos et al. [13], flavonoid derivatives induced considerably lower inhibition of XOD activity than their aglycones, and the presence of a free hydroxyl group at position C-7 exerted a significant effect on the activity of the tested compounds. In turn, Selloum et al. [24] demonstrated that the differences in the effects of flavonoids on XOD activity depended on the B-ring hydroxylation pattern. Quercetin, which contains a catechol group in the B-ring (3′,4′−OH), was found to be the most potent inhibitor of uric acid synthesis, and the hydroxyl group at position C-3 was crucial for inhibition of XOD activity. All of the compounds presented in this study contained free hydroxyl groups at carbon atoms C-5 and C-7, but did not have the −OH group at C-3 (Fig. 1); nevertheless, they exhibited variable XOD inhibitory activity (Table 2; Fig. 2). Comparison of the IC_{50} values of the analysed compounds indicated that the free hydroxyl groups at positions C-5 and C-7 determined their inhibitory activity, but the steric effect was also of similarly great importance. The highest XOD inhibitory activity was exhibited by luteolin, whereas its derivatives, which still had a free catechol group in the B-ring and free hydroxyl groups at positions C-5 and C-7, were characterised by substantially lower activity levels. Additionally, the flavonoid *C*-glycosides were weak superoxide radical scavengers (Table 2). The results obtained prove that steric effects have a stronger impact on the chemical activity than the reducing properties of the analysed compounds [13].

DPPH scavenging activity

In the presented method, the highest reducing activity was found for luteolin ($IC_{50} = 18.5 \pm 0.85$ μM), although its *C*-glycosides exhibited significant AA as well. In contrast, apigenin and its derivatives were not active in this model system (Table 2; Fig. 2). The results of the antiradical activity of the glycosides isolated from the pericarp of pepper fruits and their aglycones have confirmed the relationship between the structure and activity of the tested compounds established previously [25, 26]. Apigenin and its derivatives, which do not contain the 3′,4′-dihydroxy group in the B-ring, were inactive in this model system [25].

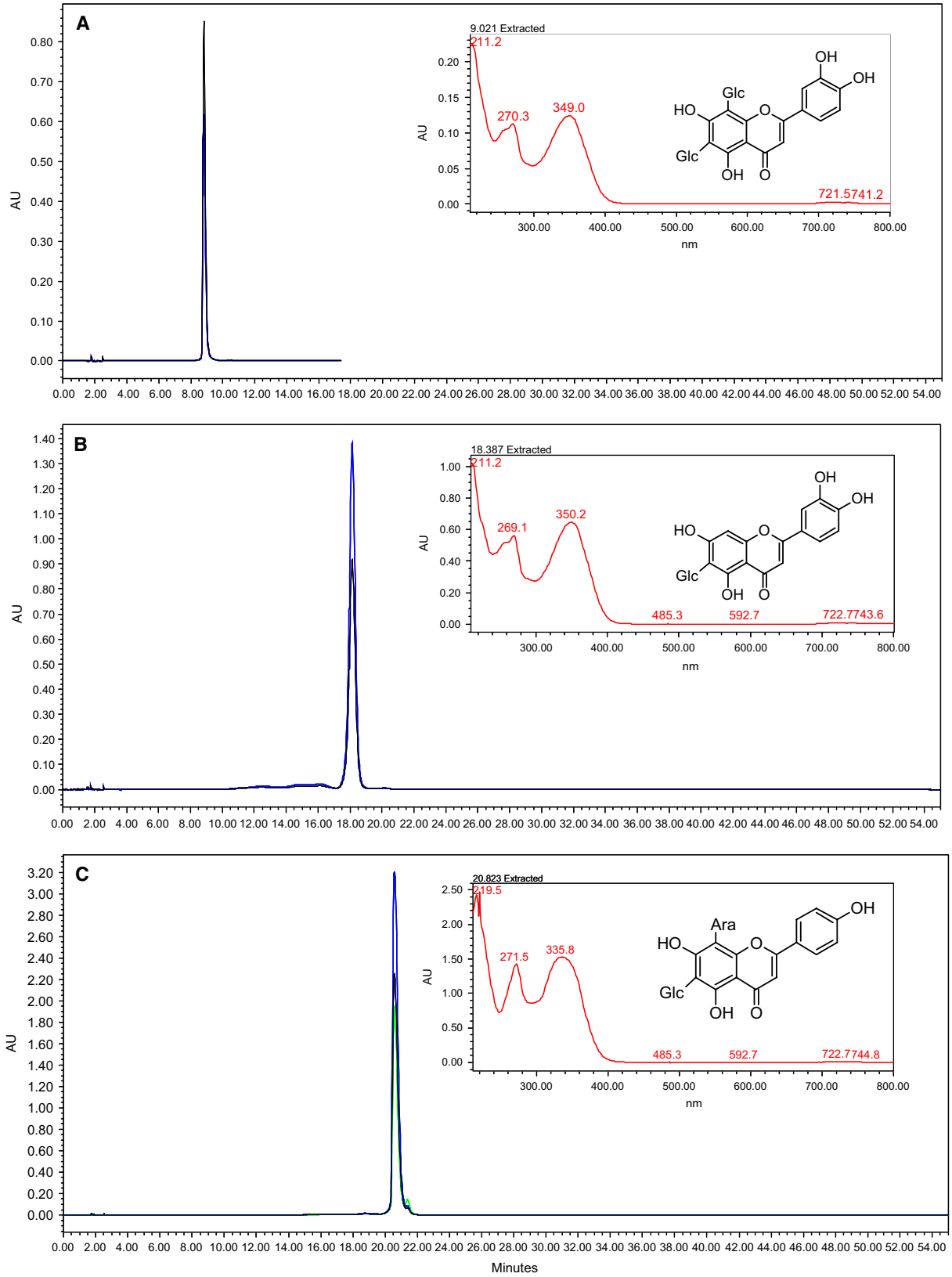

Fig. 1 Chromatograms, UV spectra and structures of analysed compounds **a** luteolin 6,8-di-*C*-glucoside, **b** luteolin 6,-*C*-glucoside and **c** apigenin-6-*C*-glucoside-8-*C*-arabinoside

β-Carotene–linoleic acid bleaching test

The β-carotene–linoleic acid method was introduced in order to compare the activity of the compounds in the lipophilic phase. This system revealed the prooxidant activity of apigenin (Fig. 2) and the relatively potent AA of its *C*-glycoside derivative, as well as a higher AA of luteolin derivatives compared with that of free aglycone (Table 2; Fig. 2). The results obtained are consistent with the literature, as the prooxidant activity of apigenin was observed in the β-carotene emulsion system [27, 28]. These properties were explained by the high single-electron oxidative potential generated in the described phenoxyl radical reaction. The contrasting chemical activity exhibited by apigenin and its *C*-glycoside derivative may be explained by the fact that the presence of sugar substituents in the glycoside molecule reduces the oxidation potential of its phenoxyl radicals or by the fact that the more hydrophilic apigenin derivatives may react in the aqueous phase of the emulsion system disrupting the β-carotene oxidation reaction already at the initial stage.

Lipophilicity calculations

The value of log *P* characterises the affinity of a compound for a lipid environment. It is expressed as the logarithm of the partition coefficient (log *P*) of a solute between two immiscible solvents (in practice, water and *n*-octanol). The importance placed on the log *P* value is a result of the fact that lipophilicity is considered a major factor in determining compound absorption and metabolism in the body. In the pharmaceutical and biotechnological industries, log *P* is used as a screening parameter for selection of compounds as drug candidates [19].

The current paper presents, for the first time, the values of log *P* as a parameter of lipophilicity of the tested compounds calculated on the basis of three algorithms (Table 2). The results obtained differ between the algorithms used; however, based on the averaged log *P* values, the compounds can be ordered according to decreasing lipophilicity as follows: A > L > L-6-*C*-Glc > A-6-*C*-Glc-8-*C*-Ara > ascorbic acid ≥ L-6,8-di-*C*-Glc. The differences in the lipophilicity of luteolin and apigenin derivatives do not correspond to the retention times of these compounds shown by the HPLC analysis (Table 1). This confirms that the retention time in the HPLC analysis is an insufficient indicator of the physical properties of the tested substances [22]. To determine the relationship between the lipophilicity of the compounds and their chemical activity assessed in in vitro systems, a linear regression analysis was performed and the coefficients of the correlation between the antiradical activity and the log *P* values were calculated (Table 3). The correlation coefficient varied depending on the methods applied for calculation and the assessment of the compound activity. Positive correlation coefficients calculated with the three algorithms were obtained for the ability of flavonoid *C*-glycosides to inhibit experimentally determined XOD activity. The highest positive correlation was observed for calculations performed using the algorithm ACD/log *P* (0.790). The result obtained may imply that the steric effects of an analysed substance have a greater impact on XOD inhibition than its hydrophilic character. Similarly, Cos et al. [13] emphasised the impact of flavonoid steric effects on the reduction of inhibition

Table 2 Antioxidant activity of flavonoid derivatives isolated from pepper fruits expressed as IC_{50} values (μM) and calculated lipophilicities (log *P*) of the individual compounds

Compound[1]	X/XO[2]	Inh. XO[3]	NADH/PMS[4]	DPPH	β-Carotene[5]	Log *P*[6]		
						milog *P*	ACD/log *P*	Ghose-Crippen
L-6,8-di-*C*-Glc	318.7 ± 7.11	345.6 ± 5.3	129.1 ± 1.98	24.4 ± 1.86	137.4 ± 5.91	−2.59	0.20	−4.24
L-6-*C*-Glc	207.8 ± 11.52	86.9 ± 4.95	76.2 ± 0.14	15.1 ± 2.28	63.7 ± 3.92	0.03	1.58	−1.61
A-6-*C*-Glc-8-*C*-Ara	>1,000	51.4 ± 0.57	>1,000	>1,000	46.3 ± 6.22	−0.94	0.04	−3.31
A	711.3 ± 13.6	69.2 ± 4.21	197.9 ± 7.41	>1,000	*[7]	2.46	2.10	1.4
L	8.9 ± 0.14	11.6 ± 0.41	55.7 ± 7.01	18.5 ± 0.85	130.1 ± 4.60	1.97	2.40	1.01
Ascorbic acid	50.1 ± 4.30	>1,000	10.6 ± 0.49	41.9 ± 0.85	>1,000	−1.40	−2.41	−2.85

[1] A: apigenin, Ara: arabinofuranoside, Glc: glucoside, L: luteolin

[2] Superoxide anion scavenging activity in the xanthine/xanthine oxidase (X/XO) system

[3] Inhibitory activity of XO

[4] Superoxide anion scavenging activity in the non-enzymatic assay (NADH/PMS)

[5] β-carotene–linoleic acid bleaching test

[6] Algorithms used for log *P* calculations: milog *P*: Molinspiration algorithm (DragonX); ACD/log *P*: fragment-based algorithm (ACD/log *P*); Ghose–Crippen: ab initio methods Hartree–Fock level with 6–31 +G** basis set

[7*] Apigenin showed prooxidant activity, and results are presented as $IC_{-50} = 158.0 \pm 4.4$

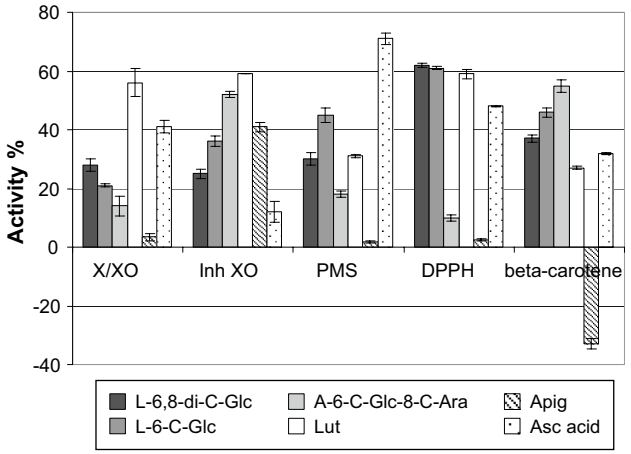

Fig. 2 Comparison of antioxidant activities of equimolar concentrations (50 μM) of luteolin and apigenin-*C*-glycosides with free luteolin and apigenin, and ascorbic acid was used as a positive control ($n = 3 \pm$ SD)

Table 3 Correlations between antioxidant activity and lipophilicities (log *P*) of the tested compounds

	X/XO	Inh. XO	NADH/PMS	DPPH	β-Carotene
milog *P*	−0.121	0.676*	−0.499	−0.342	−0.685*
ACD/log *P*	−0.177	0.790*	−0.696*	−0.023	−0.386
Ghose–Crippen	−0.043	0.594*	−0.426	−0.260	−0.725*

* Correlation significant at $p < 0.01$

of XOD activity by their derivatives. In turn, the highest negative correlations were noted for the Ghose–Crippen method and the β-carotene emulsion system (−0.725). The negative value of the correlation coefficient indicates that the decrease in lipophilicity (a log *P* parameter) is accompanied by an increase in the AA of the compound in this model system. The result obtained proves that the AA of the tested compounds in the H_2O_2/linoleic acid/β-carotene emulsion system results from their reaction with hydrogen peroxide in the aqueous phase, leading to disruption of the subsequent chain reaction of linoleic acid oxidation and β-carotene oxidation occurring in the lipid phase.

Conclusions

This paper presents the analysis of the antioxidant activities and lipophilicities of natural plant compounds.

The chemical activity of *C*-glycosidic luteolin and apigenin derivatives determined experimentally and the calculated lipophilicity of these compounds facilitate elucidation of the mechanisms of reactions taking place in in vitro systems and encourage investigation of their activity in in vivo systems.

Simultaneously, the comparison of the chemical activity of *C*-glycosidic flavonoid derivatives has distinguished apigenin 6-*C*-glucoside-8-*C*-arabinoside as a compound characterised by higher XOD inhibitory activity than apigenin, and this compound also had the highest AA determined in the β-carotene emulsion system.

Acknowledgments This work was financially supported by the Ministry of Science and Higher Education, Project No N312 021 32/1491. The author is grateful to Prof. Irena Perucka (University of Life Sciences, Lublin) for critically reviewing the manuscript and to mgr Barbara Chilczuk for technical support for the completion of the analysis.

Conflict of interest The author declares no conflict of interest.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Howard LR, Talcott ST, Brenes CH, Villalon B (2000) Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J Agric Food Chem 48:1713–1720
2. Marin A, Ferreres F, Tomas-Barberan FA, Gil MI (2004) Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). J Agric Food Chem 52:3861–3869
3. Materska M, Perucka I, Stochmal A, Piacente S, Oleszek W (2003) Quantitative and qualitative determination of flavonoids and phenolic acid derivatives from pericarp of hot pepper fruit cv. Bronowicka Ostra. Pol J Food Nutr Sci 12:72–76
4. Materska M, Piacente S, Stochmal A, Pizza C, Oleszek W, Perucka I (2003) Isolation and structure elucidation of flavonoid and phenolic acid glycosides from pericarp of hot pepper fruit *Capsicum annuum* L. Phytochemistry 63:893–898
5. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolics acids. Free Radic Biol Med 20:933–956
6. Tahir NI, Shaari K, Abas F, Parveez GKA, Ishak Z, Ramli US (2012) Characterization of apigenin and luteolin derivatives from oil palm (*Elaeis guineensis* Jacq.) leaf using LC-ESI-MS/MS. J Agric Food Chem 60:11201–11210
7. Way T, Kao M, Lin J (2004) Apigenin induces apoptosis through proteosomal degradation of HER2/*neu* in HER2/*neu*-overexpressing breast cancer cells via the phosphotidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem 6:4479–4489
8. Jiang HD, Xia Q, Wang XX, Song JF (2005) Luteolin induces vasorelaxation in rat thoracic aorta via calcium and potassium channels. Pharmazie 60:444–447
9. Zhang YH, Park YS, Yun YP (2002) Endothelium-dependent vasorelaxant and antiproliferative effects of apigenin. Gen Pharmacol 35:341–347

10. Materska M, Perucka I (2005) Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J Agric Food Chem 53:1750–1756
11. Wahyuni Y, Ballester AR, Sudarmonowati E, Bino RJ, Bovy AG (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: variation in health-related compounds and implications for breeding. Phytochemistry 72:1358–1370
12. Lin JK, Chen PC, Ho CT, Lin-Shiau SY (2000) Inhibition of xanthine oxidase and suppression of intracellular oxygen species in HL-60 cells by theaflavin-3-3′-digallate, (-) -epigallocatechin-3-gallate, and propyl gallate. J Agric Food Chem 48:2736–2743
13. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel B, Pieters L, Vlietinck AJ, Berghe DV (1998) Structure–activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 61:71–76
14. Ribeiro B, Valentao P, Baptista P, Seabra RM, Andrade PB (2007) Phenolic compounds, organic acids profile and antioxidative properties of beefsteak fungus (*Fistulina hepatica*). Food Chem Toxicol 45:1805–1813
15. Kweon MH, Hwang HJ, Sung HCh (2001) Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). J Agric Food Chem 49:4646–4655
16. Valentova K, Sersen F, Ulrichova J (2005) Radical scavenging and anti-lipoperoxidative activities of *Smallanthus sonchifolius* leaf extracts. J Agric Food Chem 53:5577–5582
17. Niki E (2010) Assessment of antioxidant capacity in vitro and in vivo. Free Radic Biol Med 49:503–515
18. Burda S, Oleszek W (2001) Antioxidant and antiradical activities of flavonoids. J Agric Food Chem 49:2774–2779
19. Koba M, Belka M, Ciesielski T, Bączek T (2012) Determination of lipophilicity for antitumor acridinone derivatives supported by gradient high-performance liquid chromatography method. Cent Eur J Chem 10:218–223
20. Meylan WM, Howard PH (2000) Estimating log *P* with atom/fragments and water solubility with log *P*. Perspect Drug Discov Des 19:67–84
21. Petrauskas AA, Kolovanov EA (2000) ACD/Log *P* method description. Perspect Drug Discov Des 19:99–116
22. Rothwell JA, Day AJ, Morgan RA (2005) Experimental determination of octanol-water partition coefficients of quercetin and related flavonoids. J Agric Food Chem 53:4355–4360
23. Valentao P, Fernandez E, Carvalho F, Andrade PB, Seabra RM, Bastos ML (2001) Antioxidant activity of *Centaurium erythraea* infusion evidenced by its superoxide radical scavenging and xanthine oxidase inhibitory activity. J Agric Food Chem 49:3476–3479
24. Selloum L, Reichl S, Muller M, Sebihi L, Arnhold J (2001) Effects of flavonols on generation of superoxide anion radicals by xanthine oxidase and stimulated neurophills. Arch Biochem Biophys 395:49–56
25. Li N, Liu JH, Zhang J, Yu BY (2008) Comparative evaluation of cytotoxicity and antioxidative activity of 20 flavonoids. J Agric Food Chem 56:3876–3883
26. Masuoka N, Matsuda M, Kubo I (2012) Characterisation of the antioxidant activity of flavonoids. Food Chem 131:541–545
27. Rietjens IMCM, Boersma MG, Haan L, Spenkelink B, Awad HM, Cnubben NHP, Zanden JJ, Woude H, Alink GM, Koeman JH (2002) The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ Toxicol Pharmacol 11:321–333
28. Skerget M, Kotnik P, Hadolin M, Hras AR, Simonic M, Knez Z (2005) Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 89:191–198