Antioxidant and anti-inflammatory activities of quercetin and its derivatives

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ABSTRACT

Quercetin is hardly bioavailable and largely transformed to different metabolites. Although little is known about their biological activities, these metabolites are crucial for explanation of health benefits associated with quercetin dietary intake. In this study, the antioxidant and anti-inflammatory activities of six quercetin derivatives (quercetin-3-O-glucuronide, tamarixetin, isorhamnetin, isorhamnetin-3-O-glucoside, quercetin-3,4′-di-O-glucoside, quercetin-3,5,7,3′,4′-pentamethylether) were compared with the activity of common onion extract as the main source of dietary quercetin and standards (butylated hydroxytoluene and aspirin). The quercetin derivatives demonstrated notable bioactivities, similar to standards and onion. Derivatization of quercetin hydroxyl groups resulted in decrease of antioxidant potency. However, the number of quercetin free hydroxyl groups was not in direct correlation with its potential to inhibit inflammatory mediators production. To conclude, quercetin derivatives present in systemic circulation after consumption of quercetin may act as potent antioxidant and anti-inflammatory agents and can contribute to overall biological activity of quercetin-rich diet.

1. Introduction

Quercetin is a natural flavonoid found abundantly in almost all edible vegetables and fruits. The daily intake of quercetin in the Western diet is high, being approximately 15 mg. As an example, it is estimated that red onion, common onion, cranberry, blueberry and fig have 39, 20, 15, 8 and 5 mg of quercetin aglycone per 100 g of fresh weight of edible portion, respectively (Bhagwat, Haytowitz, & Holden, 2014).

There is growing body of evidence showing that quercetin has great therapeutic potential in the prevention and treatment of different chronic diseases, including cardiovascular and neurodegenerative diseases, as well as cancer (Boots, Haenen, & Bast, 2008; Dajas, 2012; D'Andrea, 2015; Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012; Serban et al., 2016). It has been shown that quercetin exerts health beneficial effects in a number of cellular and animal models, as well as in humans, through modulating the signaling pathways and gene expression involved in these processes (Wang et al., 2016). Consequently, intake of a quercetin-rich diet is supported and is positively correlated with health promotion (D'Andrea, 2015). Quercetin can also be taken as a dietary supplement with daily recommended doses of 200–1200 mg,

as well as a nutraceutical through functional foods with a concentration range of 10–125 mg per serving. Dietary supplementation with quercetin and its addition into food is highly supported by data on its safety (Harwood et al., 2007; Okamoto, 2005). Bioavailability of quercetin, defined as the portion of an initially administered dose that reaches the systemic circulation unchanged (Jackson, 1997), is very low, mostly due to its extensive metabolism. Namely, quercetin is present in plants mainly in its highly hydrophilic glycosylated forms, primarily as β-glycosides of various sugars (Lee & Mitchell, 2012). Prior to absorption in the gut, flavonoids first need to be freed from plant tissue by chewing in the oral cavity and then processed by digestive juices in the intestine or by microorganisms in the colon. There are two main routes of quercetin glycosides absorption by the enterocytes. Firstly, absorption goes via a transporter, followed by deglycosylation within the enterocyte by cytosolic glycosidase. Secondly, deglycosylation can occur by luminal hydrolases, followed by transport of the aglycone into enterocyte by passive diffusion or via different transporters (Day, Gee, DuPont, Johnson, & Williamson, 2003; Walle, Otake, Walle, & Wilson, 2000; Wolffram, Blöck, & Ader, 2002; Ziberna, Fornasaro, Čvorović, Tramer, & Passamonti, 2014).

Further biotransformation of quercetin aglycone involves

glucuronidation, sulfation and methylation of hydroxyl groups, which primarily occurs in enterocytes and hepatocytes. Consequently, after the intake of food rich in quercetin glucosides and aglycone, methylated, glucuronidated, sulfated and combined derivatives of quercetin, such as isorhamnetin-3-glucuronide, quercetin diglucuronide, quercetin glucuronide sulphate, methylquercetin diglucuronide, etc., have been found in the human plasma (Terao, Murota, & Kawai, 2011). It is assumed that they are produced in the small intestine, transported into the portal vein and are further converted into other metabolites in the liver within phase II metabolism. After returning to the bloodstream they are excreted in urine via kidneys. Additionally, a portion of quercetin is converted to low molecular weight phenolic acids, such as 3-hydroxyphenylpropionic acid, 3,4-dihydroxyphenylpropionic acid and 3-methoxy-4-hydroxyphenylpropionic acid (Mullen, Edwards, & Crozier, 2006; Olthof, Hollman, Buijsman, Amelsvoort, & Katan, 2003).

In light of the efficient metabolism of quercetin, some authors consider that evidence on biological effects and mechanisms of quercetin are insufficient, as they are mainly based on *in vitro* studies with quercetin aglycone, which is hardly present in human plasma (Kroon et al., 2004; Mullen et al., 2006). In addition, some research on quercetin activities in humans have so far shown inconclusive and even conflicting results (Egert et al., 2008). On the other side, studies have shown that the biological properties of quercetin aglycone and its metabolites may be different (Amić et al., 2017; Chuang et al., 2016; Kroon et al., 2004; Terao et al., 2011). Taking into account that metabolites are the potential bioactive forms *in vivo*, more studies should be carried out on quercetin derivatives regarding their biological activities.

Bearing in mind that there is insufficient data on the antioxidant and anti-inflammatory effects of quercetin plasma metabolites (Al-Shalmani et al., 2011; Boesch-Saadatmandi et al., 2011; Cho et al., 2012; Derlindati et al., 2012; Dueñas, Surco-Laos, González-Manzano, González-Paramás, & Santos-Buelga, 2011; Justino et al., 2004; Messer, Hopkins, & Kipp, 2015; Morand et al., 1998; Santos et al., 2008; Wang et al., 2016; Wiczkowski et al., 2014), the overall goal of this study was to address these gaps in knowledge. Thus, this study was done to evaluate structure-antioxidant and anti-inflammatory activity relationships of glucuronidated and methylated quercetin metabolites commonly found in human plasma (Cialdella-Kam et al., 2013; Day et al., 2001; Wittig, Herderich, Graefe, & Veit, 2001), such as quercetin-3-*O*-glucuronide (q-glucuronide), 4′-*O*-methylquercetin (tamarixetin) and 3′-*O*-methylquercetin (isorhamnetin), as well as two forms of quercetin found in edible plants – quercetin-3,4′-di-*O*-glucoside (q-diglucoside) and isorhamnetin-3-*O*-glucoside (iso-glucoside). Besides, quercetin-3,5,7,3′,4′-pentamethylether (q-5methyl) was included in order to evaluate the importance of free hydroxyl groups in quercetin biopotential (Fig. 1). Since common onion (*Allium cepa* L.) is one of the best known sources of bioavailable quercetin (Wiczkowski et al., 2014), the extract of this plant was also examined in order to compare biopotential of dietary quercetin with biopotential of quercetin metabolites as pure compounds. In general, this research should contribute to a better understanding of factors regulating nutraceutical potency of quercetin and its role in managing different diseases.

2. Material and methods

2.1. Chemicals and reagents

Quercetin, q-glucuronide, tamarixetin, isorhamnetin, iso-glucoside, q-diglucoside and q-5methyl were purchased from Extrasynthese (Genay, France). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extract preparation

Samples of cultivated common onion (*A. cepa L. var. cepa*) were

collected in July 2009 in the village of Neradin, the Fruška Gora Mountain, Serbia. The voucher specimen No. 2-1762 was prepared, identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad Faculty of Sciences, Serbia. 30 g of fresh plant material (whole plants) was grounded and macerated with 70% aqueous methanol (8 mL per 1 g of plant material) during 72 h at 30 °C. After filtration, the solvent was evaporated to dryness under vacuum at 45 °C and dry residues were redissolved in 70% aqueous methanol to the final concentration of 200 mg/mL and used for determination of antioxidant and anti–inflammatory activities. The extract was made in triplicate.

2.3. Estimation of antioxidant potential

Antioxidant potential of quercetin, its metabolites and glycosides, as well as *A. cepa* extract, was determined using previously adapted assays for 96-well microplates related to free radical scavenging ability towards 1,1-diphenyl-2-picrylhydrazyl radical (DPPH˙), ferric reducing ability of plasma (FRAP) and inhibitory potential towards lipid peroxidation (LP; Lesjak et al., 2013, 2014). The results were compared with the synthetic antioxidant butylated hydroxytoluene (BHT). Additionally, total flavonoid content was determined in *A. cepa* extract (Lesjak et al., 2013). All compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 3.33 mg/mL stock solutions and afterwards used for estimation of antioxidant potential. Each experimental procedure is briefly explained in the text below.

2.3.1. DPPH˙ scavenging ability

Ten microliters of each sample, quercetin and its derivatives dissolved in DMSO and *A. cepa* extract dissolved in 70% aqueous methanol in series of concentrations, were added to 100 μL of 90 μmol/L DPPH solution in methanol, and the mixture was diluted with additional 190 μL of methanol. In controls, the 10 μL of sample was substituted with DMSO (for quercetin derivatives) or 70% aqueous methanol (for *A. cepa* extract). In blank probes, only methanol (290 μL) and each sample (10 μL) were mixed, while in blank probe for controls, only 300 μL of methanol were added. Measurements of absorbance were read at 515 nm after 1 h. All samples and the control were made in triplicate. The percentage of inhibition achieved by different concentrations of samples in the antioxidant assays performed was calculated by using the following equation: $I (\%) = (A_0 - A)/A_0 \times 100$, where A_0 was the absorbance of the control reaction and A was the absorbance of the examined samples, both corrected for the value of the corresponding blank probes. Corresponding inhibition-concentration curves were drawn using Origin software, version 8.0 and IC_{50} values (concentration of extract that inhibited DPPH˙ formation by 50%) were determined. For each assay final result was expressed as mean ± standard deviation (SD) of three measurements.

2.3.2. FRAP

Examined samples were tested in series of different concentrations and ascorbic acid (1.25–160 μg/mL) was used for creating a standard curve. FRAP reagent was prepared by mixing 10 mmol/L 2,4,6-tripyridyl-*S*-triazine in 40 mmol/L HCl, 0.02 mol/L $FeCl_3$, and acetate buffer (22.78 mmol/L CH_3COONa, 0.28 mol/L CH_3COOH, pH 3.6) at ratio of 1:1:10 (v/v/v), respectively. After the addition of the sample or ascorbic acid (10 μL, substituted with DMSO or 70% aqueous methanol in the control) to 290 μL of FRAP reagent and 6 min of incubation at room temperature, absorbance was read at 593 nm. In blank probes samples (10 μL) were mixed with 290 μL of distilled water. All samples and blank probes were made in triplicate and mean values of reducing power were expressed as μg of ascorbic acid equivalents per μg of dry extract or pure compound, calculated according to the standard calibration curve.
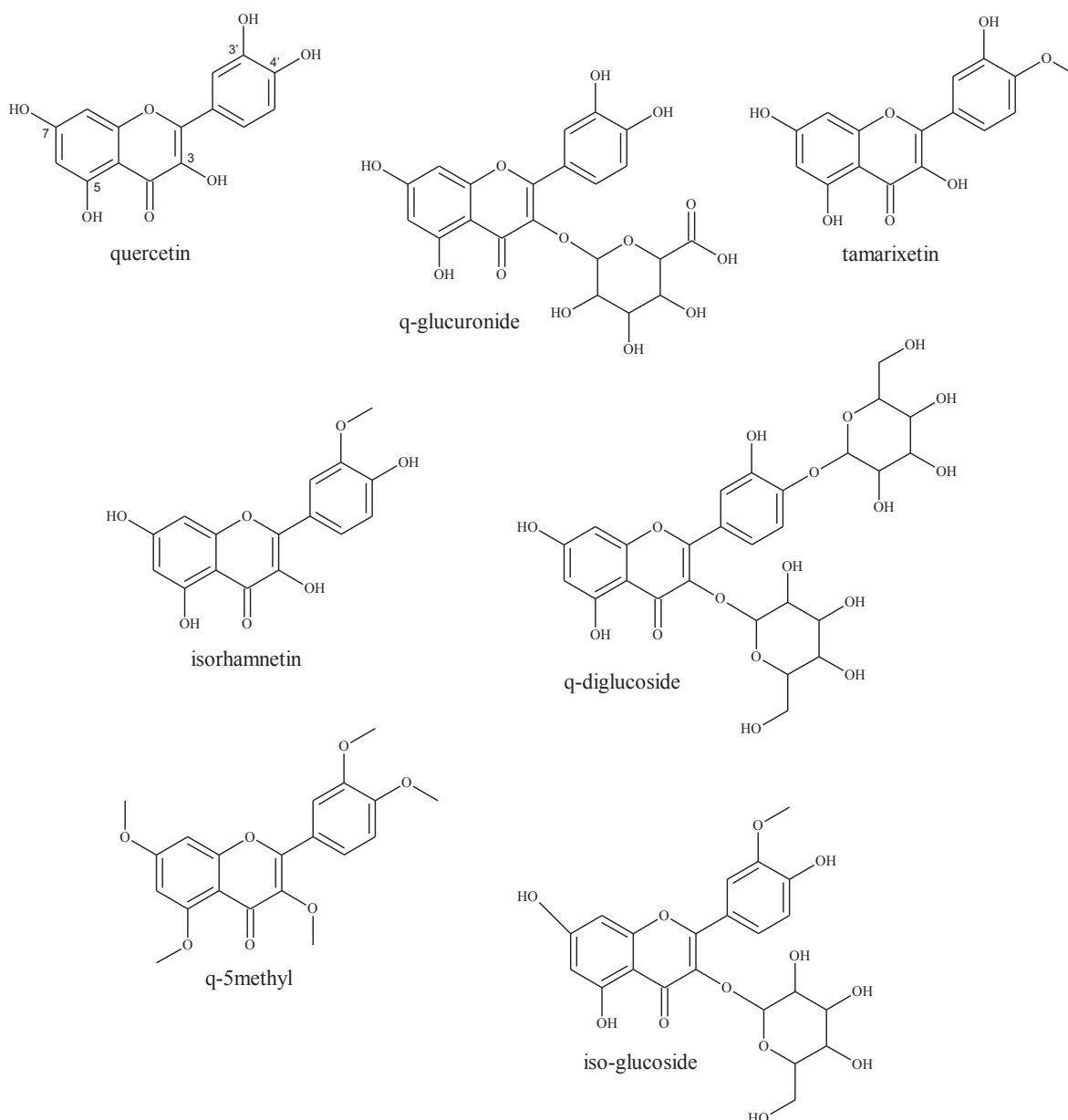
Fig. 1. Structures of examined quercetin derivatives. q-glucuronide – quercetin-3-*O*-glucuronide; tamarixetin – 4′-*O*-methylquercetin; isorhamnetin – 3′-*O*-methylquercetin; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether.

2.3.3. LP

The extent of Fe^{2+}/ascorbate induced LP was determined by thiobarbituric acid assay using polyunsaturated fatty acids, obtained from linseed by Soxhlet extraction (69.7% linolenic acid, 13.5% linoleic acid, as determined by GC–MS) as a substrate for LP induction. Fatty acids were added to phosphate buffer (12.86 mmol/L KH_2PO_4, 54.13 mmol/L Na_2HPO_4, pH 7.4) in presence of 0.25% Tween-80, to obtain a 0.035% suspension, and sonicated for 1 h. This suspension (3 mL) was mixed with 20 μL of $FeSO_4$ (4.58 mmol/L), 20 μL of ascorbic acid (87 μmol/L) and 100 μL of each tested sample concentration. In the control, instead of tested compounds or extract, 100 μL of DMSO or 70% aqueous methanol were added. Phosphate buffer (3.04 mL; pH 7.4) and 100 μL of each sample were added in blank probes. In blank probe for the controls, instead of each compound, 100 μL of DMSO or 70% aqueous methanol were added. After incubation at 37 °C for 1 h, 200 μL of ethylenediaminetetraacetic acid (EDTA) solution (37.2 mg/mL) were added to all samples followed by 2 mL of aqueous mixture containing thiobarbituric acid (3.75 mg/mL), $HClO_4$ (1.3%) and trichloroacetic

acid (0.15 g/mL). Following heating at 100 °C for 15 min, mixtures were cooled down and centrifuged at 1600*g* for 15 min, 250 μL of each mixture were transferred to 96-well microplates and absorbance was read at 532 nm. All samples and the control were made in triplicate. The percentage of inhibition achieved by different concentrations of samples in the antioxidant assays performed was calculated by using the following equation: I (%) = $(A_0 - A)/A_0 \times 100$, where A_0 was the absorbance of the control reaction and A was the absorbance of the examined samples, both corrected for the value of the corresponding blank probes. Corresponding inhibition-concentration curves were drawn using Origin software, version 8.0 and IC_{50} values (concentration of extract that inhibited LP, by 50%) were determined. For each assay final result was expressed as mean ± SD of three measurements.

2.4. Estimation of anti-inflammatory potential

In order to evaluate anti-inflammatory potential of examined samples, *ex vivo* cyclooxygenase-1 and (COX-1) and 12-lipoxygenase (12-
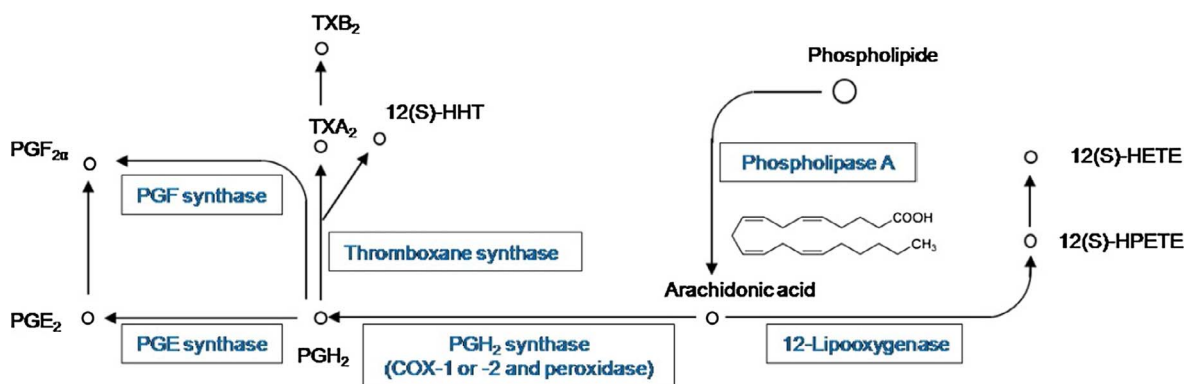
Fig. 2. COX and 12-LOX branches of AA metabolic pathway. 12-HPETE – 12-hydroperoxyeicosatetraenoic acid; PGH_2, PGE_2 and $PGF_{2\alpha}$ – prostaglandins; TXA_2 and TXB_2 – thromboxanes.

LOX) assay using human platelets was undertaken according to the method of Lesjak et al. (2013). The method is based on the inhibitory potential of compounds on the biosynthesis of eicosanoids, such as 12(S)-hydroxy(5Z,8E,10E)-heptadecatrienoic acid (12-HHT), thromboxane B_2 (TXB_2), prostaglandin E_2 (PGE_2) and 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid (12-HETE). 12-HHT, TXB_2, PGE_2 and 12-HETE are inflammation mediators derived from arachidonic acid (AA) metabolism, which is catalyzed by enzymes of inflammatory response, COX-1 and 12-LOX (Fig. 2). In brief, an aliquot of human platelet concentrate (source of COX-1 and 12-LOX enzymes), viable, but outdated for medical treatment, which contains 4×10^8 cells was suspended in buffer (0.137 mol/L NaCl, 2.7 mmol/L KCl, 2.0 mmol/L KH_2PO_4, 5.0 mmol/L Na_2HPO_4 and 5.0 mmol/L glucose, pH 7.2) to obtain the final volume of 2 mL. This mixture was slowly stirred at 37 °C for 5 min. Subsequently, 0.1 mL of standard compounds solutions (samples and aspirin were dissolved in DMSO or 70% aqueous methanol), substituted with DMSO or 70% aqueous methanol in the control and blank probe for control, and 0.1 mL of calcimycin (Calcium Ionophore A23187, 125 μmol/L in DMSO), substituted with DMSO or 70% aqueous methanol in blank probe and blank probe for the control, were added and incubated for 2 min at 37 °C, with moderate shaking. Afterwards, 0.3 mL of $CaCl_2$ aqueous solution (16.7 mmol/L), substituted with distilled water in blank probe and blank probe for the control, was added and the mixture was incubated for another 5 min at 37 °C with shaking. Acidification with cold 1% aqueous formic acid (5.8 mL) to pH 3 terminated the reaction. Internal standard prostaglandin B_2 (50 μL of 6 μg/mL solution in DMSO) was added and extraction of products was done with a mixture of chloroform and methanol (1:1 (v/v), 8.0 mL) with vigorous vortexing for 15 min. After centrifugation at 7012g for 15 min at 4 °C, organic layer was separated, evaporated to dryness, dissolved in methanol (0.5 mL), filtered, and used for further LC-MS/MS analysis of eicosanoids (12-HHT, TXB_2, PGE_2 and 12-HETE). All samples and control were made in triplicate. Percent of COX-1 and 12-LOX inhibition achieved by different concentrations of samples was calculated by using the following equation: I (%) = $100 \times (R_0 - R)/R_0$, where R_0 and R were response ratios (metabolite peak area/internal standard peak area) in the control reaction and in the samples of examined samples, respectively. Both R and R_0 were corrected for the value of appropriate blank probes. Corresponding inhibition-concentration curves were drawn using Origin software, version 8.0 and IC_{50} values (concentration of extract that inhibited COX-1 and 12-LOX metabolites formation by 50%) were determined. For each assay final result was expressed as mean ± SD of three measurements. For inhibition of COX-1 pathway, estimated IC_{50} values were compared to IC_{50} values of aspirin used as positive control.

2.5. Statistical analysis

Data were analyzed by one-way ANOVA followed by the post hoc

Tukey's HSD for multiple comparisons of means in order to determine whether antioxidant and anti-inflammatory activities of quercetin and its derivatives differed significantly between each other. Statistical significance was set at $p \leq 0.05$.

3. Results and discussion

3.1. Antioxidant activity

The antioxidant activity of six quercetin derivatives and *A. cepa* extract, as the representative of dietary quercetin source, were assessed *in vitro* by DPPH and FRAP assays, as well as by evaluation of potential to inhibit LP. Significant differences were observed for the tested compounds, as well as between the assays employed. In Figs. 3–5 the results of antioxidant activity obtained for tested samples, as well as BHT used as standard are shown. It can be clearly seen that quercetin, tamarixetin, isorhamnetin and quercetin-3-O-glucuronide displayed notable antioxidant activity, significantly higher than BHT (Figs. 3–5). Generally, quercetin aglycone was the most potent antioxidant and, as
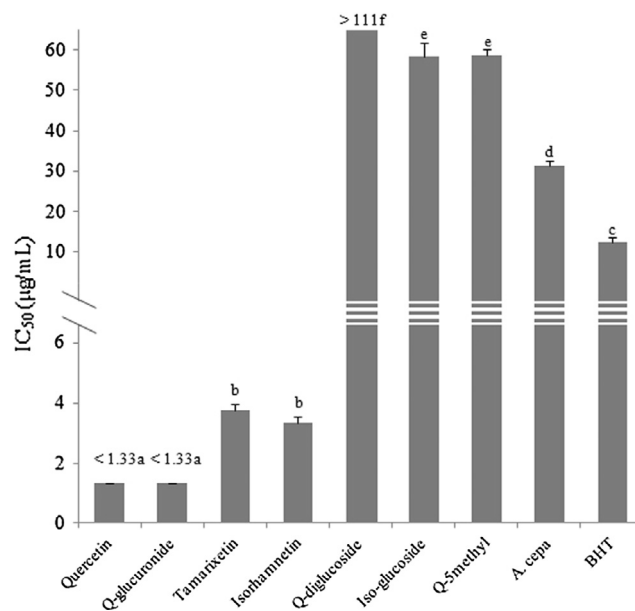

Fig. 3. Potential of examined samples to scavenge DPPH˙. Antioxidant activity of the examined samples was expressed as concentration (mg of pure compound or dry material per mL of working solution) needed to decrease initial DPPH˙ concentration by 50% (IC_{50}). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). q-glucuronide – quercetin-3-O-glucuronide; q-diglucoside – quercetin-3,4′-di-O-glucoside; iso-glucoside – isorhamnetin-3-O-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether; BHT – butylated hydroxytoluene.
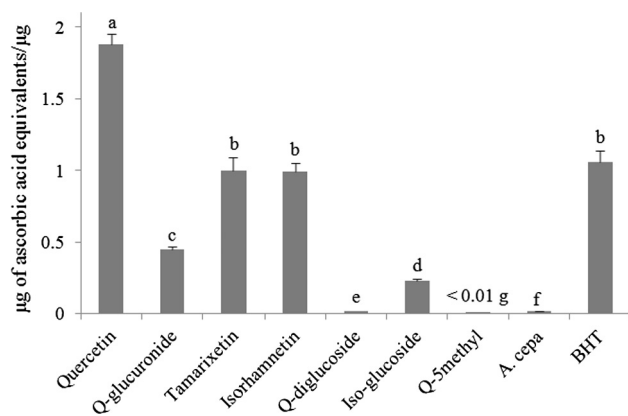
Fig. 4. Reducing power of examined samples. Reducing power of the examined samples was expressed as µg of ascorbic acid equivalents per µg of pure compound or dry extract, calculated according to the standard calibration curve. Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). q-glucuronide – quercetin-3-*O*-glucuronide; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether; BHT – butylated hydroxytoluene.
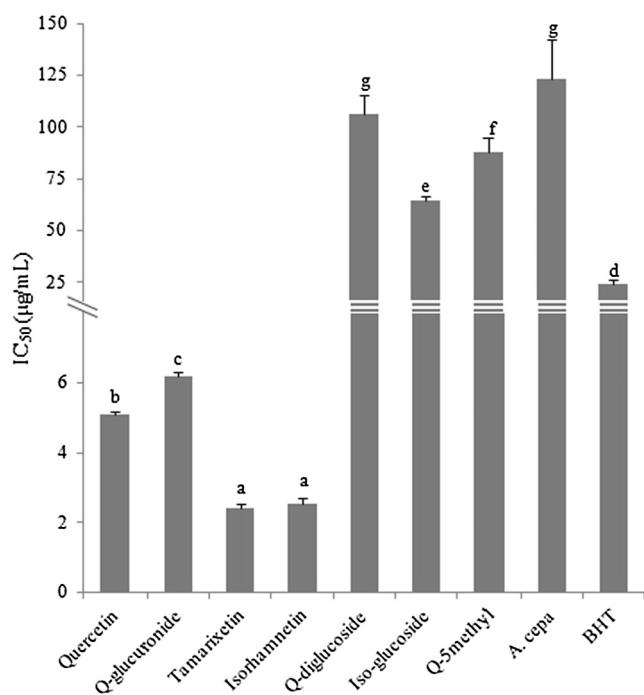

Fig. 5. Potential of examined samples to inhibit LP. Antioxidant activity of the examined samples was expressed as concentration (mg of pure compound or dry extract per mL of working solution) needed to decrease induced LP by 50% (IC_{50}). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). q-glucuronide – quercetin-3-*O*-glucuronide; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether; BHT – butylated hydroxytoluene.

expected, the derivatization of its hydroxyl groups significantly reduced the antioxidant activity of its derivatives (Dueñas et al., 2011; Messer et al., 2015; Wiczkowski et al., 2014). Thus, the overall order of antioxidant activity was as follows: quercetin > tamarixetin = isorhamnetin > quercetin-3-*O*-glucuronide > isorhamnetin-3-*O*-glucoside > quercetin-3,5,7,3′,4′-penthamethylether > quercetin-3,4′-di-*O*-glucoside. These results confirm the well known view that scavenging capacity of quercetin, as well as other flavonoids, is directly correlated to the number of free hydroxyl groups (Dueñas et al., 2011; Messer et al., 2015; Wiczkowski et al., 2014). As expected, quercetin-3,4′-di-*O*-glucoside exhibited low activity since

both hydroxyl groups that contribute most to the activity are blocked (Figs. 3–5). Surprisingly, quercetin-3,5,7,3′,4′-penthamethyl ether exhibited higher overall antioxidant activity than quercetin-3,4′-di-*O*-glucoside (Figs. 3 and 5), despite complete lack of free phenolic hydroxyl groups. In the literature, there is limited number of references which could support this result. The only assay where phenolic derivatives with less free hydroxyl groups express higher antioxidant activity is measurement of LP inhibition potential (Santos et al., 1998).

It is known that flavonoids inhibit oxidative stress *in vitro* by acting either as free radical scavengers or as metal chelating agents (Morand et al., 1998). Bearing in mind that iron chelation by quercetin and its metabolites play important role in their antioxidant potential, results of FRAP and LP assays in this study, could be discussed further. As, it has previously been shown that the preferred site for iron chelation by quercetin is between the 3-hydroxyl and 4-carbonyl group; for complexes containing one iron and one quercetin molecule, binding strength has an order 3–4 > 4–5 > 3′–4′ (Ren, Meng, Lekka, & Kaxiras, 2008). Moreover, 3–4 chelation site is also preferred for complexes which are formed between one iron and two or three quercetin molecules (Ren et al., 2008). Our data clearly indicate that the greatest decrease in antioxidant potency was observed for compounds where 3-hydroxyl group was substituted (Figs. 4 and 5). In contrast, when the 3-hydroxyl group was free (quercetin, tamarixetin and isorhamnetin) antioxidant activity was high. Also, our results could be supported with a fact that 3-hydroxyl group plays a crucial role in the oxidation process of flavonoids. Namely, flavonoids neutralize free radicals by one-step hydrogen atom or electron transfer followed by proton transfer, during which they oxidize (Williams, Spencer, & Rice-Evans, 2004). Thus, when 3-hydroxyl group is unoccupied, both flavonoid oxidation and radical neutralization is more efficient (Rice-Evans, Miller, & Paganga, 1996).

However, the most potent inhibitors of LP were methylated quercetin metabolites tamarixetin and isorhamnetin, which expressed twice higher activity than the parent quercetin molecule and significantly higher than other tested derivatives (Fig. 5). An explanation for the superiority of these metabolites, compared with quercetin and other examined derivatives may arise from the difference in their polarity. Namely, polyunsaturated fatty acids, obtained from linseed, were used as a substrate in this test. Tamarixetin and isorhamnetin are more lipophilic antioxidants, compared with quercetin and its glycosides or derivatives, and seem to interact better with fatty acids as they could incorporate themselves into lipid droplets. This location may be favorable for the trapping of peroxyl radicals originating from LP process. In contrast, quercetin and especially its polar derivatives could not locate themselves so close to fatty acids and scavenge free radical products of LP. Despite non-polar nature, LP inhibitory activity of quercetin-3,5,7,3′,4′-penthamethylether was moderate, due to lack of free hydroxyl groups (Fig. 5). Presented results are in strong correlation with previous study where methylation of the hydroxyl groups increased potential of flavonoids to inhibit LP (Santos et al., 1998). Namely, Santos et al. (1998) evidenced that isorhamnetin expressed higher anti-LP activity compared with quercetin, while quercetin-3,5,7,3′,4′-penthamethyl ether was more active than quercetin-3,7,3′,4′,-tetramethylether. The authors also concluded that an increase in the lipophilic nature of flavonoids contributed to their ability to inhibit LP.

Common onion (*A. cepa* L. var. *cepa*) is usually considered as the main source of dietary quercetin. In this study, in both DPPH and FRAP assay, the total *A. cepa* extract was significantly weaker antioxidant than quercetin aglycone, quercetin-3-*O*-glucuronide, isorhamnetin and tamarixetin, but stronger than quercetin-3,4′-di-*O*-glucoside (Figs. 3 and 4). The low activity of the extract was not surprising, since the major flavonoid component of *A. cepa* extract is quercetin-3,4′-di-*O*-glucoside (making more than 60% of total flavonoids in onion, Price & Rhodes, 1997), which expressed significantly low activity in present study. It can be assumed that the other two major components

of *A. cepa* extract – quercetin-4′-*O*-monoglucoside (making around 20% of the total flavonoids) and quercetin aglycone (up to 2% of the total flavonoids; Fredotovic et al., 2017; Price & Rhodes, 1997) are responsible for the reducing ability of the extract in FRAP and DPPH assays. In LP assay, which is more complex model system for testing antioxidant activity than DPPH and FRAP assays, and in which more factors could influence the activity, *A. cepa* extract expressed the lowest activity in comparison to all investigated pure compounds, but anyway reached 50% of inhibition (Fig. 5). While comparing IC_{50} values of extracts and pure compounds it should be taken into account that active compounds of the extract are mixed with complex matrix which enhances the mass of extract needed for 50% inhibition and also could influence the activity. Therefore, it can be concluded that *A. cepa* extract exhibited moderate antioxidant activity in assays used in this study.

3.2. Anti-inflammatory activity

While there are numerous papers confirming great anti-inflammatory potential of quercetin, particularly to inhibit AA metabolism and production of eicosanoids – potent inflammatory mediators, there are only few research papers on this particular anti-inflammatory activity of quercetin derivatives examined in this study (de Pascual-Teresa et al., 2004; During & Larondelle, 2013; Jones et al., 2004; O'Leary et al., 2004; Takano-Ishikawa, Goto, & Yamaki, 2006). Furthermore, these studies investigated the effect of quercetin and some of its derivatives only on COX-2 AA pathway, while there are no data on their potential to inhibit COX-1 and 12-LOX pathways of AA metabolism. Quercetin and its derivatives demonstrated a notable concentration-dependent inhibitory potential towards synthesis of 12-HHT, TXB_2 and PGE_2 inflammatory mediators, which is comparable with aspirin (Figs. 6–8), as well as towards 12-HETE (Fig. 9) production. Overall order of anti-inflammatory activity in this study was as follows: tamarixetin > quercetin = quercetin-3,4′-di-*O*-glucoside > isorhamnetin = quercetin-3-*O*-glucuronid > isorhamnetin-3-*O*-glucoside > quercetin-3,5,7,3′,4′-penthamethylether.

Interestingly, monomethylated quercetin metabolite tamarixetin, but not isorhamnetin, demonstrated a significantly superior anti-inflammatory potential compared with quercetin and other compounds, and similar inhibitory potential as aspirin, a well known COX-1 inhibitor (Figs. 6–8).

Results from this study point out that the number of free hydroxyl groups is not the only characteristic which defines potential of
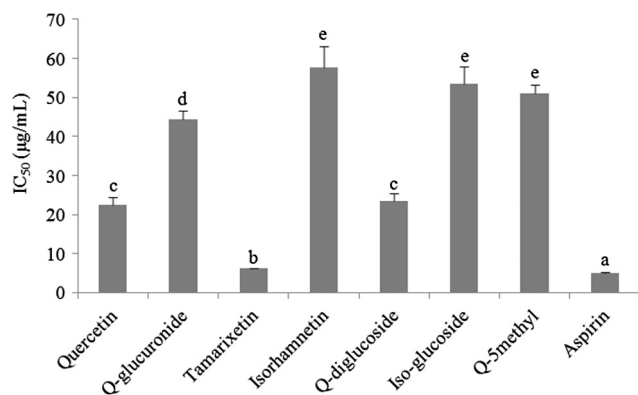
Fig. 6. Potential of examined samples to inhibit production of 12-HHT. Anti-inflammatory activity of the examined samples was expressed as concentration needed to decrease induced production of 12-HHT by 50% (IC_{50}). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). Potential of *A. cepa* extract to inhibit production of 12-HHT was not evaluated. q-glucuronide – quercetin-3-*O*-glucuronide; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether.

Fig. 7. Potential of examined samples to inhibit production of TXB_2. Anti-inflammatory activity of the examined samples was expressed as concentration needed to decrease induced production of TXB_2 by 50% (IC_{50}). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). q-glucuronide – quercetin-3-*O*-glucuronide; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether.
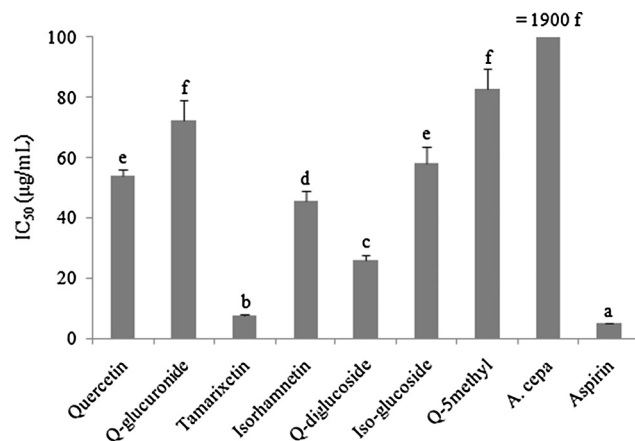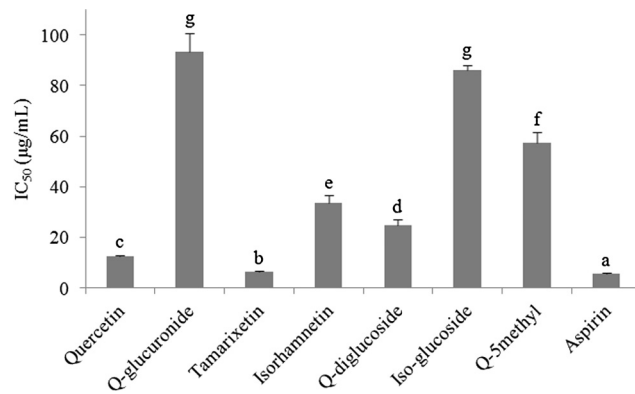
Fig. 8. Potential of examined samples to inhibit production of PGE_2. Anti-inflammatory activity of the examined samples was expressed as concentration needed to decrease induced production of PGE_2 by 50% (IC_{50}). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). Potential of *A. cepa* extract to inhibit production of PGE_2 was not evaluated. q-glucuronide – quercetin-3-*O*-glucuronide; q-diglucoside – quercetin-3,4′-di-*O*-glucoside; iso-glucoside – isorhamnetin-3-*O*-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether.

quercetin conjugates to inhibit COX-1 and 12-LOX pathway of AA metabolism, as it is with antioxidant activity. Similarly, other authors have also evidenced that the number of free hydroxyl groups of flavonoids is not directly proportional with their anti-inflammatory activity, specifically for their COX-2 inhibition potential (Chen et al., 2001; During & Larondelle, 2013; Takano-Ishikawa et al., 2006).

Even though the exact mechanism of how flavonoids inhibit COX and LOX activity is not known (Catarino, Talhi, Rabahi, Silva, & Cardoso, 2016), some assumptions can be made. Namely, both mechanisms of COX-1 and 12-LOX reactions include abstraction of hydrogen from AA leading to the formation of radical species, where flavonoids could serve as scavengers. However, hydrogen acceptor in COX-1 is tyrosyl radical (formed through tyrosine oxidation by Fe^{3+} from heme), while in 12-LOX electron is transferred directly to non-heme Fe^{3+} (Funk, Carroll, Thompson, Sands, & Dunham, 1990; Rouzer & Marnett, 2009). Quercetin and its derivatives could neutralize tyrosil radical in COX-1 or reduce Fe^{3+} to Fe^{2+} in 12-LOX, thus inactivating both enzymes. However, the difference in active site of these two enzymes could partly be the reason why all compounds express somewhat different potential towards inhibition of COX-1 and 12-LOX.
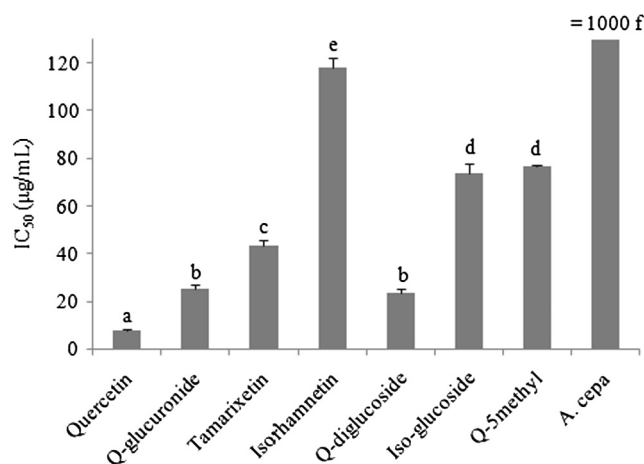
Fig. 9. Potential of examined samples to inhibit production of 12-HETE. Anti-inflammatory activity of the examined samples was expressed as concentration needed to decrease induced production of 12-HETE by 50% (IC$_{50}$). Data are mean ± SD. All samples were made in triplicate. Values with no common letters are significantly different from each other (p ≤ 0.05). q-glucuronide – quercetin-3-O-glucuronide; q-diglucoside – quercetin-3,4′-di-O-glucoside; iso-glucoside – isorhamnetin-3-O-glucoside; q-5methyl – quercetin-3,5,7,3′,4′-pentamethylether.

In addition, different quercetin derivatives would fit differently into active site of examined enzymes due to steric diversity, which could also affect results, as discussed by others for COX-2 (O'Leary et al., 2004).

Anti-inflammatory activity of *A. cepa* extract was estimated only on the basis of inhibitory potential towards TXB$_2$ and 12-HETE production, since concentrations of 12-HHT and PGE$_2$ in control and sample probes were insufficient (Figs. 7 and 9). TXB$_2$ and 12-HETE are products of two different branches of AA metabolic pathway (Fig. 2), thus by considering inhibitory potential on their production conclusion about inhibition of both COX and LOX braches can be made. Similarly as within antioxidant assays, *A. cepa* extract exhibited significantly lower anti-inflammatory potential than pure compounds, but reached IC$_{50}$ value, suggesting the presence of active principles (Figs. 7 and 9). Since in this study two of the dominant phenolic compounds of the *A. cepa* extract (quercetin-3,4′-*O*-di-glucoside and quercetin aglycone) exhibited high inhibitory potential towards COX-1 and 12-LOX enzymes (Figs. 6–9), these compounds could contribute to the anti-inflammatory activity of *A. cepa* extract. However, the extract is not purified and thus contains a lot of inactive compounds, because of which the IC$_{50}$ value is high (Figs. 7 and 9). But, if we compare the activity of *A. cepa* extract with extract of other plants, such as a well-known anti-inflammatory drug *Plantago major* L. (IC$_{50}$ = 0.65 mg/mL for 12-HHT and IC$_{50}$ = 1.73 mg/mL for 12-HETE; Beara et al., 2010), it can be concluded that the *A. cepa* extract is a potent anti-inflammatory agent.

Surprisingly, two studies evidenced that quercetin and its metabolites are activators of COX-1 *in vitro* and *in vivo* and that support COX-mediated formation of eicosanoids (Bai & Zhu, 2008; Wang, Bai, & Zhu, 2010). These studies are contradictory to our findings and to the well-known fact that quercetin possesses strong anti-inflammatory capacity. However, concentrations used in this study are smaller than those in the listed references, which could be the reason for disparity of results.

In summary, quercetin, its metabolites and glycosides exhibited significantly different antioxidant and anti-inflammatory potentials. It seems that antioxidant potential is directly proportional to the number of free hydroxyl groups in most cases, which is not the case with anti-inflammatory activity. Quercetin and its major metabolites from human plasma are shown to have better, but comparable antioxidant and anti-inflammatory potencies with *A. cepa*, a main source of dietary bioavailable quercetin. The results of this study indicate that quercetin metabolites present in systemic circulation after quercetin consumption may act as potent antioxidants and anti-inflammatory agents and should without doubt be considered when defining overall biological activity of quercetin-rich food *in vitro* and *in vivo*.

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