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Free Radical Scavenging Activity of Hydroxyflavone Sodium Salts Compared to the Activity of Hydroxyflavones Themselves

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Different studies have demonstrated that the bioactivity of hydroxyflavones is due to their antioxidant and free radical scavenging activity. Recently, most interest has been devoted to structure-activity relationships; however, the main problems encountered in these studies are the low solubility of some hydroxyflavones in aqueous solution and the pro-oxidant character of the hydroxyl group at the 3 position. In the present investigation, these problems have been resolved by preparing the corresponding hydroxyflavone sodium salts. In this way, the hydroxyl free radical $(OH)^{\bullet}$ scavenging activity of different hydroxyflavone salts were studied using the Fenton reaction model, and the superoxide $(O_2)^{\bullet -}$ scavenging activity was studied using the xanthine oxidase model. The results obtained show clearly that the $(OH)^{\bullet}$ scavenging activity of the hydroxyflavone salt form is at least two times that of the corresponding hydroxyflavone itself. Considering the $(O_2)^{\bullet -}$ scavenging activity, the salt form of hydroxyflavone is as good as the corresponding hydroxyflavone. Moreover, it was observed that for a good scavenging activity the hydroxyl at the 3' position must be free, and only the hydroxyl groups of 3 and 4' have to be substituted by sodium when the sodium salt of hydroxyflavone at position 7 does not have an important role in radical scavenging. The salt forms of hydroxyflavones are interesting free radical scavenger compounds showing a hydrophilic character.

INTRODUCTION

Hydroxyflavones are polyphenolic substances widespread among vascular plants. They have drawn particular attention for their pharmacological activity, which is due to their inhibition of certain enzymes and their antioxidant activity.[1,2] This activity can reduce the presence of free radicals responsible for many diseases, especially those caused by DNA destruction and cell damage.[1,2,3] Thus far, their mechanism of action and structural requirements have not been fully understood. Investigation into the structure-activity relationships is hampered by low solubility in most models and assays. Hence, solvents such as DMSO and ethyl alcohol are needed. These solvents possess some good radical scavenging activities themselves.[4]

Another problem, a pro-oxidant property, was encountered when investigating the hydroxyl free radical scavenging activity of several hydroxyflavones and was described by Kessler *et al.* in 2003.[5] In his experiments, he mentioned that the results for quercetin and some of

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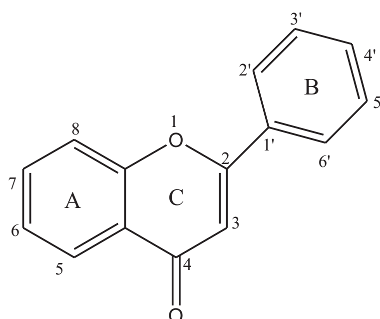
its derivatives could not be explained using the Fenton reaction system. Some of these hydroxyflavones are shown to be pro-oxidant by reduction of Fe^{2+} [6,7,8,9] and are thus considered to be unsuitable for therapeutic application. Quercetin is mentioned to be pro-oxidant (Fe^{2+} reduction or production of hydroxyl radicals).[6,7,8,9] It is one of the most abundant flavonoids in human diet. The amount of ingested quercetin was estimated at 87 mg per day.[10] This amount can be attributed to the presence of quercetin in a large range of fruits and vegetables.[11] However, it may also be due to the intestinal degradation of other flavonoids: for example, rutin which during intestinal absorption undergoes an ose-genin bond cleavage by the glycosidase enzymes of intestinal bacteria.[12]

On the other hand, quercetin has been described as a toxic compound; it has genotoxic and carcinogenic properties. These are due to its pro-oxidant activity, which can produce oxygen radical derivatives that cause DNA damage leading to cell damage.[13,14] In 1998, Jovanovic *et al.*[15] demonstrated that the decreased antioxidant activity of quercetin is due to the lowest reduction potential of the radical form, which is due to the electron donating effect of the free hydroxyl at the 3 position. When this group is substituted, like in the rutin radical, the reduction potential increases considerably and the antioxidant activity improves.

Our interest was focused on studying the salt form of quercetin in order to inverse its pro-oxidant activity to

TABLE I. Different forms of hydroxyflavones studied



Compound	Free substituents			
	–OH	–ONa	$–OC_2H_5OH$	–O-rutinose
Quercetin	3,5,7,3',4'	–	–	–
MonoHEQ[(a)]	3,5,3',4'	–	7	–
DiHEQ[(a)]	3,5,3'	–	7,4'	–
Rutin	5,7,3',4'	–	–	3
MonoHER[(b)]	5,3',4'	–	7	3
DiHER[(b)]	5,3'	–	7,4'	3
Fisetin	3,7,3',4'	–	–	–
Galangin	3,5,7	–	–	–
Kaempferol	3,5,7,4'	–	–	–
Luteolin	5,7,3',4'	–	–	–
7-Hydroxyflavone	7	–	–	–
Salt form of**:**				
Quercetin	5,3'	3,4',7	–	–
MonoHEQ[(a)]	5,3'	3,4'	7	–
DiHEQ[(a)]	5,3'	3	7,4'	–
Rutin	5,3'	4',7	–	3
MonoHER[(b)]	5,3'	4'	7	3
DiHER[(b)]	5,3'	–	7,4'	3
Fisetin	3'	3,7,4'	–	–
Galangin	5	3,7	–	–
Kaempferol	5	3,7,4'	–	–
Luteolin	5,3'	7,4'	–	–
7-Hydroxyflavone	–	7	–	–

[(a)] HEQ: hydroxyethyl quercetin.
[(b)] HER: hydroxyethyl rutin.

antioxidant activity and to reveal the responsible functional groups for this activity. Our studies were focused on several hydroxyflavones and their salts possessing structures similar to quercetin (Table I).

EXPERIMENTAL

Reagents and Chemistry

Xanthine oxidase grad III, hypoxanthine and α-naphtylamine were obtained from Sigma (St Quentin Fallavier, France). Ascorbic acid, 2-deoxy-D-ribose thiobarbituric and trichloroacetic acid, quercetin, kaempferol, fisetin and galangin were purchased from Aldrich (St Quentin Fallavier, France). Hydrogen peroxide, ascorbic acid, iron(II) ammonium sulfate and ethylenediaminetetraacetic acid disodium salt (dehydrated) were obtained from Fluka (St Quentin Fallavier, France). Rutin was obtained from Merck, luteolin and 7-hydroxyflavone were obtained from Extrasynthèse (Genay). 7-Mono(*O*-β-hydroxyethyl)rutin (monoHER), 4'-7-di(*O*-β-hydroxyethyl)rutin (diHER), 7-mono(*O*-β-hydroxyethyl) quercetin (monoHEQ) and 4'-7-di(*O*-β-hydroxyethyl) quercetin (diHEQ) were synthesized in our laboratory. All other chemicals were of analytical grade.

Hydroxyflavone salt forms were obtained by regular addition of sodium hydroxide (0.1 M aqueous solution) in order to reach pH = 10 and to obtain a concentration of 15 mmol dm^{-3}. Salt solutions were diluted in the reaction mixture using phosphate buffer (24 mmol dm^{-3} of NaH_2PO_4-Na_2HPO_4 in 15 mmol dm^{-3} of NaCl) at pH = 7.4 in order to obtain a range of concentrations between 0.05 and 0.5 mmol dm^{-3}.

The stability of all salt forms of hydroxyflavones in the reaction mixture was assessed, first by a kinetic study of the molecule's precipitation and in a second step by HPLC.

Apparatus

A microcomputer pH-vision 6072N model from Electronics Ltd. was used for pH determination of the reaction mixture.

The absorbance measurements for free radical assays were performed on a Beckman UV-Vis. Detector (Model 65), which was also used for the kinetic study of the precipitation of different molecules.

The HPLC system used to study stability consisted of a Beckman System Gold, an autosampler module 507, a pump 126 solvent module and a programmable detector module 166. The analytical column was a reverse-phase LiChrospher C8 column 250 × 4 mm containing 5 μm particles (Merck, France) and a guard column C18 10 × 4 mm (Merck, France).

Free Radical Scavenging Activity

Hydroxyl Radical Assay. – The assay procedure was described by Zhao and Jung in 1995.[16] The samples were incubated at 37 °C for 15 min in a phosphate buffer, at pH = 7.4 (24 mmol dm^{-3} NaH_2PO_4-Na_2HPO_4 in 15 mmol dm^{-3} NaCl) with deoxyribose (0.6 mmol dm^{-3}), ascorbic acid (0.6 mmol dm^{-3}), hydrogen peroxide (0.855 mmol dm^{-3}), the substances tested (0–0.5 mmol dm^{-3}) and the EDTA (0.02 mmol dm^{-3}) with $(NH_4)_2Fe(SO_4)_2$ (0.02 mmol dm^{-3}) in order to initiate the reaction. EDTA and $(NH_4)_2Fe(SO_4)_2$ were mixed prior to their addition to the reaction mixture. After 15 minutes of incubation at 37 °C, a 1.5 ml of 2.8 % cold trichloroacetic acid was added to stop the reaction. An aliquot of 1 ml of the reaction solution was mixed with 1 ml of thiobarbituric acid reagent (1 %, in 0.05 M NaOH) and then heated at 100 °C for 15 min before being left to cool to room temperature. The absorbance was determined at 532 nm against appropriate blanks.

For the hydroxyflavone acid form, we prepared a dispersion of the molecules in Arabic gum.

All experiments were carried out in triplicate and repeated three times.

The method is based on the specific reaction of deoxyribose with hydroxyl radicals generated from ascorbic acid/Fe^{2+}/EDTA/H_2O_2. This reaction produces a certain amount of malondialdehyde (MDA), which can be quantified by the reaction with thiobarbituric acid (TBA).[4]

The rate constant for the reaction of a given scavenger with hydroxyl radicals can be determined by the competition between the tested compounds and deoxyribose for hydroxyl radical. The rate constant is given by equation (1)

$$\frac{1}{A} = \frac{1}{A^\circ}\left(1 + \frac{K_s[\mathrm{S}]}{K_\mathrm{D}[\mathrm{D}] + K_\mathrm{X}}\right) \tag{1}$$

in which formula A is the absorbance value recorded at 532 nm for a given concentration of the scavenger [S], $A°$ is absorbance without the scavenger, [D] is the concentration of deoxyribose (0.6 mmol dm^{-3} in our experiments), K_D is the rate constant of deoxyribose[17] and K_s the rate constant of the studied compound; K_X is a constant dependant on the experimental conditions and represents the part of hydroxyl radicals that react with all other reagents (*e.g.*, with Fe^{2+}-EDTA, H_2O_2 …) in the assay mixture, with the exception of deoxyribose. As a result, the rate constant was expressed as:

$$\frac{A^\circ}{A} = \left(1 + \frac{K_s[\mathrm{S}]}{K_\mathrm{D}[\mathrm{D}] + K_\mathrm{X}}\right) \tag{2}$$

in which the plot of $A°/A$ against [S] allowed determining the slope a and therefore:

$$K_s = a\ (K_D \cdot [\mathrm{D}] + K_X) \tag{3}$$

Superoxide Radical Assay. – The enzymatic system of superoxide radical production was essentially described by Hu *et al.*[18] Hydroxylamine (0.2 mmol dm^{-3}), EDTA (0.1 mmol dm^{-3}) and xanthine oxidase (2.5 mg/ml) were sequentially added to a solution of hypoxanthine (0.2 mmol dm^{-3}) in a phosphate buffer (0.1 mol dm^{-3}), fixed at pH = 7.4. After incubation at 37 °C for 30 min, the dye reagent (300 μg/ml sulfanic acid, 0.75 mg/ml α-naphtylamine and 16.7 % (vol. fraction, φ) acetic acid) were added. The mix-

ture was allowed to stand for 30 min at room temperature, and the absorbance was measured at 550 nm.

For the hydroxyflavones acid form, we prepared a dispersion of the molecules in Triton X-100 (10 % (φ), in water) which does not interfere with nitrite production.

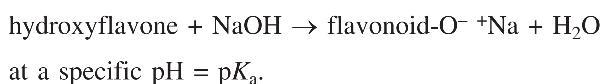
All experiments were carried out in triplicate and reproduced three times.

Values of the inhibition concentration IC_{50} of superoxide radical were determined by measuring the inhibition of the superoxide anion production at several concentrations and the concentrations of the compounds at which 50 % of the inhibition occur calculated by interpolating the 50 % inhibition point on the curve.

All hydroxyflavones and their salts were tested for their interaction with xanthine oxidase as described by Kessler *et al.* (2003).[5]

Stability of Hydroxyflavones in Alkaline Solution

Dissolution of a hydroxyflavone in alkaline aqueous solution is due to the salt formation described by the following reaction:



hydroxyflavone + NaOH $\rightarrow$ flavonoid-O^- ^+Na + H_2O

at a specific pH = pK_a.

The respective pK_as of the different –OH groups for hydroxyflavones were determined by Kadykova *et al.*,[19] Georgievskii[20] and Rybachenko.[21] The order of increasing pK_a is as follows:

8-OH < 6-OH < 3-OH < 4'-OH < 7-OH

The hydroxyflavones that we studied contained either 7-OH, and/or 4'-OH, and/or 3-OH. Their pK_as are 9.81, 8.77 and 7.65,[19] respectively.

We prepared the different hydroxyflavone salts by dissolving the appropriate hydroxyflavone in an alkaline aqueous solution at pH = 10 to avoid the risk of degradation.[19,22] The stability of hydroxyflavones in alkaline solution was studied by HPLC. In fact, two solutions of the same concentration (20 µg/ml) of the hydroxyflavone were prepared. The first solution was the methyl alcohol–water (vol. ratio 50 : 50) solution of the hydroxyflavone. The second solution was prepared as follows: the hydroxyflavone studied was dissolved in an alkaline aqueous solution at pH = 10 (formation of the corresponding salts). Two hours later, a solution of 0.1 M HCl was added until pH = 5 was obtained (recuperation of the hydroxyflavone in its acid form). The final solution was obtained by dilution of this hydroxyflavone acid solution in methyl alcohol.

The HPLC analysis of the two solutions prepared was performed under the following conditions:

- column – an RP-8 (5 µm) (Merck)
- mobile phase – a mixture of acetonitrile-water at pH = 2.3 (vol. ratio 30 : 70)
- flow rate – 1.3 ml/min
- detection with a UV/Vis detector at λ_{max} of each hydroxyflavone.

Stability of Hydroxyflavone Salts in Phosphate Buffer at pH = 7.4

The reaction mixture used to study the free hydroxyl radical scavenging was a phosphate buffer of pH = 7.4.

The stability of the different hydroxyflavone salts in the reaction mixture was studied during a fixed reaction time (15 min). We plotted the UV/Vis absorption spectrum of each hydroxyflavone salt solution in the phosphate buffer, pH = 7.4, for the two extreme concentrations of the range used in the scavenging study (0.05 mmol dm^{-3} and 0.5 mmol dm^{-3}), and at both times (t = 0 and 15 min).

RESULTS AND DISCUSSION

Study of the Stability of Hydroxyflavone Salts in Phosphate Buffer pH = 7.4

We observed that the UV/Vis spectrum plotted at time t = 15 min for quercetin salt in phosphate buffer, pH = 7.4, is exactly the same as that plotted at time t = 0 min; moreover, there is no difference between the absorbance value measured at λ_{max} at t = 15 min and t = 0. The same results were obtained for both 0.05 mmol dm^{-3} and 0.5 mmol dm^{-3} of the quercetin salt. All the other ten hydroxyflavone salts studied gave the same results as that observed for the quercetin salt. These results demonstrate clearly that the hydroxyflavone salts prepared at pH = 10 will be stable when added to the reaction mixture at pH = 7.4 during the reaction time (t = 15 min).

Stability of Hydroxyflavones in Alkaline Solution

Under the experimental conditions described above, the chromatograms obtained for the two solutions prepared for each hydroxyflavone gave the same peak with the same retention time (Table II) and the same peak area. In addition, no other peak was observed in the chromatograms obtained for the second solution, which was prepared *via* the salt form. These results demonstrate that the hydroxyflavones studied are stable under alkaline conditions used.

TABLE II. Retention time, t_{ret}, of hydroxyflavones

Compound	t_{ret}/min	Compound	t_{ret}/min
Quercetin	5.1	Fisetin	5.1
MonoHEQ	4.5	Galangin	5.3
DiHEQ	4.9	Kaempferol	5.2
Rutin	2.6	Luteolin	5.1
MonoHER	2.6	7-Hydroxyflavone	5.9
DiHER	2.6		

Scavenging Activity of Free Hydroxyl Radical

The relative antioxidant activities against aqueous phase radicals in the Fenton reaction system of the range of hydroxyflavones and their salts studied here were determined by the hydroxyl radical scavenging rate constant (Table III).

First, hydroxyflavone salts are stronger antioxidants than hydroxyflavones themselves because of their solubility and substitution of the 3-hydroxyl group, which has been described as pro-oxidant by several authors.[23,24,25] For these reasons, our discussion is focused on the salt forms of hydroxyflavones.

The most effective antioxidants in their salt forms (quercetin, rutin, monoHEQ, monoHER, diHEQ and diHER) have the same hydroxyl configuration, but substituted in different ways and all have the 3'-OH free group. Evidence for the requirement of the 3'-OH free group is seen in the effects on the antioxidant activity of specific structural changes. For example, the antioxidant activity for the quercetin salt or the monoHEQ salt is lowered when the hydroxyflavones lose the 3'-OH free group, like the kaempferol or galangin salts.

The presence of substituted 4'-OH does not change the antioxidant activity, as demonstrated in flavonols. In fact, insertion of an additional 4'-OH group into galangin produces the kaempferol configuration, which has approximately the same K_s.

3-OH is an excellent choice for substitution because of its acidity in spite of its pro-oxidant properties. Indeed, 3-OH undergoes substitution in quercetin and rutin salts by Na or rutinose, which confers the free radical scavenging activity. Furthermore, the presence of hydrogen at the 3 position in luteolin salt makes it a poorer scavenger. The type of substitution (Na or rutinose) has little effect on this activity.

Contribution of the 7,5 di-OH substitution in the A ring (Table I) is not significant, as shown by the results; the lowest antioxidant activity is attributed to the 7-hydroxyflavone salt with only one O-Na free group at the 7 position. In the case of the fisetin salt, there is clearly very little change on the insertion of the C5-OH group, like in the quercetin salt.

In conclusion, it is known that the hydroxyl radical scavenging activity of hydroxyflavones and the salts studied is due to the catechol groups, as we showed before. This activity can increase or decrease in dependence on the patterns. For example, the absence of the OH group at the 3 position decreases the scavenging activity of the salt form of quercetin. But, its presence increases the pro-oxidant activity. This fact results from two arguments. On the one hand, the presence of the OH group at the 3 position increases the energy of the highest occupied molecular orbital and leads to a very pro-oxidant molecule at the A ring. This is supported the investigations done by Rietjens *et al.* in 2001 where they showed the A ring as the glutathionyl adduct site when the hydroxyl group was present at the 3 position. In the opposite case, the site of glutathionyl addition was shown in the B ring where the catechol group was present. On the other hand, the pH of the solution or of salt formation has a very strong impact on this activity. In fact, the salt form of these compounds shows a very high hydroxyl radical scavenging activity. This can be explained by the regioselectivity of the active site, which varies in dependence on the pH of the solution or the pH of the molecular protonation. With a similar aim, Rietjens *et al.* demonstrated the pH dependence on the active site selectivity of glutathionyl addition, in the B ring at alkaline pH and in the C ring at acid or neutral pH.[26]

Moreover, these facts explained the efficient activity in the B ring at alkaline pH and thus the difference of the scavenging activity encountered between the salt and the parent hydroxyflavones.

TABLE III. Rate constants of hydroxyl radical scavenging (K_s)

Compounds	K_s/mol^{-1} dm^3 s^{-1}	Compounds	K_s/mol^{-1} dm^3 s^{-1}	
Quercetin Salt	$(1.24 \pm 0.11) \times 10^{25}$	Quercetin		u.r.[a]
MonoHEQ Salt	$(1.21 \pm 0.15) \times 10^{25}$	MonoHEQ		u.r.[a]
DiHEQ Salt	$(1.20 \pm 0.18) \times 10^{25}$	DiHEQ		u.r.[a]
Rutin Salt	$(1.32 \pm 0.18) \times 10^{25}$	Rutin	$(5.2 \pm 0.2) \times 10^{24}$	
MonoHER Salt	$(1.32 \pm 0.13) \times 10^{25}$	MonoHER	$(5.4 \pm 0.3) \times 10^{24}$	
DiHER Salt	$(1.27 \pm 0.21) \times 10^{25}$	DiHER	$(5.3 \pm 0.5) \times 10^{24}$	
Fisetin Salt	$(1.35 \pm 0.02) \times 10^{25}$	Fisetin		u.r.[a]
Galangin Salt	$(0.50 \pm 0.03) \times 10^{25}$	Galangin		u.r.[a]
Kaempferol Salt	$(0.50 \pm 0.07) \times 10^{25}$	Kaempferol		u.r.[a]
Luteolin Salt	$(0.80 \pm 0.01) \times 10^{25}$	Luteolin	$(2.5 \pm 0.6) \times 10^{24}$	
7-hydroxyflavone Salt	$(0.09 \pm 0.01) \times 10^{25}$	7-hydroxyflavone		u.r.[a]

[a] u.r., unexplainable results.

Scavenging Activity of Superoxide Radicals

All the hydroxyflavones examined in this study were capable of scavenging superoxide radicals with a certain relationship between the structure and the scavenging efficiency. Table IV shows the IC_{50} values, which represent the concentration of hydroxyflavones capable of scavenging 50 % of the superoxide radicals generated by the hypoxanthine/xanthine oxidase system.

The most effective hydroxyflavones were in this order: group 1 (luteolin, quercetin, monoHEQ, rutin, monoHER, fisetin and their salts) > group 2 (diHEQ, diHER and their salts) > group 3 (galangin, kaempferol, 7-hydroxyflavone and their salts).

As a whole, the presence of hydroxyl groups, free or substituted by ONa in the B ring (Table I), is essential for the superoxide scavenging properties of these compounds. In fact, both hydroxyflavones and the salt form of group 3 showed a poor superoxide scavenging activity due to the absence of the hydroxyl group at the 3' position. All this is further supported by the behavior of the second group, where the molecules contain the hydroxyl in the 3' position and where the 4' position is substituted by OC_2H_5OH, which showed a better scavenging effect. Moreover, the first group, where molecules contain two hydroxyl groups in the B ring (Table I) or an ONa and a hydroxyl groups, showed a stronger scavenging activity.

Introduction of OH, O-rutinose or ONa group into the 3 position of luteolin and its salt (formation of quercetin, rutin and their salt structures) did not improve the superoxide scavenging activity.

CONCLUSION

In the present study, we investigated which form (hydroxyl structure or sodium salts) is most important for a hydroxyflavone to be a good scavenger. In order to measure the true scavenging activity without interfering factors, such as solvents' activities, the pro-oxidant or antioxidant (like complexation of Fe^{2+}) activity of the OH groups, we used different salt forms of hydroxyflavones in aqueous solution. The hydroxyl scavenging activity was performed with the Fenton model; all the results for hydroxyflavone sodium salts have shown linear plots with an offset of the line practically equal to unity, which means that the rates of hydroxyl radical formation are not perturbed by the presence of the tested substance and that there is no interaction between the hydroxyflavones and Fe^{2+}, H_2O_2 and ascorbic acid. We demonstrated that the salt form of quercetin shows a very high scavenging activity without pro-oxidant activity. This result can be extrapolated to many more hydroxyflavone salts.

Thus, rutin, which is quercetin with an ose substitution at the 3 position, is a more interesting scavenger than quercetin itself. This compound has been shown to have no pro-oxidant activities either in the presence or absence of iron,[27] and its sodium salt is a better scavenger.

In conclusion, in the case of therapeutically interesting hydroxyflavones, the flavonol basic structure is not essential for good scavenging activity. However, a salt form of hydroxyflavones, especially with the 3' free hydroxyl group of catechol in ring B, is sufficient to render an interesting compound for physiological and therapeutic applications.

These salts contained the 4' and 3 hydroxyl groups substituted with sodium or a rutinose group, which allowed the compounds to become soluble in aqueous medium and possess a stronger scavenging activity. In such a case, the reduction potential was increased and the pro-oxidant activity disappeared.

The superoxide radical scavenging activity of these salts is as good as their corresponding hydroxyflavones and their hydroxyl radical scavenging activity is increased at least two times.

TABLE IV. IC_{50} values – concentration of hydroxyflavones for 50 % superoxide radical scavenging

Compounds	IC_{50}/μmol dm^{-3}	Compounds	IC_{50}/μmol dm^{-3}
Quercetin salt	18 ± 5	Quercetin	21 ± 3
MonoHEQ salt	19 ± 2	MonoHEQ	18 ± 6
DiHEQ salt	48 ± 7	DiHEQ	57 ± 9
Rutin salt	19 ± 3	Rutin	19 ± 4
MonoHER salt	19 ± 5	MonoHER	19 ± 3
DiHER salt	50 ± 4	DiHER	53 ± 6
Fisetin salt	19 ± 2	Fisetin	19 ± 3
Galangin salt	> 80	Galangin	> 80
Kaempferol salt	> 80	Kaempferol	> 80
Luteolin salt	18 ± 4	Luteolin	18 ± 7
7-Hydroxyflavone salt	> 80	7-Hydroxyflavone	> 80

[a]There is no modification of the xanthine oxidase activity by the molecules, except for MonoHEQ at 100 μmol dm^{-3}.

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SAŽETAK

Učinkovitost natrijevih soli hidroksiflavona kao hvatača slobodnih radikala u usporedbi s aktivnošću samih hidroksiflavona

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Različita istraživanja pokazala su da je bioaktivnost hidroksiflavona posljedica njihove antioksidacijske aktivnosti i sposobnosti hvatanja slobodnih radikala. Do sada je mnogo zanimanja iskazano za odnos između strukture i aktivnosti; međutim, osnovni problemi na koje se naišlo u tim istraživanjima slaba je topljivost nekih hidroksiflavona u vodenoj otopini i prooksidacijski karakter hidroksilne skupine u položaju 3. U ovom istraživanju ti problemi razriješeni su pripremom odgovarajućih natrijevih soli hidroksiflavona. Tako su proučavani učinkovitost soli raznih hidroksiflavona kao hvatača slobodnih hidroksilnih radikala $(OH)^{\bullet}$ modelom Fentonove reakcije te kao hvatača superoksid radikala $(O_2)^{\bullet-}$ modelom ksantin oksidaze. Dobiveni rezultati jasno pokazuju da soli hidroksiflavona najmanje dva puta učinkovitije hvataju hidroksilne radikale $(OH)^{\bullet}$ nego što to čine odgovarajući hidroksiflavoni. Učinkovitost hvatanja superoksid radikala $(O_2)^{\bullet-}$ jednaka je kako za soli hidroksiflavona tako i za same hidroksiflavone. Osim toga opaženo je da za dobar učinak hvatanja radikala, OH skupina u položaju 3' treba biti slobodna, da samo hidroksilne skupine 3 i 4' trebaju biti supstituirane natrijem, dok natrijeva sol hidroksiflavona u položaju 7 nema znatnijega utjecaja na hvatanje radikala. Soli hidroksiflavona, spojevi koji pokazuju hidrofilni karakter, zanimljivi su hvatači slobodnih radikala.